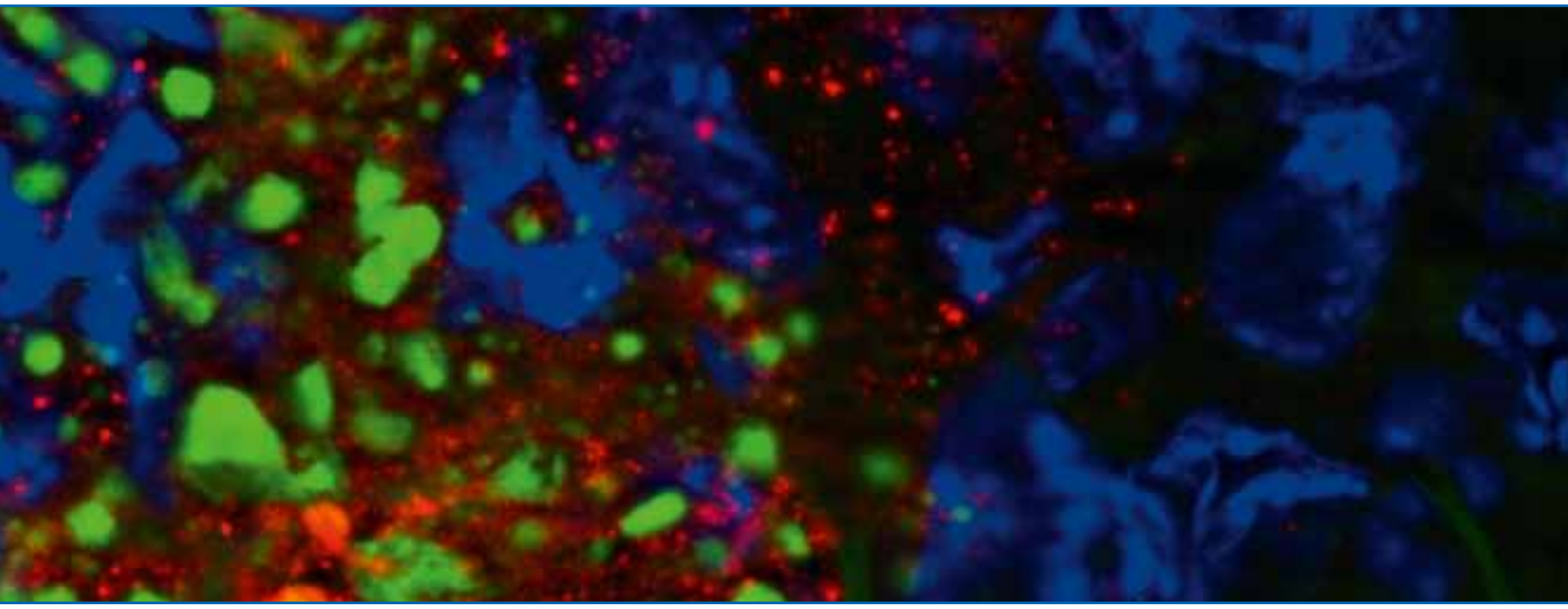
Forschungszentrum Borstel
Leibniz Lungenzentrum

FORSCHUNGSBERICHT 2017/2018



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Sehr geehrte Leserin, sehr geehrter Leser ...

Das FZB ist und bleibt - nach erfolgreicher Evaluierung im Leibniz-Verfahren - das **Lungen-Forschungszentrum der Leibniz-Gemeinschaft**. Es widmet sich der Erforschung und Behandlung von entzündlichen Lungenerkrankungen, mit einer besonderen Schwerpunktsetzung auf das allergische Asthma und die Tuberkulose (TB). Es genießt zunehmende Sichtbarkeit und Anerkennung für die Aufklärung von Mechanismen der Asthma-Prädisposition und für die molekulare Charakterisierung von Allergenen, Allergie-präventiven Substanzen sowie von bakteriellen Infektionserregern. Es ist international bekannt für seine Arbeiten zur Molekularepidemiologie, Resistenz und Pathogenese der TB und zur individualisierten Optimierung der Antibiotika-Therapie bei M/XDR-TB-Patienten. Das FZB ist Sitz des Nationalen Referenzzentrums für Mykobakterien und Supranationalen Referenzzentrums der WHO.

Die beiden letzten Jahre waren maßgeblich geprägt durch die Vorbereitungen von zwei Großprojekten: die **Baumaßnahmen auf dem Campus** und die **Evaluierung des Zentrums** im Februar 2019 durch die Leibniz-Gemeinschaft.

Die Zuwendungsgeber des Bundes (Bundesgesundheitsministerium) und des Landes Schleswig-Holstein (Ministerium für Bildung, Wissenschaft und Kunst) haben in Anerkennung der überregionalen Bedeutung des Zentrums bereits 2015/2016 entschieden, das FZB mit modernen Forschungsflächen auszustatten (Zentraler Laborneubau mit S3-Einheit, Tierzucht und Tierhaltung, Neubau des Nationalen Referenzzentrums für Mykobakterien), deren Errichtung insgesamt mindestens 53 Mio. Euro kosten und voraussichtlich 2021/2022

Dear Readers ...

The FZB is and - following a thorough evaluation according to Leibniz standards - will remain the **Lung Research Center of the Leibniz Association**. It is dedicated to the research and treatment of inflammatory lung diseases, with a particular focus on allergic asthma and tuberculosis (TB). It enjoys increasing visibility and recognition in asthma and allergy research, e.g. for elucidating the mechanisms of asthma predisposition and for the molecular characterization of allergens, allergy-preventive substances and bacterial pathogens. It is internationally renowned for its work on molecular epidemiology, resistance and pathogenesis of TB and individualized optimization of antibiotic therapy in M/XDR-TB patients. The FZB is the homestead of the National Reference Center for Mycobacteria and the Supranational Reference Center of the WHO.

The last two years were marked by the preparations of two major projects: the **construction measures on the campus** and the **evaluation of the Center** by the Leibniz Association in February 2019.

In recognition of the supra-regional importance of the Center, the sponsors of the Federal Government (Federal Ministry of Health) and the State of Schleswig-Holstein (Ministry of Education, Science and the Arts) decided as early as 2015/2016 to equip the FZB with modern research areas (new central laboratory building with S3 unit, animal breeding and husbandry, new construction of

abgeschlossen sein wird. Die verantwortliche Projektleitung liegt für den Zentralen Laborneubau bei Prof. Dr. Frank Petersen, für das NRZ bei Dr. Susanne Homolka, denen an dieser Stelle ausdrücklich für ihren unermüdlichen und hochkompetenten Einsatz gedankt sein soll.

Im Zuge der Evaluierungsvorbereitungen hat das FZB ein ambitioniertes Zukunftsprogramm in beiden Programmbereichen unter dem gemeinsamen Motto "Control and Care" (Kontrolle der Erkrankung und Versorgung der Erkrankten) entwickelt, mit Querschnittsthemen zu "evolutionärer Medizin" und "personalisierter Medizin". Zur finanziellen Umsetzung der ambitionierten Forschungsziele wird das FZB auch einen kleinen strategischen Sondertatbestand bei der Gemeinsamen Wissenschaftskommission des Bundes und der Länder beantragen. Aus ersten Rückkopplungen wissen wir (der schriftliche Bericht steht bei Drucklegung noch aus), dass sowohl die Leistungsbilanz als auch das Strategiekonzept des FZB (einschließlich der inhaltlichen Aufstellung des Sondertatbestands) sehr positiv von der Bewertungskommission beurteilt wurden.

Der vorliegende Forschungsbericht informiert in kompakter Form über die wissenschaftlichen Aktivitäten des Forschungszentrums Borstel in den Jahren 2017 und 2018. Der Magazinteil berichtet über Aktivitäten des Zentrums, besondere Initiativen, bewegende Ereignisse und wichtige Forschungsergebnisse. Exemplarisch ausgewählte Projekte der Forschungsteams zeigen sowohl die Kernkompetenzen auf als auch die erbrachten Leistungen.

Auf diese Weise möchten wir nicht nur versierten Kolleginnen und Kollegen, sondern auch einer breiteren Öffentlichkeit unsere wissenschaftlichen Fragestellungen und Ziele näherbringen, und darüber hinaus auch die Fortschritte aufzeigen, die wir in den beiden vergangenen Jahren bei der Lösung gesellschaftlich relevanter medizinischer Probleme erzielt haben.

Veränderung bleibt unser Motto – für eine exzellente Forschung zum Nutzen der Gesellschaft! Change is positive! Stolz sind wir auf das Engagement aller unserer Mitarbeiterinnen und Mitarbeiter, ein herzlicher Dank gilt allen Freunden und Förderern aus Wissenschaft, Politik und Wirtschaft. Mit Ihrer Unterstützung blicken wir weiterhin zuversichtlich in die Zukunft!

Stefan Ehlers
Susanne Krauss-Etschmann, Christoph Lange und Ulrich Schaible

the National Reference Center for Mycobacteria), the construction of which will cost a total of at least 53 million euros and is expected to be completed in 2021/2022.

Prof. Frank Petersen is responsible for the new central laboratory building and Dr. Susanne Homolka for the NRZ. We would like to take this opportunity to thank both of them for their tireless and highly competent efforts.

As part of its evaluation preparations, the FZB has developed an ambitious program for the future in both programmatic focus areas under the joint motto "Control and Care", with cross-cutting topics on "evolutionary medicine" and "personalized medicine". For the financial implementation of the ambitious research goals, the FZB will also apply for a strategic "extraordinary item of expenditure (STB)" to the Joint Science Commission of the Federal Government and the Länder. We know from initial feedback (we are still awaiting the final written report at the time of going to press) that both the FZB's current scientific performance and its strategic concept (including the STB) reached high marks with the evaluation commission.

The present research report informs in compact form about the scientific activities of the Research Center Borstel in the years 2017 and 2018. The magazine part highlights activities of the Center, special initiatives, moving events and important research results. Selected projects of the research teams show the core competences as well as the services rendered.

In this way, we want to bring not only our experienced colleagues, but also the general public closer to our scientific questions and goals, and also to demonstrate the progress we have made in the past two years in solving medical problems relevant to society.

Change continues to be our motto - for excellent research for the benefit of society! We are proud of the outstanding commitment of all our employees and would like to express our heartfelt thanks to all our friends and sponsors from science, politics and business. With your support we are confident about future developments at FZB!

Stefan Ehlers
Susanne Krauss-Etschmann, Christoph Lange und Ulrich Schaible

Nach der Evaluierung ist vor der Evaluierung!

Sieben ist die magische Zahl für Leibniz-Einrichtungen. Alle sieben Jahre erfolgt eine Prüfung auf Leistungs- und Zukunftsfähigkeit, um sicherzustellen, dass Leibniz-Institute ihre gesellschaftlich relevante Forschungsmission vollumfänglich erfüllen. Bestehen sie die Prüfung, erfolgt eine finanzielle Förderung durch Bund und die Ländergemeinschaft für weitere sieben Jahre; fallen sie durch, werden sie geschlossen.

Das Leibniz Lungenzentrum Borstel hat im Februar 2019 die Evaluierung durch eine hochkompetente Kommission mit Glanz und Gloria absolviert. Das Zentrum hatte aber auch wirklich eine bemerkenswerte Leistungsbilanz vorzuweisen: In den Jahren 2017/2018 hat das FZB 380 wissenschaftliche Artikel (peer review) mit knapp 2500 Impactfaktoren (IF) veröffentlicht. Während es in den Jahren vor 2012 am FZB nur sehr wenige **Publikationen** in hoch- und höchstrangigen Journalen gab, stieg der Anteil der hochrangigen Publikationen (IF>10) am FZB kontinuierlich an und erreichte 2017 und 2018 im Schnitt eine Quote von knapp 20 % aller Veröffentlichungen; 5 % der Veröffentlichungen haben inzwischen einen IF>20.

Das FZB ist umfangreich und langfristig durch wissenschaftliche **Drittmittel** gefördert (Volumen 2017/2018 insgesamt: 15,7 Mio. Euro), u.a. in den Deutschen Zentren für Gesundheitsforschung (Deutsches Zentrum für Lungenforschung, DZL und Deutsches Zentrum für Infektionsforschung, DZIF), in weiteren BMBF-Verbünden, EU-Konsortien, Stiftungen/NGOs, im Exzellenzcluster

Nach der Evaluierung ist vor der Evaluierung!

Stefan Ehlers

(2019-2025: Precision Medicine in Chronic Inflammation) sowie in regionalen und nationalen DFG-Verbünden. Die insgesamt eingeworbenen Drittmittel (aus der Förderung von wissenschaftlichen Projekten und aus Service-Einnahmen) für das Jahr 2017 und 2018 betrug knapp 50% der Zuwendungssumme durch Bund und Länder.

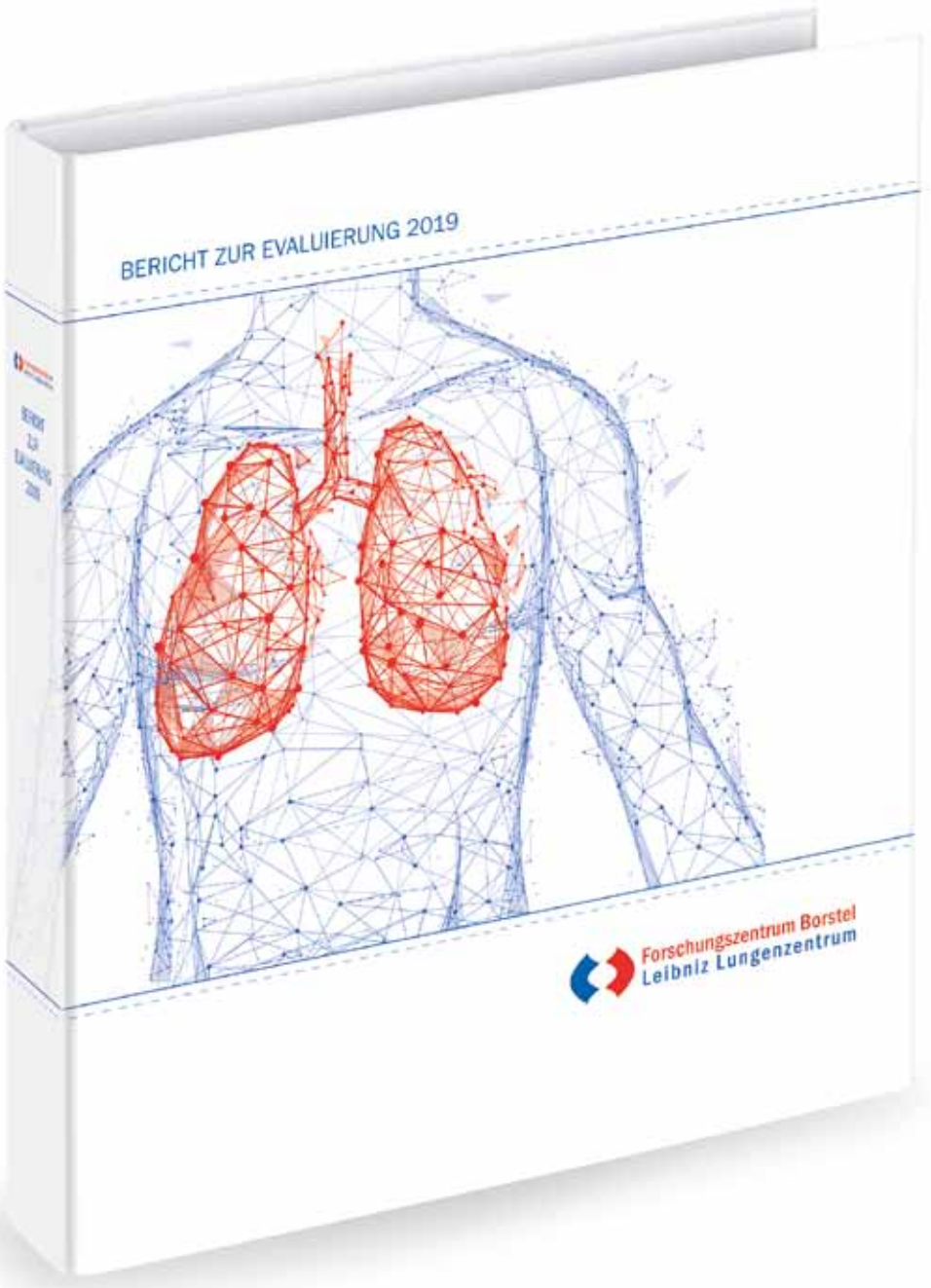
Als "Belohnung" für diese außergewöhnliche Performance und eine ausgewogene, strategische Zukunftsplanung wird nun sogar die Möglichkeit eröffnet, dass im Rahmen eines sog. Strategischen Sondertatbestands ab 2021 der Kernetat um bis zu 15% erhöht wird, damit neue passgenaue Innovations- und Strukturgruppen am Zentrum eingerichtet werden können.

Das war ein hartes Stück Arbeit für alle Beteiligten. In den letzten sieben Jahren haben sich die Programmbereiche neu aufgestellt: die Asthma und Allergieforschung hat ein einheitliches Profil entwickelt, mit Kernthemen (Initiation und Exazerbation), Schwerpunkten (life course models, mikrobielle Modulation, Autoimmunität, Allergie) und einer neuen Direktorin (Susanne Krauss-Etschmann). Die Infektionsforschung hat noch stringenter auf die Tuberkulose fokussiert, mit molekularer Epidemiologie, funktioneller Strukturanalytik, wirtsgerichteten Therapien und individualisierter Antibiotika-Behandlung als Kernthemen. Die Internationalisierung ist mit Sieben-Meilen-Schritten vorangekommen (Partnerlabore in China, Namibia, Moldau) und die Leibniz-Agenda mit Beteiligungen an Leibniz-Forschungsverbünden (INFECTIONS '21, Gesundheitstechnologien) und dem Leibniz-Wissenschaftscampus Evolutionäre Medizin der Lunge (gemeinsam mit der Uni Kiel und dem MPI für Evolutionsbiologie in Plön) umfänglich umgesetzt.

In diesem Strategieprozess hat das gesamte Zentrum **zwei Querschnittsbereiche** definiert: evolutionäre Medizin und personalisierte Medizin. Die Ausgestaltung dieser "overarching topics" wird vom Wissenschaftlichen Beirat und von der Evaluierungskommission als wegweisend betrachtet und muss nun mit Leben (und Forscherpersönlichkeiten) erfüllt werden.

Allen, die sich bei der Evaluierung eingebracht haben und die positive Rezeption des Zentrums mitgestaltet haben, möchte ich an dieser Stelle meinen **herzlichen Dank** aussprechen. Der Erfolg bei der Evaluierung war nicht nur, aber auch eine Teamleistung, und die Gutachter/innen haben zurecht die Leistungsbereitschaft der Beschäftigten gewürdigt. Für ihren nahezu übermenschlichen Einsatz bei der akribischen Vorbereitung der Unterlagen und der Durchführung der Evaluierungsveranstaltung selbst gebührt Bettina Brand höchstes Lob !

Nach der Evaluierung ist vor der Evaluierung!



Nach der Evaluierung ist vor der Evaluierung!

Stefan Ehlers

Nach der ersten **Euphorie** folgt naturgemäß **Ernüchterung**, nämlich die Erkenntnis, dass die Herausforderungen für das Zentrum in 2019 nicht kleiner geworden sind. Daher gilt: "Nach der Evaluierung ist vor der Evaluierung", und das FZB muss und wird in den nächsten Jahren die folgenden Themen prioritär bearbeiten:

1. Wissenschaft: Implementation personalisierter Medizin in Borstel und in der Welt. Neue Konzepte in der Grundlagenforschung für Surveillance, Diagnostik, Prävention bei Asthma, Allergien und Infektionen. Ein neuer Schwerpunkt ist die Prävention durch z. B. Mikrobiom-Modulation, Immunintervention und Präzisions-prophxylaxe, der auch mit einer Verstärkung im Leitungspersonal einhergehen muss.

2. Translation: Die Existenzfähigkeit der Medizinischen Klinik muss durch Verbünde und verbindliche Verträge (z.B. mit dem UKSH) langfristig gesichert werden. Gerade am Beispiel der Tuberkulose hat die Medizinische Klinik vorbildlich gezeigt, was eine Forschungsklinik für die Verbesserung der Diagnostik und Therapie beitragen kann.

3. Infrastruktur: Die Baumaßnahmen für das neue zentrale Laborgebäude (Leibniz-Respiratorium und Nationales Referenz-zentrum für Mykobakterien) sind in vollem Gange. Ein Master-plan für die Gebäude Parkallee 3-11 steht noch aus; hier ist der Umzug in eine zu sanierende Parkallee 22 avisiert – ein Vorhaben, für das allerdings (bei inzwischen fallenden Steuereinnahmen des Landes) die umfängliche Mittelakquisition nicht leichter wird.

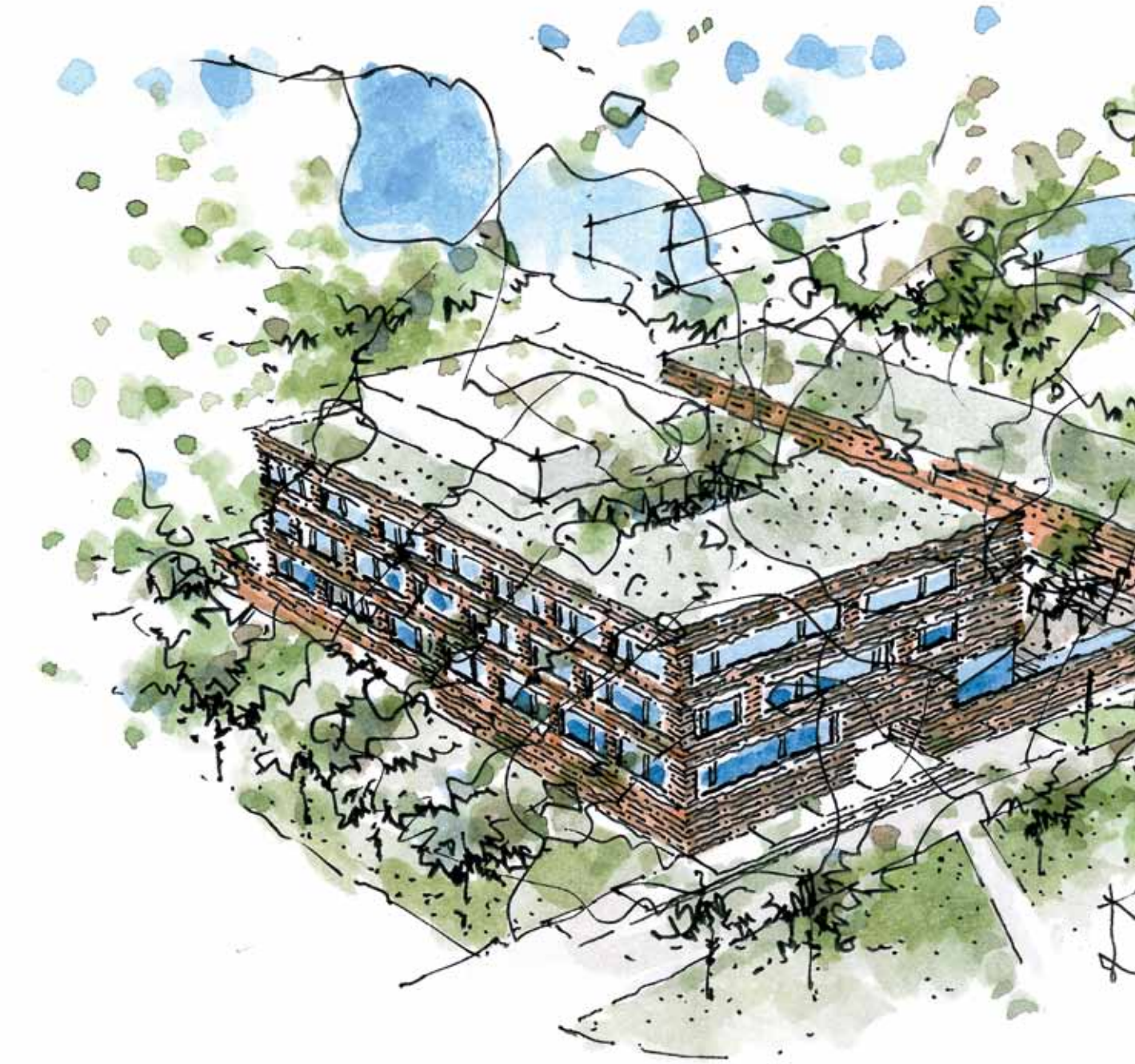
4. Zentrumskultur: die Verbindlichkeit der getroffenen Vereinbarung zur Mitarbeitergesprächen, zur Belastungs- und Gefährdungs-beurteilung, zum respektvollen Umgang miteinander, zu Verein-barkeits- und Gleichstellungsthemen, zur Unterstützung bei der Karriereplanung und Fortbildungswünschen aller Beschäftigter muss nachhaltig sichergestellt werden.

5. Governance: hier steht die Prüfung auf Managementfähigkeiten der Leitungspersonen und die Reflexion über die Konfiguration von Leitungsgremien auf dem Programm, um zielstrebig, flexibel und effektiv handlungsfähig zu bleiben.

Ich persönlich kann mit Sicherheit (und einer gewissen entspannten Genugtuung) sagen: 2019 war die letzte Evaluierung des Zentrums, die unter meiner Leitung erfolgt ist. Als Zentrumsdirektor wünsche ich mir eine reibungslose Stabübergabe an meinen Nachfolger oder meine Nachfolgerin im Amt, idealerweise schon vor dem Audit durch den Wissenschaftlichen Beirat in 2022, damit das Führungsteam im Kollegium sich rechtzeitig auf gemeinsame Positionen verständigt und das Zentrum geschlossen zu neuen Ufern lenkt. Die Ausgangslage ist jedenfalls besser als je zuvor: die Wissenschaft ist superb und strategisch bestens verankert, die Infrastruktur befindet sich in der Konsolidierung oder Erneuerung, die Finanzen werden voraussichtlich deutlich aufgestockt, und die neuen Ideen zur strukturellen Bewältigung der Herausforderungen sind zukunftsweisend.

2022 feiert das FZB sein 75-jähriges Bestehen. Wir blicken zurück auf einen glanzvollen Aufstieg einer ursprünglich als Tuberkulose-Forschungsinstitut gegründeten Einrichtung, die nach einer völligen Neuaufstellung als Lungenzentrum mit klarer Schwerpunktsetzung und Handlungsstrang-artigem Aufbau seiner im Kern translational ausgerichteten Programmbereiche sowohl die Zuwendungsgeber aus Bund und Land als auch die Gutachter/innen aus dem In- und Ausland vollends überzeugt hat. Strukturell und wissenschaftlich reicht das nicht nur für die nächsten 7 Jahre – ich finde, dass in 2022 das Motto daher sein sollte:

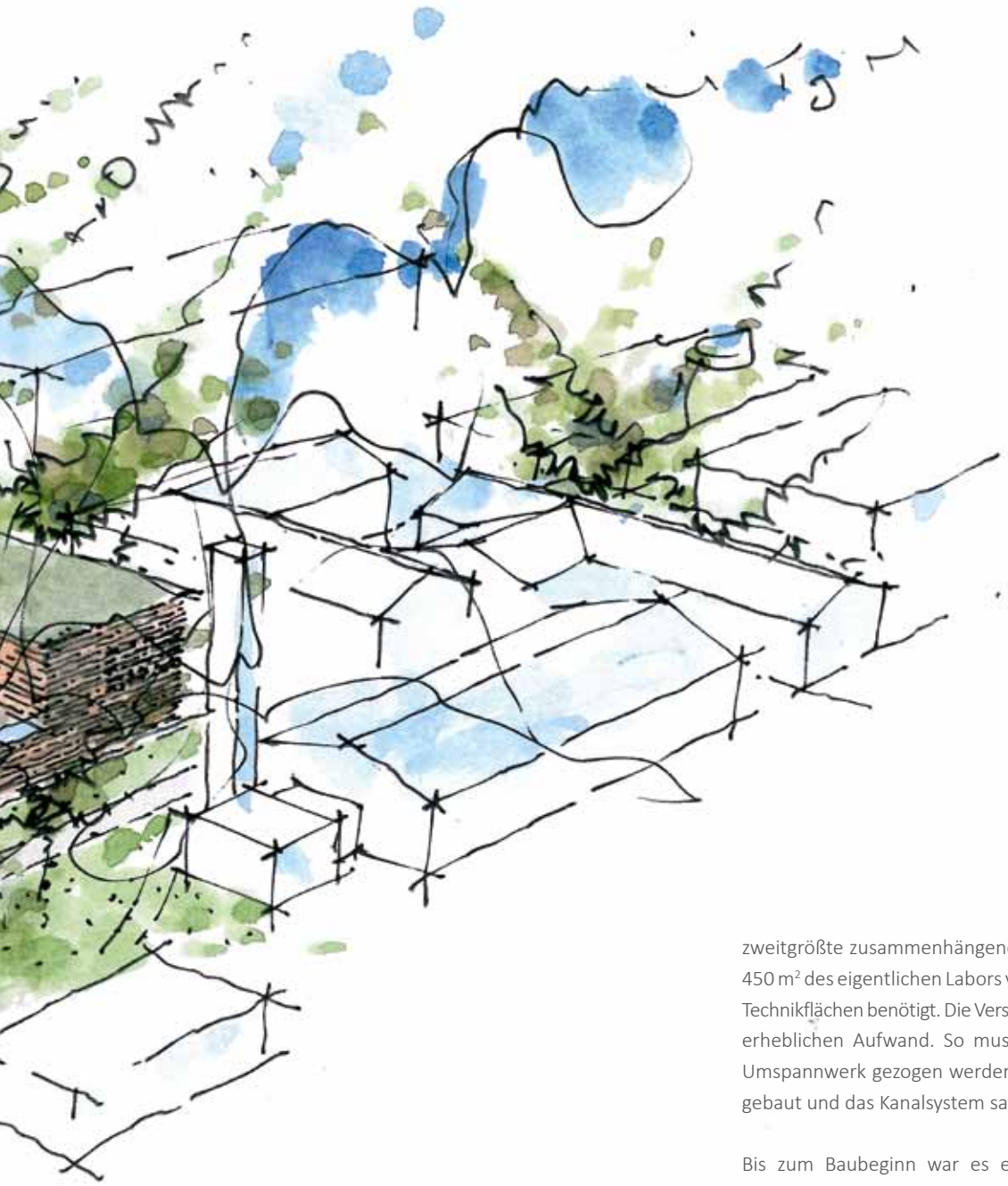
75 weitere Jahre – weil wir es wert sind !



"Parkallee X"- Finale Variante der Vorplanung
Abb. © RDS-Partner / Itten + Brechbühl

Leibniz-Respiratorium – eine kleine Chronologie

Frank Petersen



Am **15.04.2019** fand, lang erwartet und fast unbemerkt, der **Spatenstich** zu unserem neuen Laborgebäude "Leibniz-Respiratorium" statt. Damit wird Ende 2022 hier eines der modernsten und komplexesten Forschungsgebäude Norddeutschlands in Betrieb gehen. Die Grundfläche des Gebäudes wird mit ca. 3600 m^2 fast die Größe eines Fußballfeldes einnehmen. Aneinandergelegt würden die zu verbauenden Elektrokabel von Borstel bis nach Bremen reichen. Die neue S3-Anlage ist mit einer Gesamtfläche von 1350 m^2 nach dem Friedrich-Löffler Institut auf der Insel Riems wohl die zweitgrößte zusammenhängende S3-Anlage Deutschlands. Um die 450 m^2 des eigentlichen Labors versorgen zu können, werden 900 m^2 Technikflächen benötigt. Die Versorgung des LRB und des NRZ erfordert erheblichen Aufwand. So muss eine neue Starkstromtrasse vom Umspannwerk gezogen werden, eine neue Wärmeversorgung aufgebaut und das Kanalsystem saniert und ausgebaut werden.

Bis zum Baubeginn war es ein langer und komplizierter Weg, welcher hier noch einmal nachgezeichnet werden soll.

In Folge des sehr kalten Winters und sehr heißen Sommer 2013 kam es in der Parkallee 22 zu massiven technischen Problemen in der Lüftung der Tierhaltung und im Laborbetrieb, welche zu erheblichen Beeinträchtigungen bei den Beschäftigten führten. Um diese Mängel zu dokumentieren, gab das FZB im **August 2013** eine **Machbarkeitsstudie** in Auftrag, die den Sanierungsbedarf an der PA22 und deren Zukunftsfähigkeit untersuchen sollte. Nach deren Ergebnissen lag ein zwar erheblicher Sanierungsbedarf vor, dem Gebäude wurde jedoch eine grundsätzliche Sanierbarkeit

LRB - Planungsstand Bauausführung
Abb. © RDS-Partner / Itten + Brechbühl

Spatenstich am 15.04.2019
Foto: F. Petersen

Leibniz-Respiratorium – eine kleine Chronologie

Frank Petersen

attestiert. Die geschätzten Kosten für eine Sanierung in Höhe von **9,6 Mio Euro** wurden dem Kuratorium im November 2013 vorgelegt, welches ein vertieftes Sanierungskonzept beauftragte. In dem im **Sommer 2014** fertiggestellten Konzept lagen die Kosten bereits bei 15 Mio Euro. Dieser Ansatz wurde nachfolgend von den Zuwendungsgebern aus Bund und Land in einem Zuwendungsbeschluss als Kostenobergrenze festgelegt. Im Rahmen der dann über ein Jahr lang andauernden Planungen musste das FZB jedoch feststellen, dass der Kostenrahmen bereits zu diesem Zeitpunkt mit **über 18 Mio Euro** deutlich überschritten werden wurde. Die vom damaligen Generalplaner konzipierte Sanierung im laufenden Betrieb erwies sich als technisch nicht durchführbar und musste aufgegeben werden.

Am **22.09.2015** informierten Stefan Ehlers und Frank Petersen den Kurator, Herrn Staatssekretär Fischer, über den Planungszustand der PA22 und die Notwendigkeit für die Errichtung eines neuen Laborbaus. Dieses Gespräch, welches man als die **Geburtsstunde des neuen zentralen Laborgebäudes** bezeichnen kann, führte zu einem Dringlichkeitsschreiben von Herrn Fischer an das Kuratorium, welches auf seiner Sitzung am **07.12.2015** den Beschluss für den Bau eines neuen Laborgebäudes mit einer angestrebten Zuwendung in Höhe von **40 Mio Euro** traf.

Auch ohne Vorliegen eines entsprechenden Zuwendungsbescheids, welcher erst nach Prüfung der Entwurfsplanung erteilt wird, wurde vom FZB bereits im **Januar 2016** parallel zu den Ausschreibungen von Projektsteuerer und Generalplaner mit der **Leistungsphase 1** (Grundlagenermittlung) eigeninitiativ begonnen. Im **Juni 2016** stand das gesamte Planungsteam zur Verfügung und konnte seine Arbeit aufnehmen. Zeitgleich genehmigte die Bauaufsichtsbehörde grundsätzlich eine Voranfrage auf Errichtung eines Laborbaus auf dem vorgesehenen Grundstück. In dieser Phase erfolgte auch die Namensgebung: während sich der Vorschlag PA (für Parkallee) X (noch ohne Nummer), kurz PAX, nicht durchsetzen konnte, fiel die Wahl auf "Respiratorium" (von lat.: respirare = atmen, also das Gebäude, in dem für die Lungengesundheit "geatmet" wird).

Anfang **September 2016** entstand ein erster Entwurf des Gebäudes mit einem Sockelgeschoss für die Tierhaltung und vier Stockwerke für Labore und Büros. Sofort entstand die Abkürzung LRB: Leibniz Respiratorium Borstel. Aufgrund der vorgesehenen Gebäudehöhe wurde dieser Vorschlag jedoch von der Bauaufsichtsbehörde zurückgewiesen und das Gebäude musste mit einem Geschoss weniger neu geplant werden. Im Rahmen einer erneuten Bauvoranfrage wurde der nun 4-geschossige Bau (großes Bild) im **Januar 2017** von seiner Außengestaltung prinzipiell als genehmigungsfähig eingestuft (Abschluss Leistungsphase 2, Vorplanung).

Im Rahmen der dann begonnenen Entwurfsplanung (Leistungsphase 3) erfolgten in enger Abstimmung mit den zukünftigen Nutzern die Ausplanung der S3-Anlage, der Tierhaltung und der Labore sowie die Gestaltung der Außenfassade. Die vertiefte **Kostenrechnung** im **Juli 2017** ergab, dass die gegenwärtige Planung den Kostenrahmen um 1,6 Mio Euro überschreiten würde. Um die S3-Anlage und die Tierhaltung nicht ändern zu müssen, mussten im Rahmen umfassender Einsparungen unter anderem die Laborachsen im Labortrakt verkürzt und jedes Labor etwas verkleinert werden.

Parallel zur LRB-Planung durchgeführte Untersuchungen der Straßen und Abwasserkanäle erbrachten ein katastrophales Ergebnis. Sämtliche Kanäle und der überwiegende Teil der Trinkwasserversorgung im Bereich LRB, Krankenhaus und dem zukünftigen NRZ mussten saniert werden. Diese Maßnahmen mussten unverzüglich geplant und umgesetzt werden, da Wettersimulationsberechnungen eine unmittelbare Gefährdung von Baugrube und Rohbau bei fehlender Entwässerung vorhergesehen hatten. Die Baumaßnahmen, welche durch zahlreiche Behinderungen durch nicht oder falsch verzeichnete Bestandsleitungen gekennzeichnet sind, wurden ab August 2018 aufgenommen und sind voraussichtlich bis Juni 2019 abgeschlossen. Weiterhin zeigte eine umfassende Untersuchung unserer Fernwärmeversorgung, dass die bestehende Heizungsanlage und das dazugehörige Gebäude umfassend modernisiert werden müssen.

Die veränderte Entwurfsplanung wurde Ende September 2017 abgeschlossen und auf deren Basis der **Bauantrag Anfang November 2017** (Leistungsphase 4) gestellt, welcher am **23.05.2018** genehmigt wurde. In dieser Zeit wurde in Abstimmung mit der Gentechnikbehörde die Errichtung der S3-Anlage weiter geplant. Im Rahmen dieser Planungen wurden zahlreiche Mängel in der Fachplanung der technischen Gebäudeausrüstung sichtbar, die beim Generalplaner zu einem Wechsel des zuständigen Ingenieursbüros führten. Die Beseitigung der Mängel und die Optimierung des Gebäudes erforderten eine grundlegende Überarbeitung der Entwurfsplanung mit einer Vergrößerung der Grundfläche und der Technikzentralen sowie einer teilweisen Umstrukturierung innerhalb des Gebäudes. Hierfür musste Anfang März eine **erneute Baugenehmigung** eingereicht werden, die am **05.04.2019** zunächst für die Baugrube erteilt wurde.

Die Arbeiten an der Baugrube werden bis Juli abgeschlossen sein. Bis Ende Februar 2021 wird der Rohbau einschließlich Fassaden, Fenster und Dächer erstellt. Bis Ende 2021 soll der Innenausbau abgeschlossen sein und es erfolgt eine einjährige Phase der **Inbetriebnahme** bis zum **Ende 2022**. Erst dann wird nach Umzug ins Respiratorium einmal kräftig "durchgeatmet“ werden können!

Namibia

Namibia is a country with a similar size as Germany and approx. 2.3 Million inhabitants, so just 3 inhabitants per km2. It has a marvelous nature, but also big infrastructural, socio economical and public health problems. The estimated tuberculosis (TB) incidence rate was 446/100.000 in 2016, with 3.9% of multidrug resistant (MDR) TB among new and 8.7% among previously treated cases. Only 63% of them had a successful treatment outcome in a recent survey. MDR TB is likely increasing in the country, with associated social and economic costs affecting Namibian society. TB control in Namibia is challenged by several limitations that range from delayed / unavailable diagnostics, to poor treatment infrastructure and infection control. Virtually no research on molecular resistance mechanisms or transmission dynamics of M/XDR M. tuberculosis complex (Mtbc) strains is performed.

Getting ready

Our plans to perform a research stay (sabbatical) in Namibia arose from long term collaborations between the Research Center Borstel and the Medical Faculty at the University of Namibia (UNAM, Windhoek), that have been initiated by Prof. Dr. Christoph Lange and Dr. Gunar Günther. Gunar then moved to Namibia, led the TB ward at Katutura state hospital and established the first TB research laboratory at UNAM.

After longer discussions on how translational research linked to the hospital setting also leading to significant improvement of patient care can be established and sustained with a longer perspective, we developed our first ideas and then, the proposal for our six months stay in Namibia (as visiting Professor and visiting Senior Laboratory Specialist) was accepted by the board of directors at FZB in July 2016.

Tuberculosis Research in Namibia

Tanja Ubben and Stefan Niemann

This left us with a planning phase of just 4 months that, at the end appeared to be quite short and challenging. Indeed, ordering reagents in Namibia is quite unrealistic, so, we had to ship everything needed (more than 50 items, partially on dry ice). This led to intensive planning of reagent lists and logistics, but finally, the shipment arrived safely shortly before X-Mas 2016, a real present and great start for our work there. Likewise, getting the visa was a challenge; we got our work permits just on the day our flight to Namibia was scheduled – a real thrill for us, but a good start in the experience of the more African way of life.

Working and living in Windhoek

After an easy flight, we arrived safely on Tuesday, Dec 13, 2016 in Windhoek and had to face the first challenge – to drive left, but you adopt this rapidly.

We had a very warm welcome by Gunar, Dr. Emmanuelle Nepolo (Chair Biochemistry & Microbiology), and the laboratory team at UNAM and started to work together in a great team. The laboratory infrastructure at UNAM appeared to be great, and after initial checks we started to re-vitalize the 8-Capillary sequencer and to establish the laboratory workflows for targeted resistance gene sequencing from sputum and culture DNA extractions. The sequencing results were translated in easy-to-read report forms that are used for designing individualized treatment regimens for the MDR TB patients at Katutura hospital. At the end of our stay, high resolution resistance data were produced for more than 50 MDR-patients. Based on intensive trainings, the MDR TB sequencing project is continued by our Namibian friends with constant support from Borstel.

Our time was coined by the great teamwork with all members of the team at UNAM/Katutura, but also with other UNAM colleagues, as well as with colleagues working at NIP (National Institute of Pathology) or the NTP (National TB program). Getting close to patient care in a high incidence setting was another important aspect that was mind changing on a personal level and opened our eyes to real problems in TB care in high incidence settings.

We not only found superior work colleagues, but friends that welcomed us in their country and helped us with our work but also to explore life and nature in Namibia. This was quite an exciting part of our stay, you really need a 4x4 car, you can sleep in nature reserves in a roof top tent, and you can see the big five – at least in theory. At the end, we travelled more than 20.000 km in Namibia visiting the south from the Dunes of Sossusvlei to Fish River Canyon, the coast (Lüderitz, Swakopmund) and the north with the great game reserves such as Etosha National Park.

After nearly 6 months, we went back to Germany on May 23, 2017 with thousands of impressions in our minds and ready to bring TB research forward in Germany and Namibia.

Perspectives

Our stay provided an excellent basis for longitudinal collaborations and translational TB research. After getting a visiting professorship for three years at UNAM, we stayed another two months there to further sustain the laboratory setup and perform first data analysis and grant writing. At the moment, we already perform whole genome sequencing to analyze the characteristics, evolution and transmission of Mtbc strains in Namibia, with special focus on MDR-TB. Currently, three grant proposals are under review, one aiming at country-wide MDR-TB surveillance by strain genome sequencing is in final negotiations.

The collaborations between UNAM and FZB are currently further sustained in form a "Centre of excellence in infectious disease control" at UNAM with constant laboratory support and frequent personnel exchange.




Regelmäßige körperliche Betätigung und Bewegung verbessern die Lebensqualität bei Gesunden, aber auch bei Lungenkranken. Viele Menschen verbinden Fitness mit einem gesunden Herzen sowie der Reduktion von Übergewicht und Gesundheitsrisiken wie Diabetes. Aber Bewegung hält auch die Lunge fit! Das haben sich auch Borsteler Beschäftigte gesagt und laufen mit viel Spaß an den verschiedensten Veranstaltungen: mit Kolleginnen und Kollegen aus dem Bernhard-Nocht Institut und dem Heinrich-Pette Institut auf dem HSH Nordbank Run in der Hafen City, in einer gemeinsamen Staffel des FZB und der Universität zu Lübeck auf dem größten Team-Laufevent des Nordens ‚Laufen zwischen den Meeren', mit Borstelern auf dem ‚Beat Allergy Run & Walk' während des EAACI Kongresses 2018 oder auf dem lokalen Sülfelder Meilenlauf.

Im Zuge der Vorbereitungen zum Tag der Offenen Tür am 8. September 2018 entstand die Idee, den 1. Borsteler Spendenlauf zu organisieren. Nestor González Roldán aus der FG Allergobiochemie, selbst begeisterter Läufer, und Dirk Wulff vom Sportverein Sülfeld haben das Projekt in die Hand genommen und Rundstrecken von 4.5 bzw. 10 km abgesteckt. Start war das FZB, im Anschluss führte die Strecke durch den Borsteler Wald, Sülfeld und Tönningsstedt.

‚Laufen ist einfach eine tolle Sache! Sich draußen zu bewegen, mit und ohne Partner/in, mit und ohne Geplauder, auf den schönsten Laufstrecken direkt vor der Haustür und das auch noch kostenfrei: besser geht es nicht', so Nestor Gonzalez, der jeden Tag seine 8-12 km läuft.

Am 8. September hieß es dann: Auf die Plätze, fertig, los! Insgesamt 150 Läuferinnen und Läufer hatten sich angemeldet, um an dem 1. Borsteler Spendenlauf teilzunehmen. Pünktlich um 11 Uhr fiel der Startschuss und die Teilnehmerinnen und Teilnehmer machten sich bei perfektem Laufwetter auf den Weg die Rundstrecken zu bezwingen. Der schnellste Läufer des 4,5 KM Laufs lief bereits nach knapp 22 Minuten wieder im Park des Herrenhauses ein, der schnellste Läufer der 10 KM Strecke schaffte die Distanz in 36 Minuten und 33 Sekunden. Im Anschluss durften dann auch die Kleinsten zeigen, wie schnell sie sind: Bei dem 400 Meter Rundlauf durch den Park des Forschungszentrums liefen rund 50 Kinder im Alter von 4 bis 12 Jahren mit und wurden von den Besuchern lautstark unterstützt. Der Erlös von weit über 1.000 Euro kommt den Vereinen pina e.v. (Präventions- und Informationsnetzwerk Allergie und Asthma), TBC-SH e.V. (Schleswig-Holsteinische Vereinigung zur Bekämpfung der Tuberkulose und der Lungenkrankheiten e.V.) und dem Förderverein am Forschungszentrum Borstel zugute.

Fotos: N. González-Roldán, C. Lange, B. Weller

Lungengesundheit - RUN FOR YOUR LUNGS

Bettina Brand, Britta Weller

Pollen fliegen nicht nur im Frühling oder im Sommer. Sie fliegen das ganze Jahr, nur ihre Zusammensetzung und Konzentration schwankt zwischen den Jahreszeiten. Für Dr. Nestor González Roldán heißt das, ehrenamtlich bis zu 20 Stunden pro Woche die Pollenfalle auf dem Dach der Klinik zu betreuen und die Pollenanalyse durchzuführen. Hier nimmt er wöchentlich, immer zur gleichen Zeit, den Abklatsch der Natur in die Hand.

Nestor, Pollenfalle hört sich ja spektakulär an - was muss man sich darunter vorstellen?

So spektakulär ist die Falle eigentlich gar nicht: ein Elektromotor saugt ein Luftvolumen von zehn Litern pro Minute durch einen kleinen Schlitz. Das entspricht annähernd der Luftmenge, die ein erwachsener Mensch im Ruhezustand maximal einatmen würde. Die Pollen bleiben auf einer mit Vaseline präparierten Folie kleben, die auf einer Trommel angebracht ist. Die Trommel wiederum dreht sich mit einer präzisen Geschwindigkeit, so dass exakt eine Woche Pollenniederschlag auf der Folie festgehalten wird, d.h. ich muss zu einem genau festgelegten Zeitpunkt auf das Dach steigen und die Folie austauschen.

Das müssen ja nicht nur Unmengen an Pollen sein, sondern auch viele verschiedene Arten: wie wird denn die Konzentration und die Art der Pollen bestimmt?

Ich schneide die Folie in Stücke, die 24 Stunden umfassen, fixiere und färbe die Pollen. Dadurch erhalten sie unabhängig von der Art ein rosafarbenes Aussehen und sind im Mikroskop meistens gut zu erkennen. Für die Bestimmung muss man auf bestimmte Merkmale achten, wie die Größe, die Anzahl der Öffnungen, und die Oberflächenstruktur. Jeder Pollentyp hat seinen eigenen "Fingerabdruck". Anschließend werden 17% der Folie ausgewertet, um einen signifikanten Wert zu erhalten, der der Pollenanzahl pro m^3 Luft entspricht. Anschließend bestimme ich die Arten, die in die Pollendokumentation eingehen - allergologsich relevant sind insbesondere: Hasel, Erle, Esche, Gras, Roggen, Traubenkraut, Beifuß und Birke.

Soweit ich weiß, bist Du von Haus aus Immunologe und kein Botaniker – hast Du Dir die Bestimmung der Pollen selbst angeeignet?

Ja, und nicht nur das: meine Muttersprache ist spanisch! Also habe ich alle deutschen Bezeichnungen, aber auch die englischen und lateinischen Begriffe lernen müssen. Meine botanischen Kenntnisse haben sich deutlich verbessert. (Nestor schmunzelt)

Lungengesundheit - Der Borsteler Pollendetektiv

Bettina Brand

Und diese Daten werden dann zentral erfasst und kommen wem zu Gute?

Genau, ich gebe die Werte in den Deutschen Polleninformationsdienst ein, die auf europäischer Ebene in die Datenbank des European Aerobiology Networks weitergeleitet werden – es gibt sogar eine weltweite Karte aller Referenzstationen zu denen Borstel jetzt auch zählt. Dies bedeutet, dass wir jetzt das ganze Jahr messen und nicht nur während der Saison von Februar bis Oktober. Die Daten des Polleninformationsdiensts speisen eine Smartphone App, über die anhand der Postleitzahl die regionale Pollenflugvorhersage abrufbar ist. Eine wichtige Information für jeden Pollen-Allergiker. Darüberhinaus werden die Daten auch dem deutschen Wetterdienst übermittelt, sodass der Pollenflug Teil der Wettervorhersage sein kann. Die europäische Datenbank kommt einer historischen Pollenfalle gleich und und ist Basis für Studien und Modellentwicklungen von Wissenschaftlerinnen und Wissenschaftlern. Auch die Patientinnen und Patienten unserer Klinik profitieren von den Ergebnissen aus der hauseigenen Pollenfalle. So kann Tag-genau nachvollzogen werden, ob Beschwerden mit erhöhten Pollenwerte korrelieren. 2018 hatten wir z. B. nur an 2 Tagen im April hohe Konzentrationen an Birkenpollen, die Birkenpollenallergiker klagten aber bereits vor und auch nach diesem Zeitraum über starke Beschwerden. Schlussendlich stellte sich heraus, dass in dieser Zeit die Eschenpollen unterwegs waren und als wahrscheinliche Kreuzallergene die Beschwerden hervorgerufen hatten.

Ist es möglich anhand der jahrelangen Beobachtungen Vorhersagen zum Pollenflug zu treffen?

Nein, leider nicht, denn die Freisetzung von Pollen hängt vollständig von den Wetterbedingungen ab. Daher können wir nur den Zeitrahmen abschätzen, jedes Jahr hat seine eigene Pollenhandschrift – diese Jahr haben wir ein Mastjahr für die Erle d. h. die Pollenkonzentration ist 20 mal höher als normal: statt 400 - 500 Pollen/m^3 Luft haben wir 9.000 gemessen.

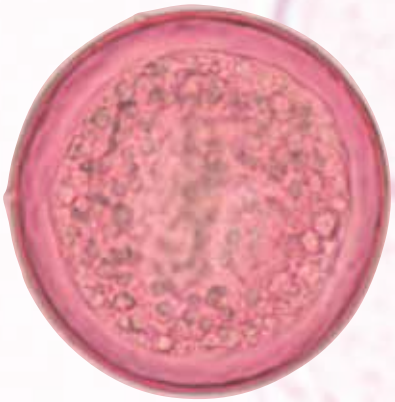
Nestor, du bist ja nicht nur Pollendetektiv, sondern bearbeitest auch Deine eigenen Forschungsprojekte – kannst Du kurz erklären worum es dabei geht?

Wir isolieren die Lipide, wasserunlösliche Naturstoffe, aus den Pollen und charakterisieren sie chemisch, um sie aufgrund der strukturellen Eigenschaften in Lipidklassen einzuordnen. Bei diesen Untersuchungen haben wir ganz neue, noch nicht beschriebene Lipide in Graspollen identifiziert. Es scheint eine Balance von Allergie-fördernden und Allergie-neutralen Lipiden mit ähnlichen chemischen Strukturen zu geben. Kleine Unterschiede in der chemischen Struktur bestimmen ihre biologische Aktivität. Dieses Gleichgewicht wird durch das Wetter beeinflusst, so dass der Gehalt an Allergie-fördernden und Allergie-neutralen Lipiden von Jahr zu Jahr variiert. Diese Balance kann durch Zugabe einer Klasse verschoben und somit die Allergenität des Pollen beeinflusst werden.

Du scheinst eine große Begeisterung für die Pollen entwickelt zu haben?

Es ist faszinierend so nah an der Natur zu arbeiten und der Entwicklung der Pflanzen mehr Aufmerksamkeit zu schenken. Es gibt wirklich erstaunliche Phänomene z.B. wenn wir regional gar keinen Pollenflug haben, die Falle aber voll ist mit Pollen. Dazu braucht es nur eine warme Luftströmung, die die Pollen bis zu 1.000 km über Land transportiert, um dann bei kühler Witterung in die Falle zu gehen. Ein anderes Phänomen sind Exotenpflanzungen in Privatgärten und Parks, deren Pollen sich dann plötzlich in unserer Falle wiederfinden oder dass Gräserpollen unter den Arten am Mikroskop nicht differenzierbar sind. Hier beschreibt man den Pollenflug in Wellen – früher Sommer und Ende Sommer. Es bleibt einfach spannend!

Fotos: N. González-Roldán, B. Weller



Matthias Merker

W1-Professur für Evolutionäre Pathogenomik von chronischen Lungenerkrankungen

Prof. Matthias Merker hat seine wissenschaftliche Karriere in Borstel mit seiner Promotion im Jahr 2011 begonnen. Sein Projekt befasste sich mit der Analyse der globalen Populationsstruktur und der Evolution der Stämme des Genotyps Beijing aus dem Mycobacterium-tuberculosis-Komplex. Diese Arbeiten hat er erfolgreich in z.T. hochkarätigen Fachzeitschriften veröffentlicht und 2014 mit der Promotion (summa cum laude) abgeschlossen.

Als PostDoc widmete er sich in den nachfolgenden Jahren (2014-2016) im Rahmen des Deutschen Gesundheitszentrums für Infektionsforschung (DZIF) der molekularen Epidemiologie und der Evolution mykobakterieller Antibiotika-Resistenzen. Seit 2017 ist er Principle Investigator im internationalen Konsortium TB-Sequel und im Wissenschaftscampus EvoLUNG. Der Schwerpunkt seiner wissenschaftlichen Arbeiten liegt weiterhin in der Erforschung pathogener Faktoren, die mit Tuberkulose-Folgeerkrankungen und schneller Adaptation an Antibiotika assoziiert sind.

2018 wurde Matthias Merker auf die W1- Professur für 'Evolutionäre Pathogenomik von chronischen Lungenerkrankungen' der Universität zu Lübeck berufen. Seine Arbeiten sind bisher in 35 Publikationen erschienen und mit mehreren Preisen u.a. dem Promotionspreis des DZIF und dem Gertrud-Meißner Award der European Society for Mycobacteriology ausgezeichnet.

Menschen / People

Florian Maurer

Leiter der Forschungsgruppe Diagnostische Mykobakteriologie und Ärztlicher Leiter des Nationalen Referenzzentrums für Mykobakterien

PD Dr. Florian Maurer verstärkt seit März 2018 die Tuberkuloseforschung am FZB und hat im Oktober 2018 die Nachfolge von Dr. Katharina Kranzer in der Leitung der FG Diagnostische Mykobakteriologie angetreten sowie die Medizinische Leitung des Referenzzentrums für Mykobakterien übernommen.

Florian Maurer hat sich nach einem B.Sc. (Honours) in Physik der Medizin zugewandt und das Studium 2010 mit dem Staatsexamen in Freiburg erfolgreich abgeschlossen, gefolgt von der Promotion 2013 an der Universität Zürich. 2016 schloss sich der Facharzt für Medizinische Mikrobiologie, Virologie und Infektionsepidemiologie an und 2017 der Abschluss als zertifizierter Experte für Antimicrobial Stewardship (Deutsche Gesellschaft für Infektiologie); beide Spezialisierungen erfolgten während seiner ärztlichen Tätigkeit am Universitätsklinikum Hamburg-Eppendorf. Vorläufiger Höhepunkt seiner Ausbildung war die Habilitation 2018 an der Universität Hamburg in der Medizinischen Mikrobiologie.

Florian Maurer ist viel gefragter Experte in internationalen Gremien wie der 'WHO Supranational Mycobacteriology Reference Laboratory task force', der 'WHO Europe European Laboratory Initiative expert core group (WHO-ELI)' und dem 'National contact point for mycobacterial diagnostics (ECDC ERLTB-Net2 consortium)'. Sein wissenschaftlicher Schwerpunkt liegt in der Diagnostik, der antimikrobiellen Resistenz, der Medikamenten-Empfindlichkeitstestung und der Genetik von Mykobakterien.

In Anerkennungen seiner Arbeiten als Wissenschaftler und Arzt hat er bereits mehrere Auszeichnungen erhalten u.a. den 'BD Research Prize' (Deutsche Gesellschaft für Hygiene und Mikrobiologie, 2019), den 'UKE prize for patient orientation and safety' (Antimicrobial Stewardship Team, Universitätsklinikum Hamburg-Eppendorf, 2018) und den ‚Semester award for the best doctoral thesis‘ der Universität Zürich.

Farewell

Katarzyna Duda

Otto Holst

Research Center Borstel (RCB) has been fortunate to witness 31 years of passionate scientific input of Prof. Dr. Otto Holst. Otto Holst who retired at the end of 2018 started his career at RCB after completing a postdoctoral fellowship at the University of Regensburg working on partial structures of a glycoprotein from *Volvox carteri*. In 1987 he joined the lab of Prof. Dr. Helmut Brade at RCB and continued the journey into the world of carbohydrates being fascinated by the core region of Gram-negative bacterial lipopolysaccharides, particularly by a sugar named Kdo (3-deoxy-D-*manno*-oct-2-ulopyranosonic acid) and its substitution. This task was not easy, as this sugar is labile, and e.g., phosphate groups can migrate. Thirty years ago structural chemists had no such wonderful sophisticated methods to solve the chemical questions as we have nowadays. But eagerness and patience were always good advisors and resulted in many findings, papers and co-operations. So, with no surprise Otto became the co-organizer of the Kdo Workshop held in Borstel annually. Since the audience grew continuously this meeting was extended to the German-Polish-Russian Meeting where Otto was one of the main contributors and decisive person. He was an open-minded, friendly and cooperative scientist. In the hard political times, he always supported scientists from central and east Europe. In 2000 he became the group leader of the Division of Structural Biochemistry. His lab was filled with guest scientists and PhD students, resulting in an atmosphere full of life, joy for science and scientific exchange. Many projects came to life. Many friendships and co-operations were established. Otto was a colleague, supervisor, mentor, but most importantly a friend, with whom excursions and dinners were always fun. Late afternoon and evening discussions were always very informative, and one could learn about the "old" techniques such as paper chromatography or the "dangerous one", i.e. sugar methylation requiring the production of explosive reagents (a hood may be useful indeed...). Later, his field of experience extended to the envelope components of Gram-positive bacteria, mycobacteria, and to the hygiene hypothesis in allergy research. In 2007 he was the chairman of the successful 14th European Carbohydrate Symposium in Lübeck, and, later, became President of the International Endotoxin and Innate Immunity Society. Now, he is enjoying retirement but has not lost the cord.... he is still active as the Editor of Innate Immunity.

Research Center Borstel, colleagues and friends wish you, dear Otto, all the best, fun and relax with your second passion – music! Rock the stages!

Fotos: T. Goldmann

Forschungszentrum Borstel
Leibniz Lungenzentrum

Forschung & Entwicklung

Aktuelles +++ Informationen +++ Nachrichten +++ Aktuelles +++ Informationen +++ Nachrichten

Media-/ Press-Clips 2017 / 2018

Deutschlandfunk
‚Der Spur der resistenten Tuberkulosekeime'

NDR Info **‚Gefährliche Erreger kennen keine Grenzen'**

RTL Nord **‚Kampf gegen Tuberkulose'**

NDR Info
‚Fehler im Immunsystem - Was hilft bei Allergien?'

Hessischer Rundfunk
‚Tuberkulose - eine Krankheit kehrt zurück'

Deutschlandfunk **‚Die Unbesiegbaren'**

NDR Info **‚Tuberkulose auf dem Vormarsch'**

ARTE **‚Die Penizillin-Story'**

NDR 3 / Visite **‚Fleischallergie: Gefahr vom Grill'**

NDR 3 / Visite **‚Pollenallergie'**

NDR / Schleswig-Holstein Magazin
‚Neues Beatmungsgerät: Hilfreich, aber zu teuer? '

ARTE **‚Die WHO - im Griff der Lobbyisten?'**

NDR / Visite ,
Ambrosia-Allergie durch Auto-Abgase:
Pollen werden durch Luftverschmutzung aggressiver'

NDR / Schleswig Hostein Magazin
‚Der schwere Kampf gegen Tuberkulose'

Der Tagesspiegel
‚Die Schwindsucht schwindet nicht'

Stern , **Essen mit Nebenwirkungen'**

TAZ **‚Nationaler Notstand'**

Lübecker Nachrichten
‚Borsteler Institut führt neuen Feldzug gegen Tuberkulose an'

Hamburger Abendblatt
‚Borsteler Forscher im Afrika-Einsatz'

Medical Tribune
‚Multiresistente TB bereitet Kopfzerbrechen'

Lübecker Nachrichten
‚Neuer Behandlungs-Ansatz bei Erdnuss-Allergie '

Hamburger Abendblatt
‚Gefahr beim Fest: Forscherin warnt vor Nuss-Allergien'

Welt
‚Multiresistente Tuberkulose-Keime bei Flüchtlingen'

Focus **‚Ausbruch während Flüchtlingskrise: Multiresistente Tuberkulose-Keime entdeckt'**

Hamburger Abendblatt ,
51 Millionen Euro für Forschungszentrum'

Spektrum der Wissenschaft
‚Neue Medikamente werden das Problem nicht lösen'

Lübecker Nachrichten
‚Forscherin sagt Allergien den Kampf an'

Stuttgarter Nachrichten **‚Tuberkulose bleibt ein Risiko'**

Deutsches Ärzteblatt **‚Quelle für weltweite Infektionen nach Herz-OPs gefunden'**

Hamburger Abendblatt
‚Neuer Chef im Forschungszentrum in Borstel

Die Zeit **‚Drei Tote pro Minute'**

Gut vernetzt: Kick-Off Meeting des **Leibniz Postdoc Networks** in Borstel. Am 26. und 27.10.2017 trafen sich Postdoc Vertreter von 45 Leibniz-Instituten in Borstel zur offiziellen Gründung des Leibniz Postdoc Networks und zur Festlegung der Struktur des Netzwerkes.

Well networked: Kick-off meeting of the **Leibniz Postdoc Network** in Borstel. On 26 and 27 October 2017, postdoc representatives from 45 Leibniz Institutes met in Borstel for the official founding of the Leibniz Postdoc Network and to determine the structure of the network.

Der **Forschungsverbund ANTI-TB** erhält in den nächsten drei Jahren Fördergelder des BMBF in Höhe von 2,8 Millionen Euro. Ziel des Projektes ist es, die Therapiesituation bei resistenten Tuberkuloseerregern mit Hilfe von Nanotransportern zu verbessern.

The **ANTI-TB research network** will receive BMBF funding of 2.8 million Euros over the next three years. The aim of the project is to improve the therapeutic situation of resistant tuberculosis pathogens with the help of nano-transporters.

Christian Schwager, FG Klinische und Molekulare Allergologie, hat den **DGAKI-Nachwuchsförderpreis der Deutschen Gesellschaft für Allergologie und klinische Immunologie** verliehen bekommen. Der mit 5.000 Euro dotierte Preis würdigt seine Arbeit an der Aufklärung von Allergenen der Erdnuss.

Christian Schwager, FG Clinical and Molecular Allergology, has been awarded the **DGAKI Young Investigators Award of the German Society for Allergology and Clinical Immunology**. The prize, worth 5,000 Euros, honours his work in the clarification of allergens of peanuts.

Best of 2017

Helmut Salzer, FG Klinische Infektiologie, hat das **Gilead Stipendium** in Höhe von 49.000 Euro erhalten, um chronisch pulmonale Aspergillose in Namibia zu erforschen.

Helmut Salzer, Clinical Infectious Diseases, has received the **Gilead Fellowship** of 49,000 Euros to study chronic pulmonary aspergillosis in Namibia.

Tag der offenen Tür in Borstel zum **Welt-Asthmatag 2017** mit zahlreichen Forschungs- und Klinikständen sowie geführten Besichtigungen durch die Labore des FZBs und die unterschiedlichen Abteilungen der Medizinischen Klinik.

Open Day in Borstel on World Asthma Day 2017 with numerous research and clinic stands as well as guided tours through the laboratories of the FZB and the various departments of the Medical Clinic.

Matthias Merker, FG Experimentelle und Molekulare Mykobakteriologie, erhielt auf der Jahrestagung der European Society for Mycobacterioloy (ESM) den **Gertrud Meißner Preis** für seine exzellenten Forschungsarbeiten auf dem Gebiet der mykobakteriellen Epidemiologie. Der Preis ist mit 2.000 Euro dotiert.

Matthias Merker, FG Experimental and Molecular Mycobacteriology, received the **Gertrud Meißner Prize** at the annual meeting of the European Society for Mycobacterioloy (ESM) for his excellent research in the field of mycobacterial epidemiology. The prize is endowed with 2,000 Euros.

Leibniz-Wirkstoff des Jahres 2017 (Candidalysin) - ein Gift des krankheitserregenden Hefepilzes Candida albicans. Der Leibniz-Forschungsverbund "Wirkstoffe und Biotechnologie" zeichnete Dr. Duncan Wilson, Dr. Selene Mogavero und Prof. Bernhard Hube vom Hans-Knöll-Institut in Jena sowie Thomas Gutsmann, FG Biophysik, für ihre bedeutende Forschung auf dem Gebiet von bioaktiven Substanzen aus.

Leibniz-Wirkstoff of the year 2017 - a toxin (candidalysin) of the pathogenic yeast fungus Candida albicans. The Leibniz Forschungsverbund "Wirkstoffe und Biotechnologie" awarded Dr. Duncan Wilson, Dr. Selene Mogavero and Prof. Bernhard Hube from the Hans Knöll Institute in Jena and Thomas Gutsmann, FG Biophysics, for their important research in the field of bioactive substances.

Bencard Next Generation Award geht an Sabine Bartel, FG Frühkindliche Asthmaprägung.

Bencard Next Generation Award goes to Sabine Bartel, Early Origin of CLD.

2017

Funktionsfähiges molekularbiologisches **Forschungslabor in Namibia** auf dem Campus der UNAM eingeweiht, um Antibiotikaresistenzen der Tuberkulosebakterien rasch und präzise zu erfassen. Dadurch kann in der Zukunft die Behandlung der Patienten in dem Hochinzidenzland maßgeschneidert erfolgen. Am 23.02.2017 fand die offizielle Eröffnung des gemeinsamen Forschungslabors in Anwesenheit des Pro-Vizekanzler der UNAM, Professor Kenneth Matengu, des Dekans der School of Medicine, Professor Peter Nyarang´o, dem Referenten für Wirtschaftliche Zusammenarbeit der Deutschen Botschaft in Windhoek, Herrn Christian Grün, und Vertretern der UNAM, des Forschungszentrums Borstel und der Universität zu Lübeck mit einem Tuberkulosesymposium an der UNAM statt.

Functional molecular biological **research laboratory in Namibia** inaugurated on the UNAM campus to detect antibiotic resistance of tuberculosis bacteria quickly and precisely. In this way, the treatment of patients in the high incidence country can be tailor-made in the future. On February 23rd, 2017 the joint research laboratory was officially opened with a tuberculosis symposium at UNAM in the presence of Professor Kenneth Matengu, Vice-Chancellor of UNAM, the Dean of the School of Medicine, Professor Peter Nyarang´o, Christian Grün, Speaker for Economic Cooperation of the German Embassy in Windhoek, and representatives of UNAM, the Research Center Borstel and the University of Lübeck.

Der **Master Preis des Fördervereins am Forschungszentrum Borstel** geht an Dörte Nischkowski für ihre Arbeiten am Nichtkleinzelligen Lungenkarzinom.

The **Master Prize of the Förderverein am Forschungszentrum Borstel** goes to Dörte Nischkowski for her work on non-small cell lung cancer.

Best of 2017

Die **"SH-Chairs"** sind spezielle Professuren, mit denen exzellente Wissenschaftlerinnen und Wissenschaftler an die schleswig-holsteinischen Standorte gebunden werden sollen. Sie werden jeweils für einen Zeitraum von maximal sechs Jahren gefördert. Einen dieser acht "SH-Chairs" hat Stefan Niemann, FG Experimentelle und Molekulare Mykobakteriologie, inne.

The **"SH-Chairs"** are special professorships with which excellent scientists are to be bound to the Schleswig-Holstein locations. They are each funded for a maximum period of six years. One of these eight "SH-Chairs" is held by Stefan Niemann, Experimental and Molecular Mycobacteriology.

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anTBiotic – die EU fördert die klinische Tuberkuloseforschung in Borstel. Gemeinsam mit Kolleginnen und Kollegen von GlaxoSmith-Kline in Tres Cantos (Spanien), den Universitäten von Cape Town und Stellenbosch in Südafrika und der Universität von Tromsö in Norwegen werden jetzt Ärzte und Wissenschaftler der Klinik am FZB von 2017-2021 mit annähernd 6 Mio. Euro gefördert, um bessere Antibiotika für die Behandlung der Tuberkulose zu entwickeln und die Therapie der Tuberkulose zu individualisieren.

anTBiotic – the EU supports clinical tuberculosis research in Borstel. Together with colleagues from GlaxoSmithKline in Tres Cantos (Spain), the Universities of Cape Town and Stellenbosch in South Africa and the University of Tromsö in Norway, doctors and scientists from the FZB Clinic in 2017-2021 are now receiving nearly 6 million Euros to develop better antibiotics for the treatment of tuberculosis and individualize the treatment of tuberculosis.

Partnerschaft mit der Universität Xiamen in Fujian, China: 1. Deutsch-chinesisches Symposium zur Bekämpfung chronischer nicht-infektiöser Lungenerkrankungen.

Partnership with the University of Xiamen in Fujian, China: 1st German-Chinese Symposium to Combat Chronic Non-Infectious Lung Diseases.

Den **Promotionspreis Kreis Segeberg** erhalten Laura Paulowski, FG Biophysik, und Viola Dreyer, FG Experimentelle und Molekulare Mykobakteriologie, für ihre exzellenten Dissertationen.

Laura Paulowski, Biophysics, and Viola Dreyer, Experimental and Molecular Mycobacteriology, were awarded the **doctoral prize of the Segeberg district** for their excellent dissertations.

Publikation / Publication Blockbuster: Nature Communications (Bioanalyt. Chemistry), European Respiratory Journal (Clin. Infect. Disease, Exp. & Mol. Mycobacteriology, Study Center, Diagnost. Mycobacteriology, Biobank), American Journal of Respiratory and Critical Care Medicine (Clin. Infect. Disease, Early Origin of CLD), Lancet Respiratory Medicine (Exp. & Mol. Mycobacteriology, Clin. Infect. Diseases), Journal of Allergy and Clinical Immunology (Clin. & Mol. Allergology, Exp. Pneumology, Early Origin of CLD, Innate Immunity), Lancet Infectious Diseases (Exp. & Mol. Mycobacteriology), Science (Cell. Microbiology).

2017

Ausgezeichnet als **"paper of the month 01/2018"** der Deutschen Gesellschaft für Hygiene und Mikrobiologie: Forscher der Universität Greifswald und des FZB entschlüsseln einen grundlegenden Mechanismus der Zellwandbiosynthese beim wichtigsten bakteriellen Erreger von Lungenentzündungen.

Awarded **"paper of the month 01/2018"** by the German Society for Hygiene and Microbiology: Researchers from the University of Greifswald and the FZB decipher a fundamental mechanism of cell wall biosynthesis in the most important bacterial pathogen of pneumonia.

Der **Promotionspreis Kreis Segeberg** geht an Christian Schwager, FG Klinische und Molekulare Allergologie, und Patrick Beckert, FG Experimentelle und Molekulare Mykobakteriologie.

The **Segeberg District Doctoral Award** goes to Christian Schwager, Clinical and Molecular Allergology, and Patrick Beckert, Experimental and Molecular Mycobacteriology.

Imke Storm, Borsteler Biologielaborantin, bei der **IHK-Bestenehrung** in Lübeck als Prüfungsbeste ausgezeichnet.

Imke Storm, Borstel biology laboratory assistant, was awarded **best exam at the IHK awards** ceremony in Lübeck.

Leibniz-Präsident, Prof. Matthias Kleiner, besucht den **WissenschaftsCampus EvoLUNG**.

The President of the Leibniz Association, Prof. Matthias Kleiner, visited the **ScienceCampus EvoLUNG**.

Best of 2018

Paradigmenwechsel in der Tuberkulosebehandlung - Genomsequenzierung ersetzt klassische Resistenztests. Eine groß angelegte Analyse des Erbguts von über 10.000 Erregerstämmen hat gezeigt, dass der Einsatz von Genomsequenzierungen die Behandlung von Tuberkulose-Patienten verbessern kann.

Paradigm shift in tuberculosis treatment - genome sequencing replaces classical resistance tests. A large-scale analysis of the genetic material of over 10,000 pathogen strains has shown that the use of genome sequencing can improve the treatment of tuberculosis patients.

Großes Interesse beim **Tag der offenen Tür** am FZB. Über 400 Besucher kamen am 8. September ans Forschungszentrum, um einen Blick hinter die Kulissen der Lungenforschung und des Klinikalltags zu werfen. Neben einem spannenden und abwechslungsreichen Programm rund um die Arbeit des Forschungszentrums fand an diesem Tag zudem der 1. Borsteler Spendenlauf statt.

Great interest at the **Open Day** at the FZB. Over 400 visitors came to the research centre on 8 September to take a look behind the scenes of lung research and everyday clinical life. In addition to an exciting and diversified program all around the work of the research center, the 1st Borsteler Fund Raising Run took place on this day.

Sebastian Marwitz wirbt 12-monatiges **Forschungsstipendium der DFG** (40.000 Euro) ein. Er führt seine Untersuchungen des TGF-β-Signalwegs bei Lungenkrebs als Gastwissenschaftler am Providence Portland Medical Center in Portland/Oregon (USA) fort.

Sebastian Marwitz is awarded a 12-month **research fellowship from the DFG** (40,000 euros). He continues his research on the TGF-β signalling pathway in lung cancer as a visiting scientist at the Providence Portland Medical Center in Portland/Oregon (USA).

Gute wissenschaftliche Praxis: **Leibniz-Führungskolleg** trifft sich am FZB.

Good scientific practice: **Leibniz-Führungskolleg** meets at the FZB.

Uta Jappe, Oberärztin der Medizinischen Klinik Borstel und Leiterin der FG Klinische und Molekulare Allergologie am FZB, ist erneut auf der Liste der **Top Mediziner der renommierten Zeitschrift Focus-Gesundheit** vertreten.

Uta Jappe, senior physician at the Medical Clinic Borstel and head of the Clinical and Molecular Allergology group at the FZB, is once again on the **list of top physicians** of the renowned journal Focus-Gesundheit.

2018

Großer Erfolg für Sabine Bartel: Der Borsteler Postdoktorandin ist es gelungen, einen der renommierten **"Marie Skłodowska-Curie Research Fellowships (RESPIRE 3)"** einzuwerben.

Great success for Sabine Bartel: The Borstel postdoctoral researcher has successfuly applied for one of the renowned **"Marie Skłodowska-Curie Research Fellowships (RESPIRE 3)"**.

Erste **Internationalen Asthma- und Allergiekonferenz** in Borstel. Im Fokus der zweitägigen Veranstaltung standen die Mechanismen der Vererbung von Asthma über mehrere Generationen, der Einfluss der mikrobiellen Flora auf die Gesundheit und Asthmaentwicklung, die verschiedenen Krankheitsbilder von Asthma und wie man diese auf molekularen Ebene unterscheiden kann, sowie die molekularen Mechanismen der allergischen Reaktion, neue Formen von Allergenen und deren Einsatz in neuartigen Diagnostiktestsystemen.

First International Asthma and Allergy Conference in Borstel. The two-day event focused on the mechanisms of inheritance of asthma predisposition over several generations, the influence of microbial flora on health and asthma development, the different disease patterns of asthma and how these can be differentiated at the molecular level, as well as the molecular mechanisms of allergic reactions, new forms of allergens and their use in novel diagnostic test systems.

Best of 2018

Innovatives Borsteler Immuntherapie-Konzept von Uta Jappe und Christian Schwager mit dem **Kanert-Preis für Allergieforschung** ausgezeichnet.

Innovative Borsteler immunotherapy concept by Uta Jappe and Christian Schwager awarded the **Kanert Prize for Allergy Research**.

Weltweit einmalig: Korrekte Diagnose der Lungentuberkulose in nur drei Tagen. Am FZB ist es gelungen, ein neues und schnelles Verfahren für die Diagnose der Lungentuberkulose zu entwickeln. Diese innovative Methode ist ein wichtiger Baustein für eine optimale Diagnostik und könnte in Zukunft zu einer Verbesserung der Tuberkulose-Behandlung führen.

Worldwide unique: Correct diagnosis of pulmonary tuberculosis in only three days. The FZB has succeeded in developing a new and rapid method for the diagnosis of pulmonary tuberculosis. This innovative method is an important building block for optimal diagnostics and could lead to an improvement in tuberculosis treatment in the future.

Der **Memento Forschungspreis** für vernachlässigte Krankheiten wurde an Martina Sester von der Universität des Saarlandes und an Christoph Lange, FG Klinische Infektiologie, verliehen.

The **Memento Research Prize for Neglected Diseases** was awarded to Prof. Dr. Martina Sester from Saarland University and Christoph Lange, Clinical Infectious Diseases.

Publikation / Publication Blockbuster: Science (Pathology), Journal of Allergy and Clinical Immunology (Exp. Pneumology, Study Center, Clin. & Mol. Allergology, Biochem. Immunology), Lancet Respiratory Medicine (Clin. Infect. Disease), European Respiratory Journal (Exp. & Mol. Mycobacteriology, Exp. Pneumology, Clin. Infect. Diseases, Pathology, Biobank), New England Journal of Medicine (Exp. & Mol. Mycobacteriology), American Journal of Respiratory and Critical Care Medicine (Study Center, Clin. Infect. Diseases), Lancet (Clin. Infect. Diseases), Lancet Infectious Diseases (Exp. & Mol. Mycobacteriology, Clin. Infect. Diseases), Nature Communications (Pathology), Lancet Oncology (Study Center).

2018

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Asthma and Allergy**

Asthma Exacerbation & Regulation

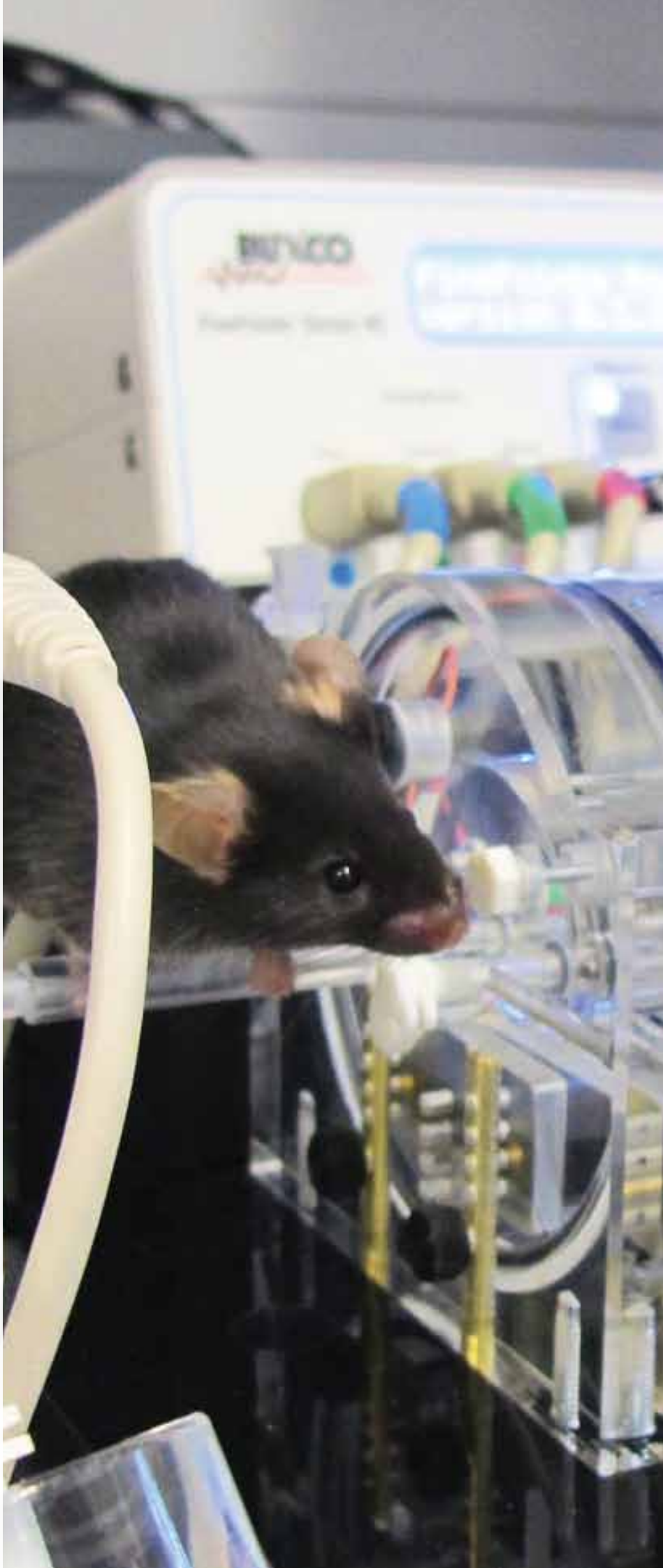
LUNG FUNCTION

ANIMAL MODELS

AIRWAY INFLAMMATION

EXACERBATION

REGULATION



Head

- Dr. Michael Wegmann

Members

- Dr. Lars Lunding
- Dr. Sina Webering
- Dr. Alexandra Schröder
- Rebecca Bodenstein-Sgró
- Linda Lang
- Vanessa Simon
- Lena Voges

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Asthma and Allergy**

Asthma Exacerbation & Regulation

Mission

Unter Verwendung geeigneter Mausmodelle, die verschiedene Krankheitsstadien widerspiegeln, wollen wir die Prozesse und Mechanismen entschlüsseln, die der Exazerbation, Progression und Chronifizierung der allergischen Atemwegsentzündung und damit des allergischen Asthma unterliegen.

To elucidate the processes and mechanisms underlying acute exacerbation, progression and chronification of allergic airway inflammation and, thus, the formation of allergic bronchial asthma by using appropriate mouse models mimicking the respective disease stages.

Most important findings

Bronchial asthma is a heterogenous and chronic disease of the airways that is characterized by airway hyperresponsiveness, episodes of broncho-obstruction, respiratory distress, and productive cough. Except from the rare cases of clearly intrinsic asthma these symptoms arise on the basis of an dysegulated, chronic inflammation of the airways. Thus, understanding dysregulation of this inflammatory response is a key to unravel the pathogenesis of bronchial asthma.

We have previously shown that interleukin (IL-) 37 is an anti-inflammatory cytokine that is able to regulate allergic immune responses and that local application of this cytokine improves all major aspects of experimental asthma in mice. Together with the finding that children with asthma display an impaired production of IL-37, we hypothesized that an inherent deficiency of this regulatory cytokine could predispose towards development of a chronic inflammatory disease like asthma. In turn, compensation of this deficiency could be a novel treatment option for the respective disease. In order to make use of these insights the mode of action of IL-37 has to be elucidated. After figuring out that the receptor chains IL-18Rα and SIGIRR form the so far unknown IL-37 receptor, we investigated the interference of IL-37 with a proinflammatory IL-18-signal in-vitro and in-vivo. By using mouse strains deficient for IL-18, IL-18Rβ, and IL-18BP we could exclude that the therapeutic effects of IL-37 on experimental asthma originate from a competitive inhibition of IL-18 or interference with its decoy receptor IL-18BP. In contrast, we could clearly show that IL-37 counterbalances the proinflammatory effects of IL-1β on TH2-type immune responses as well as on experimental asthma. In contrast to IL-38 – a near relative to IL-37 – the use of an IL-37-fc-fusion protein mimicked and even exceeded the therapeutic effect of IL-37.

Highlights

IL-37 counteracts proinflammation signals independent of IL-18 signaling

The matrikine, CP17, protects from airway neutrophilia during acute experimental asthma exacerbation

IL-6 is of critical importance for acute experimental asthma exacerbation

Selected publications

Wegmann M. Targeting cytokines in asthma therapy: could IL-37 be a solution? Expert Rev Respir Med. 2017;11:675-677.

George L, Mitra A, Thimraj TA, Irmler M, Vishweswaraiah S, Lunding L, Hühn D, Madurga A, Beckers J, Fehrenbach H, Upadhyay S, Schulz H, Leikauf GD, Ganguly K. Transcriptomic analysis comparing mouse strains with extreme total lung capacities identifies novel candidate genes for pulmonary function. Respir Res. 2017 Aug 9;18(1):152.

Fehrenbach H, Wagner C, Wegmann M. Airway remodeling in asthma: what really matters. Cell Tissue Res. 2017;367:551-569.

Nissen G, Hollaender H, Tang FSM, Wegmann M, Lunding L, Vock C, Bachmann A, Lemmel S, Bartels R, Oliver BG, Burgess JK, Becker T, Kopp MV, Weckmann M. Tumstatin fragment selectively inhibits neutrophil infiltration in experimental asthma exacerbation. Clin Exp Allergy. 2018;48:1483-1493.

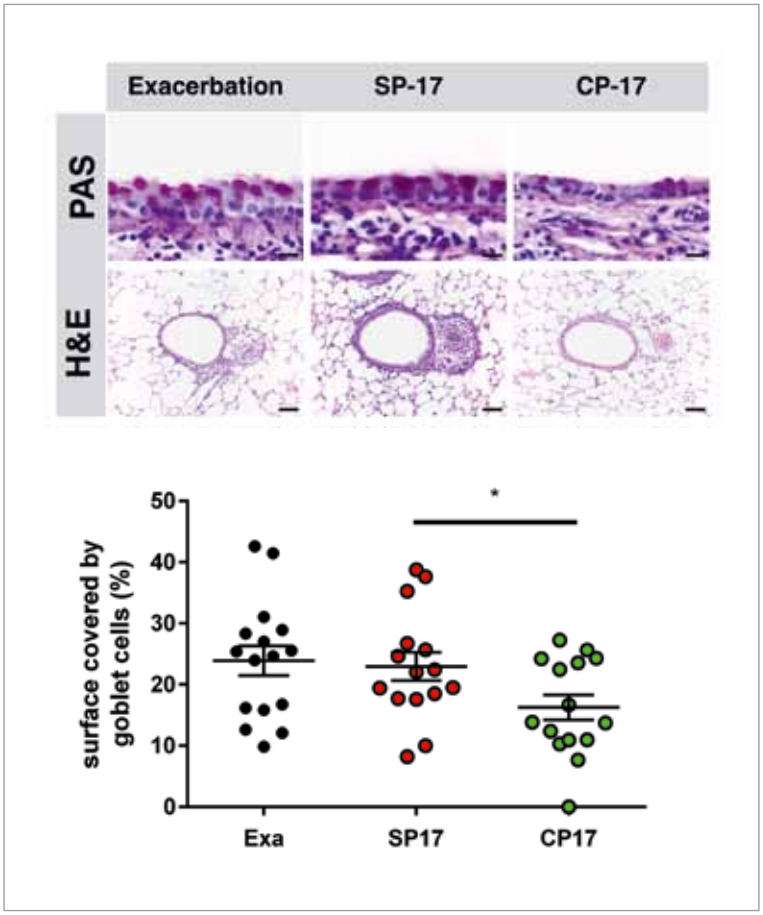


Figure 1. The peptide CP17, a collagen type IV cleavage product, selectively decreases neutrophil infiltration in a mouse model of asthma exacerbation. Histological analysis shows reduced mucus production in CP17 treated lung cells (upper left panel, PAS). H&E staining displays the reduction in neutrophil infiltration with reduced interstitial accumulation of cells (lower left panel, black arrows). Assessment of mucus producing cells using a newCAST system shows significantly reduced number of mucus producing cells after CP17 treatment. * $P < 0.05$ (published in Clin Exp Allergy. 2018 Nov;48(11):1483-1493.)

Priority Research Area **Asthma and Allergy**

Asthma Exacerbation & Regulation

While asthma patients inherently display a dysbalance between the release of proinflammatory and regulatory mediators that is usually controlled by the use of corticosteroids (CS), exogenous triggers such as high allergen load or respiratory infection have the ability to overwhelm this equilibrium and to lead to acute aggravation of the disease. Unfortunately, to date the only way to regain disease control during this acute exacerbation is quadrupling or even quintupling the CS use with the well-known side effects. Thus, it represents an unmet medical need to recognize an upcoming exacerbation as soon as possible in order to take countermeasures and to avert aggravation of the stable disease. It is the aim of the EXASENS consortium to identify, validate and detect marker molecules that predict an upcoming exacerbation. Using a well-established mouse model of virus-induced asthma exacerbation, we performed comprehensive time kinetics and found at least three candidates, namely IL-6, IL-8 and tumor necrosis factor (TNF), that are a) released in high and detectable amounts, b) at a very early time-point after infection, c) with high discrepancy to the stable disease, and that d) disappear during the process of remission. By fulfilling these criteria these candidates will now enter the technical development process as markers predicting an upcoming exacerbation.

Having identified these candidates we started to investigate their functional relevance for the pathogenesis of acute, virus-triggered asthma exacerbations. We started with IL-6, which appeared in highest concentrations in BAL fluid as soon as two hours after infection and remained at peak levels until full formation of the exacerbation with aggravated airway inflammation, mucus production, and further decreased lung function. Therefore, up-regulation of IL-6 precedes IL-17 release by NK cells, which is critically important for the exacerbation. Interestingly, animals lacking IL-6 are completely protected from induction of acute, virus-triggered asthma exacerbation, indicating an important role of this cytokine for the formation of this disease stage. Interestingly, the same is true for animals that are deficient for the cytokine IL-15, which regulates proliferation of NK cells.

Airway neutrophilia is a common feature of the aggravated airway inflammation during acute asthma exacerbation. In this situation among various cytotoxic mediators also enzymes are released in order to destroy pathogens and to remove necrotic tissues. In collaboration with the Department of Pediatric Pneumology & Allergology, University Medical Center Schleswig-Holstein, we found that these enzymes also digest collagen fibers and thereby release collagen fragments with biological activity. Hence, the collagen fragment tumstatin decreased cytotoxic activity and migration of neutrophils in vitro. Consequently, local application of tumstatin to mice with acute, virus-triggered asthma exacerbation resulted in markedly reduced airway neutrophilia and improved aggravated pathophysiological hallmarks such as mucus hyperproduction.

Internal and external collaboration

Research Center Borstel: the Divisions of Biochemical Immunology, Cellular Pneumology, Clinical & Molecular Allergology, Experimental Pneumology, Infection Immunology, Innate Immunity, Invertebrate Models, Mucosal Immunology & Diagnostics and the Fluorescence Cytometry Core Facility.

External national cooperations: DZL partner sites at the Department of Pediatric Pneumology & Allergology, University Medical Center Schleswig-Holstein, Campus Centrum Lübeck, the Department of Clinical Chemistry and Molecular Diagnostics of the Clinic of the Philipps-University Marburg, the Department of Pulmonary & Allergy, University Children's Hospital Munich, LMU Munich, the Department Translational Pulmonology, University of Heidelberg, The Center of Medical Microbiology & Hygiene, University Clinics Heidelberg, the Heinrich-Pette Institute in Hamburg, the University Medical Center Hamburg (UKE), the Institute of Biochemistry at the University of Kiel as well as with the Clinic of Internal Medicine V at the University Clinic of the Saarland at Homburg.

International cooperations: the Division of Infectious Diseases at the University of Colorado, Denver, USA, the Department of Experimental Immunopathology, and the Ospedale a Milano, Italy.

Grant support

BMBF DZL DA-AA-TFP

BMBF DZL LI-JRG-1

BMBF FKZ: 13N13857

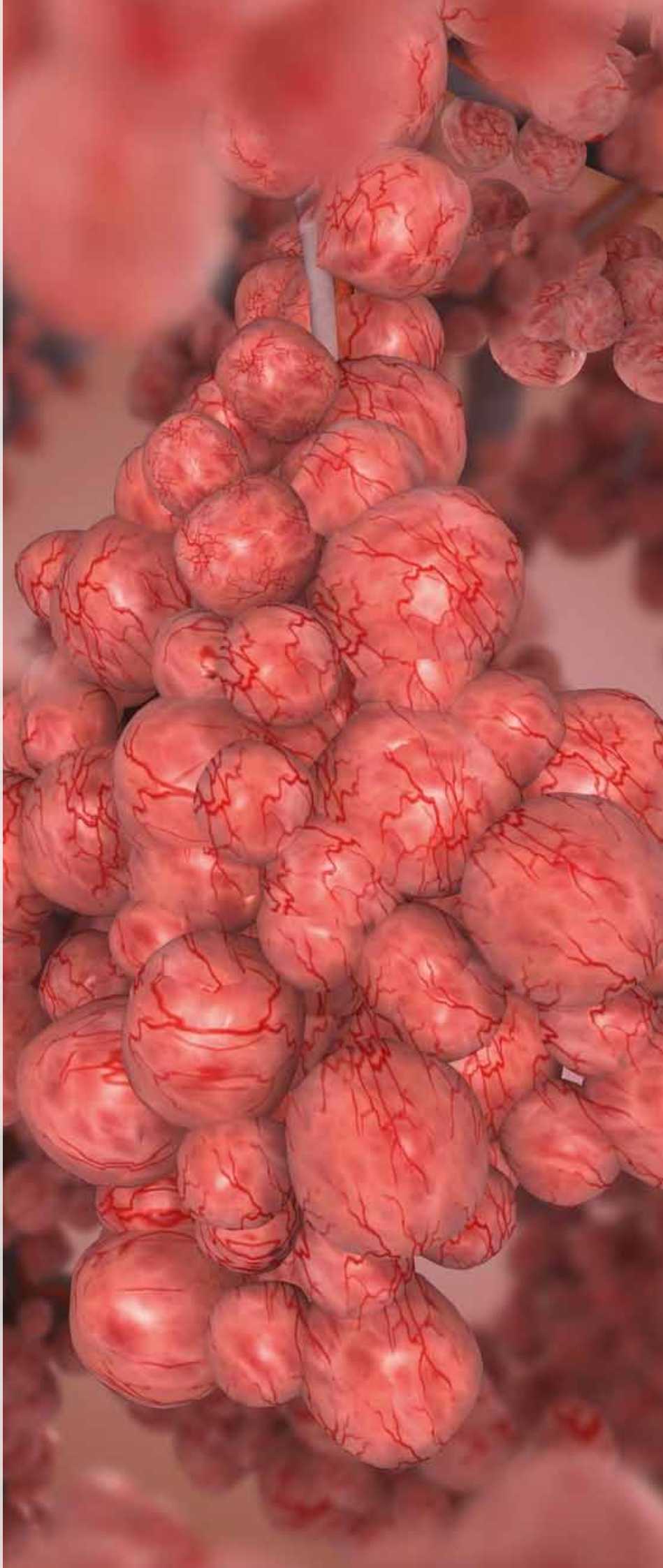
AUTOIMMUNITY

PRIMARY SJÖGREN'S SYNDROME

SYSTEMIC SCLEROSIS

PULMONARY INFLAMMATION

EXPERIMENTAL MODELS



Priority Research Area **Asthma and Allergy**

Autoimmunity in the Lung

Head

- Prof. Dr. Xinhua Yu
- Prof. Dr. Gabriela Riemekasten, University of Lübeck

Members

- Xiaoyang Yue
- Junping Yin
- Yaqing Shu

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Asthma and Allergy**

Autoimmunity in the Lung

Mission

Der Forschungsfokus der Liaison-Gruppe "Autoimmunität in der Lunge" liegt in der Untersuchung von autoimmunbedingten Lungenerkrankungen wie systemischer Sklerose (SSc) und primärem Sjögren-Syndrom (pSS). Wir wollen die pathogenen Mechanismen der Remodellierung von pulmonalem Interstitium und Gefäßen dieser Erkrankungen untersuchen. Wir hoffen, dass das Verständnis der zugrundeliegenden pathologischen Vorgänge uns nicht nur helfen wird, neue therapeutische Wege in der Behandlung der systemischen Autoimmunität zu entwickeln, sondern auch entsprechende Mechanismen in anderen chronisch-entzündlichen Lungenkrankheiten wie Asthma und COPD zu identifizieren.

The Liaison group "Autoimmunity in the Lung" focuses on the investigation of autoimmune-related lung diseases such as systemic sclerosis (SSc) and primary Sjögren's syndrome (pSS). We aim to investigate the pathogenic mechanisms underlying the remodeling of the pulmonary interstitium and vessels of those diseases. We hope that understanding the underlying pathological principles will not only help us to develop novel therapeutic strategies in the treatment of systemic autoimmunity but also to identify corresponding mechanisms in other chronic inflammatory lung disease like asthma and COPD.

Most important findings

Primary Sjögren's syndrome (pSS) is an autoimmune disorder mainly targeting salivary and lacrimal glands and leading to xerostomia (dry mouth) and exerophthalmia (dry eye). In some patients with pSS, autoimmunity-mediated disease manifestations are also observed in inner organs, including the lung. immunologically, pSS is characterized by lymphocytic infiltrates into target tissues and ectopic expression of MHC II molecules on glandular epithelial cells as well as by a specific panel of circulating autoantibodies. In contrast to the unknown disease-related autoreactive T cells, many autoantibodies have been identified in patients with pSS, including anti-SSA/Ro and anti-SSB/La autoantibodies, rheumatoid factor, anti-nuclear antibody, anti-muscarinic type 3 acetylcholine receptors (M3R) and anti-α fodrin antibodies. Although B cell hyper-activation resulting hypergammaglobulinemia and production of autoantibodies is a predominant feature of pSS, it is not clear whether B cells and autoantbodies are indispensible for the development of the disease. Animal models provide a powerful tool for understanding the pathogenesis of disease.

Recently, we have established a novel mouse model for pSS by immunizing mice with the Ro60_316-335 peptide containing a predominant T cell epitope emulsified in TiterMax® as an adjuvant. After immunization, mice develop several symptoms mimicking pSS, including a decreased secretion of tears, lymphocytic infiltration into the lacrimal glands, autoantibodies and increased levels of inflammatory cytokines (Figure 1). Disease susceptibility to this novel

Highlights

1. Establishment of a novel mouse model for primary Sjögren's syndrome.

2. Identification of ectopic expression of MHC II molecules as an early presymptomatic feature of the mouse model of primary Sjögren's syndrome.

3. Establishment of a novel mouse model for systemic sclerosis by immunizing mice with human AT1R.

Selected publications

Yin J., Zheng J., Deng F., Zhao W., Chen Y., Huang Q., Huang R., Wen L., Yue X., Petersen F. and Yu X. Gene Expression Profiling of Lacrimal Glands Identifies the Ectopic Expression of MHC II on Glandular Cells as a Presymptomatic Feature in a Mouse Model of Primary Sjögren's Syndrome. Front. Immunol., 2018 Oct 31;9:2362. doi: 10.3389/fimmu.2018.02362. eCollection 2018.

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Yu X, Riemekasten G, Petersen F. Autoantibodies against muscarinic acetylcholine receptor M3 in Sjogren's syndrome and corresponding mouse models. Front Biosci (Landmark Ed). 2018 Jun 1;23:2053-2064.

Yu X, Petersen F. A methodological review of induced animal models of autoimmune diseases. Autoimmun Rev. 2018 May;17(5):473-479. doi: 10.1016/j.autrev.2018.03.001.

Yu X, Kasprick A, Hartmann K, Petersen F.The Role of Mast Cells in Autoimmune Bullous Dermatoses. Front Immunol. 2018 Feb 28;9:386

Wen L, Krauss-Etschmann S, Petersen F, Yu X. Auto-antibodies in Chronic Obstructive Pulmonary Disease. Wen L, Krauss-Etschmann S, Petersen F, Yu X.Front Immunol. 2018 Jan 25;9:66.

Deng F, Chen Y, Zheng J, Huang Q, Cao X, Zillikens D, Petersen F, Yu X.CD11b-deficient mice exhibit an increased severity in the late phase of antibody transfer-induced experimental epidermolysis bullosa acquisita. Exp Dermatol. 2017 Dec;26(12):1175-1178

Zheng J, Huang Q, Huang R, Deng F, Yue X, Yin J, Zhao W, Chen Y, Wen L, Zhou J, Huang R, Riemekasten G, Liu Z, Petersen F, Yu X. B Cells Are Indispensable for a Novel Mouse Model of Primary Sjögren's Syndrome. Front Immunol. 2017 Oct 24;8:1384.

Petersen F, Yue X, Riemekasten G, Yu X. Dysregulated homeostasis of target tissues or autoantigens - A novel principle in autoimmunity. Autoimmun Rev. 2017 Jun;16(6):602-611.

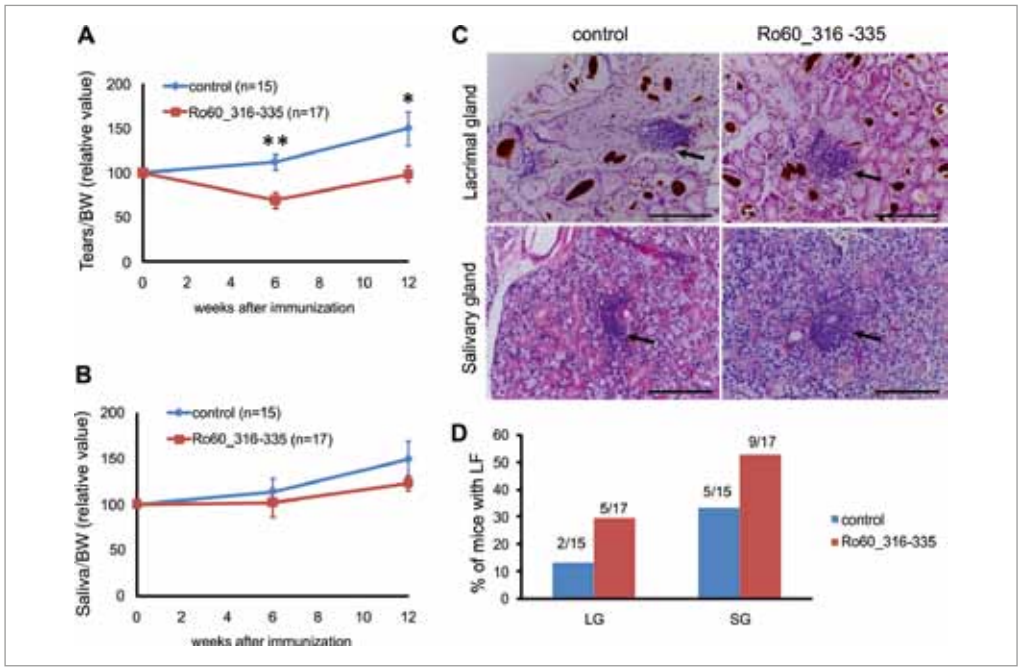


Figure 1. Immunization with the Ro60_316-335 peptide induces a pSS-like disease in C3H/He mice. C3H/HeJ mice were immunized with Ro60_315-336 (n=17) or treated with PBS as control (n=15) and secretion of tears (A) and saliva (B) was determined after pilocarpine stimulation. Values were normalized to the respective body weights and subsequently to the levels of secretion determined before immunization. (C) Representative sections with lymphocytic foci (LF) derived from lacrimal (upper panel) and salivary glands (lower panel) of Ro60_315-336 immunized mice or controls after H&E staining. Black arrows indicate LF. Bars, 100 µm. (D) Incidence of mice with LF in lacrimal and salivary glands. Numbers above bars indicate the ratio of number of mice with LF/total number of mice examined. (* p<0.05 and ** p<0.01).

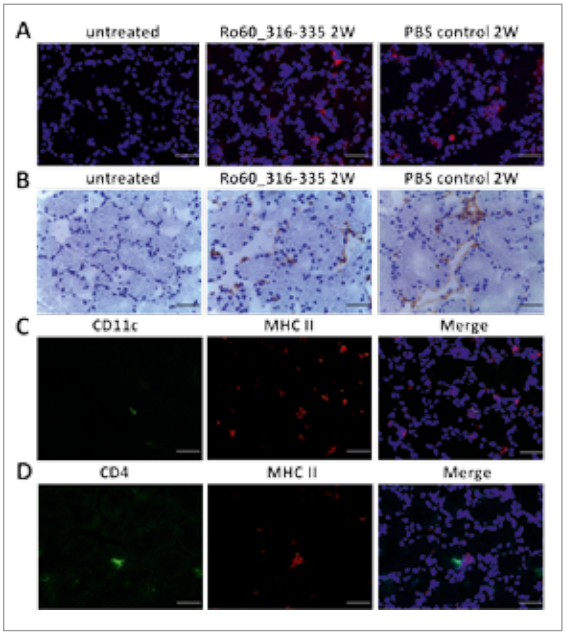


Figure 2. Ectopic expression of MHC II molecules on glandular cells in lacrimal glands of TiterMax®-treated mice. A: Representative micrographs of MHC II molecules on lacrimal glands from mice immunized with Ro60_316-335 peptides or PBS emulsified in TiterMax® at the week 2 after immunization and on untreated mice (week 0). MHC II molecules were detected on cryosections of the lacrimal glands by direct immunofluorescence staining using Alexa-647 conjugated rat-anti-mouse I-A/I-E Antibody. B: Representative micrographs of MHC II molecules on lacrimal glands of mice. Expression of MHC II molecules was determined by immunohistochemistry on cryosections. C: Co-staining for CD11c and MHC II molecules on cryosections of lacrimal glands from mice immunized with Ro60_316-335 peptides. D: Co-staining for CD4 and MHC II molecules on cryosections of lacrimal glands from mice immunized with Ro60_316-335 peptides. Bars, 50um.

Priority Research Area **Asthma and Allergy**

Autoimmunity in the Lung

mouse model varies among strains, where C3H/He (H2-k) was susceptible while DBA/1 (H2-q) and C57BL/6 (H2-b) were resistant. Furthermore, depletion of B cells using anti-CD20 monoclonal antibodies could prevent C3H/He mice from development of the pSS-like disease. Therefore, this provides a novel mouse model for pSS and reveals an indispensable role of B cells in this model. Moreover, it suggests that T cell epitope within Ro60 antigen is potentially pathogenic for pSS.

Although it is well known that ectopic expression of MHC II molecules on glandular cells is a feature of pSS, the cause of this ectopic expression and its potential role in the pathogenesis of the disease remains elusive. By detecting gene expression profiling of lacrimal glands at different phases of the disease in the novel mouse model, we have found that the expression of MHC II gene in lacrimal glands of diseased mice is highly upregulated compared with that in untreated mice. Using immunohistochemistry and immunofluorescence staining, we have confirmed the ectopic expression of MHC II molecules at the protein level on glandular epithelial cells. Our study has also demonstrated that the ectopic expression of MHC II is caused by TiterMax® and appears before the onset of disease. In addition, co-localization of CD4+ T cells and MHC II expressing cells has been observed in lacrimal glands, indicating that glandular cells may have the ability to present antigen to corresponding T cells (Figure 2). Therefore, our results suggest that ectopic expression of MHC II molecules on glandular cells represents a presymptomatic feature of pSS and is a potentially pathogenic event.

Internal and external collaboration

Petersen F, Division of Biochemical Immunology, Research Center Borstel.

Krauss-Etschmann S, Division of Early Life Origins of CLD, Research Center Borstel.

Frey A, Division of Mucosal Immunology and Diagnostic, Research Center Borstel.

Scholzen T, Core Facility Fluorescence Cytometry, Research Center Borstel.

Zillikens D, Institute of Dermatology, University of Lübeck.

Ludwig R, Institute of Dermatology, University of Lübeck.

Ibrahim S, Institute of Dermatology, University of Lübeck.

Aiping Ma, the department of Rheumatology, the First Affiliated Hospital of Xiamen University.

Juan Chen, the department of Rheumatology, the First Affiliated Hospital of Xiamen University .

Wei Qiu, Department of Neurology, The Third Affiliated Hospital of Sun Yat-Sen University, China

Junfeng Zheng, Institute of Psychiatry and Neuroscience, Xinxiang Medical University, China.

Grant support

DFG Cluster of Excellence "Inflammation at Interfaces"

DFG GRK 1727 "Modulation von Autoimmunität"

BMBF Deutsches Zentrum für Lungenforschung (DZL), ARCN

MAST CELL-NEUTROPHIL INTERACTION

CELL ADHESION

TRANSMIGRATION

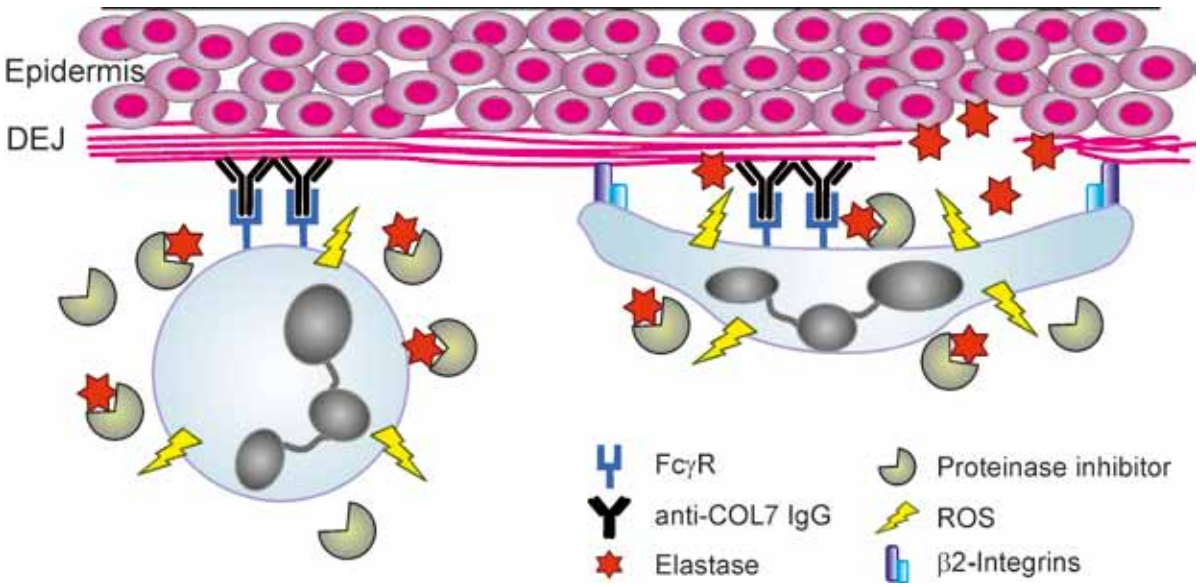
TISSUE DAMAGE

AIRWAY EPITHELIUM

ASTHMA

AUTOIMMUNITY

PROTEASES



Priority Research Area **Asthma and Allergy**

Biochemical Immunology

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Cell adhesion is a prerequisite for neutrophil-mediated tissue damage. Neutrophils are activated e.g. by immune complexes (here: COL7/anti-COL7) via FcγR. Consequently, the activated neutrophils generate ROS and release elastase and other proteinases. When neutrophil adhesion is prevented (left panel), proteinases will be bound to inhibitors and ROS will be deactivated by scavengers resulting in tissue protection. If neutrophils can properly adhere to the target structure during activation (right panel), a β_2-integrin-stabilized closed space will prevent the access of inhibitors and scavengers to proteinases and ROS resulting in uncontrolled activity of the latter mediators which leads to consequent tissue damage.

Priority Research Area **Asthma and Allergy**

Biochemical Immunology

Mission

Unser Ziel ist es, die pathophysiologischen Prozesse in der Effektorphase des Asthmas zu verstehen und dadurch neue therapeutische Angriffspunkte zur Behandlung dieser Erkrankung zu identifizieren. Wir untersuchen dabei insbesondere die regulatorischen Mechanismen, welche dem Zusammenspiel von Rezeptor-aktivierenden Autoantikörpern, Mastzellen und Neutrophilen in der Pathogenese des Asthmas zugrunde liegen.

Our goal is to understand the pathophysiological processes in the effector phase of asthma and COPD and thereby to identify new therapeutic targets for the treatment of these diseases. In particular, we investigate the regulatory mechanisms underlying the interaction of receptor-activating autoantibodies, mast cells and neutrophils in the pathogenesis of asthma.

Most important findings

Despite intensive research and significant advances in treatment, **asthma** and its underlying pathomechanisms are still largely unclear. A central question here is how the disease ultimately manifests itself in the organ and how tissue damage and remodeling are mediated in the lungs.

Neutrophils play an essential role as first-line effector cells in host defense against microbial invaders. In a study we were able to show that neutrophil-deficient animals are protected against the development of experimental asthma. Since no differences in cytokine pattern between neutropenic and wild type mice in disease was found, a role for direct cell-cell contact and short-range components such as **proteases** or reactive oxygen metabolites can be suggested. Although the relevance of proteinases in the pathogenesis of asthma and COPD has been known for a long time, therapeutic approaches with corresponding inhibitors have been largely unsuccessful. We are trying to understand the reasons for this and aim to increase the efficiency of biologically effective proteinase inhibitors through new strategies. In a study we could show in vitro as well as in animal models in vivo that the adherence of neutrophils has a key function here (Fig.1). Cell **adherence** induced by the activation of neutrophils creates a closed space between cell and substrate in which proteinases and reactive oxygen species can exert their tissue-damaging effect without being accessible to exogenous inhibitors (Fig. 2). In current studies, we are investigating whether the simultaneous pharmacological attack on cell adherence and proteinases could be a new therapeutic approach in the treatment of chronic obstructive pulmonary disease.

Highlights

Identification of neutrophil adherence as a key-process in protease-mediated tissue damage

Detection of auto-antibody signatures in asthma and COPD patients

First generation of monoclonal antibodies that functionally activate GPCR

Selected publications

Yu X, Akbarzadeh R, Pieper M, Scholzen T, Gehrig S, Schultz C, Zillikens D, König P, Petersen F. Neutrophil adhesion is a prerequisite for antibody-mediated proteolytic tissue damage in experimental epidermolysis bullosa acquisita. JOURNAL OF INVESTIGATIVE DERMATOLOGY 2018, 138: 1990-1998

Epp A, Hobusch J, Bartsch YC, Petry J, Lilienthal G-M, Koeleman CAM, Eschweiler S, Möbs C, Hall A, Morris SC, Braumann D, Engellenner C, Bitterling J, Rahmöller J, Leliavski A, Thurmann R, Collin M, Moremen KW, Strait RT, Blanchard V, Petersen A, Gemoll T, Habermann JK, Petersen F, Nandy A, Kahlert H, Hertl M, Wuhrer M, Pfützner W, Jappe U, Finkelman FD, Ehlers M. Sialylation of IgG antibodies inhibits IgG-mediated allergic reactions. THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY 2018, 148: 399-402

Yu X, Kasprick A, Hartmann K, Petersen F. The Role of Mast Cells in Autoimmune Bullous Dermatoses. FRONTIERS IN IMMUNOLOGY 2018, 9: 386

Petersen F, Yue X, Riemekasten G, Yu X. Dysregulated homeostasis of target tissues or autoantigens- A novel principle in autoimmunity. AUTOIMMUNITY REVIEWS 2017, 16:602-611.

Zhao W, Yue X, Liu K, Zheng J, Huang R, Zou J, Riemekasten G, Petersen F, Yu X. The status of pulmonary fibrosis in systemic sclerosis is associated with IRF5, STAT4, IRAK1, and CTGF polymorphisms. RHEUMATOLOGY INTERNATIONAL 2017, 37:1303-1310.

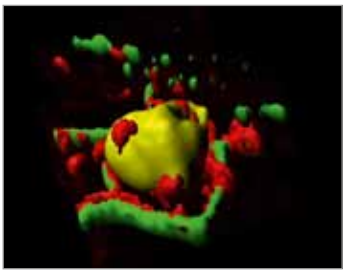

Figure 1. A neutrophil attached to a lung epithelial cell. The neutrophil adhere via its integrins (visualized in yellow) to the tight junctions (green) of the epithelial cells. The cell-cell interaction is stabilized by actin filaments (red).

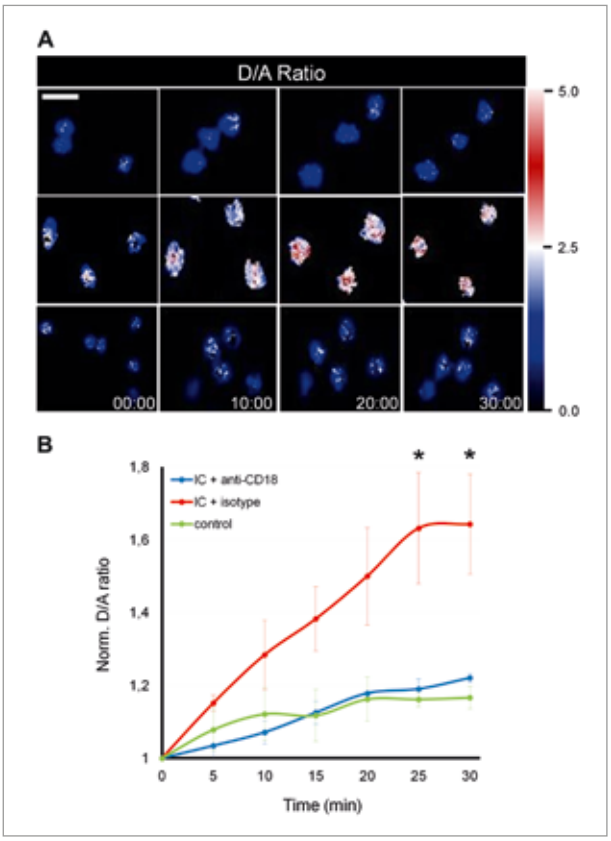

Figure 2. Neutrophil adhesion enables cell-free protease-activity in the presence of protease-inhibitors. Neutrophils were loaded with the elastase FRET probe NEmo-2 in presence of 100 µM of the inhibitor alpha-1-antitrypsin and subsequently exposed to uncoated surfaces (**A**, upper panel), to immobilized immune complexes (IC) in presence of unrelated IgG (**A**, middle panel), or IC in presence of adhesion-blocking anti-CD18 antibodies (**A**, lower panel). Elastase enzyme-activity was determined by the loss of the FRET signal, indicated as color change from dark blue to red. Donor (Coumarin 343) and sensitized acceptor (TAMRA) emission was collected for different time points and D/A ratio images were calculated using the ImageJ 1.38r software. Normalized D/A ratios of three independent experiments were quantified and compared among these groups (**B**). Data are presented as mean ± SD. Statically significant differences were indicated. Activation-induced adhesion of neutrophils allows protease activity even in the presence of inhibitors (middle panel). Blocking of adhesion results in the inhibition of protease activity (lower panel) although the release of proteases is not changed.

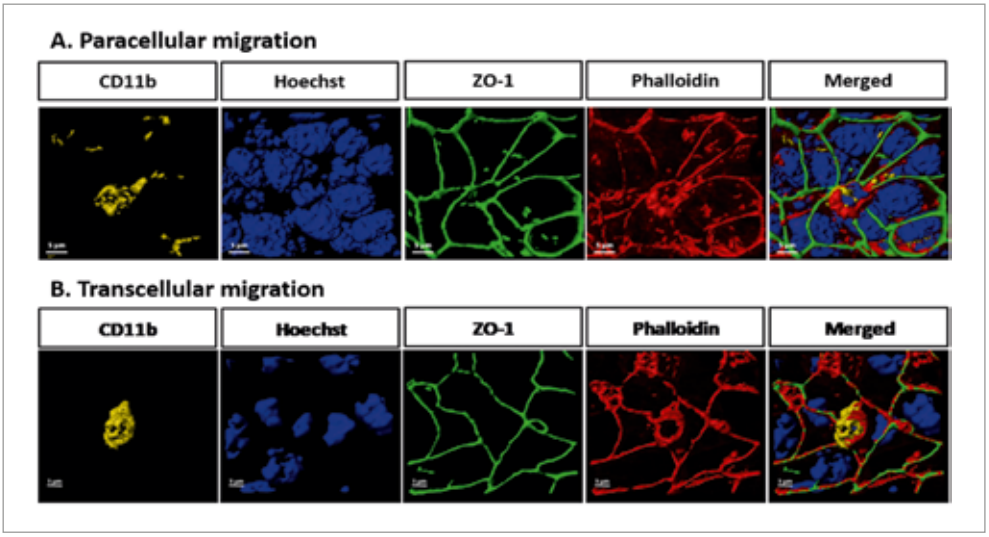

Figure 3. Paracellular and transcellular transmigration of neutrophils across the epithelial barrier. Neutrophils were stimulated with fMLP in an inverted transwell system to allow transmigration in a basolateral to apical direction. CD11b indicates the neutrophils, ZO-1 the presence of tight junctions, and phalloidin identifies actin filaments. In case of paracellular transmigration, the tunnel consists of tight junction proteins and actin filaments (A) while in transcellular migration the tunnel is formed solely by actin filaments (B).

Priority Research Area **Asthma and Allergy**

Biochemical Immunology

Cell adherence and protease activity are not only essential in tissue damage, they also provide the basis for the **transmigration** of neutrophils through endo- and epithelial barriers. According to our hypothesis, transmigration represents a pro-inflammatory signal leading to reprogramming of the epithelium. First results show that in this case neutrophils follow beside the classical paracellular migration through tight junctions a recently discovered transcellular pathway directly through the epithelial cells (Fig. 3).

The pro-pathogenic function of **mast cells** and their mediators in allergic asthma has long been known. Interestingly, most studies on the function of these cells refer to the period of acute reaction after contact with an allergen, during which their role in chronic disease is much less understood. According to our hypothesis, the biological function of certain mediators can change completely during the course of the disease, so that an initial pathogenic effect can develop into a protective function and vice versa. The consideration of chronicity in the effect of a mediator therefore appears to be of central importance for the assessment of its usability as a therapeutic point of attack. We investigate this function change using the example of mast cell chymase and its murine equivalent MCP-4. Using MCP-4-deficient mice, we were able to show in a chronic asthma model that the effect of chymase changes from protective to pro-pathogenic function during inflammation. In further studies, we clarify causes and mechanisms of MCP-4 functional change during the course of the disease and examine the potential use of the enzyme as a potential therapeutic target in the treatment of allergic asthma.

While in the past **autoimmunity** was primarily associated with rheumatic diseases, more recent findings point to the involvement of autoimmune processes in the pathogenesis of other chronic diseases, too. The focus of the investigations is on the role of so-called functional autoantibodies, which intervene in immunoregulation via a direct activation of cytokine and chemokine receptors. We are currently conducting a clinical pilot study within the DZL in asthmatics and COPD patients to determine whether corresponding functional autoantibodies can be detected in these diseases. Initial results indicate that each disease has a specific signature of autoantibodies. The further characterization of these antibodies will attempt to establish a new class of prognostic and diagnostic biomarkers for asthma and COPD. Since these antibodies can play a role in the regulation of disease processes, we also hope to identify new therapeutic targets. The efficacy of competing receptor agonists and antagonists as adjuvant therapeutics will be tested in novel animal models.

Internal and external collaboration

Internal:
Yu X, Clinical Liaison Group Autoimmunity in the Lung; Wegmann M, Division of Asthma Exacerbation & Regulation; Hölscher C, Division of Infection Immunology; Frey A, Division of Mucosal Immunology and Diagnostic; Heine H, Division of Innate Immunity; Goldmann T, Division of Clinical and Experimental Pathology; Scholzen T, Core Facility Fluorescence Cytometry; Gaede K, Biobank North.

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Grant support

DFG Cluster of Excellence "Inflammation at Interfaces"

DFG RTG1727 "Modulation of Autoimmunity"

DFG IRTG1911 "Immunoregulation of Inflammation in Allergy and Infection"

BMBF Deutsches Zentrum für Lungenforschung (DZL), ARCN

LIPOPHILIC ALLERGENS

BREAST MILK

BIOLOGICALS

PEANUT OLEOSINS

BASOPHIL ACTIVATION TEST

GLYCANS

INHALANT ALLERGENS

THERAPEUTIC ANTIBODIES

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Priority Research Area **Asthma and Allergy**

Clinical and Molecular Allergology

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Asthma and Allergy**

Clinical and Molecular Allergology

Mission

Die FG Klinische und Molekulare Allergologie erforscht die Aufklärung von Pathomechanismen der Allergie- und Asthmaentstehung. Dazu werden neue Allergene aus diversen Allergenquellen identifiziert, charakterisiert und sowohl für die mechanistische Aufklärung als auch für die Verbesserung der Diagnostik genutzt. Durch die Anbindung an 2 Allergie-Ambulanzen (Borstel und Lübeck) kommen (A) neueste Forschungsergebnisse den Patienten zugute (Translation), und kann (B) die Grundlagenforschung mit Primärmaterial klinisch gut charakterisierter Patienten in Ethik-gestützten Studien durchgeführt werden.

The Division of Clinical and Molecular Allergology is focusing on the identification of pathomechanisms leading to allergy and asthma development. Allergens in different allergen sources are identified, characterized and used as tools for both, mechanistic research and improvement of diagnostic tests. Because of the association with 2 allergy outpatient clinics (Borstel and Luebeck), (A) patients are provided with the latest research results (translation of basic science), and (B) basic science is performed with primary material from well-characterized patients in studies with ethical approval.

Most important findings

Identification of oleosins as markers for symptom severity in peanut allergy

Peanuts are the leading cause of food-induced anaphylaxis in Europe, the US and Australia. As patients experience the whole spectrum of allergic symptoms including dyspnea and allergic asthma, we investigated the association of single allergens with symptom severity and "organ specificity" with a particular emphasis on lipophilic peanut allergens, the oleosins. Oleosins are absent from defatted aqueous extracts used for diagnosis and experimental therapies, and therefore might be one reason for misdiagnosis and treatment failure. In a study cohort of 120 participants, among them 90 peanut-allergic individuals, an association between oleosin sensitization and severe allergic symptoms was observed. A flow cytometric diagnostic test was established that is able to discriminate perfectly between peanut-allergic subjects and peanut-sensitized individuals, and thus might reduce the number of oral provocation test in future.

Highlights

development of a highly-sensitive ELISAs for the determination of the peanut allergens Ara h 2 and Ara h 6 in breast milk

identification of single HDM-allergens potentially associated with respiratory symptoms

identification of immunogenic epitopes on infliximab with pharmacological impact

identification of peanut oleosins as potential biomarker for symptom severity

election of Prof. Jappe as member of the Collegium Internationale Allergologicum (CIA) and member of the EAACI interest group Biologicals

election of Prof. Jappe as member of the executive board of the WHO/IUIS Allergen Nomenclature Committee

Prizes

Kanert-Price for Allergy Research (Kanert Foundation), 2018

Price for the best PhD Thesis of the district of Bad Segeberg, 2018

Young talent advancement award of the German Society for Allergology and Clinical Immunology (DGAKI), 2017

Selected publications

Jappe, U., Minge, S., ..., Becker, W. M., Goldmann, T, ..., Homann, A: Meat allergy associated with galactosyl-α-(1,3)-galactose (α-Gal)-Closing diagnostic gaps by anti-α-Gal IgE immune profiling, ALLERGY, 2018, 73(1), p. 93-105

Epp, A., ..., Jappe U., ..., Ehlers M.: Sialylation of IgG antibodies inhibits IgG-mediated allergic reactions, JACI, 2018, 141(1), p. 399-402

Schocker, F., Scharf, A., Kull, S. & Jappe, U.: Detection of the Peanut Allergens Ara h 2 and Ara h 6 in Human Breast Milk, Int Arch Allergy Immunol . 2017, 174, p 17-25

Homann, A., ..., Jappe, U.: Glycan and Peptide IgE Epitopes of the TNF-alpha Blockers Infliximab and Adalimumab, THERANOSTICS, 2017, 7(19), p. 4699-4709

Homann, A., Schramm, G. & Jappe, U.: Glycans and Glycan-specific IgE in Clinical and Molecular Allergology-Sensitization, Diagnosis and Clinical Symptoms, JACI, 2017, 140(2), p. 356-368

Schwager, C., Kull, S., Behrends, J., Röckendorf, N., Schocker, F., Frey, A., Homann, A., Becker, W-M. & Jappe, U.: Peanut oleosins associated with severe peanut allergy, JACI, 2017, 140(5), p. 1331-1338

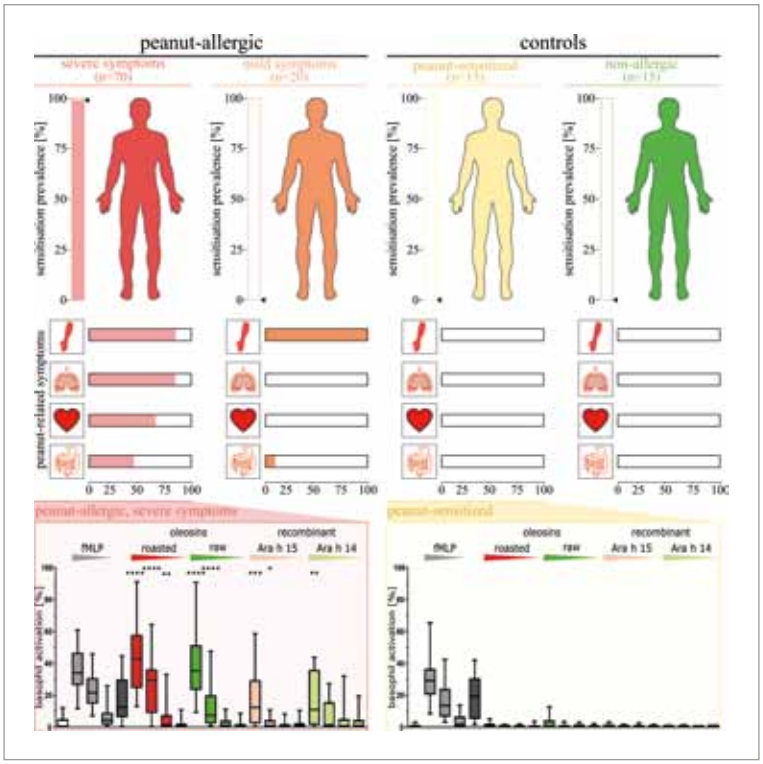

Figure 1. Characteristics of the investigated study population with regard to sensitization prevalence to oleosins (top), frequency of peanut-related symptoms affecting different organs (skin, respiratory tract, cardiovascular system and gut) (middle). Comparison of oleosin-induced basophil activation in peanut-allergic individuals (n=20) and peanut-sensitized subjects (n=15). Mann-Whitney U test ****P < .0001, ***P < .001, and **P < .01, *P < .05.

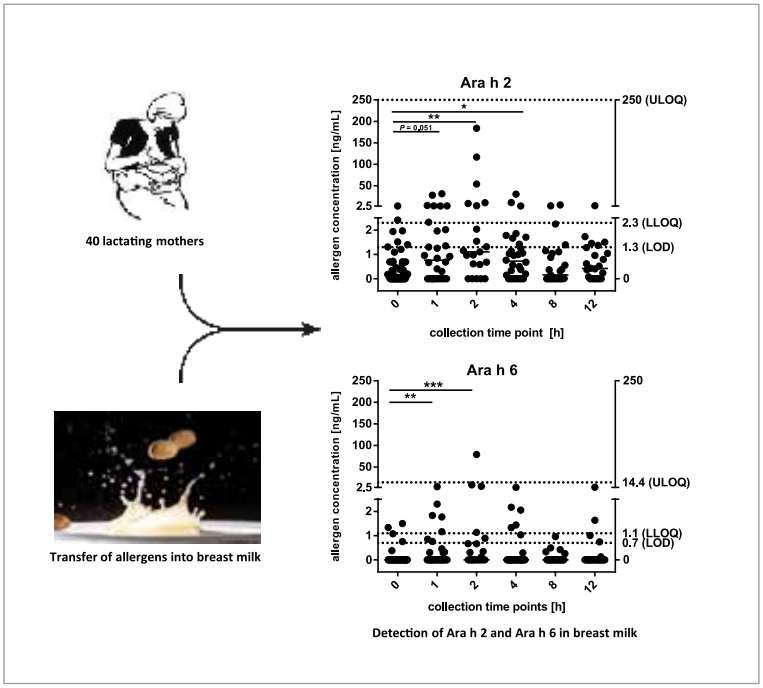

Figure 2. Ara h 2 and Ara h 6 concentrations in breast milk samples of the German study group determined by developed and validated ELISA assays. Schocker, F., Scharf, A., Kull, S. & Jappe, U.: *Detection of the Peanut Allergens Ara h 2 and Ara h 6 in Human Breast Milk: Development of Two Sensitive and Specific Sandwich ELISA Assays*, INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY. 2017, 174, p 17-25.

Priority Research Area **Asthma and Allergy**

Clinical and Molecular Allergology

Marker allergen for severe peanut allergy detected in breast milk

In the literature, statistics concerning the development of peanut allergy via breast feeding are missing. However, recent findings suggest that early contact to peanuts via breast feeding may play a role for tolerance induction to peanuts in the breast-fed child. Therefore, quantification tools for the reliable determination of the clinically relevant allergens Ara h 2 and Ara h 6 are mandatory in the complex lipid-rich matrix breast milk. We were able to develop and validate well-characterized and highly sensitive ELISA assays. By this means, we could show that Ara h 2 was detectable in 14/40 of the participants of our German breast milk study group between 2.3-250 ng/mL and Ara h 6 in 9/40 lactating mothers between 1.1-9.7 ng/mL. The kinetics of secreted Ara h 2 and Ara h 6 appeared to be similar but with a difference in concentration. Follow-up studies on the tolerogenic or sensitizing properties of the allergens in breast milk now become accessible.

Molecular phenotyping of House dust mite (HDM) allergics for a better characterization of patients

The DZL-flagship project "basic science" coordinated by Prof. Jappe focuses A. on the effect of allergenic structure on allergenicity with particular emphasis on allergen-lipid interaction and B. on molecular phenotyping to investigate the sensitization progress to HDM allergens and the association between certain sensitization patterns and organ-specific symptoms. For the investigation of patients from diverse cohorts (ALLIANCE, Hannover and Borstel/Lübeck), the HDM allergens Der p 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 15, 18, 20, 21 and 23 were included in a serum saving multiplex system which allows the simultaneous identification of specific IgE. In general, sensitization patterns were very diverse, however, HDM-allergic patients often showed a sensitization to the major HDM allergens Der p 1 and Der p 2. Moreover, sensitization to Der p 20 and Der p 21 seems to be more often associated with bronchial symptoms.

Identification of immunogenic glycans

In 2008, a new allergy entity, a carbohydrate (galactose-alpha-1,3-galactose (alpha-GAL)) -associated delayed anaphylaxis to red meat, gelatine and some drugs, particularly biologicals (chimeric target antibodies (Cetuximab)), has been reported in the USA. In 2010, Uta Jappe has initiated a nationwide German multicentre study on this issue with the aim to improve the diagnostic procedure in this so far unknown severe allergy. A new diagnostic method was developed to identify patients with this disease among the large group of those who have the diagnosis "idiopathic anaphylaxis", meaning that no culprit has been found via routine measures.

Internal and external collaboration

Internal

P Zabel (Clinics Borstel & Lübeck), H Heine (Innate Immunity), A Frey (Mucosal Immunity and Diagnostic), K Duda (Allergobiochemistry), J Behrends & T Scholzen (Core Facility Fluorescence), D Schwudke (Bioanalytical Chemistry), T Goldmann (Clinical and Experimental Pathology), T Gutsmann (Biophysics), G Schramm (Experimental Pneumology), K Gaede (Biobank), A Schromm (Immunbiophysics)

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Networks

DZL (German Center for Lung Research); ARCN (Airway Research Center North); NORA (Network for Online-Registration of Anaphylaxis); CIA (Collegium Internationale Allergologicum); EAACI: Interest Group Biologicals; IG Food Allergy; IG Insect Venom Allergy; IG In vitro Allergy Diagnostic; IRTG-1911 (International Research Training Group); Cluster of Excellence: Inflammation at Interfaces; ZIEL (Zentrum für Infektions- und Entzündungsforschung, Lübeck); CCAD (Comprehensive Center for Allergic Diseases, Lübeck)

Grant support

Structural and functional analyses of lipophilic peanut allergens with special emphasis on oleosins (DFG JA1007/2-1)

Multicenter study on Birch Associated Soy Allergy and Immuno-Therapy (BASALIT_Jappe)

Disease Area Asthma & Allergy, DZL, 2010-2020

Förderprogramm Zentrales Innovationsprogramm Mittelstand (ZIM), Fördermodul Kooperationsprojekte ZIM-KF2784701AJ0: Development of tests for the detection of specific IgE-antibodies directed against therapeutic monoclonal antibodies (biologicals) (Uta Jappe)

Förderprogramm Zentrales Innovationsprogramm Mittelstand (ZIM), Fördermodul Kooperationsprojekte ZIM-KF2784702SB4: Development of an innovative test to prevent secondary treatment failure due to hypersensitivity reaction to a target treatment with biologicals (Uta Jappe)

Kanert Foundation for allergological research

Priority Research Area **Asthma and Allergy**

DZL-Junior Group of Allergobiochemistry

BIOACTIVE LIPIDS

BIOMARKER

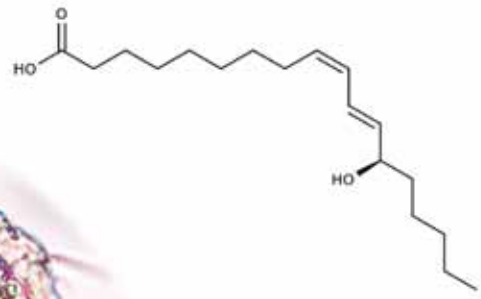
ALLERGIC INFLAMMATION

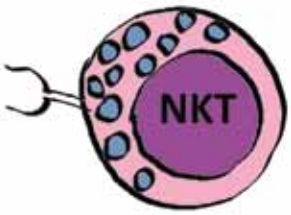
STRUCTURE-FUNCTION-STUDIES

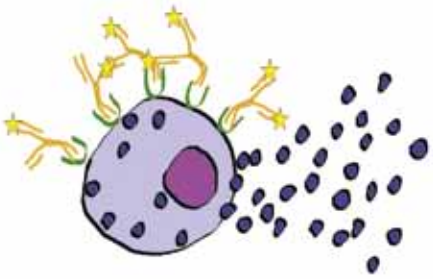
INKT CELLS

CHEMICAL ANALYSES

AEROALLERGENS







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Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Asthma and Allergy**

DZL-Junior Group of Allergobiochemistry

Mission

In der DZL-Nachwuchsgruppe Allergobiochemie haben wir uns zum Ziel gesetzt, bioaktive Lipide und antigene Kohlenhydrate, die aus Allergenquellen und deren assoziierten Bakterien stammen, und die für die Dynamik allergischer Atemwegsentzündungen relevant sind, zu identifizieren und chemisch und funktionell zu charakterisieren.

The DZL-Junior Research Group of Allergobiochemistry is committed to the identification, the chemical and functional characterization of bioactive lipids and antigenic carbohydrates derived from airborne environmental sources of allergens and associated bacteria that are relevant for the dynamics of allergic airway inflammation.

Most important findings

Glycolipids from allergen sources as natural adjuvants for allergen immunotherapy.

The glycoglycerolipid isolated from Timothy grass pollen and its synthetic derivatives induced proliferation of murine Invariant Natural Killer T cells (iNKT) cells ex vivo and secretion of IFN-γ and high amount of IL-13 in a structure dependent manner. Additionally, fractions containing glyceroglycolipids were recognized by human iNKT and γδT cells from peripheral blood, evidenced by the expression of the activation marker CD69.

Identification of lipid-mediated pathomechanisms in allergic inflammation

Lipid mediators are small molecular compounds known for their pro-inflammatory and chemotactic activity. These mediators are known to be rapidly released from the source of allergen upon contact with aqueous media, such as at mucosal surfaces.

We performed chemical analyses of the Timothy pollen associated lipids, that are rapidly released upon hydration. As main components we have identified different types of phytoprostanes (PhytoPs), and for the first time phytofurans (PhytoFs), with predominating 16-F1t-PhytoPs, 9-F1t-PhytoPs, 16-E1t-PhytoPs and 9-D1t-PhytoPs, and 16(RS)-9-epi-ST-Δ14-10-PhytoFs. Interestingly 16-E1t-PhytoP and 9-D1t-PhytoPs were found to be bound to glycerol. Lipid-containing samples (aqueous pollen extract, APE) induced murine mast cell chemotaxis and IL-6 release and enhanced their IgE-dependent degranulation (Fig. 1, A, B, C performed in cooperation with Z. Orinska), demonstrating a role for these lipids in the immediate effector phase of allergic inflammation. Noteworthy, mast cell degranulation seems to be dependent on glycerol-bound, but not free phytoprostanes (Fig. 1, E). On

Highlights

We isolated and structurally characterized the bioactive lipid mediators related to allergic asthma, namely 9- and 13-HODEs from house dust mites (HDM).

HDM-HODEs and the HDM major allergen (Der p2) have a strong synergistic effect on DC/Epithelial cell network.

HDM-HODEs enhance the allergic inflammatory response initiated by mast cell degranulation.

We isolated and structurally characterized lipid mediators (ester bound phytoprostanes and phytofuranes) from Grass pollen that prime dendritic cells for glycolipid presentation through overexpression of CD1d and active glycoglycerolipids.

We developed a high-throughput-screening system based on the cryopreservation of whole peripheral blood and flow cytometry for innate-like lipid-reactive T cells, suitable as biomarkers to better characterize and monitor allergic patients.

Selected publications

Pallach, M., Di Lorenzo, F., Facchini, F.A., Gully, D., Giraud, E., Peri, F., Duda, K.A., Molinaro, A., Silipo, A. 2018. Structure and inflammatory activity of the LPS isolated from Acetobacter pasteurianus CIP103108. Int J Biol Macromol. 8;119:1027-1035. doi: 10.1016/j.ijbiomac.2018.08.035. [Epub ahead of print]

Bönisch, E., Oh, Y.J., Anzengruber, J., Hager, F.F., López-Guzmán, A., Zayni, S., Hinterdorfer, P., Kosma, P., Messner, P., Duda, K.A.*, Schäffer, C*. 2018. Lipoteichoic acid mediates binding of a Lactobacillus S-layer protein. Glycobiology. 1;28(3):148-158. doi: 10.1093/glycob/cwx102.
* corresponding authors

Ling Choy, S., Bernin, H., Aiba, T., Bifeld, E., Lender, S.C., Mühlenpfordt, M., Noll, J., Eick, J., Marggraff, C., Niss, H., González Roldán, N., Tanaka, S., Kitamura, M., Fukase, K., Clos, J., Tannich, E., Fujimoto, Y. & Lotter, H. 2017. Synthetic analogs of an Entamoeba histolytica glycolipid designed to combat intracellular Leishmania infection. Scientific Reports, 25;7(1):9472DOI:10.1038/s41598-017-09894-8

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Di Lorenzo F., Palmigiano A., Al Bitar-Nehme S., Sturiale L., Duda K.A., Gully D., Lanzetta R., Giraud E., Garozzo D., Bernardini M.L., Molinaro A., Silipo A. 2017. The Lipid A from *Rhodopseudomonas palustris* Strain BisA53 LPS Possesses a Unique Structure and Low Immunostimulant Properties. Chemistry. 23(15):3637-3647.

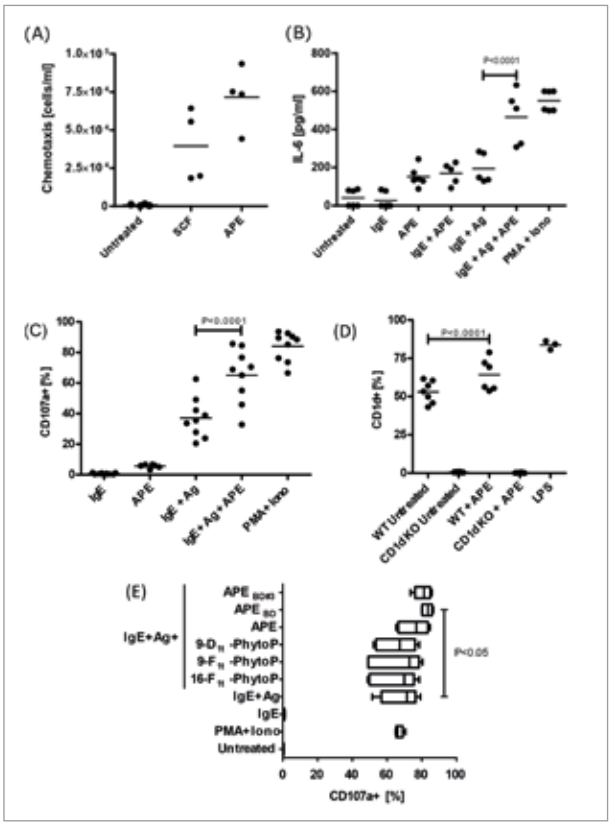

Figure 1. Lipid mediators from Timothy grass induce BMMC chemotaxis (A), IL-6 production (B) enhances IgE/Ag-mediated effects (C) and selectively induce expression of CD1d on dendritic cells (D). Statistical significance was calculated using the 1-way ANOVA analysis followed by Bonferroni's post-test for selected pairs of columns (IgE+Ag vs. IgE+Ag+APE). Ag stands for antigen DNP-HAS. PMA and Ionomycin were used as positive control. Further experiments revealed that glycerol-bound phytoprostanes are responsible for the enhancement of MC degranulation (E). APEB/D (containing enriched Gro-phytoprostanes) and enriched Gro-16E1t-PhytoP/Gro-9-D1t-PhytoP (APEB/D#3) fraction but not free PhytoP led to enhanced IgE/Ag-induced degranulation of BMMCs as measured by CD107a translocation to plasma membrane. Statistical significance was calculated by One-way ANOVA followed by Dunnett's multiple comparison post-test to a control group (IgE+Ag). Means ± SD are shown as lines.

murine dendritic cells, APE selectively induced the upregulation of CD1d, likely preparing lipid-antigen presentation to iNKT cells (Fig. 1, D).

In frame of DZL flagship project we studied utilizing various gas chromatography and mass spectrometry analyses the, unknown to date, lipid composition of the major indoor-allergen inducing allergic asthma, namely house dust mite (HDM). Interestingly 9-HODE and 13-HODE (9- and 13-hydroxy-octadecadienoic acid), lipid metabolites of enzymatic oxidation of linoleic acid (LA, 18:2n-6) were found (Fig. 2 A,B). HODEs belong to the so-called endogenous lipid mediators and are produced by different cell types in the lungs. Increased levels of 13-HODE in extracellular fluids of human atopic asthmatics were also reported. HDM-HODEs enhanced murine mast cell degranulation (Fig. 2, C) and acted synergistic in DC/Epithelial cell network (in cooperation with H. Heine).

Priority Research Area **Asthma and Allergy**

DZL-Junior Group of Allergobiochemistry

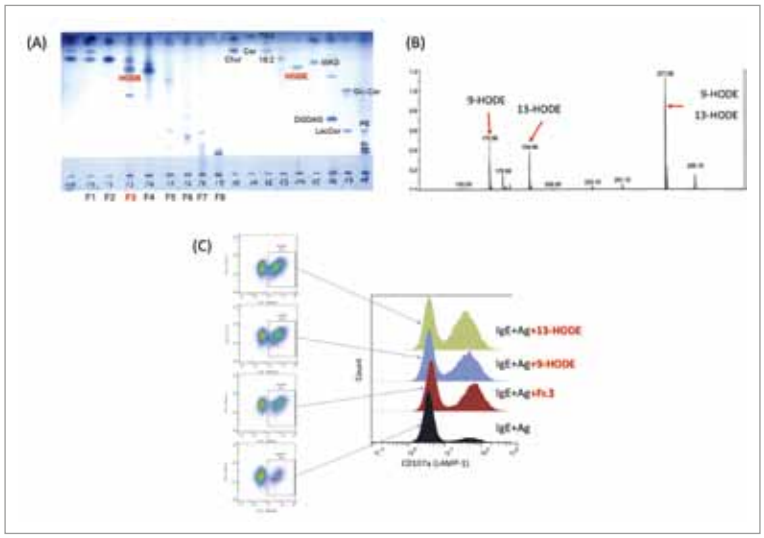

Figure 2. Thin layer chromatography of HDM-derived lipids. indicating the presence of different classes of lipids, among them the proinflammatory lipid mediators - HODEs (A). For comparison, synthetic standards were run simultaneously. Mass spectroscopic analyses of HDM lipid fraction 3 confirmed the presence of HDM-HODEs, 9- and 13-HODE (B). Electrospray ionization MS analysis was performed in negative ion mode. 9- and 13-HODEs enhance BMMC degranulation (C). Mast cell degranulation was induced with IgE and antigen, in the presence or absence of 9- or 13-HODE or HDM-derived lipid fraction 3 (containing mostly 9- ad 13-HODE). Left, representative pseudo color plots show the frequency of mast cells positive for the degranulation marker CD107a (LAMP-1), analyzed by flow cytometry. On the right, the increase of mast cell degranulation is shown as histograms after the indicated treatment (right).

Identification of lipid-related cellular biomarkers in allergic asthma: innate-like glycolipid-reactive lymphocytes.

One fundamental gap relevant for the improvement of asthma treatment, is the identification of biomarkers allowing to confirm the diagnosis, to define specific phenotypes, predict outcomes and follow up the response to therapy.

iNKT and γδ T cells are innate-like lymphocytes reacting to lipophilic antigens. Both cell types represent pre-expanded populations readily detectable in whole peripheral blood by flow cytometry. In addition, several studies have shown that the proportion of these cell populations in peripheral blood varies during disease, such as infection with virus, bacteria and parasites, as well as a result of changes in the microbiome.

To validate the concept of monitoring iNKT and γδ T cells as biomarkers relevant for asthma, we developed a workflow suitable for high-throughput-screening analyses, consisting on the optimized cryopreservation of whole peripheral blood samples, and the use of multiparametric flow cytometry.
Our preliminary results show that iNKT and γδ TCR+Vd1+CD8+ cell populations are either strongly reduced or absent in allergic patients.

Internal and external collaboration

Internal
U. Jappe (Division of Clinical and Molecular Allergology),
H. Heine (Division of Innate Immunity),
S. Krauss-Etschmann (Early life origins of CLD),
Z. Orinska (Division of Experimental Pneumology),
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Jens Duus, Technical University of Denmark
Mariola Pasciak, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences

Grant support

BMBF

LI-JRG-2 (Junior Group of Allergobiochemistry)
(PI: K. Duda)

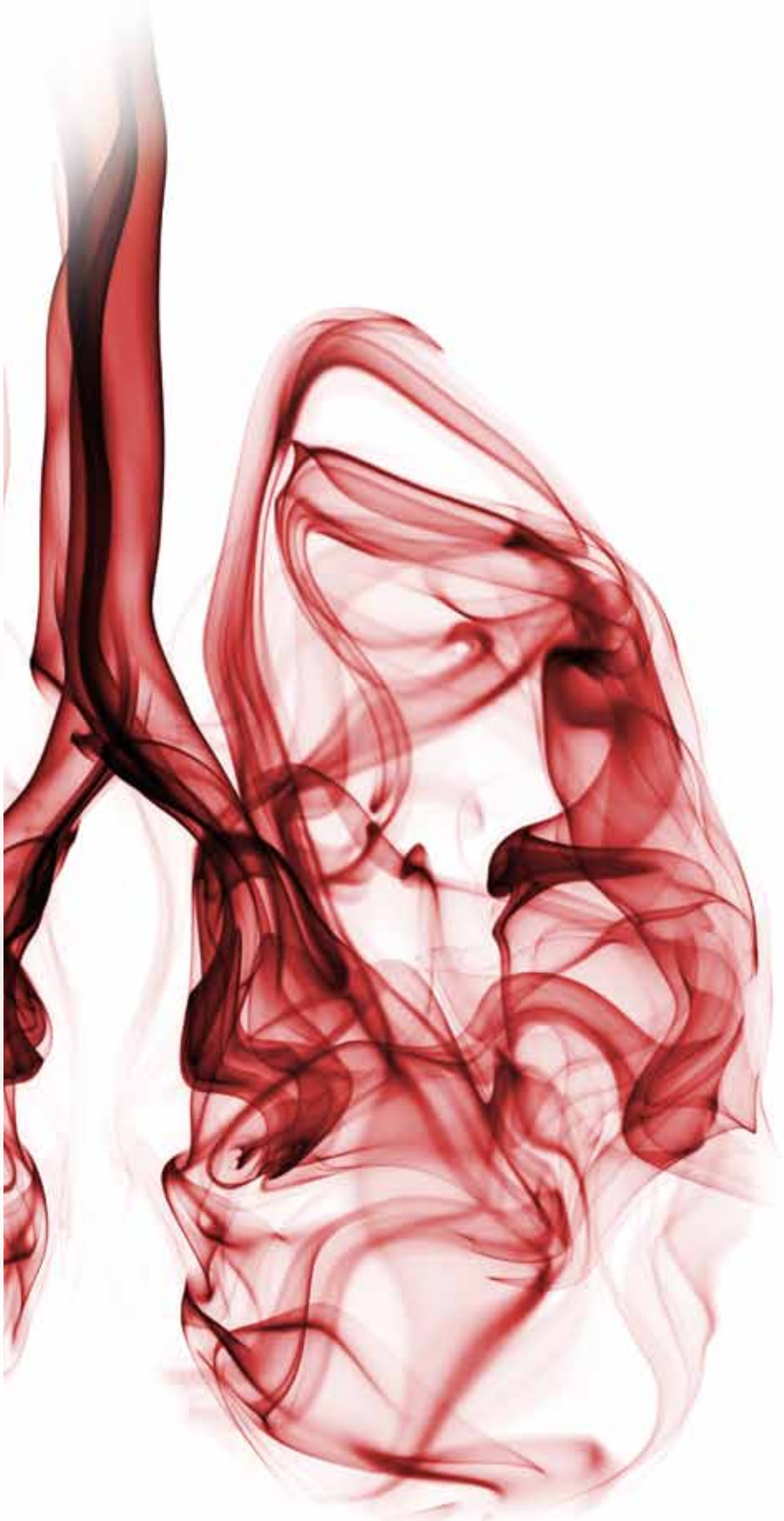
DA-AA-BFP1 (Basic Flagship AA)
(Coordinator: Prof. U. Jappe)

TRANSGENERATIONAL
SMOKING
EXACERBATIONS

ASTHMA
COPD

Priority Research Area **Asthma and Allergy**

Early Life Origins of Chronic Lung Disease



Head

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  Susanne Krauss-Etschmann

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- Barbara Hammer
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- Joni Lund
- Gregor Jatzlauk
- Janin Braun
- Masha Spauszus

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Asthma and Allergy**

Early Life Origins of Chronic Lung Disease

Mission

Wir wollen verstehen, wie präkonzeptionelle, pränatale und frühe Lebensexpositionen gegenüber Umweltfaktoren die Lungen- und Immunentwicklung beeinflussen und wie diese das Risiko der Entstehung von Asthma und chronisch obstruktiver Lungenerkrankung (COPD) im späteren Leben determiniert. In diesem Zusammenhang betrachten wir das Lungenmikrobiom als "Zielorgan" für die zukünftige Entwicklung therapeutischer und präventiver Maßnahmen. In einem zweiten Themenfeld identifizieren und validieren wir Biomarkersignaturen, um COPD-Exazerbationen vorherzusagen.

We aim to understand how preconceptional, prenatal and early life exposures to environmental factors shape lung and immune development and how thes determines the risk to develop of asthma and chronic obstructive pulmonary disease (COPD) later in life. In this context we consider the lung microbiome as a target "organ" for future development of therapeutic and preventative interventions. To this end we aim to identify microbial metabolites to be used for such interventions. In a second topic, we identify and validate biomarker signatures to predict COPD exacerbations.

Most important findings

1. Maternal smoking in pregancy

Epidemiological studies have demonstrated an association of *in utero* smoke exposure with reduced birth weight and impaired lung function development which are themselves risk factors for asthma development in the offspring. Mothers often underestimate their own smoking behaviour such that mild smoking might not be recorded. We asked if mild maternal smoking - while not leading to reduced birth weight or lung function deficits - still negatively affects immune development thereby predisposing to asthma. The answer to this question is important as it helps to decide if public resources to prevent smoking in women should be mainly allocated to heavily smoking women or need to target all smoking women. To answer this question, pregnant female mice were exposed daily cigarette smoke (CS) or room air (RA) until delivery. Thereafter, thymic T-cells were quantified in offspring and isolated $CD4^+$ and $CD8^+$ thymocytes were further examined by smallRNA sequencing three weeks after birth. This revealed a decrease in T cells of prenatally smoke-exposed offspring with increased $CD4^+$ T cells and a prominent decrease of $CD8^+$ T cells (Fig. 1.). RNA sequencing suggested alterations in gene expression levels related to the hematological system as well as to developmental disorders and immune cell trafficking. Single genes of interest were *interleukin 4 receptor (IL4R), runt-related transcription factor 3 (RUNX3), forkhead box protein P1 (Foxp1)* and *IL10RB* which were upregulated whereas *phasphoinositide-3-kinase (PI3K)* was downregulated. These genes are involved in the differentiation of innate

Selected publications

S. Dehmel, P. Nathan, S. Bartel, N. El-Merhie, H. Scherb, K. Milger, G. John-Schuster, AO. Yildirim, M. Hylkema, M. Irmler, J. Beckers, B. Schaub, O. Eickelber, S. Krauss-Etschmann. Intrauterine smoke exposure deregulates lung function, pulmonary transcriptomes, and in particular insulin-like growth factor (IGF)-1 in a sex-specific manner. Sci Rep. 2018 May 15;8(1):7547.

S. Bartel, G. Carraro, F. Alessandrini, S. Krauss-Etschmann, FLM Ricciardolo, S. Bellusci. miR-142-3p is associated with aberrant Wingless/Integrase I (WNT) signaling during airway remodeling in asthma. Am J Physiol Lung Cell Mol Physiol. 2018 ;315(2):L328-L33

M. Kostric, K. Milger, S. Krauss-Etschmann, M. Engel, G.Vestergaarda, M. Schloter, A. Schöler. Development of a stable lung microbiome in healthy neonatal mice. Microb Ecol. 2018 75(2):529-542;doi: 10.1007/s00248-017-1068

I. Kepert, J. Fonseca, C. Müller, K. Milger, K. Hochwind, M. Kostric, M. Fedoseeva, C. Ohnmacht, S. Dehmel, P. Nathan, S. Bartel, O. Eickelberg, M.Schloter, A. Hartmann, P. Schmitt-Kopplin, S. Krauss-Etschmann. D-tryptophan from probiotic bacteria influences the gut microbiome and allergic airway disease.J Allergy Clin Immunol 2017;139(5):1525-1535.

C. Svanes*, J. Koplin*, (...) S. Krauss-Etschmann, (...) F. Gomez Real. Father's environment before conception and asthma risk in his children: A multi-generation analysis of the Respiratory Health In Northern Europe study. Int J Epidemiol. 2017 Feb 1;46(1):235-245

Peer reviewed reviews

B. Hammer, C. Wagner, A. Divac Rankov A, S. Reuter. S. Bartel, MN. Hylkema, A. Krüger, C. Svanes, S. Krauss-Etschmann. In utero exposure to cigarette smoke and effects across generations: a conference of animals on asthma. Clin Exp Allergy. 2018 48(11):1378-1390.

E. Melén, R. Barouki, M. Barry, M. Bouzen, B. Hoffmann, S. Krauss-Etschmann, G.Koppelman, B. Forsberg. Promoting public health activities through epigenetics research. An ERS Environment Health Committee Workshop Report. Eur Resp J 4;51(4).2018

F. Sommer*, M. Rühlemann*, C. Bang, M. Höppner, A. Rehman, C. Kaleta, Ph. Schmitt-Kopplin, A,Dempfle, S. Weidinger, E. Ellinghaus, S. Krauss-Etschmann, D. Schmidt-Arras, K. Aden, D. Schulte, D. Ellinghaus, S. Schreiber, A. Tholey, J. Rupp, M. Laudes, J .Baines, Ph. Rosenstiel & A. Franke. Microbiomarkers in Inflammatory Bowel Diseases – Caveats come with Caviar. Gut 2017 66(10):1734-1738

G. Jatzlauk*, S. Bartel*, H. Heine, M. Schloter, S. Krauss-Etschmann. Influences of environmental bacteria and their metabolites on allergies, asthma and host microbiota. Allergy 2017 72(12):1859-1867. *equal contribution

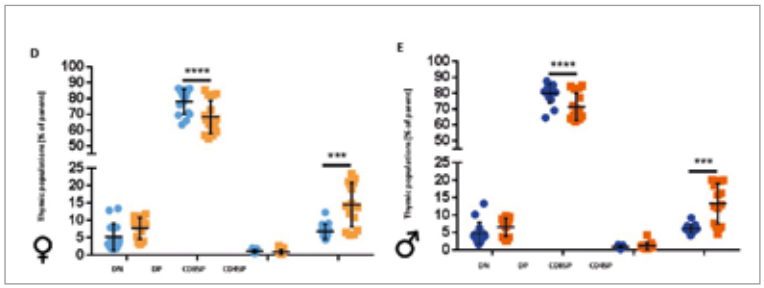
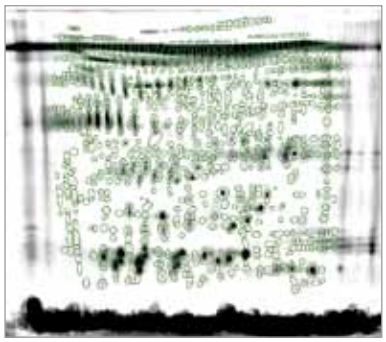


Figure 1. Thymic T-cells of *in vitro* cigarette smoke exposed 21-day old female and male offspring. Percentages of thymic T cell populations based on expression of CD4 and CD8; DN = double negative for CD4 and CD8; DP = double positive for CD4 and CD8.



Mucin-5B
Lipocalin 2
Prolactin-inducible protein
Plasminogen
Titin
GAPDH
Alpha-enolase
Annexin A1
Cathepsin D
Alpha-1 antitrypsin

Figure 2a (left). 2D-gel analysis of murine BAL revealed 906 well-resolved protein spots.

Figure 2b (right). Proteins identified both in mice and human COPD transcriptomes. Green: upregulated; red: downregulated.

Early Life Origins of Chronic Lung Disease

memory-like $CD8^+$ thymocytes, which however needs further confirmation. Previous studies suggest that these cells support the immune defense in neonates and young children before peripheral memory $CD8^+$ T cells are established. Taken together these results demonstrate pronounced alterations of the immune development of mice born to mildly smoking mothers.

Currently, we explore to what extent fathers smoking during own adolescence affects postnatal growth of their offspring born later.

2. Biomarker development for prediction of COPD exacerbations.

Cigarette smoking is the main risk factor for the development of COPD. The course of COPD is often aggravated by acute exacerbations which are mostly triggered by viral or bacterial infections. COPD exacerbations result in a lasting deterioration of respiratory health thus leading to hospitalization and in the worst case to death. In order to identify the mediators released during the early stages of COPD exacerbations, we developed a mouse model of heavy smoking and used a viral mimic to trigger an exacerbation. We observed a typical exacerbation phenotype characterized by an increase in concentrations of pro-inflammatory cytokines such as IL-6, GM-CSF, IFN-γ, TNF-α and KC/GRO (an IL-8 analogue) in bronchoalveolar fluid (BALF) of exacerbated mice. As these markers are common to many inflammatory diseases, we wished to identify novel markers which might show more specificity for the lung. To this end, we performed a proteome analysis in BALF which identified 906 protein spots in a 2D-protein gel (Fig. 2a): From these we selected the six most promising candidate spots for mass spectrometric analysis (Fig. 2b). We next tested if these proteins are also present in BALF transcriptomes of COPD patients. This comparison revealed jointly up- and down- regulated candidates, see (Fig. 2c), in both mice and humans. We suggest that our model could not only be used to reveal novel candidate biomarkers predicting exacerbations but also to further understand the mechanisms underlying viral-induced exacerbations.

Internal and external collaboration

Internal

Innate Immunity, Asthma Exacerbation and Regulation, Pathology, Mucosal Immunology and Diagnostics.

External

German Center for Lung Research: Bianca Schaub University Children's Hospital Munich; Saverio Bellusci University of Gießen; A. Önder Yildirim, Helmholtz-Center Munich; Michael Schloter Helmholtz Center Munich.
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Ceciliie Svanes; Randi Jacobsen Bertelsen, both University of Bergen, Norway
Machteld Hykema University of Groningen, Netherlands

Grant support

2014-today
German Center for Lung Research (DZL) "Development of asthma phenotypes: predictors and mechanisms"; 347; BMBF

2015- 2018
BMBF-Consortium EXASENS Leibniz Research Alliance EXASENS – "POC-Sensor Platform for chronic-inflammatory Airway Diseases"; Subproject, FKZ 13N13857, FZB "On-site exacerbation diagnostics in asthma & COPD: probes, models, analytes and capture systems"; total vol 1.5 Mio

2016- 2020;
Leibniz Campus Evolutionary Medicine of the Lung; Subproject total vol 4 Mio;

2016- 2019;
Leibnitz Competition 2016 "The lung Microbiota at the Interface between airway epithelium and the environment" (PI) vol 1.1 Mio;

2019 – 2022
Oral and Environmental Microbiota, Endotoxin and Lung health: the 'United Airways' concept extended; R. Bertelsen (PI)- ERC Starting grant

2019 – 2022
C. Svanes, 1.5 Mio (total) 80T allocated to SKE; Norwegian Research Council: Program "Better Health and Quality of Life" (BEDREHELSE)

AIRWAY REMODELING

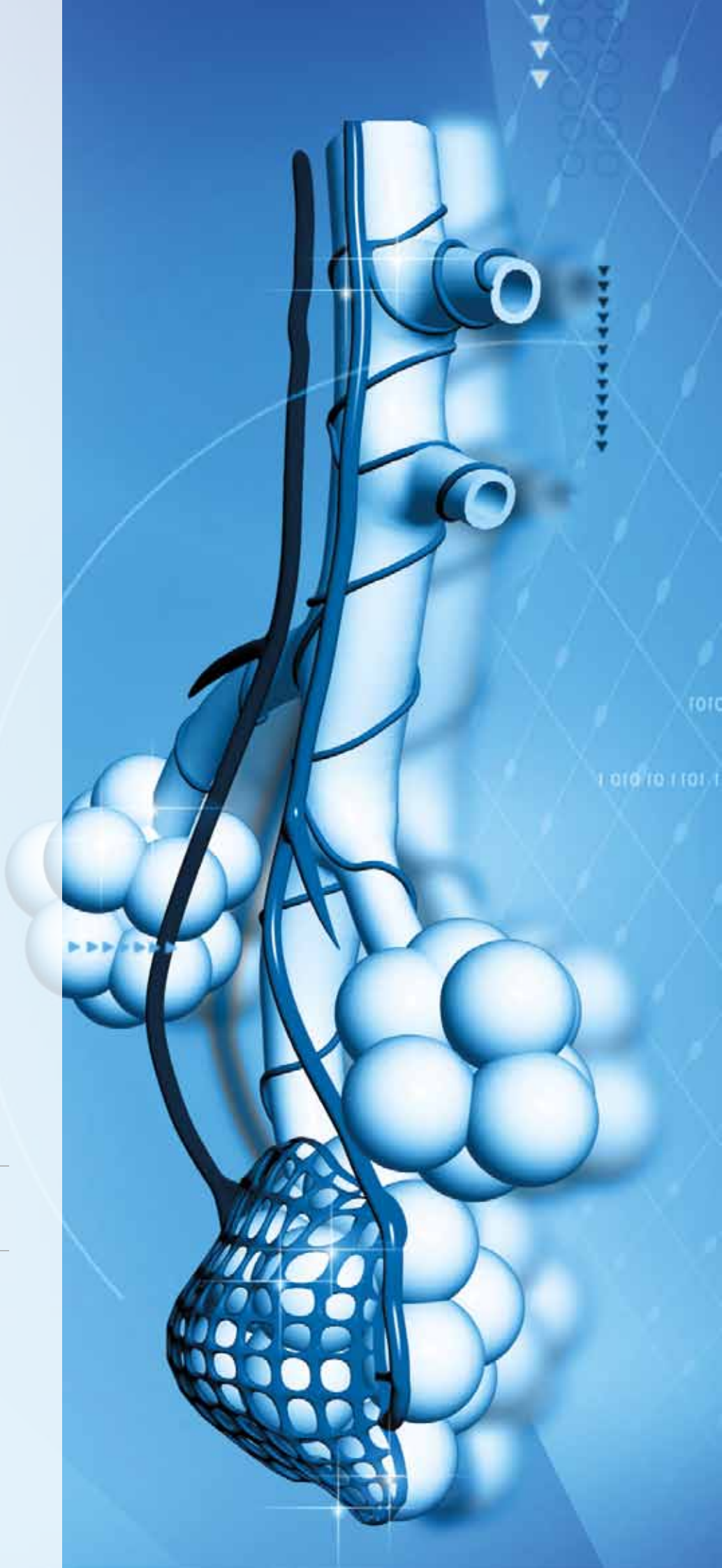
EPITHELIUM

BASOPHILS

BRONCHIAL ASTHMA

MAST CELLS

DESIGN-BASED STEREOLOGY



Priority Research Area **Asthma and Allergy**

Experimental Pneumology

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- Gesine Rode
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- Kerstin Viertmann
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Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Asthma and Allergy**

Experimental Pneumology

Mission

Ziel unserer Arbeiten ist es zu erforschen, auf welche Weise in die Entstehung bzw. Exazerbation chronisch-entzündlicher Atemwegserkrankungen, wie zum Beispiel allergisches Asthma bronchiale, durch Modulation der entzündlichen Prozesse eingegriffen werden kann. Im Fokus stehen für uns Untersuchungen von pulmonalem Epithel, Mastzellen und basophilen Granulozyten.

Our research studies aim at investigating how to influence the pathogenesis of initiation and exacerbation of chronic inflammatory lung diseases such as allergic bronchial asthma by modulation of inflammatory processes. The focus of our studies is on the role of pulmonary epithelium, mast cells and basophilic granulocytes.

Most important findings

The **airway epithelium** is the initial target of environmental factors such as viruses, cigarette smoke, and particles, which may trigger the development of airway diseases and exacerbations. Therefore, studying the responses of airway epithelial cells to such triggers is a major focus of the group. For investigating epithelial immune functions and remodeling of the epithelium, we make primarily use of Air-Liquid-Interface (ALI) cultures of murine and human airway epithelial cells (Fig. 1). In addition, mast cells and basophilic granulocytes are of particular significance in the effector phase in allergic bronchial asthma. Therefore, our studies additionally address the functional relevance of these immune cells.

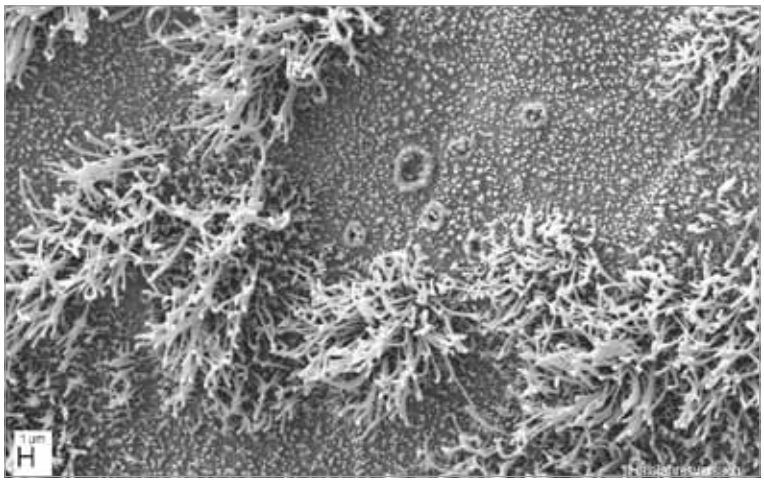

Figure 1. Scanning electron micrograph of the apical surface of primary human airway epithelial cells after 6 months of in-vitro culture at air-liquid-interface (ALI). The epithelium still exhibits different types of cells such as a number of ciliated cells with long and slender cilia (micrograph by courtesy of Peter König & Harry Manfeldt, Institute of Anatomy, University of Lübeck).

Selected publications

Bonniaud P, Fabre A, Frossard N, Guignabert C, Inman M, Kuebler WM, Maes T, Shi W, Stampfli M, Uhlig S, White E, Witzenrath M, Bellaye PS, Crestani B, Eickelberg O, Fehrenbach H, Guenther A, Jenkins G, Joos G, Magnan A, Maitre B, Maus UA, Reinhold P, Vernooy JHJ, Richeldi L, Kolb M. Optimising experimental research in respiratory diseases: an ERS statement. Eur Respir J 2018 May 17;51(5). pii: 1702133

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Knuhr K, Langhans K, Nyenhuis S, Viertmann K, Kildemoes AMO, Doenhoff MJ, Haas H, Schramm G. Cross-Reactivity between *Schistosoma mansoni* antigens and the latex allergen Hev b 7: putative implication of cross-reactive carbohydrate determinants (CCDs). Front Immunol. 2018 Oct 10;9:2293.

Kordowski A, Reinicke AT, Wu D, Orinska Z, Hagemann P, Huber-Lang M, Lee JB, Wang YH, Hogan SP, Köhl J. C5a receptor 1-/- mice are protected from the development of IgE-mediated experimental food allergy. Allergy. 2019 Apr;74(4):767-779.

Lindner K*, Webering S*, Stroebele M, Bockhorn H, Hansen T, König P* & Fehrenbach H*. Low dose Carbon Black nanoparticle exposure does not aggravate allergic airway inflammation in mice irrespective of the presence of surface polycyclic aromatic hydrocarbons. Nanomaterials 2018, 8(4), 213. (*equal contribution).

Infection of airway epithelial cells with respiratory viruses such as human respiratory virus (HRV) and respiratory syncytial virus (RSV) are main triggers of asthma exacerbations. In the context of the BMBF funded research consortium EXASENS, a pilot project of Leibniz Health Technologies, we established a human in-vitro model of asthma exacerbations. Primary human airway epithelial cells cultured at ALI were first incubated with recombinant human (rHu) interleukin (IL-) 13 to trigger an epithelial phenotype of Th2-type bronchial asthma. Once established, this epithelial phenotype was additionally treated with poly(I:C), a surrogate of double-stranded viral RNA, to trigger an exacerbated epithelial phenotype. This epithelial exacerbation phenotype was characterized by significantly increased expression levels of exacerbation-related interleukins such as IL-6, IL-8, and TNF-α. Currently, RNA micro array data are being mined to reveal novel genes related to the development of an asthma exacerbation and to identify biomarkers allowing for the early prediction of an exacerbation.

Helminth-derived molecules have been identified as a new therapeutic approach for various immune-mediated diseases. We aim at developing novel preventive strategies against allergic diseases such as allergic asthma by making use of potential anti-inflammatory factors isolated from the eggs of the helminth *Schistosoma mansoni*. One of these factors is IPSE/alpha-1 that triggers the release of IL-4 and IL-13 from **basophilic granulocytes** (Fig. 2). We could demonstrate that IL-4 released from IPSE/alpha-1 stimulated basophils inhibits the production of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α from LPS-stimulated human monocytes. Monocytes adopt an alternatively activated phenotype. These results suggest an anti-inflammatory role of IPSE/alpha-1 as well as of basophils (Knuhr et al., *Frontiers Immunol*. 2018).

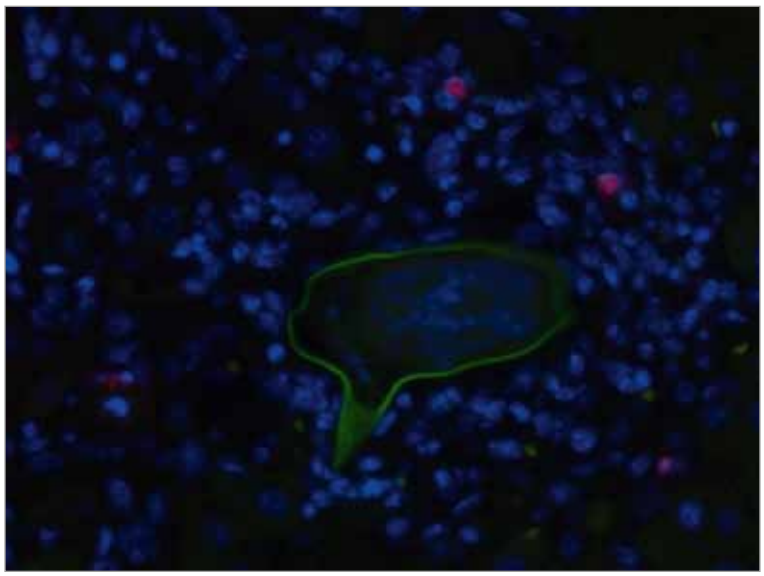


Figure 2. Detection of basophils (magenta) by the basophil-specific antibody anti-mMCP-8 and an egg of *S. mansoni* (green autofluorescence of the egg shell) in a granuloma in *S. mansoni*-infected mouse liver by immunofluorescence. Nuclei are stained with DAPI (blue).

Priority Research Area **Asthma and Allergy**

Experimental Pneumology

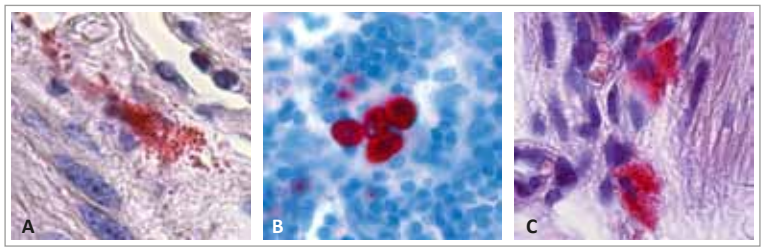


Figure 3. Micrographs of naphtholesterase-stained mast cells from different organs of the mouse. Trachea (A), lymph node (B), stomach (C).

Mast cells (MCs) are long-living sentinel cells located in skin and mucosal tissues (Fig. 3), interacting with endothelial cells and nerves. MCs can sense different immunological and chemical cues and respond in stimulus-specific way by production of cytokines, chemokines and lipid mediators or by release of granule-stored proteases or biogenic amines thus contributing to innate and adaptive immunity. Overwhelming MC response is a key feature of allergic diseases. IgE-independent G-protein coupled receptor-mediated MC degranulation in response to polycationic compounds is the second type of MC-mediated hyperresponsiveness. MC degranulation in IgE-independent manner is induced by binding of different chemical ligands to MrgprB2- transmembrane protein of MAS-related G-protein receptor family. The mechanisms controlling MrgprB2-mediated MC degranulation are unknown. Here we identify tetraspanin CD37 as regulator of MrgprB2-mediated MC response. MC degranulation induced by polycationic compound- or phorbol ester/ Ca2+ ionophore-stimulation leads to translocation of granular membrane proteins (CD107a, CD107b, CD63) to the cell surface and release of granule-stored beta-hexosaminidase. CD37-deficient MCs display a much higher sensitivity to selective antibiotics of the fluoroquinolone family and respond with strongly enhanced degranulation, indicating the existence of the stimulus-specific regulatory mechanisms of MC hypersensitivity. Therefore, our results indicate that tetraspanin CD37 is involved in the regulation of MrgprB2-mediated MC sensing of small chemical molecules and MC-mediated IgE-independent hypersensitivity reactions.

Internal and external collaboration

Internal: Junior Research Groups Asthma-Exacerbation & Regulation, Invertebrate Models; Divisions Early Life Origins of CLD, Mucosal Immunology and Diagnostics.

University of Lübeck: Matthias Kopp & Markus Weckmann, Division of Pediatric Pneumology and Allergology, University of Lübeck, Lübeck.

External:

collaborators in the German Center for Lung Research: Bianca Schaub (University Children's Hospital Munich); Carsten Schmidt-Weber (ZAUM, Munich); Katharina Sewald (Fraunhofer ITEM, Hannover).

other national collaborators: Helmut Haas (*helmin* Guard, Borstel); Marcus Maurer and Martin Metz (Charité, Berlin); Minka Breloer (Bernhard-Nocht-Institute for Tropical Medicine, Hamburg)

international collaborators: Maria Yazdanbakhsh, Hermelijn H. Smits, D. Cornelis H. Hokke (all Department of Parasitology, LUMC, Leiden); Michael J. Doenhoff (University of Nottingham & BioGlab Ltd., Nottingham); Marc Vendrell (University of Edinburgh); Rodolfo Lavilla (University of Barcelona); Young-Tae Chang (National University of Singapore); Matthias Clauss (Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis).

Grant support

German Center of Lung Research, Disease Area Asthma/Allergy, Platform Imaging (H.F.)

BMBF-Consortium EXASENS "POC-Sensorplattform für chronisch-entzündliche Atemwegserkrankungen" (FKZ 13N13857) (H.F.)

DFG-GRK1727 "Modulation of Autoimmunity", TPB5 "Modulation of autoimmunity by CD37-specific antibodies" (Z.O.)

TOLL-LIKE RECEPTOR

TLR8

RNA

INNATE IMMUNITY

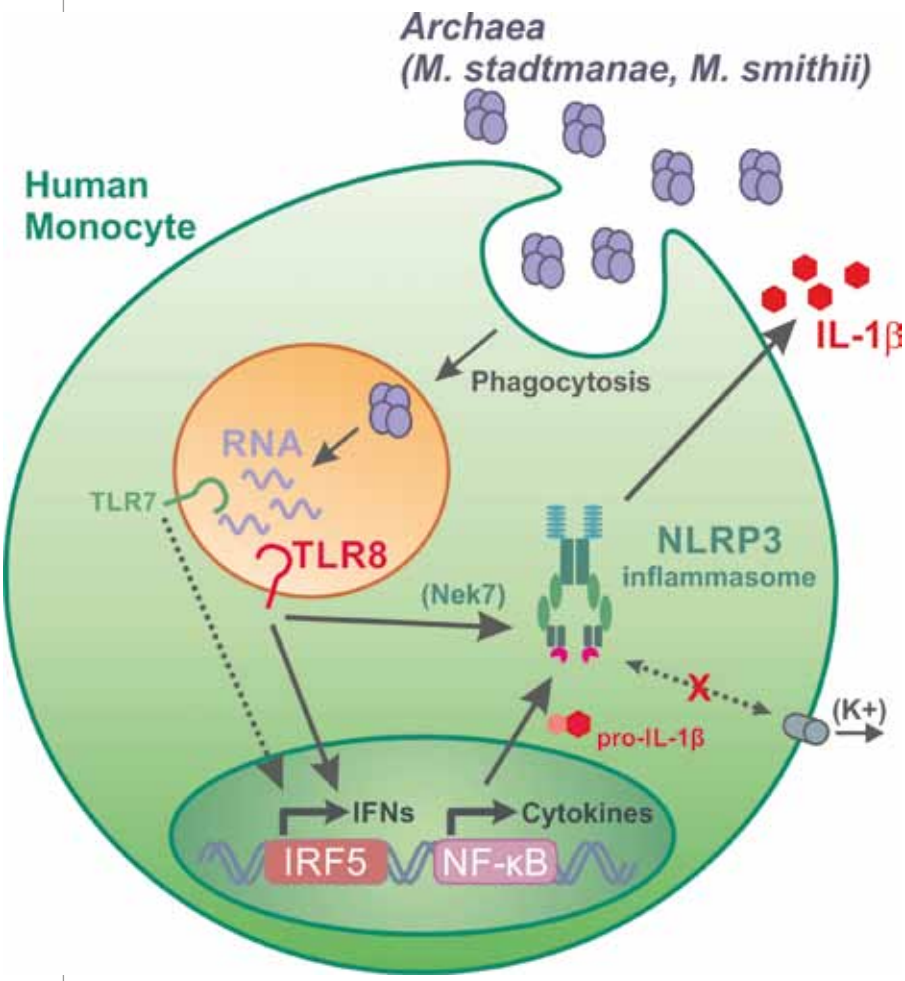
3D CO-CULTURE MODEL

ALLERGY-PROTECTION

EPITHELIAL CELLS

DENDRITIC CELLS

ARCHAEA



Priority Research Area **Asthma and Allergy**

Innate Immunity

Head

- Prof. Dr. Holger Heine

Members

- Marten Holtermann
- Katrin Böhnstedt
- Ina Goroncy
- Tanja Mengden
- Dr. Tim Vierbuchen
- Dr. Karina Stein

Former members

- Liesa Hofmann
- Hanna Rosigkeit
- Dr. André Jenckel
- Prof. Dr. Artur J. Ulmer

Priority Research Area **Asthma and Allergy**

Innate Immunity

Mission

Die Mission der Forschungsgruppe Angeborene Immunität ist die Untersuchung, Analyse und Charakterisierung von Aktivierungsmechanismen des angeborenen Immunsystems durch Mikroben, mikrobielle Strukturen und Allergene. Insbesondere interessieren wir uns für das Zusammenspiel und die Kommunikation von Epithel- und dendritischen Zellen untereinander und mit dem adaptiven Immunsystem sowie die sich daraus ergebenden Konsequenzen für Lungenerkrankungen wie Allergie und das Asthma.

The mission of the division of Innate Immunity is the investigation, analysis and characterization of activation mechanisms of the innate immune system through microbes, microbial structures and allergens. Particularly, we examine the interplay and communication of epithelial and dendritic cells with each other and the adaptive immune system and the consequences of this activation for lung diseases such as allergy and asthma.

Most important findings

i) One of the main topics of the group is the study of the interaction of various types of innate and adaptive immune cells in the initiation phase and during ongoing allergic asthma. The airway epithelium is the first entry site for airborne environmental factors and its role not only as physical barrier but as part of the innate immune system got strengthened by several studies over the last years. Dendritic cells are known key regulators in the allergic sensitization process and the initiation of allergic immune responses. Signals derived from the airway epithelium can decisively affect DC function. However, understanding the importance of the interaction of DCs and airway epithelial cells by cell-cell contact or soluble mediators in this context is just at the beginning. A 3D co-culture model involving the bronchial epithelial cell line Calu-3 cultured under air-liquid interface conditions and monocyte-derived DCs shows an interdependent regulation of the immune response e.g. towards allergens. Treatment of the epithelial cells with T cell-derived cytokines before co-culturing, such as IL-4, IL-13 and IFN-γ, imprints a well-defined gene expression pattern which is most likely universal since the regulation of selected candidates was comparable in several investigated bronchial airway epithelial cell lines and in primary cells (cooperation with ZAUM; Fig.1). Gene expression analysis of these epithelial cells identified new molecular candidates which might be of importance in the process of cytokine-induced polarization. Specific gene knockouts were generated in Calu-3 cells through CRISPR/Cas9, and first results show their contribution not only in the cytokine-induced imprint of the epithelial cells themselves but also in the modulatory effect these cells convey to co-cultured dendritic cells.

Selected publications

Bang C, Vierbuchen T, Gutsmann T, Heine H, Schmitz RA: Immunogenic properties of the human gut-associated archaeon Methanomassiliicoccus luminyensis and its susceptibility to antimicrobial peptides. PloS one 2017, 12(10):e0185919.

Jatzlauk G, Bartel S, Heine H, Schloter M, Krauss-Etschmann S: Influences of environmental bacteria and their metabolites on allergies, asthma, and host microbiota. Allergy 2017, 72(12):1859-1867.

Lindner K, Strobele M, Schlick S, Webering S, Jenckel A, Kopf J, Danov O, Sewald K, Buj C, Creutzenberg O, Tillmann T, Pohlmann G, Ernst H, Ziemann C, Huttmann G, Heine H, Bockhorn H, Hansen T, Konig P, Fehrenbach H: Biological effects of carbon black nanoparticles are changed by surface coating with polycyclic aromatic hydrocarbons. Part Fibre Toxicol 2017, 14(1):8.

Stein K, Brand S, Jenckel A, Sigmund A, Chen ZJ, Kirschning CJ, Kauth M, Heine H: Endosomal recognition of Lactococcus lactis G121 and its RNA by dendritic cells is key to its allergy-protective effects. The Journal of allergy and clinical immunology 2017, 139(2):667-678 e665.

Vierbuchen T, Bang C, Rosigkeit H, Schmitz RA, Heine H: The Human-Associated Archaeon Methanosphaera stadtmanae Is Recognized through Its RNA and Induces TLR8-Dependent NLRP3 Inflammasome Activation. Front Immunol 2017, 8:1535.

Adanitsch F, Shi J, Shao F, Beyaert R, Heine H, Zamyatina A: Synthetic glycan-based TLR4 agonists targeting caspase-4/11 for the development of adjuvants and immunotherapeutics. Chem Sci 2018, 9(16):3957-3963.

Borio A, Holgado A, Garate JA, Beyaert R, Heine H, Zamyatina A: Disaccharide-Based Anionic Amphiphiles as Potent Inhibitors of Lipopolysaccharide-Induced Inflammation. Chemmedchem 2018, 13(21):2317-2331.

Vierbuchen T, Stein K, Heine H: RNA is taking its Toll: Impact of RNA-specific Toll-like receptors on health and disease. Allergy 2019, 74(2):223-235.

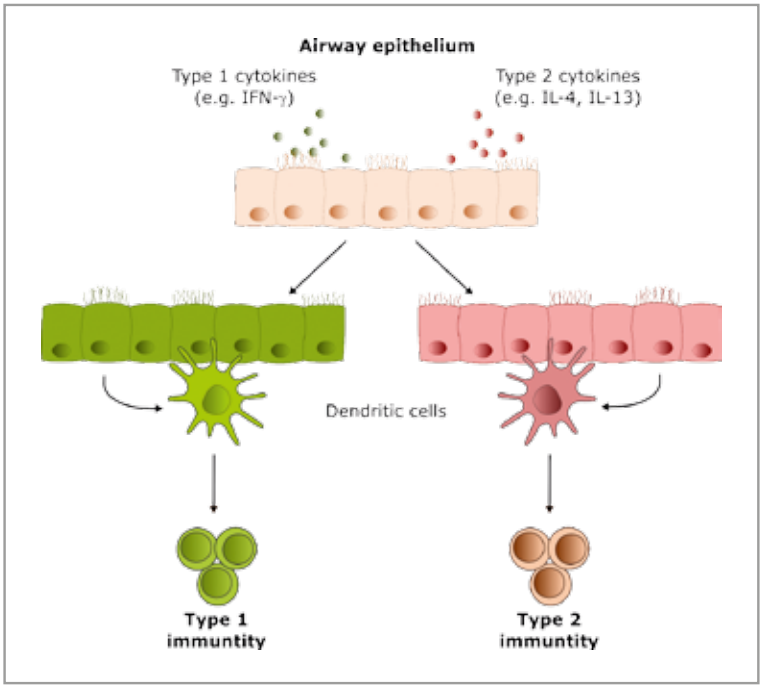


Figure 1. T cell-derived cytokines polarize human bronchial epithelial cells in type 1 and type 2 cells which in turn convey this imprint to human monocyte-derived dendritic cells.

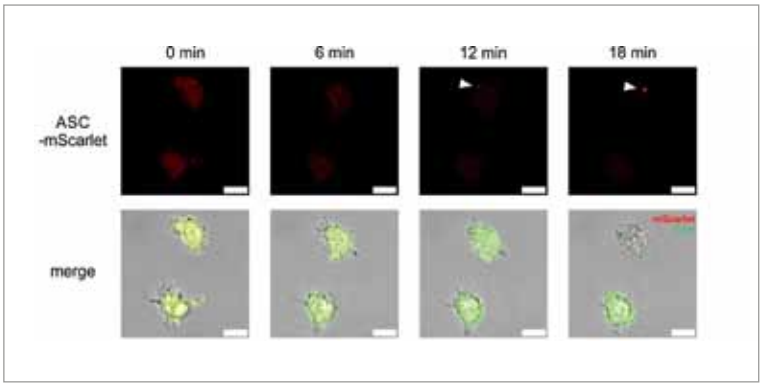


Figure 2. CRISPR/Cas9-based generation of an ASC-mScarlet reporter cell line in BLaER1 monocytes. BLaER1 ASC-mScarlet monocytes were pre-incubated for 3 h with 50 ng/ml LPS before 13.4 µM nigericin has been added Cells were analyzed directly after addition of nigericin via live cell confocal microscopy.

Innate Immunity

ii) Inflammatory signaling induced by the Gram-negative cell wall constituent LPS remains a two-sided sword: it can lead to immune protective TLR4-dependent signaling as well as detrimental caspase-dependent pyroptosis and cell death. In cooperation with A. Zamyatina´s group in Vienna we analyzed the ability of glycan- and disaccharide-based synthetic structures to act as either agonists or antagonists of LPS signaling and tried to disentangle the structural requirements for either type of cellular response.

III) Although methanogenic archaea are part of the human microbiota, their immunological function has not been elucidated so far. Recently, we were able to demonstrate that gut-derived strains such as *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* can induce inflammatory immune responses in human DCs. However, the molecular basis of this recognition was not known until now. We were able to show for the first time that these archaeal strains are recognized through their RNA. In order to achieve this recognition, archaeal cells must be phagocytosed and processed in acidified endosomes. Using a newly established CRISPR/Cas9 platform in our lab to either knockout or modify (s. Fig.2) molecules involved in innate recognition and signaling, we could demonstrate that the archaeal RNA is then recognized by the two Toll-like receptors that are specific for single-stranded RNA, TLR7 and TLR8. The recognition is predominantly achieved through TLR8, in particular the induction of type I interferons and IL-1β release depends on the expression of TLR8. The *M. stadtmanae*-induced IL-1β release requires the formation of a NLRP3-dependent inflammasome. In general, inflammasome activation can be achieved via canonical, non-canonical and the recently described LPS/TLR4-dependent alternative pathway. By studying archaea we identified a so far unknown new RNA- and TLR8-dependent inflammasome activation pathway resembling mostly the alternative pathway (supported by DFG).

Internal and external collaboration

Otto Holst, Kasia Duda, Susanne Krauss-Etschmann, Heinz Fehrenbach, Uta Jappe, Nicolas Gisch, Thomas Gutsmann

Thomas Roeder, CAU Kiel
Ruth Schmitz-Streit, CAU Kiel
Carsten Schmidt-Weber, ZAUM & TU Munich
Alla Zamyatina, BOKU Wien
Christian Keller, Uni Marburg

Grant support

BMBF DZL, disease area AA2.2
DFG HE 2758/4-2

DROSOPHILA MELANOGASTER

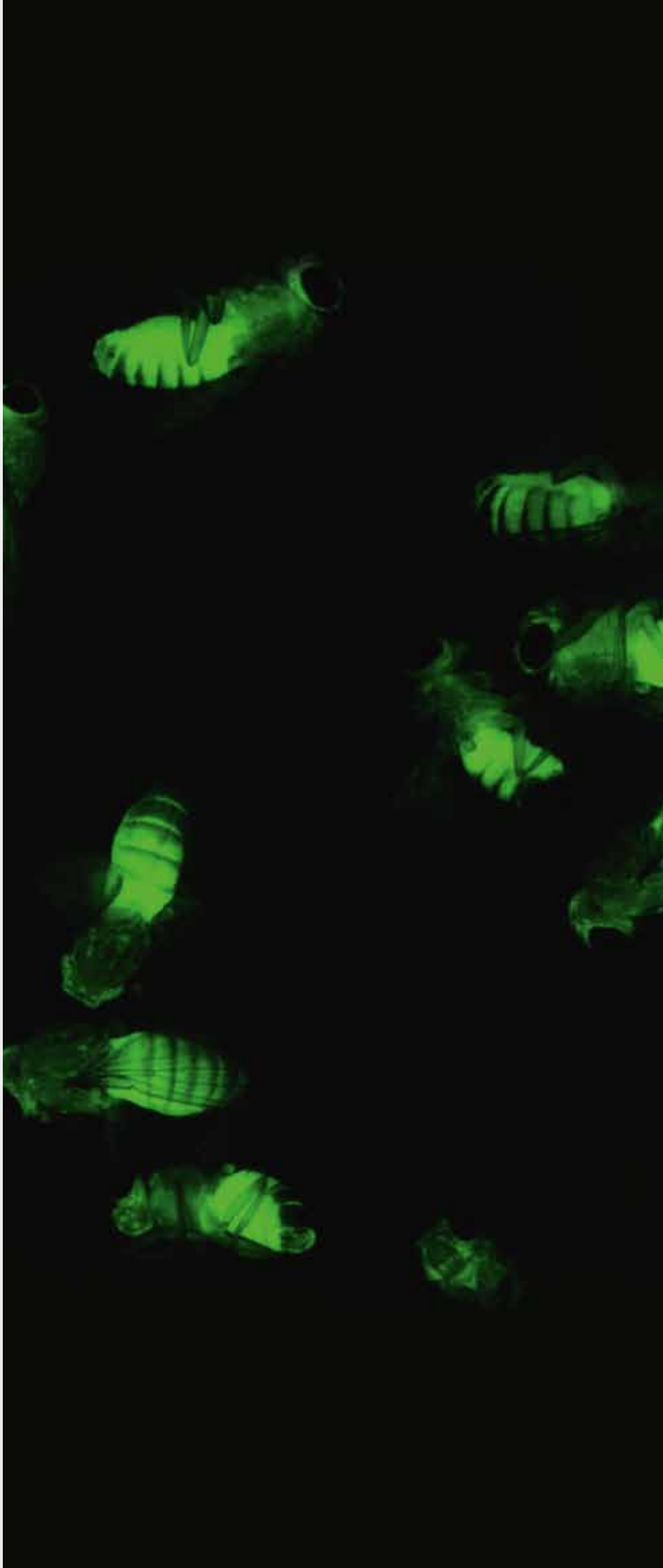
ASTHMA SUSCEPTIBILITY GENES

INNATE IMMUNITY

AIRWAY EPITHELIUM

AIRWAY MICROBIOME

CIGARETTE SMOKING



Priority Research Area **Asthma and Allergy**

Invertebrate Models

Head

- Dr. Christina Wagner

Members

- Hanna Angstmann
- Beate Hoeschler
- Arne Jodlauk
- Dr. Stephanie Papenmeier
- Karolina-Theresa Sirocko
- Dr. Karin Uliczka

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Asthma and Allergy**

Invertebrate Models

Mission

Ziel der Nachwuchsgruppe **Invertebratenmodelle** ist es zu verstehen, inwieweit die Pathogenese von chronisch-entzündlichen Atemwegserkrankungen durch umweltbedingte bzw. genetische Risikofaktoren beeinflusst wird. Hierbei konzentrieren wir uns in erster Linie auf Untersuchungen zur angeborenen Immunabwehr und Barrierefunktion des Atemwegsepithels. Unter Zuhilfenahme des Modellsystems *Drosophila melanogaster* möchten wir evolutionär hoch konservierte Gene und Signalwege identifizieren, deren Fehlregulationen die Pathogenese der Erkrankungen maßgeblich beeinflussen und somit Ziel für die Entwicklung neuer therapeutischer Interventionen darstellen könnten.

The objective of the junior research group **Invertebrate Models** is to better understand how the pathogenesis of chronic inflammatory airway diseases is affected by environmental and genetic factors. Current investigations focus on the innate immune response and barrier function of the airway epithelium. Using the model system *Drosophila melanogaster*, we are primarily interested in identifying genes and molecular pathways whose dysregulations impair disease pathogenesis and are therefore ideal targets for new therapeutic interventions.

Most important findings

Epidemiological studies suggest that effects of maternal smoking on asthma development can also be transferred to subsequent generations, even if the unborn child is not directly exposed to maternal tobacco use. So far the molecular and epigenetic changes induced by maternal smoking during pregnancy are mostly unknown. This also includes their underlying key regulators and pathways crucial for proper fetal airway development and growth. In order to identify and analyze them more closely, we use the model system *Drosophila melanogaster* as a screening tool. So far, we could successfully establish a *Drosophila* smoking model reflecting key features of an antioxidant phenotype. This is mainly characterized by the airway epithelial-specific expression of *Cyp18A1* (a murine and human cytochrome P_{450} 1a1 (*Cyp1a1*) homologue) which demonstrates that also in the airways of the fly highly toxic tobacco smoke components such as polycyclic aromatic hydrocarbons are detoxified by cytochrome P450 homologues. Furthermore, it is featured by a significant upregulation of different antioxidant enzyme genes which are involved in glutathione metabolism (e.g. *GstD4*), Cyp P_{450} metabolism (e.g. *Cyp6a2*), and in the oxidative stress response (e.g. *hsp70*). Interestingly, their expression is not restricted to the epithelium of the posterior airways facing the air, but also extends to the anterior one implicating that smoke enters the whole airway tree (Figure 1). While a single exposure towards cigarette smoke (CS) at the juvenile (larval) stage does not affect the survival rate of adults, the survival rate of

Highlights

Establishment of a *Drosophila* smoking model.

Selected publications

Prange, R, Thiedmann, M, Bhandari, A, Mishra, N, Sinha, A, Häsler, R, Rosenstiel, P, Uliczka, K, Wagner, C, Yildirim, AÖ, Fink, C & Roeder, T 2018, 'A Drosophila model of cigarette smoke induced COPD identifies Nrf2 signaling as an expedient target for intervention' Aging, Jg. 10, Nr. 8, S. 2122-2135.

Hammer, B*, Wagner, C*, Divac Rankov, A*, Reuter, S, Bartel, S, Hylkema, MN, Krüger, A, Svanes, C & Krauss-Etschmann, S 2018, 'In utero exposure to cigarette smoke and effects across generations:a conference of animals on asthma' Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, Jg. 48, Nr. 11, S. 1378-1390.
* equally contributed.

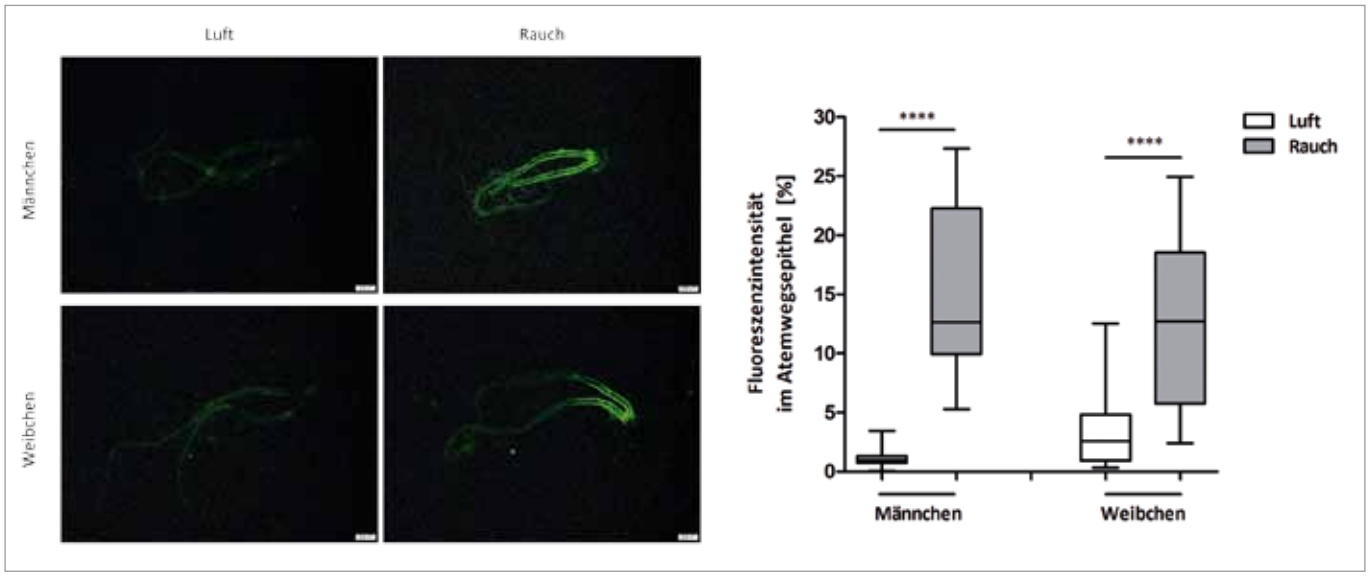


Figure 1. Visualization and quantification of *hsp70* expression in dissected airways of cigarette-smoke exposed male as well as female larvae carrying GFP (green fluorescence protein) under the control of an hsp (heat shock protein) 70 gene promotor.

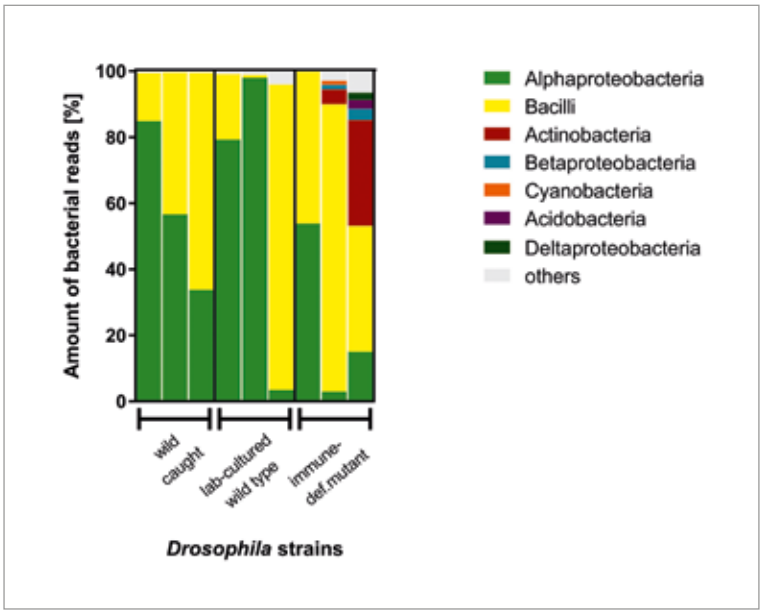


Figure 2. Airway microbiome of Drosophila wild-caught individuals and laboratory-cultured wild type as well as immunodeficient strains (diagram shows 16S rRNA gene sequencing results of three biological replicates).

Invertebrate Models

smoke-exposed male larvae is significantly reduced compared to female ones. Interestingly, such sex-specific differences in mortality rates triggered by an increased vulnerability towards cigarette smoke exposure can be also observed in humans with chronic obstructive lung diseases.

Beside environmental factors also genetic factors have been associated with an increased risk of developing asthma. These genetic factors include variants of two serine protease inhibitor (serpins) genes, namely *spink5* and *scca1*. Even though it was shown that the expression of both genes is dysregulated in the airway epithelium of asthmatics, little is known about their physiological role as well as interaction with environmental exposures. In our lab, these issues are currently being investigated in fly models for *scca1* and *spink5* mimicking the situation found in human carriers. So far, we have shown that airway-specific dysexpression of *scca1* affects airway number or length. Moreover, it results in an increased vulnerability towards hypoxic stress. While *scca1* dysexpressing larvae generally showed a developmental delay, exposure to hypoxia during the larval stage does not affect the pupal as well as adult development even further. It is most likely that airway morphological changes induced by *scca1* dysexpression are responsible for an increased vulnerability towards environmental stressors. Next, larvae will be repeatedly exposed to CS for testing this hypothesis.

Since it is known that an altered airway microbiome may be a feature of asthma, we asked whether variants of asthma susceptibility genes are substantially involved in shaping it. However, until recently it was unclear whether *Drosophila* has an airway microbiome at all. First 16S rRNA sequencing runs indicate that an airway microbiome exists in wild-caught as well as laboratory-cultured wild type strains (Figure 2). Surprisingly, their airway microbiome is of low diversity and primarily consists of gram-negative α-proteobacteria as well as gram-positive bacilli. As expected, only airways from immunodeficient mutants (devoid of TNFα signalling) show a higher diversity in their microbiome composition.

Internal and external collaboration

Internal

Heinz Fehrenbach, Division of Experimental Pneumology; Susanne Krauss-Etschmann, Early Life Origin of CLD; Holger Heine, Division of Innate Immunity; Michael Wegmann, Division of Asthma Exacerbation and Regulation

External

Thomas Roeder, Research Group Molecular Physiology, University of Kiel; Sabrina Schreiner, Institute of Virology, TUM/ Helmholtz Center Munich; Annette Kraegeloh, Research Group Nano Cell Interactions, Leibniz Institute for New Materials, Saarbrücken; PD. Dr. Klaus Unfried, Research Group Environmentally-induced Skin and Lung Aging, Leibniz Research Institute for Environmental Medicine, Düsseldorf; Michael Schloter, Research Unit Comparative Microbiome Analysis, German Research Center for Environmental Health; Inke Koenig, Institute of Medical Biometry and Statistics, University of Lübeck; Ulrich Theopold, Department of Molecular Biology and Functional Genomics, Stockholm University, Sweden.

Grant support

Leibniz Research Alliance EXASENS; Subproject FKZ 13N13857 (BMBF/ Leibniz): "On-site exacerbation diagnostics in asthma & COPD: probes, models, analytes and capture systems"; Coordinator: Prof. Heinz Fehrenbach.

Leibniz ScienceCampus EvoLUNG (FZB, CAU, MPI-EB); Subproject RAII.2: "Deciphering the pathophysiological significance of serine peptidase inhibitor gene variants in asthma"; Coordinator: Prof. Dr. Stefan Niemann.

Leibniz Research Alliance Nanosafety (INM, IUF, IfADo, FZB, FIZ KA, KMRC); Subproject A3a: "Effects of nanomaterials on epithelial immune responses in the non-vertebrate model system *Drosophila melanogaster*"; Coordinator: Dr. Annette Kraegeloh.

Priority Research Area **Asthma and Allergy**

Mucosal Immunology and Diagnostics

MOLECULAR EVOLUTION

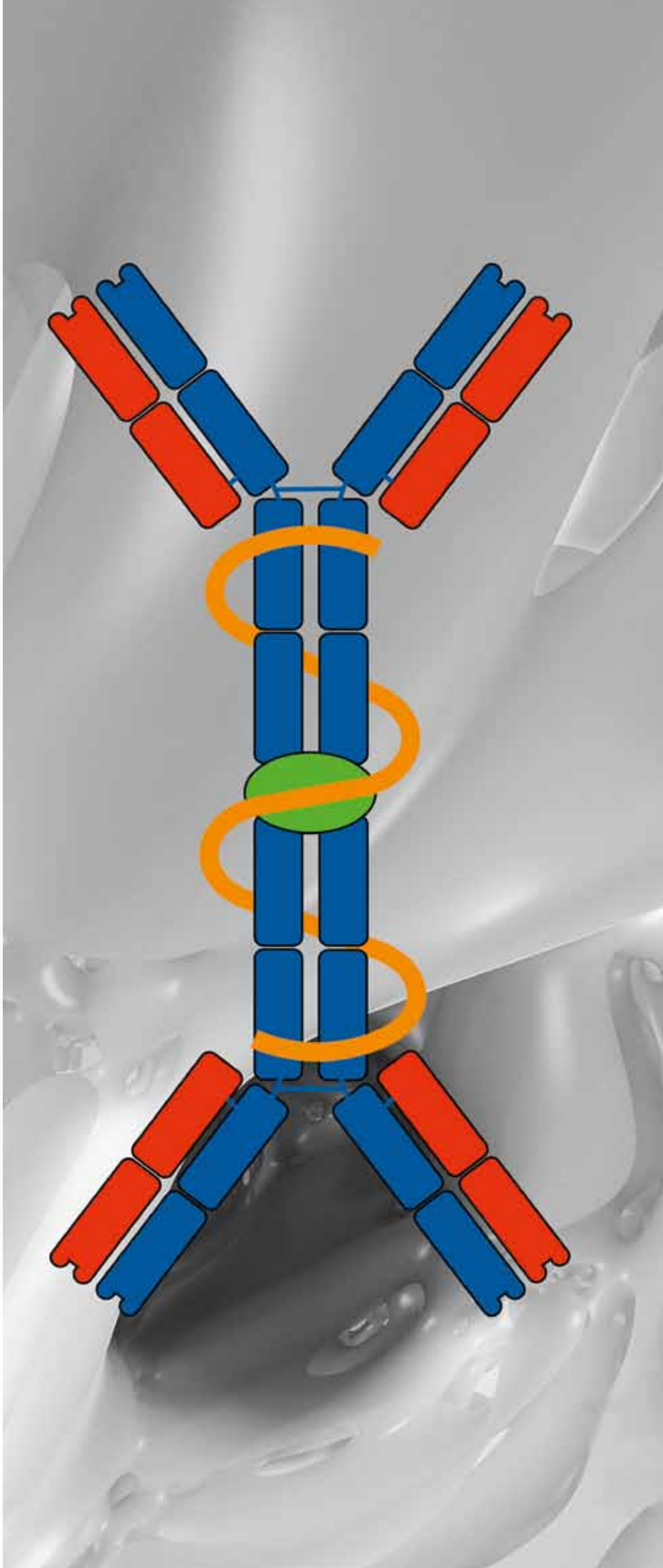
HIGH THROUGHPUT PEPTIDE ASSAYS

DIAGNOSTIC MARKERS

MUCOSAL BARRIERS

POINT-OF-CARE DIAGNOSTICS

IMMUNOGLOBULIN RECOGNITION MAPPING



Head

- PD Dr. Andreas Frey

Members

- Dr. Barbara Frey
- Dr. Thorsten Krause
- Dr. Katrin Ramaker
- Dr. Niels Röckendorf
- Dr. Kristof Tappertzhofen (until Juli 2018)
- Meike Göddeke (until December 2018)
- Sina Hennings
- Jürgen Sarau
- Özge Ulupinar-Kök (until April 2017)
- Alheidis von Quast

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Asthma and Allergy**

Mucosal Immunology and Diagnostics

Mission

Die Forschungsgruppe Mukosale Immunologie & Diagnostik erforscht auf molekularer und zellulärer Ebene Auslöser, Mechanismen und therapeutische Ansatzmöglichkeiten bei Schleimhaut-assoziierten Erkrankungen, insbesondere auf den Gebieten Asthma/COPD und Allergien. Wir wollen Marker für Ausbruch, Verlauf und Schwere dieser Krankheiten identifizieren, Wege finden, um diese Marker für diagnostische, prognostische and therapeutische Ansätze zu nutzen, und neue Maßnahmen aufzeigen, um präventiv vor Ausbruch oder Verschlimmerung dieser Erkrankungen zu schützen.

The Division of Mucosal Immunology and Diagnostics focusses its research on molecular and cellular structures which can give us insight into the cause and mechanisms of mucosa-associated diseases and may help us create novel therapeutic interventions, especially in the fields of asthma/COPD and allergic disorders. Specifically, we want to identify and characterize markers for the initiation, course and severity of these diseases, provide tools to survey them for diagnostic, prognostic and therapeutic purposes and develop means to prevent or alleviate disease aggravation.

Most important findings

One of our research topics is the humoral and **secretory immune response at the airway mucosa** with an emphasis on the balance of the different immunoglobulin classes and the role of pathogen-specific IgA and IgE in allergy and asthma. To identify hitherto unknown reactivities of IgE and / or IgA we have established a procedure to screen millions of potential epitopes in parallel. We use one-bead-one-compound libraries, where random peptide sequences are synthesized onto microparticles which can subsequently be analyzed for the binding of immunoglobulins from human serum or mucosal secretions. We were able to successfully identify specific IgE-epitope-particles in a library of irrelevant peptide-particles when screening with serum from allergic patients with known IgE-specificity. The method can now be applied to reveal novel epitopes and/or cross-reactivities of immunoglobulins potentially associated with asthma or allergies.

The **delivery of drugs** into cells is a key requirement in molecular medicine. To carry the reagent across the cell membrane, small, usually cationic peptides are often chosen as a transporter and linked to the drug in question. Over the past years, a large number of these so-called **cell penetrating peptides** (CPPs) have been described, with varying across-the-membrane transport capabilities being reported for different set-ups and assay conditions. We have conducted as systematic investigation of 474 CPPs published in a special database and have analyzed and compared their cargo transport capabilities in a standardized assay under uniform conditions, generating a ranking of all CPPs according to their performance in our uptake test.

Highlights

Extensive validation and optimization of cell penetrating peptides as transmembrane drug carriers

Definition of "pre-exacerbation markers" for point-of-care-monitoring of asthma and COPD

Chip-based diagnostic assays for the measurement of pre-exacerbation markers

Selected publications

Krause, T., Röckendorf, N., Gaede, K.I., Ramaker, K., Sinnecker, H., Frey, A. (2017) Validation of antibody reagents for mucin analysis in chronic inflammatory airway diseases. mAbs 9:333-341.

Schwager, C., Kull, S., Behrends, J., Röckendorf, N., Schocker, F., Frey, A., Homann, A., Becker, W.-M., Jappe, U. (2017). Peanut oleosins associated with severe peanut allergy – importance of lipophilic allergens for comprehensive allergy diagnostics. J. Allergy Clin. Immunol. 140:1331-1338.

Röckendorf, N., Meckelein, B., Scherf, K. A., Koehler, P., Frey A. (2017). Identification of novel antibody-reactive detection sites for comprehensive gluten monitoring. PLoS ONE 12:e181566 (doi: 10.1371/journal.pone.0181566

Homann, A., Röckendorf, N., Kromminga, A., Frey, A., Platts-Mills, T.A., Jappe, U. (2017). Glycan and peptide IgE epitopes of the TNF-alpha blockers infliximab and adalimumab – precision diagnostics by cross reactivity immune profiling of patient sera. Theranostics 7:4699-4709

Ramaker, K., Henkel, M., Krause, T., Röckendorf, N., Frey, A. (2018) Cell penetrating peptides: a comparative transport analysis for 474 sequence motifs. Drug Delivery 25:928-937.

Krause, T., Röckendorf, N., El-Sourani, N, Ramaker, K., Henkel, M., Hauke, S., Borschbach, M., Frey, A. (2018). Breeding cell penetrading peptides: optimization of cellular uptake by a function-driven evolutionary process. Bioconjugate Chem. 29:4020-4029.

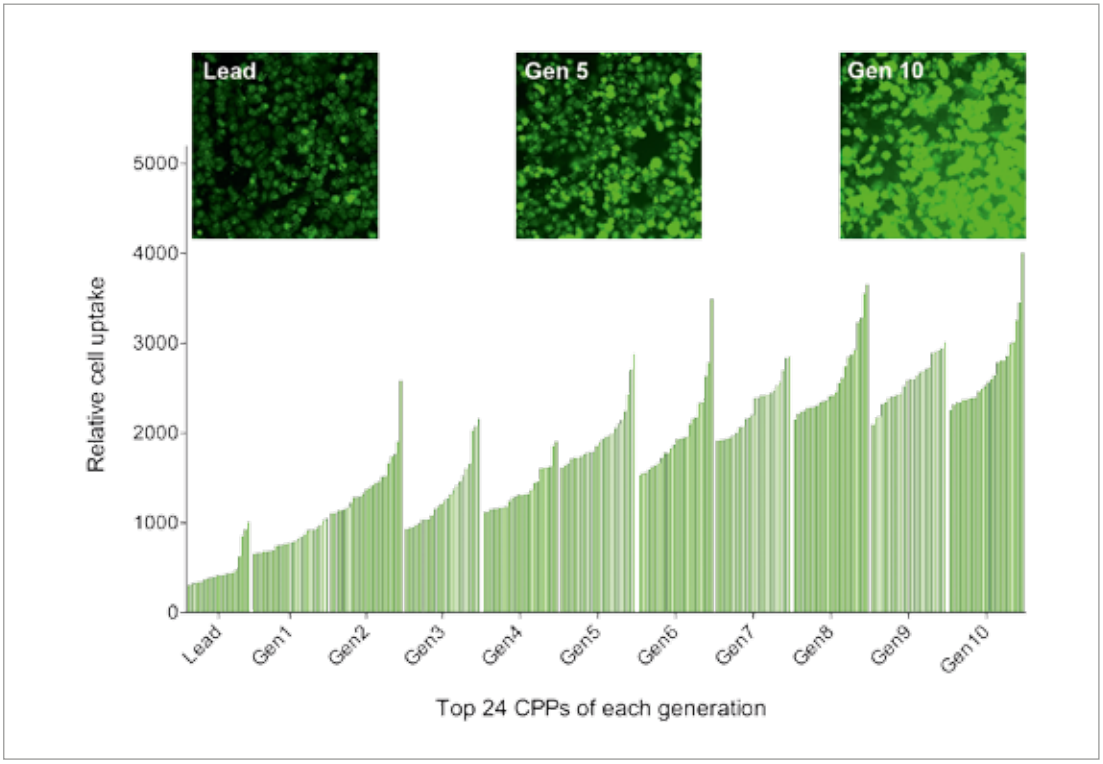


Figure 1. Evolutionary improvement of the performance of cell penetrating peptides (CPPs). CPP candidates were equipped with a fluorophore cargo and transport of the dye into cultured HeLa cells was monitored by fluorescence microscopy. The freight transport capabilities of the CPPs was improved by molecular evolution applying a genetic algorithm over a total of 10 generations.

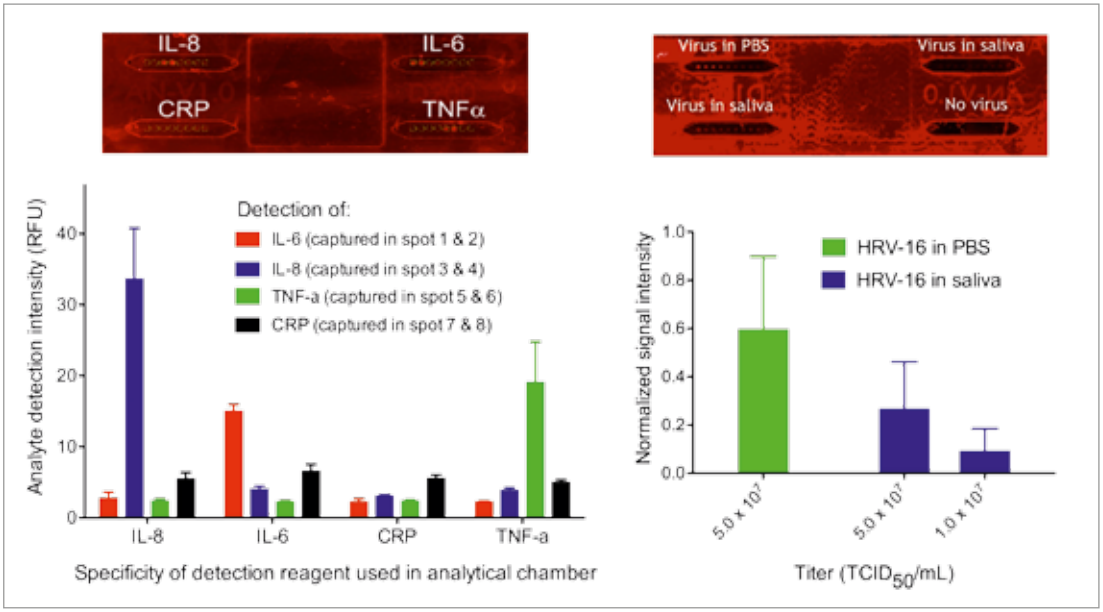


Figure 2. On-chip detection of potential pre-exacerbation markers. The cytokines IL-6, IL-8 and TNF-α as well as C-reactive protein CRP (left) or the human rhinovirus HRV-16 (right) were selectively captured out of human saliva samples onto defined areas of a glass chip. The glass chip was subsequently processed in a newly developed point-of-care device with four separate analytical chambers using one individual analyte-specific detection reagent in each chamber.

Priority Research Area **Asthma and Allergy**

Mucosal Immunology and Diagnostics

To further improve the CPP transport capabilities and generate an optimized carrier molecule we applied a proprietary "evolution platform". Starting out with the best performers in our previous screening as "lead peptides", we submitted the CPPs to successive rounds of *in silico* recombination and experimental validation. In the course of 10 generations, we managed to improve the transport capabilities of our top CPP candidates six-fold compared to the best CPPs known before (figure 1). We now have at hand several new peptides optimized for their use as carriers of drugs, diagnostics or other molecules into cells.

In a collaborative project involving a total of nine Leibniz institutions we are developing a versatile **diagnostic platform** to monitor the disease status in asthma and COPD in a point-of-care manner and to assess the risk of potential upcoming exacerbations of these diseases. For this task, we have identified a panel of cytokines, chemokines and acute phase proteins which show a marked change in their concentration in the lung and associated body fluids in association with an acute worsening of asthma or COPD. In a selective process we have singled out a limited number of these molecules to serve as potential **pre-exacerbation** biomarkers, along with typical airway infectious agents such as rhinoviruses or *Streptococci pneumoniae* which are known inducers of aggravations in chronic inflammatory lung diseases.

To enable the parallel analysis of these markers in e.g. saliva samples with the aid of the envisioned point-of-care diagnostic device, we have devised methods to capture the marker molecules and the pathogens out of biological samples, immobilize them onto the surface of a diagnostic chip and determine their concentration by a combination of specific detection reagents and a sophisticated optical read-out procedure. We have achieved to establish the necessary assay systems and have integrated them in a demonstrator for the point-of-care device such that we can measure the marker content of saliva samples (figure 2).

Internal and external collaboration

Inhouse:

Heinz Fehrenbach, Experimental Pneumology
Karoline Gaede, BioMaterialBank North
Thomas Gutsman, Biophysics
Uta Jappe, Clinical and Molecular Allergology
Susanne Krauss-Etschmann, Early Life Origins of CLD
Michael Wegmann, Asthma Exacerbation & Regulation
Xinhua Yu, Autoimmunity of the Lung
Christian Herzmann, Medical Clinic Borstel

External:

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Christian Wenger, Leibniz-Institut für Innovative Mikroelektronik, Frankfurt / Oder
Olaf Kniemeyer, Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie, Jena
Erika von Mutius, Ludwig-Maximilians-Universität München
Markus Borschbach, Fachhochschule der Wirtschaft Nordrheinwestfalen, Bergisch Gladbach

Grant support

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Bundesministerium für Bildung und Forschung (BMBF), German Center for Lung Research DZL, Projects AA1.1 & COPD-2.8

Volkswagenstiftung, Grant Program Experiment!

NMR SPECTROSCOPY

BACTERIAL GLYCOLIPIDS

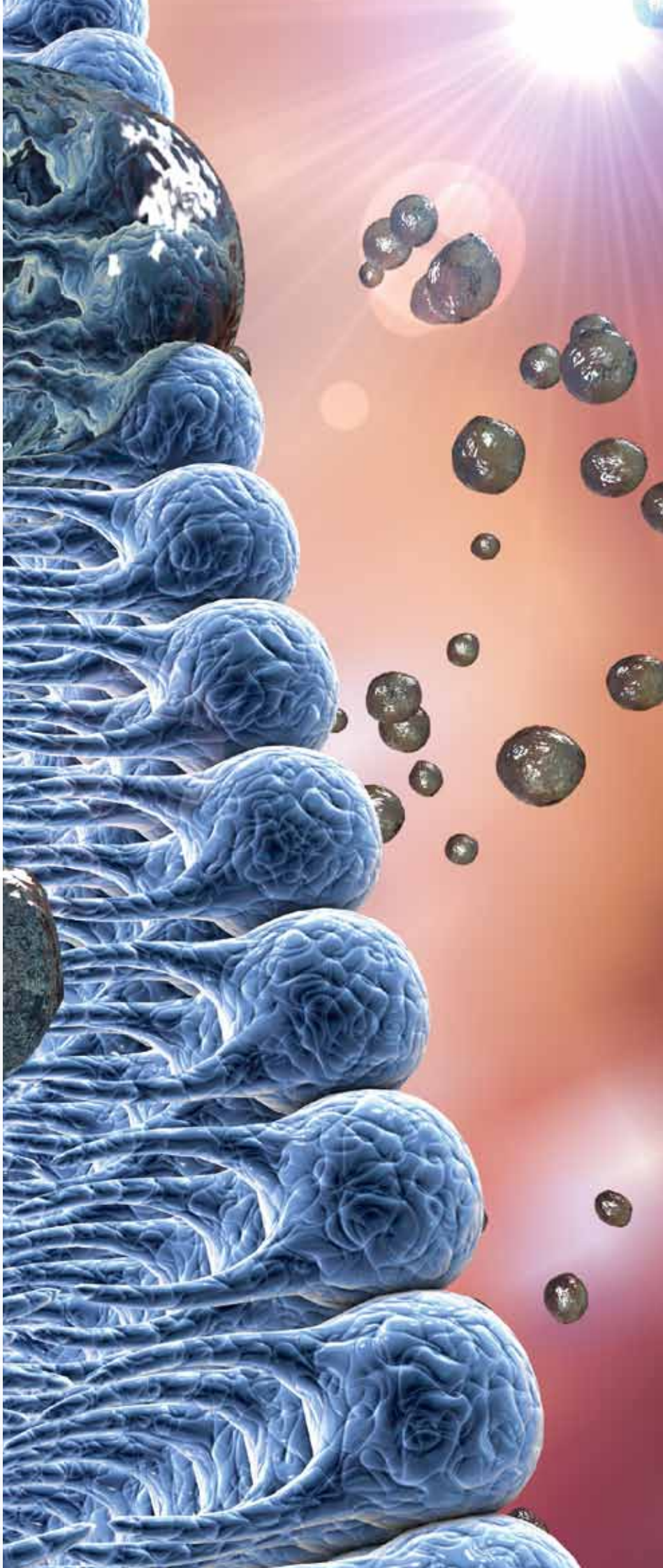
LUX SCORE

HOST-PATHOGEN INTERACTION

LIPIDOMICS

MASS SPECTROMETRY

LIPID MEDIATORS



Head

- PD Dr. Dominik Schwudke

Members

- Dr. Fadi Al Machot
- Birte Buske (until 08/2017)
- Lisa M. Deter (until 10/2017)
- Lars F. Eggers (until 01/2018)
- Dr. Nicolas Gisch
- Dr. Hande Karaköse
- Heiko Käßner
- Daniel Krause (from 07/2018)
- Brigitte Kunz
- Verena Scholz
- Ursula Schombel
- Simone Thomsen
- Franziska Waldow
- Michael Weinkauf
- Michelle Wröbel
- Dr. Adam Wutkowski

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Infections**

Bioanalytical Chemistry

Mission

Wir untersuchen die Rolle von Zellwandbestandteilen in bakteriellen Infektionen und metabolische Pathogen-Wirts-Interaktionen im Hinblick auf potenzielle therapeutische und diagnostische Anwendungen. Wir vereinen Kompetenzen zur Anwendung der Gaschromatographie (GC), Massenspektrometrie (MS) und der Kernmagnetischen Resonanzspektroskopie (NMR), um detaillierte Analysen metabolischer Prozesse in biologischen Modellsystemen sowie in der klinischen Anwendung durchzuführen. Um die Forschung zur Antibiotika-Resistenzentwicklung in der Tuberkulose zu unterstützen, haben wir eine Plattform zur Quantifizierung von Antibiotika implementiert, die für PK/PD Ansätze im Tiermodell und in der Klinik angewandt wird. In Kombination mit Lipid-basierten diagnostischen Markern tragen wir zur Etablierung personalisierter Therapien in der Tuberkulose bei. Des Weiteren analysieren wir das Lungen-Lipidom in verschiedenen Mausmodellen und des Menschen, um die Regulation des Lipidmetabolismus der Lunge im Kontext der Entwicklung von Asthma, COPD und Lungenkrebs zu untersuchen. Ergebnisse unserer Lipidom-Forschung werden systembiologisch interpretiert und für weitere bioinformatische Analysen in einer Datenbank der Öffentlichkeit zur Verfügung gestellt.

Our group studies the role of cell wall components in bacterial infections and explores metabolic pathogen-host-interactions for potential therapeutic and/or diagnostic applications. We unite competences in gas chromatography (GC), mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy to perform in-depth studies of metabolic processes in biological model system as well as for clinical application. To investigate the development of antibiotic resistance in tuberculosis, we implemented a platform for the quantification of antibiotics, which is used for studying PK/PD in animal models and clinical investigations. Further, we contribute to the establishment of personalized therapies in tuberculosis by identifying and evaluating lipid-based diagnostic markers. To explore the regulation of the lung lipid metabolism in the context of asthma, COPD and lung cancer, we study the lung lipidome in different mouse models and in humans. The results of our lipidome-based research are interpreted with systems biology approaches and provided to the public as a resource for future bioinformatics studies.

Most important findings

Therapeutic Drug Monitoring

We established an analytical platform within DZIF and for TB research in general to quantify all antibiotics required for treating M/XDR-TB. The platform is well suited for testing new administration concepts in animal models and cell systems but is also applicable for clinical samples. Sample preparation procedures were standardized for plasma samples, lung tissue and cell culture. Liquid chromatography conditions as well as mass spectrometric parameters were optimized so that within 20 minutes

Highlights

Publication of Heß, Waldow, Kohler et al. (Nat. Commun., 2017) was awarded as "Paper of the month" by the DGHM (01/2018)

Successful continuation of the Lipidomics Forum conference series in cooperation with Robert Ahrends (ISAS Dortmund): 3rd meeting 12.-14.11.2017 at the RCB; 4th conference 11-13.11.2018 at the ISAS.

Habilitation, Dr. Dominik Schwudke, University of Lübeck 2017

Selected publications

Wutkowski A, Krajewski M, Bagwan N, Schäfer M, Paudyal BR, Schaible UE, Schwudke D. Software-aided quality control of parallel reaction monitoring based quantitation of lipid mediators. ANAL CHIM ACTA. 2018; 1037: 168-176.

Hofmann S, Krajewski M, Scherer C, Scholz V, Mordhorst V, Truschow P, Schöbel A, Reimer R, Schwudke D, Herker E. Complex lipid metabolic remodeling is required for efficient hepatitis C virus replication. BIOCHIM BIOPHYS ACTA MOL CELL BIOL LIPIDS. 2018; 1863: 1041-1056.

Gisch N*, Auger J-P*, Thomsen S, Roy D, Xu J, Schwudke D#, Gottschalk M#. Structural analysis and immunostimulatory potency of lipoteichoic acids isolated from three *Streptococcus suis* serotype 2 strains. J BIOL CHEM 2018; 293: 12011-12025.

Heß N*, Waldow F*, Kohler TP*, Rohde M, Kreikemeyer B, Gómez-Mejia A, Hain T, Schwudke D, Vollmer W, Hammerschmidt S#, Gisch N#. Lipoteichoic acid deficiency permits normal growth but impairs virulence of *Streptococcus pneumoniae*. NAT COMMUN 2017; 8: 2093.

Eggers LF, Müller J, Marella C, Scholz V, Watz H, Kugler C, Rabe KF, Goldmann T, Schwudke D. Lipidomes of lung cancer and tumour-free lung tissues reveal distinct molecular signatures for cancer differentiation, age, inflammation, and pulmonary emphysema. SCI REP 2017; 7: 11087.

Sezin T, Krajewski M, Wutkowski A, Mousavi S, Chakievska L, Bieber K, Ludwig RJ, Dahlke M, Rades D, Schulze FS, Schmidt E, Kalies K, Gupta Y, Schilf P, Ibrahim SM, König P, Schwudke D, Zillikens D, Sadik CD. The leukotriene B4 and its receptor BLT1 act as critical drivers of neutrophil recruitment in murine bullous pemphigoid-like epidermolysis bullosa acquisita. J INVEST DERMATOL 2017; 137: 1104-1113.

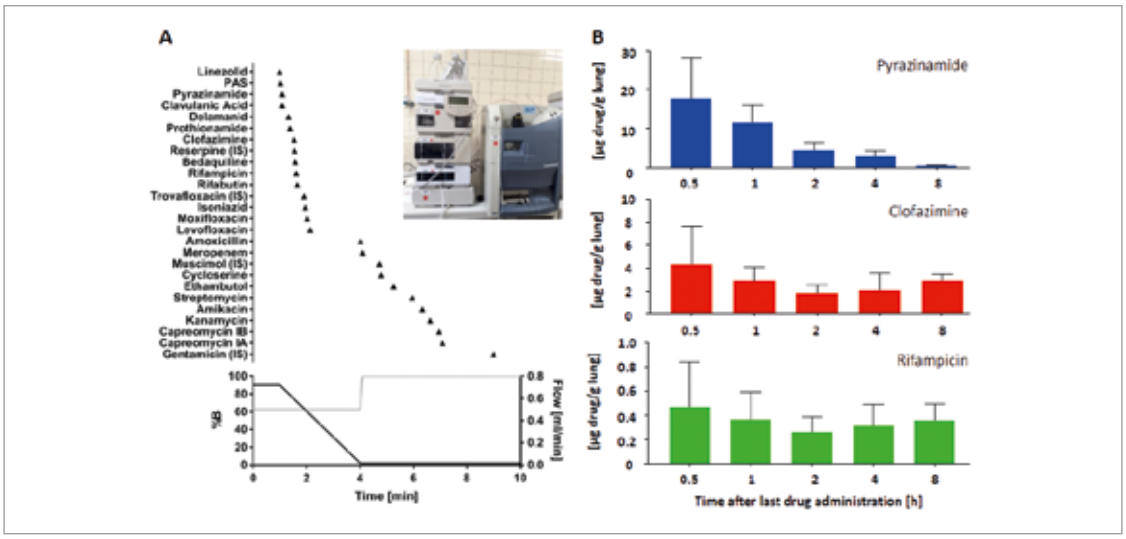


Figure 1. Platform for Quantitation of Antibiotics. A) Liquid chromatography – tandem mass spectrometry system (LC-MS/MS) to quantify 22 anti-TB drugs for MDR/XDR regimens. Complete LC-MS/MS run time is 20 minutes and regimens with up to eight drugs can be analysed in one run. B) Exemplary PK analysis in uninfected BALB/C mice for a combination of Pyrazinamide, Clofazimine and Rifampicin quantified in lung tissue homogenates (N = 3 per time point; error bars represent one standard deviation). In collaboration with K. Walter (Infection Immunology).

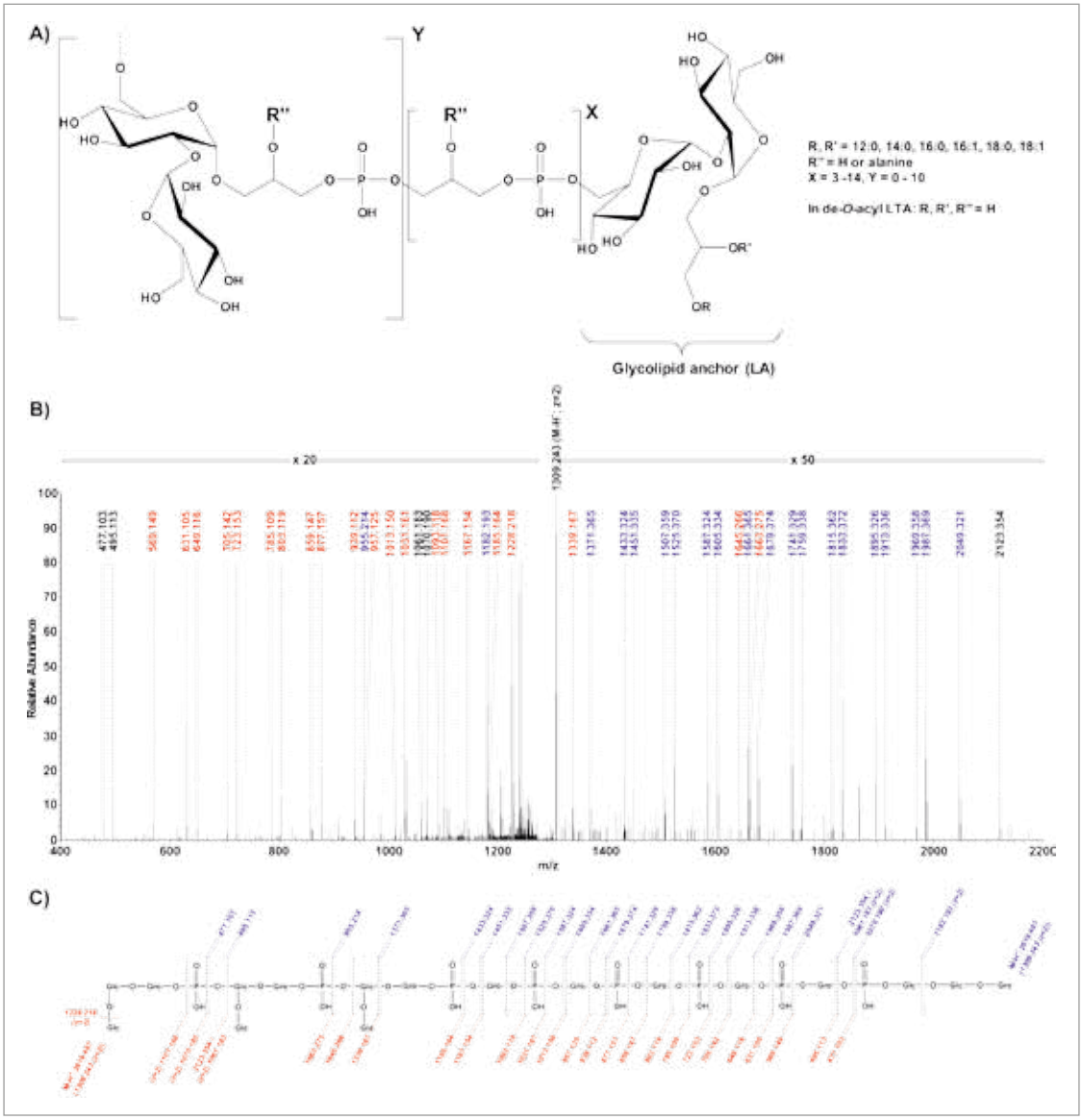


Figure 2. *S. suis* is the first bacterium identified that possesses two different LTA types. A) Chemical structures of LTA isolated from *S. suis* serotype 2 strains P1/7 (ST1) and SC84 (ST7). B,C) MS/MS analysis of de-O-acylated LTA molecule Y_3X_5A of *S. suis* strain P1/7 enabled the proof of the consecutive order of the two different repeating units. (ST – sequence type) (Gisch *et al.*, J. Biol. Chem. 2018)

Priority Research Area **Infections**

Bioanalytical Chemistry

run time up to 8 drugs can be quantified in parallel (Fig 1. A). Individual patients drug regimens can be monitored by combination of previously optimized single reaction monitoring (SRM) channels. Furthermore, the LC-MS/MS system is inherently expandable to novel drugs and the related PK/PD analyses. In this way, we support the complete translational pipeline for implementing individualized therapies. Exemplarily, we show that PK of Pyrazinamide clearly differs from Clofazimine and Rifampicin. Main collaborators for TB drug research are the groups of C. Lange (Clinical Infectious Diseases), U. Schaible (Cellular Microbiology) and C. Hölscher (Infection Immunology). The development of the platform has helped to acquire funding for research on novel nano-carrier based administration of antibiotics (ANTI-TB). Ongoing research is focussed on integrating lipid based diagnostic markers with PK/PD analyses to foster individualized therapy monitoring.

Recent progress in the analysis of lipoteichoic acid structures, biosynthesis, and their impact on host-pathogen interaction

Teichoic acids (TA) are important structures to maintain cell integrity and cell morphology of Gram-positive bacteria. In the past two years, we gained significant new insights into lipoteichoic acid (LTA) structures as well as their biosynthesis and importance for bacterial pathogenicity, especially in *Streptococcus pneumoniae* and *Streptococcus suis*. In close collaboration with the group of Prof. Dr. S. Hammerschmidt (University of Greifswald), we identified the so far unknown lipoteichoic acid ligase TacL in pneumococci (e. g. SPD_1672 in strain D39/SP_1893 in strain TIGR4). The analysis of the *tacL* KO-mutants and complemented strains showed that TacL is responsible for the ligation of pnTA precursor chains onto the glycolipid anchor to form the LTA. Absence of TacL led to a complete loss of LTAs but retained pnWTAs, which in consequence strikingly decreased virulence in murine infection models, although the mutants grow normally in culture. Hence, LTA is important for *S. pneumoniae* to establish systemic infections. First findings indicate that this might be the result of a significantly decreased ability of the TacL-deficient strain to adhere on human lung epithelial cells. Together with the group of Prof. Dr. M. Gottschalk (University of Montreal), we characterized the LTA structures from three *S. suis* serotype 2 strains differing in genetic background and virulence. Our analyses revealed that these strains possess – in addition to the typical type I LTA present in other streptococci – a second, mixed-type series of LTA molecules of high structural complexity. We observed a sequence type (ST) specific difference in the incorporation of glycosyl residues into these mixed-type LTAs. By MS/MS experiments, we could verify the consecutive order of two different repeating unit types in those LTA molecules (see Fig. 2). In *S. suis* strains P1/7 and SC84, we found a defined subset of LTA molecules with 3–10 GroP repeats to be elongated with 1–10 repeats of the more complex glycosyl residue-containing units. To the best of our knowledge, this is the first example of a regulated synthesis of a mixed-type LTA in bacteria. Moreover, our recent data suggest that the glycosylation of these LTA molecules has a high impact on the virulence of *S. suis*.

Internal and external collaboration

Inhouse

T. Goldmann, T. Gutsmann, C. Hölscher, K. Walter, U. Jappe, C. Lange, U. Mamat, S. Niemann, S. Homolka, S. Malm, N. Reiling, J. Brandenburg, U. Schaible, A. Schromm, K. Brandenburg

National

S. Hammerschmidt, T. Kohler (Greifswald), G. Hölzl (Bonn), S. Ranf (München), S. Bülow (Regensburg), B. Kreikemeier (Univ. Rostock), Manfred Rohde (HZI, Braunschweig), T. Hain (Univ. Gießen), I. Bekeredjian-Ding (PEI, Langen), P. König, C. Sadik, (Lübeck), A. Haas (Bonn), E. Herker (Hamburg), S. Hebbar, A. Shevchenko (Dresden), R. Ahrends (Dortmund), J. Ziebuhr, C. Müller (Gießen)

International

M. Gottschalk (Montreal, Canada), J. Hermoso (Madrid, Spain), W. Vollmer (Newcastle, Great Britain), A.M. Di Guilmi (Toulouse, France), Gregg Silverman (New York, USA), C. Sohlenkamp (Cuernavaca, Mexico), Turska-Szewczuk, Komaniecka (Lublin, Poland), Jesús Vázquez (Madrid, Spain)

Grant support

DZIF TTU Tuberculosis: ClinTB, Personalised Medicine

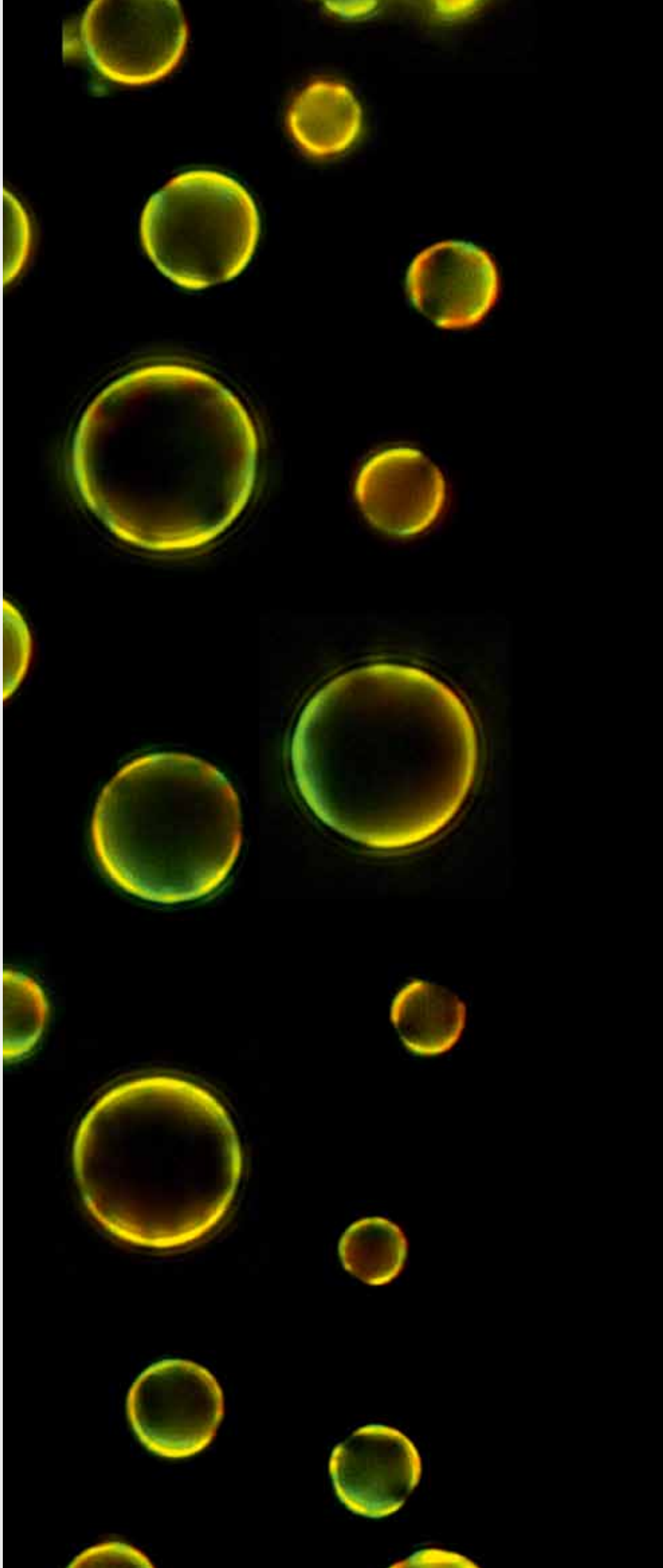
Lipidomics Informatics for Life-Science (LIFS, de.NBI - FKZ 031L0108B; 2016-2019)

Antibiotika Nanocarrier zur therapeutischen Inhalation gegen Tuberkulose (ANTI-TB)

DFG (GI 979/1-1; 2014-2017), "Molekulare und strukturelle Analyse der Teichonsäurebiosynthese bei *Streptococcus pneumoniae* und Bedeutung veränderter Teichonsäuren für die bakterielle Pathophysiologie"

Exzellenzcluster Inflammation at Interfaces (DFG – EXC 306 – CL X, 2013-2017)

IMI JU ENABLE 115583 (02/2017 – 01/2018)



Priority Research Area **Infections**

Biophysics

Head

- Prof. Dr. Thomas Gutsmann

Members

- Ute Agge
- Dr. Wilmar Correa-Vargas
- Sabine Dabelstein
- Sabrina Groth
- Dr. Sven Müller-Loennies
- Dr. Christian Nehls
- Kerstin Stephan
- Veronika Susott
- Dr. Laura Paulowski
- Elisabeth Pfrommer

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Infections**

Biophysics

Mission

Die FG Biophysik widmet sich der Struktur-Funktions-Analyse bakterieller und humaner Membranen, deren Wechselwirkung mit natürlichen und synthetischen antimikrobiellen Peptiden und membranaktiven Substanzen von Pathogenen. Der Focus liegt dabei auf Mykobakterien und Gram-negativen Bakterien. Die Entwicklung und Charakterisierung neuer antimikrobieller und anti-septischer Wirkstoffe auf Peptidbasis wird bis hin zu ersten klinischen Versuchen vorangetrieben. Die molekularen Grundlagen der bakteriellen Resistenzen gegenüber natürlichen und synthetischen AMPs werden analysiert. Ein neu entstandener Arbeitsschwerpunkt ist die Interaktion zwischen membranaktiven Komponenten von Pathogenen, insbesondere intrazellulärer Bakterien, und Membranen humaner Zellen.

The RG Biophysics is dedicated to the structure-function analysis of bacterial and human membranes, their interaction with natural and synthetic antimicrobial peptides and membrane-active substances of pathogens. The focus is on mycobacteria and Gram-negative bacteria. The development and characterization of new antimicrobial and anti-septic peptide-based active substances will be advanced up to first clinical trials. The molecular basis of bacterial resistance to natural and synthetic AMPs will be analysed. A new focus of our work is the interaction between membrane-active components of pathogens, especially intracellular bacteria, and membranes of human cells.

Most important findings

i) It has long been established that binding of sialic acid (Sia) is essential for influenza A virus (IAV) to establish an infection and has an influence on host tropism. Yet, binding assays employing a novel shotgun N-glycan microarray of human lung which was developed in Rick Cummings group at Harvard Medical School revealed that all tested isolates of IAV bind also to phosphorylated N-glycans. Using the unique recombinant monoclonal antibody fragment scFv M6P-1 isolated by phage display we were able to help establishing that mannose 6-phosphate (M6P) containing glycans are receptors for IAV. Such binding to phosphorylated glycans could explain the previously reported observation that desialylation of cells by neuraminidase treatment failed to block infection and suggests a possible role for alternative ligands, such as phosphorylated glycans, as co-factors or facilitators of virus entry, possibly opening new or additional routes to Influenza treatment.

Highlights

Influenza binds phosphorylated glycans from human lung

Aggregation of membrane-active peptides modifies their activity

ADAM 10 and 17 specifically interact with phosphatidylserine

Selected publications

Coupling killing to neutralization: combined therapy with ceftriaxone/Pep19-2.5 counteracts sepsis in rabbits. Bárcena-Varela S, Martínez-de-Tejada G, Martin L, Schuerholz T, Gil-Royo AG, Fukuoka S, Goldmann T, Droemann D, Correa W, Gutsmann T, Brandenburg K, Heinbockel L. Exp Mol Med. 2017 Jun 16;49(6).

Testing cathelicidin susceptibility of bacterial mastitis isolates: Technical challenges and data output for clinical isolates. Langer MN, Blodkamp S, Bayerbach M, Feßler AT, de Buhr N, Gutsmann T, Kreienbrock L, Schwarz S, von Köckritz-Blickwede M. Vet Microbiol. 2017 Oct;210:107-115.

Characterisation of Plasmodium falciparum populations selected on the human endothelial receptors P-selectin, E-selectin, CD9 and CD151. Metwally NG, Tilly AK, Lubiana P, Roth LK, Dörpinghaus M, Lorenzen S, Schuldt K, Witt S, Bachmann A, Tidow H, Gutsmann T, Burmester T, Roeder T, Tannich E, Bruchhaus I. Sci Rep. 2017 Jun 22;7(1):4069.

Immunogenic properties of the human gut-associated archaeon Methanomassiliicoccus luminyensis and its susceptibility to antimicrobial peptides. Bang C, Vierbuchen T, Gutsmann T, Heine H, Schmitz RA. PLoS One. 2017 Oct 5;12(10):e0185919.

Virulence-associated protein A from Rhodococcus equi is an intercompartmental pH-neutralising virulence factor. von Bargen K, Scraba M, Krämer I, Ketterer M, Nehls C, Krokowski S, Repnik U, Wittlich M, Maaser A, Zapka P, Bunge M, Schlesinger M, Huth G, Klees A, Hansen P, Jeschke A, Bendas G, Utermöhlen O, Griffiths G, Gutsmann T, Wohlmann J, Haas A. Cell Microbiol. 2018 Sep 24:e12958.

Peptide drug stability: The anti-inflammatory drugs Pep19-2.5 and Pep19-4LF in cream formulation. Kuhlmann N, Heinbockel L, Correa W, Gutsmann T, Goldmann T, Englisch U, Brandenburg K. Eur J Pharm Sci. 2018 Mar 30;115:240-247.

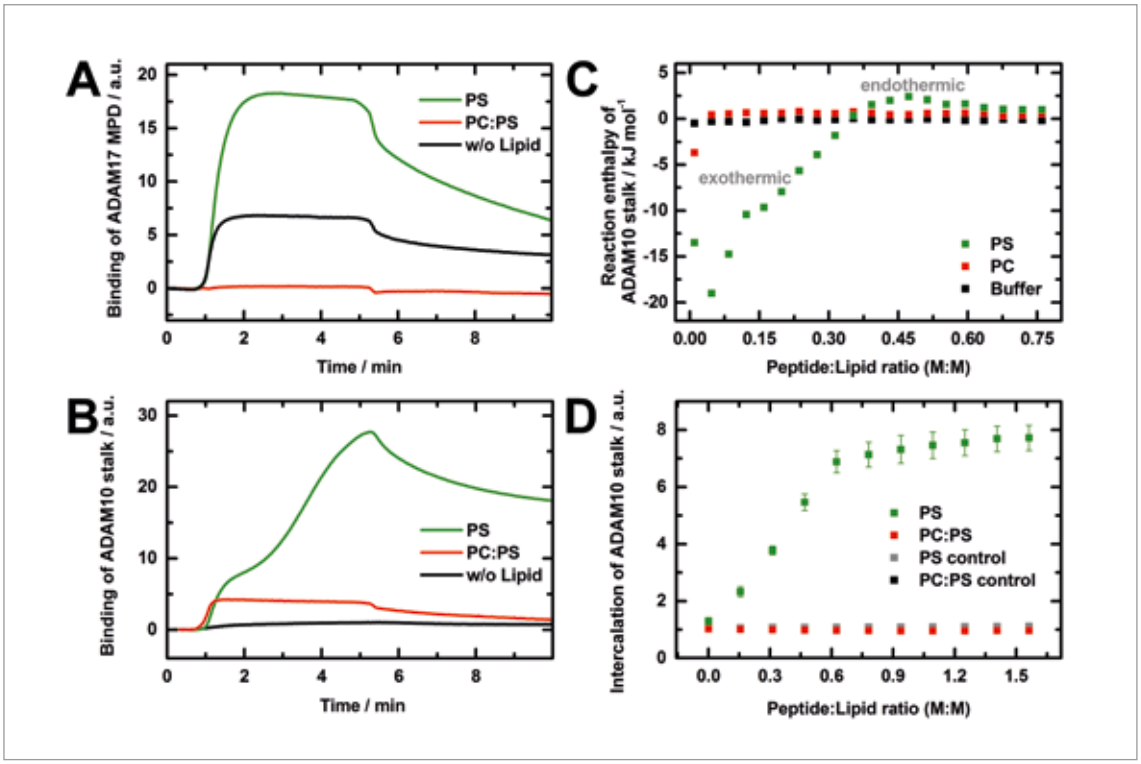


Figure 1. Investigation of the PS-dependent interaction between ADAM protein PS detecting domains and model membranes. A: Biosensor measurements with solid-supported membranes show a clear binding of ADAM17 MPD to membranes with 100% (green) but not 10% PS content (red). B: For the stalk region of ADAM10 a two-step binding process occurred at 100% PS concentration, whereas at 10% PS only a moderate binding was visible. C: Isothermal titration calorimetry on lipid liposomes was used to specify the two-stage binding as an initially exothermic reaction with transition to an endothermic reaction. D: By Förster Resonance Energy Transfer (FRET) measurements on liposomes, the second binding step was associated with an insertion between the lipid molecules.

ii) Disintegrin-like metalloproteinases (ADAMs) belong to the group of cell-bound proteinases and regulate a number of essential cell functions through cleavage of membrane-associated substrates. In a collaboration within the Excellence Cluster Inflammation at Interfaces, we have demonstrated that the activation of shedase function of two important members of the ADAM family is controlled by cell membrane asymmetry. If phosphatidylserine (PS), is transported locally into the outer layer by phospholipid translocators, a functional activation of the ADAM proteins occurs through the specific interaction of a PS-detecting protein domain. For ADAM17, this is the membrane proximal domain (MPD). The closely related protease ADAM10, which lacks an MPD domain, hosts the PS-binding domain in the stalk region. For both protein domains, biophysical methods have been used to characterize the interaction with model membranes. For ADAM17 MPD we could identify a pronounced binding to high PS membranes, which supports the hypothesis of functional PS recognition. We suggests that the ADAM10 stalk region might not only be responsible for the recognition of PS microdomains in the cell membrane, but also for the subsequent functional reorientation of the protein towards the membrane.

Priority Research Area **Infections**

Biophysics

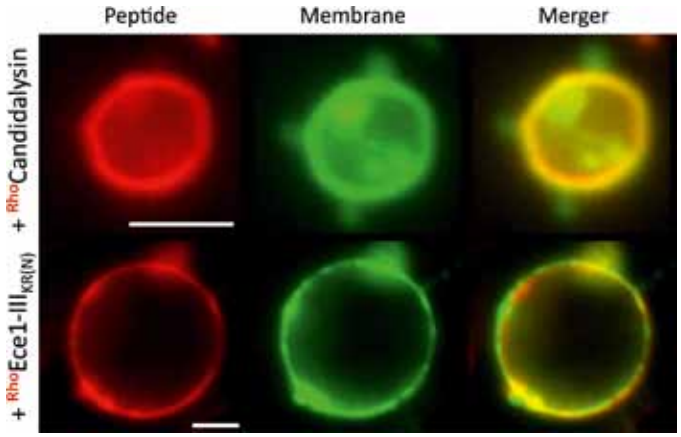


Figure 2. Fluorescence microscopy images of giant vesicles (GUVs) composed of DOPC after exposure to pre-mature (Ece1-III$_{KR(N)}$) or mature candidalysin. Top: Mature candidalysin is distributed homogenously on the membrane (left) and induces small lesions as visible by the faint green fluorescence (middle). Bottom: Pre-mature candidalysin induces aggregates on the membrane. The heterogeneous fluorescence along the membrane shows small lipid aggregates (middle) in close proximity to larger peptide aggregates (left).

III) Candidalysin, secreted by *Candida albicans*, is the first cytolytic peptide toxin identified in a human pathogenic fungus, exerts strong membranolytic activity. Proteolytic processing by the fungal Golgi proteases Kex2p and Kex1p generates two peptide variants of candidalysin with either Lys or Lys Arg at the C-terminus. This step is essential for the autoregulation of candidalysin's biological activity. The C terminal structure and the membrane composition defines the heterogeneity of peptide aggregates formed in membranes at peptide-specific critical concentrations. Recently, we found a novel membrane-peptide mechanism that we call "peptide-aggregate induced lesion (PAIL) mechanism". PAIL is playing a key role as consecutive step to carpet like peptide binding acting as a trigger point for membrane permeabilization. We successfully showed that an irregular number of peptide molecules (>10) induces lesions and complete loss of membrane integrity. This makes candidalysin creating a new class of peptide toxins.

Internal and external collaboration

Norbert Reiling, Ulrich Schaible, Holger Heine, Uta Jappe, Dominik Schwudke, Andra Schromm, Nicolas Gisch, Niels Röckendorf, Torsten Goldmann, Susanne Homolka
Regina Scherließ, CAU Kiel
Karina Reiss, CAU Kiel
Christian Hübner, Univ. Lübeck
Walter Mier, Univ. Heidelberg
Bernhard Hube, UKI
Iris Bruchhaus, BNITM
Maria Mroginski, TU-Berlin
Julian Naglik, King´s College London
Ulrich Keyser, Uni. Cambridge
Anne S. Ulrich, KIT Karlsruhe
Albert Haas, Univ. Bonn

Grant support

Phospholipid Research Center; Project title: "Bottom-up designed synthetic bacteria – a tool to develop new antibiotic strategies"

ZIM Project of the BMWi; Project title: "Quantitativer Nachweis matrixassoziierter, antimikrobieller Peptide durch Massenspektrometrie ihrer Aminosäuren"

Cluster of Excellence *Inflammation at Interfaces* (I@I); Project title: "Anti-inflammatory regulation of immune cells by membrane active host defense peptides"

Leibniz Research Alliance *Infections'21*

Innovative Medicine Initiative (IMI) ENABLE

ZIM Project of the BMWi; Project title: "Calciumhaltige Implantatoberfläche mit antibakteriellen, antiinflammatorischen Wirkstoffdepots zur Vermeidung periprothetischer Infektionen"

DRUG-NANOCARRIER

MICROBIOTA

MACROPHAGE

MYCOBACTERIUM

HOST-DIRECTED THERAPY

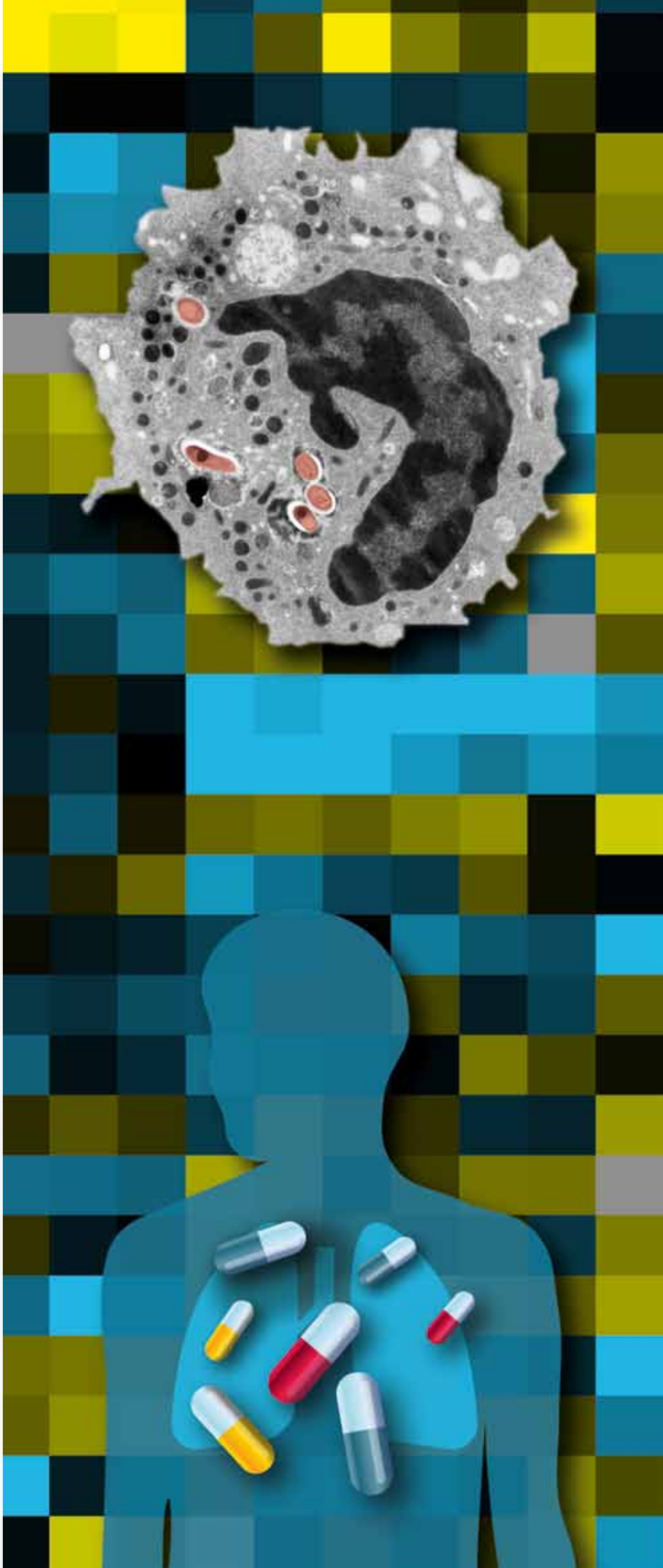
NEUTROPHIL

TUBERCULOSIS

PHAGOSOME

T CELL

LIPIDS



Priority Research Area **Infections**

Cellular Microbiology

Head

- Prof. Dr. Ulrich E. Schaible

Members

- Dr. Natalja Redinger
- Dr. Christian Alexander
- Dr. Tobias Dallenga
- Dr. Matthias Hauptmann
- Dr. Uwe Mamat
- Lara Linnemann
- Jessica Ojong
- Tijani Hawanot Olaitan
- Elisabeth Pfrommer
- Christoph Leschczyk
- Jacqueline Eich
- Dörte Grella
- Nina Grohmann
- Kristine Hagens
- Manuel Hein
- Dagmar Meyer
- Marion Schuldt
- Vivien Sparr

Guest scientists

- Prof. Dr. Avinash Sonawane, Humboldt Professor
- Sumanta Naik, DAAD PhD student

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Infections**

Cellular Microbiology

Mission

Die FG Zelluläre Mikrobiologie untersucht Wirts-Erreger-Interaktionen in der Tuberkulose. *Mycobacterium tuberculosis* ist ein fakultativ intrazellulärer Keim, der sich in Makrophagen vermehren kann. Wir fragen uns inwieweit die intrazelluläre Nische des Erregers Vermehrung und Übertragung, aber auch angeborene und erworbene Immunreaktionen, die Pathogenese und Effizienz anti-mikrobieller Therapien beeinflusst. Unser ganzheitlicher Blick schließt auch das Mikrobiom als wichtigen Immunregulator ein. Neu haben wir den aufkommenden und antibiotikaresistenten Opportunisten, *Stenotrophomonas maltophilia*, in unser Portfolio aufgenommen. Unsere Erkenntnisse nutzen wir um neue Prophylaxen und Therapien gegen bakterielle Lungeninfektionen zu entwickeln.

The Cellular Microbiology group studies host-pathogen interactions in tuberculosis. *Mycobacterium tuberculosis* is a facultative intracellular pathogen able to proliferate in macrophages. We ask how the intracellular niche of *M. tuberculosis* influences the pathogens survival, growth and transmission, as well as innate and acquired immunity, pathogenesis and anti-microbial drug efficacy. Our holistic view on the host also includes its microbiota as immune regulator. We recently added the emerging and drug resistant opportunist, *Stenotrophomonas maltophilia*, to our research portfolio. The knowledge gained provides the basis to develop prophylactic and therapeutic strategies against bacterial lung infections.

Most important findings

Tuberculosis (TB) caused by *Mycobacterium tuberculosis (Mtb)* is a leading cause of morbidity and mortality worldwide. Increasing multi and extensively drug-resistant cases prompted us to explore novel approaches to support antibiotic therapy: I) host-directed therapies, II) nano-containers for drug delivery and III) targeting the microbiota.

We found that neutrophils quickly succumb to necrotic cell death upon infection by *Mtb* through their own reactive oxygen intermediates (ROI). Subsequent uptake of infected necrotic neutrophils by macrophages promoted *Mtb* growth and necrosis in these cells. The *Mtb* protein ESAT-6 is singly responsible for this virulence trait. A host-directed therapy targeting the ROI generating neutrophil myeloperoxidase (MPO) reduced necrotic cell death and rendered macrophages able to control *Mtb* upon uptake of infected, but now, apoptotic neutrophils (Fig.1b, Dallenga et al., 2017). We also identified the macrophage receptor for apoptotic cells, MINCLE, to be involved in controlling inhibition of mycobacterial phagosome maturation by interaction with the cell wall lipid, trehalose dimycolate (Patin et al., 2017). Necrotic neutrophil and macrophage associated signatures identified by our studies are evaluated as biomarkers to monitor treatment success and disease state in TB patients to develop a quick point-of-care sputum test. For this project we cooperate with partners in *DZIF*

Highlights

Novel host-directed therapies in tuberculosis targeting neutrophils (Dallenga et al. 2017)

Innovative nano carriers to target antibiotics to mycobacteria (ANTI-TB (antitb.fz-borstel.de), 2017-2020)

Selected publications

Dallenga, T., Repnik, U., Corleis, B., Eich, J., Reimer, R., Griffiths, G. W. & Schaible, U. (2017) *M. tuberculosis*-induced necrosis of infected neutrophils promotes bacterial growth following phagocytosis by macrophages. *Cell Host & Microbe*. 22, 4, S. 519-530.

Steinmann,J., Mamat, U., Abda, E.M., Kirchhoff, L., Streit, W.R., Schaible,U.E., Niemann, S., and Kohl, T.A. (2018) Analysis of phylogenetic variation of *Stenotrophomonas maltophilia* reveals human-specific branches. *Front. Microbiol*. 9: 806.

Patin, E. C., Geffken, A. C., Willcocks, S., Leschczyk, C., Haas, A., Nimmerjahn, F., Lang, R., Ward, T. H. & Schaible, U. E. (2017) Trehalose dimycolate interferes with FcγR-mediated phagosome maturation through Mincle, SHP-1 and FcγRIIB signaling. PLOS ONE. 12(4):e0174973

Patin EC, Orr SJ, Schaible UE. (2017) Macrophage inducible C-type lectin as a Multifunctional player in immunity. Front Immunol. 8:861

Rein, V., Meschkov, A., Hagens, K., Redinger, N., Schepers, U., Mehlhorn, H., Schaible, U.E. & Feldmann, C. (2019) Zirconyl hydrogenphosphate nanocontainers for flexible transport and release of lipophilic cytostatics, insecticides and antibiotics. Ad. Funct. Mat., in press

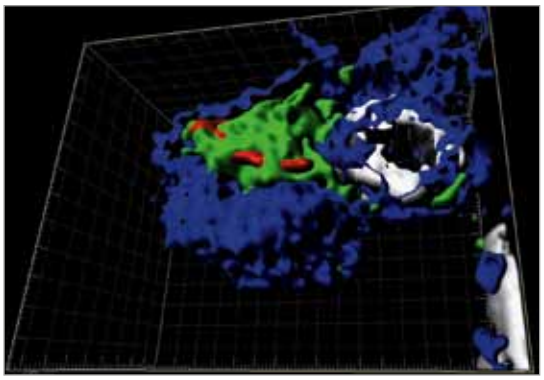


Figure 1a. 3D reconstruction based on stacks of confocal microscopy pictures of a human macrophage (nucleus in blue), which had engulfed a necrotic neutrophil (green) infected with *M. tuberculosis* (in red). The bacterium is still partially enwrapped by the neutrophil remnants. (Dallenga et al. 2017)

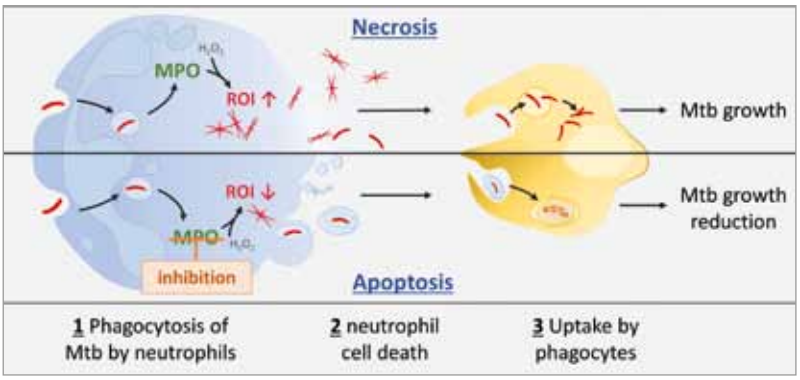


Figure 1b. Schematic drawing (courtesy of Lara Linnemann) of how a host-directed therapy targeting neutrophil myeloperoxidase can deviate necrotic into apoptotic neutrophil cell death and subsequently facilitate the macrophage to eliminate the mycobacteria.

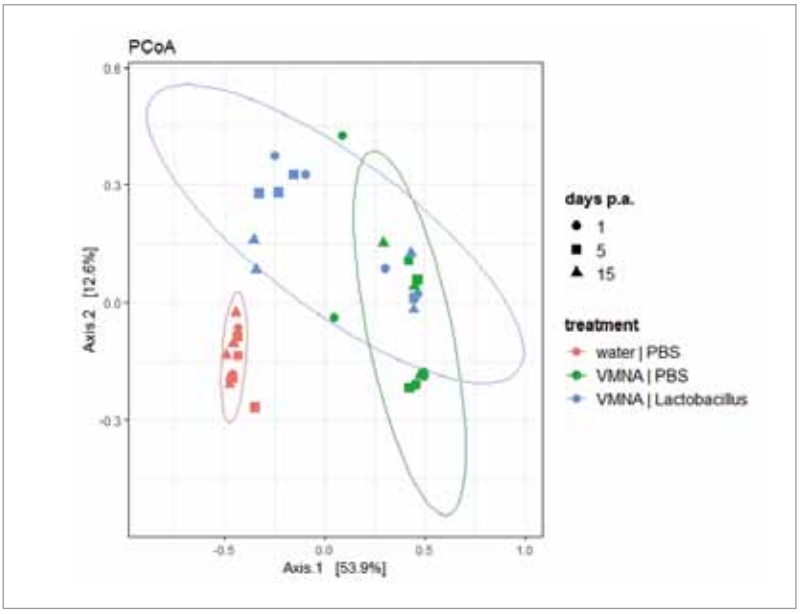


Figure 2a. Lactobacilli can readjust the microbiota after antibiotic therapy. Mice were treated **for 12 days with VMNA antibiotics via drinking water or left untreated. 6 days later mice were** given 10^5 Lactobacilli or PBS as control. Mice were sacrificed at indicated time points after application of Lactobacilli (p.a.) and caecum microbiota were characterized by 16S rRNA sequencing. PCoA plot shows Bray-Curtis dissimilarities between groups of mice. (Hauptmann et al. in prep.)

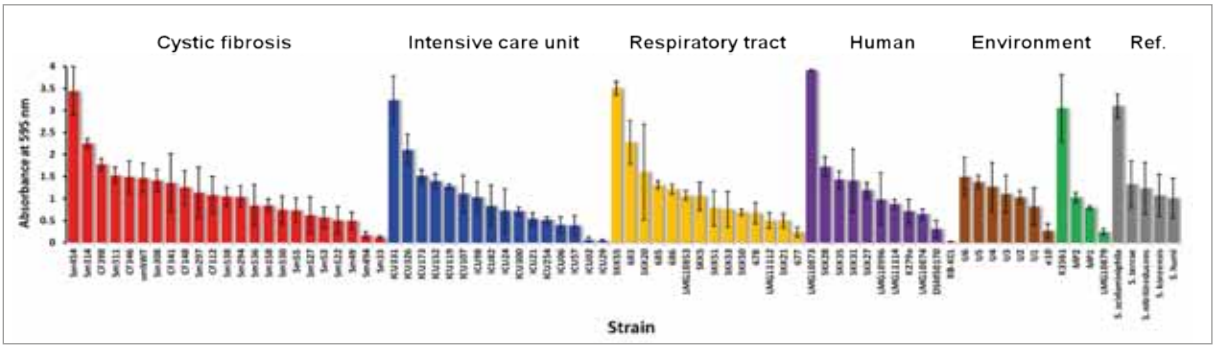


Figure 2b. Biofilm formation by clinical and environmental *S. maltophilia* isolates. The ability of the strains to form biofilms on polystyrene was investigated by the microtiter plate assay using crystal-violet for biofilm staining, followed by absorbance readings at 595 nm. For comparison, reference strains of the genus Stenotrophomonas were included. (Steinmann et al. 2018)

Priority Research Area **Infections**

Cellular Microbiology

TTU-TB and *TB-Sequel* including sites in Bucharest, The Gambia, Mozambique and Tanzania.

Current anti-TB treatment is effective, but takes a long time and is associated with severe adverse effects, which limits compliance to therapy and results in increasing numbers of cases with drug-resistant *Mtb*. To enhance local drug concentrations at site of infection but lower ones in the periphery, we explore different nanocarriers for targeted drug delivery together with partners in NAREB and ANTI-TB. Biodistribution and efficacy analyses in a mouse model highly susceptible to *Mtb* which is and develops necrotic granulomas, revealed that Lipidots, HSA and Chitosan nanocarriers were selectively transported to the lung of infected mice and showed equal or even better efficacy than the free drugs (Patel et al., in revision; Rein et al., 2019). Successful nanocarriers will be formulated for pulmonary delivery by aerosols or dry powder inhalation.

Increasing awareness has recently been given to host microbiota as influencer of lung diseases, and therapies targeting microbiota have entered the clinic. Mechanisms, how microbiota accomplish beneficial effects are yet unclear. We have identified three bacterial strains, which can ameliorate dysbiosis following antibiotic treatment by diversifying the host caecum microbiota (Fig. 2a; Hauptmann *et al.*, in prep.).

The Gram-negative *Stenotrophomonas maltophilia (Sm)*, is found in the environment but has increasingly been recognized as nosocomial pathogen causing bacteremia and pneumonia in immunocompromised patients with a high rate of mortality such as cystic fibrosis patients. Identification in healthy donors indicated *Sm* to be considered as pulmonary commensal but putative opportunistic pathogen. Its virulence traits, including secreted proteases, lipases, hemolysin contribute to inflammation. *Sm* can also form biofilms leading to transient phenotypic antimicrobial resistance and persistence. As part of whole-genome sequencing based phylogenetic characterization of *Sm*, the ability to form biofilms of a collection of human and environmental *Sm* isolates was assessed (Fig.2b; Steinmann et al., 2017). The ability to form biofilms differed between individual strains, but was not restricted to human isolates suggesting that formation of biofilms is irrelevant for human colonization.

Internal and external collaboration

Internal collaborations

Thomas Gutsmann, Holger Heine, Christoph Hölscher, Barbara Kalsdorf, Susanne Krauss-Etschmann, Thomas Kohl, Christoph Lange, Florian Maurer, Stefan Niemann, Norbert Reiling, Dominik Schwudke

External collaborations

Anti-TB drug-Nanocarrier: Lea Ann Dailey, Halle; Claus Feldmann, KIT; Robert Gieseler, RBT, Hannover; Claus-Michael Lehr, HZI-HIPS, Saarbrücken; Katharina Schwarz, ITEM, Hannover; NAREB Consortium
Iron: Esther Meyron-Holtz, Haifa Univ.
Microbiota & S. maltophilia: John Baines, MPI Plön; Wolfgang Streit, Hamburg; Jörg Steinmann, Klinikum Nürnberg, Germany; Daniel Vero, Barcelona, Spain
Phagocyte Biology and Immunity: Alexander Apt, Central Institute for Tuberculosis, Moscow, Russia; Moran Benhar, Haifa Univ.; Gareth Griffiths, Oslo Univ.; Albert Haas, Univ. Bonn; Tamas Laskay, UzL; Christina Stallings, Washington University, St. Louis, USA; Vladimir Yeremeev, Central Institute for Tuberculosis, Moscow, Russia
Infectious Aerosols: Gülsah Gabriel, HPI; Kerstin Schepanski, TROPOS

Grant support

BMBF: German Center of Infection (DZIF), TTU-TB (WP4 Neutrophil Signatures); BMBF Africa, TB-Sequel; ANTI-TB, Antibiotics nanocarrier for therapeutic inhalation against tuberculosis

DFG: SPP 1580 Intracellular compartments as places of pathogen-host-interactions; EXC Inflammation@ Interfaces (Lysosomal disorders and bacteria-induced inflammation; Cluster Lab 9); IRTG 1911 Immuno-regulation of Inflammation in Allergy and Infection; Germany - Russia ("Correlates of exacerbated and ameliorated pathogenesis")

Leibniz: Leibniz-Forschungsverbund INFECTIONS'21; Leibniz-Wissenschafts Campus EVOLung

EU FP7: NAREB, Nanotherapeutics to treat bacterial infectious diseases

Industry: Clinic La Prairie

IMMUNOMETABOLISM
COLLECTIN-MEDIATED SIGNALTRANSDUCTION

SURFACTANT PROTEIN A

ALVEOLAR MACROPHAGES

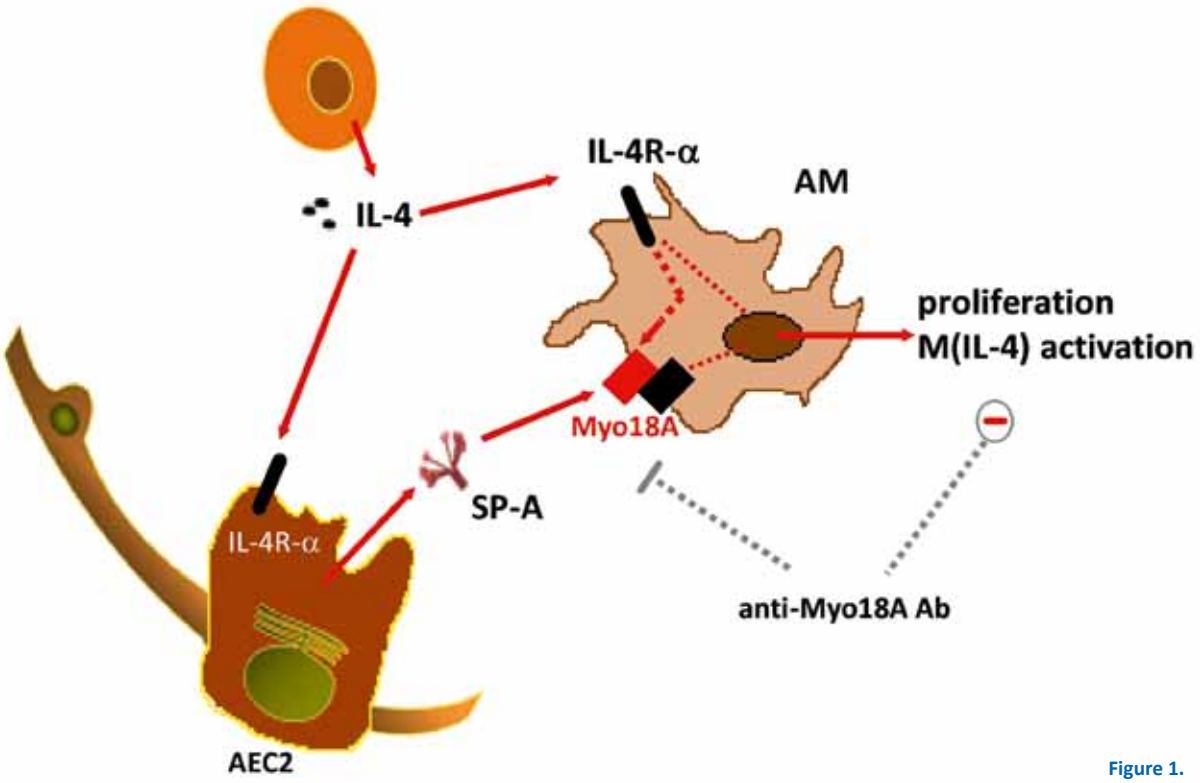


Figure 1.

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- Katja Freundt
- PD Dr. med. Patrick Kellner
- Kristina Gloystein
- Robin Orlikowsky-Rein

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Infections**

Cellular Pneumology

Mission

Pulmonaler Surfactant, ein intraalveolärer Lipoproteinkomplex, verbindet zwei vitale Lungenfunktionen: Atmung und Immunabwehr. Die Collectine Surfactant Protein (SP)-A und SP-D regulieren die pulmonale Immunantwort auf eine Infektion unter anderem über eine spezifische Modulation der funktionellen Plastizität von Alveolarmakrophagen und Lungenepithelzellen. Unsere Studien auf zellulärer, molekularer und tierexperimenteller Ebene untersuchen Mechanismen der Collectin-vermittelten Immunprägung der Infektion mit Fokus auf Zell/Collectin Interaktion und konsekutiver Zellaktivierung/-deaktivierung, um Targets für prophylaktische oder therapeutische Interventionsmöglichkeiten zu identifizieren.

Lung function requires the production and homeostasis of pulmonary surfactant, a unique lipoprotein complex that combines two vital functions: breathing and immune defense. The soluble collectins surfactant protein (SP)-A and SP-D regulate lung immune responses in part by specifically modulating the functional plasticity of alveolar macrophages and alveolar epithelial cells. Our studies at the cellular, molecular and organismal level investigate mechanisms of immune imprinting by SP-A and SP-D to pulmonary infection with the focus on lung cell-specific/lectin interactions and downstream effects on cell activation/deactivation to target for development of therapeutic interventions.

Most important findings

Whereas the role of lung collectins SP-A and SP-D in innate immunity is well established, their function in adaptive immune responses to infection has been unknown. In a collaborative project with the Universities of Madrid and Edinburgh we have demonstrated for the first time that SP-A and its structural homologue C1q are organ specific enhancer of IL-4-dependent type 2 effector functions and thereby limit infection in lung and liver, respectively, and reinforce tissue repair. The underlying mechanisms are schematically depicted in Fig. 1. In the lung, rather than IL-4 signaling to AM alone, IL-4 induces a second signal for full IL-4 activation and proliferation. IL-4 enhances the production of SP-A by alveolar epithelial type II cells and the expression of its receptor, Myo18, on AM. The identification of this tissue specific, IL-4-amplifying loop via endogenous proteins is subject of ongoing work on PI3K/Akt/mTORC-dependent signaltransduction pathways and the metabolic requirements involved (DFG-funded Clinician Scientist School Lübeck and Coop. Microbial Interface Biology and Bioanalytical Chemistry). The activation status of macrophages is inherently linked to metabolic remodeling. Surfactant-directed functional metabolic pathways, e.g., real time changes of oxygen consumption rates (OCR, fatty acid oxidation) and glycolysis in primary AM are not yet identified. Within preliminary work applying Extracellular Flux Analysis, we show that OCR is

Highlights

SP-A and its structural homologue C1q are organ specific enhancers of IL-4-dependent type 2 effector functions and thereby limit infection in lung and liver and reinforce tissue repair

Volatile anesthetics improve oxygenation and attenuate the inflammatory response in a long-term sedation model of lung injury in rats.

Selected publications

Minutti CM, Jackson-Jones LH, Garcia-Fojeda B, Knipper JA, Sutherland TE, Logan N, Rinqvist E, Guillamat-Prats R, Ferenbach DA, Artigas A, Stamme C, Chroneos ZC, Zaiss DM, Casals C, Allen JE. Local amplifiers of IL-4Rα-mediated macrophage activation promote repair in lung and liver. Science 2017; June 9;356 (6342):1076-1080. doi:10.1126/science.aaj2067.

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Stelzner M, Lau FL, Freundt K, Büther F, Linh Nguyen M, Stamme C, Ebers S. Precise detection and treatment of human diseases based on nano networking. Proceedings 11th International Conference on Body Area Networks, 2017, pp 58-64, doi: 10.4108/eai.15-12-2016.2267585.

Schmidt C, Heringlake M, Kellner P, Berggreen AE, Maurer H, Brandt S, Bucsky B, Petersen M, Charitos EI. The effects of systemic oxygenation on cerebral oxygen saturation and its relationship to mixed venous oxygen saturation: A prospective observational study comparison of the INVOS and ForeSight Elite cerebral oximeters. Can J Anaesth 2018 Jul;65(7):766-775. doi 10.1007/s12630-018-1093-3.

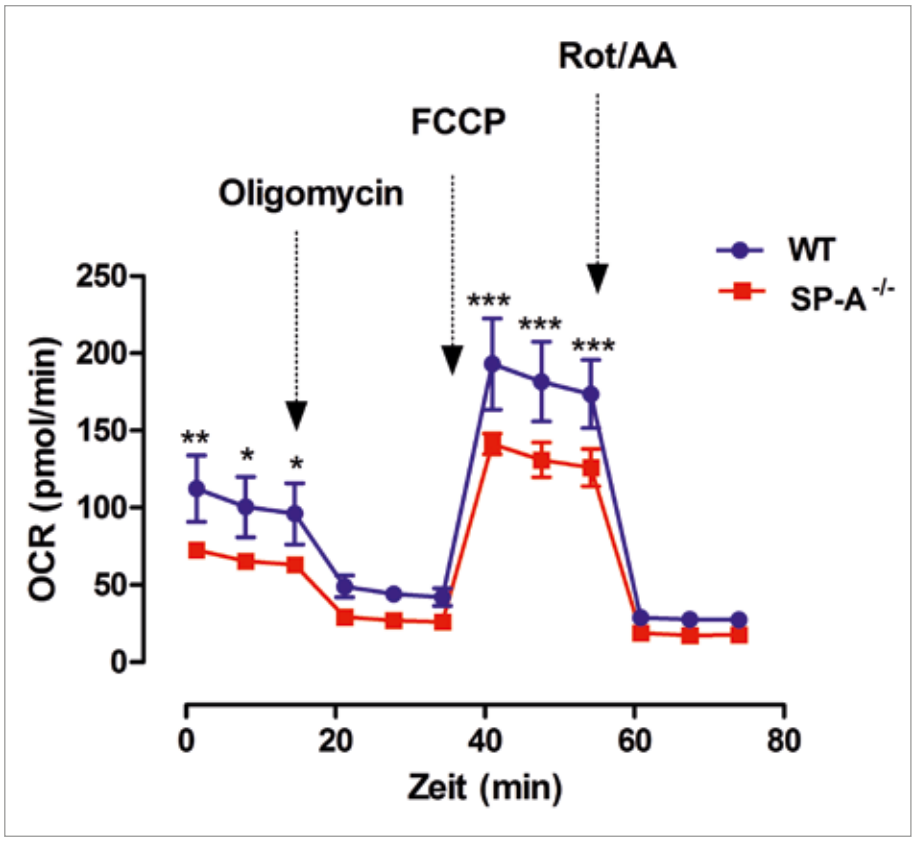

Figure 2. Primary AM from WT and SP-A$^{-/-}$ mice were sequentially treated with oligomycin (inhibits the mitochondrial ATP synthase), FCCP (uncouples ATP synthesis from oxygen consumption), and rotenone plus antimycin (inhibit the electron transport chain). Mitochondrial respiration was determined by OCR.

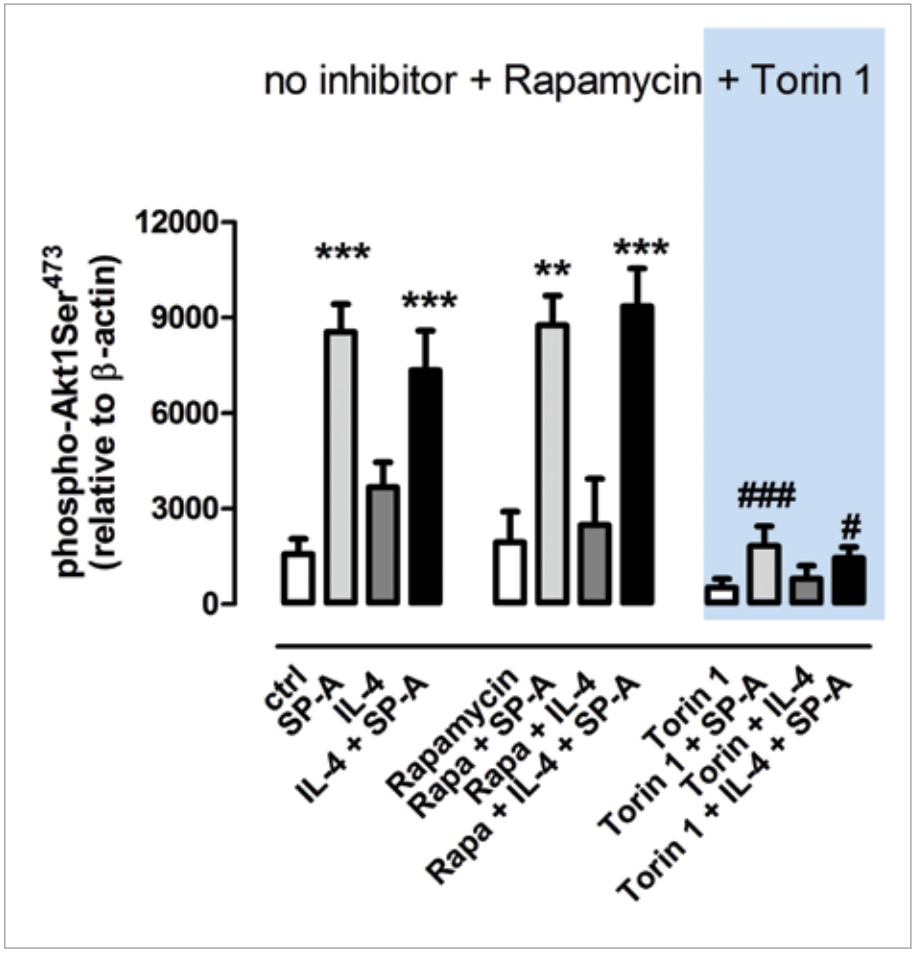

Figure 3. Primary rat AM were left untreated or were treated with SP-A, IL-4, or both in the absence or presence of rapamycin or torin 1. Whole cell lysates were subjected to SDS-PAGE and immunoblotted for phospho-Akt1Ser473 and β-actin. Data of six to eight independent experiments were normalized to β-actin and analyzed by one-way ANOVA with Bonferroni's post-test (mean ± SEM). ** p< 0.01, *** p< 0.001 versus control in the corresponding treatment group. # p < 0.05, ### p < 0.001 versus in the absence of inhibitors.

Priority Research Area **Infections**

Cellular Pneumology

significantly higher in AM from wild-type *versus* SP-A$^{-/-}$ mice (Fig. 2) reflecting a lower mitochondrial activity in AM in the absence of endogenous SP-A.

Central to both the metabolic control and polarization of eukaryotic cells is the mechanistic target of rapamycin (mTOR), that occurs in mTORC1 and mTORC2 complexes, the former regulating cell growth and metabolism, the latter controlling cell proliferation and survival act in a tissue-specific manner. The signals that provide lung specific control of mTORC activity in AM under basal and infectious conditions are not yet identified. The serine/threonine kinase Akt has a central role in the mTORC circuit. We have previously shown that SP-A induces a rapid and transient PI3K-dependent Akt^{Ser473} phosphorylation in primary rat and mouse AM that is functionally required for protective host defense functions of SP-A. *In vitro*, both functions of SP-A, enhancement of M(IL-4) and proliferation of AM, strictly depend on the activation of the PI3K/Akt pathway (coop. Casals et al., manuscript in prep). Further we found that SP-A-enhanced pAkt^{Ser473} is inhibitable by torin 1 (inhibits both mTORC1 and mTORC2), but not rapamycin (inhibits mTORC1) (Fig. 3), providing first evidence for the involvement of mTORC2 in SP-A-enhanced phosphorylation of Akt^{Ser473}. *In vivo* studies, however, on SP-A-mediated changes in AM metabolism to promote M(IL-4) and proliferation are pending.

A direct translational focus of our work investigates the immunomodulatory effects of volatile anesthetics in bacterial lung infections requiring mechanical ventilation (Dept. of Anesthesiology, UKSH, Campus Lübeck). We have shown that volatile anesthetics improve oxygenation and attenuate the inflammatory response in a long-term sedation model of LPS-induced acute lung injury in rats. Current *in vitro* studies reveal that the long-term application of volatile anesthetics can inhibit the growth of clinically relevant pulmonary pathogens and stabilize surfactant-associated proteins.

Internal and external collaboration

Asthma Exacerbation & Regulation (M. Wegmann, L. Lunding), Microbial Interface Biology (N. Reiling, J. Brandenburg), Cellular Microbiology (U. Schaible, M. Hauptmann), Biobank (K.I. Gaede), Clinical Study Center (C. Herzmann), Bioanalytical Chemistry (D. Schwudke), Experimental Pneumology (H. Fehrenbach, Z. Orinska)

C. Casals, University Madrid
Minutti, University Edinburgh
Garcia-Fojeda, University Madrid
M. Damme, CAU Kiel
S. Karnati, University Würzburg

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Promotionsstipendium Lübecker Exzellenzmedizin, University Lübeck

Clinician Scientist School Lübeck

Priority Research Area **Infections**

Clinical Infectious Diseases

BIOMARKERS
CPA INTERNATIONAL HOSPITAL PARTNERSHIPS
PERSONALIZED MEDICINE
CLINTB
MDR-TB
DZIF
NTM-PD
GLOBAL HEALTH
MEMENTO AWARD
CAPACITY BUILDING
NEW DRUGS
XDR-TB
TB NET
TUBERCULOSIS
TB INFO

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- Dr. Janne Kandulla
- Niklas Köhler
- Carolin Oertel
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- Dr. Patricia Sanchez Carballo
- Dagmar Schaub

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Infections**

Clinical Infectious Diseases

Mission

Wir möchten die Prävention, Diagnostik und Therapie von pulmonalen Infektionskrankheiten (Tuberkulose, Erkankungen durch nicht-tuberkulöse Mykobakterien und der chronischen pulmonalen Aspergillose) substantiell verbessern und wissenschaftliche Fortschritte in die klinische Praxis übertragen.

We wish to contribute to a substantial improvement in the prevention, diagnosis and treatment of pulmonary infectious diseases (tuberculosis, non-tuberculous mycobacteria pulmonary diseases, chronic pulmonary aspergillosis) and to translate scientific advances into clinical practice.

Most important findings

Personalized treatment improves outcome in MDR-TB

We performed a prospective cohort study, analyzing management and treatment outcomes stratified by incidence of patients with MDR-TB in Europe. 380 patients with MDR-TB were recruited and followed-up between 2010 and 2014 in 16 European countries. Patients in high-incidence countries compared with low-incidence countries were treated more frequently with standardized regimen (83.2% vs. 9.9%), had delayed treatment initiation (median 111 vs. 28 days), developed more additional drug resistance (23% vs. 5.8%), and had increased mortality (9.4% vs. 1.9%). Only 20.1% of patients using pyrazinamide had proven susceptibility to the drug. Applying WHO outcome definitions, frequency of cure (38.7% vs. 9.7%) was higher in high-incidence countries. Simplified outcome definitions that include one year of follow-up after the end of treatment showed similar frequency of relapse-free cure in low- (58.3%), intermediate- (55.8%) and high- incidence (57.1%) countries, but highest frequency of failure in high-incidence countries (24.1% vs. 14.6). In conclusion, conventional standard MDR-TB treatment regimens resulted in a higher frequency of failure compared to individualized treatments. Overall, cure from MDR-TB is substantially more frequent than previously anticipated, and poorly reflected by WHO outcome definitions. PMID: 29509468

Highlights

1. When modern molecular and immunological methods are combined, more than 98% of patients with pulmonary TB can be correctly identified within a few days after presentation.

2. In MDR-TB standardized treatment regimens lead to higher failure rates than individualized treatment regimens.

3. High cure rates from MDR-TB can be achieved on personalized therapies, indistinguishable from those in pan drug-susceptible TB.

Selected publications

Günther G, et al. Clinical management of multidrug-resistant tuberculosis in 16 European countries. Am J Respir Crit Care Med. 2018 Mar 6. doi: 10.1164/rccm.201710-2141OC. PMID: 29509468

Heyckendorf J, et al. Relapse-free cure from multi-drug-resistant tuberculosis in Germany. Eur Respir J. 2018 Feb 21;51(2). pii: 1702122. doi: 10.1183/13993003.02122-2017. PMID: 29467205

Jafari C, et al. Rapid diagnosis of pulmonary tuberculosis by combined molecular and immunological methods. Eur Respir J. 2018 Mar 29. pii: 1702189. doi: 10.1183/13993003.02189-2017. PubMed PMID: 29599184.

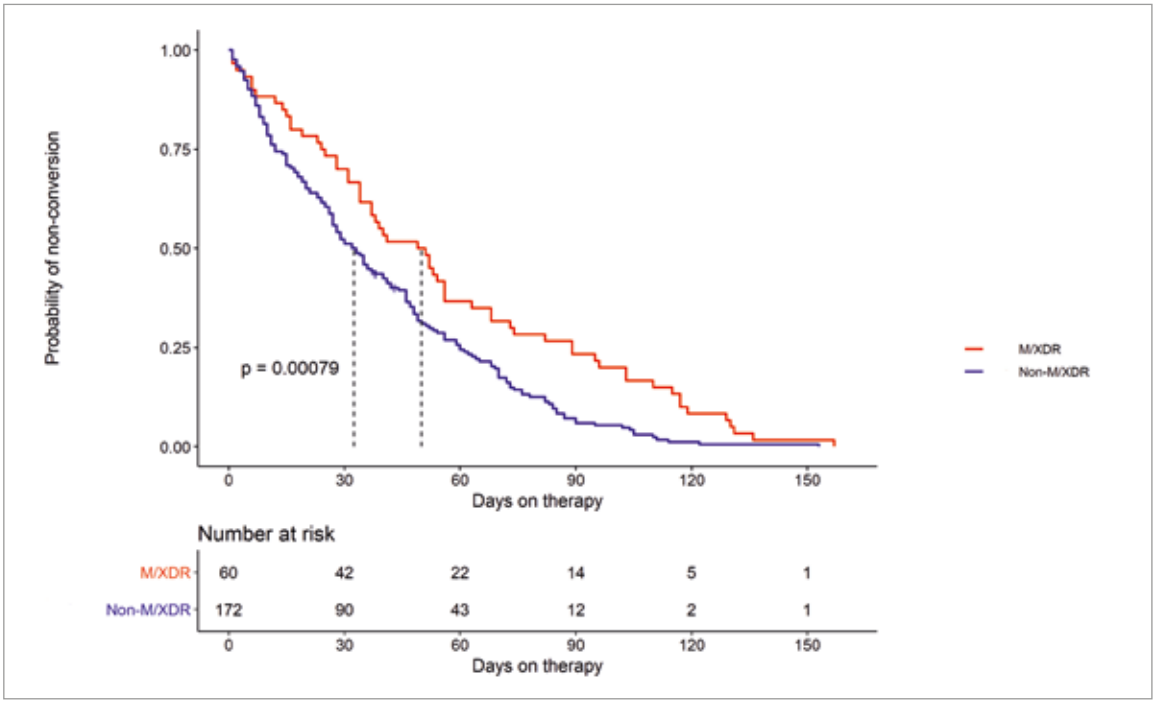

Figure 1. High rates of successful treatment responses by personalized patient management at the Medical Clinic of the Research Center Borstel. M/XDR-TB = multi/extensively-drug resistant tuberculosis. Non-M/XDR-TB = pan-drug-susceptible, mono-drug-resistant or poly-drug-resistant TB.

Finding optimal treatment regimens for patients with MDR-TB

We aimed to estimate the association of treatment success and death with the use of individual drugs, and the optimal number and duration of treatment with those drugs in patients with MDR-TB and searched MEDLINE, Embase, and the Cochrane Library to identify potentially eligible observational and experimental studies published between Jan 1, 2009, and April 30, 2016. Using propensity score-matched generalised mixed effects logistic, or linear regression, we calculated adjusted odds ratios and adjusted risk differences for success or death during treatment, for specific drugs currently used to treat MDR-TB, as well as the number of drugs used and treatment duration. Of 12 030 patients from 25 countries in 50 studies, 7346 (61%) had treatment success, 1017 (8%) had failure or relapse, and 1729 (14%) died. Compared with failure or relapse, treatment success was positively associated with the use of linezolid (adjusted risk difference 0•15, 95% CI 0•11 to 0•18), levofloxacin (0•15, 0•13 to 0•18), carbapenems (0•14, 0•06 to 0•21), moxifloxacin (0•11, 0•08 to 0•14), bedaquiline (0•10, 0•05 to 0•14), and clofazimine (0•06, 0•01 to 0•10). There was a significant association between reduced mortality and use of linezolid (–0•20, –0•23 to –0•16), levofloxacin (–0•06, –0•09 to –0•04), moxifloxacin (–0•07, –0•10 to –0•04), or bedaquiline (–0•14, –0•19 to –0•10). Compared with regimens without any injectable drug, amikacin provided modest benefits,

Clinical Infectious Diseases

Figure 2. Tuberculosis is the leading cause of morbidity attributed to a single microorganism worldwide

but kanamycin and capreomycin were associated with worse outcomes. The remaining drugs were associated with slight or no improvements in outcomes. Treatment outcomes were significantly worse for most drugs if they were used despite in-vitro resistance. The optimal number of effective drugs seemed to be five in the initial phase, and four in the continuation phase. Treatment outcomes were significantly better with use of linezolid, later generation fluoroquinolones, bedaquiline, clofazimine, and carbapenems for treatment of MDR-TB. PMID: 30215381

The Collaborative Group for the analysis of individual patient data in MDR-TB treatment.

Treatment correlates of successful outcomes in pulmonary multidrug-resistant tuberculosis– an individual patient data meta-analysis of 12,030 patients from 25 countries. The Lancet. 2018 Sep 8;392(10150):821-834. doi: 10.1016/S0140-6736(18)31644-1. PMID: 30215381

Forschungszentrum Borstel
Leibniz Lungenzentrum

Internal and external collaboration

Local: Prof. Dr. M. Addo, Dr. S. Andres, Prof. Dr. T. Goldmann, Dr. D. Hillemann, Prof. Dr. A. Katalinic, Prof. Dr. K-F. Klotz, PD Dr. F. Maurer, Prof. Dr. M. Merker, Prof. Dr. S. Niemann, Prof. Dr. J Rupp/ Center for Infectious Diseases Borstel-Lübeck (DGI), Dr. S. Schmiedel, Dr. D. Schwudtke, Prof. Dr. U. Schaible,. Excellence Cluster Precision Medicine.

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DZIF M/XDR-TB network, TBNET.

International: Dr. S. Alexandru (Chisinau, Moldova), Prof. Dr. C. Barry (Bethesda, USA/Cape Town, South Africa), Dr. V. Crudu (Chisinau, Moldova), Prof. Dr. K. Dheda, (Cape Town, South Africa), Prof. Dr. A. Diacon (Stellenbosch, South Africa), Dr. Gunar Günther (Windhoek, Namibia), Prof. Dr. R. Horsburgh (Boston, USA), Dr. E. Ibrahim (Bucharest, Romania), Dr. F. v. Leth (Amsterdam, Netherlands), Dr. A. Mandalakas (Houston, USA), Prof. Dr. D. Menzies (Montreal, Canada), E. Noroc (Chisinau, Moldova), Dr. M. Olegovna (Montreal, Canada), Prof. Dr. E. Schurr (Montreal, Canada), Dr. A. Skrahina (Minsk, Belarus).

EU-H2020 anTBiotic, EU-H2020 CARE, EDCTP ClicTB, Collaborative Group for Meta-Analysis of Individual Patient Data in MDR-TB; Medicines sans frontiers (MSF), Tuberculosis Network European Trialsgroup (TBNET), RESIST-TB.

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Grant support

AID, BMBF, Cluster of Excellence: Inflammation at Interfaces (Precision Medicine), DAAD, DZIF, EDCTP, EU-H2020, S.-H. TB Society, TBNET

COINFECTION

MOUSE MODELS

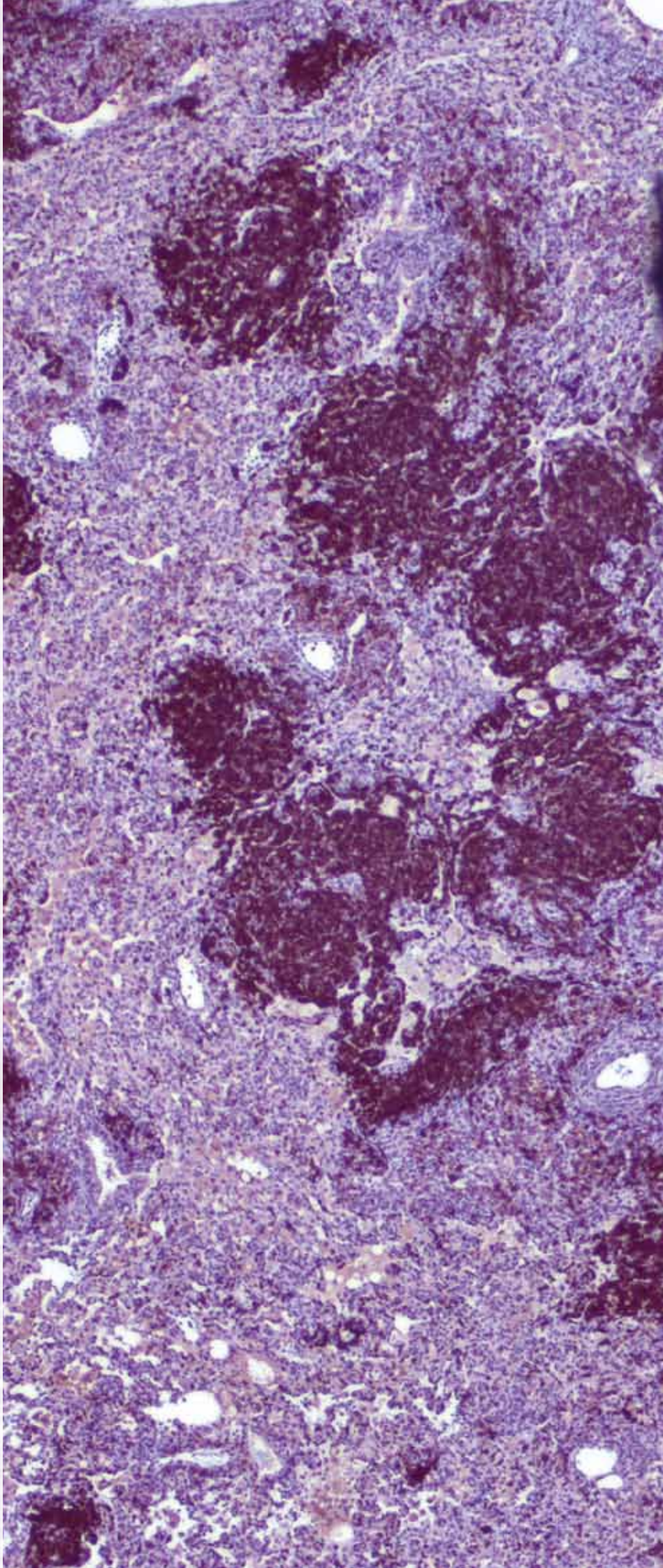
TUBERCULOSIS

HETEROLOGOUS EFFECTS OF BCG

SEX DIFFERENCES

INFLUENZA

MALARIA



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- David Hertz
- Lone Hoop
- Sarah Ring
- Julia Witschkowski

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- Linda von Borstel

Priority Research Area **Infections**
Coinfection

Priority Research Area **Infections**

Coinfection

Mission

Die Nachwuchsgruppe Koinfektion untersucht mit Hilfe experimenteller Mausmodelle, wie intrinsische und extrinsische Faktoren das empfindliche immunologische Gleichgewicht beeinflussen, das eine langfristige Kontrolle einer Infektion mit *Mycobacterium tuberculosis* ermöglicht. Dabei liegt unser Fokus auf der Erforschung von Koinfektionen und dem biologischen Geschlecht.

The overall goal of our research is to understand the mechanisms driving the immunological control or control failure of *Mycobacterium tuberculosis (Mtb)* infection. Tuberculosis (Tb) disease cannot be seen as a single entity but is influenced by multiple intrinsic and extrinsic factors that affect the host immune system and host-pathogen interplay. We use experimental mouse model to address the role of coinfections and the biological sex in these processes.

Most important findings

Influenza impairs host control of *Mtb* via the induction of IL-10

Epidemiological findings indicate that coinfection with influenza viruses is associated with an increased risk of death in patients suffering from Tb but the underlying pathomechanisms are not well understood. We sought to elucidate the consequences of influenza on *Mtb* infection and found that influenza A virus (IAV) coinfection rapidly impairs control of *Mtb* in C57BL/6 mice. Viral coinfection was associated with significantly increased bacterial loads (Fig. 1A), reduced survival (Fig. 1B) and a substantial modulation of innate and adaptive immune defenses (Ring et al. 2019). Our findings strongly indicate that IAV coinfection compromises the host's ability to control *Mtb* infection via the production of IL-10 which was rapidly induced upon viral infection (Fig. 1C). High levels of IL-10 were accompanied by an impaired onset and development of *Mtb*-specific CD4+ T cell responses and the accumulation of macrophages with increased arginase-1 production in the lungs. The blockade of IL-10 receptor signaling reduced the bacterial load in coinfected mice to a level comparable with that in *Mtb*-only-infected animals (Fig. 1D). *Mtb*-specific T cell responses were not restored by anti-IL-10 receptor treatment. Therefore, IL-10 most likely impaired control of *Mtb* infection by changing macrophage polarization. While our study yet again supports a detrimental role of IL-10 during *Mtb* infection, we show for the first time that it is a major driver of influenza-mediated disease exacerbation.

Highlights

Influenza impairs host control of *Mtb* via the induction of IL-10

Increased male susceptibility to *Mtb* infection is associated with a marked decrease in lymphoid follicles in male lungs

BCG vaccination partly protects mice from experimental cerebral malaria

Selected publications

Dibbern J, Eggers L, Schneider BE (2017) Sex differences in the C57BL/6 model of Mycobacterium tuberculosis infection. Sci Rep. Sep 8;7(1):10957 DOI: 10.1038/s41598-017-11438-z

Hertz D & Schneider B. Sex differences in tuberculosis. Semin Immunopathol (2018). https://doi.org/10.1007/s00281-018-0725-6 [Epub ahead of print]

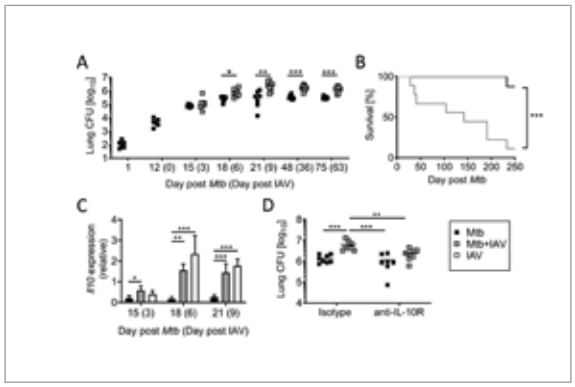

Figure 1. Influenza impairs host control of *Mtb* via the induction of IL-10.
A) Lung CFU, B) survival, and C) *Il10* expression in lungs of mice infected with *Mtb* alone or *Mtb* and IAV. D) A single dose of anti-IL-10 receptor antibody reduced the bacterial load in coinfected mice to a level comparable with that in *Mtb*-only-infected animals.

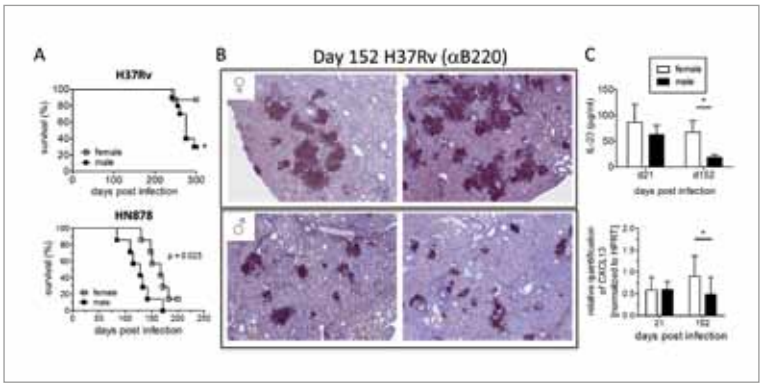

Figure 2. Sex-dependent differences in the quality of the granulomatous lesions.
A) Survival of C57BL/6 mice after low-dose aerosol-infection with *Mtb* H37Rv or HN878. B) B220 stain of lung sections 152 days after *Mtb* H37Rv infection. C) IL-23 protein and CXCL-13 mRNA levels in lungs of *Mtb* H37Rv infected mice.

Sex differences in Tb

Globally, Tb notification data show a male-to-female ratio of 2:1. Both gender- and sex-related factors likely contribute to higher Tb rates in men, but the role of the latter has been largely ignored in scientific investigations. Studies in our lab revealed an increased male susceptibility towards *Mtb* H37Rv and HN878, a clinical isolate of the Beijing lineage, in C57BL/6 mice (Fig. 2A). Premature death of males was associated with striking differences in the quality of the granulomatous lesions compared to females. B220+ B cell follicles, which are correlates of protection in Tb because their formation is a consequence of proper T cell localization within the lung tissue, are significantly smaller in males (Fig. 2B). In good agreement, the expression of factors involved in follicle formation such as CXCL-13 and IL-23 are reduced in males (Fig. 2C). We hypothesize that impaired formation and/or maintenance of lymphoid follicles are responsible for accelerated disease progression in males. However, the molecular underpinnings to date remain elusive. In the future, we aim to explore the role of (epi)-genetic and hormonal influences on T cell subsets in males and females to decipher the molecular pathways governing sexual dimorphism in Tb. Identifying factors associated with sex-differences in Tb-induced T cell responses and granuloma formation will be particularly valuable for future Tb vaccine design and may facilitate the development of individualized vaccination approaches, which will be critical to adequately protect both sexes against *Mtb* infection.

Coinfection

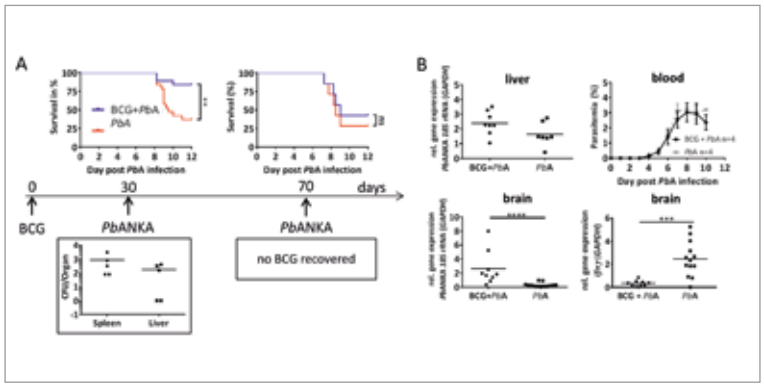

Figure 3. BCG confers partial and time-dependent protection from ECM.
A) C57BL/6 mice were infected with *Plasmodium berghei* ANKA (*Pb*ANKA) 30 or 70 days after BCG vaccination. Survival of mice and recovered BCG from spleen and liver are shown. B) BCG-mediated protection was not associated with a reduction in parasitic load but in IFN-γ expression in the brain (*Pb*ANKA infection 30 days after BCG vacc).

Non-specific effects of BCG vaccination towards unrelated pathogens

Evidence suggests that BCG immunization has a number of additional beneficial non-specific effects including a reduction in overall child mortality attributable to causes other than Tb. It has been proposed that BCG mediates its non-specific effects by the induction of long-term changes in the innate immune system called trained immunity. In mice, we found that BCG vaccination confers partial protection against *Plasmodium*-induced experimental cerebral malaria (ECM; Fig. 3A). However, protection waned over time and was associated with the recovery of viable BCG from liver and spleen (Fig. 3A). Surprisingly, BCG did not reduce parasitic load in blood, liver or brain but the expression of pro-inflammatory mediators associated with the development of ECM (Fig. 3B). In conclusion, our data suggest immunomodulatory effects by viable BCG rather than trained immunity to be responsible for the partial protection from ECM.

Internal and external collaboration

Internal collaborations:
Jochen Behrends, Dominik Schwudke, Adam Wutkowski, Christoph Hölscher, Susanne Homolka, Stefan Niemann

External collaborations:
Ann-Kristin Müller, Roland Frank (University Hospital Heidelberg); Thomas Jacobs (BNITM Hamburg); Gülsah Gabriel (HPI Hamburg); Andrea Kröger (University Magdeburg), Hanna Lotter (BNITM)

Grant support

DFG: SCHN 1150/4-1 "Studying the protective efficacy of radiation-attenuated Plasmodium sporozoites as anti-infective malaria vaccine during concurrent infection with Mycobacterium tuberculosis"

Leibniz Graduate School Model Systems for Infectious Diseases: "Impact of an influenza virus infection on the outcome of experimental tuberculosis"

Priority Research Area **Infections**

Diagnostic Mycobacteriology (NRC)

WHO SUPRANATIONAL REFERENCE LABORATORY

INTERNATIONAL COLLABORATION
NEXT-GENERATION DIAGNOSTICS
CAPACITY BUILDING
ERLTB2-NET EUROPEAN REFERENCE LABORATORY
NOVEL ANTIMYCOBACTERIALS
PHENOTYPIC DRUG SUSCEPTIBILITY TESTING
NONTUBERCULOUS MYCOBACTERIA
DZIF
TBINFO
MDR-TB
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TRANSLATIONAL RESEARCH
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PERSONALIZED MEDICINE

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- Anne-Katrin Wit
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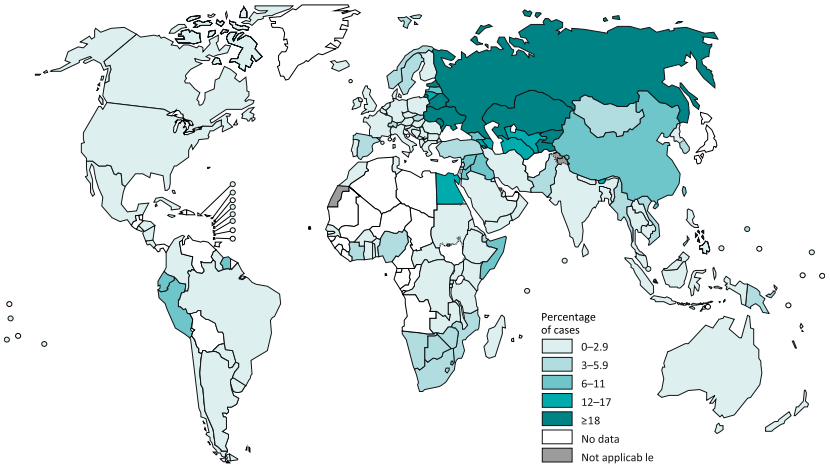
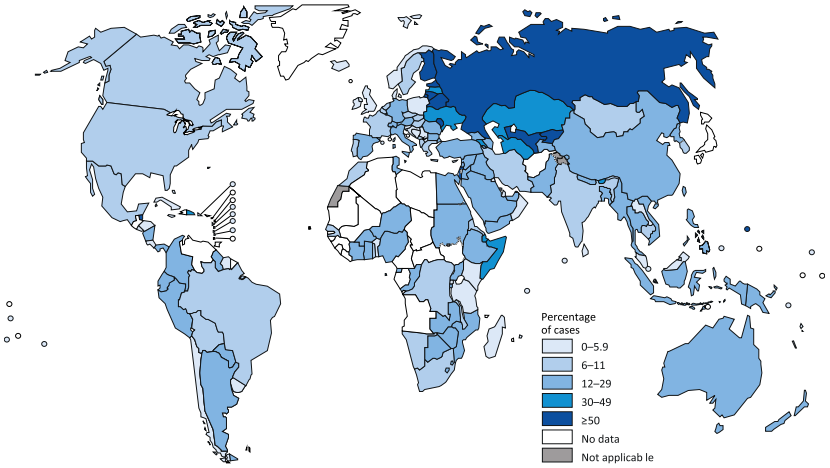
Percentage of new TB cases with MDR/RR-TB [a]



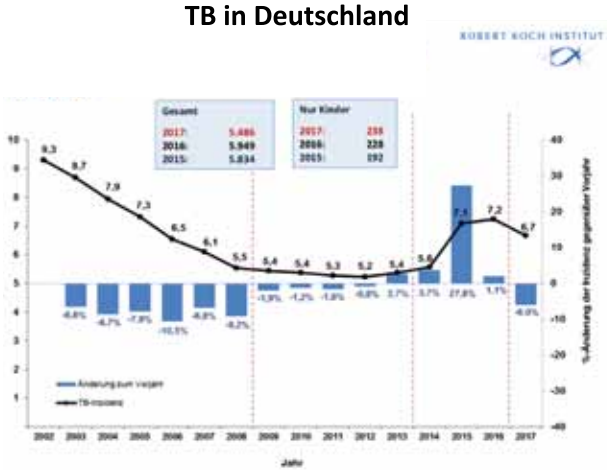
[a] Figures are based on the most recent year for which data have been reported, which varies among countries. Data cover the period 2002–2018.

Percentage of previously treated TB cases with MDR/RR-TB [a]



[a] Figures are based on the most recent year for which data have been reported, which varies among countries. Data cover the period 2005–2018. The high percentages of previously treated TB cases with RR-TB in Belize, Guam and Sao Tomé and Principe refer to only a small number of notified cases (range: 1–8 notified previously treated TB cases).

TB in Deutschland



Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Infections**

Diagnostic Mycobacteriology (NRC)

Mission

Als Nationales Referenzzentrum für typische und atypische Mykobakterien bieten wir innovative, deutschlandweit und international führende Diagnostik und Therapieberatung auf fachlich höchstem Niveau. Wir verstehen uns als zentrales Bindeglied zwischen Grundlagenforschung, angewandter Mykobakteriologie und klinischer Infektiologie.

As German national reference center for mycobacteria we operate at the interface between fundamental research, routine diagnostics and clinical infectious diseases. We provide cutting-edge mycobacterial diagnostics and therapeutic decision support to all our clients both nationally and internationally.

Most important findings

Emerging resistance to all newly released antimycobacterials

Multidrug-resistant tuberculosis (MDR-TB) is defined by resistance to isoniazid and rifampicin. XDR-TB is defined as MDR-TB plus resistance to at least one fluoroquinolone and one second-line injectable agent (amikacin, capreomycin, or kanamycin). According to the World Health Organization (WHO), 6.2% of the 490,000 newly diagnosed MDR-TB cases met the criteria for XDR-TB in 2016. Treatment of XDR-TB is extremely challenging due to limited therapeutic options, poor drug tolerability associated with frequent adverse events, and high treatment failure and mortality rates. The recent approval of two new antitubercular drugs, bedaquiline (BDQ) and delamanid (DLM), has expanded treatment options for patients with MDR- and XDR-TB. BDQ has recently been added by WHO to the group A category of recommended agents, and should now be administered to all patients suffering from MDR-TB unless contraindications prohibit its use. To date, only few cases of acquired resistance to both BDQ and DLM have been described. However, a very limited number of laboratories globally is capable of performing antimicrobial susceptibility testing (DST) for BDQ and DLM. Since we introduced testing of BDQ and DLM into our diagnostic routine in early 2018, we observed 7 patients from Eastern Europe with acquired resistance to BDQ, which were treated for MDR-/XDR-TB in Germany or Austria. In some of the M. tuberculosis complex (MTBC) strains isolated from these patients, the drug resistant phenotype could be linked to known genetic markers by whole-genome sequencing. In others, this was not the case and it is likely that yet unknown determinants affect susceptibility to BDQ in these strains. These findings are of utmost relevance, as capacities for DST of BDQ and companion drugs in high-incidence countries are insufficient. Efforts are currently ongoing to provide support to our partner reference laboratories in four such countries (Moldova, Armenia, Azerbeijan, Pakistan) with regard to establishing such capacities. PMID: 30933266

Selected publications

Polsfuss, S, Hofmann-Thiel, S, Merker, M, Krieger, D, Niemann, S, Rüssmann, H, Schönfeld, N, Hoffmann, H & Kranzer, K. Emergence of low-level Delamanid and Bedaquiline resistance during extremely drug-resistant tuberculosis treatment' Clin. Infect. Dis. 2019, Epub, PMID: 30933266

Maurer FP, Pohle P, Kernbach M, Sievert D, Hillemann D, Rupp J, Hombach M, Kranzer K. Differential drug susceptibility patterns of *Mycobacterium chimaera* and other members of the Mycobacterium avium-intracellulare complex. Clin Microbiol Infect. 2019 Mar;25(3):379.e1-379.e7. PMID: 29906595

Schön T, Matuschek E, Mohamed S, Utukuri M, Heysell S, Alffenaar JW, Shin S, Martinez E, Sintchenko V, Maurer FP, Keller PM, Kahlmeter G, Köser CU. Standards for MIC testing that apply to the majority of bacterial pathogens should also be enforced for *Mycobacterium tuberculosis* complex. Clin Microbiol Infect. 2019 Apr;25(4):403-405. PMID: 30771527

Nikolayevskyy V, Maurer FP, Holicka Y, Taylor L, Liddy H, Kranzer K. Novel external quality assurance scheme for drug susceptibility testing of non-tuberculous mycobacteria: a multicentre pilot study. J Antimicrob Chemother. 2019 Feb 11, Epub, PMID: 30753511

Walker TM, Merker M, Knoblauch AM, Helbling P, Schoch OD, van der Werf MJ, Kranzer K, Fiebig L, Kröger S, Haas W, Hoffmann H, Indra A, Egli A, Cirillo DM, Robert J, Rogers TR, Groenheit R, Mengshoel AT, Mathys V, Haanperä M, Soolingen DV, Niemann S, Böttger EC, Keller PM; MDR-TB Cluster Consortium. A cluster of multidrug-resistant *Mycobacterium tuberculosis* among patients arriving in Europe from the Horn of Africa: a molecular epidemiological study. Lancet Infect Dis. 2018 Apr;18(4):431-440. PMID: 29326013

van Ingen J, Kohl TA, Kranzer K, et al. Global outbreak of severe *Mycobacterium chimaera* disease after cardiac surgery: a molecular epidemiological study. Lancet Infect Dis. 2017 Oct;17(10):1033-1041. PMID: 28711585

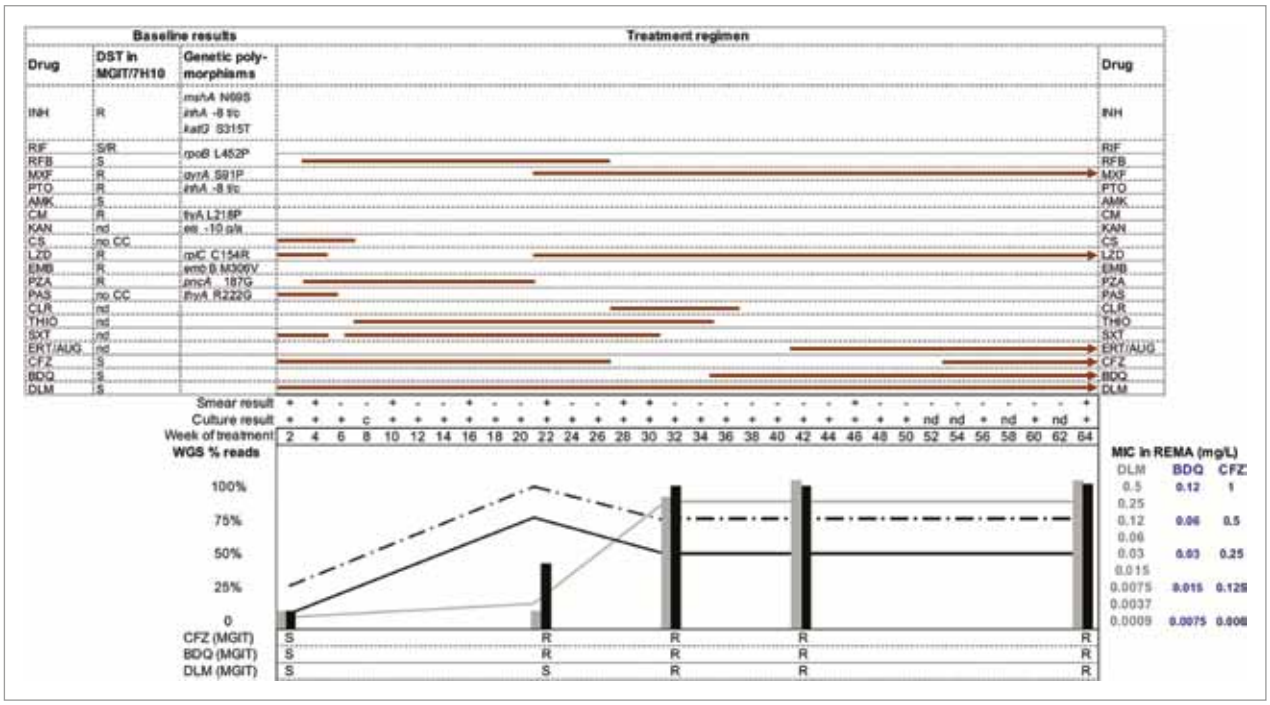


Figure 1. Results of smear microscopy, culture, whole genome sequencing and phenotypic drug susceptibility testing in a patient with acquired resistance to bedaquiline.

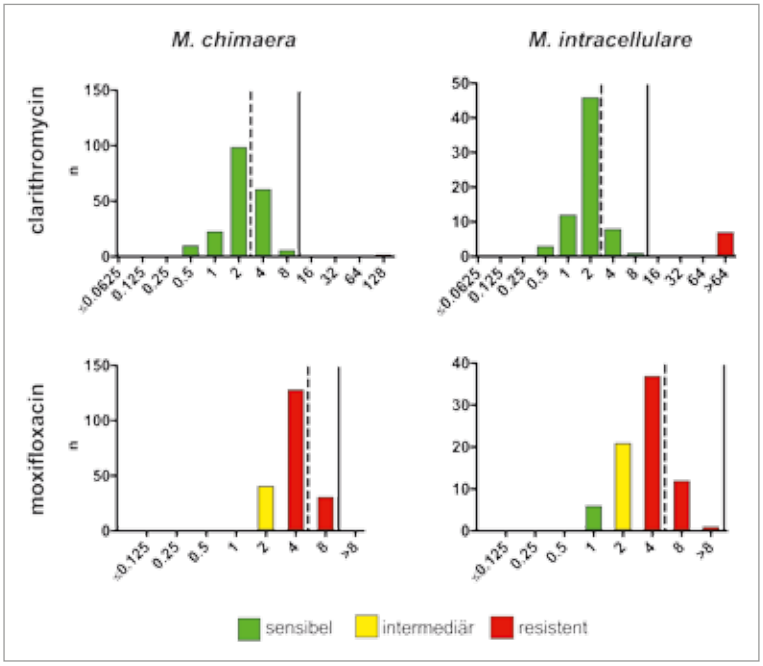


Figure 2. *M. chimaera* drug susceptibility patterns are comparable to those of *M. intracellulare*. Split wild-type populations such as with moxifloxacin lead to false resistant and false susceptible test results and require revision.

Priority Research Area **Infections**

Diagnostic Mycobacteriology (NRC)

Breakpoints used for drug-susceptibility testing of mycobacteria require substantial revision

In the light of the ongoing spread of ever more antibiotic-resistant MTBC strains and the increase of infections with highly drug-resistant non-tuberculous mycobacteria (NTM), accurate and affordable DST is key for control of both TB and infections caused by NTM. For pragmatic and historical reasons, the vast majority of routine phenotypic DST for the MTBC has traditionally been done by testing only the breakpoints, which are known as critical concentrations and which were first defined in the 1960s. This contrasts with other bacterial pathogens, for which quantitative DST results are often routinely obtained using Minimal Inhibitory Concentration (MIC) testing. Indeed, clinical breakpoints, as defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), cannot be set unless MIC distributions tested with a standardized method in multiple laboratories are pooled to define epidemiological cut-off (ECOFF) values, and pharmacokinetic/pharmacodynamic and clinical outcome data are evaluated (PMID 30771527). For NTM, MIC determination by the broth microdilution method is well established. However, it is hampered b y lack of intra- and interlaboratory reproducibility and weak correlation with clinical outcome. The slow-growing NTM species *M. chimaera* currently receives much attention in the context of a global outbreak of severe postsurgical bloodstream infections which has been described in detail under participation of both ourselves and the research group of Prof. Niemann. Using DST data for 203 nonduplicate *M. chimaera* isolates and a comparator dataset of 480 other *M. avium* complex isolates as an example, we were able to demonstrate that current breakpoints released by the Clinical and Laboratory Standards Institute (CLSI) to translate MIC values into "susceptible", "intermediate" or "resistant" categories, are to a large extent fraught with technical inadequacies. In particular, many of these breakpoints split bacterial populations with the same genetic background, leading to both major (false resistant) and very major (false susceptible) errors as well as categorization changes upon retesting. This work, which is currently expanded to other NTM and also MTBC, will pave the way for a concerted European effort to redefine the fundamentals of DST in mycobacteriology based on state-of-the-art MIC distribution analysis leading to well-supported, transparent decision criteria and, thereby, to a better correlation of DST results with therapeutic response in vivo.
PMIDs: 30771527, 30753511, 29906595

Internal and external collaboration

Local:
S. Niemann, C. Lange, U. Schaible, T. Gutsmann, M. Wilmanns (EMBL / CSSB Hamburg), M. Aepfelbacher (UKE Hamburg), H. Rohde (UKE Hamburg), M. Merker, S. Homolka, S. Feuerriegel, D. Schwudke, T. Kohl
Infectophysics Consortium

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Robert-Koch-Institut, DZIF M/XDR-TB network, TBNET, DGHM.

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C. Köser (Cambridge), B. Aldridge (Tufts, USA), T. Schön (Kalmar, Sweden), D. Cirillo (Milan, Italy), E. Cambeau (Paris, France), G. Kahlmeter (Stockholm, Sweden), L. Kremer (Montpellier, France), J.-L. Hermann (Versailles, France), V. Nikolayevsky (London, UK), F. Drobniewski (London, UK), K. Kranzer (London, UK), K. Kombrink (Laredo, USA), V. Crudu (Chisinau, Moldova), E. Kabasakalyan (Yerevan, Armenia), R. Tahirli (Baku, Azerbeijan), B. Savic (Belgrade, Serbia), M. Zolnir (Golnik, Slovenia), M. Omari (Accra, Ghana), A. Indra (Vienna, Austria), J. Abazovic (Sarajevo, Bosnia and Herzegowina), L. Foray (Sierra Leone), M. Dojki (Karachi, Pakistan)
F. Maurer is a member of the WHO Europe European Laboratory Initiative expert core group, a consortium member of the ERLTB2-Net European Network of Mycobacteria Reference Laboratories and the supervisor of the Instand EQA mycobacteria scheme with >250 participating laboratories.

Grant support

RKI, BMG, WHO, ECDC, BMBF, DZIF, Joachim-Herz-Foundation

3D LIPID STRUCTURES

INFLAMMATION CONTROL

BACTERIAL LIPIDS

MEMBRANE BIOPHYSICS

MACROPHAGE BIOLOGY

PULMONARY SURFACTANT

Priority Research Area **Infections**

Immunobiophysics

Head

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Members

- Irina von Cube
- Sarah Kupsch
- Kristian Kuzmicki

Trainees

- Sarah Griesoph
- Eike Heydorn
- Joe Knoof
- Stefanie Reese

Priority Research Area **Infections**

Immunobiophysics

Mission

Wir kombinieren hochauflösende 3D Strukturanalytik und Membranbiophysik mit immunologischer Expertise in der Makrophagenbiologie, um die strukturellen Grundlagen und molekularen Mechanismen Lipid-vermittelter Entzündungsreaktionen aufzuklären. Ziel unserer Arbeiten ist die Entwicklung neuer Strategien für Wirts-orientierte anti-entzündliche Therapien.

We combine high-resolution 3D structure analysis and membrane biophysics with immunological expertise in macrophage biology to elucidate the structural basis and molecular mechanisms of lipid-mediated inflammatory reactions. The aim of our studies is to develop new strategies for host-directed anti-inflammatory therapies.

Most important findings

Lipids in anti-inflammatory immune therapy

By the immunological and biophysical characterization of inflammatory bacterial lipids we want to elucidate how lipids are recognized as danger signals by the immune system. In addition to the chemical building blocks, the supramolecular structure of lipids plays a central role in immunological activity. By combining immunological characterization and structure analysis we have elucidated the molecular mode of action of a number of bacterial pathogenicity factors, activators and inhibitors of immunological reactions. We work with natural and reconstituted membrane systems and use biophysical methods such as fluorescence and IR spectroscopy to characterize membrane properties. We investigate the 3D organization of lipids in solution with the method of small angle X-ray diffraction (SAXS) at the beamline P12 of EMBL at DESY (Hamburg). On this basis we are currently working on the analysis **of the mechanisms of action of natural and new synthetic lipids for targeted immune modulation** (Facchini et al. J Med Chem 2018). Strategies for fine-tuning immune modulation through the targeted combination of different active substances is a new approach that is currently proving to be of particular interest. The investigation of lipid mixtures and complex membranes has so far hardly been addressed. In the medical context, this basic research has many possible applications. A further project in this field of research that was performed under a grant of the Cluster of Excellence Inflammation at Interfaces in collaboration with clinicians from the department of pediatrics at the University Hospital in Kiel and colleagues in Borstel is concerned with the **modification of surfactant preparations used in neonatology** by anionic phospholipids (Spengler et al. AJPLung 2018; Kupsch et al. manuscript in preparation) (Fig. 1). The aim is to positively influence the inflammatory processes in acute respiratory failure of newborn infants suffering from nARDS. Decisive progress has been made in the improvement of therapeutic surfactant preparations.

Highlights

Our 10th Annual Meeting of the North German Biophysicists took place on January 13, 2017. Organized by Thomas Gutsmann (RCB), Christian Hübner (University of Lübeck) and Matthias Winterhalter (Jacobs University Bremen) and Andra Schromm (RCB) this meeting is a fruitful platform that brings together scientists from the fields of biophysics, membrane biology, molecular modelling and structural biology.

The structural basis of function of new synthetic compounds for targeted immune modulation of Toll-like receptor mediated inflammation was characterized.

Phosphatidyglycerol and-inositol phospholipid species were positively evaluated as potent inhibitors of inflammation in vitro and in vivo in a collaborative study on surfactant fortification in a model of neonatal acute respiratory distress syndrome. Our results support an advantage of phospholipid fortification of surfactant preparations in anti-inflammatory therapy of nARDS.

Selected publications

Facchini FA, Zaffaroni L, Minotti A, Rapisarda S, Calabrese V, Forcella M, Fusi P, Airoldi C, Ciaramelli C, Billod JM, Schromm AB, Braun H, Palmer C, Beyaert R, Lapenta F, Jerala R, Pirianov G, Martin-Santamaria S, Peri F. Structure-Activity Relationship in Monosaccharide-Based Toll-Like Receptor 4 (TLR4) Antagonists. J Med Chem. 2018, 61(7):2895-2909.

Spengler D, Winoto-Morbach S, Kupsch S, Vock C, Blöchle K, Frank S, Rintz N, Diekötter M, Janga H, Weckmann M, Fuchs S, Schromm AB, Fehrenbach H, Schütze S, Krause MF. Novel therapeutic roles for surfactant-inositols and phosphatidylglycerols in a neonatal piglet ARDS model: A translational study. AJP lung Cell Mol Physiol 2018, 314:L32-L53.

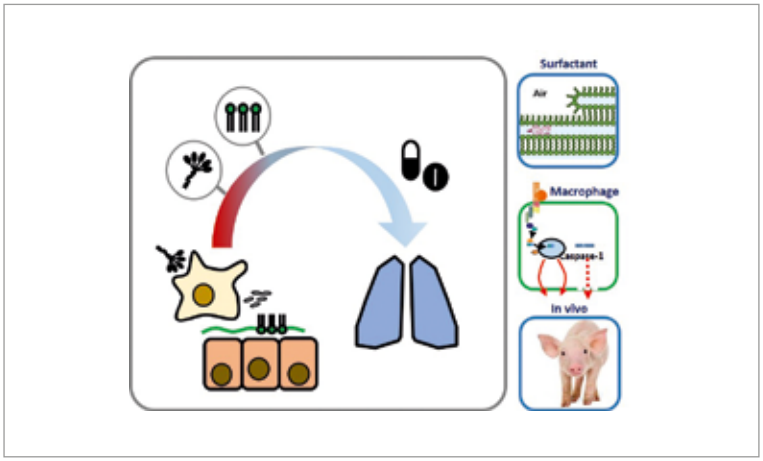

Figure 1. Evaluation of the anti-inflammatory potential of modified surfactant preparations in vitro (biophysical characterization and function on alveolar and monocyte-derived macrophages from piglets and human) and *in vivo* (piglet model of nARDS).

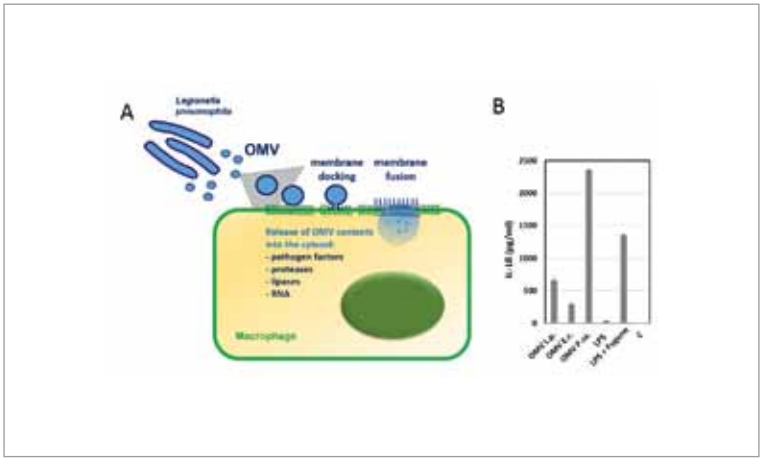

Figure 2. a) Self-promoted transport of bacterial membrane vesicles enables delivery of pathogen factors to host cells and activates intracellular signaling cascades b) OMV-mediated activation of cytoplasmic caspases initiates inflammasome activation and IL-1β release. In contrast to OMV, LPS requires transfection to activate this pathway.

Priority Research Area **Infections**

Immunobiophysics

Bacterial membrane vesicles -
transporters between microbes and immune cells

Outer membrane vesicles (OMV) are important bacterial shuttles for virulence-associated molecules. The relevance of this transport process for pathogen-host interaction is increasingly recognized. In studies on OMVs from Legionella pneumophila we could show that membrane vesicles, in contrast to isolated lipids from the bacterial membrane, are able to penetrate immune cells without lipid transport proteins and thus can transfer virulence factors in the cell interior. Studies on human macrophages and model membranes have shown that this transport is based on membrane fusion. This entry mechanism is the basis for the activation of the inflammasome, a recently discovered mechanism of intracellular endotoxin recognition (Fig. 2). In cooperation with microbiologists and structural biologists, we are currently investigating the composition, membrane structure, uptake mechanisms and function of membrane vesicles more comprehensively performing comparative studies on different pathogens (E.coli, Ps.aeruginosa, and L.pneumophila). Work on strains of the Mycobacterium tuberculosis complexes are in preparation. This topic is addressed as part of a cross-group focus within the priority area infections. The clinical significance of OMVs in the context of infections, pathomechanisms and qualification as diagnostic markers has hardly been investigated so far and is therefore of great importance in the medical context.

Internal and external collaboration

Internal collaborations:
Flow Cytometry Unit; Heinz Fehrenbach, Division of Experimental Allergology; Nicolas Gisch, Division of Bioanalytical Chemistry; Torsten Goldmann, Division of Clinical and Experimental Pathology; Thomas Gutsmann, Division of Biophysics; Susanne Homolka and Stefan Niemann, Division of Molecular and Experimental Mycobacteriology; Uta Jappe, Division of Clinical and Molecular Alergology; Uwe Mamat, Division of Cellular Microbiology; Dominik Schwudke, Division of Bioanalytical Chemistry

External collaborations:
Klaus Brandenburg, Brandenburg Antiinfektiva; Koichi Fukase, Department of Chemistry, University of Osaka, Japan; Martin Krause, Clinic of Pediatrics, University Clinic Kiel; Kay Grünewald, HPI/CSSB, Hamburg; Jörg Labahn, Center for Applied Systems Biology (CSSB), c/o DESY, Hamburg; Francesco Peri, Department of Biotechnology and Biosciences, University of Milano, Italy; Stefan Schütze, Institute of Immunology, University of Kiel; Michael Steinert, Institute of Microbiology, Technical University Braunschweig; Guido Stichtenoth, Clinic of Pediatrics, University Hospital UKSH, Lübeck; Guillermo Martinez de Tejada, University of Pamplona, Spain

Grant support

Beamtime grants SAXS-690 and SAXS-807 for measurements at the German Electron Synchrotron DESY (EMBL beamline P12@ PETRA III, c/o DESY)

MICE

ARGINASE-1

PROTECTIVE GRANULOMA

GRANULA NECROSIS

TUBERCULOSIS

INTERLEUKIN-13

INTERLEUKIN-17A

INTERLEUKIN-27

MULTIFUNCTIONAL T CELLS

Priority Research Area **Infections**

Infection Immunology

Head

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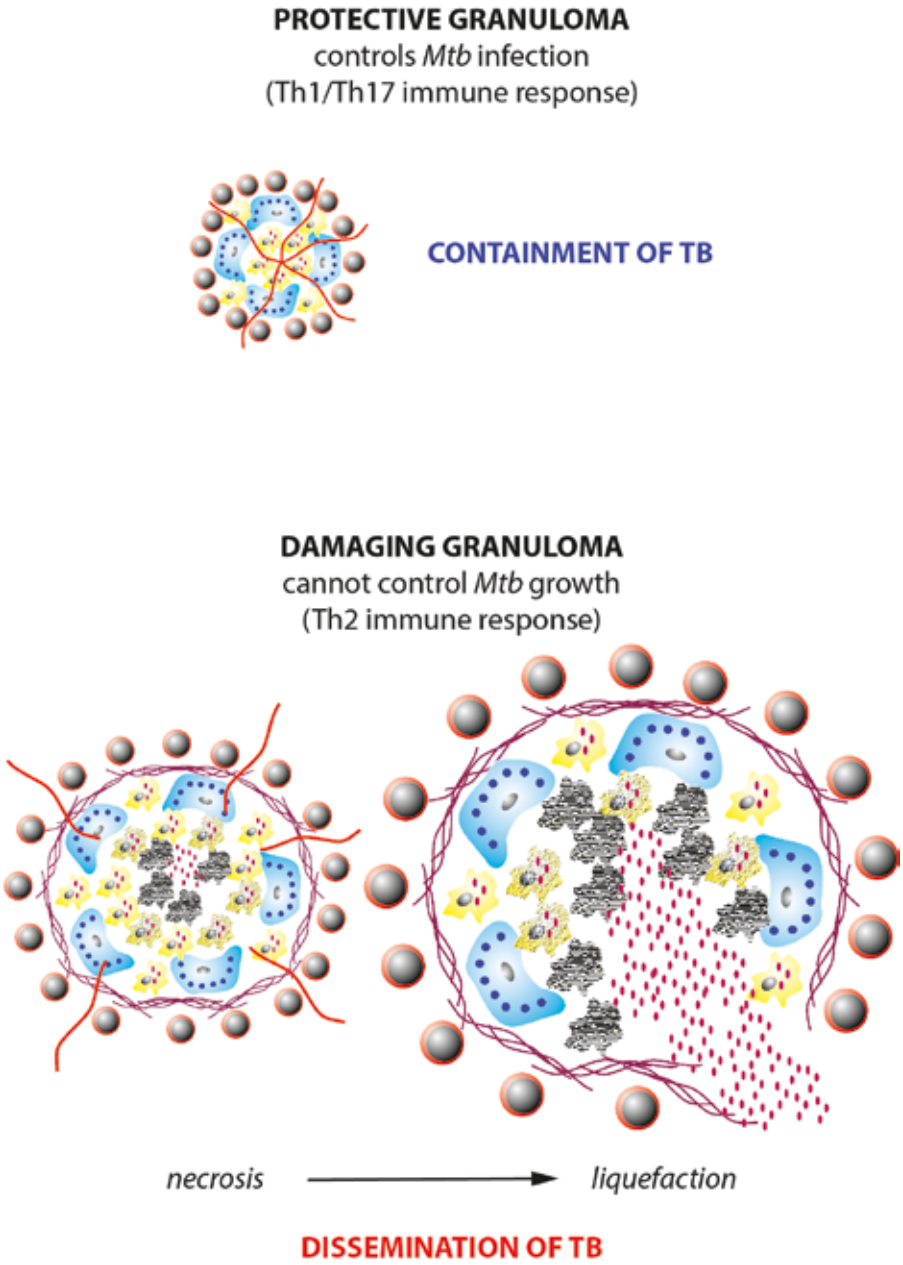


Protective and damaging, centrally necrotizing granuloma.

Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Infections**

Infection Immunology

Mission

Die Ausbildung von Granulomen ist ein typisches Merkmal einer Infektion mit *Mycobacterium tuberculosis (Mtb)* und stellt das histologische Korrelat einer Entzündungsreaktion des Gewebes dar, welche üblicherweise mit einer schützenden Immunantwort verbunden ist. In den meisten Fällen, ist der Wirt in der Lage, den Erreger in diesen schützenden Granulomen in Schach zu halten. Dennoch entwickelt sich in einigen infizierten Individuen die Krankheit Tuberkulose (TB), wenn ein ursprünglich schützendes Granulom seine Fähigkeit verliert, das Wachstum der Mykobakterien zu kontrollieren. Es nimmt an Größe zu und transformiert in ein zerstörendes Granulom, welches letzten Endes nekrotisiert. Die Forschungsgruppe Infektionsimmunologie ist daher daran interessiert, die Zytokin-vermittelte Regulation von (i) Schutz und (ii) Pathologie in diesem beiden Typen von Granulomen zu verstehen, um schützende und schädliche Mechanismen für adjuvante Vakzinierungs- und Therapieansätze voneinander zu entkoppeln.

Granuloma formation is a hallmark of *Mycobacterium tuberculosis (Mtb)* infection and represents the histological correlate of inflammatory tissue responses generally associated with protective immunity. In most cases the immune system of the host is capable to control the pathogen in these protective lung granulomas. However, in some infected individuals the disease tuberculosis (TB) develops when a formerly protective granuloma loses the capability to keep mycobacterial replication in check. It increases in size and transforms in a damaging granuloma that eventually necrotizes. The Infection Immunology research group wants to understand the cytokine-mediated regulation of (i) protection and (ii) pathology within these two types of TB-associated granulomas in order to dissect protective and pathology-promoting mechanisms to develop adjuvant vaccination and therapy strategies. (887)

Most important findings

(i) Interferon-gamma (IFN-γ) and interleukin (IL)-17A are two prototype T helper (Th) 1 and Th17 cytokines, respectively, that play a critical role in protection against infections with intracellular pathogens. As we have previously shown, mice lacking IL-17A production are highly susceptible to infection identifying a Th17 immune reaction as an important effector arm of protective immune responses. On the other hand, Th17 cells also play an essential role in the development of chronic inflammatory diseases, especially those mediated by T effector cells. This proverbial function of Th17 cytokines as a "double-edged sword" is highlighted by our studies of TB in IL-27 receptor-alpha (Rα)-deficient mice. In these animals, an enhanced inflammatory immune response to Mtb infection results in a better control of mycobacterial growth but also lead to immunopathology and premature death. We could now show that these opposed effects are exclusively mediated by an elevated expression of

Highlights

Arginase-1 mediates central granuloma necrosis in experimental TB

Arginase-1 is involved in IL-13-dependendent susceptibility to intracellular pathogens

SOCS3 keeps IL-6-dependent divergent macrophage responses expression under control and safeguard protective macrophage effector mechanisms against Mtb

ACC1-dependent fatty acid synthesis is a crucial mechanism in T cells to fight against mycobacterial infection

After Mtb infection, IL-27 suppresses the IL-17A-dependent formation of protective granuloma

Selected publications

Müller U, Schaub GA, Mossmann H, Köhler G, Carsetti R, Hölscher C. Immunosuppression in Experimental Chagas Disease Is Mediated by an Alteration of Bone Marrow Stromal Cell Function During the Acute Phase of Infection. FRONTIERS IN IMMUNOLOGY 2018; 9: 2794.

Abad Dar M, Hölscher C. Arginase-1 Is Responsible for IL-13-Mediated Susceptibility to Trypanosoma cruzi Infection. FRONTIERS IN IMMUNOLOGY 2018; 9: 2790.

Holz K, Prinz M, Brendecke SM, Hölscher A, Deng F, Mitrücker H-W, Rose-John S, Hölscher C. Differing Outcome of Experimental Autoimmune Encephalitis in Macrophage/Neutrophil- and T Cell-Specific gp130-Deficient Mice. FRONTIERS IN IMMUNOLOGY 2018; 9: 836.

Erdmann H, Behrends J, Ritter K, Hölscher A, Volz J, Rosenkrands, I, Hölscher C. The increased protection and pathology in *Mycobacterium tuberculosis*-infected IL-27R-alpha-deficient mice is supported by IL-17A and is associated with the IL-17A-induced expansion of multifunctional T cells. MUCOSAL IMMUNOLOGY 2018; 11: 1168.

Stüve P, Minarrieta L, Erdmann H, Arnold-Schrauf C, Swallow M, Guderian M, Krull F, Hölscher A, Ghorbani P, Behrends J, Abraham W-R, Hölscher C, Sparwasser TD, Berod L. Fatty Acid Synthesis During Mycobacterial Infection Is a Prerequisite for the Function of Highly Proliferative T Cells, But Not for Dendritic Cells or Macrophages. FRONTIERS IN IMMUNOLOGY 2018; 9: 495.

Schmok E, Abad Dar M, Behrends J, Erdmann H, Rückerl D, Endermann T, Heitmann L, Hessmann M, Yoshimura A, Rose-John S, Scheller J, Schaible UE, Ehlers S, Lang R, Hölscher C. Suppressor of Cytokine Signaling 3 in Macrophages Prevents Exacerbated Interleukin-6-Dependent Arginase-1 Activity and Early Permissiveness to Experimental Tuberculosis FRONTIERS IN IMMUNOLOGY 2017; 8: 1537.

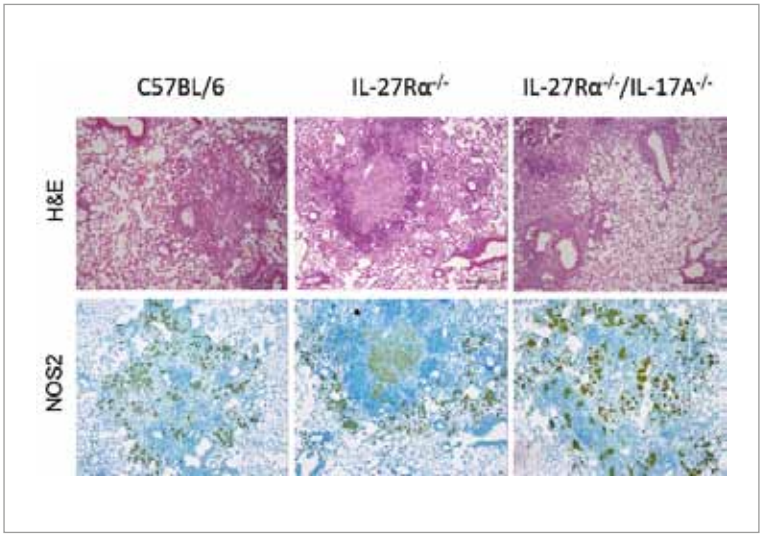


Figure 1. IL-17A promotes the formation of highly stratified granuloma.
C57BL/6, IL-27Rα$^{-/-}$ and IL-27Rα$^{-/-}$/IL-17A$^{-/-}$ mice were infected with 100 CFUs *Mtb* via the aerosol route. After 43 days, formalin-fixed lung sections were stained with H&E or for NOS2.

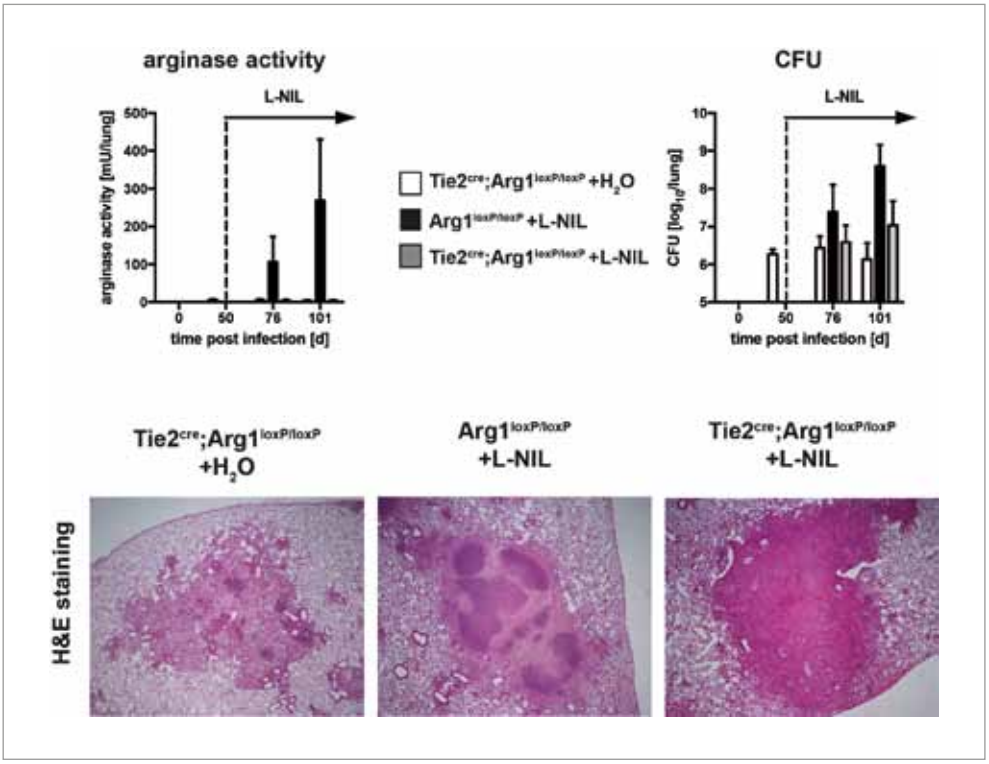


Figure 2. *Mtb* recrudescence and development of centrally necrotizing granuloma is dependent on arg-1.
NOS2 activity was blocked by treating arg-1-competent Arg1$^{loxP/loxP}$ or arg-1-deficient LysM^{cre}; Arg1$^{loxP/loxP}$ mice 50 days after aerosol infection with 100 CFU *Mtb* with L-NIL administered in drinking water; as control infected LysM^{cre}; Arg1$^{loxP/loxP}$ mice received untreated water. At different time points, arginase enzyme activity and CFU were determined in lung homogenates. At day 100 of infection, the granulomatous response in formalin-fixed lung sections from untreated LysM^{cre}; Arg1$^{loxP/loxP}$ mice and L-NIL-treated Arg1$^{loxP/loxP}$ mice or LysM^{cre}; Arg1$^{loxP/loxP}$ mice mice was analysed.

Priority Research Area **Infections**

Infection Immunology

IL-17A. Of importance, however, is that the increased levels of IL-17A lead to the formation of highly structured "protective" granulomas (Fig. 1). The exact mechanisms how these granulomas are induced by IL-17A and which mechanism expressed in these lesions in fact promote protection is not clear at the moment. Given that an increased expression of IL-17A fosters protective immune responses, a controlled increase in IL-17A or of downstream effects may represent an immunomodulatory approach for a host directed therapy.

(ii) Centrally necrotizing granuloma, strictly stratified in a fibrous capsule and a layer of foamy macrophages that separate the necrotic core from the adjoining tissue, are features of human post-primary TB. By comparative genetic analysis in TB patients and healthy individuals the Infection Immunology research group identified a mutation in the IL-4Rα chain linked to the degree of pathology. This genetic association study supported our hypothesis that IL-13/IL-4Rα-dependent mechanisms are involved in tissue pathology of human TB and we developed a mouse model in which increased IL-4Rα-mediated signalling after *Mtb* infection results in granuloma necrosis strongly resembling the pathology of human TB. Strikingly, in these IL-13-overexpressing mice, arginase (arg)-1-expressing alternatively activated macrophages, were prominently found within foamy macrophages located between the central necrotic core and the outer fibrous rim. To prove that arg-1 contributes to granuloma necrosis during experimental TB, we infected arg-1-competent and arg-1-deficient mice and prompted arg-1 expression independently of IL-13 during chronic infection. Because the formation of a fibrous rim and granuloma necrosis was only induced in the presence of arg-1 (Fig. 2), this enzyme appears to be critically involved in mediating TB pathology. Hence, targeting this pathway may constitute an approach to medicate TB tissue pathology and to prevent dissemination of *Mtb*.

Internal and external collaboration

Ehlers S, Schaible U, Niemann S, Reiling N, Dominik Schwudke, Petersen F, Research Center Borstel; Thye T, Breloer M, Lotter H, Jacobs T, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg; Berod L, Sparwasser T, TWINCORE, Braunschweig; Lang R, University Hospital Erlangen; Köhl J, University of Lübeck; Diefenbach A, University of Mainz Medical Center; Triantafyllopoulou A, Freiburg University Medical Center; Hölscher M, Klinikum der Universität München; Römpp A, University of Bayreuth; Tobin D, Duke University, Durham, USA; Brombacher F, University of Cape Town, South Africa; Hildeman D, Cincinnati Children's Hospital Medical Center, Cincinnati, OH USA.

Grant support

BMBF; DZIF TTU-TB 02.705 Infrastructure "Myco Drug and Trials"

BMBF; DZIF TTU-TB 02.806 Project "New Drugs and Regimen"

BMBF; DZIF TTU-TB 02.901 Flexible Fund "BTZ - A Novel TB Drug"

BMBF; DZIF TTU-TB 02.902 Flexible Fund "Development of a new immunostimulatory compound for the treatment of Leishmaniasis and Tuberculosis"

BMBF; DZIF TTU-TB 02.906 FlexFund "Optimization of mycobacterial thioredoxin reductase inhibitors, novel lead compounds against *M. tuberculosis*"

BMBF; DZIF TTU- TI 07.003 MD05 MD Stipend "A molecular bacterial load (MBL) assay, for measuring viable Mycobacterium tuberculosis in mouse models of tuberculosis as an alternative to solid agar and liquid culture systems."

BMBF; DZIF TTU-TI 07.003 MD09 Project "Local activity of anti-TB therapeutics in highly stratified human-like lesions of a novel pre-clincial mouse model"

DFG; IRTG191: "Immunoregulation of Inflammation in Allergy and Infection": Project B6 "ProThe impact of Tr1 cells in aged mice on susceptibility and vaccination efficacy in TB"

DFG; IRTG191: "Immunoregulation of Inflammation in Allergy and Infection": MD Project "Spatial analysis of host and pathogen responses in highly structured tuberculous granulomas after laser capture microdissection"

DFG; GRK 1727: "Modulation von Autoimmunität": Projekt A3: "Cell type-specific effects of interleukin-17 in Epidermolysis bullous acquisita"

Priority Research Area **Infections**

Microbial Interface Biology

MYCOBACTERIUM TUBERCULOSIS

PATHOGEN VARIABILITY

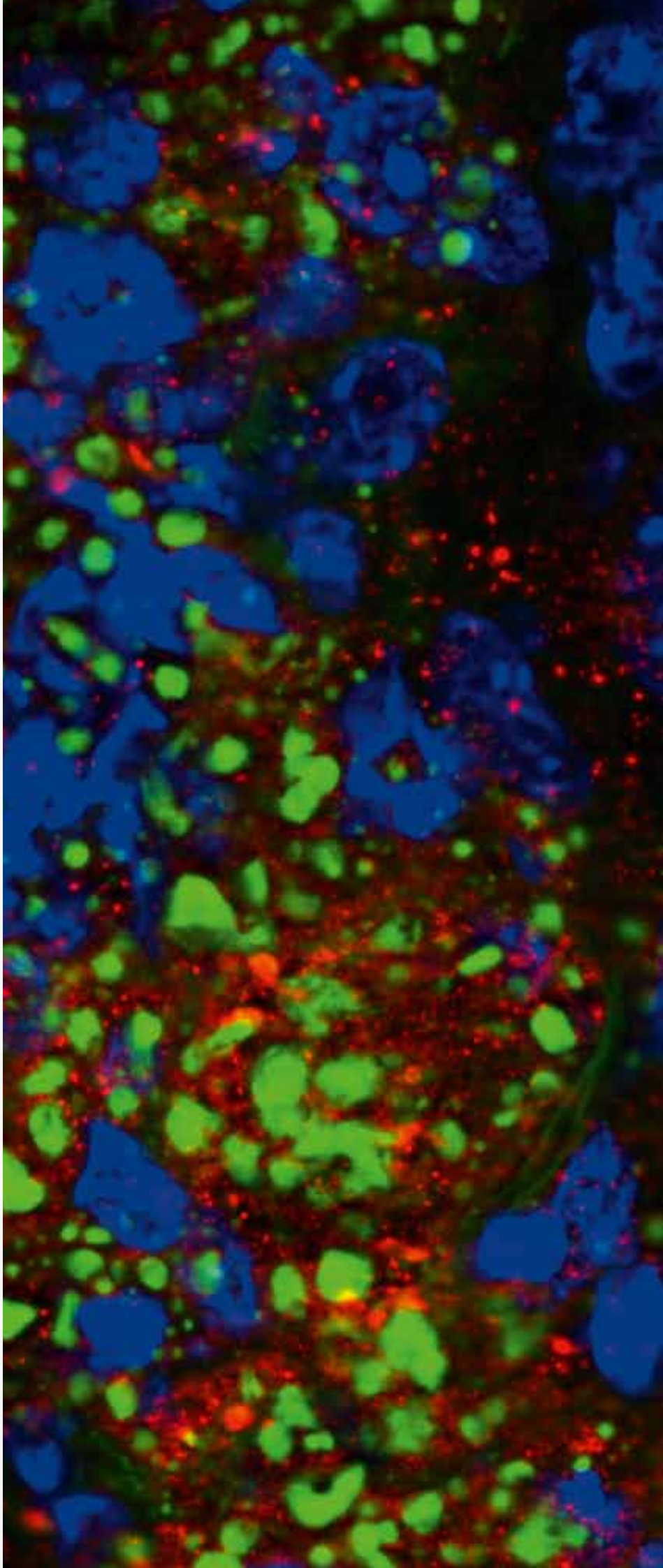
PHAGOSOME ISOLATION

MACROPHAGE

WNT SIGNALING

IN VITRO DRUG TESTING

LIPID METABOLISM



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Forschungszentrum Borstel
Leibniz Lungenzentrum

Priority Research Area **Infections**

Microbial Interface Biology

Mission

Der Fokus der FG Mikrobielle Grenzflächenbiologie liegt auf der detaillierten Charakterisierung der Interaktion von primären Makrophagen mit pathogenen Erregern des *Mycobacterium tuberculosis* Komplex (MTBC) sowie der Identifikation neuer anti-mykobakterieller Signalwege und Substanzen. Hierbei kommen verschiedene in der FG entwickelte Verfahren zur Charakterisierung von intrazellulären Kompartimenten aus Makrophagen (Phagosomen, Makropinosomen und Lipidkörper) zum Einsatz. Darüber hinaus nutzt die FG verschiedene selbst entwickelte Testsysteme zur Identifikation neuer anti-mykobakterieller Wirkstoffe. Die Analyse und die Modulation des Lipid-Metabolismus in der Interaktion zwischen Wirtszelle und Erreger bilden hierbei einen Schwerpunkt.

The main focus of the RG Microbial Interface Biology is the detailed characterization of the interaction between pathogenic mycobacteria of the *M. tuberculosis* complex (MTBC) and their prime target cells, the macrophages in order to identify novel anti-mycobacterial pathways and compounds. The RG has developed novel approaches to isolate und characterize intracellular compartments from uninfected and *M. tuberculosis*-infected macrophagen (phagosomes, macropinosomes and lipid droplets) and various test systems to identify anti-mycobacterial lead compounds. Several projects focus on the analysis and the modulation of the lipid metabolism in the interaction between host cells and *M. tuberculosis*.

Most important findings

Shaping the niche in macrophages: Genetic diversity of the *M. tuberculosis* complex and its consequences for the infected host.

Pathogenic mycobacteria of the *M. tuberculosis* complex (MTBC) have co-evolved with their individual hosts and are able to transform the hostile environment of the macrophage into a permissive cellular habitat. The impact of MTBC genetic variability has long been considered largely unimportant in TB pathogenesis. Members of the MTBC can now be distinguished into three major phylogenetic groups (Figure 1) consisting of 7 phylogenetic lineages and more than 30 so called sub-lineages/subgroups. MTBC genetic diversity indeed influences the transmissibility and virulence of clinical MTBC isolates as well as the immune response and the clinical outcome. In this manuscript we review the genetic diversity and epidemiology of MTBC strains and describe the current knowledge about the host immune response to infection with MTBC clinical isolates using human and murine experimental model systems in vivo and in vitro. We discuss the role of innate cytokines in detail and portray two in our group recently developed approaches to characterize the intracellular niches of MTBC strains. Characterizing the niches and deciphering the strategies of MTBC strains to transform an antibacterial effector cell into a permissive cellular

Highlights

Discovery of Novel Enhancers of Isoniazid Toxicity in *Mycobacterium tuberculosis*

Strengthening of anti-TB drug research: Successful grant applications with national and international partners focusing on drug discovery

Defining Mtb´s intracellular niches: Leibniz Center Infection (LCI) funded project to characterize lipid droplet composition in Mtb infected macrophages

Selected publications

Böscke R, Vladar EK, Könnecke M, Hüsing B, Linke R, Pries R, Reiling N, Axelrod JD, Nayak JV, Wollenberg B. Wnt Signaling in Chronic Rhinosinusitis with Nasal Polyps. Am J Respir Cell Mol Biol. 2017 May;56(5):575-584.

Reiling N, Homolka S, Kohl TA, Steinhäuser C, Kolbe K, Schütze S, Brandenburg. Shaping the niche in macrophages: Genetic diversity of the *M. tuberculosis* complex and its consequences for the infected host. Int J Med Microbiol. 2018 Jan;308(1):118-128.

Lentz F, Reiling N, Martins A, Molnár J, Hilgeroth A. Discovery of Novel Enhancers of Isoniazid Toxicity in *Mycobacterium tuberculosis.* Molecules. 2018 Apr 4;23(4). pii: E825.

Radloff J, Heyckendorf J, van der Merwe L, Sanchez Carballo P, Reiling N, Richter E, Lange C, Kalsdorf B. Mycobacterium Growth Inhibition Assay of Human Alveolar Macrophages as a Correlate of Immune Protection Following *Mycobacterium bovis* Bacille Calmette-Guérin Vaccination. Front Immunol. 2018 Jul 24;9:1708.

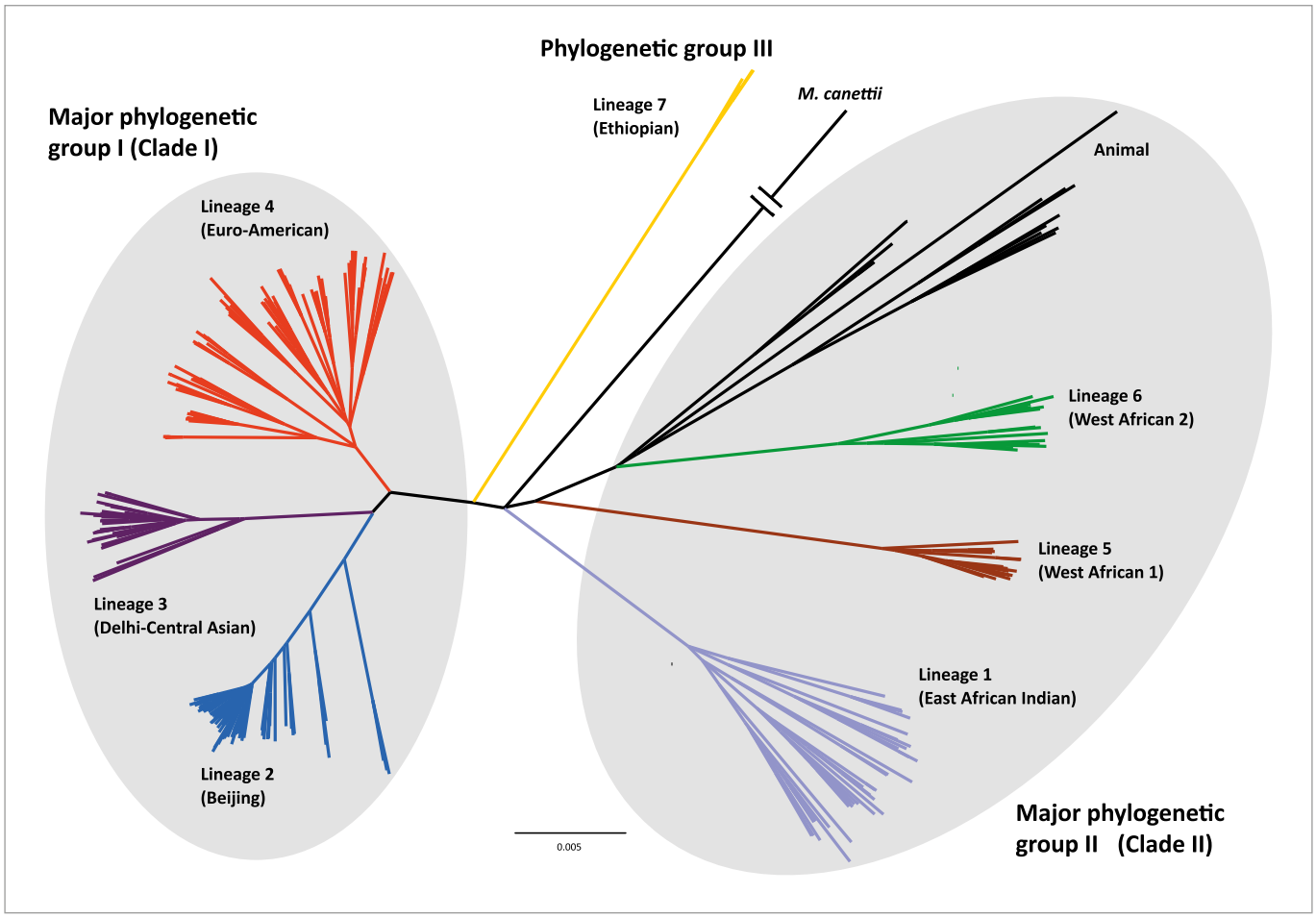

Figure 1. Genetic diversity of the MTBC complex. Maximum likelihood tree built from 960 SNP positions of 287 isolates covering the *M. tuberculosis* complex, with 3,936,656 out of 4,411,532 bp of the reference genome meeting criteria to be included (by T. Kohl; FZB). (Reiling N, et al., *Int J Med Microbiol.* 2018)

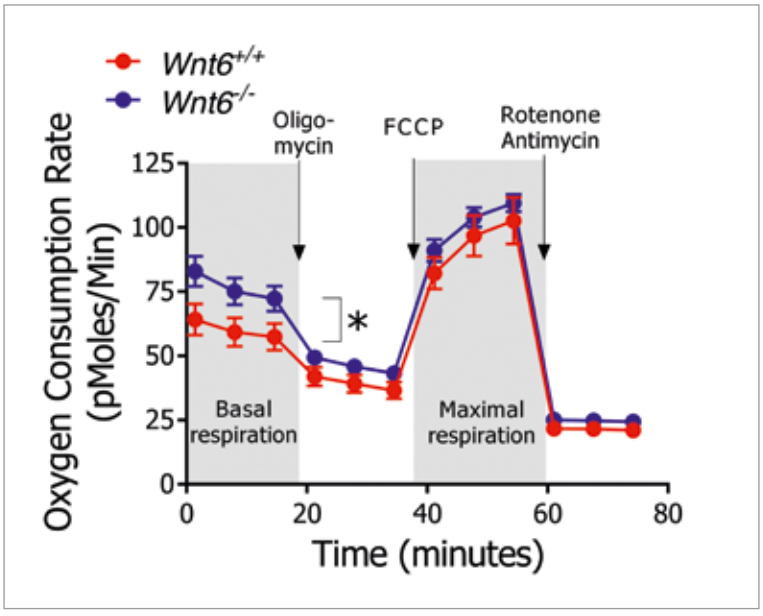

Figure 2. WNT6-dependent metabolic changes in macrophages incubated with heat-inactivated *M. tuberculosis.* Bone-marrow derived macrophages were cultivated in the presence of heat-inactivated *M. tuberculosis* for 24h and subjected to a Seahorse XFe96 Extracellular Flux Analyzer. Oxygen consumption rates (OCR) were determined in real-time during sequential injection of the mitochondria active compounds oligomycin, Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) and roteone/antimycin. Depicted is the mean +/- SEM of 3 independent experiments.

Priority Research Area **Infections**

Microbial Interface Biology

habitat offers the opportunity to identify strain- and lineage-specific key factors, which may represent targets for novel antimicrobial or host directed therapies for tuberculosis.

Discovery of novel enhancers of Isoniazid toxicity in *M. tuberculosis*.

The number of effective first-line antibiotics for the treatment of *Mycobacterium tuberculosis* infection is strongly limited to a few drugs. Due to emerging resistance against those drugs, second- and third-line antibiotics are now being used in TB therapy, however they often show severe side effects. In addition an increasing number of resistant strains against these drugs is already reported. An alternative to such novel drugs or combined therapeutic regimes, which may reduce resistance development, is finding enhancers of mycobacterial drug effectiveness, especially enhancers that counteract resistance development mechanisms. Such enhancers may reduce the extracellular drug efflux mediated by bacterial efflux pumps and thus enhance the intracellular drug toxicity. We developed novel 1,4-dihydropyridines (DHPs) as potential efflux pump inhibitors with some determined P-gp affinities. The influence on the antituberculotic drug toxicity was investigated for three prominent antituberculotic drugs. Exclusive and selective toxicity enhancing effects have been detected for isoniazid (INH) which could be related to certain substituent effects of the 1,4-DHPs. Thus, promising enhancers could be identified and a suggested efflux pump inhibition is discussed.

Novel technologies implemented to study *M. tuberculosis* infection in macrophages

Focus metabolism: With the newly acquired Seahorse XFe96 Metabolic Flux Analyzer (Agilent), now located in the Biosafety Level 3 laboratory, we are able to perform realtime metabolic flux analyses of primary macrophages during the infection with MTBC strains (Figure 2).

Focus gene knock down: We have successfully developed transdifferentiated BlaER1 monocytes/macrophages into a novel and valuable human Mtb-infection model system. These cells are easy to handle and can be used for CRISPR/Cas9-based gene manipulation e.g. gene knock-down. This represents a major technologic advance when addressing the functional role of specific factors during Mtb infection of human macrophages and ideally complements the use of pharmacological inhibitors.

Internal and external collaboration

Internal

J. Behrends, N. Gisch, T. Goldmann, T. Gutsmann, H. Heine, C. Herzmann, J. Heyckendorf, C. Hölscher, B. Kalsdorf, C. Lange, S. Marwitz, S. Niemann, U.E. Schaible, B. Schneider, T. Scholzen, D. Schwudke, C. Stamme.

External

T. Lindhorst, S. Schütze (University of Kiel), J. Rupp, B. Wollenberg (University Clinics Schleswig Holstein, Campus Lübeck), E. Herker (Heinrich Pette Institut for Virology, Hamburg), A. Kispert (Hannover Medical School, Hannover), P. Imming, A. Hilgeroth (University of Halle-Wittenberg), M. Bastian, A. Dorhoi (Friedrich Löffler Institute, Riems), G. van Zandbergen (Paul Ehrlich Institute, Langen), J. Rybniker (University of Cologne), A. Hirsch, J. Herrmann (Helmholtz-Institute for Pharmaceutical Research, Saarbrücken), O. Koch (University of Dortmund), W. Wohlleben (University of Tübingen), Felix Hausch, (Technical University of Darmstadt), A. Blumenthal (University of Queensland, Brisbane, Australia), J. Enninga (Institute Pasteur, Paris, France), K. Prasad (Yenepoya Research Centre Mangalore, India), M. Leitges (University of Oslo, Oslo, Norway), T.H. Flo (NTNU, Trondheim, Norway), M. Lerm (Linköping University, Sweden), K. Kolbe, D. Barber, K. Mayer-Barber (National Institutes of Health, Bethesda, USA)

Grant support

BMBF, Program: Targetvalidation for pharmaceutical drug development "Validation of the γ-glutamylpolyamine synthetase GlnA3Mt as a promising target for development of novel anti-tubercular drugs (GPS-TBT)"

DFG Priority Program 1580 "Intracellular Compartments as Places of Pathogen-Host-Interaction" (Re 1228/5-2)

DFG Cluster of Excellence 306 "Inflammation at Interfaces": Project "Lipid Disorders"

Deutsches Zentrum für Infektionsforschung (DZIF): Grant "TTU02.806: New Drugs & Regimens"

Leibniz Center Infection (LCI): "Isolation and characterization of *M. tuberculosis*-induced lipid droplets from human primary macrophages"

Olav Thon Foundation (Norway): "Discovering new therapeutic targets and drugs to combat AMR tuberculosis"

Priority Research Area **Infections**

Molecular and Experimental Mycobacteriology

Head

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MYCOBACTERIUM TUBERCULOSIS COMPLEX
MOLECULAR EPIDEMIOLOGY
LUNG PATHOGENS
WHOLE GENOME SEQUENCING
MYCOBACTERIA
HOST-PATHOGEN INTERACTION
EVOLUTION
VIRULENCE
PERSONALIZED MEDICINE

TUBERCULOSIS

PATHOBIOLOGY
POPULATION STRUCTURE
DRUG RESISTANCE
RESISTANCE MECHANISMS

Forschungszentrum Borstel
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Priority Research Area **Infections**

Molecular and Experimental Mycobacteriology

Mission

Die Bekämpfung der Tuberkulose (TB) und anderer Lungenerkrankungen durch ein besseres Verständnis der Erreger aus dem *Mycobacterium tuberculosis* Komplex (Mtbk) und weiterer bakterieller Lungenpathogene ist das zentrale Ziel der Forschungsgruppe (FG). Die translationale Forschungsagenda der FG umfasst folgende Schwerpunkte: lokale und globale Transmissionsdynamik, Aufklärung von Resistenz- und kompensatorischen Mechanismen, Populationsstruktur und Evolution der Mtbk Stämme bzw. anderer Mykobakterien, Virulenz, Physiologie und Pathobiologie von Mykobakterien, Anwendung von Hochdurchsatztechnologien in Forschung und Diagnostik, Implementierung neuer Technologien in Hochinzidenzländern, sowie individualisierte Therapie und Evolutionäre Medizin.

The fight against tuberculosis (TB) and other lung diseases based on a better understanding of the causative agents is the central aim of the Research Group (RG). In the main focus are *Mycobacterium tuberculosis* complex strains (Mtbc) and other bacterial lung pathogens. The translational research agenda of the RG comprises the following topics: local and global transmission dynamics, determination of resistance and compensatory mechanisms, population structure and evolution of Mtbc strains and other mycobacteria, virulence/physiology/pathobiology of mycobacteria, application of high-throughput technologies in research and diagnostics, implementation of new technologies in high incidence countries, as well as for individualized therapy and evolutionary medicine.

Most important findings

By using high resolution genotyping techniques, the work performed during the last years has significantly contributed to the **current understanding of transmission dynamics, population structure and evolution of sensitive, and esp. of multidrug/extremely drug resistant (MDR/XDR) TB strains in low and high incidence settings**. In fact, the transmission of MDR/XDR-TB strains could be demonstrated in several geographical settings including cross-border spread e.g. in Africa, from Africa to Europe, or in Eastern Europe. Contrary to previous assumptions, active transmission of MDR-TB strains is the main driver of the MDR-TB epidemic. In several cases, we identified dominant MDR-TB clones/outbreaks with extensive resistance profiles across different countries. The onset of these outbreaks can be traced back to the 1980s with multiple subsequent steps of drug resistance acquisition, e.g. during non-programmatic treatments in the former Soviet Union. These strains also acquired mutations that compensate for a fitness deficit caused by resistance mutations rendering them highly transmissible in Eastern Europe, Russia and Central Asia. Their genetic background e.g. extensive drug resistance profiles potentially jeopardizes the success of new drug regimens and molecular drug resistance assays. Consequently,

Selected publications

CRyPTIC Consortium and the 100,000 Genomes Project (Beckert P, Kohl TA, Merker M, Niemann S, Utpatel C) Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing. N Engl J Med. 2018 11;379(15):1403-1415.

Merker M, Barbier M, Cox H, Rasigade JP, Feuerriegel S, Kohl TA, Diel R, Borrell S, Gagneux S, Nikolayevskyy V, Andres S, Nübel U, Supply P, Wirth T, Niemann S. Compensatory evolution drives multidrug-resistant tuberculosis in Central Asia. Elife. 2018 Oct 30;7. pii: e38200.

Kohl TA, Harmsen D, Rothgänger J, Walker T, Diel R, Niemann S. Harmonized Genome Wide Typing of Tubercle Bacilli Using a Web-Based Gene-By-Gene Nomenclature System. EBioMedicine. 2018 Aug;34:131-138.

Meehan CJ, Moris P, Kohl TA, et al., Niemann S*, de Jong BC*. The relationship between transmission time and clustering methods in Mycobacterium tuberculosis epidemiology. EBioMedicine. 2018 Oct 16. pii: S2352-3964(18)30424-9. *equal contribution

van Ingen J*, Kohl TA*, Kranzer K*, et al., Spröer C, Bunk B, Nübel U, Bloemberg GV*, Böttger EC*, Niemann S*, Wagner D*, Sax H*. Global outbreak of severe Mycobacterium chimaera disease after cardiac surgery: a molecular epidemiological study. Lancet Infect Dis. 2017 17(10):1033-1041. *equal contribution

Walker TM*, Merker M*, et al., Niemann S*, Böttger EC*, Keller PM*; MDR-TB Cluster Consortium. A cluster of multidrug-resistant Mycobacterium tuberculosis among patients arriving in Europe from the Horn of Africa: a molecular epidemiological study. Lancet Infect Dis. 2018 Apr;18(4):431-440. *equal contribution

Awards

Thomas A. Kohl. DGHM Förderpreis 2019

Patrick Beckert. PhD Award "Kreis Segeberg" 2018.

Viola Schleusener. PhD Award "Kreis Segeberg" 2017.

Prof. Dr. Stefan Niemann. Visiting Professor University of Namibia.

Stefan Niemann. Schleswig-Holstein-Excellence-Chair.

Stefan Niemann. Speaker Leibniz Science Campus "Evolutionary Medicine of the Lung".

Matthias Merker. W1 professorship.

Matthias Merker. Gertrud Meissner Award of the European Society of Mycobacteriology 2017.

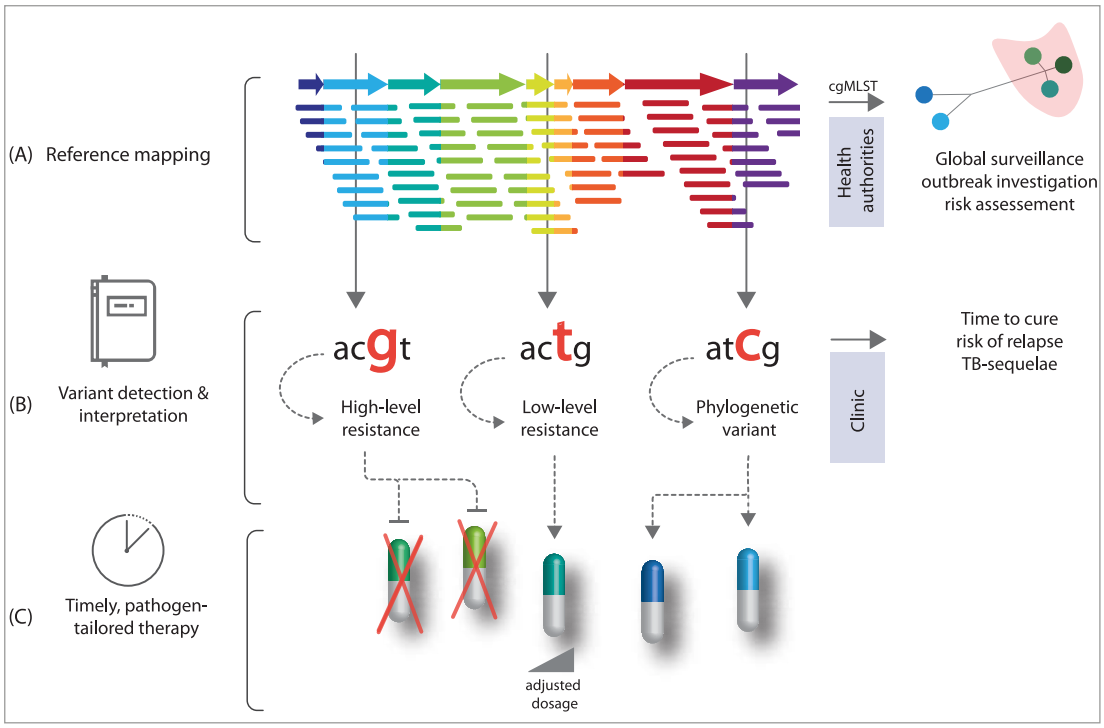

Figure 1. Principle of pathogen-tailored individualized treatment design. (A) Mutations are obtained from a whole genome sequencing reference mapping approach which can be also transferred into a core genome multi locus sequencing type (cgMLST) for molecular outbreak surveillance reasons. (B) Individual mutations are further interpreted towards their biological phenotype employing a validated consensus mutation catalogue. (C) When canonical/high-level resistance-conferring mutations are present, this drug should not be used. However, mutations associated with a moderate/intermediate resistance level may allow for the application use of drugs at increased doses. Moreover, mutations can be used to predict different treatment outcomes. Thus, by also considering phylogenetic benign mutations that do not confer resistance, a comprehensive molecular drug susceptibility profile could be inferred for a pathogen-tailored individualized treatment regimen in the future (Gröschel et al. PLoS Path 2018).

our data challenge current TB control concepts and underline the need to establish effective measures to stop MDR/XDR-TB strain transmission with suitable intervention strategies currently being developed in large collaboration projects (Walker et al. Lancet ID 2017, Malm et al. EID 2017, Makhado et al. Lancet ID 2018, Merker et al. Elife 2018).

We further developed concepts for using whole genome sequencing (WGS) as **prime technology for Mtbc transmission analysis and genotypic resistance prediction**. In another set of studies, key parameters for the interpretation of genome-based data for tracing transmission of Mtbc strains, as well as tools for the standardization of next generation sequencing(NGS)-based molecular epidemiology employing "core genome multi locus sequence typing (cgMLST)" technology linked with a web-based nomenclature server were established. In fact, we could demonstrate that whole genome analysis relying on single nucleotide polymorphisms (SNPs) and the cgMLST gene-by-gene approach offer a comparable discriminatory power for genotyping Mtbc strains. As cgMLST genotypes are inherently standardized, easy to communicate, and require little computational power even for large scale comparisons, they can form the basis for **highest-resolution national and international surveillance efforts and nomenclature systems to identify strains globally.** These technologies are now being employed for integrated genome-based TB surveillance projects in low and high incidence settings (Kohl et al. EBioMedicine 2018, Meehan et al. 2018, Fig. 1).

Moreover, we pioneered the use of NGS genome analysis to **identify resistance determinants** and predict resistance in individual patients by defining the "**Resistome**". This paves the ground for comprehensive molecular TB resistance

Priority Research Area **Infections**

Molecular and Experimental Mycobacteriology

diagnostics which are crucially important for MDR-TB patients with complex resistance patterns, and thus enabling genome-based individualized treatment regimens. This was combined with the implementation of **automated analysis tools such as PhyResSE allowing for automated data analysis and standardized reporting** and is now further developed towards **integrated approaches for precision medicine of TB** (CRyPTIC Consortium et al. NEJM 2018, Miotto et al. Eur Respir J 2018, Heyckendorf et al. Antimicrob Agents Chemother 2018, Gröschel et al. PLoS Pathog 2018, Schleusener et al. Sci Rep 2017, Fig. 1).

We transferred our competence in NGS, phylodynamics and molecular resistance prediction to other lung pathogens. As paramount example, we were able to reconstruct **a global Mycobacterium chimaera outbreak** caused by contaminated heater-cooler units (HCU) from our comprehensive WGS analysis. Currently, investigations of further HCU related cases as well as of the global population structure and hospital related transmission of *M. chimaera* and other non-tuberculous mycobacteria are ongoing. (Plate et al. 2018, van Ingen et al. Lancet ID 2017, Fig. 2).

The research program is conducted in several national and international collaborative networks such as the German Center for Infection Research (DZIF), the Wellcome Trust funded Cryptic consortium, or the ECDC initiative EUSeqMyTB. Implementation of future technologies in high incidence settings is carried out e.g. in the frame of the Supranational Reference Laboratory Network of the WHO and the SeqMDRTB_NET funded by the BMG Global Health Protection program.

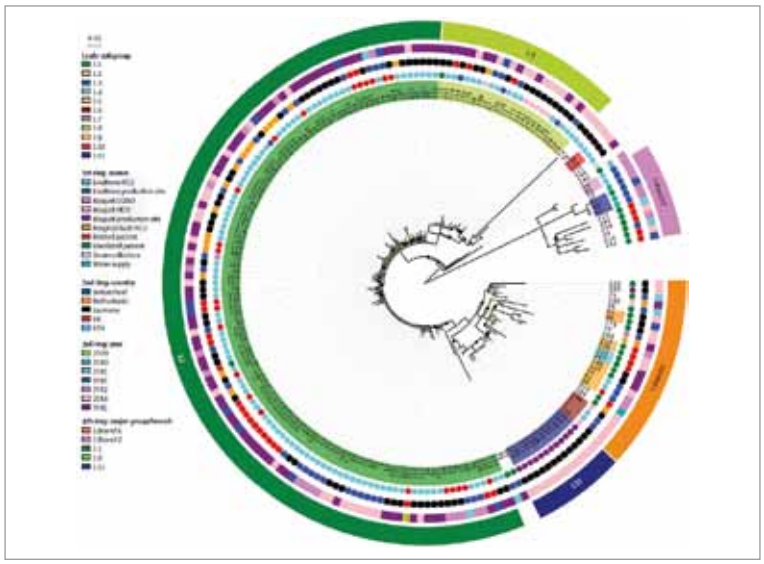

Figure 2. Maximum likelihood tree built from 860 SNP positions of the 200 group 1 isolates mapped to the genome of *Mycobacterium chimaera* ZUERICH-1 shown as a circular phylogram. The maximum SNP distance-based classification, as well as isolate source, country of origin, year of isolation, and major groups or branches are indicated by subsequent colored circles. Circles on nodes indicate resampling support of at least 90% (green circles) or at least 70% (black circles). Each leaf of the tree is labelled by sample ID and colored according to its subgroup, with ungrouped samples in white. ECMO=extracorporeal membrane oxygenation. HCU=heater-cooler unit. SNP=single nucleotide polymorphism.

Internal and external collaboration

FZB: *Pathobiology:* Bioanalytical Chemistry, Infection Immunology, Microbial Interface Biology, Cellular Microbiology, Fluorescence Cytometry. Diagnostics, *Epidemiology:* Diagnostic Mycobacteriology, Clinical Infectious Diseases. *Bioinformatics, Data analysis*: Central Unit IT
Individual external collaborations: H. Schulenburg, T. Dagan, A. Franke, University of Kiel; J. Bains, MPI Plön; D. Harmsen University Münster; H. Hoffmann, IML Red Gauting; M. Hölscher, LMU München; E. Nepolo, University of Namibia, Windhoek; U. Nübel DSMZ; S. Gagneux, Swiss TPH, Basel; K. Rhode University of Central Florida; P. Supply, Institute Pasteur, Lille; T. Wirth, Muséum National d'Histoire Naturelle, Paris; C. Köser, University of Cambridge; D. Crook, University of Oxford; D. Cirillo, San Raffaele Scientific institute; N. Zetola, uPenn, Bostwana; M. Zignol, WHO Geneva.
Networks: Cryptic: Global; EUseqMyTB: TB National Reference Laboratories in Europe; Excellence Cluster PMI: Borstel, Kiel, Lübeck and Plön; Leibniz Center Infection: FZB, BNI, and HPI; Leibniz Science Campus EvoLUNG: Borstel, Kiel, and Plön; Leibniz Research Alliance INFECTIONS´21: 14 Leibniz Institutions and external partners; German Center for Infection Research: 35 partner institutions in Germany; SeqMDRTB_NET: Partners in Europe and Africa; WANETAM: Excellence Network in West Africa; WHO SRL Network: Global.

Grant support

CRyPTIC: Comprehensive Resistance Prediction for Tuberculosis: an International Consortium, Wellcome Trust (PI).
EUseqMyTB: Pilot study on the use of Whole Genome Sequencing for molecular typing and characterization of M. tuberculosis in the EU/EEA, ECDC (PI)
German Center for Infection Research, BMBF. Coordinator of the Translational Thematic Unit "Tuberculosis".
EvoLUNG: Leibniz Science Campus Evolutionary Medicine of the Lung, Leibniz Association (Speaker).
MDR-TBNet: Host-pathogen interactions in Multidrug-Resistant Tuberculosis (MDRTB): Study of the Molecular Epidemiology, Host Immune Responses and Genome-Based Prediction and Early Identification of MDRTB in High Tuberculosis Burden Settings, EU FP7 (PI).
NRZ_{Myc}: National Reference Center for Mycobacteria, BMG (Co-Head).
RaPaed-AIDA-TB: Rapid and Accurate Diagnosis of Paediatric TB- An AIDA (Assessment of Innovative Diagnostics and Algorithms for Early and Sensitive Detection of Acute TB) platform study, H2020.
TB Sequel: Comorbidities, risk factors and longterm sequelae defining the individual outcome and public health impact of TB disease (PI, co-lead "the pathogen").
TB-seqDISK: molecular test based on Next Generation Sequencing for rapid diagnosis and comprehensive resistance determination in tuberculosis, BMBF (PI).
SeqMDRTB_NET: Network for application of sequencing technology for the fight against resistant tuberculosis in high incidence settings, BMG (Coordinator).
WANETAM: West Africa Network of Excellence for TB, AIDS and Malaria, H2020.

ALLERGIE
ASTHMA

STUDIEN
PATIENTEN

BIOBANK

INFEKTION

Highlights

Gesunde Kohorte des FZB: Dieses interne Kooperationsprojekt des FZB wurde zur Generierung von Biomaterialproben von gesunden Freiwilligen initiiert. Im Rahmen dieses ersten knapp 1/3 der Forschergruppen übergreifenden Kooperationsprojektes konnten Zellen aus bronchoalveolären Lavagen, Spülflüssigkeit, Urin-, Speichel-, Sputum- und unterschiedliche Blutproben zur Verfügung gestellt werden. Die Proben der 40 ausführlich phänotypisierten Probanden können für unterschiedlichste aktuelle und zukünftige Forschungsprojekte genutzt werden. Finanziert wurde das Projekt durch den PB Allergie & Asthma, den PB Infektionen und den Bereich Medizin.

Head

- PD Dr. Karoline I. Gaede

Members

- Steffi Fox
- Birgit Kullmann
- Romina Pritzkow
- Rasmus Hecht

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Forschungszentrum Borstel
Leibniz Lungenzentrum

Medicine

BioMaterialBank Nord

Mission

Die BioMaterialBank Nord (BMB Nord) unterstützt die biomedizinische Forschung in der Lungenheilkunde. Sie wird gemeinsam betrieben vom Forschungszentrum Borstel, der LungenClinic Grosshansdorf, der Medizinischen Klinik III - Pneumologie/ Infektiologie sowie der Sektion Pädiatrische Pneumologie und Allergologie am Universitätsklinikum Schleswig-Holstein, Campus Lübeck.

Die dezentrale BMB Nord gewinnt, verarbeitet, sammelt, lagert und verwaltet Biomaterialproben wie Blut, Gewebe, Zellen von Probandinnen und Probanden, die an Studien ihrer Mitglieds-Institutionen teilnehmen. Die Biomaterialproben werden von Wissenschaftlerinnen und Wissenschaftlern analysiert. So übernimmt die BMB Nord die Funktion einer Schnittstelle zwischen Patientenversorgung und Grundlagenwissenschaft. Darüber hinaus berät sie Wissenschaftlerinnen und Wissenschaftler bei der Planung von translationalen Forschungsprojekten im Bereich der Lungenheilkunde. Auf Antrag stellt sie Forschergruppen klinische Informationen in pseudonymisierter Form zur Verfügung. Durch deren Analyse lassen sich Rückschlüsse auf Krankheiten und deren Verläufe ziehen, was eine Verbesserung der Diagnostik und Therapie ermöglicht.

Die aus Mitteln der öffentlichen Hand geförderte BioMaterialBank Nord ist auf die Unterstützung von Probenspenden angewiesen. Ohne die Bereitschaft von Patientinnen, Patienten oder freiwilligen Probandinnen und Probanden kann die medizinische Forschung keinen Beitrag zum Verständnis, der Erkennung und der Therapie von Krankheiten leisten.

Der Schwerpunkt der BioMaterialBank Nord liegt auf übertragbaren und nichtübertragbaren Erkrankungen der Atemwege und der Lunge.

Die BMB Nord bietet:

- Proben- und datenbezogene Beratung und Planung für Wissenschaftlerinnen und Wissenschaftler bzw. Konzepterstellung für Forschungsprojekte (Antragstellung)
- Erarbeitung von umfassenden Einverständniserklärungen wie der sog. breiten Einwilligungserklärung für Erwachsene sowie Eltern, Kinder und Jugendliche
- Verarbeitung von Biomaterialproben entsprechend vorliegender Standardarbeitsanweisungen oder nach Anpassung an das jeweilige Forschungsprojekt
- Verarbeitung von Blutproben und deren Derivaten (z. B. Vollblut, Buffy Coat, Serum, Plasma, Blutzellen) verschiedenen Abstrichen, Nasen- und Lungenbürstungen, Lungenspülflüssigkeit und -zellen, Gewebeproben (Kryoproben, Formalin- oder HOPE-fixiert), Urinproben, Sputum- und Speichelproben, Pathogenen und deren DNA und anderen.
- Präanalytik wie Blutbild, Zytologie von Lungenspülungen etc.
- Lagerung, Verwaltung und Bereitstellung für wissenschaftliche Einzel- und Verbundprojekte
- Isolierung von Primärzellen

Selected Publications

Krause T[1], Röckendorf N[1], Gaede KI[2,3], Ramaker K[1], Sinnecker H[1], Frey A[1]. MAbs. Validation of antibody reagents for mucin analysis in chronic inflammatory airway diseases. 2017 Feb/Mar;9(2):333-341. doi: 10.1080/19420862.2016.1264551. Epub 2016 Dec 2.

Rhinovirus infections change DNA methylation and mRNA expression in children with asthma
Oliver Fuchs, Barbara Roesler, Nils Welchering, Naschla Kohistani-Greif, Katja Landgraf-Rauf, Kristina Laubhahn, Nicole Maison, Claudia Liebl, Bianca Schaub, Markus Ege, Erika von Mutius, Isabell Ricklef, Gesa Diekmann, Laila Sultansei, Markus Weckmann, Matthias V Kopp, Gyde Nissen, Inke R. König, Dominik Thiele, Thomas Bahmer, Anne-Marie Kirsten, Frauke Pedersen, Henrik Watz, Benjamin Waschki, Klaus F. Rabe, Christian Herzmann, Annika Opitz, Karoline I. Gaede, Peter Zabel, Folke Brinkmann, Anna-Maria Dittrich, Christine Happle, Ruth Grychtol, Aydin Malik, Nicolaus Schwerk, Christian Dopfer, Mareike Price, Gesine Hansen, Michael Zemlin, Matthias Müller, Ernst Rietschel, and Silke van Koningsbruggen-Rietschel. , Herzmann, C., Gaede, K. I. & Zabel, P., 28.11.2018, in : PLOS ONE. 13, 11, S. e0205275

Evaluation of Galactomannan Testing, the Aspergillus-Specific Lateral-Flow Device Test and Levels of Cytokines in Bronchoalveolar Lavage Fluid for Diagnosis of Chronic Pulmonary Aspergillosis
Salzer, H. J. F., Prattes, J., Flick, H., Reimann, M., Heyckendorf, J., Kalsdorf, B., Obersteiner, S., Gaede, K. I., Herzmann, C., Johnson, G. L., Lange, C. & Hoenigl, M., 02.10.2018, in : Frontiers in Microbiology. 9, S. 2223

Barbara Roesler, Nils Welchering, Naschla Kohistani-Greif, Katja Landgraf-Rauf, Kristina Laubhahn, Bianca Schaub, Markus Ege, Claudia Liebl, Erika von Mutius, Johanna Kurz, Oliver Fuchs, Isabell Ricklefsd, Gesa Diekmann, Laila Sultanseid, Markus Weckmannd, Gyde Nissend, Matthias V Kopp, Inke R. König, Dominik Thiel, Folke Brinkmann, Anna-Maria Dittrich, Christine Happle, Aydin Malik, Nicolaus Schwerk, Christian Dopfer, Mareike Price, Ruth Grychtol, Gesine Hansen, Michael Zemlin, Matthias Müller, Ernst Rietschel, Silke van Koningsbruggen-Rietschel, Thomas Bahmer, Anne-Marie Kirsten, Frauke Pedersen, Henrik Watz, Benjamin Waschki, Klaus F. Rabe, Christian Herzmann, Annika Opitz, Karoline I. Gaede, Peter Zabel, Fuchs, O., Bahmer, T., Weckmann, M., Dittrich, A-M., Schaub, B., Rösler, B., Happle, C., Brinkmann, F., Ricklefs, I., König, I. R., Watz, H., Rabe, K. F., Kopp, M. V., Hansen, G., von Mutius, E., Gaede, K. I., Herzmann, C. & Zabel, P., The all age asthma cohort (ALLIANCE)- from early beginnings to chronic disease: a longitudinal cohort study. 20.08.2018, in : BMC PULMONARY MEDICINE. 18, 1, S. 140

Schupp, J. C., Freitag-Wolf, S., Bargagli, E., Mihailović-Vučinić, V., Rottoli, P., Grubanovic, A., Müller, A., Jochens, A., Tittmann, L., Schnerch, J., Olivieri, C., Fischer, A., Jovanovic, D., Filipovic, S., Videnovic-Ivanovic, J., Bresser, P., Jonkers, R., O'Reilly, K., Ho, L-P., Gaede, K. I. &41 mehr, Phenotypes of organ involvement in sarcoidosis. 25.01.2018, in : The European respiratory journal. 51, 1

Zimmermann, A., Knecht, H., Häsler, R., Zissel, G., Gaede, K. I., Hofmann, S., Nebel, A., Müller-Quernheim, J., Schreiber, S. & Fischer, A., Atopobium and Fusobacterium as novel candidates for sarcoidosis-associated microbiota. 2017, in : The European respiratory journal. 50, 6

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Medicine

BioMaterialBank Nord

The BioMaterialBank Nord (BMB Nord) supports biomedical research in pulmonary medicine. The BMB North gains, processes, collects, stores and manages biomaterial such as blood, tissue, cells of patients and volunteers who participate in studies conducted by their member institutions. The biomaterial is analyzed by scientists.

The BMB Nord acts as link between patient care and basic science. Moreover, BMB North offers consulting in planning of translational research projects in the field of pulmonary medicine. Upon request, BMB North provides research groups with pseudonymised clinical information. Scientific analysis lead to conclusions about lung diseases and their progression, which enables improvement of diagnostics and therapy.

The publicly funded BioMaterialBank Nord relies on the support of sample donors. Without the support of patients and volunteers medical research cannot improve understanding, recognition and treatment of diseases.

The focus of BioMaterialBank North is on transmissible and non-communicable lung diseases.

The BMB Nord offers:

- Sample and data-related consulting and planning for scientists and concept development for research projects (grant application)
- Development of comprehensive consent declarations, such as the so-called broad consent for adults as well as parents, children and adolescents
- Processing of biomaterial samples in accordance with existing standard operating procedures or after adaptation to the respective research project
- Processing of blood samples and their derivatives (e.g. whole blood, buffy coat, serum, plasma, blood cells) various smears, nasal and lung brushing, bronchoalveolar lavage fluid and cells, tissue samples (cryosamples formalin or HOPE-fixed), urine samples, sputum and saliva samples, pathogens and their DNA and others.
- Pre-analytics such as blood count, lung lavage cytology etc.
- Storage, administration and provision for individual and collaborative science projects
- Isolation of primary cells

Internal and external collaboration

Internal collaboration
Bionanalytische Chemie: D. Schwudke
Immunbiophysik: A. Schromm
Mikrobielle Grenzflächen: N. Reiling
Zelluläre Pneumologie: C. Stamme
Studienzentrum: C. Herzmann
Klinische und Molekulare Allergologie: U. Jappe
Mukosale Immunologie & Diagnostik: A. Frey
Frühkindliche Asthmaprägung: S. Krauss-Etschmann
Experimentelle Pneumologie: H. Fehrenbach
Biochemische Immunologie: F. Petersen
Asthma-Exazerbation & Regulation: M. Wegmann

Selected External collaboration
J. Müller-Quernheim, Freiburg
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M. Nauck, Greifswald
M. Krawczak, Kiel
PD Dr. D. Drömann, Lübeck
H. Watz, Großhansdorf
J. Habermann, Lübeck
T. Illig, Hannover
K. Günther, Bremen
M. Kiehntopf, Jena

Funding

Deutsches Zentrum für Lungenforschung (BMBF)
Probenqualität in Biobanken (DFG)
DZL CT PLBV ‚Lippenbremse' (DZL, BMBF)

TRANSLATIONAL RESEARCH

NTM
COPD

CLINICAL TRIALS

ASTHMA
TUBERCULOSIS

Head

- PD Dr. Christian Herzmann

Members

- Study Nurses:
  Andrea Glaewe
  Johanna Döhling
  Lenka Krabbe
- Technical Assistance:
  Laura Gruner

Forschungszentrum Borstel
Leibniz Lungenzentrum

Medicine

Center for Clinical Studies

Highlights

Pursed Lip Breathing Ventilation Trial started

The German Center for Lung Research granted funds for a prospective randomised trial to evaluate the effect of a new non-invasive ventilation mode imitating the pursed lip breathing technique in patients with advanced COPD. The ventilation algorithm was developed and is patented in the department of sleep medicine of the Medical Clinic Borstel (Dr. Rüller). Four German hospitals participate in this ongoing study, co-ordinated by the Center for Clinical Studies and the BioMaterialBank North. First results are expected in 2021.

DZIF DOPPIO Study initiated

The DZIF study DOPPIO is a prospective multicenter study of vaccine efficacy in haemodialysis patients. The aim is to compare pneumonia rates between newly vaccinated patients and those vaccinated against pneumococcal infection 2 years ago. 884 patients will be enrolled until May 2019. The Center for Clinical Studies in Borstel is a member of the DZIF CTU. It is the second largest centre in this study.

Lung microbiota at the interface between airway epithelium and environment

This study is funded by the Leibniz Association. It investigates respiratory microbiota in smokers and ex-/non-smokers. Bronchoalveolar lavage samples from non-smoking volunteers and from smokers who quit smoking were analysed.

Selected publications

Silkoff PE, […] Herzmann C et al. Toll-like receptor 3 blockade in rhinovirus-induced experimental asthma exacerbations: A randomized controlled study. J Allergy Clin Immunol. 2018 Apr;141(4):1220-1230

Herzmann C, Ernst M, Lange C et al. Pulmonary immune responses to Mycobacterium tuberculosis in exposed individuals. PLoS One. 2017 Nov 10;12(11):e0187882

Salzer HJF, […] Herzmann C et al. Evaluation of Galactomannan Testing, the Aspergillus-Specific Lateral-Flow Device Test and Levels of Cytokines in Bronchoalveolar Lavage Fluid for Diagnosis of Chronic Pulmonary Aspergillosis. Front Microbiol. 2018 Oct 2;9:2223. doi: 10.3389/fmicb.2018.02223.

Grant support

VolkswagenStiftung, German Center for Infection Research, German Center for Lung Research, Niedersächsischer Verein zur Bekämpfung der Tuberkulose und Lungenerkrankungen, Bundesministerium für Bildung und Forschung, INSMED Pharmceuticals, Otuska Pharmaceuticals, Leibniz Association

Internal and external collaboration

Internal collaborations

Experimental Asthma Research (S. Krauss-Etschmann); Mouse Models Asthma (M. Wegmann); Mucosal Immunology (A. Frey, B. Frey); Clinical and Experimental Pathology (T. Goldmann); Bioanalytical Chemistry (D. Schwudtke); Immunobiophysics (A. Schromm); Microbial Interface Biology (N. Reiling); Cellular Pneumonology (C. Stamme); Clinical Infectious Diseases (C. Lange); BioMaterialBank North (K. Gaede)

External collaborations

Medical Laser Center Lübeck; Hypertech Laser Lübeck; Insmed, Bridgewater, NJ, USA; University Hospital Marburg / Gießen; German Center for Infection Research; German Center for Lung Research; LungenClinic Großhansdorf; Brandenburg Antiinfektiva GmbH; Thoraxklinik Heidelberg; Medizinische Hochschule Hannover; University Hospital Schleswig-Holstein Campus Lübeck; Otsuka Pharma GmbH; Leibniz-Institute German Collection of Microorganisms and Cell Cultures; Technical University Munich; Leibniz Institute of Photonic Technology Jena

PRIMARY HUMAN CELLS

TGF-SIGNALING

EPIGENETICS

TRANSCRIPTOMICS

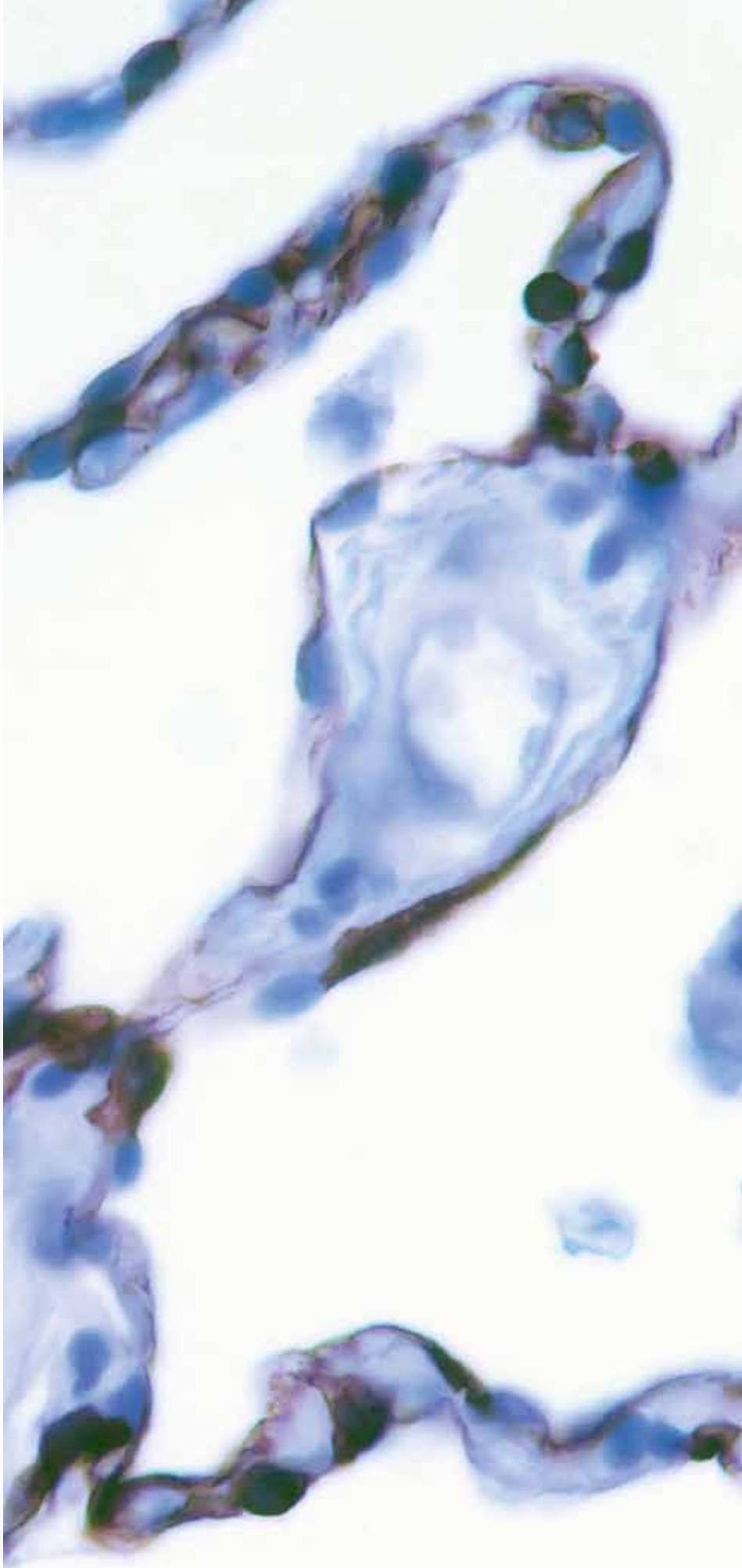
MOLECULAR DIAGNOSTICS

IMMUNE CHECKPOINT INHIBITORS

EX VIVO

LIPIDOMICS

MULTISPECTRAL IMAGING



Head

- Prof. Dr. Sven Perner

Members

- Prof. Dr. Torsten Goldmann
- PD Dr. Verena Sailer
- Dr. Florian Stellmacher
- Dr. Rosemarie Krupar
- Dr. Sebastian Marwitz
- Dörte Nitschkowski
- Ulrike Büchsler
- Gabriele Cornehls
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- Iris Jonas
- Angela Kelp
- Ghanima Alexa Korf
- Sousanna Kotanidou
- Britta Liebe
- Bettina Lühr
- Vladimira Oldenburg
- Silke Perner
- Christian Rosero
- Jasmin Tiebach
- Wenzel Vogel
- Rolf Warneke

Forschungszentrum Borstel
Leibniz Lungenzentrum

Medicine

Pathology

Mission

Exzellente Diagnostik und Forschung in der Pneumologie.
Zentrales Werkzeug sind funktionelle Studien im humanen System wobei verschiedene Gewebekulturmodelle und humane Primärzellen zum Einsatz kommen. Screening-Untersuchungen auf Transkriptom-Ebene dienen zur Identifizierung von Biomarkern und therapeutischen Zielstrukturen. Die Pathologie ist ein interdisziplinäres Fachgebiet mit großen Berührungsflächen zu beiden Programmbereichen des Zentrums.

We are devoted to excellent diagnostics and research in pneumology.
Central experimental tools remain functional human tissue culture models and primary human cells. For biomarker identification and target development, we apply inhouse transcriptome analyses.
Pathology is an integrative field with a broad interface to other disciplines, supporting both priority areas in our center and beyond.

Most important findings

Identification of new biomarkers for COPD

As part of a large screening study in different human materials, we conducted a study in COPD-tissues applying transcriptomics and epigenetics (DZL-COPD-GE2). We identified six new target genes, Aquaporin 3 (AQP3), extracellular matrix protein 1 (ECM1), four and a half LIM domain 1 (FHL1), milk fat globule epidermal growth factor 8 (MFGE8, lactadherin), phosphodiesterase 4D-interacting protein (PDE4DIP), and creatine transporter SLC6A8. All six proteins were allocated to distinct cell types by immunohistochemistry (Figure 1). Upon stimulation by cigarette smoke extract, human type II pneumocytes showed a dose-dependent down-regulation of MFGE8, while ECM1 and FHL1 also tended to be down-regulated. MFGE8 (image on the left) turned out to be an interesting new candidate gene in COPD deserving further studies (1).

The role of Alveolar Epithelial Cells Type II in EMT and fibrosis

We investigated TGFβ-signaling in cytoskeleton remodeling and Epithelial-Mesenchymal-Transition by Alveolar Epithelial Cells Type II and tested the hypothesis if these cells are capable of trans-differentiation and production of pro-fibrotic collagen. A TGFβ-responsive fingerprint was found and investigated for mutual interactions. Interaction modules exhibited enrichment of genes that favor actin cytoskeleton remodeling, differentiation processes and collagen metabolism. Cross-validation of the TGFβ-responsive fingerprint in an independent IPF dataset revealed overlap of genes and supported the direction of regulated genes and TGFβ-specificity. Primary human alveolar epithelial cells type II seem undergo a TGFβ-dependent phenotypic change, exhibit differential expression of EMT markers in vitro and acquire the potential to produce collagen (2).

Selected publications

1. Heinbockel L, Marwitz M, Schromm A, Watz H, Kugler Ch, Ammerpohl O, Schnepf K, Rabe KF, Droemann D, Goldmann T. Identification of novel target genes in human lung tissue involved in Chronic Obstructive Pulmonary Disease. Int J COPD 2018:13 2255–2259.
2. Goldmann T, Zissel G, Watz H, Drömann D, Reck M, Kugler Ch, Rabe KF, Marwitz M. Human alveolar epithelial cells type II are capable of TGFβ-dependent Epithelial-Mesenchymal-Transition and collagen-synthesis. Resp Res 2018 19:138
3. Marwitz S, Scheufele S, Perner S, Reck M, Ammerpohl O, Goldmann T. Epigenetic modifications of the immune-checkpoint genes CTLA4 and PDCD1 in Non-Small Cell Lung Cancer results in increased expression. Clinical Epigenetics 2017, 9:51.
4. Marwitz M, Heinbockel L, Scheufele S, Kugler Ch, Reck M, Rabe KF, Perner S, Goldmann T, Ammerpohl O. Fountain of youth for squamous cell carcinomas? On the epigenetic age of NSCLC and corresponding tumor-free lung tissues. 2018 Int J Cancer.
5. George J, et al., Perner S, Travis WD, Haas SA, Olivier M, Foll M, Büttner R, Hayes DN, Brambilla E, Fernandez-Cuesta L, Thomas RK. Integrative genomic profiling of large-cell neuroendocrine carcinomas reveals distinct subtypes of high-grade neuroendocrine lung tumors. Nat Commun. 2018 03 13;9(1):1048
6. Schmitt A, Knittel G, Welcker D, Yang TP, George J, Nowak M, Leeser U, Buttner R, Perner S, Peifer M, Reinhardt HC. ATM deficiency is associated with sensitivity to PARP1 and ATR inhibitors in lung adenocarcinoma. Cancer Res. Jun 1;77(11):3040-3056.

Grant support

Deutsche Forschungsgemeinschaft (DFG)
PE1179/15-1. Die Rolle der TRIM-Proteine in der Kolonisierung des Knochens und der Modulation des Knochenmilieus im metastasierten Prostatakarzinom. *Sven Perner*
PE1179/11-1. 1. The Biological and Clinical Relevance of EVI1 Expression in Prostate Carcinogenesis. Sven Perner
PE 1179/9-1: The role of the Mediator complex subunits MED12 and MED15 in the development of androgen-dependent prostate cancer into androgen-independent castration resistant prostate. *Sven Perner*
DR797/3-1. Die Rolle des TGF-ß Pseudorezeptors Bambi bei der Lungenfibrose. *Daniel Drömann, Torsten Goldmann*
BMBF
Deutsches Zentrum für Lungenforschung (DZL), Disease Area Lung Cancer (LC, COPD, Asthma/Allergy). *Torsten Goldmann*
Deutsches Zentrum für Lungenforschung (DZL), Platform Biobanking. *Karoline Gaede, Torsten Goldmann*
Deutsches Zentrum für Lungenforschung (DZL), DZL-Labor für humane Primärzellen und Gewebekulturmodelle. *Torsten Goldmann, Heinz Fehrenbach*
Andere
EUROIMMUN Medizinische Labordiagnostika AG: "EUROPathologie System". *Sven Perner*
Bristol-Myers Squibb, Nr. CA209-8C7. "PD-L1 expression as the hallmark of differential genomic and transcriptomic profile of "hot" and "cold" NSCLC". *Sven Perner*

Figure 1. Epigenetic and gene expression analyses of immune checkpoint molecules in human NSCLC and corresponding control tissues.
HumanMethylation450k Bead Chip data, obtained from 39 tumor tissues and their corresponding controls, identified multiple CpG loci differentially methylated in CTLA4 (A) and PDCD1 (B) genes (FDR < 0.01, T-test). For data validation bisulfite pyrosequencing (C) of an independent patient cohort (n=20) was performed, confirming differential methylation of tumor tissues detected previously via HumanMethylation450k BeadChip (D) at selected loci (CTLA4: cg08460026 and cg26091609, PDCD1: cg25798782). Heatmap.2 was utilized for hierarchical cluster analysis and data visualization. Blue bars on top of heatmaps: tumor-free lung tissue, yellow bars: tumor tissue. Transcriptome analyses of 18 patients showed elevated transcript expression of CTLA4 (E), PDCD1 (F). Transcriptome data was analyzed via paired T-test of quantile-normalized, relative gene expression values with p≤0.05 (=*) regarded as significant.
Clin Epigenetics 2017, 9:51

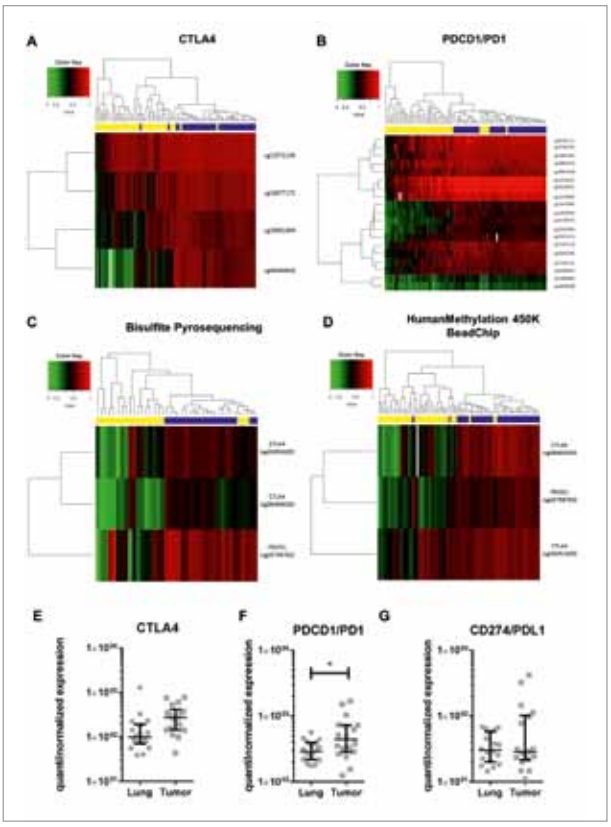
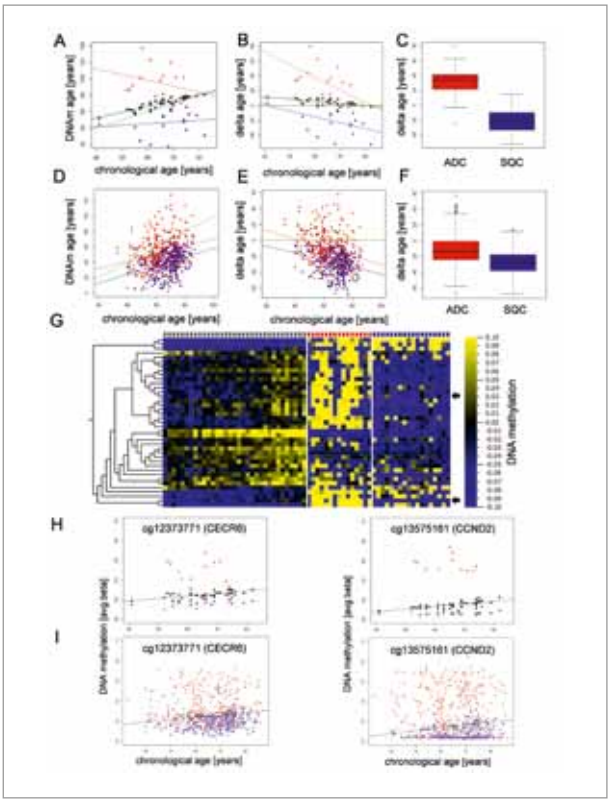


Figure 2. NSCLC tissues display epigenetic age distortions.
Dot plot (A) and Bland-Altman-Plot (B) showing correlations between the chronological age of the host and the epigenetic DNAm age of matched tumor tissues from normal control specimens (black dots), adenocarcinomas (red) and squamous cell carcinomas (blue). (C) Boxplot of the deviations of DNAm age from the chronological age in adenocarcinomas (ADC, red) and squamous cell carcinomas (SQC, blue). The delta age of cancer entities differs significantly (p-value<1.32x10-5, Wilcoxon rank sum test). **Validation cohort (public available TCGA data set):** Dot plot (D) and Bland-Altman-Plot (E) showing correlations between the chronological age and the epigenetic DNAm age of adenocarcinomas (red) and squamous cell carcinomas (blue) in an independent validation cohort. (F) The delta age of adenocarcinomas (ADC, red) and squamous cell carcinomas (SQC, blue) differs significantly (p-value<2.2x10-16, Wilcoxon rank sum test). The delta age is calculated as DNAm age [years]- chronological age [years]. (A, B, C, D) Black, red and blue lines represent the regression lines for control samples, adenocarcinomas and squamous cell carcinomas, respectively. The green line indicates a perfect match of chronological age and DNAm age (chronological age=DNAm age). **DNA methylation changes of distinct CpG loci associated with aging and alterations of these patterns in lung cancer (G-I).** Heatmap (G) of CpG loci with changing DNA methylation during aging. Pearson correlation analysis of avg. beta values of all CpG loci included in this study in normal lung tissue (controls) with the chronological age of the sample donors identified 39 loci with |Pearson correlation coefficients|>0.6 (Table 1). The DNA methylation values of these loci are presented separately for controls (gray squares, top lane), adenocarcinoma (red squares) and squamous cell carcinoma (blue squares). Samples are ordered according to donors' chronological ages. Heatmap: blue, low DNA methylation values; yellow, high DNA methylation values, for visualization purposes, the DNA methylation values were normalized to zero for each locus, and the bar below the heatmap indicates the range and color code. Arrows indicate randomly selected loci detailed in H and I. (H) Detailed presentation of data from 2 randomly selected loci. DNA methylation of cg12373771 (CECR6) and cg13575161 (CCND2) increases during aging in control samples (black spheres). Compared to the controls, squamous cell carcinoma (blue spheres) are characterized by lower DNA methylation values, while DNA methylation is increased in adenocarcinoma (red spheres), putatively reflecting decelerated and accelerated aging, respectively. (I) A similar pattern is seen also in an independent dataset. Int. J. Cancer: 143, 3061–3070 (2018) © 2018



Epigenetics and transcriptomics of immune checkpoint molecules in NSCLC

We analyzed epigenetic modifications of PDCD1 (PD1), CD274 (PD-L1), and CTLA4 in NSCLC tissues from 39 patients. Results were correlated with transcriptome data. Significant differences in the CpG-methylation patterns between tumor tissues and matched controls were observed for CTLA4 and PDCD1 (PD1) showing a decreased methylation of these genes compared to matched tumor-free tissues from the same

patients (Figure 1). Results were confirmed by bisulfide sequencing in an independent validation cohort. Hypomethylation also resulted in increased expression of these genes as shown by transcriptome data. These epigenetic pathways as a hallmark of NSCLC might be useful to generate more precise diagnostic approaches in the future (3).

Epigenetics and transcriptomics of aging in NSCLC

Recently, it was shown that the methylation of certain CpG dinucleotides strongly correlates with chronological age. We investigated these molecular age loci in non-small cell lung cancer (NSCLC) tissues from patients with adenocarcinomas (AC) and squamous cell carcinomas (SQC) as well as in matched tumor-free lung tissue. In both NSCLC subtypes, the calculated epigenetic age did not correlate with the chronological age. In particular, SQC exhibited rejuvenation compared to the corresponding normal lung tissue as well as with the chronological age of the donor (Figure 2). Moreover, the younger epigenetic pattern was associated with a trend toward stem cell-like gene expression patterns. These findings show deep phenotypic differences between the tumor entities AC and SQC, which will serve for a better understanding of the biology to develop new therapeutic and diagnostic approaches (4).

Distinct subtypes of high-grade neuroendocrine lung tumors

Pulmonary large-cell neuroendocrine carcinomas (LCNECs) have similarities with other lung cancers, but their precise relationship has remained unclear. Here we perform a comprehensive genomic (n=60) and transcriptomic (n=69) analysis of 75 LCNECs and identify two molecular subgroups: "type I LCNECs" with bi-allelic TP53 and STK11/KEAP1 alterations (37%), and "type II LCNECs" enriched for bi-allelic inactivation of TP53 and RB1 (42%). Despite sharing genomic alterations with adenocarcinomas and squamous cell carcinomas, no transcriptional relationship was found; instead LCNECs form distinct transcriptional subgroups with closest similarity to SCLC. While type I LCNECs and SCLCs exhibit a neuroendocrine profile with ASCL1high/DLL3high/NOTCHlow, type II LCNECs bear TP53 and RB1 alterations and differ from most SCLC tumors with reduced neuroendocrine markers, a pattern of ASCL1low/DLL3low/NOTCHhigh, and an upregulation of immune-related pathways. In conclusion, LCNECs comprise two molecularly defined subgroups, which may allow stratified targeted treatment of high-grade neuroendocrine lung tumors (5).

ATM Deficiency and PARP1- and ATR Inhibitors in Lung Adenocarcinoma

Here we demonstrate that bi-allelic Atm deletion in mouse models of Kras-mutant lung adenocarcinoma does not affect cisplatin responses. In marked contrast, Atm-deficient tumors displayed an enhanced response to the topoisomerase-II poison etoposide. Moreover, Atm-deficient cells and tumors were sensitive to the PARP inhibitor olaparib. This actionable molecular addiction to functional PARP1 signaling was preserved in models that were proficient or deficient in p53, resembling standard or high-risk genetic constellations, respectively. Atm deficiency also markedly enhanced sensitivity to the ATR inhibitor VE-822. Our results provide a rationale to profile human tumors for disabling ATM mutations, given their impact on PARP1 and ATR inhibitors (6).

Internal and external collaboration

Inhouse

C. Lange, S. Krauss-Etschmann, S. Niemann, T. Gutsmann, A. Schromm, D. Schwudke, H. Heine, N. Reiling, U. Schaible, F. Petersen, H. Fehrenbach, U. Jappe, K. Duda, A. Frey, P. Zabel, J. Behrends

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International

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Yerevan State University, Armenia, A. Mkhitaryan
University of Dohuk, Iraq, M. Abdullah
Universitätsspital Basel, C. Lengerke
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Universitätsspital Zürich, A. Soltermann, H. Moch

Core Facility

Fluorescence Cytometry

FLOW CYTOMETRY
LIVE CELL IMAGING

JOINT RESEARCH PROJECTS

SUPPORT

MULTIPARAMETER ANALYSIS

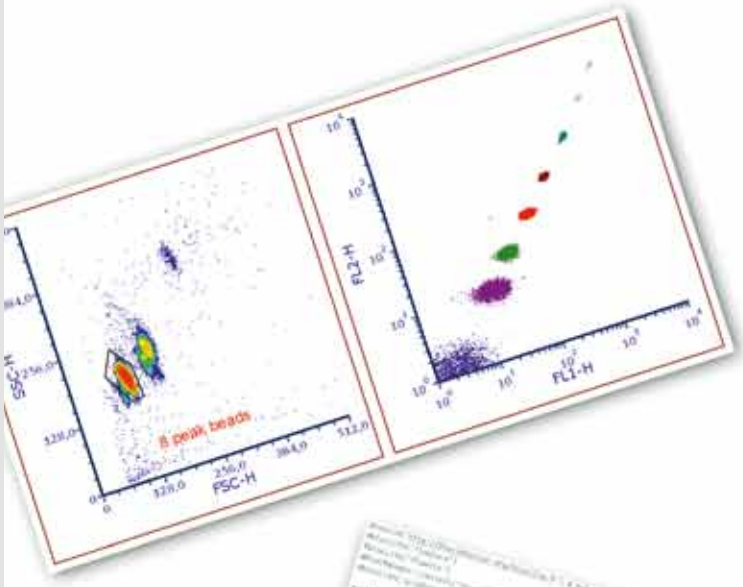
FRET/FRAP

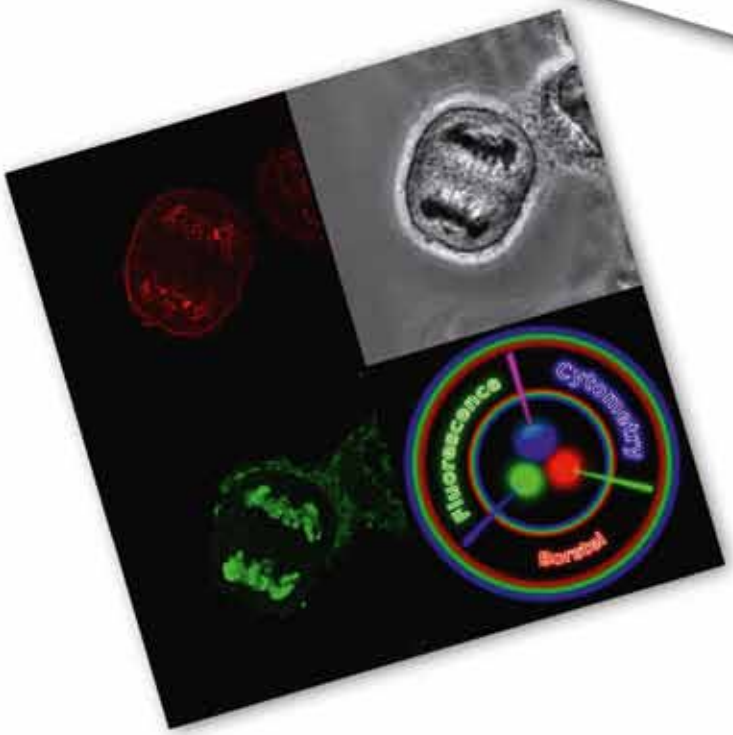
CONFOCAL MICROSCOPY

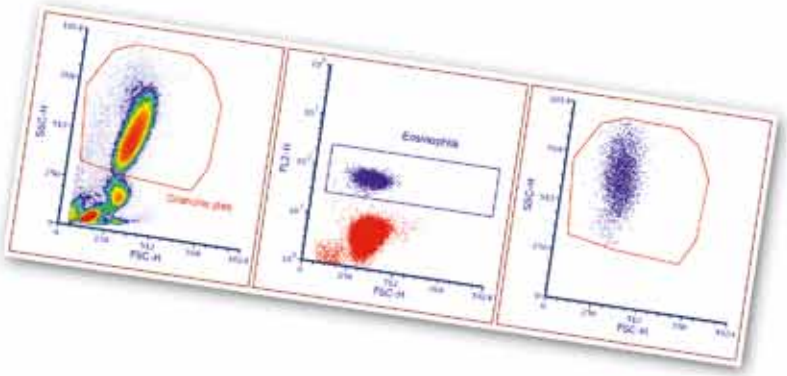
CELL SORTING

BSL3

LECTURES
CONSULTATION
PRACTICAL TRAINING







Head

- Dr. Jochen Behrends
- Dr. Thomas Scholzen

Members

- Martina Hein

Forschungszentrum Borstel
Leibniz Lungenzentrum

Core Facility

Fluorescence Cytometry

Mission

Die Zentrale Einheit Fluoreszenz-Zytometrie bietet FZB-Mitarbeiterinnen und Mitarbeitern Zugang zu modernen Fluoreszenz-basierten Techniken für die Analyse von molekularen und zellulären Wechselwirkungen in den Bereichen Entzündungs-, Infektions- und Allergieforschung. Zur phänotypischen Charakterisierung einzelner Zellen ist die Einheit mit einer Reihe unterschiedlicher Durchflusszytometer und Fluoreszenzmikroskope ausgestattet. Die Fluoreszenzmikroskopie eignet sich gut, um eine begrenzte Anzahl von Zellen im Detail zu untersuchen. Im Gegensatz dazu erlaubt die Durchflusszytometrie eine große Anzahl von Suspensionszellen quantitativ zu analysieren. Darüber hinaus ermöglichen Zellsortierer, einzelne Populationen lebender Zellen nach phänotypischen Markern zu trennen. Die Zentrale Einheit bietet nicht nur Zugang zu den Instrumenten, sondern auch praktische Schulungen vor Ort an, ergänzt durch individuelle Beratung und eine umfangreiche theoretische Schulungsreihe.

The core facility Fluorescence Cytometry provides access to modern fluorescence-based techniques for the analyses of molecular and cellular interactions in the fields of inflammation, infection and allergy. To phenotypically characterize individual cells, the facility is equipped with various flow cytometers and fluorescence microscopes. Fluorescence microscopy is well suited to study a limited number of cells in great detail. In contrast, flow cytometry allows analyzing large numbers of suspension cells in a quantitative manner. Moreover, cell sorters can be used to separate individual populations of living cells according to phenotypic markers. The core facility offers access to the instruments including on-site training for RCB employees. This is complimented by theoretical training courses and individual consulting conducted by members of the Fluorescence Cytometry.

Most important features

Fluorescence Microscopy

The microscopes of the Fluorescence Cytometry core facility are equally well equipped for the visualization of fixed and living cells. The Olympus IX-81 is an imaging system with a motorized stage and a highly sensitive CCD camera. It can obtain images with transmission light and four fluorescence channels. Due to an incubation chamber with CO_2 gassing, temperature and humidity regulation, it is especially well suited for live cell imaging. The Leica TCS SP5 is an inverse confocal laser scanning microscope equipped with five fluorescence detectors. Three of these are conventional photomultipliers, while two are highly sensitive hybrid detectors. The SP5 also contains an incubation chamber with temperature regulation and CO_2 gassing. The system can not only be used for cell and tissue imaging but also for the active manipulation of cells with laser light. An example of this is the FRAP (fluorescence recovery after photobleaching) technique that allows the determination of protein mobility within living cells. A recently purchased deconvolution workstation featuring the innovative Leica "Lightning" software allows the generation of sharper images with increased resolution (see figure 1).

Service

The core facility Fluorescence Cytometry offers service for scientists to carry out high speed cell sorting on our BD FACSAriaIIu flow cytometer as well as assistance for using the S3 Cell Sorter within the BSL3 facility. The core facility is also available for consultation about the design of individual experiments, the analysis of data and can also take on joint research projects.

Selected publications

Erdmann H, Behrends J, Ritter K, Hölscher A, Volz J, Rosenkrands I, Hölscher C. The increased protection and pathology in Mycobacterium tuberculosis-infected IL-27R-alpha-deficient mice is supported by IL-17A and is associated with the IL-17A-induced expansion of multifunctional T cells. Mucosal Immunol. 2018 May 4.

Stüve P, Minarrieta L, Erdmann H, Arnold-Schrauf C, Swallow M, Guderian M, Krull F, Hölscher A, Ghorbani P, Behrends J, Abraham WR, Hölscher C, Sparwasser TD, Berod L. De Novo Fatty Acid Synthesis During Mycobacterial Infection Is a Prerequisite for the Function of Highly Proliferative T Cells, But Not for Dendritic Cells or Macrophages. Front Immunol. 2018 Apr 5;9:495.

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Offermann A, Vlasic I, Syring I, Vogel W, Ruiz C, Zellweger T, Rentsch CA, Hagedorn S, Behrends J, Nowak M, Merseburger A, Bubendorf L, Kirfel J, Duensing S, Shaikhibrahim Z, Perner S. MED15 overexpression in prostate cancer arises during androgen deprivation therapy via PI3K/mTOR signaling. Oncotarget. 2017 Jan 31;8(5):7964-7976.

Schmok E, Abad Dar M, Behrends J, Erdmann H, Rückerl D, Endermann T, Heitmann L, Hessmann M, Yoshimura A, Rose-John S, Scheller J, Schaible UE, Ehlers S, Lang R, Hölscher C. Suppressor of Cytokine Signaling 3 in Macrophages Prevents Exacerbated Interleukin-6-Dependent Arginase-1 Activity and Early Permissiveness to Experimental Tuberculosis. Front Immunol. 2017 Nov 10; 8:1537.

Schwager C, Kull S, Behrends J, Röckendorf N, Schocker F, Frey A, Homann A, Becker WM, Jappe U. Peanut oleosins associated with severe peanut allergy - Importance of lipophilic allergens for comprehensive allergy diagnostics. J Allergy Clin Immunol. 2017 Mar 22.

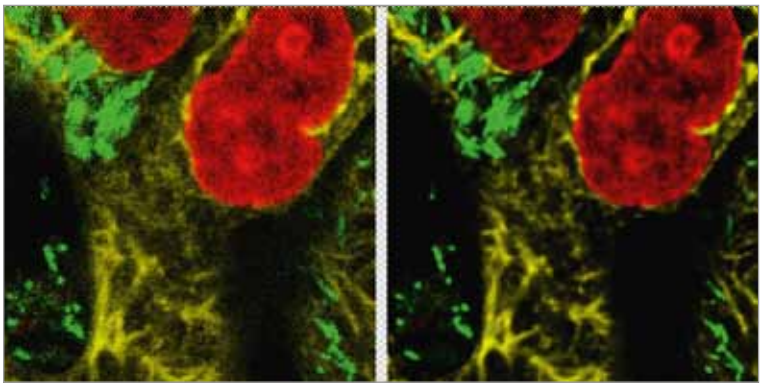


Figure 1. High-resolution micrograph enlargement (20 µm length) obtained by confocal microscopy (left). Individual *Stenotrophomonas maltophilia* bacteria (green) can be better distinguished after "*lightning*" deconvolution (right image). The noise is reduced and the cytokeratin fibers (yellow) appear clearer. Nuclei were counterstained in DAPI (red). Micrograph courtesy of Dr. Uwe Mamat.

Flow cytometry

For the quantitative analysis of suspension cells, five flow cytometers are available. The multicolor “flagship” is the BD LSR II, which has been upgraded in summer 2018. With its new detectors, it can now analyze up to 18 fluorescence channels in parallel. On top of this, an additional used BD LSR II was acquired and upgraded to provide an identical backup system (see figure 2). If fewer channels are needed, analysis can also be performed using a BD FACSCalibur cytometer (four fluorescence channels), a MACSQuant Analyzer 10 (eight fluorescence channels) and a BD FACSArray bioanalyzer (four channels). Additionally, within the BSL3 facility a BD FACSCantoII cytometer (eight fluorescence channels) is available. However, for functional studies it is often not sufficient to merely analyze cells but also to separate them according to phenotypic markers. If highly purified cell populations are required, the core facility Fluorescence Cytometry offers a cell sorting service employing a BD FACSAria IIu sorter. In addition, for cell sorting within the BSL3 facility, a user operated Bio-Rad S3 Cell Sorter is provided. To achieve cost-cutting operation of the various flow cytometers within the facility, part of the maintenance and repair is carried out by the Fluorescence Cytometry staff.

Core Facility

Fluorescence Cytometry

Figure 2. Two high-end analyzers BD LSRII - The multicolor "flagship", which has been upgraded with its new detectors. It can now analyze up to 18 fluorescence channels in parallel. An additional used BD LSR II was acquired and upgraded to provide an identical backup system.

Training

Practical and theoretical training is an integral part of the Fluorescence Cytometry service concept. The practical training is provided at the instruments for individuals or small groups. For most flow cytometers an initial training of two hours is required, while the basic introduction to the confocal microscope takes at least 3 hours. In addition to the practical instructions, the facility offers a seminar program comprising topics from the fields of flow cytometry and microscopy. This program currently includes 19 different lectures given by members of the Fluorescence Cytometry staff. These lectures are regularly complemented by seminars given by invited speakers.

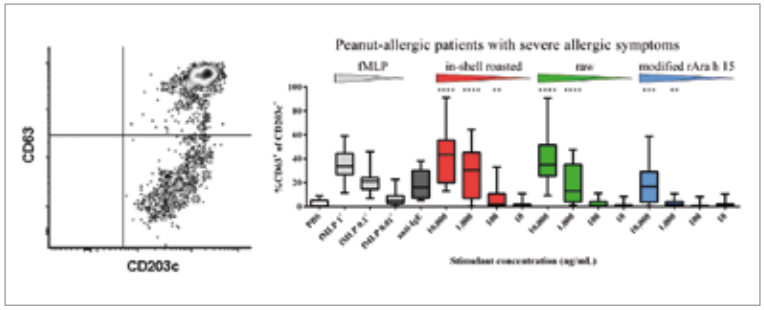


Figure 3. Using a flow cytometric basophil activation test (BAT) to improve the diagnosis of peanut-allergic patients with severe symptoms from peanut-sensitized but tolerant subjects (the CD63+ percentage of CD203+ cells is calculated; joint research project with the research group Clinical and Molecular Allergology, Prof. U. Jappe).

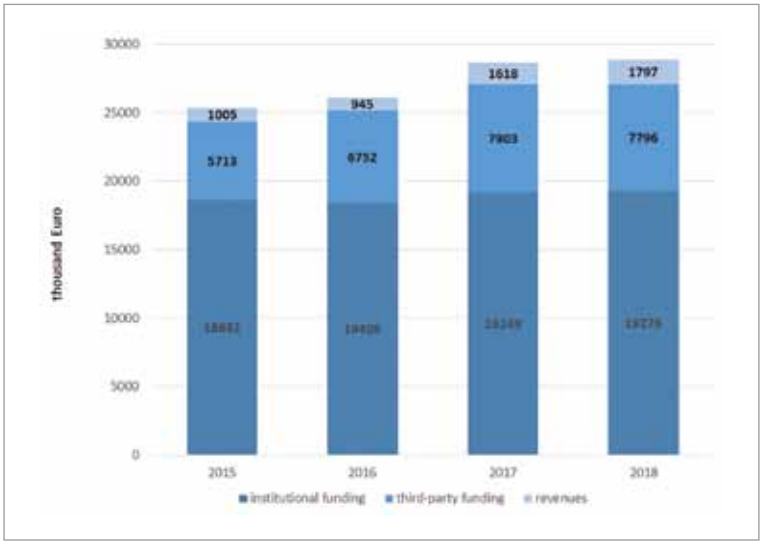
Selected methods

multiparameter analysis of cell populations; cell proliferation/viability assays; FRET/FRAP; co-localization analysis; sorting of different cell types, e.g. DCs, B cells, T cells and various cell lines; metabolic analysis of different Mtb strains; measuring calcium influx.

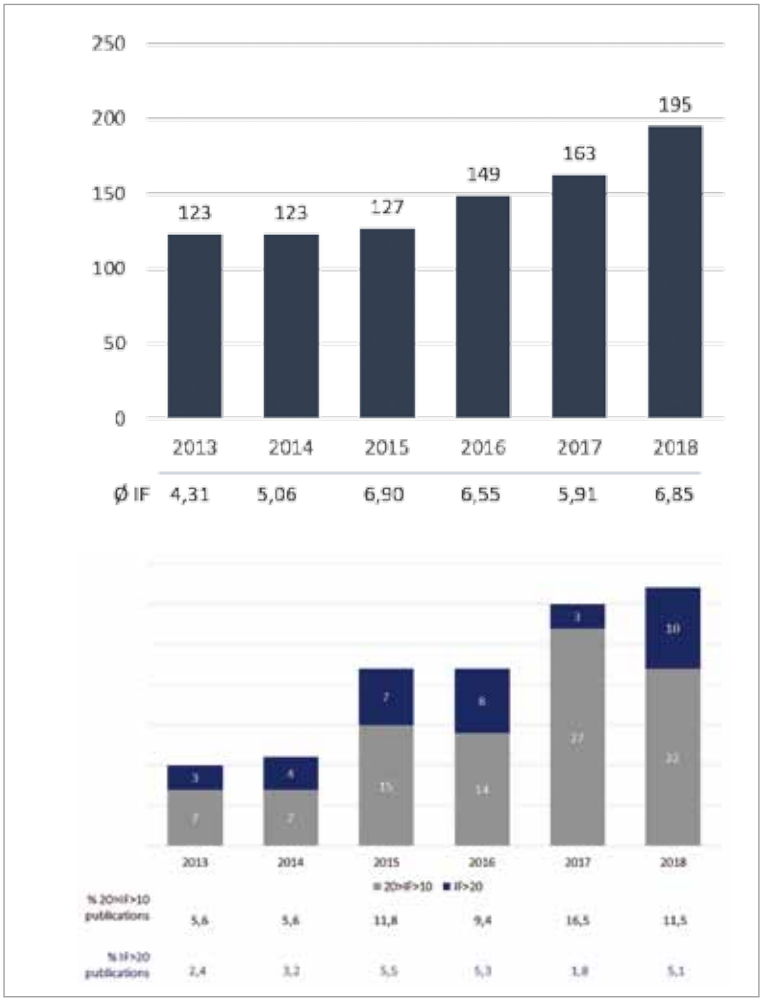
Internal and external collaboration

Internal: Priority area Asthma and Allergy (Innate Immunity, Autoimmunity of the Lung, Biochemical Immunology, Experimental Pneumology, Early Life Origins of CLD, Clinical and Molecular Allergology, Mucosal Immunology and Diagnostic); Priority area Infections (Biophysics, Immunobiophysics, Infection Immunology, Cellular Microbiology, Coinfection, Microbial Interface Biology, Molecular and Experimental Mycobacteriology); Medicine (Pathology).

External: M. Ehlers (University of Lübeck and Medical Center Schleswig-Holstein, Lübeck); W. Handke (DRK-Blutspendedienst NSTOB, Springe); R. Rahmanzadeh (University of Lübeck and Medical Center Schleswig-Holstein, Lübeck); M. Reck, I. Watermann (LungenClinic Grosshansdorf, Großhansdorf); K. Schütze (Johannes Gutenberg-University Mainz, Mainz); T. Schulze (DRK-Blutspendedienst NSTOB, Springe); A. Verschoor (University of Lübeck and Medical Center Schleswig-Holstein, Lübeck).



Funding 2015- 2018



Peer Reviewed Publications

Facts & Figures

National Networks 2017/2018

DFG
- Cluster of Excellence 306 'Inflammation at Interfaces'
- SPP 1580 'Intracellular compartments as places of pathogen-host interactions'
- SPP 2084 µBONE 'Kolonisierung und Interaktionen von Tumorzellen innerhalb der Knochenmikroumgebung'
- IRTG 1911 'Immunregulation der Entzündung bei Allergien und Infektionen'
- GRK 1727 'Modulation von Autoimmunität'

BMBF
- Deutsches Zentrum für Lungenforschung
- Deutsches Zentrum für Infektionsforschung
- NanoColt - "Langzeitwirkung modifizierter Carbon Black Nanopartikel auf gesunde und vorgeschädigte Lungen"
- BMBF Forschungsverbund ANTI-TB
- Förderinitiative "Vor Ort Analytik mit photonischen Verfahren für den Einsatz in den Lebenswissenschaften", Projekt Agadi

Leibniz Agenda
- Leibniz Center Infection
- Leibniz Forschungsverbund (LFV) 'INFECTIONS'21'
- LFV 'Gesundheitstechnologien'
- LFV 'Wirkstoffe und Biotechnologie'
- LFV 'Nanosicherheit'
- EvoLUNG - Leibniz-WissenschaftsCampus, Universität Kiel
- Leibniz WissenschaftsCampus InterACt, Heinrich-Pette-Institut, Universität Hamburg
- Leibniz WissenschaftsCampus KiSOC, Universität Kiel
- Leibniz-Projekt vernetzte Forschung 'Lung microbiota at the interface between airway epithelia and environment'

International Networks 2017/2018

7th EU Frame Program / Horizon 2020
- NAREB - nanotherapeutics for antibiotic resistant emerging bacterial pathogens
- MDR- TBNet (ERANET)
- anTBiotic - Entwicklung von Antibiotika für die Therapie der Tuberklose und neuer Ansätze für die individualisierte Therapie
- WANETAM - Westafrica-Network of Excellence für TB, AIDS und Malaria
- RaPaed-AIDA-TB - Rapid and accurate diagnosis of paediatric TB

European Centre for Disease Prevention and Control
- EUseqMyTB

Gates Foundation
- CrYptic/WellComeTrust - comprehensive resistance prediction of tuberculosis: an international consortium

USAID
- Stop TTH

BMBF
- TB Sequel - Tansania, Mozambique, Gambia, Südafrika
- TB-seqDISK: Molecular test based on next generation sequencing for rapid diagnosis and comprehensive resistance determination in tuberculosis.

BMG
- SeqMDRTB_NET: Network for application of sequencing technology for the fight against resistant tuberculosis in high incidence settings.

International Partner Laboratories
- University of Namibia School of Medicine, Windhoek, Namibia
- Medical College of Xiamen University, China

Facts & Figures

Guest Scientists

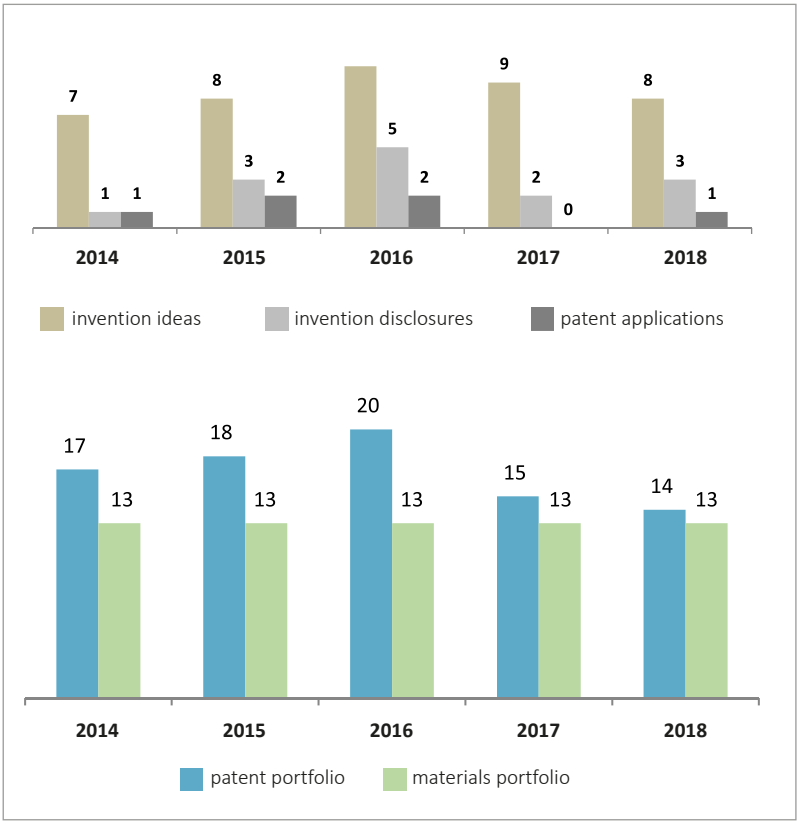
	2017	2018
national	6	5
international	19	9

Conferences / Workshops

2017	2018
37	23

Academic Degree / Professional Qualifications

	2017	2018
Dissertation	10	7
Master of Science	9	8
Bachelor of Science	1	2
Habilitation	1	1
Technicians	11	8



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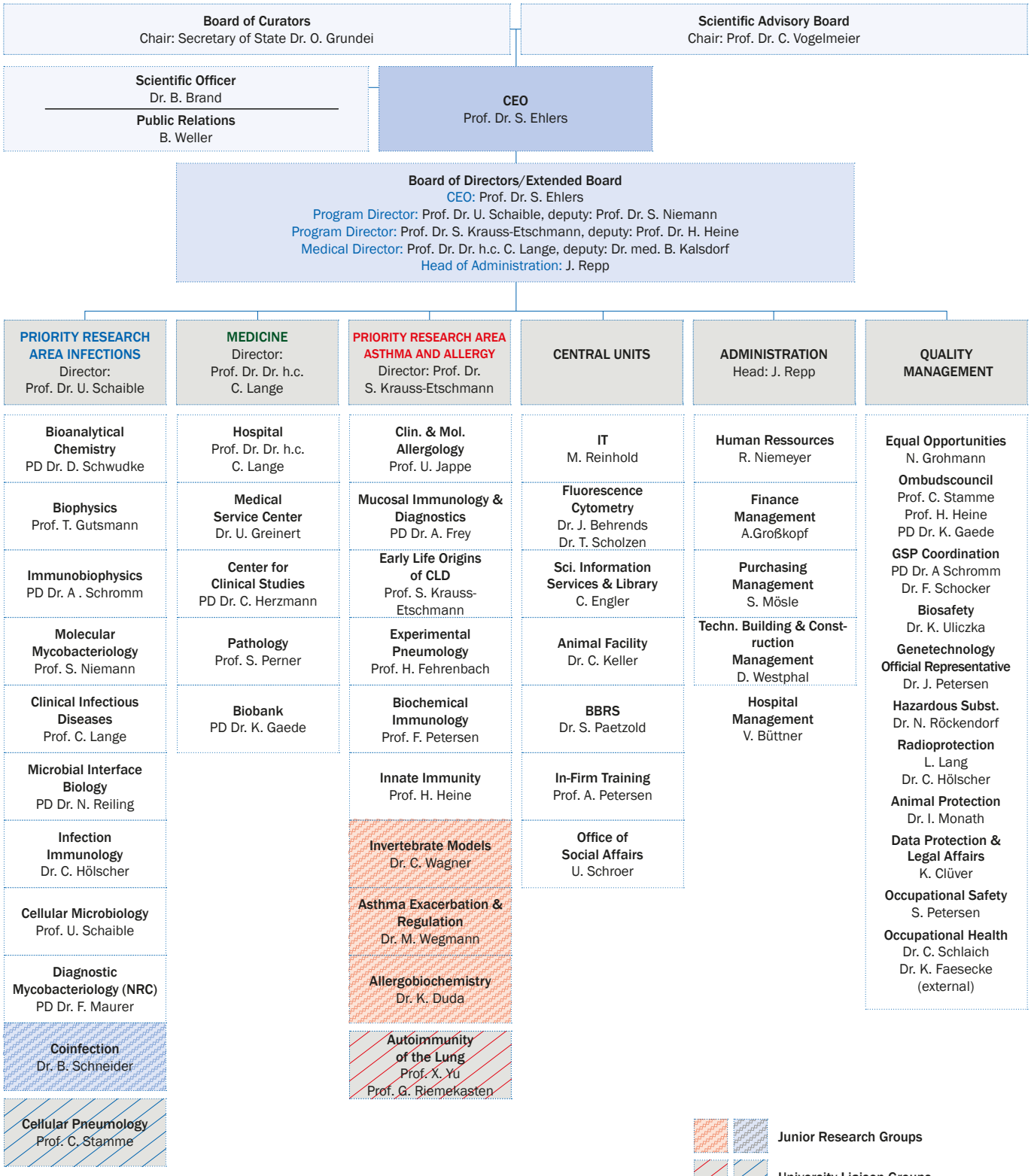
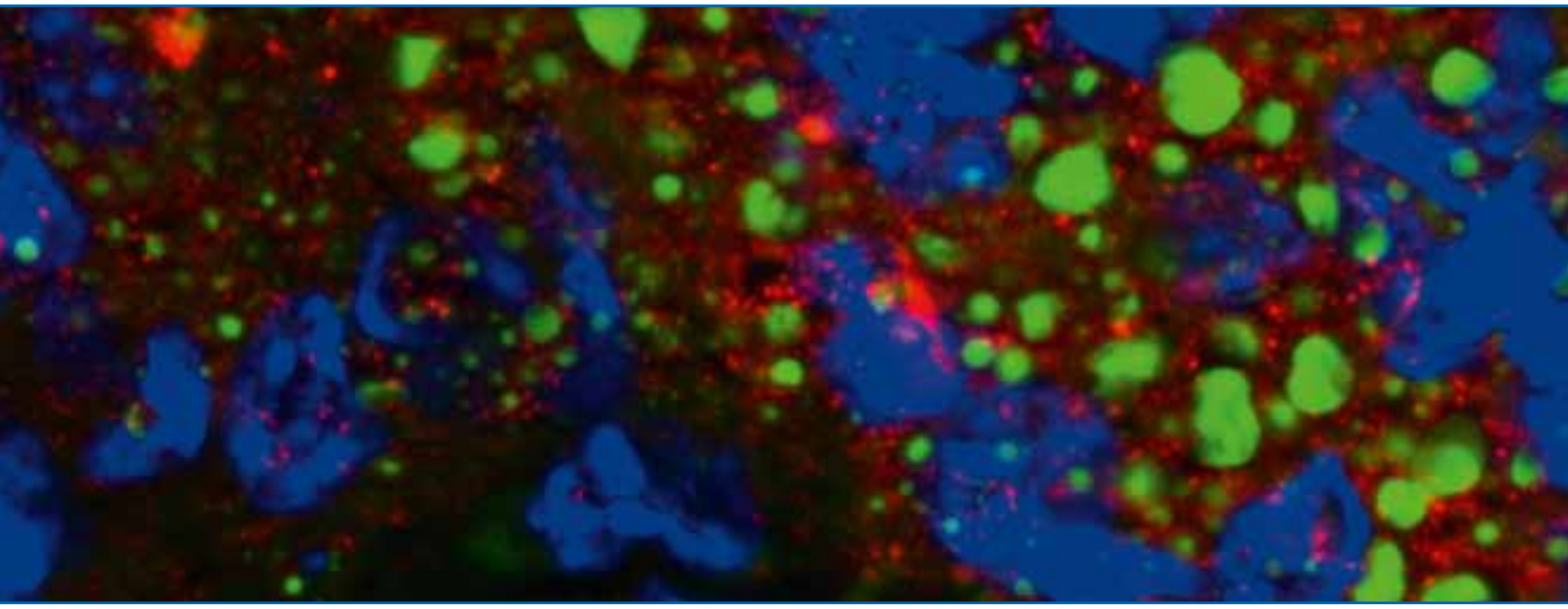
Organization Chart



BBRS - Borstel Biomedical Research School
GSP - Good Scientific Practice



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Juni 2019