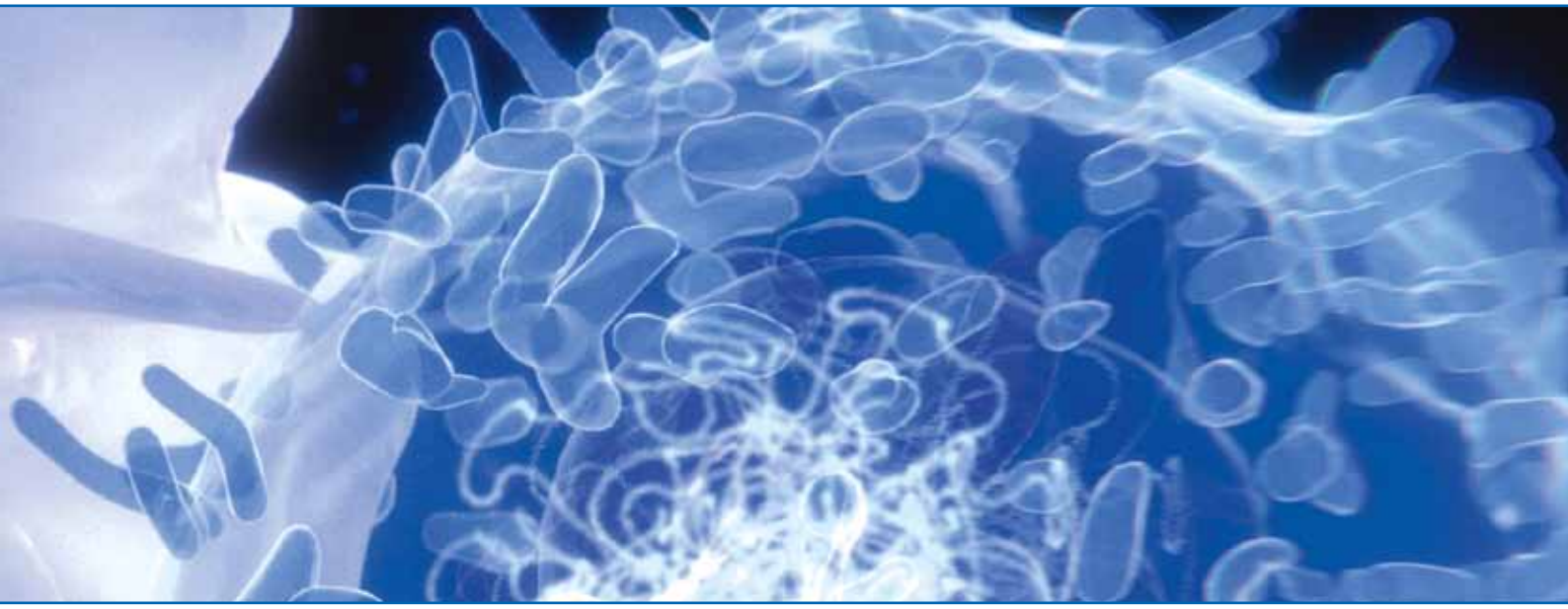
Forschungszentrum Borstel
Leibniz-Zentrum für Medizin und Biowissenschaften

FORSCHUNGSBERICHT 2015/2016



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Sehr geehrte Leserin, sehr geehrter Leser ...

Das Forschungszentrum Borstel (FZB) ist das Lungenforschungszentrum der Leibniz-Gemeinschaft. Sein Erfolgskonzept beruht darauf, dass die im Kern translationale Forschung auf wenige, definierte Fragen zu Pathogenese, Diagnostik und Therapie chronisch-entzündlicher Lungenerkrankungen fokussiert ist. Das FZB verfügt über eine ausgeprägte, interdisziplinäre Expertise im Bereich der molekularen Mikrobiologie und der Strukturbiologie mikrobieller Bestandteile und Allergene. Diese Expertise wird kombiniert mit einer Vielfalt zellbiologischer und tierexperimenteller Modellsysteme für die Asthma- und Tuberkulose-Forschung. Die wissenschaftlichen Schwerpunkte (Asthma/Allergien und infektionsbedingte, chronische Lungenerkrankungen) werden in zwei Programmbereichen (PB) von der Grundlagen- bis in die Klinische Forschung bearbeitet. Die zum FZB gehörende pneumologische Fachklinik und der Bereich Medizin (Klinische Pathologie, Biobank, Klinisches Studienzentrum) ermöglichen Studien nicht nur an humanem Untersuchungsmaterial, sondern auch am Patienten.

Das FZB hat in den letzten zwei Jahren die qualitativ hochkarätigsten Veröffentlichungen seit seiner Gründung vor 70 Jahren erstellt und ist mit seiner Verbundforschung weiterhin überaus erfolgreich in der strategischen Drittmitteleinwerbung. Diese Leistungsfähigkeit und –bereitschaft hat die Zuwendungsgeber überzeugt, dem FZB Investitionsmittel in Höhe von € 40 Millionen zuzusichern, um bis zum Jahre 2021 ein neues zentrales Laborgebäude zu errichten, das modernsten Erfordernissen an Tierhaltung, biologischer Sicherheit, Laborqualität und Nachhaltigkeit entspricht.

Der vorliegende Forschungsbericht informiert in kompakter Form über die wissenschaftlichen Aktivitäten des Forschungszentrums Borstel in den Jahren 2015 und 2016. Der Magazinteil berichtet über Aktivitäten des Zentrums, besondere Initiativen, bewegende Ereignisse und wichtige Forschungsergebnisse, Verbundaktivitäten und neue Forschungsgruppen. 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 näher bringen, und darüber hinaus auch die Fortschritte aufzeigen, die wir in den beiden vergangenen Jahren bei der Lösung gesellschaftlich relevanter medizinischer Probleme erzielt haben.

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

Dear Reader ...

The Forschungszentrum Borstel (FZB) is the Lung Research Center of the Leibniz Association. Its continued success is based on translational research focusing on a few, clearly defined questions regarding pathogenesis, diagnostics and therapy of chronic inflammatory lung diseases. The FZB has a highly reputable interdisciplinary expertise in molecular microbiology and structural biology of microbial components and allergens. These capabilities are combined with a variety of cell biological and animal models in asthma and tuberculosis research. The core themes (asthma/allergies and chronic infectious lung diseases) are covered in two research priority areas connecting basic science and clinical research. In addition, the medical department (clinical pathology, biobank, clinical study center) and the specialty clinic for respiratory diseases provide access not only to human tissues but also to patients.

In the past two years, the FZB published the highest number of papers in high ranking journals since it was founded 70 years ago and continues to be highly successful in networking politics and the acquisition of third party funding. Both, the performance and the commitment convinced the funding regional and federal authorities to invest 40 Mio. Euro for constructing a new central laboratory building which will meet the highest standards for animal housing, biological safety, laboratory quality and sustainability.

The present report informs about the scientific activities of the Forschungszentrum Borstel in the years 2015 and 2016 in a compact form. The magazine section reports on activities, specific initiatives, important research results, scientific networks and new research teams. By way of examples, hand-picked projects of the research groups and clinical units illustrate both the core competences and the achieved accomplishments.

In this way, we would like to “give a feel” for our research approach and objectives not only to experienced colleagues but also to a broader public. In addition, this report shows the progress we have made during the last two years in contributing to solving medical problems relevant to society.

Change is positive – and excellent research will ultimately benefit society! We are proud of the excellent work of all of our colleagues, and we are deeply grateful to all friends and supporters from science, politics, and industry. With your support we are looking optimistically into the future!

Stefan Ehlers
Heinz Fehrenbach, Susanne Krauss-Etschmann, Stefan Niemann, Frank Petersen, Ulrich Schaible, Peter Zabel

Skizze des neuen Laborgebäudes ‚Leibniz-Respiratorium‘,
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Leibniz Lungenzentrum - nomen est omen -

Ein Interview mit Zentrumsdirektor Prof. Dr. Stefan Ehlers

Prof. Dr. Ehlers, das Forschungszentrum Borstel erhält Mitte 2017 einen neuen Namenszusatz. Was hat es damit auf sich? Zumal die Änderung einer Marke eher unüblich ist, frei nach dem Motto ‚never change a running system'.

Das FZB ist faktisch DAS Lungenzentrum der Leibniz-Gemeinschaft. Schon in früheren Evaluierungen hatten die Gutachter den Wunsch geäußert, dass sich das Profil des Zentrums auch im Namen widerspiegeln sollte. Nach einer Bereiche-übergreifenden Konzeptschärfung in den letzten Jahren und strategischen Beteiligungen an langfristigen, Lungen-zentrierten Forschungsverbünden zeigt das FZB mit dem Namenszusatz Leibniz Lungenzentrum, dass es translationaler denn je aufgestellt ist – denn das Leibniz-Motto lautet ja: „theoria cum praxi", also anwendungsorientierte Grundlagenforschung, am FZB eben insbesondere in Bezug auf die Lunge. Wir haben bei dieser Namenserweiterung auf vielfachen Wunsch der Mitarbeiterinnen und Mitarbeiter des Zentrums einerseits wertekonservativ gehandelt (Forschungszentrum Borstel als Marke bleibt erhalten), aber die Thematik im Namenszusatz noch einmal fokussiert.

2012 haben wir bereits ein Interview geführt nachdem sich das Zentrum einer programmatischen Reform unterzogen hat: weg von Abteilungsstrukturen hin zu Handlungssträngen. Vier Jahre später ist es Zeit für eine erste Bilanz.

Ich hätte nicht gedacht, dass die programmatisch orientierte Neuausrichtung und das Denken in Handlungssträngen so schnell Fuß fassen und Erfolge zeitigen würde. Seit 2012 hat sich jedoch, nicht ausschließlich aber auch im Zuge dieser von den Programmdirektoren gemanagten Profilschärfung, der kumulative Impact-Faktor der FZB-Publikationen mehr als verdoppelt, was vor allem auf qualitativ höherwertige Veröffentlichungen zurückzuführen ist. Im Vergleich mit ähnlichen Einrichtungen wie dem HPI, dem HKI, dem BNI und dem RKI steht das FZB zudem meist besser da, was die Höhe der Drittmittelförderung in strategischen Verbundprojekten angeht (Quelle: DFG 2015). In seinem Audit-Bericht hat der Wissenschaftliche Beirat die strategischen Weichenstellungen und auch die Berufungspolitik des Zentrums als rundherum gelungen gewürdigt. Wir haben also ein Erfolgsmodell, um das uns - wie ich aus vielen Gesprächen mit anderen Institutsleitern innerhalb der Leibniz-Gemeinschaft weiß - viele beneiden.

Leibniz Lungenzentrum - nomen est omen -

Erfolgreiche wissenschaftliche Einrichtungen leben auch von einer modernen funktionstüchtigen Infrastruktur. Nach der inhaltlichen Reform hat das Zentrum nun eine infrastrukturelle Inventur vorgenommen. Welche Ergebnisse und Konsequenzen haben sich daraus ergeben?

Vor allem: Große Baustellen ! Es ist leider wahr, dass über lange Zeit der enorme Renovierungs- und Erneuerungsbedarf nicht ernst genug genommen wurde, dass aber nun die Grenzen der technischen Gebäudeausrüstungen erreicht sind und damit auch die Genehmigungsfähigkeit einiger Labore in Frage gestellt ist. Dies ist das wirklich problematische an der Situation: der transparente Umgang mit den infrastrukturellen Unzulänglichkeiten hat es auch erforderlich gemacht, mit den Aufsichtsbehörden (Arbeitsschutz, Gesundheitsamt, Gentechnik usw.) ins Gespräch zu kommen, um Übergangsregelungen bis zur Neuerrichtung der Laboratorien zu erhalten. Naturgemäß müssen Behörden dabei vor allem auf die Einhaltung von Vorschriften und gesetzlichen Regelungen drängen, was den Zeitdruck, Maßnahmen zur Behebung der infrastrukturellen Unzulänglichkeiten umzusetzen, zusätzlich erhöht.

Hat das Zentrum sozusagen die Büchse der Pandora geöffnet?

Leider ja, aber es hilft ja nichts – man muss sich diesen Herausforderungen stellen und darf die Defizite nicht unter den Teppich kehren, auch wenn dieser Weg, vor allem für die direkt beteiligten Sicherheits- und Arbeitschutzbeauftragten sowie die Technische Leitung, sehr beschwerlich ist. Nüchtern betrachtet haben wir aber schon einiges bewirkt: die Zuwendungsgeber haben die Not erkannt und uns EUR 40 Mio für einen zentralen Laborneubau mit Tierhaltung und S3-Anlage genehmigt, der von 2018-2021 errichtet wird. Sie haben auch grundsätzlich anerkannt, dass die Laboratorien des Nationalen Referenzzentrums für Mykobakterien neu gebaut werden müssen, da nur so die langfristige Arbeits- und biologische Sicherheit gewährleistet werden kann. Und schließlich sind wir uns einig, dass ein Masterplan für den Campus aufgestellt werden muss, der z.B. die Sanierung der PA22 beinhalten könnte, so dass im Anschluss weitere Laboratorien in das sanierte Gebäude einziehen könnten. Allerdings ist das FZB nicht die einzige Einrichtung im Land Schleswig-Holstein, die unter schwerschwiegenden Mängeln im Infrastrukturbereich leidet: an den Universitäten und Kliniken des Landes herrscht Investititonsstau, so dass das FZB nicht immer die oberste Priorität bei der finanziellen Unterstützung durch das Land genießen kann.

2022 wird das Zentrum 75 Jahre alt und hat sich bis dahin eine wissenschaftliche Agenda gegeben. Wo sehen Sie die Meilensteine, die das Zentrum in den nächsten fünf Jahren erreichen sollte?

75jährige gehören heute zu den Best Agers: hochmotiviert, leistungsfähig, mobil und aufklärerischen Werten verpflichtet. Wir können das Niveau unserer Arbeiten und damit unsere internationale Anerkennung sicher noch einmal steigern und müssen dann dauerhaft im oberen Drittel der Bundesliga spielen: Exzellenzcluster, Deutsche Zentren für Gesundheitsforschung, Leibniz-Verbünde und –Wissenschaftscampi. Das Thema Personalisierte Medizin muss noch konkretisiert werden – diese Ausrichtung der Medizinischen Klinik soll ein neuer Markenkern werden: eine „Leibniz-Klinik“ eben. Internationalisierung ist auch ein Kernthema, das wir fortentwickeln müssen – über Partnerlaboratorien in China, Studienzentren in Namibia, Rumänien und Moldawien, aber auch über die aktive Rekrutierung von ausländischen Mitarbeiterinnen und Mitarbeitern aus der Wissenschaft. Wenn wir doch nur so gut betucht wären wie viele Best Agers heute ! Denn auch das ist leider wahr: wir müssen sparen, wir kommen mit dem verminderten jährlichen Finanzaufwuchs von 1,5% nicht zurecht, wir müssen vor allem in unserer Personalentwicklung schmerzhafte Einschnitte machen. All das muss bis 2022 konsolidiert werden, dann wird es Zeit für den nächsten „Großen Sprung“ in der wissenschaftlichen Fortentwicklung. Aber darüber müssen meine Nachfolger und Nachfolgerinnen entscheiden.....

... und was machen Sie 2022?

Das Kuratorium muss mich ja 2017 im Amt bestätigen oder absetzen – von daher bin ich nicht in der Lage, dies sicher zu sagen. Ob und wie lange ich noch im Amt bin, hängt auch davon ab, wie das Evaluierungsergebnis 2019 ausfällt und auch davon, ob bereits ein guter Nachfolger/eine gute Nachfolgerin sichtbar wird, der/die vielleicht schon 2020 oder 2021, also zwei bis drei Jahre vor der geplanten Pensionierung, bereit stünde – wenn es Borstel nützt, das habe ich immer gesagt, wäre ich auch bereit früher zu gehen.

Interviewpartnerin: Dr. Bettina Brand

Der Leibniz-WissenschaftsCampus ist ein zukunftsweisendes Modell der regionalen Zusammenarbeit zwischen Leibniz-Einrichtungen und Hochschulen. Als gleichberechtigte Partner bearbeiten diese klar definierte Fragestellungen, entwickeln dazu eine gemeinsame Strategie und interdisziplinäre Forschungsansätze. WissenschaftsCampi bieten ideale Voraussetzungen, um gesellschaftlich relevante Fragestellungen zu bearbeiten, Forschungsbereiche weiter zu entwickeln und somit Profil und Sichtbarkeit der Standorte zu erhöhen.

Der Leibniz-WissenschaftsCampus „Evolutionary Medicine of the Lung“ (EvoLUNG) erhält in den vier Jahren von 2016 bis 2020 eine Förderung von insgesamt rund 4 Millionen Euro, davon 1,2 Millionen von der Leibniz-Gemeinschaft und 0,5 Millionen vom Land Schleswig-Holstein. FZB, CAU und das Max-Planck-Institut für Evolutionsbiologie in Plön (MPI-EB) sind Träger des Verbundes, der gemeinsam mit dem Evolutionsmedizinischen Zentrum der CAU Kiel und dem SFB 1182 „Origin and Function of Metaorganisms“ einen neuen Schwerpunktbereich der biomedizinischen Forschungslandschaft in Schleswig-Holstein darstellt.

> EvoLUNG ist ein weiteres herausragendes Beispiel für die interdisziplinäre Spitzenforschung im Norden - und die Zusammenarbeit verschiedener Forschungseinrichtungen. Die Entscheidung der Leibniz-Gemeinschaft ist ein Meilenstein für den Wissenschaftsstandort Schleswig-Holstein.
>
> *Wissenschaftsministerin Kristin Alheit*

EvoLUNG

Neuer Leibniz WissenschaftsCampus zur Erforschung von Lungenkrankheiten

Ziele des neuen WissenschaftsCampus EvoLUNG sind die interdisziplinäre Erforschung von schweren Lungenerkrankungen anhand evolutionswissenschaftlicher Methoden gefolgt von der Entwicklung neuer Therapien für Erkrankungen wie Asthma, Tuberkulose, Mukoviszidose oder chronische Bronchitis. Im Mittelpunkt stehen Strategien, die die Entwicklung von Antibiotika-Resistenzen nicht nur vorhersagen, sondern auch verhindern sollen, sowie die Nutzung von Bestandteilen oder Metaboliten organspezifischer Mikrobiome, um chronisch-entzündliche Erkrankungen zu behandeln.

Die wissenschaftliche Aufgabenstellung von EvoLUNG umfasst drei Hauptthemen: Der erste Forschungsbereich beschäftigt sich mit der Verbreitung und Herkunft von behandlungsresistenten Krankheitserregern in der Lunge. Der zweite Schwerpunkt erforscht die Evolution von krankheitsauslösenden Genen des Menschen, insbesondere solchen Krankheitsgenen, die Lungenerkrankungen begünstigen. Das dritte Forschungsgebiet untersucht das Zusammenspiel von Krankheitsgenen, Mikroorganismen, Krankheitserregern und Umwelt als Faktoren der Krankheitsentstehung in der Lunge.

> Die Standorte Borstel, Plön und die CAU stehen für besondere Forschungserfolge auf den Gebieten der Evolutionstheorie, der experimentellen Evolution und der evolutionären Genomik. Hinzu kommen besondere Kenntnisse in der funktionellen Analyse von Krankheitsgenen, in der Erforschung der Interaktionen von Wirt und Krankheitserregern und von chronisch-entzündlichen Lungenkrankheiten, vor allem der Tuberkulose und des Asthmas. Im EvoLUNG-Projekt vereinen sie diese Stärken, um die Erforschung schwerwiegender Lungenkrankheiten entscheidend voranzubringen.
>
> *Professor Stefan Niemann, Sprecher des Campus*

> Trotz großer Fortschritte in Diagnostik und Behandlung sind Lungenkrankheiten weltweit weiterhin auf dem Vormarsch und gehören zu den häufigsten Todesursachen. Mit der interdisziplinären Erforschung der zugrundeliegenden evolutionären Mechanismen der Krankheitsentstehung eröffnen wir eine völlig neue Perspektive, dieser dringlichen medizinischen und gesellschaftlichen Herausforderung zu begegnen.
>
> *Professor Stefan Ehlers, Zentrumsdirektor FZB*

Zusätzliche zur Bearbeitung von wissenschaftlichen Fragestellungen hat sich EvoLUNG die Karriereförderung von Postdocs und die strukturierte Ausbildung von Doktoranden/innen auf die Fahne geschrieben. Spezifisches, auf die Karrierestufe abgestimmtes Mentoring, Einbindung in die Lehre oder disziplinübergreifende Teamerfahrung an der Schnittstelle von Evolutionsbiologie und Biomedizin sind nur einige der anvisierten Inhalte.

Postdocs - zwischen Baum und Borke?

Forschungszentrum Borstel
Postdoc Initiative

Ein Interview mit Dr. Tobias Dallenga und Dr. Sabine Bartel

Der Begriff „Postdocs“ steht für die Phase zwischen der Promotion und einer möglichen Professur. In dieser Zeit müssen sich die Nachwuchsforscherinnen und Nachwuchsforscher für ihre wissenschaftliche Karriere durch innovative Projekte, hochrangige Publikationen und Aufenthalte in den großen Instituten ihres Forschungsthemas weiter qualifizieren. Gleichzeitig ist diese Phase aber geprägt von befristeten Verträgen, dem Zwang zur Mobilität und ungewissen Jobchancen. Die Folge ist mangelnde Planbarkeit und eine große Unsicherheit über die eigene Zukunft und das in der Phase des Lebens, in der normalerweise die Weichen für Karriere und Privatleben gestellt werden.

Ist somit ein Plan B, eine Strategie für den Ausstieg vom Einstieg in die Wissenschaft, zwingend für jede Nachwuchswissenschaftlerin und jeden Nachwuchswissenschaftler erforderlich?

Am Forschungszentrum Borstel hat sich 2015 eine Postdoc-Initiative gebildet, die sich für ein Programm zur gezielten Förderung von Postdocs am FZB engagieren und die Interessen dieser Gruppe innerhalb des Zentrums stärker vertreten wollen.

Postdocs - zwischen Baum und Borke?

Die Postdoc-Initiative am FZB ist ja noch relativ jung. Wie kam es überhaupt zu diesem Zusammenschluss und was sind Eure genauen Ziele?

Sabine B.: Der Anstoß wurde auf dem letzten Retreat des Zentrums gegeben als das Thema „Karriereförderung“ diskutiert wurde. Grundlage der Diskussion war die Umfrage eines Kollegen unter den Postdocs zu ihrer beruflichen Situation. Zur Gründung der Postdoc Initiative am Zentrum gab es auch ein Treffen mit dem Zentrumsdirektor (ZD) Stefan Ehlers, der von Beginn an unsere Initiative nicht nur von der Idee her unterstützt hat, sondern auch die Notwendigkeit sah, die Initiative vorerst für 2 Jahre finanziell auszustatten. Dieser Rückenwind hat uns beflügelt wohl wissend, dass finanziell besser ausgestattete Institutionen zum Teil gar nichts für ihre PostDocs tun.

Tobias D.: An dieser Stelle sollte auch gesagt werden, dass der ZD ohne Umschweife klar gemacht hat, dass die Chancen für befristete Postdocs am FZB, eine Entfristung in absehbarer Zeit angeboten zu bekommen, nahe Null sind. Genau aus diesem Grund findet er es so enorm wichtig, dass wir Aktivitäten entwickeln und gezielte Angebote machen, die hilfreich sein können, die berufliche Zukunft zu gestalten.

Sabine B.: Ein erstes Highlight war sicher das Kick-off Meeting mit Liz Elvidge vom Imperial College in London. Sie leitet dort ein professionelles Postdoc Development Center. Die Diskussionen mit ihr waren enorm hilfreich und haben auch deutlich gemacht, dass Postdocs nicht in einer Art Seifenblase leben sollten, sondern dass es darum geht, vom wissenschaftliche Umfeld ernst genommen zu werden und sichtbarer zu sein: ein Postdoc ist eben mehr als nur ein/e emsige/r Laborarbeiter/in, der/die zudem willens sein sollte, Aufgaben, die z.B. im Bereich Labormanagement liegen, zu übernehmen. Wir möchten ein Programm entwickeln, das sich aus verschiedenen Angeboten zusammensetzt und somit eine Art Tool-Box für die Gestaltung und Erkennung der beruflichen Zukunft darstellt. Das können u.a. Coachings sein, Seminare zur Führungskompetenz, Entwicklung eines Plans B.

Und die Förderung der Orientierung nach ‚draußen‘ ist enorm wichtig. Damit meine ich, dass die aktive Teilnahme an Kongressen Meetings, Workshops, AGs in Gesellschaften unerlässlich ist, um eigene Kontakte aufzubauen, die sich dann zu einem wertvollen Netzwerk entwickeln können.

Gibt es unter den Kolleginnen und Kollegen regen Zulauf?

Tobias D.: Der Kreis der aktiven Postdocs und derer, die uns ein Feedback geben begrenzt sich fast ausschließlich auf die befristet angestellten Postdocs. Postdocs sind eben ein bunt gemischter Haufen: es gibt jüngere und ältere Postdocs, die sich z.B. gar nicht als Postdocs verstehen. Es gibt befristete und unbefristete. Es gibt Postdocs, die aufgrund ihrer Finanzierung aus einem Drittmittel mehr Möglichkeiten haben, sich fortzubilden als andere. Große Grants bieten z.T. sehr elitäre und auch kostspielige Weiterbildungsangebote an. Da ist ein Postdoc, der auf einer Stelle aus einem DFG Einzelantrag sitzt schon benachteiligt. Auf diesem Kollegenkreis liegt unser Augenmerk.

Und was sagen die Gruppenleiter/leiterinnen zu Eurer Initiative?

Sabine B.: Auch diese Gruppe scheint sehr heterogen zu sein. Manche begrüßen die Initiative, manche sind offen interessiert, andere zeigen weniger bis gar kein Interesse.

Fürchtet man den Aufstand der Postdocs?

Sabine B. (grinst): Wohl eher nicht – außerdem ist das nicht unser Interesse! Wir wollen nicht den Aufstand proben, motzig über den Campus laufen und unmögliche Forderungen stellen. Wir leben in der Realität. Was wir uns wünschen und wofür wir arbeiten ist, dass ein Postdoc ein voll anerkanntes Berufsbild ist, das durch Interessensgemeinschaften vertreten ist und damit an Sichtbarkeit gewinnt.

Tobias, Du sagtest gerade, dass die Postdocs eine bunt gemischte Truppe sind. Wie wird eigentlich ein Postdoc definiert? Es scheint, dass Inhalte, Dauer und Grenzen der Postdoc-Phase völlig undefiniert sind. Das Wissenschaftszeitvertragsgesetz begrenzt die befristete Tätigkeit in der Wissenschaft nach der Promotion zwar auf sechs Jahre doch auf Drittmittelforschungsstellen ist es möglich, noch lange jenseits des 40. Lebensjahres zum „wissenschaftlichen Nachwuchs“ gezählt zu werden. Ist das nicht absurd? Dabei soll das Gesetz ja dem Nachwuchs verlässlichere Karrierewege ermöglichen?

Tobias D.: Postdoc ist so eine ‚europäische‘ Bezeichnung, die nichts über den Aufgaben- und Verantwortungsbereich sagt. Im anglo-amerikanischen Raum ist diese Bezeichnung eher

Einige Mitglieder der Postdoc Initiative mit ihrem Postdog: Karin Uliczka, Arne Homann, Kristof Tappertzhofen, Tobias Dallenga, Thorsten Krause, Katrin Ramacker und Karina Stein.

selten: dort findet man Positionen wie ‚Research Associate' oder ‚Principle Investigator'. Ich selbst z.B. habe die Verantwortung für den Arbeitsbereich von 2 TAs, betreue Studenten, übernehme zentrale Aufgaben im Rahmen des Labormanagements und bin der Stellvertreter meines Gruppenleiters. Ich fasse das übrigens als Vertrauensbeweis und Wertschätzung meines Chefs auf.

Sabine B.: Da bin ich Deiner Meinung. Man ist durchaus stolz, wenn man als Vertreterin der Gruppenleitung zu einer Konferenz geschickt wird … und das Selbstbewusstsein wächst.

D.h. Ihr habt einen Aufgaben- und Verantwortungsbereich der einem Nachwuchsgruppenleiter gleichkommt, aber eben nicht so heißt.

Tobias D.: Ja schon, dennoch bin ich gerne Postdoc und würde auch gerne weiter Postdoc bleiben. Gruppenleiter zu werden ist sicher erstrebenswert, sollte aber nicht die einzig mögliche Option sein.

Postdocs - zwischen Baum und Borke?

Die Klausel des Wissenschaftszeitgesetzes, das die Beschäftigung auf befristeten Verträgen auf 6 Jahre begrenzt, kann in diesem Zusammenhang mit einem Genickbruch für ein gesamtes Berufsfeld verglichen werden. Man könnte auch sagen, dass dieses Gesetz einem Berufsverbot gleichkommt. Starker Tobak? Ja, aber so fühlt es sich an und ich beanspruche für mich schon selbst entscheiden zu können, ob ich mit befristeten Arbeitsverträgen leben kann oder nicht und wie lange. Kein Unternehmen würde und muss wertvolle Mitarbeiter ziehen lassen und damit auch einen Wissensverlust verkraften, nur weil ein Gesetz es vorschreibt und jegliche Flexibilität vermissen lässt. Den Mittelbau sterben zu lassen zugunsten einer Professureninflation ist in meinem Augen ein Fehler. Der Mittelbau war und ist essentiell für das wissenschaftliche Fundament und die Nachhaltigkeit der Wissenschaft und zwar in Forschung und Lehre und bietet eine Zukunftsperspektive für innovative Wissenschaftler und Wissenschaftlerinnen – eben die besten Köpfe!

Sabine B.: Die Wissenschaftslandschaft ist in manchen Punkten ebenso starr. Warum kann die universitäre Lehre nicht für Postdocs geöffnet werden? Und damit meine ich nicht als Vertreter oder Lückenbüßer, sondern als professionelle Lehrkräfte, die Gefallen an der Lehre finden und das gern und mit Herzblut tun so wie andere mit Leidenschaft forschen.

Zurück zu Eurer Postdoc-Initiative - seid ihr mit anderen vergleichbaren Initiativen vernetzt? Könnte man sich eine breite Initiative auf Leibniz-Ebene vorstellen.

Tobias D.: Tatsächlich gibt es an einigen wenigen Orten schon sehr professionell aufgestellte Initiativen, die hoffentlich Vorreiter – und Modellfunktion haben werden. Beispiel Uetrecht – dort gibt es eine Initiative seit 2007, die ein Geschäftsmodell entwickelt haben, das landesweit agiert und sehr erfolgreich ist- auch finanziell.

Sabine B.: In der Tat spielen wir gerade mit der Idee in Borstel ein großes Treffen der verschiedenen Postdoc Interessensvertretungen zu organisieren.

Aha, das klingt nach höheren Aggregationsebenen, die dann auch politisch aktiv werden? Lobbyarbeit im besten und positiven Sinne?

Beide (grinsen): … vielleicht … obwohl das Wort Lobby doch eher negativ besetzt ist.

Laut einer ZEIT Studie in 2015 spielen über 80% der Nachwuchswissenschaftler/innen mit dem Gedanken auszusteigen. Ihr auch? Wie würdet Ihr eure persönliche Situation beurteilen? Reicht die Leidenschaft für Forschung für ein ganzes Leben aus?

Sabine B.: Ob die Leidenschaft für ein ganzes Leben reicht, weiß ich nicht. Sicher ist, man braucht eine gehörige Portion davon, um Forschung zu betreiben. Trotzdem können wirtschaftliche und soziale Zwänge ein Grund sein, den Exit aus der Wissenschaft zu wählen und z.B. in der Wirtschaft zu arbeiten, in welcher Position auch immer. Auch die fehlende Perspektive, Wissenschaft ohne Leitungsfunktion auf Dauer betreiben zu können, ist sehr frustrierend und für manchen Grund genug den sogenannten Plan B aus der Tasche zu ziehen.

Tobias D.: Die Leidenschaft ist oft so groß, dass mancher Postdoc sehenden Auges in die berufliche Sackgasse geht.

Eine letzte Frage: Schafft Ihr es Beruf und Privatleben in dieser Phase Eures Berufs in Einklang zu bringen?

Tobias D.: Mit einem befristeten Vertrag zu arbeiten ist wirtschaftlich ein erhebliches Risiko. Da ist es zweifelsfrei hilfreich wenn z.B. der Partner oder die Partnerin einen „sicheren“ Job hat. Diese Unsicherheit hat selbst Leute, die für die Wissenschaft gebrannt haben, dazu gebracht in die Wirtschaft zu gehen und als Produktmanager zu arbeiten. Ich persönlich weiß das breite Angebot des FZB zur ‚Vereinbarkeit von berufundfamilie‘ sehr zu schätzen. Eine eigene KiTa, Vertrauensarbeitszeit und Home Office sind Möglichkeiten, die es nicht an vielen Einrichtungen gibt.

Sabine B.: Es ist schon eine sehr gute Kommunikation und eine echte Partnerschaft notwendig, um ganz ‚normale‘ Dinge wie am Wochenende an der Publikation zu arbeiten oder Ergebnisse auszuwerten, bei dem einen oder anderen Experimente bis spät abends zu arbeiten oder mal wieder umzuziehen, um die nächste Postdoc Stelle anzunehmen, nicht zum Problem werden zu lassen.

Das Interview führte Dr. Bettina Brand

Sven Perner

Leiter der Verbundpathologie Borstel-Lübeck

Ressourcen bündeln und die Qualität in Krankenversorgung, Forschung und Lehre weiter steigern waren gute Gründe für die Universität Lübeck und das Forschungszentrum Borstel eine Standort-übergreifende Verbundpathologie zu etablieren. Diese gewährleistet u. a., dass Untersuchungsmaterialien, die zur lungenpathologischen Diagnostik eingesandt werden, auch für Forschungsprojekte des DZL und der Forschungsgruppen am FZB sowie für die Biobank-Archivierung fachgerecht aufgearbeitet werden können.

Neuer Direktor des Instituts für Pathologie am Campus Lübeck und Leiter der Forschungsgruppe Klinische und Experimentelle Pathologie in Borstel ist seit 1. August 2015 Prof. Dr. Sven Perner.

Zuvor war Sven Perner als geschäftsführender Oberarzt des Instituts für Pathologie am Universitätsklinikum Bonn tätig. Dort leitete er auch die Sektion für Prostatakarzinom-Forschung der Rudolf-Becker-Stiftung. Sein Studium absolvierte der 44-Jährige an der Universität Ulm. Weitere Stationen waren das Institut für Pathologie des Universitätsklinikums Ulm, die Harvard Medical School in Boston, das Weill Cornell Medical Center in New York und das Institut für Pathologie des Universitätsklinikums Tübingen.

Der Fokus der wissenschaftlichen Arbeit Prof. Perners liegt auf der translationalen Tumorforschung und der molekularen Pathogenese und Genetik solider Tumoren mit Schwerpunkt auf den Karzinomen der Lunge und Atemwege sowie auf dem Prostatakarzinom. Für seine wissenschaftlichen Leistungen erhielt Prof. Perner 2009 den Württembergischen Krebspreis, 2010 den Rudolf-Virchow Preis sowie den Wissenschaftspreis der Konrad-Morgenroth-Fördergesellschaft 2015.

Menschen / People

Xinhua Yu & Gabriela Riemekasten

A Chinese-German tandem oversees the projects of the new liaison group „Autoimmunity of the Lung“

2015 the Group „Autoimmunity of the Lung“ started out in liaison with the University of Lübeck and the University Xiamen (China). The group focuses on autoimmune interstitial diseases of the lung such as systemic sclerosis and Sjögren Syndrome. Both disorders are characterized by severe courses of disease based on the formation of pulmonary fibrosis and hypertension. The causative pathomechanisms for remodeling lung and vessels remain so far unresolved and will be investigated side by side with corresponding processes in allergic asthma and COPD.

Prof. Dr. Gabriela Riemekasten accepted a full professorship for Rheumatology and Systemic Inflammatory Disorders at the University of Lübeck in March 2015. Her prime scientific interest is the regulation of the immune system and the pathogenesis of autoimmune diseases, the improvement of therapies, both with a clear focus on systemic sclerosis. Gabriela Riemekasten studied medicine at the Humboldt-University Berlin. She is an internist and consulting rheumatologist and, until 2015, worked as adjunct professor at the Charité heading the day hospital Rheumatoloy and Clinical Immunology, as well as working as an independent Group Leader at the Deutsches Rheumaforschungszentrum.

Prof. Dr. Xinhua Yu is adjunct professor at the Medical College of Xiamen University and has worked as an independent researcher in the RG ‘Biochemical Immunology’ at the FZB since 2008. Xinhua Yu studied at the University of Wuhan, finished his Master’s degree in genetics at the Peking Union Medical College, Beijing in 2002 and graduated at the University of Rostock with a PhD degree in genetics in 2006. His main focus are autoimmune diseases with manifestations in the lung. Using both patient samples and experimental mouse models, he aims to explore the pathogenesis of autoimmune diseases as well as to search for novel therapeutics.

Katharina Kranzer

Leiterin des Nationalen Referenzzentrums für Mykobakterien

Dr. Katharina Kranzer verstärkt seit Oktober 2015 die Tuberkuloseforschung am FZB und leitet nicht nur die FG ‚Diagnostische Mykobakteriologie', sondern auch das Nationale und Supranationale Referenzzentrum für Mykobakterien am FZB. Der Status „Referenzzentrum" wurde nach Beantragung durch Dr. Katharina Kranzer und ihren Stellvertreter Prof. Stefan Niemann im Juni 2015 dem Zentrum erneut für weitere 4 Jahre durch das Bundesgesundheitsministerium zuerkannt.

Dr. Kranzer hat sich 2003 direkt nach ihrem Studium der Medizin in Regensburg und München, das sie erfolgreich mit der Promotion abschloss, der Infektions- und Tropenmedizin zugewandt. In London, das für viele Jahre ihre Heimat werden sollte, hat sie an der renommierten London School of Hygiene & Tropical Medicine ihren Master of Science und ihren Doctor of Philosophy (PhD) abgelegt. Zudem ist Dr. Kranzer Fachärztin für Klinische Mikrobiologie und Infektionshygiene.

In Anerkennung ihrer hervorragenden wissenschaftlichen Arbeiten erhielt Katharina Kranzer den Young Investigator Award (2011) auf der 18th Conference on Retroviruses and Opportunistic Infections, Boston, USA sowie die Woodruff Medal (2013). Neben ihren erfolgreichen Forschungsarbeiten auf dem Gebiet der Tuberkulose und der Koinfektion mit HIV hat Katharina Kranzer den klinischen Alltag nie aus den Augen verloren. Sie war sowohl in London als auch in Malawi und Südafrika als Ärztin tätig. Sie ist Mitglied der Leitlinienkomitees der Weltgesundheitsorganisation zur Diagnose und Therapie der latenten und aktiven Tuberkulose und Ko-Autorin der deutschen Leitlinien zur Tuberkulose-Diagnostik.

Menschen / People

Katarzyna Duda

Head of the Junior Research Group ‚Allergobiochemistry' funded by Deutsches Gesundheitszentrum für Lungenforschung (DZL).

Dr. Katarzyna Duda studied biology at the University of Silesia, Poland, and received her Doctor's degree in 2007. She joined the FZB in 2009 as a postdoctoral fellow (DFG) in the RG ‚Structural Biochemistry' focusing on the isolation and structural analysis of bacterial cell envelope components. In 2012 she received funding from the DZL and took over the group ‚Analytics' as part of the RG ‚Structural Biochemistry'. Katarzyna Duda changed her scientific focus to (glyco)-lipid compounds from grass pollen and dust mites addressing the central research agenda of the Research Priority Area Asthma and Allergy.

In 2016 Katarzyna Duda built up her own group ‚Allergobiochemistry', still funded by the DZL. Her team is devoted to the isolation, chemical and functional characterization of important structural or antigenic carbohydrate, lipid or glycolipid moieties of environmental or bacterial origin relevant for the dynamics of allergic inflammation.

Guntram Graßl

Ruf an die Medizinische Hochschule in Hannover auf die Professur für „Medizinische Mikrobiomforschung“

Prof. Dr. Guntram Graßl war als Juniorprofessor für Entzündungsmodelle von 2009 bis 2015 im Exzellenzcluster Entzündungsforschung an der CAU, in Kiel und am FZB tätig. Zuvor arbeitete er als Postdoc an der University of British Columbia, Vancouver, Kanada.

Sein wissenschaftliches Interesse galt und gilt den chronisch-entzündlichen Darmerkrankungen (ECD) Colitis Ulcerosa und Morbus Crohn. Beide kennzeichnet eine chronische Entzündung, schwere Darm-Pathologie und Veränderungen in der Darm-Physiologie.

In seiner Forschung zielte Guntram Graßl auf die Aufklärung der Wirts- und bakteriellen Faktoren ab, die zur Entwicklung der chronischen Entzündung und intestinalen Fibrose führen. Sein Augenmerk lag auf der Identifizierung der bakteriellen Faktoren in Salmonellen, die zur Entzündung und Fibrose führen, der Charakterisierung der Wirts-Mechanismen in der chronischen Darm-Entzündung und Fibrose, sowie mit besonderem Schwerpunkt auf der Charakterisierung der beteiligten Zelltypen und der produzierten pro- und antifibrotischen Faktoren.

Guntram Graßl war und ist ein sehr geschätzter Kollege, guter Netzwerker und außerordentlich adaptionsfähiger Bayer bei uns im Norden. Wir haben ihn nicht gern nach Hannover ziehen lassen, freuen uns aber mit ihm über seinen Ruf an die MHH und wünschen ihm viel Erfolg.

Farewell

Ekkehard Vollmer

The man who brought the HOPE-technique to Borstel

Prof. Dr. Dr. Dr. h.c. Ekkehard Vollmer retired after 23 years at the Research Center Borstel in January 2016 and took leave with a farewell symposium in the Manor House on April 18, 2016.

Ekkehard Vollmer graduated in Medicine 1977 at the University of Marburg and 1984 in Veterinary Medicine at the FU Berlin. He received his Venia Legendi in 1992 at the University of Münster. In 1993 he joined the Research Center Borstel as the head of the RG Clinical and Experimental Pathology.

His first activities focused on the establishment of a reliable immunohisto-chemistry and a computer-based system to store and monitor patient data. At the same time he initiated and sustained a long lasting collaboration with the LungenClinic Grosshansdorf.

Ekkehard Vollmer's prime scientific interest was to build up a modern molecular pathology with a powerful portfolio of methodologies, including FISH, several sequencing approaches and others which are still state of the art, strengthening his team in a rapidly developing and competitive field.

As one central aspect of his scientific career, he propagated the development of the HOPE-technique, a fixation technique which enables comprehensive molecular read out in archived specimens. This outstanding technique is renowned internationally and is one of Borstel's flagships. He and his coworkers published 174 peer reviewed publications.

Ekkehard Vollmer was also an active teacher and mentor organizing help for pathologists in Eastern Europe: Two DAAD-summer schools at the Borstel campus were held in 2009 and 2010. A two-weeks' visit with workshops in molecular diagnostics was held in Tblisi, Georgia and Yerevan, Armenia in 2011. Candidates from Iraq and Mongolia received their PhD at the University of Lübeck after being trained in Borstel. Numerous guest scientists from all over the world have visited the group. Finally, Ekkehard Vollmer received an honorary doctorate from the University of Craiova in Romania in 2011 for his continuous support.

We wholeheartedly thank Ekkehard Vollmer for his long-term commitment, his dedication and support. Although he is now retired, we still have the pleasure of occasionally meeting him and receiving advice in his inimitable fashion. We wish him all the best for a long and prosperous retirement.

Forschung & Entwicklung

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

Media-/ Press-Clips 2015 / 2016

NDR Info / Logo Wissenschaftsmagazin
‚**Interview zum Weltkrebstag**'

NDR / Radio Visite ‚**Hilfe für COPD Patienten**'

NDR /
Radio Visite ‚**Pollen machen Allergikern zu schaffen**'

NDR Info /
Logo Wissenschaftsmagazin ‚**Wie sicher ist Nano?**'

NDR 3 / Visite ‚**Allergische Reaktion durch Wespenstiche vermeiden**'

NDR 3 / Abenteuer Diagnose ‚**Rückenwirbeltuberkulose**'

NDR / Schleswig-Holstein Magazin
‚**Können Asthmaanfälle bald vorhergesagt werden**'

Neue Osnabrücker Zeitung
‚**Trendumkehr bei Tuberkulose: Mehr Fälle in Deutschland**'

Süddeutsche Zeitung ‚**Der fremde Patient**'

Hamburger Abendblatt
‚**40 Millionen Euro für medizinische Forschung**'

Spektrum der Wissenschaft
‚**Kehrt die Tuberkulose zurück?**'

Hamburger Abendblatt
‚**Forschungszentrum kooperiert mit Xiamen**'

Deutsches Ärzteblatt ‚**Tuberkulosevakzine: Blackbox, die wir noch nicht verstehen**'

Hamburger Abendblatt ‚**Spinoza Lehrstuhl in Medizin für Forscher in Borstel**'

Union TV
‚**National TB Reference Laboratory**'

SPIEGEL-ONLINE ‚**Tuberkulose: Genetisches Lexikon entlarvt gefährliche Resistenzen**'

FOCUS online ‚**Atemhilfe statt Ritalin**'

europe
online magazin ‚**Gefährliche Flecken auf der Lunge**'

RP online
‚**Tuberkulosefälle in Deutschland auf Rekordhoch**'

Science and Technology in Society *forum* 2016 Kyoto, Japan

Ein Bericht von Dr. Susanne Homolka,
FG Molekulare und Experimentelle Mykobakteriologie

Auf Einladung unseres Leibniz-Präsidenten Prof. Kleiner bekam ich die Möglichkeit, am diesjährigen STS*forum* (Science and Technology in Society *forum*) in Kyoto teilzunehmen. Das vor 13 Jahren von Koji Omi ins Leben gerufene Forum bietet „a new mechanism for open discussions on an informal basis and to build a human network that would, in time, resolve the new types of problems stemming from the application of science and technology." (Koji Omi 2016).

Mit einer wagen Idee, was mich in Kyoto erwarten würde, durfte ich als eine von 5 Leibnizianer/innen im Rahmen des sogenannten „Future Leader Program" das Forschungszentrum Borstel vertreten. Zusammen mit insgesamt 100 Nachwuchswissenschaftler/innen aus 25 Ländern hatten wir zunächst die Möglichkeit mit 10 Nobelpreisträgern unterschiedlicher Disziplinen ins Gespräch zu kommen. In intensiven Diskussionsrunden mit Prof. Jerome Issac Friedmann, Nobelpreisträger in Physik 1990, sowie Dr. Eng. Ryoji Noyori, Nobelpreisträger in Chemie 2001, wurden Themengebiete wie Erfolg in der Wissenschaft, gute wissenschaftliche Praxis, Ethik, Interdisziplinarität und Originalität sowie Wissenschaft mit Leitungsfunktion erarbeitet und kritisch durchleuchtet.

Aus dem breiten Erfahrungsschatz und persönlichen Einschätzungen der Nobelpreisträger wurden nicht nur Ideen entwickelt wie man als junger Mensch durch Wissenschaft unsere Gesellschaft verändern kann, sondern auch wie man in schwierigen Zeiten sich für Wissenschaft begeistern und diese erleben kann. „Das Geheimnis guter Wissenschaft ist die Geduld, die Leidenschaft und der Glauben daran, etwas in einer signifikanten Art und Weise zu verändern" (frei zitiert nach Jerome Friedmann).

Nach diesem inspirierenden Auftakt standen auch die nächsten Tage im Zeichen intensiven Austauschs über große Themengebiete wie erneuerbare Energien, Klimawandel, „Genome Engineering", Infektionskrankheiten, „Big Data" aber auch Wissenschaft und Technologie in Entwicklungsländern oder „Smart Cities". Über eintausend Vertreter unterschiedlicher Disziplinen aus Industrie, Politik und Wissenschaft entwickelten in kleineren Workshops Lösungsansätze und Ideen, Menschen, die vermutlich nur im Rahmen dieses Forums zusammenkommen.

Für mich als Nachwuchswissenschaftlerin stellte es eine fantastische Möglichkeit dar, nicht nur andere Themenbereiche zu diskutieren, sondern auch Zusammenhänge großer gesellschaftlicher Probleme besser zu verstehen, was ich zuvor noch nie so erlebt habe. „Wissenschaft ist wie ein Orchester, es muss dirigiert werden, damit es harmonisch klingt." (frei zitiert nach Ryogi Noyari).

Leibniz-Gründerpreis geht an Medizin-Start-up „Brandenburg Antiinfectiva GmbH“ für eine Medikamentenentwicklung gegen Blutvergiftungen.
„Brandenburg Antiinfectiva GmbH“ is awarded with the **Leibniz Award for Business Start-ups** for the development of a new drug combating sepsis.

Start des Forschungsverbundes „**INFECTIONS´21**“ mit 14 Leibniz Partnerinstitutionen. Primäres Ziel ist, eine Kultur der interdisziplinären Kommunikation zu etablieren, und dadurch neue Strategien und Methoden für Frühwarnsysteme auch unter Beteiligung der Öffentlichkeit sowie ein verbessertes Management von Ausbrüchen und eine optimierte Eindämmung der Erregerausbreitung zu entwickeln.
The interdisciplinary Leibniz Research Alliance „**INFECTIONS'21**“ is set to work. 14 Leibniz institutions are devoted to developing innovative strategies and methods for early warning systems to improve the management of the outbreak and the control of infectious diseases.

„**TB-Sequel**“: Netzwerk zur Erforschung der Tuberkulose und deren Auswirkungen auf die öffentliche Gesundheit in afrikanischen Ländern, gefördert durch das BMBF. Am Netzwerk beteiligt sind die Länder Tansania, Mozambique, Gambia und Südafrika.
„**TB-Sequel**“ is an international network to foster research of tuberculosis and its ramifications for public health on the African continent.

Best of 2015

Dr. Nicolas Gisch, FG Bioanalytische Chemie, erhält - gemeinsam mit Dr. Stefanie Ranf vom Wissenschaftszentrum Weihenstephan der TU München - den mit 20.000 Euro dotierten „**Wissenschaftspreis Weihenstephan der Stadt Freising**". Die beiden Wissenschaftler haben einen wichtigen Beitrag zum Verständnis der Immunabwehr bei Pflanzen geleistet: Es ist Ihnen erstmals gelungen, einen Sensor zu identifizieren, mit dem Pflanzen Krankheitserreger anhand deren Lipopolysacchariden (LPS) erkennen und eine Immunantwort auslösen.
Dr. Nikolas Gisch, RG Bioanalytical Chemistry and his colleague, Dr. Stefanie Ranf, Wissenschaftszentrum Weihenstephan, receive the „**Science Award Weihenstephan der Stadt Freising**" endowed with 20.000 Euro. Both scientists successfully identified a sensor enabling plants to recognize pathogenic agents via lipopolysaccharides and to trigger an immune response.

Erfolgreiche Wiederbeantragung des **Nationalen Referenzzentrums für Mykobakterien** für vier weitere Jahre.
Successful application for hosting the **National Reference Center for Mycobacteria** for another four years.

Auf gute Zusammenarbeit! Das Forschungszentrum Borstel baut **Kooperation mit der Universität in Xiamen** (China) im Bereich Asthma- und Allergieforschung aus.
Initiative to set up a **cooperative partnership with the University of Xiamen** (China) in allergy and asthma research.

„**Atemgas-Analysator zur Krebsfrühdiagnose**": Das BMBF fördert ein Forschungskonsortium im Raum Lübeck mit einem Projektvolumen von 1,1 Mio. € zur Entwicklung einer Methode, die Analyse von Atemluft von Patienten für eine Frühdiagnose von Lungenkrebs ermöglicht.
„**Breathing gas analyser in early diagnosis of cancer**". A regional research network, funded by the BMBF with 1,1 Mio. Euro to develop a device for analyzing breathing air of patients providing access to early diagnosis of lung cancer.

Lisa-Marie Johannssen erhält den **Leibniz-Auszubildenden-Preis 2015**.
Lisa-Marie Johannssen receives the **Trainees-Award 2015 of the Leibniz Association**.

Der **Promotionspreis des Deutschen Zentrums für Infektionsforschung** geht an Dr. Matthias Merker, FG Molekulare und Experimentelle Mykobakteriologie.
Dr. Matthias Merker, RG Molecular and Experimental Mycobacteriology, receives the **„Best Thesis Award" of the German Center for Infection Research**.

Den **Promotionspreis des Kreises Segeberg für die beste Promotion 2015** erhält Dr. Julius Brandenburg, FG Mikrobielle Grenzflächenbiologie.
Dr. Julias Brandenburg, RG Microbial Interface Biology, receives the **„Best Thesis Award" of the district of Segeberg**.

Der **Förderpreis des ‚Vereins zur Erforschung infektiologischer und allergischer Prozesse'** für die beste Masterarbeit geht an Miriam Hiller, FG Mikrobielle Grenzflächenbiologie.
The **Förderverein (supporter's asscociation) VEIAP** honors Miriam Hiller, RG Microbial Interface Biology, for her excellent scientific work (MSc).

Publikation / Publication Blockbuster: Nature Immunology (Infection Immunology, Bioanalytical Chemistry), New England Journal of Medicine (Clin. Infectious Diseases; Mol. Exp. Mycobacteriology), Nature Genetics (Mol. Exp. Mycobacteriology), Lancet Infectious Diseases (Mol. Exp. Mycobacteriology), J. of Allergy and Clin. Immunology (Innate Immunity, Clin. Mol. Allergology, Structural Biology), Am. J. Respir. and Crit. Care Medicine (Structural Biology).

40 Millionen Euro für das neue zentrale Laborgebäude. Die Zuwendungsgeber des Landes Schleswig-Holstein und des BMG investieren in die Expertise und Zukunft des FZB.
40. Mio. Euro to build a new main laboratory building. The awarding authorities State Government Schleswig-Holstein and Federal Ministry of Health invest in the longterm development of the FZB.

Deutsches Zentrum für Lungenforschung hervorragend begutachtet
German Center for Lung Research assessed as excellent: „The original mission and strategy of combining five individually powerful centres into a coherent whole has been implemented in an excellent fashion at multiple levels, and has achieved a game change as a result."

Expertisen aus neun Leibniz-Instituten vereinigen sich in einem Chip im Pilotprojekt „**EXASENS**", der als Point-of-Care-Technologie zur Vorhersage und Diagnose von chronisch-entzündlichen Atemwegserkrankungen eingesetzt werden soll. Der Verbund wird vom BMBF mit 6,25 Millionen Euro gefördert.
EXASENS - The expertises of nine Leibniz institutes are pooled in one chip to be utilised in the prediction and diagnosis of chronic inflammatory lung diseases. The alliance is funded by the BMBF with 6,25 Mio. Euro.

Deutsche Lungenstiftung zeichnet Borsteler Wissenschaftlerin aus!
Im Rahmen des 57. Jahreskongresses der Deutschen Gesellschaft für Pneumologie und Beatmungsmedizin in Leipzig erhält Dr. Sabine Bartel den vom Pharmakonzern Boehringer Ingelheim mit insgesamt 6.000 Euro dotierten Doktorandenpreis der Deutschen Lungenstiftung.
Dr. Sabine Bartel is honored with the **„Thesis Award" of the Deutsche Lungenstiftung for the best experimental work.** The award includes prize money of 6.000 Euro sponsored by Boehringer Ingelheim.

Best of 2016

Tag der offenen Tür in Borstel zum **Weltasthmatag 2016**
Open house at FZB on the occasion of **World Asthma Day 2016**.

Große Ehre: Prof. Uta Jappe zum Mitglied des ‚**Collegium Internationale Allergologicum**' ernannt.
Great honor: Prof. Uta Jappe is inducted as a member of the ‚**Collegium Internationale Allergologicum**'.

Prof. Christoph Lange erhält den **Spinoza Lehrstuhl für Medizin** der Universität von Amsterdam für das akademische Jahr 2016/2017.
Prof. Christoph Lange is appointed **"Spinoza Chair" of medicine** 2016/2017 at the University of Amsterdam.

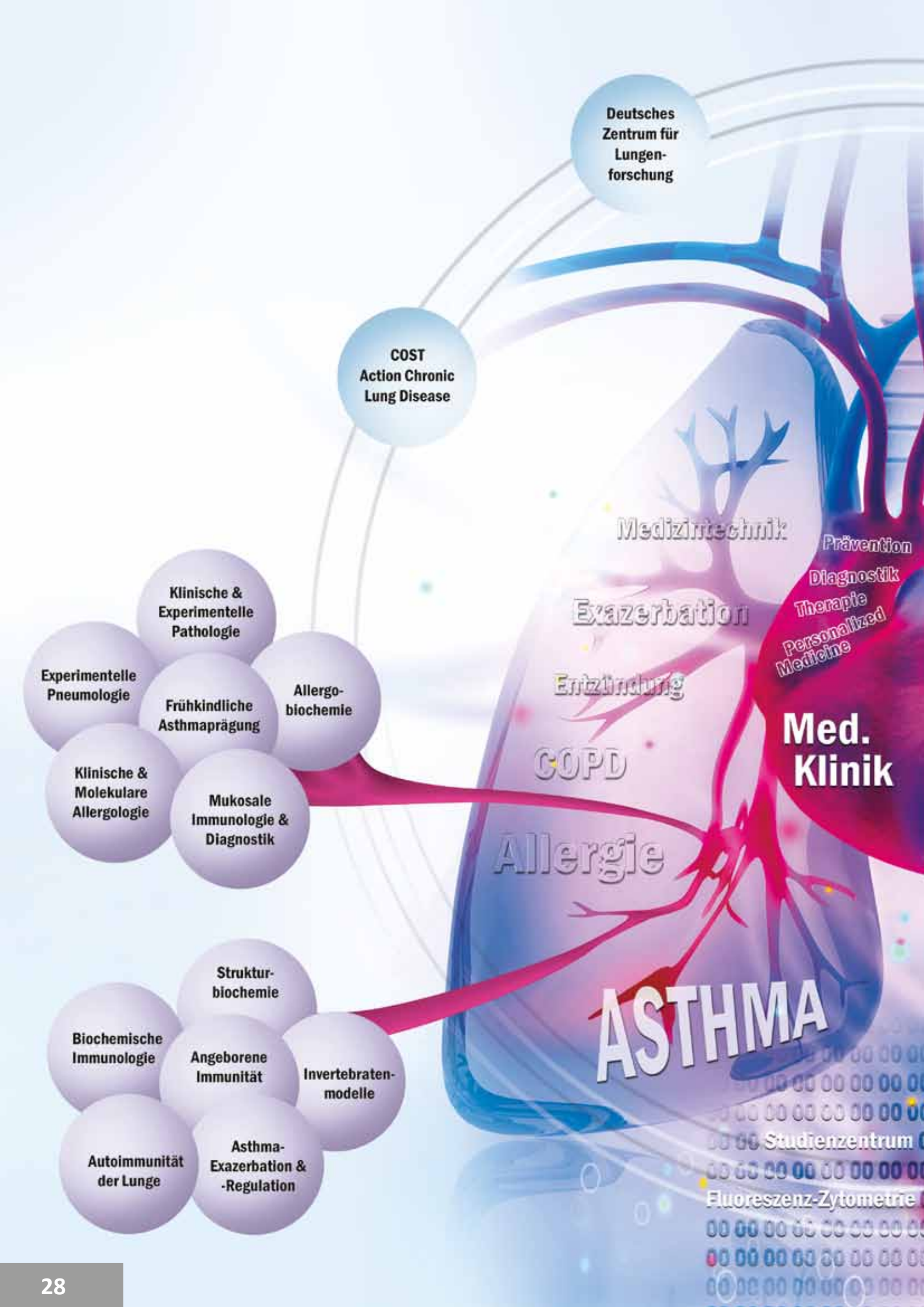
Das FZB schließt sich im Rahmen einer **assoziierten Partnerschaft der CSSB (Zentrum für Strukturelle Systembiologie)-Gemeinschaft** auf der Jagd nach Infektionserregern an.
The FZB joins the **CSSB (Centre for Structural Systems Biology) partnership as an associated member** to jointly hunt for pathogenic agents.

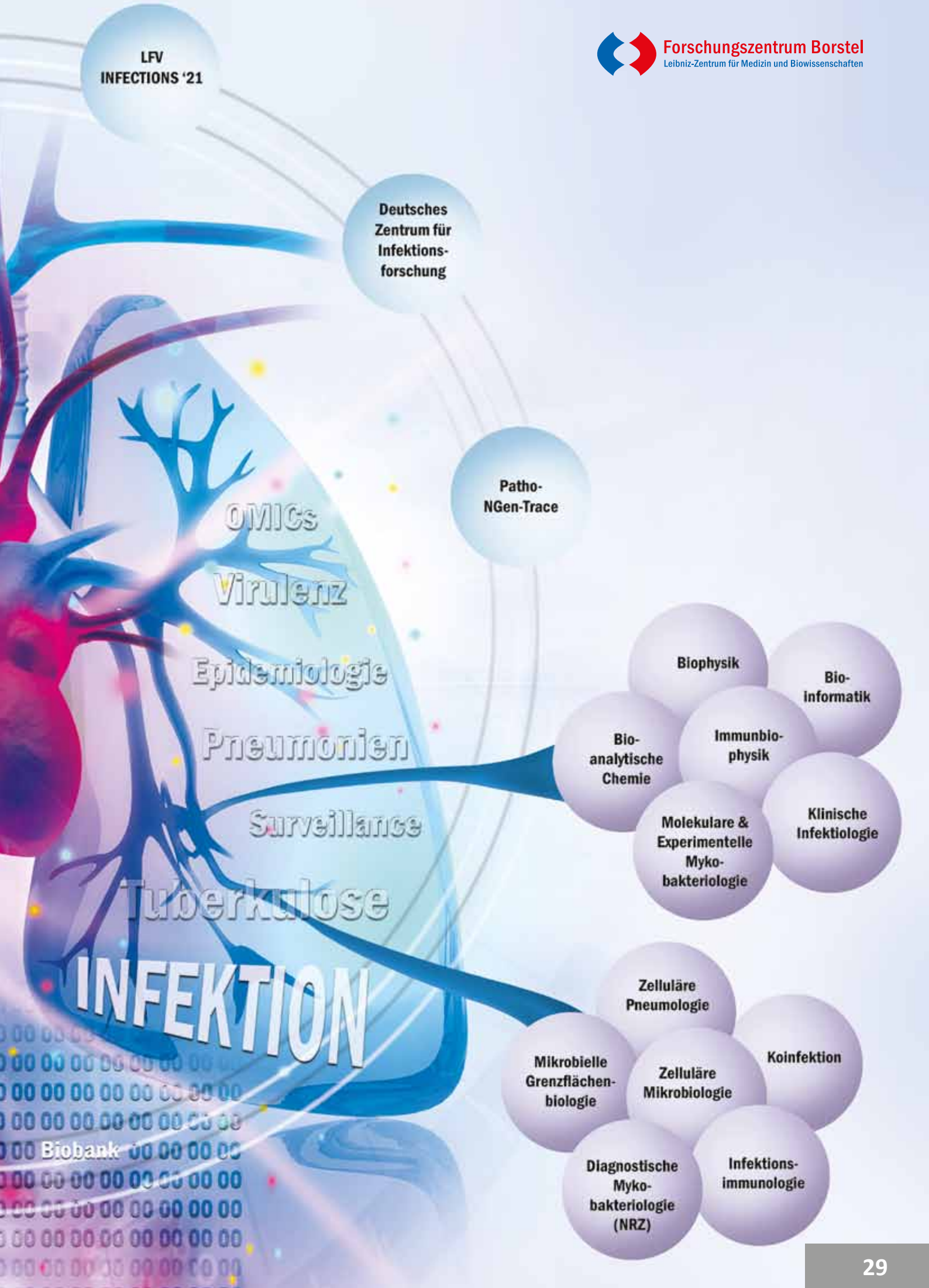
Prof. Stefan Niemann erhält den **„Hauptpreis" der Deutschen Gesellschaft für Hygiene und Mikrobiologie** und einen **Schleswig-Holstein Exzellenzchair**.
Prof. Stefan Niemann receives the **„Main Award" of the German Society for Hygiene and Microbiology** and is appointed as one of the **Schleswig-Holstein Excellence Chairs**.

Dr. Julia Wernecke (FG Biophysik) und Dr. Jannike Dibbern (FG Koinfektion) erhalten den **Promotionspreises des Kreises Segeberg**. Arabella Karstedt (FG Klinische u. Molekulare Allergologie), und Anna-Belen Erazo (FG Koinfektion) erhalten den Preis für die beste Masterarbeit des **Vereins für die Erforschung infektiologischer und allergologischer Prozesse (VEIAP)**.
Dr. Julia Wernecke (RG Biophysics) and Dr. Jannike Dibbern (RG Coinfection) receive the **„Best Thesis Award" of the district Segeberg**. **The Förderverein (supporter's asscociation) VEIAP** honors Arabella Karstedt (RG Clinical and Molecular Allergology) und Anna-Belen Erazo (RG Coinfektion) for their excellent MSc theses.

Publikation / Publication Blockbuster: Nature Communications (Clin. Exp. Pathology, Mol. Exp. Mycobacteriology, Biophysics), J. Allergy and Clin. Immunology (Innate Immunity, Early Life Origins of CLD), Am. J. Respir. Crit. Care Medicine (Early Life Origins of CLD, Clin. Infect. Diseases, Mol. Exp. Mycobacteriology), New England J. Medicine (Clin. Infect. Diseases), Lancet Respir. Medicine (Mol. Exp. Mycobacteriology), Nature Genetics (Mol. Exp. Mycobacteriology), Lancet (Diagn. Mycobacteriology).

2016


Deutsches Zentrum für Lungen-forschung
COST Action Chronic Lung Disease
Medizintechnik
Prävention
Diagnostik
Therapie
Personalized Medicine
Exazerbation
Entzündung
COPD
Allergie
Med. Klinik
ASTHMA
Klinische & Experimentelle Pathologie
Experimentelle Pneumologie
Frühkindliche Asthmaprägung
Allergo-biochemie
Klinische & Molekulare Allergologie
Mukosale Immunologie & Diagnostik
Struktur-biochemie
Biochemische Immunologie
Angeborene Immunität
Invertebraten-modelle
Autoimmunität der Lunge
Asthma-Exazerbation & -Regulation
Studienzentrum
Fluoreszenz-Zytometrie


LFV
INFECTIONS '21
Deutsches Zentrum für Infektions-forschung
Patho-NGen-Trace
OMICs
Virulenz
Epidemiologie
Pneumonien
Surveillance
Tuberkulose
INFEKTION
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Biophysik
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Bio-analytische Chemie
Immunbio-physik
Molekulare & Experimentelle Myko-bakteriologie
Klinische Infektiologie
Zelluläre Pneumologie
Mikrobielle Grenzflächen-biologie
Zelluläre Mikrobiologie
Koinfektion
Diagnostische Myko-bakteriologie (NRZ)
Infektions-immunologie

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
- Alexandra Schröder
- Linda Lang
- Steffi Hahn

Priority Research Area **Asthma and Allergy**

Asthma Exacerbation & Regulation

(former: Mouse Models of Asthma)

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 zugrunde liegen.

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

Allergic bronchial asthma arises on the basis of a chronic inflammatory response in the airways causing destruction and remodeling of airway tissues. The consequences are mucus hypersecretion and airway hyperresponsiveness (AHR) manifesting in the patient as cough, respiratory distress, the typical asthma attack, and could also lead to death. Since chronic airway inflammation represents the basis of all these hallmarks, understanding its regulation is a key to understand the pathogenesis of the disease.

A major aspect of this regulation is the balanced production of proinflammatory cytokines and its anti-inflammatory counterparts. Because in asthmatic patients allergen inhalation leads to enhanced release of proinflammatory cytokines and a shift towards inflammation, if the release of counterbalancing anti-inflammatory cytokines is overwhelmed. We introduced interleukin (IL) 37 as such a cytokine regulating allergic inflammation in asthma, since we found a significantly lower production of this new cytokine in peripheral blood mononuclear cells from asthmatic children. Moreover, local treatment with IL-37 after allergen provocation significantly down-regulated allergic airway inflammation, mucus hypersecretion, and AHR in mice with experimental allergic asthma. We were able to provide the first data on the mode of action of this cytokine and discovered its hetero-dimeric receptor consisting of IL-18Rα and SIGIRR. These data further enabled us to identify the target cells of IL-37 including leukocytes such as naïve T helper (TH) cells, TH2 cells, and dendritic cells as well as structural cells of the airway like epithelial and smooth muscle cells.

Highlights

First description IL-37 as a regulator of an allergic immune response

Discovery of the IL-37 receptor formed by IL-18Rα and SIGIRR

Identification of IL-17 producing NK cells as drivers of virus-induced asthma exacerbation

Selected publications

Zimmer J, Weitnauer M, Boutin S, Küblbeck G, Thiele S, Walker P, Lasitschka F, Lunding L, Orinska Z, Vock C, Arnold B, Wegmann M, Dalpke A. Nuclear Localization of Suppressor of Cytokine Signaling-1 Regulates Local Immunity in the Lung. Front Immunol. 2016;7:514. (IF 5.695)

Lunding L, Wegmann M. NK cells in asthma exacerbation. Oncotarget. 2015;6:19932-3. (IF 6.195)

Voss M, Wolf L, Kamyschnikow A, Honecker A, Herr C, Lepper PM, Wegmann M, Menger MD, Bals R, Beisswenger C. IL-17A contributes to maintenance of pulmonary homeostasis in a murine model of cigarette smoke-induced emphysema. Am J Physiol Lung Cell Mol Physiol. 2015;309:L188-95. (IF 4.041)

Vock C, Yildirim AÖ, Wagner C, Schlick S, Lunding LP, Lee CG, Elias JA, Fehrenbach H, Wegmann M. Distal airways are protected from goblet cell metaplasia by diminished expression of IL-13 signaling components. Clin Exp Allergy. 2015;45:1447-1458. (IF 4.324)

Lunding L, Schöder A, Wegmann M. Allergic Airway Inflammation: Unravelling the Relationship between IL-37, IL-18Ra and Tir8/SIGIRR. Expert Rev Respir Med 2015;9:739-50. (IF 2.333)

Lunding LP, Webering S, Vock C, Behrends J, Wagner C, Hölscher C, Fehrenbach, Wegmann M. pIC-triggered exacerbation of experimental asthma depends on IL-17A produced by NK cells. J Immunol 2015;194:5615-25. (IF 5.362)

Lunding L, Webering S, Vock C, Schröder A, Raedler D, Schaub B, Fehrenbach H, Wegmann M. IL-37 requires IL-18Rα and SIGIRR/IL-1R8 to diminish allergic airway inflammation in mice. Allergy 2015;70:366-73. (IF 5.995)

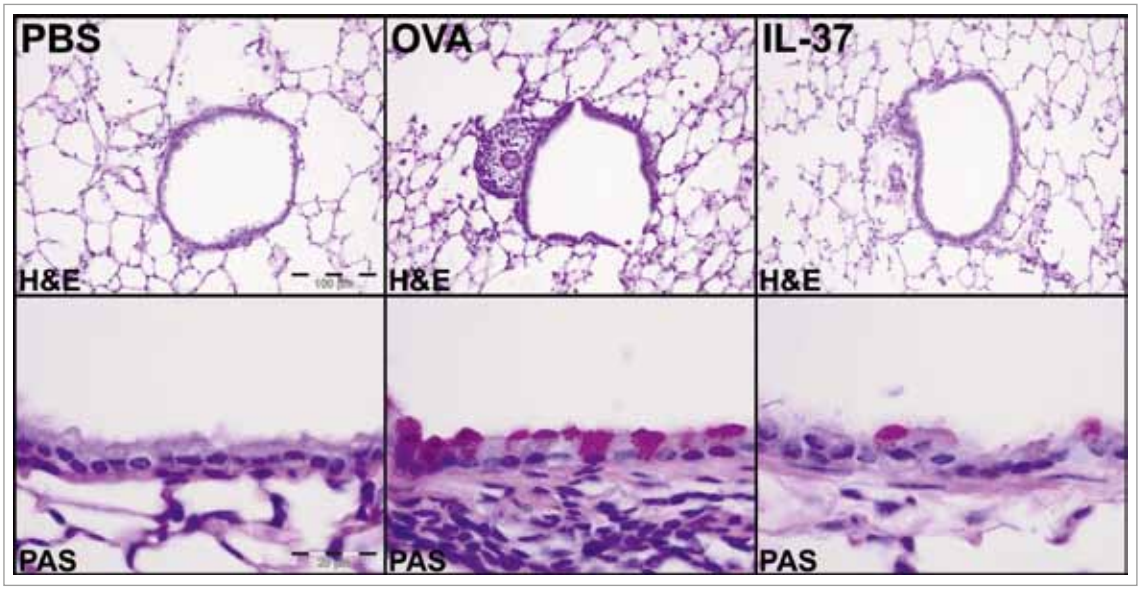


Figure 1. Local treatment with IL-37 reduces allergic airway inflammation and mucus hyperproduction. H&E or PAS-staining of airway cross sections of sham-treated animals with experimental asthma (OVA), IL-37 treated animals with acute asthma (IL-37) and sham-treated healthy controls (PBS) to identify allergic airway inflammation and mucus-producing goblet cells.

Additionally, we identified the α-melanocyte stimulating hormone (α-MSH), a neuro-peptide hormone genually desdcribed in the skin, to be also involved in the regulation of allergic airway inflammation. Thus, asthma patients and mice with experimental allergic asthma display significantly enhanced levels of α-MSH. Interestingly, neutralization of α-MSH results in aggravation of experimental asthma and vice versa local application of α-MSH markedly reduced airway eosinophilia and improved hallmarks of the disease indicating that this hormone exerts regulating effects on the allergic immune response. Such beneficial effects could not be achieved in animals lacking the melanocortin-receptor 5 suggesting that α-MSH requires this receptor to deploy its activity. Based on these data, we have demonstrated that both mediators, IL-37 and α-MSH, have the capacity to counterbalance allergic airway inflammation and to improve asthma symptoms suggesting them as promising targets for the development of a new asthma therapy.

While these two mediators emerged as examples for new regulators of airway inflammation in asthma pathogensis, exogenous triggers such as high allergen load or respiratory infection have the ability to overwhelm such regulatory mechanisms and to lead to acute aggravation of the disease. Hence, respiratory viral infections have been shown to be by far the most important factor triggering acute exacerbation of allergic bronchial asthma, as characterized by dramatic worsening of asthma hallmarks and the requirement of acute medication and/or health care measures. We used the fact that all respiratory viruses that have been associated with such an event (eg rhino-, respiratory syncytial-, influenza

Priority Research Area **Asthma and Allergy**

Asthma Exacerbation & Regulation

viruses, etc.) display double-stranded RNA-motifs in the tertiary structure of their genome or as an intermediate during viral replication, which in turn is recognized intra-cellularly by toll-like receptor 3 (TLR-3), to establish a new mouse model of acute asthma exacerbation. Thus, local activation of TLR-3 by polyIC (pIC) resulted in a dramatic increase of airway inflammation, airway neutrophilia, mucus hyperproduction and AHR comprising the typical features of an acute exacerbation. Using this new model we further demonstrated that this exacerbation is mainly mediated by IL-17A and have identified infiltrating NK cells as the major source of this proinflammatory cytokine. Consequently, neutralization of either IL-17A or NK cells completely prevented induction of acute asthma exacerbation by pIC in mice suggesting them as novel targets for therapeutic intervention of this disease stage.

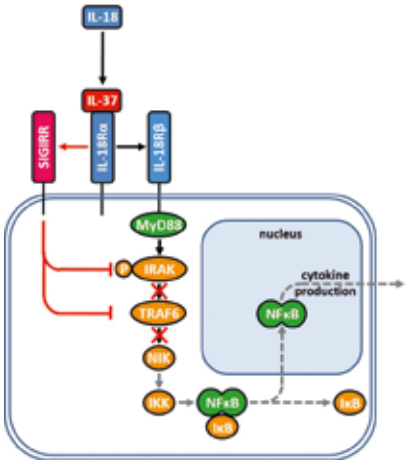


Figure 2.
Proposed mechanism of IL-37 signaling. IL-37 interferes with pro-inflammatory cytokine signaling of members of the IL-1 cytokine family (e.g. IL-1, IL-18, etc.) by binding to IL-18Rα. This leads to recruitment of SIGIRR as a co-receptor and subsequent blocking of pro-inflammatory cytokine signaling at the level IRAK and TRAF-6, which inhibits translocation of NFκB and, thus, further expression of pro-inflammatory cytokines.

Recurrent viral infections and, thus, recurrent acute exacerbations also represent the major risk factor for disease progression towards a severe asthma endotype, which is characterized by serious impairment of lung function, insensitivity towards corticoid (CS) therapy, and the requirement of intensive medical care, high dose systemic medication, and mechanical ventilation. Alike during episodes of acute exacerbation airway inflammation of such patients typically reveals not only large numbers of infiltrating neutrophils but also increased levels of IL-17. The production of this proinflammatory cytokine is controlled by the transcription factor transcription factor RORγt. So we hypothesized that targeting this molecule is an option to regulate allergic airway inflammation and, thus, a promising candidate for therapeutic intervention. Indeed, mice that are lacking functional expression of RORγt display an impaired production of IL-17A and are nearly refractory towards the induction of experimental severe asthma underlining the importance of this transcription factor in this setting. Using target-specific small-interference (si) RNA we could demonstrate that downregulation of RORγt for example in in-vitro generated TH17 cells results in dramatically reduced secretion of IL-. Consequently, local treatment with this RORγt-specific siRNA markedly reduced airway neutrophilia, IL-17A production and lung function in a mouse model of experimental severe asthma.

Internal and external collaboration

At the Research Center Borstel we cooperate with the Divisions of Biochemical Immunology, Cellular Microbiology, Early Life Origins of CLD, Experimental Pneumology, Invertebrate Models and the Fluorescence Cytometry Core Facility.

External national cooperations have been established with the 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, and the Department Translational Pulmonology, University of Heidelberg as well as with the Clinic of Internal Medicine V at the University Clinic of the Saarland at Homburg, and Department of Dermatology, Hospital of the University of Münster.

International cooperations have been established with the Division of Infectious Diseases at the University of Colorado, Denver, USA, the Department of Experimental Immunopathology, Ospedale a Milano, Italy, and the Institute of Pathophysiology and Allergy Research at the Medical University of Vienna, Austria.

Grant support

BMBF DZL DA-AA-TFP

BMBF DZL LI-JRG-1

BMBF FKZ: 13N13857

AUTOIMMUNITY

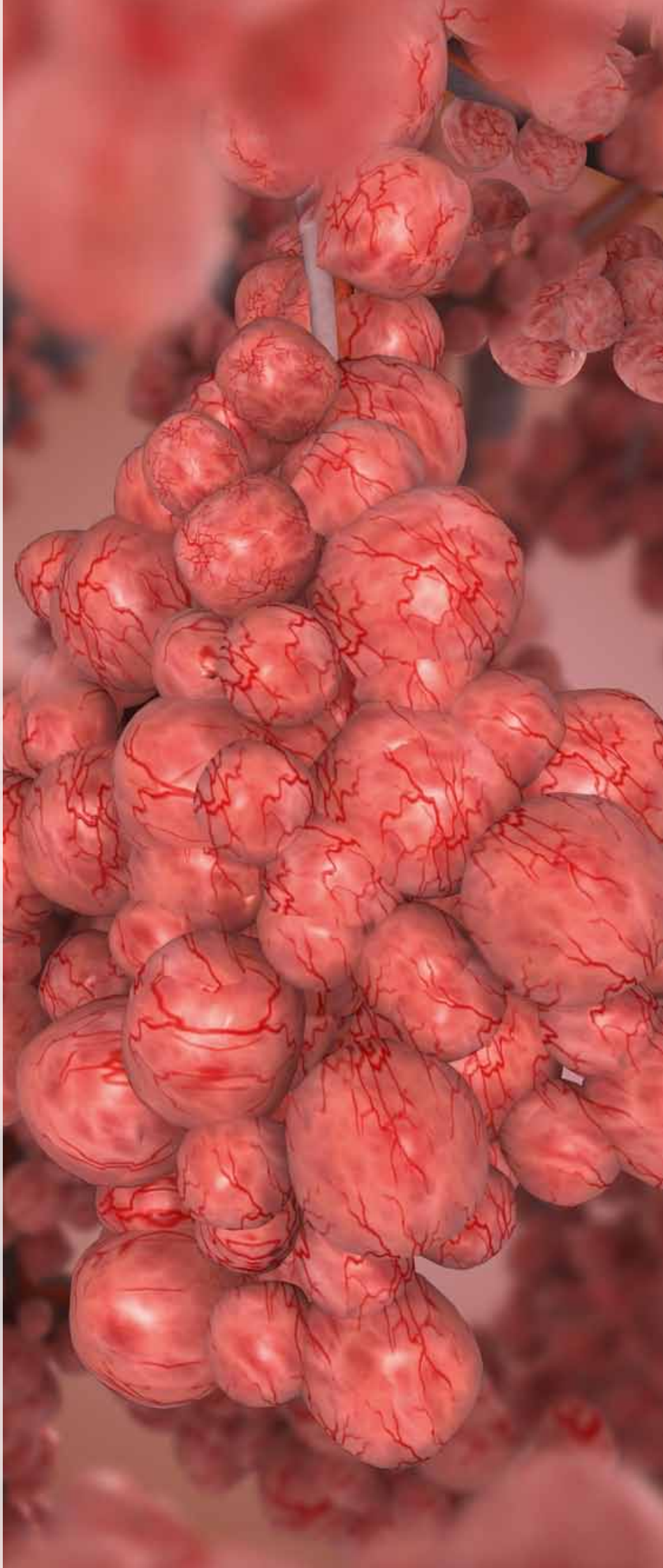
SYSTEMIC SCLEROSIS

NEUTROPHIL

PULMONARY FIBROSIS

EXPERIMENTAL MODELS

TISSUE DAMAGE



Priority Research Area **Asthma and Allergy**

Autoimmunity in the Lung

Head

- Prof. Dr. Xinhua Yu
- Prof. Dr. Gabriela Riemekasten

Members

- Dr. Junie Tchudjin Magatsin
- Xiaoyang Yue
- Kai Yang

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

Neutrophils play an essential role in host defence against microbial invaders. However, due to their lacking specificity, these defence mechanisms always bear the risk of self-tissue damage. As a consequence, the range of action of mediators like reactive oxygen species (ROS) or proteolytical enzymes is limited and tightly controlled by a variety of scavenger and inhibitor molecules abundantly present in blood and tissues. However, this fine balance in neutrophil activation between host defence and tissue protection can be disturbed under some pathological conditions. Therefore, the understanding of the different molecular steps of neutrophil activation, e.g. neutrophil adhesion in disease could help to develop new strategies to prevent the host from harmful attacks by neutrophils.

Previously, we demonstrate that immune complex (IC)-induced neutrophil adhesion is an indispensable prerequisite in the process of tissue damage. Based on our findings, we propose a model of how IC-induced neutrophils adhesion contribute to tissue damage (Fig 1). After recruitment into the peripheral tissue, neutrophils are activated by immobilized IC via FcγR. During activation, neutrophils tightly adhere to the target tissue and form a closed space preventing a molecular exchange with the environment. As a consequence, uncontrolled elastase activity inaccessible to exogenous inhibitors will attack and destroy the structurally important proteins of the tissue. Given

Highlights

Establishment of the Clinical Liaison Group „Autoimmunity in the Lung” (Gabriela Riemekasten, Dep. of Rheumatology, University of Lübeck, and Xinhua Yu, Priority Area Asthma Allergy, Research Center Borstel), May 2015

Memorandum of Understanding between the Medical College of Xiamen University (China) and the Research Center Borstel. Establishment of the Xiamen-Borstel Joint Laboratory of Autoimmunity in Xiamen. Start of the Xiamen - Borstel students exchange program, Sept. 2015

Selected publications

Chen Y, Zheng J, Huang Q, Deng F, Huang R, Zhao W, Yin J, Song L, Chen J, Gao X, Liu Z, Petersen F, Yu X. Autoantibodies against the Second Extracellular Loop of M3R Do neither Induce nor Indicate Primary Sjögren‘s Syndrome.PLOS ONE 2016, 22: e0149485.

Zheng J, Huang R, Huang Q, Deng F, Chen Y, Yin J, Chen J, Wang Y, Shi G, Gao X, Liu Z, Petersen F, Yu X. The GTF2I rs117026326 polymorphism is associated with anti-SSA-positive primary Sjögren‘s syndrome. RHEUMATOLOGY (Oxford). 2015, 54:562-4.

Yu X, Kasprick A, Petersen F. Revisiting the role of mast cells in autoimmunity. AUTOIMMUNITY REVIEWS 2015, 14: 751-9

Kasprick A, Yu X, Scholten J, Hartmann K, Pas HH, Zillikens D, Ludwig R J, Petersen F. Conditional depletion of mast cells has no impact on the severity of experimental epidermolysis bullosa acquisita. EUROPEAN JOURNAL OF IMMUNOLOGY 2015, 45: 1462-70

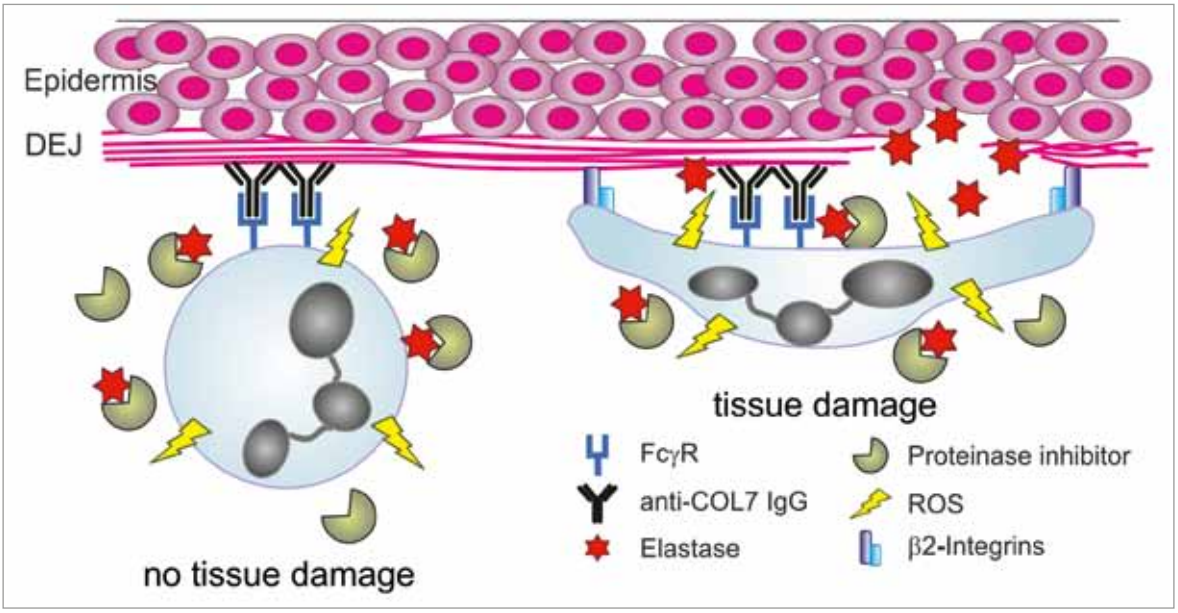


Figure 1. An indispensible role of IC-induced neutrophil adhesion to the target surface in tissue damage.

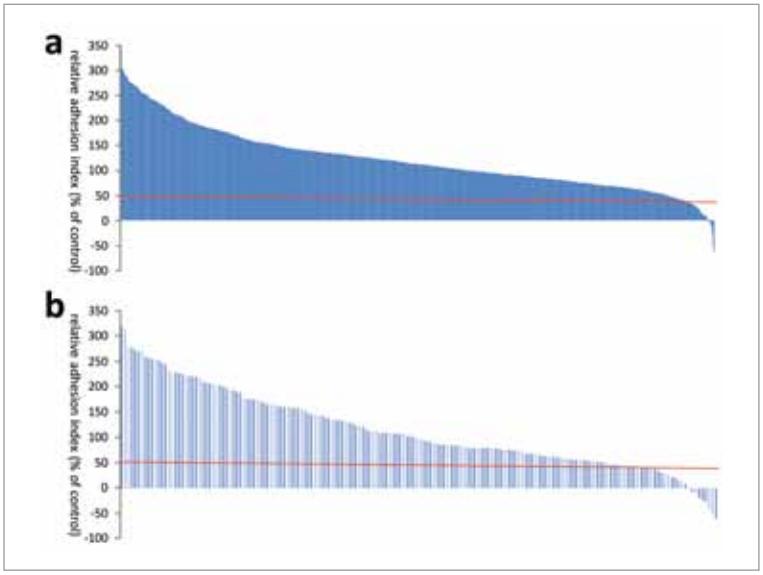


Figure 2. Inhibition effect of chemical components on IC-induced neutrophil adhesion. IC-induced neutrophil adhesion was quantified by using xCELLigence system. Relative adhesion index was calculated using IC stimulation as positive control (100%) and without stimulation (PBS) as negative control (0%). The effect of each molecule from the Prestwick Chemical Library (a) and the Target-Selective Inhibitor Library (b) on neutrophil adhesion was evaluated individually in presence of IC stimulation.

Autoimmunity in the Lung

that neutrophil adhesion represents an essential prerequisite in the tissue damage process in many inflammatory disease, such as autoimmune blistering diseases and COPD, it provide a chance to treat the diseases by specifically targeting neutrophil adherence without affecting other neutrophil functions. Consequently, we are currently identifying therapeutic molecules which can specifically inhibit neutophil adhesion by screening two chemical libraries, the Prestwick Chemical Library containing 1280 approved drugs and the Target-Selective Inhibitor Library containing 141 target-selective inhibitors. The results from the first round of screening shows that 83 out of 1053 molecules from the Prestwick Chemical Library and 34 out of 141 molecules from the Target-Selective Inhibitor Library have considerable inhibitory effect (more than 50% inhibition) on neutrophil adhesion (Figure 2). Therefore, these 117 molecules will be further evaluated for their toxicity, specificity and therapeutic efficacy.

Systemic sclerosis (SSc) is a severe autoimmune disease with a reduction of life expectancy by 34 years in female SSc patients and by 16 years in male patients compared to age- and sex-matched controls. Lung manifestation is one clinical hallmark of the disease, leading to pulmonary hypertension and lung fibrosis, which represent the two major leading causes of death in SSc patients. The pathogenesis of autoimmunity associated lung manifestation needs to be explored. To investigate the pathogenic mechanism of SSc, we are currently establishing **experimental models** for SSc via passive transfer IgG or PBMC from SSc patients or via active immunization. Very recently, we have established a novel mouse model of SSc by immunizing mice with antigen. The immunized mice develop many disease symptoms mimicking SSc, including skin fibrosis, skin and lung inflammation, while such symptoms were not observed in the control mice, suggesting that this is **a novel mouse model** of SSc. Since this novel mouse model of SSc is under the process of patent application, detailed information are not shown here.

Internal and external collaboration

Petersen F, Division of Biochemical Immunology, Research Center Borstel; Hölscher C, Division of Infection Immunology, Research Center Borstel; Frey A, Division of Mucosal Immunology and Diagnostic, Research Center Borstel; Heine H, Division of Innate Immunity, Research Center Borstel; Scholzen T, Core Facility Fluorescence Cytometry, Research Center Borstel; Riemekasten G, Department of Rheumatology, University of Lübeck; König P, Institute of Anatomy, University of Lübeck; Zillikens D, Institute of Dermatology, University of Lübeck; Ludwig R, Institute of Dermatology, University of Lübeck; Ibrahim S, Institute of Dermatology, University of Lübeck, Zhou J, the Medical College of Xiamen University; Shi G, the department of Rheumatology, the First Affiliated Hospital of Xiamen University .

Grant support

DFG Cluster of Excellence „Inflammation at Interfaces"; Research Area H: IRN Autoimmunity to Type VII Collagen.

DFG GRK 1727 „Modulation von Autoimmunität"

BMBF Deutsches Zentrum für Lungenforschung (DZL), ARCN

MAST CELL-NEUTROPHIL INTERACTION

ASTHMA

PROTEASES

TISSUE DAMAGE

AIRWAY EPITHELIUM AUTOIMMUNITY

Priority Research Area **Asthma and Allergy**

Biochemical Immunology

Head

- Prof. Dr. Frank Petersen

Members

- Marjan Ahmadi
- Reza Akbarzadeh
- Nestor Gonzalez Roldan
- Christine Engellenner
- Cindy Hass
- Diana Heinrich
- Brigitte Kasper
- Carola Schneider
- Xiaoqing Wang
- Jacqueline Wax

Figure 1. On the way to in vitro models of the lung surface - Human primary epithelial cells can form 3D-cell spheroies in which cells express mucus (red) and ciliated surfaces. (PAS/HE staining, 100fold magnification).

Priority Research Area **Asthma and Allergy**

Biochemical Immunology

Mission

Die Forschungsarbeiten der Gruppe widmen sich der Untersuchung von pathophysiologischen Prozessen in der Effektorphase chronisch entzündlicher Prozesse der Lunge. Wir untersuchen über welche regulatorischen Prinzipien und pathologische Mechanismen Neutrophile und Mastzellen zur Pathogenese des Asthmas sowohl während der Initiation als auch bei Exazerbationsepisoden beitragen.

The work of the division is focused on the analysis of pathophysiological processes in the effector phase of chronic inflammatory processes in the lung. We are investigating the regulatory principles and pathological mechanisms underlying the interaction of mast cells and neutrophils in the initiation and exacerbation of asthma.

Most important findings

Two major processes of asthma disease transition, from ‚healthy to diseased' and from ‚controlled **asthma** to aggravated disease', have been recently identified as major fields of research in the Priority Areas Asthma & Allergy. We postulate a **mast cell - neutrophil axis** which represents a fundamental pathophysiological element essentially involved in the execution and regulation of the asthma pathology.

Increased numbers of **neutrophils** in the lung are characteristic for asthma especially during late-phase reactions and in cases of severe and steroid-resistant forms of the disease. Surprisingly, their basic pathological role remains largely unclear. In our studies, we used an adjuvant-free mouse model of asthma where either a transient neutropenia was induced by neutrophil depletion during effector phase or by the use of (constitutively) neutrophil-deficient mice. Airway hyperreactivity (AHR), airway inflammation, and cytokines were analyzed in the presence or absence of neutrophils. In both models we found that AHR, goblet cell hyperplasia, and mucus production were significantly reduced in the absence of neutrophils indicating a pathogenic impact of these cells on the airway epithelium. However, neutropenia did not alter inflammatory cell infiltration or expression of Th2 cytokines in restimulated lung cells. Unexpectedly, neutropenic mice produced increased amounts of IFNγ and IL-17A, indicating a previously unrecognized regulatory role of neutrophils in cytokine production. To define neutrophil-mediated pathomechanisms the interaction between neutrophils and **epithelial cells** as well as **T cells** were analyzed in new model systems *in vitro* (Figure 1). Here, neutrophils provoked a dose-dependent loss of epithelial cell integrity which became visible in a reduction of cell adhesion (detachment) and changes in epithelial cell morphology. Furthermore, cytokine expression by activated T cells was strongly and dose-dependently reduced in the presence of neutrophils

Highlights

Establishment of the Clinical Liaison Group „Autoimmunity in the Lung" (Gabriela Riemekasten, Dep. of Rheumatology, University of Lübeck, and Xinhua Yu, Priority Area Asthma Allergy, Research Center Borstel), May 2015

Memorandum of Understanding between the Medical College of Xiamen University (China) and the Research Center Borstel. Establishment of the Xiamen-Borstel Joint Laboratory of Autoimmunity in Xiamen. Start of the Xiamen - Borstel students exchange program, Sept. 2015

Renewal of the German Center of Lung Research, Jan. 2016

Selected publications

Akbarzadeh R, Yu X, Vogl, T, Ludwig, RJ, Schmidt E, Zillikens D, Petersen F. Myeloid-related proteins-8 and -14 are expressed but dispensable in the pathogenesis of experimental epidermolysis bullosa acquisita and bullous pemphigoid. JOURNAL OF DERMATOLOGICAL SCIENCE 2016, 81: 165-72

Yu X, Kasprick A, Petersen F. Revisiting the role of mast cells in autoimmunity. AUTOIMMUNITY REVIEWS 2015, 14: 751-9

Kasprick A, Yu X, Scholten J, Hartmann K, Pas HH, Zillikens D, Ludwig R J, Petersen F. Conditional depletion of mast cells has no impact on the severity of experimental epidermolysis bullosa acquisita. EUROPEAN JOURNAL OF IMMUNOLOGY 2015, 45: 1462-70

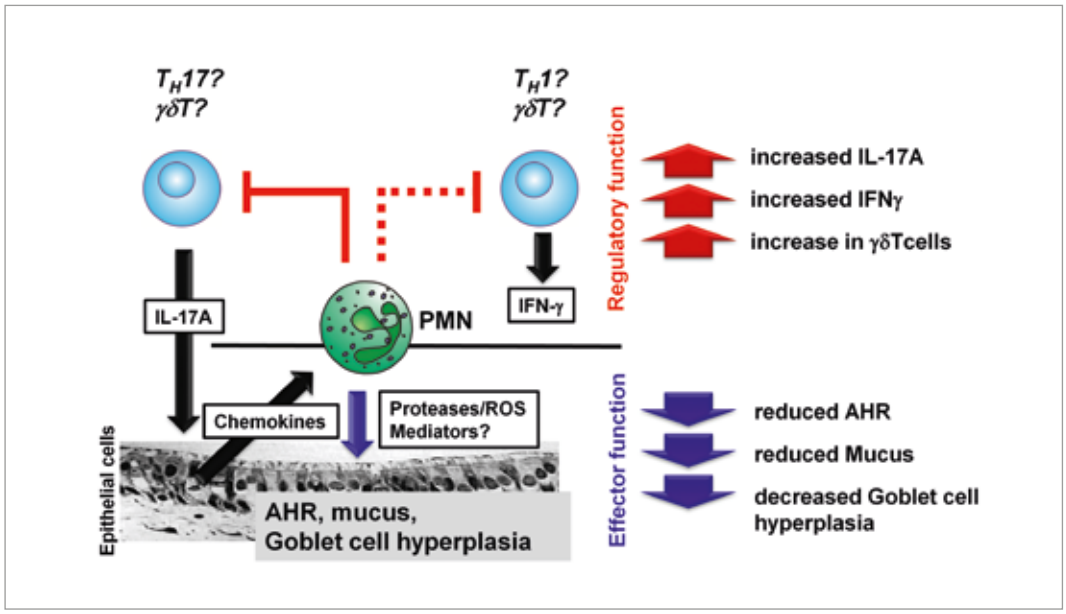


Figure 2. Regulatory and effector functions of neutrophils in allergic asthma. Airway hyperreactivity, mucus production, and goblet cell hyperplasia are dramatically reduced in neutropenic mice demonstrating a fundamental pro-inflammatory role of these cells in the effector phase of asthma. However, Th1 and Th17 cytokines as well as γδT cells are elevated in these mice indicating a previously unrecognized regulatory function of neutrophils on T cells during allergic airway inflammation.

All effects were dependent on cell-cell contact, indicating the involvement of neutrophil proteases. Together, these results provide evidence for a dual role for neutrophils in experimental asthma identifying them as essential promotors of the disease pathology and regulators of (Th1 and Th17) cytokine production (Figure 2).

Mast cells (MC) have been shown to be essentially involved in allergic diseases by releasing a variety of mediators upon activation. Among these, human MC chymase and its murine homologue Mcpt4 are involved in several inflammatory processes like tissue damage or proteolytical processing of cytokines. Surprisingly, recent studies suggest a potent protective role of Mcpt4 in a model of acute asthma. In this study, we examined the role of MC and Mcpt4 in a chronic model of experimental allergic asthma. Mice of the strains B6, B6-Mcpt4$^{-/-}$, MC-deficient Kit$^{W\text{-}sh}$ as well as Kit$^{W\text{-}sh}$ reconstituted either with wild type MC or Mcpt4$^{-/-}$-MC were sensitized with OVA alum-free i.p. followed by weekly repeated OVA challenge i.t. up to week 10. In this model, airway hyperreactivity (AHR) to metacholine was unexpectedly higher in MC-deficient Kit$^{W\text{-}sh}$ mice than in the corresponding WT controls (Figure 3A). This effect could be reverted to control levels by reconstitution of Kit$^{W\text{-}sh}$ mice with WT MC indicating a protective role of MC in our chronic model. Moreover, Mcpt4$^{-/-}$ mice as well as Kit$^{W\text{-}sh}$ mice reconstituted with Mcpt4$^{-/-}$-MC were found to be resistant to an induction of AHR in experimental asthma demonstrating the essential role of Mcpt4 in the disease (Figure 3BC). Further analysis revealed that goblet cell hyperplasia and mucus production was increased in all sensitized groups irrespective whether MC or Mcpt4 were present or not. MC-derived Mcpt4 represents a specific promoter of AHR in chronic experimental asthma, while WT MC act protective in disease. Targeting MC chymase in chronic asthma could be a promising new strategy to reduce airway limitations and to prevent asthma exacerbation.

Priority Research Area **Asthma and Allergy**

Biochemical Immunology

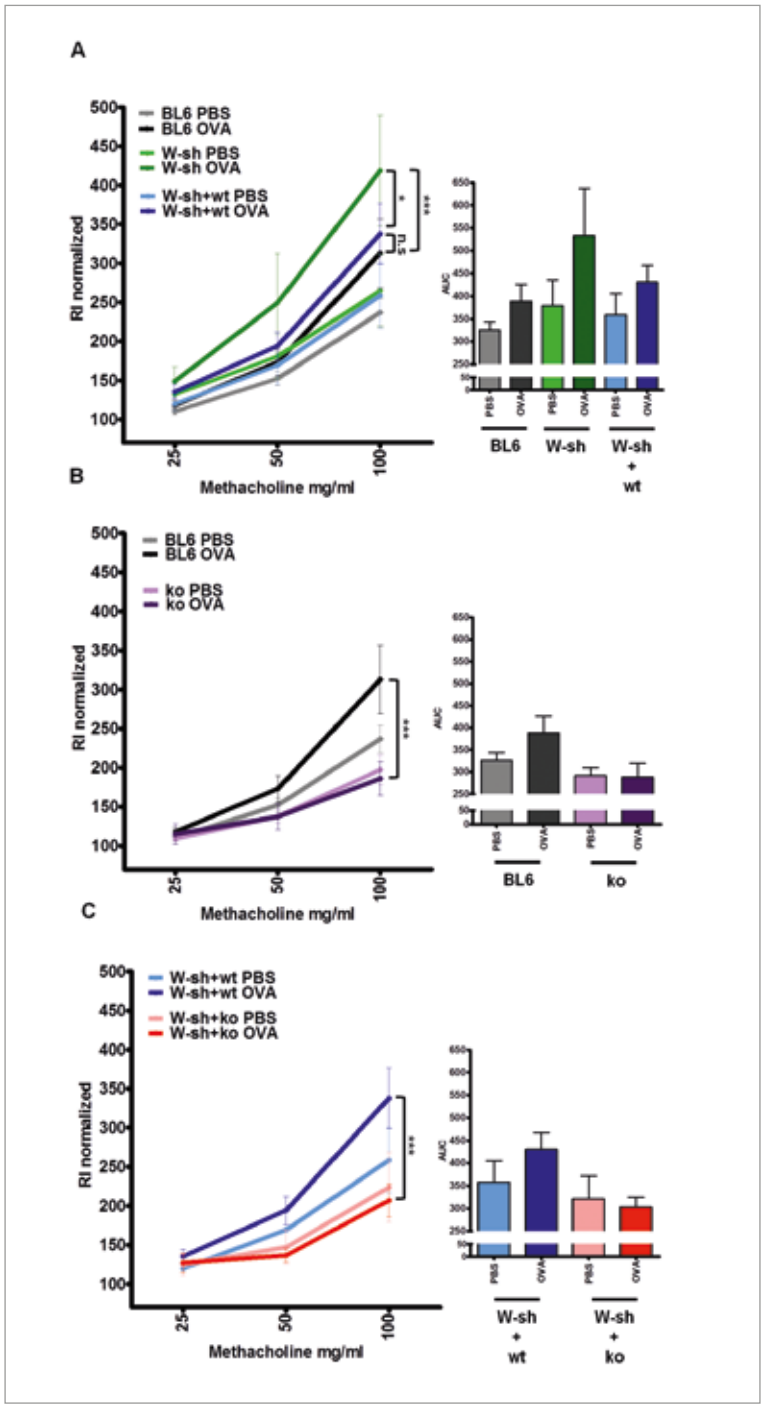


Figure 3. Antagonistic effects of mast cells and Mcpt-4 on airway hyperreactivity (AHR) in chronic experimental asthma. **A)** AHR was determined in WT and MC-deficient W-sh mice. AHR was elevated in W-sh mice as compared to WT or W-sh mice reconstituted with WT MC, indicating a protective role of MC in asthma. **B)** AHR in Mcpt-4 deficient mice. Mice lacking Mcpt-4 do not develop AHR in chronic experimental asthma. **C)** W-sh mice reconstituted with BMMC derived from Mcpt-4$^{-/-}$ mice (ko), but not with MC from WT animals, are resistant to an induction of AHR in chronic asthma. Data are expressed as airway resistance (left panels) or as area under the curve (right panels) of these data. Statistically significant differences are indicated by asterisks.

Internal and external collaboration

Yu X, Clinical Liaison Group Autoimmunity in the Lung, Research Center Borstel; Wegmann M, Division of Asthma Exacerbation & Regulation, Research Center Borstel; Hölscher C, Division of Infection Immunology, Research Center Borstel; Frey A, Division of Mucosal Immunology and Diagnostic, Research Center Borstel; Heine H, Division of Innate Immunity, Research Center Borstel; Goldmann T, Division of Clinical and Experimental Pathology, Research Center Borstel; Scholzen T, Core Facility Fluorescence Cytometry, Research Center Borstel; Riemekasten G, Department of Rheumatology, University of Lübeck; Köhl J, ISEF, University of Lübeck; Ehlers M, ISEF, University of Lübeck; König P, Institute of Anatomy, University of Lübeck; Weckmann M, Department of Pediatric Allergy and Pulmonology, University of Lübeck; Zillikens D, Institute of Dermatology, University of Lübeck; Ludwig R, Institute of Dermatology, University of Lübeck; Schmidt E, Institute of Dermatology, University of Lübeck ; Vogl T, Division of Immunology, University of Münster, Schultz C, EMBL Heidelberg; Chen Y, Hogan SP, Cincinnati Children's Hospital, USA; Fillipi MD, Cincinnati Children's Hospital, USA

Grant support

DFG Cluster of Excellence „Inflammation at Interfaces"; Research Area H: IRN Autoimmunity to Type VII Collagen.

DFG GRK 1727 „Modulation von Autoimmunität"

DFG IRTG 1911 „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

Priority Research Area **Asthma and Allergy**

Clinical and Molecular Allergology

Head

- Prof. Dr. Uta Jappe

Members

- Marisa Böttger
- Maren Hohn
- Dr. Arne Homann
- Dr. Skadi Kull
- Carolin Murawski
- Daniel Rosero
- Dr. Frauke Schocker
- Marion Schuldt
- Christian Schwager
- Daniela Warneke

Physicians

- Dr. Annika Opitz
- Tahmina Kolaly
- Dr. Mareike McIntyre

Master students

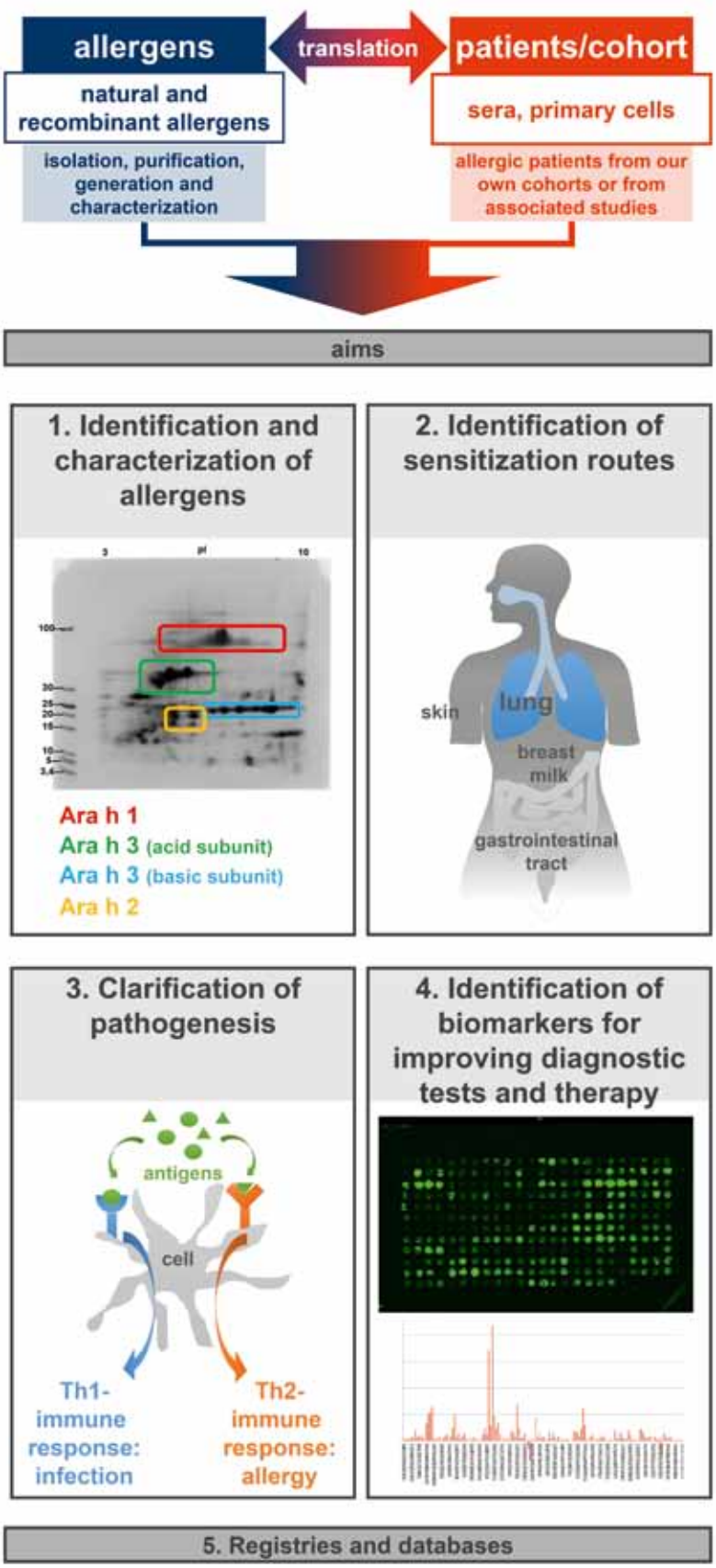
- Alexandra Scharf
- Arabella Karstedt

Apprentices

- Imke Storm
- Lea Bendler
- Masha-Melissa Spauszus

Mission/Overall concept

„From beside to bench and back“



Priority Research Area **Asthma and Allergy**

Clinical and Molecular Allergology

Mission

Ziel unserer translationalen Forschung ist, zur Aufklärung von Pathomechanismen der Allergie- und Asthmaentstehung und möglicher Sensibilisierungswege beizutragen. Dazu werden neue Allergene sowie Allergie-relevante Epitope identifiziert und hinsichtlich ihrer molekularen Struktur und deren Einfluss auf ihre Funktion charakterisiert. Auf diese Weise sollen Marker identifiziert werden, die die Allergiediagnostik zielgenauer gestalten und es erlauben, das individuelle Risiko einzelner Betroffener zu erfassen.

Main aspects within the translational research are the isolation, identification and structural characterization of allergens in order to improve allergy diagnostic tests (individualized diagnostic) and to use them as tools for investigating the influence of allergen structures, for example, on sensitization routes, allergy and asthma pathomechanisms, and on the severity and localization (organ involvement) of the individual allergic symptom development.

Most important findings

Characterization of peanut oleosins as novel candidates for in vitro routine diagnostic

For investigating the „organ specificity" of certain single allergens, peanut allergy is excellently suited as a model disease because patients experience the whole spectrum of allergic symptoms including lung-associated symptoms. These respiratory symptoms include allergic rhinitis, dyspnea and allergic asthma. In addition, peanut allergy is one of the most severe class I food allergies with increasing prevalence in Europe.

In the course of the identification of lipophilic peanut allergens, we developed a novel strategy for the isolation and purification of peanut oil body proteins, present only in the lipid fraction. For the first time we were able to purify the 8 known peanut oleosins simultaneously. Moreover, we identified new oil body integral proteins, termed caleosins, and confirmed the presence of steroleosins. IgE binding to purified oleosins was observed in 45 of 63 sera from peanut-allergic patients by western blot analysis. Thus, oleosins have been officially accepted as new allergens by the WHO/IUIS allergen nomenclature subcommittee and were denominated as Ara h 14 and Ara h 15 (Schwager et al., Plos One 2015). In parallel, together with the Core Facility Fluorescence Cytometry, a flow cytometric basophil activation test was established which allows us to investigate the biological activity of our newly identified oleosin allergens (Fig. 1).

Highlights

Characterization and registration of novel peanut oleosins Ara h 14 and Ara h 15

Detection of the peanut allergen Ara h 2 in breast milk

Immunogenic epitopes on infliximab with pharmacological impact

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

Prizes: Poster & Talk Award, Summer School, Cluster of Excellence and Best Presentation Award plus Travel Grant of the World Immune Regulation Meeting (WIRM), Davos to Dr. A Homann

VEIAP Master-Award 2015 to A. Scharf; VEIAP Master Award 2016 to A. Karstedt

Selected publications

Schocker F, Baumert J, Kull S, Petersen A, Becker WM, Jappe U. Prospective investigation on the transfer of Ara h 2, the most potent peanut allergen, in human breast milk. PAI 2016,27(4):348-355 (Editor`Choice Pediatr Allergy Immunol 2016,27(4):337)

Jappe U, Nikolic J, Opitz A, Homann A, Zabel P, Gavrovic-Jankulovic M. Apparent IgE-negative anaphylactic reaction to banana combined with kiwi-allergy – Complementary diagnostic value of purified single banana allergens. JEADV 2016,30:1195-1252

Schwager C, Kull S, Krause S, Schocker F, Petersen A, Becker WM, Jappe U. Development of a novel strategy to isolate lipophilic allergens from peanuts. Plos One 2015, 10(4):e0123419

Petersen A, Kull S, Rennert S, Becker WM, Krause S, Ernst M, Gutsmann T, Bauer J, Lindner B, Jappe U. Peanut defensins: novel allergens isolated from lipophilic peanut extract. JACI 2015, 136(5):2195-1301

Homann A, Röckendorf N, Kromminga A, Frey A, Jappe U. B cell epitopes on infliximab identified by oligopeptide microarray with unprocessed patient sera. J Transl Med 2015,13:339

Kull S, Vrtala S, Jappe U. Bekannte Einzelallergene der Hausstaubmilben: Struktur, Funktion und Relevanz, Allergologie 2015,38:55–63

Treudler R, Franke A, Schmiedeknecht A, Ballmer-Weber B, Worm M, Werfel T, Jappe U, Biedermann T, Schmitt J, Brehler R, Kleinheinz A, Kleine-Tebbe J, Brüning H, Ruëff F, Ring J, Saloga J, Schäkel K, Holzhauser T, Vieths S and Simon J. Double blind placebo-controlled allergen-specific immunotherapy with the hypoallergenic folded variant of rBet v 1-FV in birch related soy allergy. Allergy 2016 Dec 20. doi: 10.1111/all.13112. [Epub ahead of print]

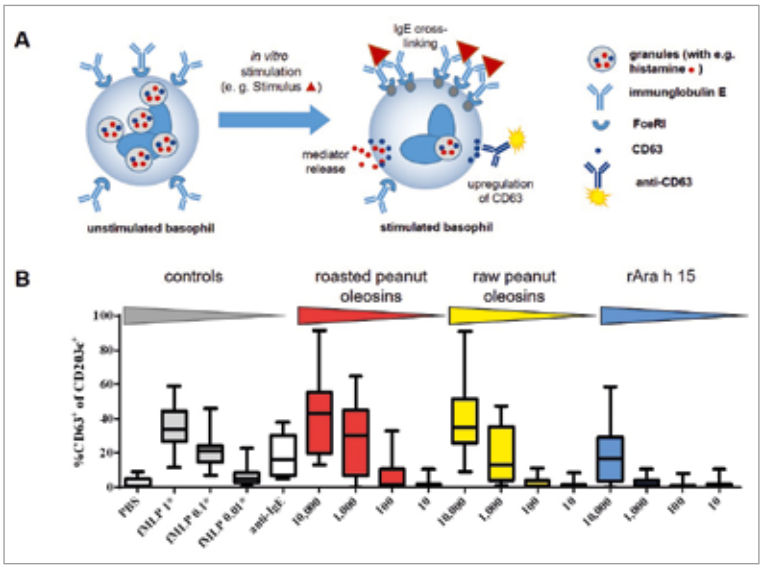


Figure 1. A) Schematic view of basophil activation modified from T. Gentinetta et al. Pipette 2011,5:13. B) Basophil activation peanut oleosins.

Marker allergen for severe peanut allergy detected in breast milk

Breast feeding may predispose at risk-babies to sensitization to peanuts. However, it may also act as a vehicle for tolerance induction. In our study we investigated the transfer of Ara h 2, the most potent peanut allergen and a predictor for severe allergic reactions, into human breast milk assessing the time kinetics of Ara h 2 appearance and its concentrations. Therefore, we investigated 32 lactating, non-peanut allergic women from Germany, the largest study population studied prospectively thus far, analysing the secretion of Ara h 2 into breast milk at different time points after consumption of a defined amount of dry roasted peanuts (Fig. 2).

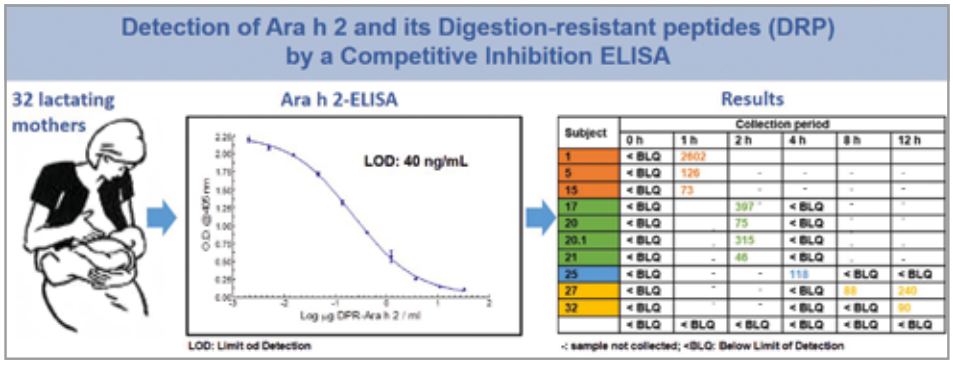


Figure 2. Transfer of Ara h 2 into Human Breast Milk modified from Schocker F et al. Pediatr Allergy Immunol 2016, 27(4):348-355

Priority Research Area **Asthma and Allergy**

Clinical and Molecular Allergology

It could be shown that Ara h 2 was transferred into human breast milk and that time and concentration of secreted Ara h 2 is individually regulated (Schocker et al., PAI 2016). The identification of Ara h 2 allows us to design follow-up investigations studying breast feeding as a sensitization route or a vector that can be used to induce tolerance to peanut very early in life if introduced at the relevant time interval (critical window of opportunity) and in the correct dose.

Allergy against Biologicals - Identification of immunogenic glycan & peptide epitopes

This research project focuses on the detection of immunogenic epitopes on biologicals widely used as therapeutic antibodies in precision medicine, e.g. for the treatment of asthma. The clinical relevance is immense since many patients develop anti-drug antibodies which results either in hypersensitivity and/or loss of therapeutic efficacy and in discontinuation of the biological therapy. Thus, a test for pre-existing IgE as well as antibody treatment monitoring has been developed. In cooperation with the Research Group Mucosal Immunology and Diagnostics, head PD Dr. Frey, we were able to identify anti-drug antibodies against the TNF-alpha blockers infliximab and adalimumab. The epitopes are all located in the TNF-alpha binding region thus most likely interfering with the TNF-alpha neutralization (Homann et al., J Transl Med 2015).

The characterization of immunogenic epitopes on therapeutic monoclonal antibodies using unprocessed patient sera shall lead to direct translational aspects for the development of less immunogenic therapeutic antibodies. Patients benefit from less adverse events and longer lasting drug effects. A second funding period of three years has been granted for a BMWi (AiF-ZIM) project in July 2015.

Internal and external collaboration

Inhouse

FZB: 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
University of Lübeck: M Ehlers, Institute for Systemic Inflammation Research; G Riemekasten, Dept. of Rheumatology; M Kopp, Pediatric Pneumology and Allergology

National

A Kromminga, IPM Biotech; T Bahmer, Lung Clinic Grosshansdorf; M Worm, Allergy Center, Charité Berlin; R Treudler, University of Leipzig

International

T Platts-Mills, University of Virginia, Charlottesville, USA; J Baumert, University of Nebraska,
Lincoln, USA; M Gavrovic-Jankulovic, University of Belgrade, Belgrade, Serbia; S Vrtrala, University of Wien, Austria; C Akdis, SIAF, University of Zurich, Davos; E Spillner, University of Aarhus, Denmark

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), ZIM-KF2784701AJ0: Development of tests for the detection of specific IgE-antibodies directed against therapeutic monoclonal antibodies (biologicals); ZIM-KF2784702SB4: Development of an innovative test to prevent secondary treatment failure due to hypersensitivity reaction to a target treatment with biologicals (Uta Jappe)

Priority Research Area **Asthma and Allergy**

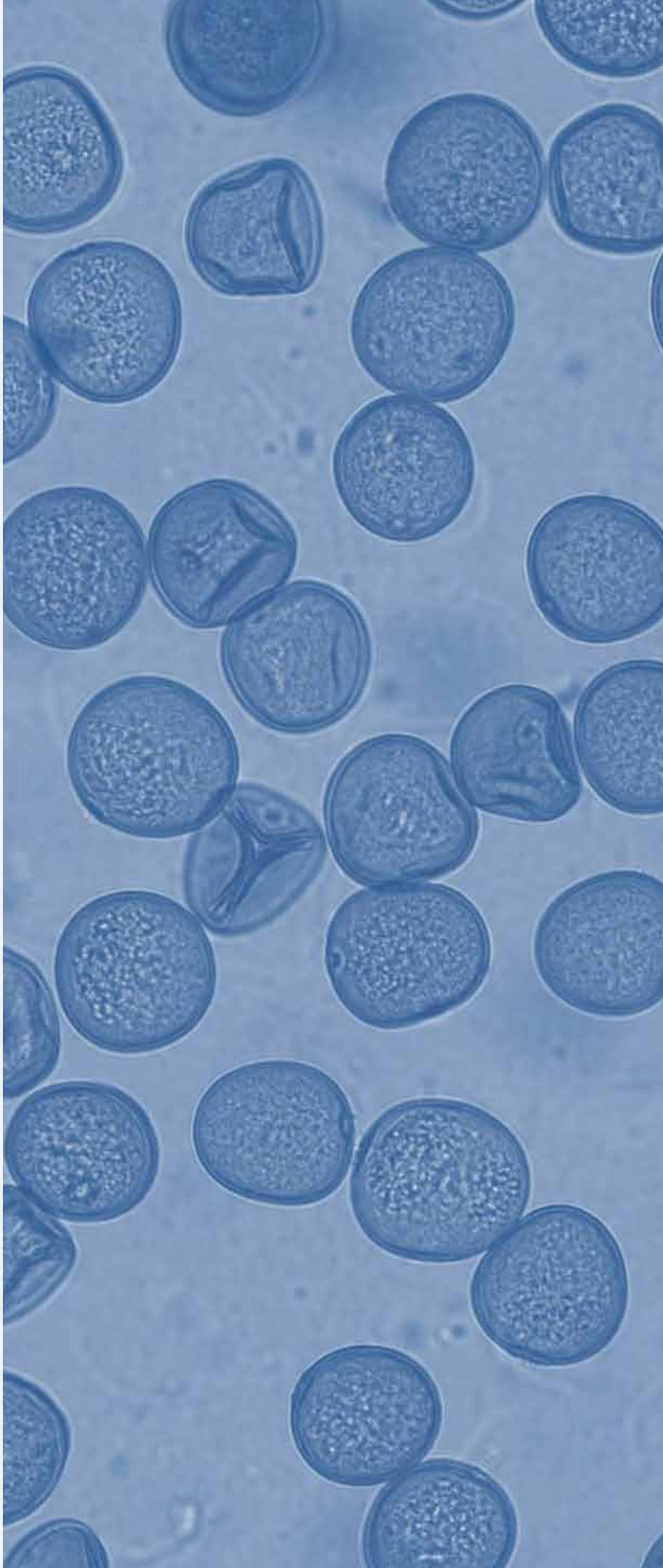
DZL-Junior Group of Allergobiochemistry

LIPIDS

ALLERGIC INFLAMMATION

STRUCTURE-FUNCTION

NKT CELLS



Head

- Dr. Katarzyna Duda

Members

- Regina Engel
- Dr. Nestor Gonzalez Roldan

Priority Research Area **Asthma and Allergy**

DZL-Junior Group of Allergobiochemistry

Mission

Die DZL-Nachwuchsgruppe Allergobiochemie widmet sich der Struktur-Funktions-Analyse von lipophilen Verbindungen aus Allergenquellen oder Bakterien, die in Zusammenhang mit der Entstehung und/oder Exazerbation von allergischen Reaktionen relevant sind.

Following the structure-function principle the DZL-junior group of Allergobiochemistry is devoted to the isolation, chemical and functional characterization of lipid moieties of environmental or bacterial origin relevant for the origin and/or exacerbation of allergic inflammation.

Most important findings

The DZL-junior group of Allergobiochemistry has been established in January 2016. Since then, the work of the group has focused on the chemical and functional analyses of lipophilic compounds isolated from human-relevant airborne allergens, such as pollen and house dust mite (HDM).

During atopic (allergic) asthma, an IgE-mediated immune response is triggered against proteins such as pollen and house dust mite allergens. Thus, most of the studies on allergy have focused on the role of protein antigens as allergens. However, allergens do not come alone. Their delivery to the nose is exclusively on particles, which carry a wide range of chemically different molecules, including lipids. Importantly, the occurrence of hydrophobic allergens, having ability to bind or interact with lipids, has been shown. Lipids have a potential to activate invariant natural killer T cells (NKT cells) via the CD1 antigen presenting molecules. Due to their very fast response and release of Th1/Th2 cytokines, this specialized T cell population owns a powerful immunoregulatory potential.

To date, there are only few examples of allergy-related lipids, and no reports on allergen-lipid interactions.

We hypothesized that lipids with different structures target different cells and receptors of the innate immunity. Doing so, they can modulate the dynamics of allergic responses to certain proteins as adjuvants; or trigger allergic inflammation on they own.

To approach this complex research question, we adapted a workflow, that we previously developed within the group of Structural Biochemistry, allowing us not only to extract lipids from various allergen sources, but also their purification and full chemical characterization. Timothy grass pollen (*Phleum pratense*, Greer), house dust mite (HDM, *Dermatophagoides pteronyssinus*, Greer), their feces or their aqueous extracts were subjected to chloroform/methanol/water extraction. The organic compounds were further fractionated on silica gel 60

Highlights

The group has successfully established diverse flow cytometry-based high throughput screening systems using murine cells for functional characterization of lipids from various allergen sources.

Selected publications

Steffens T.*, Duda K. *, Lindner B., Vorhölter F.J., Bednarz H., Niehaus K., Holst O. 2016. The lipopolysaccharide of the crop pathogen *Xanthomonas translucens* pv. translucens: chemical characterization and determination of signaling events in plant cells. *Glycobiology*. Doi: 10.1093/glycob/cww093
* Contributed equally

Gonzalez Roldan N, Orinska Z, Ewers H, Bulfone-Paus S. CD252 regulates mast cell mediated, CD1d-restricted NKT-cell activation in mice. *Eur J Immunol*. 2016 Feb; 46(2):432-9.

Duda K. A., Petersen S., Holst O. 2016. Structural characterization of the lipoteichoic acid isolated from Staphylococcus sciuri W620. *Carbohydr* Res. 430:44-47.

Kenyon* J. J., Duda* K. A., De Felice A., Cunneen M. M., Molinaro A., Laitinen J., Skurnik M., Holst O., Reeves P. R. and De Castro C. 2016. Serotype O:8 isolates in the *Yersinia pseudotuberculosis* complex have different O-antigen gene clusters and produce various forms of rough LPS. *Innate Immunity.* 22(3):2015-217.
* Contributed equally

Diederich A. K., Duda K. A., Romero-Saavedra F., Engel R., Holst O., Huebner J. 2016. Deletion of fabN in Enterococcus faecalis results in unsaturated fatty acid auxotrophy and decreased release of inflammatory cytokines. *Innate Immunity.* pii: 1753425916639669. [Epub ahead of print]

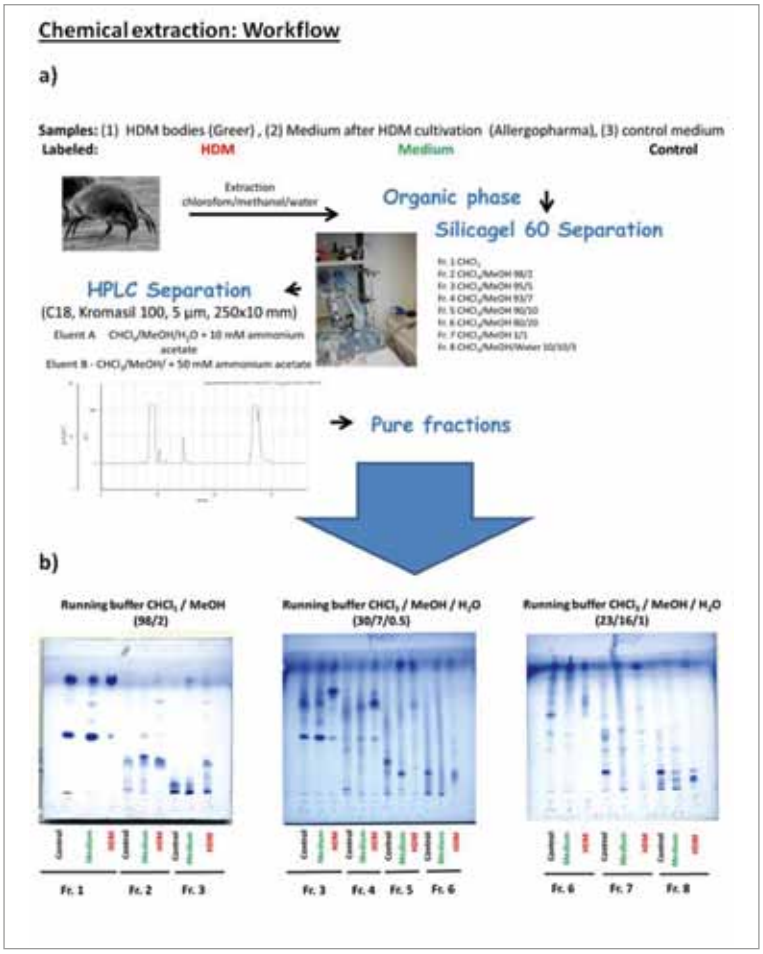


Figure 1. Workflow of isolation and purification of lipids from allergen sources (a), visualization of different lipid fraction after silica gel separation from HDM on HPTLC stained with molybdenum blue (b).

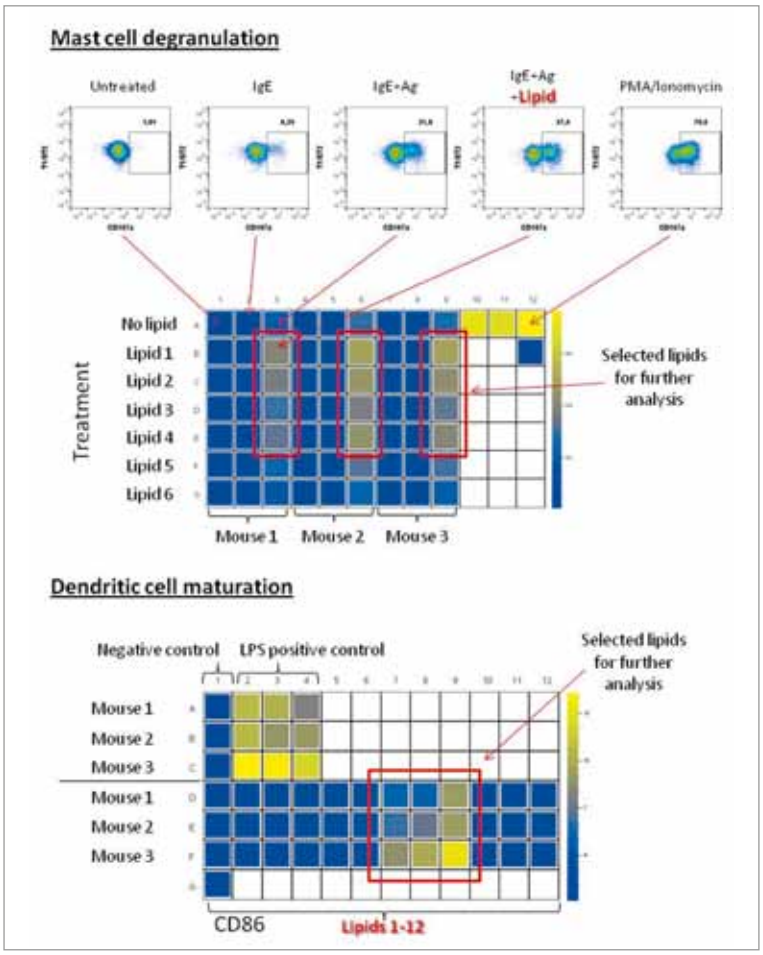


Figure 2. Example of flow cytometry high throughput analyses of lipids in bone marrow-derived mast cells (upper panel) and dendritic cells (lower panel). A high volume of data can be analyzed and displayed as heat maps, which enables the fast identification of active samples.

Priority Research Area **Asthma and Allergy**

DZL-Junior Group of Allergobiochemistry

(Merck) and by HPLC (C-18 5 µm; 250 x 10 mm, Kromasil) (Fig. 1a). Obtained lipid compounds were characterized by HPTLC, GC/MS, partially by ESI MS (in cooperation with D. Schwudke) and NMR.

Since allergen sources are highly heterogenous, we have obtained a high number of chemically different lipid fractions, in very low quantities (Fig. 1b). In a next step, following our structure-function principle we established diverse murine systems to pre-test all the samples to identify the biologically active candidates, on which the further structural analyses will be focused (Fig. 2).

Noteworthy, we developed a multiparameter staining panel for flow cytometry for the profiling of all lipid-reactive cell populations present on peripheral blood mononuclear cells (PBMC) of healthy donors and allergic patients (Fig. 3). We aim to evaluate the potential use of such profile as a biomarker of the allergic disease. With this translational approach, we head towards personalized medicine.

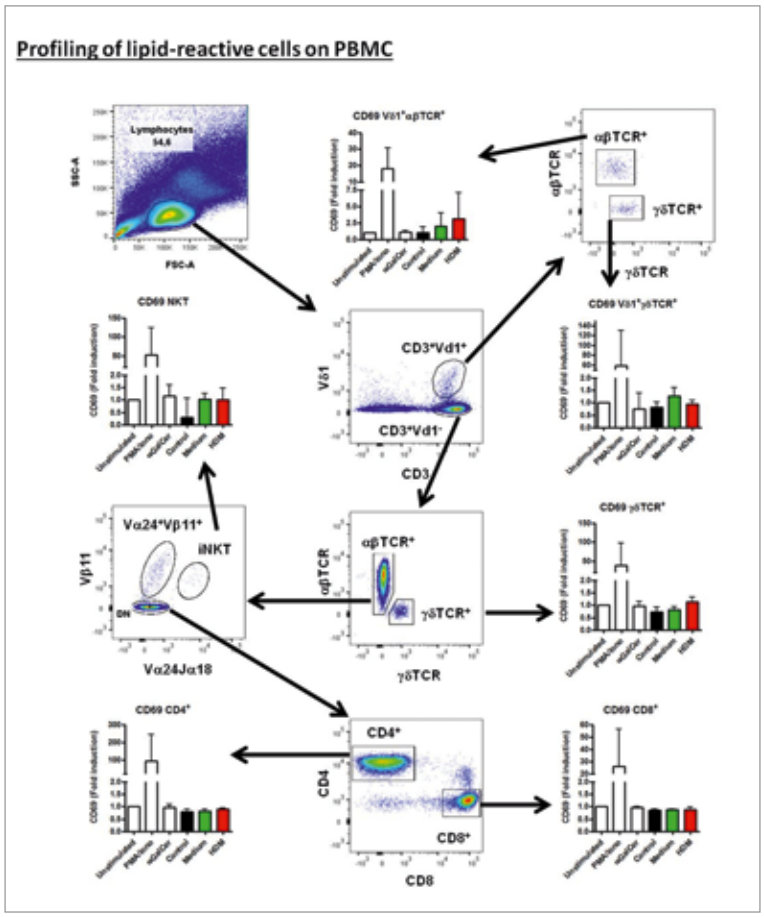


Figure 3. Multiparameter flow cytometry analysis of peripheral blood mononuclear cells collected from allergic patients. This approach allows the simultaneous detection and determination of the activation status of all lipid-reactive cell populations.

Internal and external collaboration

Internal

U. Jappe (Division of Clinical and Molecular Allergology)
H. Heine (Division of Innate Immunity)
F. Petersen (Division of Biochemical Immunology)
D. Schwudke (Division of Bioanalytical Chemistry)
Z. Orinska (Division of Experimental Pneumology)

National

H. Garn (Institute of Laboratory Medicine, Philipps-University Marburg)
A.M. Dittrich (Department for Pediatric Pneumology, Allergology and Neonatology, Hannover School of Medicine)
M. Werchan (Stiftung Deutscher Polleninformationsdienst)

International

L. O'Mahony, Molecular Immunology, Swiss Institute of Allergy and Asthma Research
A. Molinaro, University of Napoli Federico II, Dipartimento di Chimica, Napoli, Italy.
M. Skurnik, Haartman Institute, Department of Bacteriology and Immunology, University of Helsinki, Finland
Z. Kaczyński, Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland
A. Choma, I. Komaniecka, A. Turska-Szewczuk, Department of Genetics and Microbiology, Maria Curie-Sklodowska University, Lublin, Poland
C. Schäffer, Department of Nanobiotechnology, University for Agricultural Sciences, Vienna, Austria
J. Thomas-Oates, Department of Chemistry, University of York, UK
P. Kosma, Institute for Organic Chemistry, University for Agricultural Sciences, Vienna, Austria

Grant support

BMBF

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

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

Priority Research Area **Asthma and Allergy**

Early Life Origins of Chronic Lung Disease

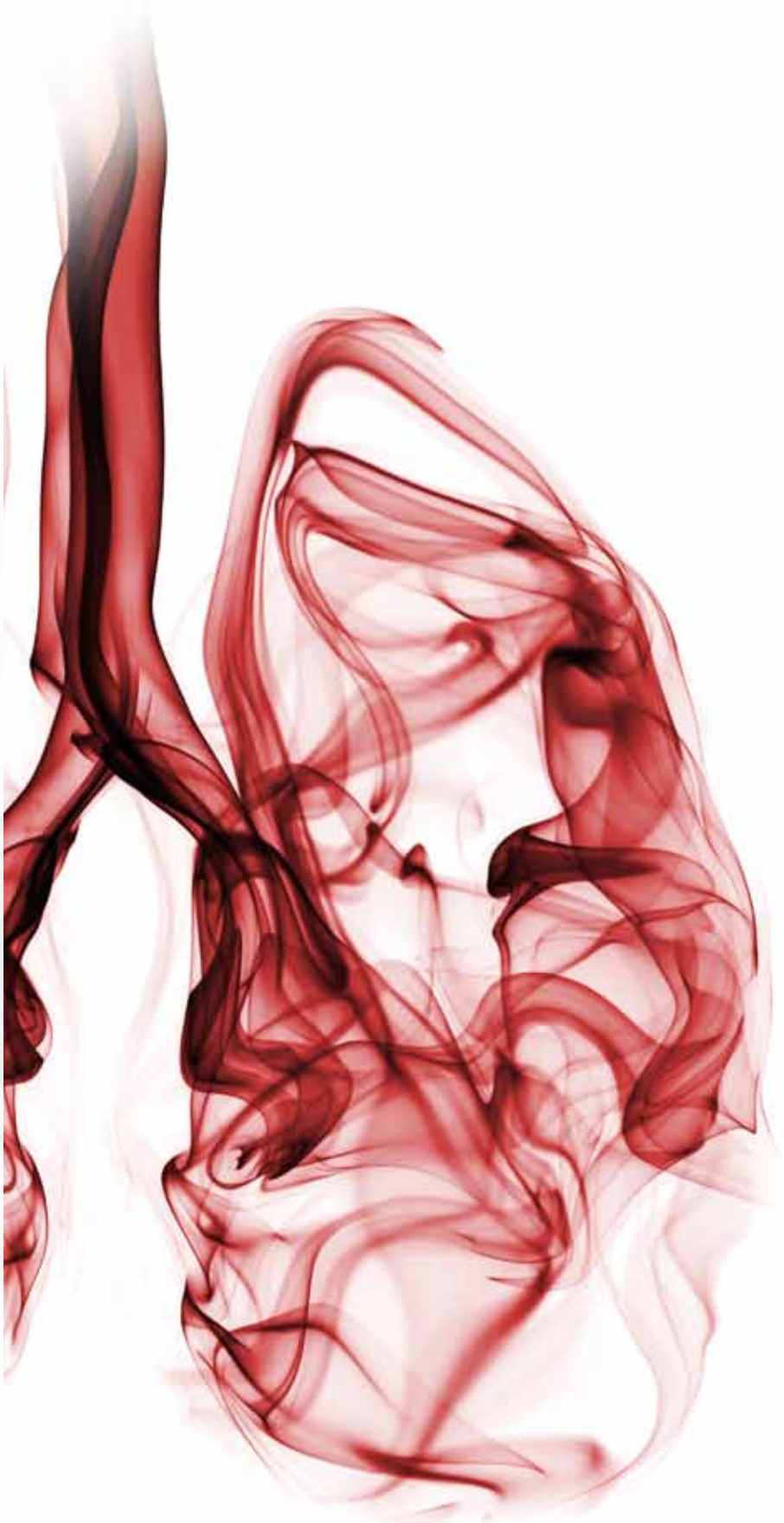
NICOTINE
MICROBIOTA
CIGARETTES

ASTHMA AND COPD RISK

EARLY LIFE

TRANSGENERATIONAL
TRANSMISSION OF DISEASE RISKS

PARENTAL EXPOSURE



Head

- Prof. Dr.
  Susanne Krauss-Etschmann

Members

- Dr. Sabine Bartel
- Janin Braun
- Barbara Hammer
- Gregor Jatzlauk
- Gabriele Huß
- Draginja Kovacevic
- Arne Krüger
- Joni Lund
- Dr. Sebastian Reuter
- Martin Wolff

Priority Research Area **Asthma and Allergy**

Early Life Origins of Chronic Lung Disease

Mission

Vorrangiges Ziel unserer Arbeiten ist es die Entwicklungsursprünge chronischer Lungenerkrankungen besser zu verstehen, um daraus neue Maßnahmen zu deren Prävention abzuleiten. Da die Exposition gegenüber Tabakrauch einen hohen Risikofaktor darstellt im späteren Leben an Asthma oder Chronisch Obstruktiver Bronchitis (COPD) zu erkranken, untersuchen wir in Tiermodellen wie sich Zigaretten bzw. Nikotin-Konsum während der Schwangerschaft auf die Lungen- und immunentwicklung der Nachkommen auswirkt.

The main aim of our group is to contribute to a better understanding of early life origins of chronic lung disease as this will allow to develop novel preventative strategies against these diseases. As prenatal exposure to cigarette smoke is a major risk factor to develop asthma or Chronic obstructive pulmonary Disease (COPD) in later life, we investigate in animal models how cigarette or nicotine abuse during pregnancy affects lung- and immune development n offspring.

Most important findings

1. Prenatal exposures and models

Epidemiological studies have demonstrated an association of environmental exposures during critical developmental windows with the risk for impaired lung function development and respiratory disease in later life. However, the mechanisms underlying this phenomenon are incompletely understood, thus hampering the development of innovative preventative or therapeutic strategies. A recognized risk factor for infant wheeze and childhood asthma is maternal smoking during pregnancy. Therefore, we have set up murine models of maternal smoking: moderate smoking of pregnant Balb/c mice led to intrauterine growth restriction, airway remodeling and impaired lung function in the offspring. Additionally, lung growth was selectively reduced in female offspring only. Molecular studies demonstrated deregulated pulmonary growth hormones (IgF-1, IGBP3) in females which could explain lung growth deficits. In another model of milder maternal smoking, offspring had normal lung function and somatic growth. Nonetheless, thymic T cell development was impaired resulting in a reduced production of regulatory T cells, which are required for maintaining immune tolerance and hence protection from asthma.

So far it has been postulated that prenatal and early postnatal life are the most critical life time windows for shaping disease risks in later life. Thus, the formation of disease risks has been thought to occur essentially via environmental exposures of the pregnant mother only.

Selected publications

I. Kepert, ... S. Bartel, ... S. Krauss-Etschmann. D-tryptophan from probiotic bacteria influences the gut microbiome and allergic airway disease. J Allergy Clin Immunol 2016 [Epub ahead of print].

E. Bergroth, ... S. Krauss-Etschmann, ... J. Pekkanen, and the PASTURE study group Enhanced T helper 1 and 2 cytokine responses at birth associate with lower risk of middle ear infections in infancy. Pediatr Allergy Immunol 2016. [Epub ahead of print].

C. Svanes, ... 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. 2016 [Epub ahead of print].

M. Bogdan, ... S. Bartel,.... E.N.M. Nolte-'t Hoen. Obstacles and Opportunities in the Functional Analysis of Extracellular Vesicle RNA, Journal of Extracellular Vesicles 2016 [accepted].

S. Altmäe,... S. Bartel, ... S. Krauss-Etschmann, Cristina Campoy. Maternal pre-pregnancy obesity is associated with altered placental transcriptome. PLoSONE [accepted].

I.E. Kammerl, ... S. Krauss-Etschmann,..... S. Meiners. „Impairment of immunoproteasome function by cigarette smoke and in COPD". Am J Resp Crit Care Med 2016 Jun 1;193(11):1230-41.

B. Oehrle, G. Burgstaller, M. Irmler, S. Dehmel, J. Grün, T. Hwang, S. Krauss-Etschmann, J. Beckers, S. Meiners, O. Eickelberg. Validated prediction of pro-invasive growth factors using a transcriptome-wide invasion signature derived from a complex 3D invasion assay. Sci Rep. 2015 Aug 5;5:12673.

S. Reuter, J. Maxeiner, H. Meyer-Martin, A. Michel, P. Baars, T. Bopp, A. Waisman, S. Reissig, TC. Wehler, H. Schild, C. Taube, M. Stassen. Cylindromatosis (Cyld) gene mutation in T cells promotes the development of an IL-9-dependent allergic phenotype in experimental asthma. Cellular immunology, 2016, 308, 27-34.

C. Berti,... S. Krauss-Etschmann, ... B. Koletzko. Pregnancy and infants' outcome: nutritional and metabolic implications. Crit Rev Food Sci Nutr. 2016 Jan 2;56(1):82-91.

Bousquet J, ... S. Krauss-Etschmann, ...M. Zins. „Operational Definition of Active and Healthy Ageing (AHA): A Conceptual Framework." J Nutr Health Aging. 2015 Nov;19(9):955-60.

S. Krauss-Etschmann, K.F. Meyer, S. Dehmel, Machteld N. Inter- and Transgenerational Epigenetic Inheritance; Evidence in Asthma and COPD? Clin Epigenetics 2015;7(1):53.

Reuter S, Beckert H & Taube C. Take the Wnt out of the inflammatory sails: modulatory effects of Wnt in airway diseases' Laboratory Investigation 2015 96 (2) 177-85. 2015.143

A very recent study where we participated (Svanes et al 2106), provided for the first time evidence that paternal exposures occurring around puberty are also important for shaping asthma risk in offspring: in this multinational study the risk to develop early-onset non-allergic (NA) asthma was almost tripled in offspring of fathers who started smoking cigarettes around puberty. This increase of risk was independent from the duration of smoking, as fathers who smoked since very long time (i.e. > 10 years) before conception but had started smoking after puberty were less likely to have children with NA asthma. Of note, this effect was not restricted to cigarette smoking: offspring from fathers having been exposed around puberty to metal fumes from welding also had an increased risk to develop NA asthma. Thus, first it a new critical window has been identified that is important to shape disease risks even many years before a child is born. A hypothetical explanation is that paternal priming of asthma risk occurs through exposure induced disturbed development of primordial germ cells into spermatogonial stem cells during puberty (Fig. 1). This hypothesis is currently tested in animal models inn our lab. Second, it can be assumed that exposure to any type of air pollution, from occupational to chemical exposures, could also have a detrimental effect. This has clear implications for public health strategies: it will be important for policymakers to focus on interventions targeting young men and warning them of the dangers of smoking and other exposures to their unborn children in the future.

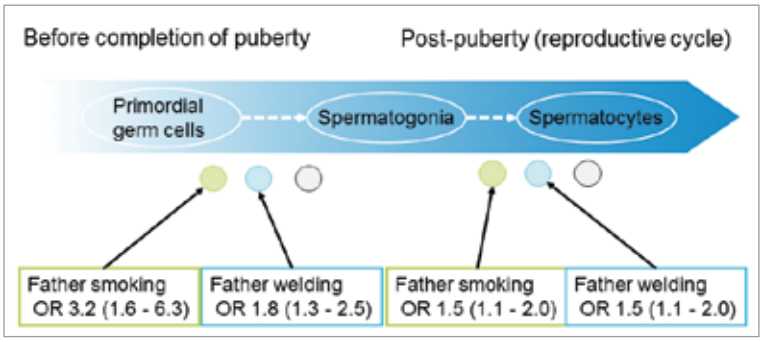


Figure 1. Adapted from: C. Svanes et al. *Int. J. Epidemiol.* 2016

Priority Research Area **Asthma and Allergy**

Early Life Origins of Chronic Lung Disease

2. Preclinical intervention of allergic asthma

Both for individual health and society it is of utmost importance to lobby for the prevention of smoking and tobacco use. However, smoking bans will never be complete. Therefore, means to protect the growing lung of an unborn child is mandatory. Probiotics have been proposed to prevent allergic eczema in children when applied to pregnant mothers and babies, but clinical trials yielded conflicting results. However, the interaction of living bacteria with the hosts´ microbiome and immune system is extremely complex and difficult to predict. We assumed that application of chemically defined single bacterial products might lessen this problem. In a close and multidisciplinary collaboration with Prof. Hartmann (retired head of Dept. Mikrobe-Plant Interaction, Helmholtz-Zentrum München (HMGU)), Prof. P. Schmitt-Kopplin, (Analytical Biogeochemistry, HMGU) and Prof. Schloter (Terrestrial Ecogenetics, HMGU) we isolated the dextrorotatory amino acid D-tryptophan from supernatants of probiotic bacteria. This amino acid cannot be produced by humans and - different from L-tryptophan – is not proteinogenic. D-trypophan reduced the production of the "pro-allergic" chemokine TARC in a human T cell line in vitro. When fed to mice via drinking water, D-tryptophan ameliorated their asthmatic phenotype by reducing airway inflammation and improving lung function. D-tryptophan further increased the gut microbial diversity, which was otherwise reduced in „asthmatic mice". We therefore currently assume that D-tryptophan can act directly on immune cells but also indirectly via stabilization of the gut microbiome.

Internal and external collaboration

Internal

Junior Research Group Asthma Invertebrate Models; Divisions Fluorescence Cytometry (J Behrens), Innate Immunity (H. Heine), Innate Immunity and Mucosal Immunology and Diagnostics (A. Frey), and Cellular Microbiology (U. Schaible; Clinical Study Center (C. Herzmann) and Biobank (K. Gaede)

National

collaborators in the German Center for Lung Research: H. Garn (Department of Clinical Chemistry and Diagnostics, Marburg); B. Schaub (University Children's Hospital Munich); N. Kahn/F.Herth (Department of Pneumology, Heidelberg); S. Meiners (Comprehensive Pneumology Center, Munich); S. Bellusci (University of Gießen); H. Ehrhardt (Neonatology, University of Gießen); H. Schulz (Epidemiology I, HMGU)
J. Heinrich (Munich Center of Health Sciences, Ludwig-Maximilians-University, Munich; B. Koletzko (University Children's Hospital Munich); M. Schloter (Terrestrial Ecogenetics, HMGU). P. Schmitt-Kopplin, (Analytical Biogeochemistry, HMGU). A. Hartmann (retired; formerly Microbe plant Interaction, HMGU)

International

Network of COST Action BM1201, in particular C. Svanes (University of Bergen, NO), R. J. Bertelsen, B. Benediktsdottir, D. Norbäck, T. Sigsgaard, V, Schlünssen, J.W. Holloway (University of Southampton, UK), R. Gosens (University of Groningen, NL), M. Hylkema R. Gosens (University of Groningen, NL), S. La Grutta Pediatrc Allergology, ,Palermo University, IT), A. Divac Rankov (University of Belgrade, Serbia)

Grant support

German Center of Lung Research, Disease Areas Asthma/Allergy, and COPD (SKE)

BMBF-Consortium EXASENS „POC-Sensorplattform für chronisch-entzündliche Atemwegserkrankungen" (FKZ 13N13857) (SKE)

Leibniz Competition „The lung Microbiota at the Interface between airway epithelium and the environment (SKE)

Leibniz Science Campus „Evolutionary Medicine of the Lung". Subproject „Host genetics and Lung microbiota" (SKE)

Sino-German Society for Science (SKE)

AIRWAY REMODELING

EPITHELIUM

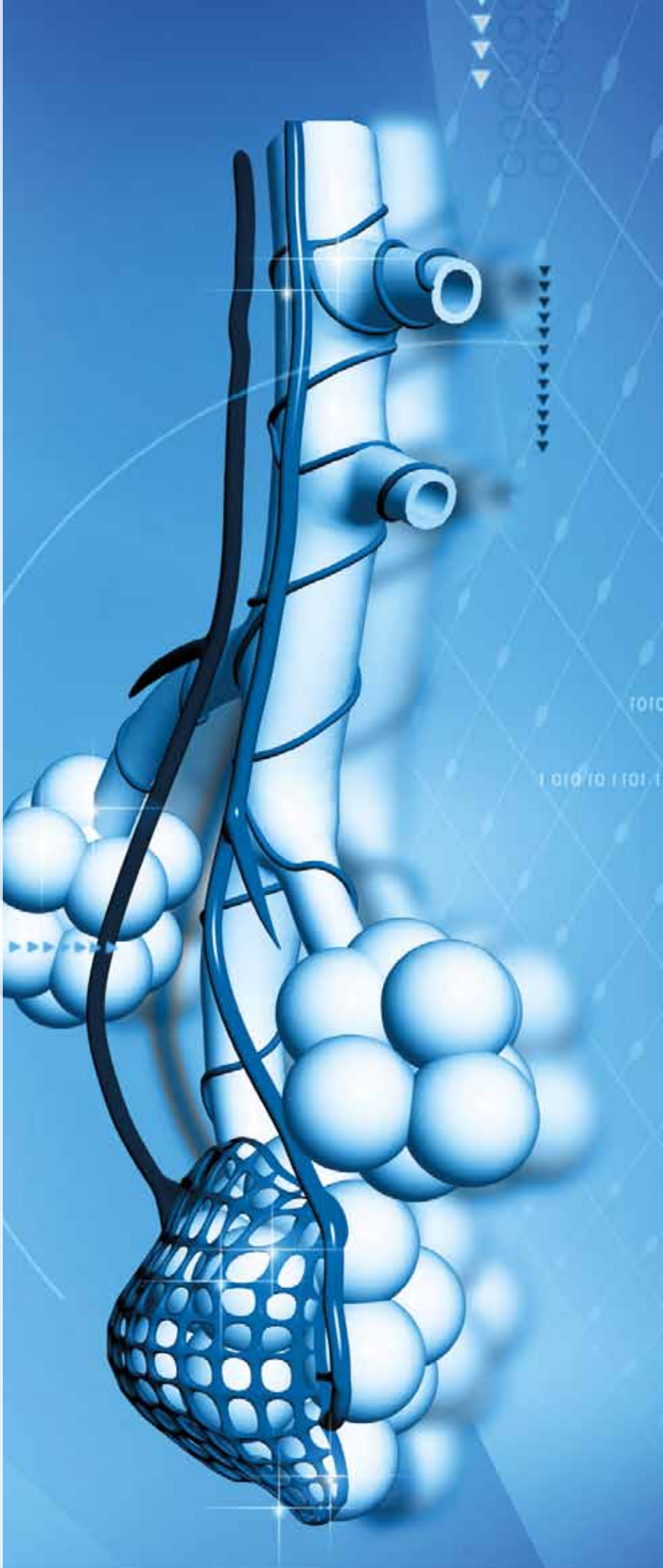
BASOPHILS

BRONCHIAL ASTHMA

MAST CELLS

DESIGN-BASED STEREOLOGY

NANOTOXICOLOGY



Priority Research Area **Asthma and Allergy**

Experimental Pneumology

Head

- Prof. Dr. Heinz Fehrenbach

Members

- Juliane Artelt
- Franziska Beyersdorf
- Johanna Ehlers
- Philipp Hagemann
- Frauke Koops
- Sandra Nyenhuis
- Patricia Prilla
- Gesine Rode
- Dr. Gabriele Schramm
- Kerstin Viertmann
- Dr. Christina Vock
- Dr. Sina Webering
- Dr. Zane Orinska

PhD students who completed their studies in 2016:

- Mylène Divivier
- Kathrin Knuhr
- Kristina Langhans

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 well-known risk factors (e.g. viruses, cigarette smoke, particles) of airway diseases and of trigger factors for disease exacerbations. Therefore, studying the effects of airborne triggers on the airway epithelium is a major focus of the group. In turn, the epithelial responses to such triggers translate into immunological responses. In allergic diseases such as allergic bronchial asthma, mast cells and basophilic granulocytes are of particular significance and therefore, our studies will additionally address the functional relevance of these cell types. By establishment of Air-Liquid-Interface (ALI) cultures of murine and human alveolar as well as of airway epithelial cells (**Figure 1**) we are investigating the role of the airway epithelium in asthma both in mice and humans.

Figure 1. Immunohistochemical stainings for marker proteins reveal that primary human airway epithelial cells differentiated into major epithelial cell types (left to right: basal, ciliated, goblet cells) after culture at the air-liquid-interface (micrographs by courtesy of Johanna Ehlers).

Selected publications

Vock C, Yildirim AÖ, Wagner C, Schlick S, Lunding LP, Lee CG, Elias JA, Fehrenbach H*, Wegmann M*. Distal airways are protected from goblet cell metaplasia by diminished expression of IL-13 signalling components. *Clin Exp Allergy* 2015;45:1447-58. (*equal contribution)

Lunding LP, Webering S, Vock C, Behrends J, Wagner C, Hölscher C, Fehrenbach H*, Wegmann M*. Poly(inosinic-cytidylic) acid-triggered exacerbation of experimental asthma depends on IL-17A produced by NK cells. *J Immunol* 2015;194:5615-25. (*equal contribution)

Lunding L, Webering S, Vock C, Schröder A, Raedler D, Schaub B, Fehrenbach H, Wegmann M. IL-37 requires IL-18Rα and SIGIRR/IL-1R8 to diminish allergic airway inflammation in mice. *Allergy* 2015; 70:366-73.

Meyer NH, Mayerhofer H, Tripsianes K, Blindow S, Barths D, Mewes A, Weimar T, Köhli T, Bade S, Madl T, Frey A, Haas H, Mueller-Dieckmann J, Sattler M, Schramm G. A crystallin fold in the interleukin-4-inducing principle of *Schistosoma mansoni* eggs (IPSE/alpha-1) mediates IgE binding for antigen-independent basophil activation. *J Biol Chem* 2015; 290:22111-26.

Reimers N, Homann A, Höschler B, Langhans K, Wilson RA, Pierrot C, Khalife J, Grevelding CG, Chalmers IW, Yazdanbakhsh M, Hoffmann KF, Hokke CH, Haas H, Schramm G. Drug-induced exposure of *Schistosoma mansoni* antigens SmCD59a and SmKK7. *PLoS Negl Trop Dis* 2015; 9:e0003593.

Doenhoff MJ, El-Faham M, Liddell S, Fuller HR, Stanley RG, Schramm G, Igetei JE. Cross-Reactivity between *Schistosoma mansoni* antigens and the latex allergen Hev b 7: putative implication of cross-reactive carbohydrate determinants (CCDs). *PLoS One* 2016;11(7):e0159542.

Polansky JK, Bahri R, Divivier M, Duitman EH, Vock C, Goyeneche-Patino DA, Orinska Z, Bulfone-Paus S. High dose CD11c-driven IL15 is sufficient to drive NK cell maturation and anti-tumor activity in a trans-presentation independent manner. *Sci Rep* 2016; 6:19699.

Zimmer J, Weitnauer M, Boutin S, Küblbeck G, Thiele S, Walker P, Lasitschka F, Lunding L, Orinska Z, Vock C, Arnold B, Wegmann M, Dalpke A. Nuclear localization of suppressor of cytokine signaling-1 regulates local immunity in the lung. *Front Immunol* 2016; 7:514.

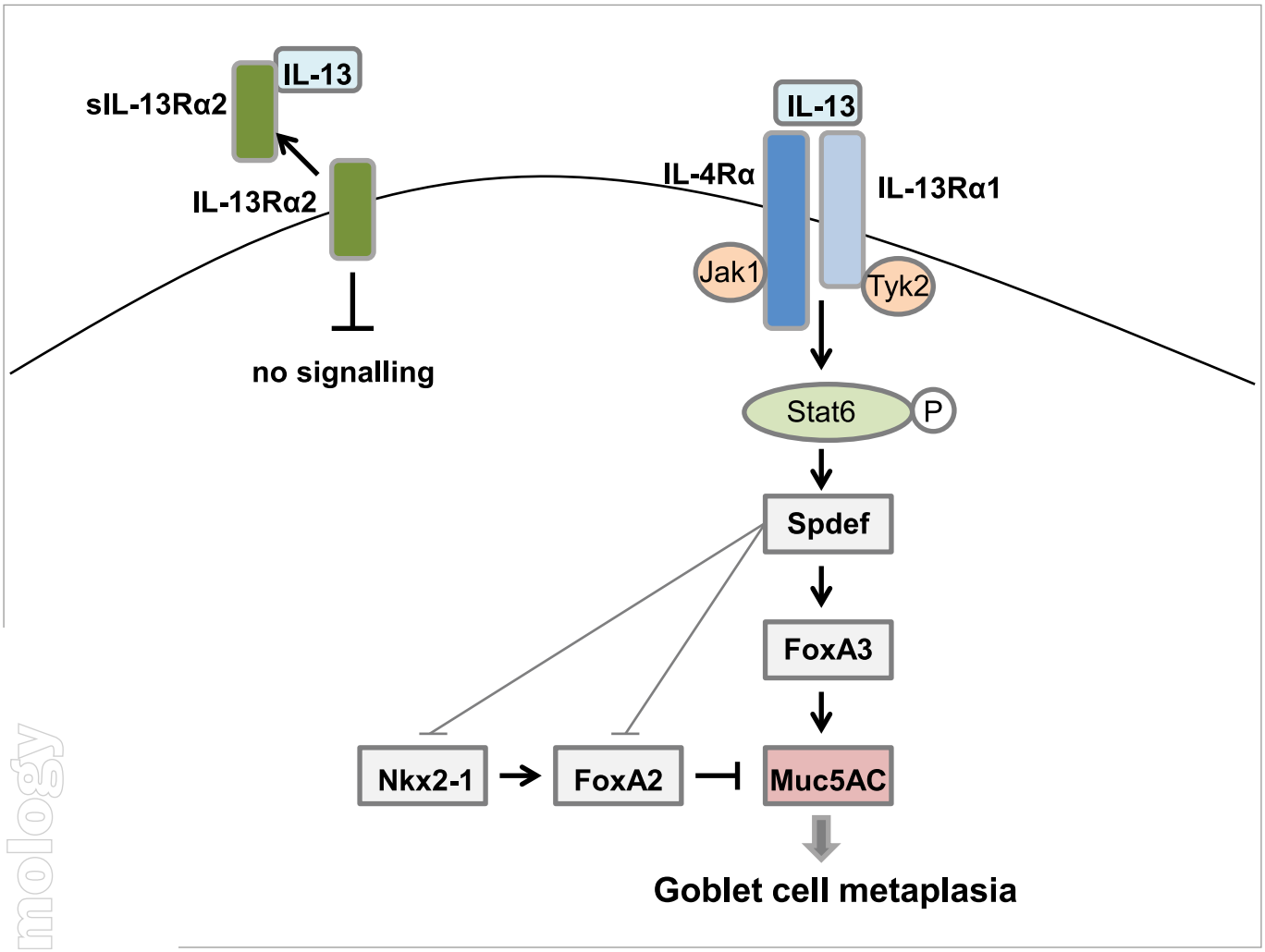


Figure 2. Major components of the IL-13 signalling pathway involved in goblet sell metaplasia (Fig. 6a from Vock et al. 2015)

The T helper type 2 cytokine interleukin (IL) 13 is a major regulator of goblet cell metaplasia and mucus production in the airways. We could recently demonstrate an airway-region-specific regulation of goblet cell metaplasia and mucus production in healthy mice and three mouse models of experimental asthma. As the expression of factors stimulating the mucus production revealed no region-specific differences, we suggest that this is dependent on the lower expression of IL-13Rα1 in distal airways, which is the major receptor for IL-13-dependent goblet cell metaplasia and mucus production. Additionally, IL-13Rα1-dependent activation of the downstream transcription factors Spdef and FoxA3, which are functionally associated with goblet cell and mucus production, was diminished in distal airways. Thus, a regulatory mechanism was proposed that renders distal airways less sensitive to IL-13-induced goblet cell metaplasia and mucus production and, thus, could protect airways from mucus plugging and impaired ventilation of the attached alveoli (**Figure 2**).

Priority Research Area **Asthma and Allergy**

Experimental Pneumology

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 the anti-inflammatory potential of factors isolated from the eggs of the helminth *Schistosoma mansoni*. In two PhD projects funded by the DFG we could substantiate the immunomodulatory activity of the schistosome egg-derived factor IPSE/alpha-1. Kathrin Knuhr could demonstrate that IL-4 released from **basophilic granulocytes** that were stimulated with IPSE/alpha-1 inhibits the release of pro-inflammatory cytokines such as IL-1β and IL-6 from LPS-stimulated human monocytes. Kristina Langhans showed a potential role of IPSE/alpha-1 as an immunoglobulin-binding factor in the induction of regulatory B cells during chronic schistosome infection. Recently, the administration of *S. mansoni* soluble egg antigens was demonstrated to reduce the severity of colitis in an adoptive transfer mouse model characterized by an increased Th2 response and a suppressed Th17 response.

Mast cells (MCs) are long-living cells contributing to innate and adaptive immunity. IgE-mediated FcεRI-dependent mast cell degranulation is the main effector reaction in allergies. IgE-independent G-protein coupled receptor-mediated mast cell degranulation in response to polycationic compounds is the second type of hyperresponsiveness described for MCs so far. Furthermore MC degranulation could be induced by different venoms and pharmacological agents e.g. calcium ionophores. MC degranulation is a complex and precisely regulated process, leading to local and systemic inflammatory response. The exocytosis of granules is orchestrated by membrane receptor complexes responding to the environmental signals, intracellular signalling modules transducing the incoming signals, cytoskeletal proteins and granule membrane protein complexes controlling exocytosis. Tissue-type specific mechanisms controlling MC degranulation are the main focus of our investigation. We aim at characterizing specific regulatory mechanisms that act on a cell membrane level towards prevention of MC hyperresponsiveness. Members of the tetraspanin family CD37 and CD81 are of particular interest as regulators of MC degranulation. Analysis of receptor expression is combined with measurement of intracellular histamine content. Thus, the multiparametric analysis of stimulus-specific receptor expression/translocation will dissect new functional populations of MCs and help to identify environmental signals required for successful MC tolerization.

Internal and external collaboration

Internal: Junior Research Groups Allergobiochemistry, Asthma Exacerbation & Regulation, Invertebrate Models; Divisions Clinical and Experimental Pathology, Early Life Origins of CLD, Innate Immunity and Mucosal Immunology and Diagnostics.

University of Lübeck: Peter König, Institute of Anatomy; Gereon Hüttmann, Institute for Biomedical Optics; Matthias Kopp, Department of Pediatrics, University Medical Center Schleswig-Holstein, Lübeck.

External:

collaborators in the German Center for Lung Research: Holger Garn (Department of Clinical Chemistry and Diagnostics, Marburg); Bianca Schaub (University Children's Hospital Munich); Carsten Schmidt-Weber (ZAUM, Munich); Armin Braun (Fraunhofer ITEM, Hannover); Matthias Ochs (Institute for Applied and Functional Anatomy, Hanover); Marcus Mall (Department of Translational Pulmonology, Heidelberg)

other national collaborators: Henning Bockhorn (Karlsruhe Institute of Technology), Bernd Müller (Philipps-University of Marburg), Tanja Hansen (Fraunhofer ITEM, Hannover); Helmut Haas (helminGuard, Borstel); Alexander Dalpke (Department of Infectious Diseases, Medical Microbiology and Hygiene, Heidelberg); 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); Jack A. Elias (Warren Alpert School of Medicine, Brown University, Providence).

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.)

BMBF-Consortium nanoCOLT (FKZ 03X0093A) (H.F.)

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

TOLL-LIKE RECEPTOR

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

- Suhad Al-Badri
- Katrin Böhnstedt
- Ina Goroncy
- Dr. Andre Jenckel
- Tanja Mengden
- Hanna Rosigkeit
- Dr. Karina Stein
- Prof. Dr. Artur J. Ulmer
- Tim Vierbuchen

Former members

- Mandy Mai
- Dr. Anna Störmer
- Dr. Karin Uliczka

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, Allergene und Carbon-Black-Nanopartikel. 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 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, allergens and carbon black nanoparticles. 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

Studying the interaction of various types of innate and adaptive immune cells in the initiation and during ongoing allergic asthma is one of the main topics of the group. Dendritic cells (DC) are known key regulators in this context, however, the airway epithelium is the first entry site for airborne environmental factors and signals derived from these cells can affect DC function decisively. The establishment of a 3D co-culture model involving the bronchial epithelial cell line Calu-3 (EC), cultured under air-liquid interface conditions, and monocyte-derived dendritic cells enables studies of the complex cell interaction when challenged with environmental factors (Fig. 1).

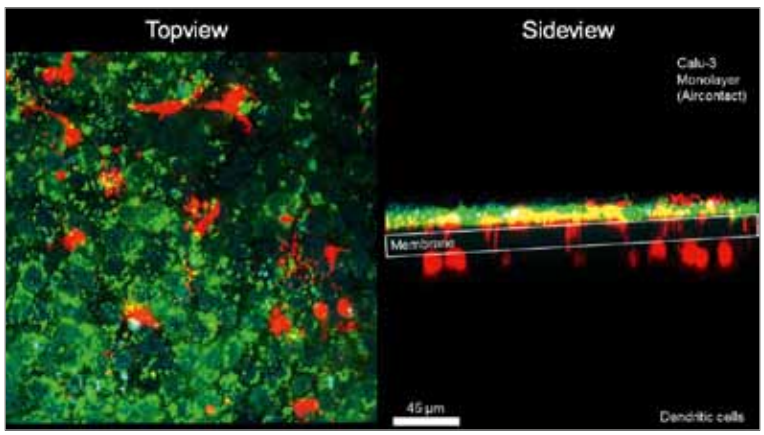


Figure 1. 3D cell culture model consisting out of the human bronchial epithelial cell line Calu-3 (green) and human primary monocyte-derived dendritic cells (red). Left: topview, right: sideview.

Selected publications

Toll-Like Receptor 2 recognizes *Orientia tsutsugamushi* and increases susceptibility to murine experimental scrub typhus.
Gharaibeh, M., Hagedorn, M., Lilla, S., Hauptmann, M., Heine, H., Fleischer, B. & Keller, C. 12.09.2016 in : Infection and immunity .

Endosomal recognition of *Lactococcus lactis* G121 and its RNA by dendritic cells is key to its allergy-protective effects.
Stein, K., Brand, S., Jenckel, A., Sigmund, A., Chen, Z. J., Kirschning, C. J., Kauth, M. & Heine, H. 15.07.2016 in : The Journal of allergy and clinical immunology.

Interleukin-4 and interferon-γ orchestrate an epithelial polarization in the airways.
Zissler, U. M., Chaker, A. M., Effner, R., Ulrich, M., Guerth, F., Piontek, G., Dietz, K., Regn, M., Knapp, B., Theis, F. J., Heine, H., Suttner, K. & Schmidt-Weber, C. B. 07.2016 in : Mucosal immunology. 9, 4, S. 917-26

ORMDL deregulation increases stress responses and modulates repair pathways in *Drosophila* airways. Kallsen, K., Zehethofer, N., Abdelsadik, A., Lindner, B., Kabesch, M., Heine, H., Roeder, T. 2015 in: J Allergy Clin Immunol 136(4): 1105-1108.

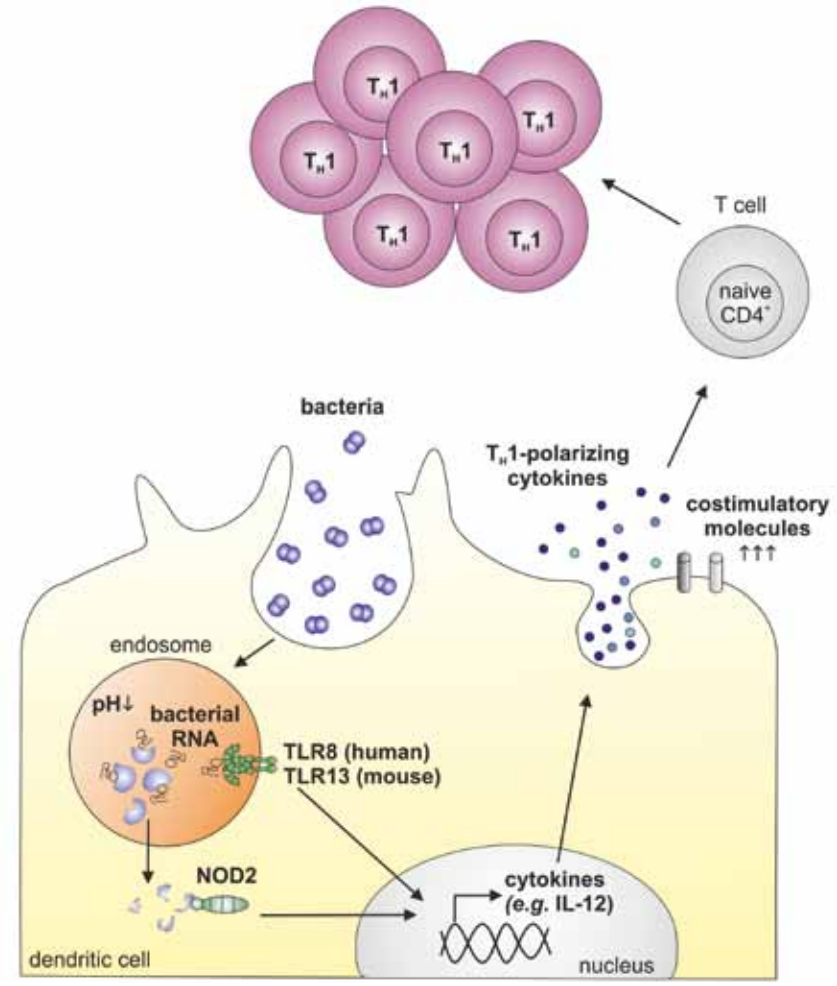


Figure 2. Allergy protection by ***Lactococcus lactis*** G121

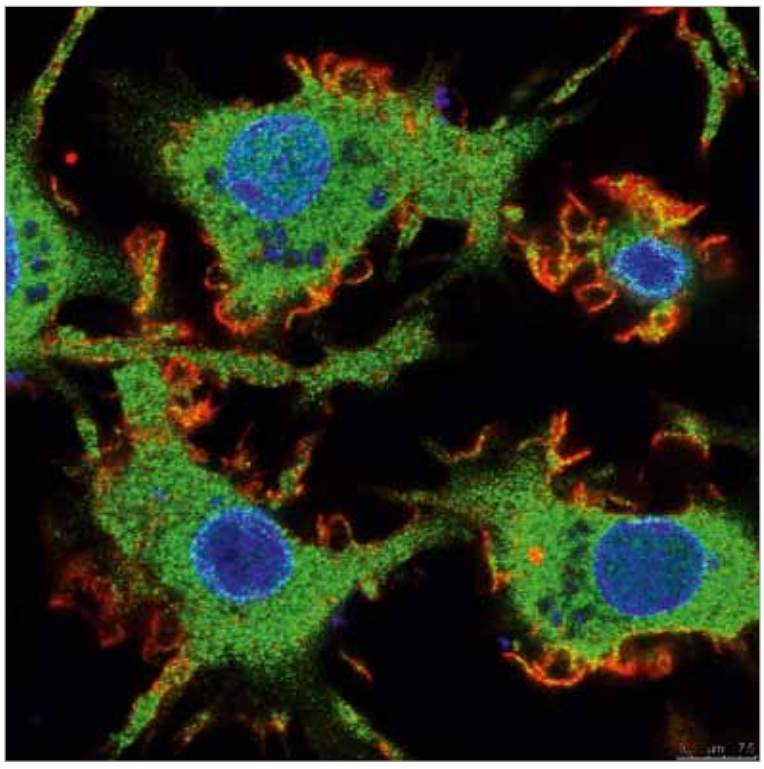


Figure 3. Uptake of the archeon *Methanosphaera stadtmanae* in human dendritic cells does not lead to the formation of ASC speck structures (blue: DNA, green: ASC, red: actin).

Priority Research Area **Asthma and Allergy**

Innate Immunity

i) The first project deals with synthetically produced carbon black nanoparticles (CBNP) which are used in industrial products like printer toner or car tires. Released CBNP are part of the air we breathe and have high binding capacities, thus, complexes with other harmful substances in the air can be found. However, the effects on the health due to incorporation are poorly investigated. Following treatment of DC, CBNP were recovered within intracellular compartments, but had only a minor impact on the expression of T cell co-stimulatory molecules. While CBNP-treatment of DC resulted in the release of the chemokine CXCL8, treatment of EC only led to an increasing IL-6 amount when particles were coated with the polycyclic aromatic hydrocarbon benzo[a]pyrene, or acetylene soot. However, we observed no adverse effects on cell growth and survival upon CBNP-treatment (supported by BMBF).

ii) The influence of airborne allergens, e.g. Bet v1 or house-dust mite components, on cell interactions is the essential element of the second project. In the 3D system, we observed induction of immune stimulatory cytokines without direct EC/DC contact and even more, when contact was enabled, indicating the importance of soluble factors together with direct cell interaction. Further, the induction of asthma related molecules, e.g. IL-1 family members, was profoundly changed when the epithelium was polarized with T_H1- or T_H2-cytokines. Therefore, the 3D co-cultures underline the importance of EC on immunomodulatory processes and consequently, on the outcome of an asthmatic inflammation.

iii) Several studies indicate an important role for environmental microorganisms and their products in the asthma-preventive effect of farming environment. We recently identified the ligands and pattern recognition receptors through which the cowshed isolate *Lactococcus lactis* G121 confers allergy protection. Inhibiting phagocytosis and endosomal acidification impaired the release of T_H1-specific cytokines, co-stimulatory molecule expression, and T cell activation upon *L. lactis* challenge. *In vivo*, allergy protection was dependent upon endosomal acidification and we identified TLR13 as the receptor for *L. lactis* RNA in the mouse. The T_H1-polarizing activity of *L. lactis* G121-treated human DCs was blocked by TLR8-specific inhibitors, mediated by *L. lactis* G121 RNA, and synergistically enhanced by activation of NOD2 (supported by BMBF, DZL AA).

In the past decade, there has been a growing interest in the influence of the human microbiota on health and immune homeostasis. Although methanogenic archaea are known to be an important part of the natural human microbiota, the research is biased towards bacteria. During earlier studies in our laboratory we observed that the gut-derived archaeal strains *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* can induce inflammatory immune responses in human DCs. Our study aims to elucidate the cellular receptors and mediators involved in signal processes leading to immune responses and to clarify potential differences in signaling pathways for different archaeal strains. To achieve that, we will generate clonal human monocytic knockout cell lines utilizing the CRISPR/Cas9 system. In addition, a novel RNA sequencing approach (dual RNA-seq) will be used to detect temporal changes in the transcriptome of both the human host and methanoarchaeal species during contact. This method will offer a comprehensive understanding of host-methanoarchaea interaction (supported by DFG).

Internal and external collaboration

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

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

Grant support

BMBF DZL, disease area AA2.2
BMBF NanoCOLT, TP4
DFG HE 2758/4-2
EKFS 2013-A130

DROSOPHILA MELANOGASTER

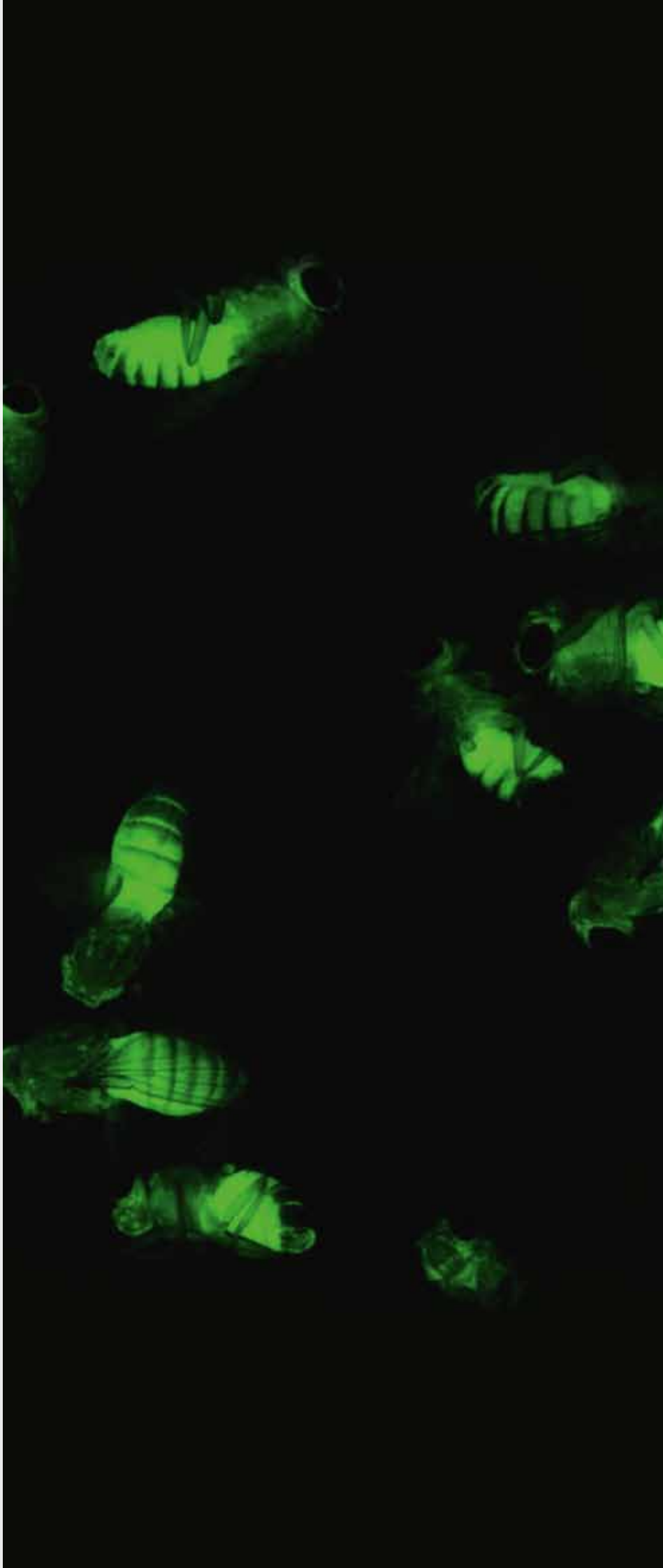
ASTHMA RISK FACTORS

INNATE IMMUNITY

AIRWAY EPITHELIUM

STRESS HORMONES

TRANSGENERATIONAL STUDIES



Priority Research Area **Asthma and Allergy**

Invertebrate Models

Head

- Dr. Christina Wagner

Members

- Hanna Angstmann
- Katharina Bösing
- Beate Höschler
- Torge Keup
- Carina Krützmann
- Karolina-Theresa Neumann
- Stephanie Papenmeier
- Dr. Karin Uliczka

Priority Research Area **Asthma and Allergy**

Invertebrate Models

Mission

Die Nachwuchsgruppe (NWG) **Invertebratenmodelle** widmet sich ausschließlich der Erforschung des angeborenen Immunsystems und seiner Beteiligung an der Pathogenese des Asthma bronchiale. Hierbei liegt der Fokus insbesondere auf der Immunantwort von Atemwegsepithelzellen und ihrer Modulation durch Risikofaktoren, die die Entstehung und Verschlimmerung der Erkrankung begünstigen. In diesem Kontext nutzt die NWG vorrangig als Modellsystem die Taufliege *Drosophila melanogaster,* um evolutionär hoch konservierte Gene und Signalwege zu identifizieren, deren Fehlregulationen die Pathogenese der Erkrankung maßgeblich vorantreiben.

The junior research group (JRG) **Invertebrate Models** concentrates on research of the innate immune system and its impact on the pathogenesis of bronchial asthma. The focus lies on immune functions of the airway epithelium and its modulation by factors that have been described to increase the risk of asthma development and exacerbation. For this purpose the JRG primarily makes use of the model *Drosophila melanogaster* to identify highly conserved genes and pathways whose deregulations contribute to disease pathogenesis.

Most important findings

In the last two years, we elucidated the effect of stress-induced signaling pathways and transcription factors mediating the epithelial immune response in flies and men. One example is the transcription factor FoxO (forkhead members of the O subclass), which can be stimulated in the airway epithelium of the fly by stress factors such as cold and oxidative stress but also by an infection. Epidemiological studies suggest especially these factors as triggers of acute asthma exacerbations and also activate human FoxO homologues (FoxO1, FoxO3a & FoxO4) in different alveolar and bronchial epithelial cell lines (Figure 1). Preliminary work carried out in a mouse model of acute experimental asthma indicates that these proteins (e.g. FoxO3a) also play a crucial role in the acute phase of an asthma episode.

Tobacco smoke has also been described as a factor for asthma development and acute exacerbations. Epidemiological studies further suggest that the effects of tobacco smoking on disease development can even be propagated to the following generation(s). This indicates that beside genetic changes also epigenetic changes are involved in disease pathogenesis. By using a transgenerational Drosophila smoking model, we would like to identify epigenetic changes that occur exclusively in airway epithelial cells due to prenatal exposure to parental smoking.

Highlights

Establishment of a *Drosophila* smoking model.

Selected publications

Fehrenbach H, Wagner C, Wegmann M. Airway remodeling in asthma - what really matters. Manuscript accepted in CTR Special Issue on „Development, remodeling & regeneration of the lung".

Lunding L, Webering S, Vock C, Behrends J, Wagner C, Hölscher C, Fehrenbach H, Wegmann M. (2015) pIC-triggered exacerbation of experimental asthma depends on IL-17A produced by NK cells. *J Immunol.* 194:5615-25.

Vock C, Yildirim AÖ, Wagner C, Schlick S, Lunding LP, Lee CG, Elias JA, Fehrenbach H & Wegmann M. (2015) Distal airways are protected from goblet cell metaplasia by diminished expression of IL-13 signaling components. *Clin Exp Allergy.* 45:1447-1458.

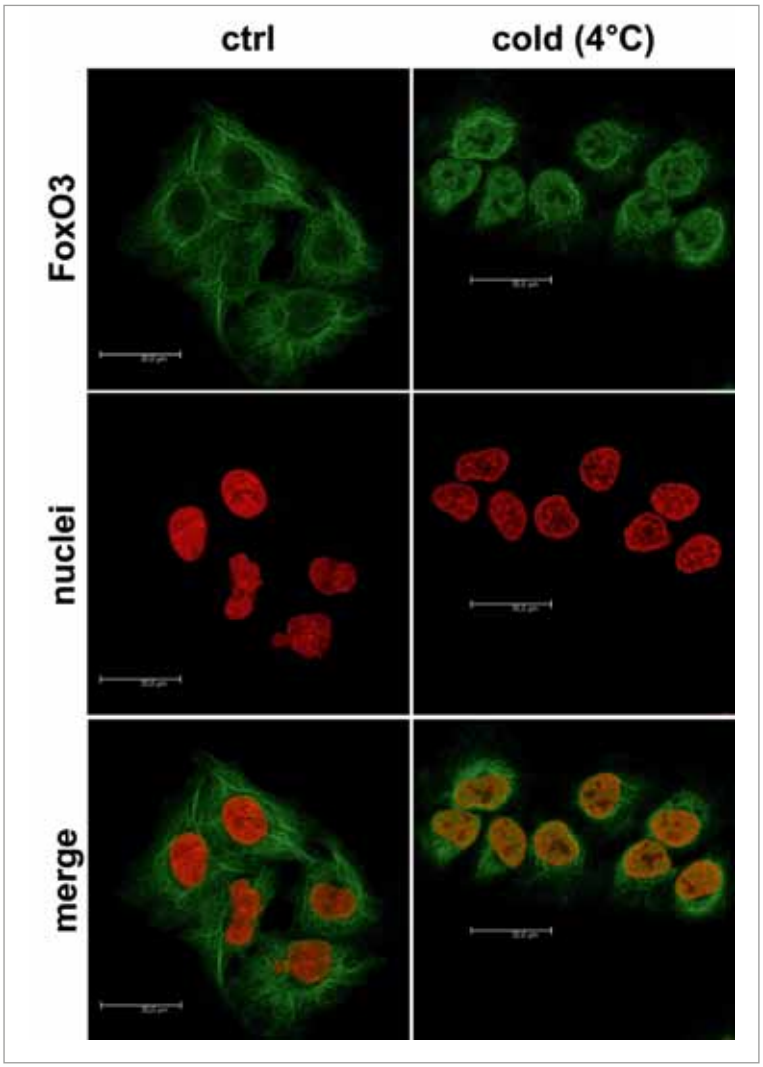


Figure 1.
Cold air, a trigger for asthma exacerbations, activates members of the FoxO family (FoxO3) in human epithelial cell lines (A549).

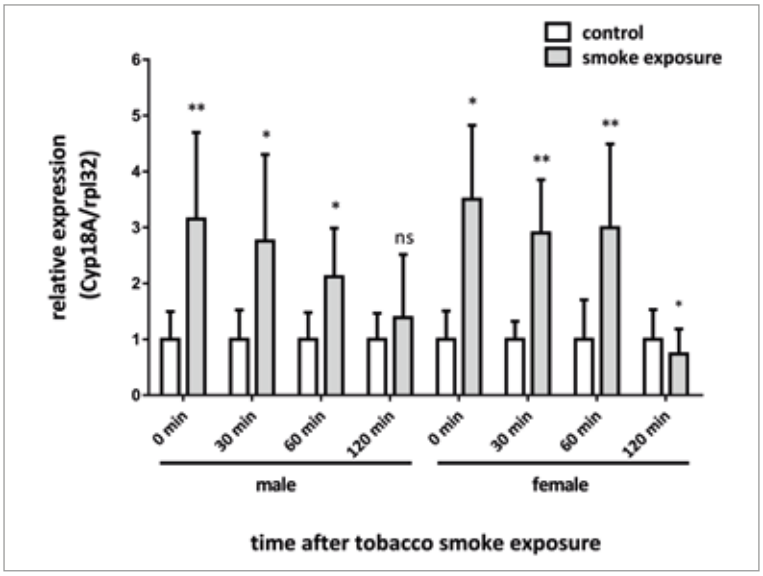


Figure 2. Comparable to humans, tobacco smoking induces the expression of the cytochrome P450 1a1 homologue Cyp18A1 in *Drosophila* larvae.

Priority Research Area **Asthma and Allergy**

Invertebrate Models

So far, a *Drosophila* smoking model has been successfully established reflecting key features of an antioxidant phenotype. So similar to men and mice, Drosophila larvae degrade nicotine to the major metabolite cotinine after entering the body, indicating that in general tobacco smoke is more readily absorbed by the larvae. Furthermore, the expression level of Cyp18A1 - a murine and human cytochrome P450 1a1 (Cyp1A1) homologue - is markedly increased in tobacco smoke exposed larvae as well as their isolated airways demonstrating that also in flies highly toxic tobacco smoke components such as poly-aromatic hydrocarbons are detoxified by cytochrome P450 homologues (Figure 2). While tobacco smoke exposure at the juvenile stage does not affect the survival rate of the emerged adult flies, the mortality rate of tobacco smoke exposed male but not female larvae is significantly increased which indicates that tobacco smoke exposure modulates gender-specifically the expression profile of developmentally relevant genes.

Since it is well known that stress in general is an exacerbating factor of asthma, we are also interested in the immunomodulatory effect of stress hormones such as biogenic amines (e.g. noradrenalin). In vertebrates, they are secreted during a stress response and modulate the immune response of numerous immune cells such as alveolar macrophages. In this context, we could gain first insights, that octopamine, the fly structural analogue of noradrenalin, has rather an immunoenhancing effect at least when being systemically released. While all major immunocompetent tissues of the fly including macrophage-like hemocytes express all three octopaminergic receptor subtypes, only immune-challenged mutants deficient for Octß1R and Octß2R show a significantly lower survival rate compared to their respective controls. It can therefore be assumed that octopamine mediates its immunoenhancing effect though Octß1R and Octß2R, respectively. So far we have no evidence that octopamine modulates hemocyte-dependent immune functions. However, first in-vitro studies suggest that similar to non-immune-cells octopaminergic signaling increases the intracellular cAMP level through Gs-mediated activation of adenylyl cyclase pathway and that cAMP in general decreases significantly the phagocytic rate of hemocytes from *Drosophila* larvae.

Internal and external collaboration

Internal

Prof. Dr. Heinz Fehrenbach (Experimental Pneumology); Prof. Dr. Susanne Krauss-Etschmann (Early Life Origin of CLD); Prof. Dr. Holger Heine (Innate Immunity); Dr. Michael Wegmann (Asthma Exacerbation and Regulation); Dr. Jochen Behrends & Dr. Thomas Scholzen (Fluorescence Cytometry).

External

Prof. Dr. Thomas Roeder, Research Group Molecular Physiology, University of Kiel; PD. Dr. Sabrina Schreiner, Institute of Virology, TUM/ Helmholtz Center Munich; Dr. 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; Prof. Dr. Ulrich Theopold, Department of Molecular Biology and Functional Genomics, Stockholm University, Sweden.

Grant support

WA 2972-2/2 (DFG): „Regulation and modulation of the innate immune response via neuronal and endocrine signals in the fruit fly Drosophila melanogaster".

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; in cooperation with PD Dr. Andreas Frey (Mucosal Immunology and Diagnostic), Prof. Susanne Krauss-Etschmann (Early Life Origin), Dr. Michael Wegmann (Asthma Exacerbation and Regulation).

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

DIAGNOSTIC MARKERS

MOLECULAR EVOLUTION

HIGH THROUGHPUT PEPTIDE ASSAYS

MUCOSAL BARRIERS

POINT-OF-CARE DIAGNOSTICS

IMMUNOGLOBULIN RECOGNITION MAPPING

Head

- PD Dr. Andreas Frey

Members

- Dr. Barbara Frey
- Dr. Maik Henkel (bis September 2015)
- Dr. Thorsten Krause
- Dr. Katrin Ramaker
- Dr. Niels Röckendorf
- Jürgen Sarau
- Dr. Heike Sinnecker
- Dr. Kristof Tappertzhofen
- Özge Ulupinar-Kök
- Alheidis von Quast
- Geraldine Wiese (bis Juli 2016)

Priority Research Area **Asthma and Allergy**

Mucosal Immunology and Diagnostics

Mission

Die Forschungsgruppe Mukosale Immunologie & Diagnostik erforscht auf molekularer und zellulärer Ebene die Auslöser und Abläufe von Schleimhaut-assoziierten Erkrankungen, insbesondere auf den Gebieten Asthma/COPD und Allergien. Basierend auf unseren Erkenntnissen über Krankheits-spezifische Stukturen und Mechanismen wollen wir Marker für Ausbruch, Verlauf und Schwere dieser Krankheiten identifizieren und Wege finden, um diese Marker für diagnostische, prognostische and therapeutische Ansätze zu nutzen.

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, especially in the fields of asthma/COPD and allergic disorders. Based on our findings on specific disease features and processes, we want to identify and characterize markers for the initiation, course and severity of these diseases and provide means to survey them for diagnostic, prognostic and therapeutic purposes.

Most important findings

The „Immunology“ wing of our group explores the humoral and structural armament of mucosal barriers and aberrations thereof which may be involved in pathologic developments.

Inadequate mounting of an immune defence against „innocuous“ antigens may result in severe allergic and inflammatory disorders. The responsible immunologic responses are complex events, depending on the B- and T-cell repertoire at the time of antigen contact, the physical integrity of the antigen, the site of contact, and on the presence and status of various cofactors, cytokines and humoral mediators. Consequently, the type of immunoglobulin induced as well as its specific antigen recognition may differ from individual to individual and from situation to situation. We want to elucidate the interconnection between different immunoglobulin (sub)classes, antigen recognition profiles and disease manifestation and severity. We have established a high-throughput epitope recognition assay based on synthetic peptide libraries and are using these libraries in collaboration with the Research Group Clinical and Molecular Allergology to identify immunogenic epitopes of different allergens by mapping the immunoglobulin profile from sera of patients suffering from allergic disorders. Our findings will help to devise and to monitor individual, personalized therapeutical approaches for each patient. In an additional project, we use particle-bound random peptide libraries („one-bead-one-compound libraries“) to identify novel immunoreactive epitopes with relevance to the development of asthma.

Highlights

Versatile approach to generate switchable fluorescent probes for the sensitive detection of analytes in diagnostic assay systems.

Novel reducing agents for specific diagnostic and biochemical applications.

Extensive validation of mucin antibodies for applicability in diagnostic settings.

Selected publications

Meyer, N.H., Mayerhofer, H., Tripsianes, K., Blindow, S., Barths, D., Mewes, A., Weimar, T., Köhli, T., Bade, S., Madl, T., Frey, A., Haas, H., Mueller-Dieckmann, J., Sattler, M., and Schramm, G. (2015) A cristallin fold in the interleukin-4-inducing principle of *Schistosoma mansoni* eggs (IPSE/α-1) mediates IgE binding for antigen-independent basophil activation. *J. Biol. Chem.* 290: 22111-22126.

Homan, A., Röckendorf, N., Kromminga, A., Frey, A., and Jappe, U. (2015) B cell epitopes on infliximab identified by oligopeptide microarray with unprocessed patient sera. *J. Transl. Med.* 13:339-348

Henkel, M., Röckendorf, N., and Frey, A. (2016) Selective and efficient cysteine conjugation by maleimides in the presence of phosphine reductants. *Bioconjugate Chem.* 27:2260-2265

Krause, T., Röckendorf, N., Gaede, K.I., Ramaker, K., Sinnecker, H., and Frey, A. (2016) Validation of antibody reagents for mucin analysis in chronic inflammatory airway diseases. *mABs.* DOI 10.1080/19420862.2016.1264551

Ramaker, K., Bade, S., Röckendorf, N., Meckelein, B., Vollmer, E., Schulz, H., Fröschle, G.-W., and Frey, A. (2016) Absence of the epithelial glycocalyx as potential tumor marker for the early detection of colorectal cancer. PLoS One; DOI:10.1371/journal.pone.0168801

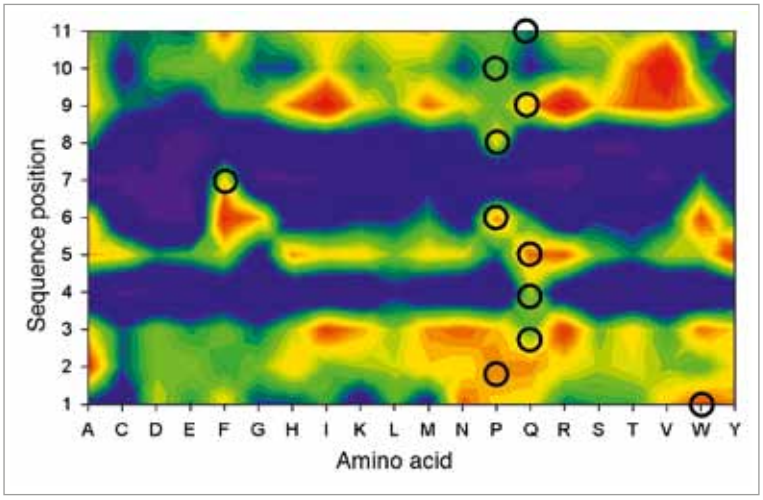


Figure 1. „Heat map" for affinity adjustment of selected antibody-binding peptides. Sequential substitution of every amino acid in every position in a 15mer peptide epitope leads to defined changes in affinity. The antibody binding strength of each peptide is reflected by the color of the spot corresponding to the respective sequence motif. Black circles depict the original epitope sequence. Blue regions indicate substitutions with low, red regions substitutions with high affinity.

In the „Diagnostics" wing, we investigate novel diagnostic tools for the analysis of pathological conditions at mucosal surfaces. Our work concentrates on the identification and characterization of diagnostically utilizable marker features and on the creation of reagents and probes for in-vitro point-of-care diagnostics.

In order to expand the tool box for diagnostic systems, we have developed an elegant immunooptical procedure for the quantification of a diagnostic analyte by utilizing a switchable fluorescent probe. To implement this system we have devised a new method for the specific, site-directed fluorescent modification of an antibody and have performed a fine-tuned affinity-adjustment of a set of binding peptides using an affinity heat map approach (figure 1). By providing a battery of antibodies with specific fluorescent labels and simultaneously adjusting the corresponding binding peptides to defined affinities, we were able to devise a switchable multiplex system for the parallel assessment of different antigen epitopes or multiple analytes.

As one promising marker candidate for the point-of-care diagnostics in COPD and asthma, we have pinpointed airway mucins, asserting that amount, composition and glycosylation of the respiratory mucins may reflect different stages and conditions of inflammatory lung disorders. We therefore want to provide reagents and probes appropriate for a rapid and meaningful mucin analysis. Our new sample preparation procedure employing specific novel reducing agents shall enable the diagnostically conclusive analysis of airway mucus characteristics. Moreover, we have evaluated all currently available

Priority Research Area **Asthma and Allergy**

Mucosal Immunology and Diagnostics

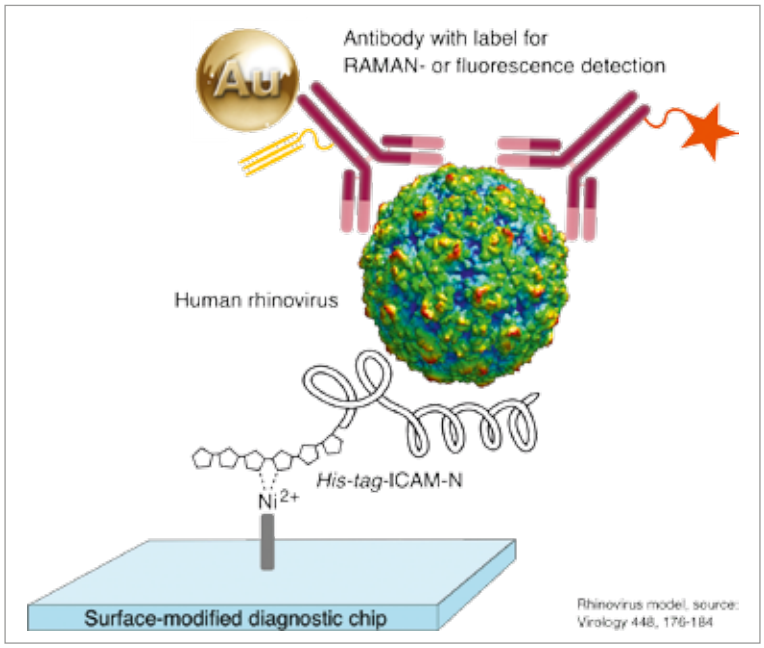


Figure 2. Rhinovirus capture- and detection assay for on-chip diagnostic systems.

antibodies directed against the major airway mucin MUC5AC and have defined the specific requirements for such an antibody to be utilizable in the mucin diagnosis of patient samples.

In a collaborative project involving a total of nine Leibniz institutions we want to develop a versatile diagnostic platform to monitor the disease status in asthma and COPD in a point-of-care manner and to define an approach for assessing the risk of potential upcoming exacerbations of these diseases. For this task, we have identified human rhinoviruses as a potential marker for asthma exacerbations, and have devised a method to capture rhinoviruses out of biological samples, immobilize them onto the surface of a diagnostic chip and equip them optimally for analysis by sophisticated optical detection procedures (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

External:

- Jürgen Popp, Leibniz-Institut für Photonische Technologien, Jena
- 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
- Peter Köhler, Deutsche Forschungsanstalt für Lebensmittelchemie, Freising
- Markus Borschbach, University of Applied Sciences, Paderborn
- Mario Bürger, GeSiM, Dresden
- Thomas Weiss, R-Biopharm, Darmstadt
- Maren Wiese, Hermann Kröner Stärke, Ibbenbüren

Grant support

Bundesministerium für Bildung und Forschung (BMBF), Collaborative grants GLUTEVIS (13GW0042) and EXASENS (13N13857)

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

STENOTROPHOMONAS MALTOPHILIA

ALLERGEN-PROTECTIVE BACTERIA AND COMPOUNDS

ANTIBODY/ LECTIN/ PROTEIN-CARBOHYDRATE INTERACTION

Priority Research Area **Asthma and Allergy**

Structural Biochemistry

Head

- Prof. Dr. Otto Holst

Members

- Ute Agge
- Helga Bartels
- Rainer Bartels
- Petra Behrens
- Sylvia Düpow
- Dörte Grella
- Volker Grote
- Manuel Hein
- Katharina Jakob
- Gudrun Lehwark-Yvetot
- Dr. Uwe Mamat
- PD Dr. Sven Müller-Loennies
- Sandra Petersen
- Wiebke Schnoor
- Veronika Susott

Priority Research Area **Asthma and Allergy**

Structural Biochemistry

Mission

Strukturelle Analyse von hoch gereinigten, biologisch relevanten, bakteriellen und ökologischen Verbindungen.
Untersuchungen zur phänotypischen Heterogenität von *Stenotrophomonas maltophila.*
Strukturelle und funktionelle Analyse von Antikörpern und Lektinen, die an mikrobielle Glykane binden.
Identifizierung von Kohlenhydrat-Epitopen für den Einsatz in Diagnostik und Therapie sowie in der Aufklärung von Bindungsmechanismen.

Structural analysis of highly purified, biologically relevant, bacterial and environmental compounds.
Phenotypic heterogeneity in *Stenotrophomonas maltophilia*.
Structural and functional analyses of antibodies and lectin binding to microbial glycans.
Identification of carbohydrate epitopes to be used for diagnostic and therapeutic purposes and to reveal binding mechanisms.

Most important findings

One of the important cell envelope compounds of Gram-positive bacteria is lipoteichoic acid (LTA). We isolated LTA from allergy-protective *Staphylococcus sciuri* W620 and characterized its structure by chemical analyses, as well as 1D and 2D NMR spectroscopy. The compound comprised glycerol (Gro), phosphate-Gro, alanine-Gro, glucose (Glc) and fatty acids, and the LTA possessed a backbone composed of glycerol-phosphate repeating units only substituted with D-alanine (Ala) and a lipid anchor with the structure β-D-Glcp(1→6)-β-D-Glcp(1→3)-1,2-diacyl-sn-Gro. Such anchor is typical for the genus Staphyloccocus.

Stenotrophomonas maltophilia is found ubiquitously in the environment, but is increasingly recognized as an important opportunistic nosocomial pathogen that can cause bacteremia and pneumonia in immunocompromised patients with a high rate of mortality. *S. maltophilia* is considered a clinically relevant pathogen in cystic fibrosis patients. Since it has also been isolated from lung specimen of healthy donors, it is thought to represent a lung-associated commensal with the potential of an opportunistic pathogen. The bacterium intrinsically carries resistance genes to a broad spectrum of antimicrobials, which makes treatment a difficult clinical task and most likely promotes the increasing incidence of *S. maltophilia* infections worldwide.

Selected publications

Haji-Ghassemi O, Müller-Loennies S, Rodriguez T, Brade L, Grimmecke HD, Brade H, Evans SV. The combining sites of anti-lipid A antibodies reveal a widely utilized motif specific for negatively charged groups. JOURNAL OF BIOLOGICAL CHEMISTRY. 2016; 291:10104-10118.

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Rueda F, Céspedes MV, Sanchez-Chardi A, Seras-Franzoso J, Pesarrodona M, Ferrer-Miralles N, Vázquez E, Rinas U, Unzueta U, Mamat U, Mangues R, Garcia-Fruitos E, Villaverde A. Structural and functional features of self-assembling protein nanoparticles produced in endotoxin-free *Escherichia coli.* MICROBIAL CELL FACTORY. 2016; 15:59.

Torrealba D, Seras-Franzoso J, Mamat U, Wilke K, Villaverde A, RoherN, Garcia-Fruitos E. Complex particulate biomaterials as immunostimulant-delivery platforms. 2016. PLoS ONE 11:e0164073.

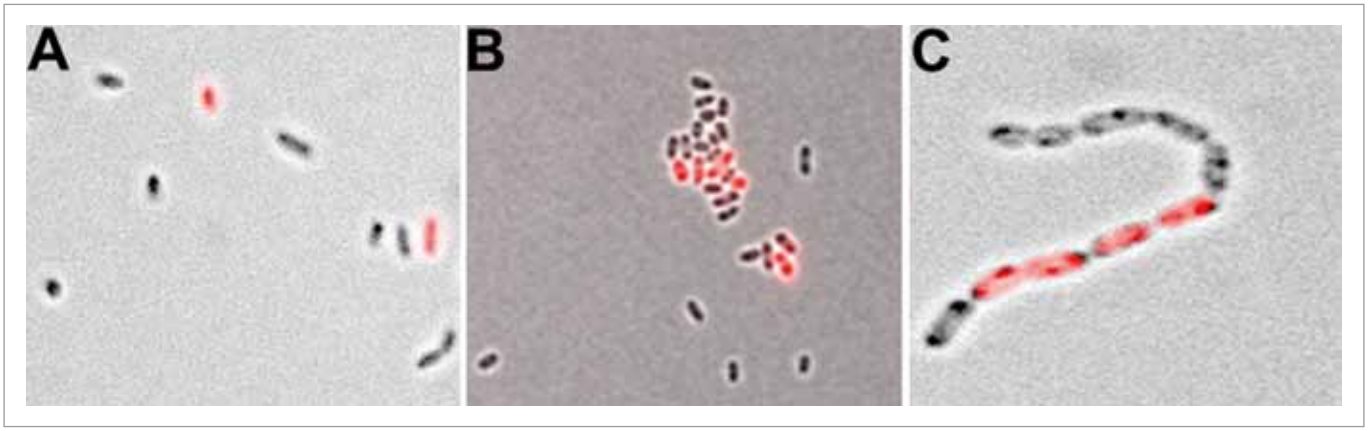

Figure 1. Analysis of single cell fluorescence of P*bla*$_{L1}$ and P*bla*$_{L2}$ promoter gene fusions. Expression of *bla*$_{L1}$ (A) and *bla*$_{L2}$ (B) promoters fused to the *rfp* gene in *S. maltophilia* K279a, and phenotypic heterogeneity as observed in P*bla*$_{L2}$::*rfp* cells forming long cell chains (C). The cells were grown at 30°C for 17 h under aerobic conditions (200 rpm) in LB medium containing 100 µg/ml ampicillin.

Non-genetic variations within an isogenic bacterial population contribute to the survival and fitness of the population in response to various stresses such as antibiotic treatment. In a clonal population, cell-to-cell variation may result in a measurable phenotype termed phenotypic heterogeneity, which is a way to enhance the ability to adapt to changing environmental conditions via heterogeneous gene expression. Earlier, we reported phenotypic heterogeneity in *S. maltophilia* K279a upon exposure to β-lactam antibiotics. In the presence of ampicillin, *S. maltophilia* K279a cells showed heterogeneity in colony and cell morphology, which was accompanied by colony-specific patterns of

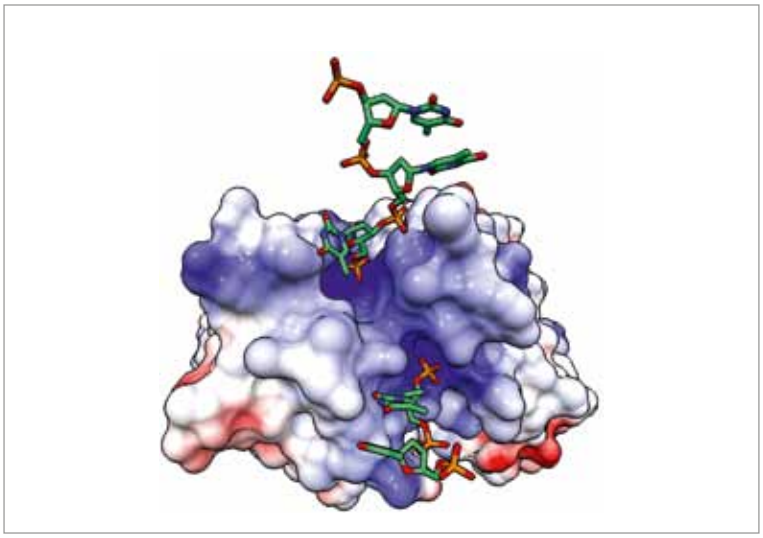

Figure 2. α-Carbon alignment between DNA-specific BV04-01 Fv (blue) and lipid A specific S1–15 Fv (white). S1-15 binds two nucleotides at distinct sites based on 3'- and 5'-phosphate recognition.

Priority Research Area **Asthma and Allergy**

Structural Biochemistry

differentially expressed genes, including expression of the β-lactam resistance genes bla_{L1} and bla_{L2}. As demonstrated with reporter gene fusions, bla_{L1} and bla_{L2} were subject to differential regulation even at the single cell level (Fig. 1). We suggested that phenotypic heterogeneity in *S. maltophilia* K279a cells provides a selective advantage in natural environments and during infection of human epithelia to respond against antimicrobial effectors. This adaptation is probably also relevant during acute and chronic human infections associated with *S. maltophilia* and effectiveness of antibiotic treatment.

In continuation of our efforts to decipher the role of antibodies (Abs) in endotoxin neutralization and diagnostics we have succeeded in resolving the structures of a panel of Abs in complex with derivatives of free lipid A, the endotoxic principle of LPS. Sequence analyses together with binding assays and comparative X-ray crystal structure analyses revealed that such Abs are highly specific despite distinct germ line precursors. The structures of antigen-binding fragments of two homologous mAbs specific for lipid A, S55-3 and S55-5, identified a conserved positively charged pocket formed within the complementarity determining region H2 loops that binds the terminal phosphates of lipid A. Significantly, this motif occurs in unrelated Abs where it mediates binding to negatively charged moieties through a range of epitopes, including phosphorylated peptides used in diagnostics and therapeutics. The observed motif may have significant immunological implications as a tool for engineering recombinant Abs.

Reports on polyspecificity of anti-lipid A Abs toward single-stranded DNA indicate that they may play a role in the development of autoimmune diseases such as systemic lupus erythematosus or thyroiditis. The observed homology of S1-15 and A6 and reports of several single-stranded DNA-specific mAbs such as BV04-01 and Dna-1 prompted the determination of the structure of S1-15 in complex with single-stranded DNA fragments. The crystal structure of S1–15 Fab in complex with p5(dT)p showed two different oligonucleotide fragments bound at each phosphate-binding site, with a total of 14 hydrogen bonds and several hydrophobic interactions between ssDNA ligands and residues of the combining site. Auto-Ab BV04-01 does recognize the backbone phosphate groups in its complex with the trinucleotide p5(dT), with a central thymidine forming stacking interactions with a Tyr and a Trp residue on either side of the thymidine. Additionally, the 5′P and central phosphate groups of the trinucleotide contribute to binding through hydrogen bonds. The 5′P-binding site of BV04-01 contains many interactions that correspond to the 5′P (and the 4′P lipid A)-binding site of S1–15 (Fig. 2), marking its importance for dual recognition of ssDNA and lipid A.

Internal and external collaboration

Internal Collaborations

D. Schwudke, RG Bioanalytical Chemistry
T. Gutsmann, RG Biophysics
A. Schromm, RG Immunobiophysics
U. Schaible, RG Cellular Microbiology
S. Niemann, RG Molecular Microbiology
T. Goldmann, RG Clinical and Experimental Pathology
U. Jappe, RG Clinical and Molecular Allergology
N. Reiling, RG Microbial Interface Biology
H. Heine, RG Innate Immunity
K. A. Duda, Junior RG Allergobiochemistry

External Collaborations

M. Perbandt, Laboratory for Structural Biology of Infection and Inflammation at DESY, University of Hamburg, Germany
W. Streit, Microbiology & Biotechnology, University of Hamburg, Biocenter Klein Flottbek, Germany
F. Schmidt, Interfaculty Institute for Genetics and Functional Genomics, Department of Functional Genomics, EMA-University of Greifswald, Germany
S. Gröger, Periodontology Clinic, Justus-Liebig-University Giessen, Germany
R. Woodard, Department of Medicinal Chemistry, University of Michigan, USA
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S. Evans, Institute for Biochemistry & Microbiology, University of Victoria, BC, Canada
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G. Alter, The Ragon Institute, Massachusetts General Hospital, Cambridge, MA, USA

Grant support

Deutsches Zentrum für Lungenforschung (DZL)

NMR SPECTROSCOPY

BACTERIAL GLYCOLIPIDS

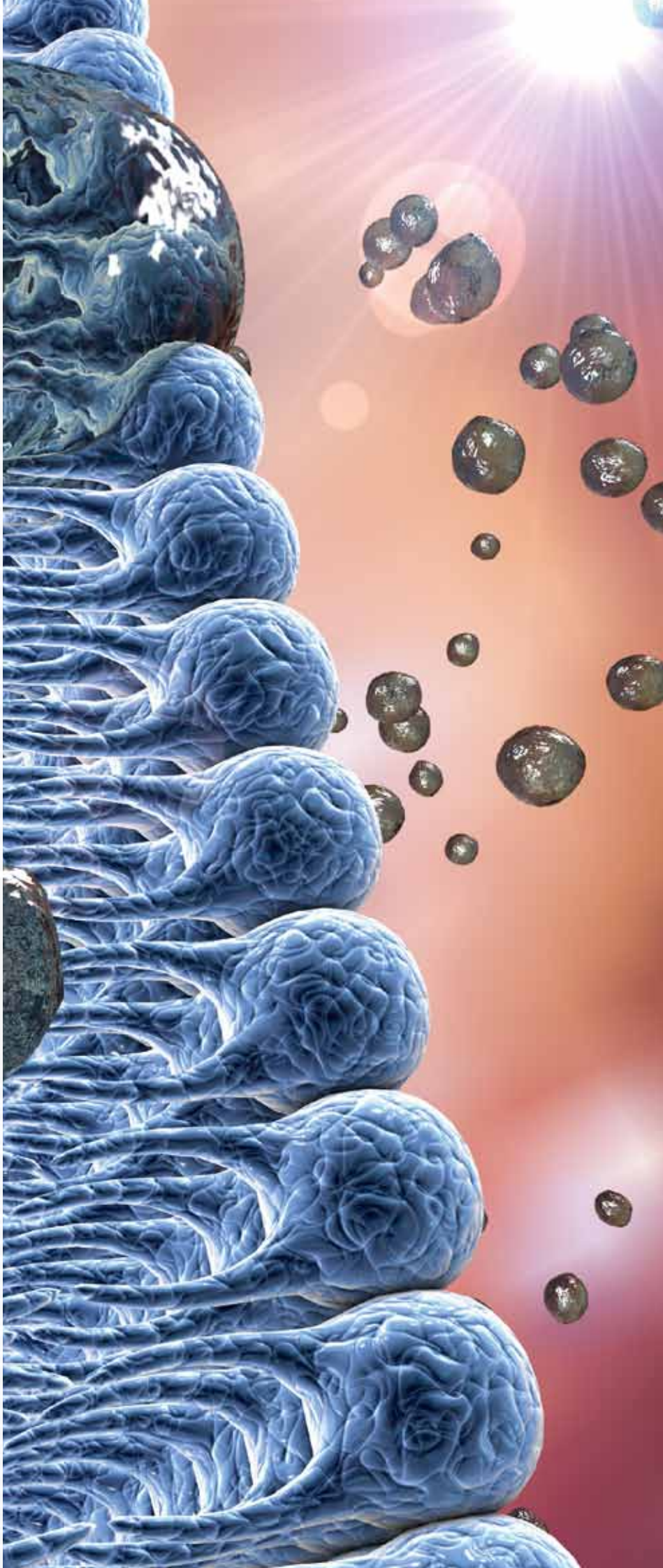
LUX SCORE

HOST-PATHOGEN INTERACTION

LIPIDOMICS

MASS SPECTROMETRY

LIPID METABOLIC INTERACTION



Priority Research Area **Infections**

Bioanalytical Chemistry

Head

- Dr. Dominik Schwudke

Members

- Birte Buske
- Lisa Marie Deter
- Lars Florian Eggers
- Dr. Nicolas Gisch
- Dr. Hande Karaköse
- Heiko Käßner
- Brigitte Kunz
- Chakravarthy Marella
- Verena Scholz
- Ursula Schombel
- Simone Thomsen
- Franziska Waldow
- Michael Weinkauf
- Dr. Adam Wutkowski

Priority Research Area **Infections**

Bioanalytical Chemistry

Mission

Das primäre Ziel der Forschungsgruppe Bioanalytische Chemie ist es Signalkaskaden sowie Biosynthesewege, die im Zuge von Infektionen und Entzündungsprozessen verändert sind, auf molekularer Ebene aufzuklären. Hierfür entwickeln wir OMICs-basierte Arbeitsabläufe sowie Methoden zur Strukturaufklärung, die vor allem auf die Analyse von Lipiden, Glyko-Konjugaten und bakteriellen Zellwandbestandteilen abgestimmt sind. In unserer FG vereinen wir Kompetenzen zur Anwendung der Massenspektrometrie (MS) und der Kernmagnetischen Resonanzspektroskopie (NMR), um detaillierte Analysen metabolischer Prozesse in biologischen Modellsystemen sowie in der klinischen Anwendung zu realisieren. Innerhalb des **Programmbereiches Infektion** untersuchen wir die Rolle von Zellwandbestandteilen im Zuge bakterieller Infektionen, wobei das Hauptaugenmerk auf der Analyse von *M. tuberculosis* und *S. pneumoniae* liegt. Während einer Infektion finden metabolische Wechselwirkungen zwischen Pathogen und Wirt statt, die wir im Hinblick auf potenzielle therapeutische Anwendungen untersuchen. Um ein besseres Verständnis für die Entstehung von Asthma und COPD zu erlangen, untersuchen wir gemeinsam mit Gruppen aus dem **Programmbereich Asthma und Allergie** sowie der Abteilung für **Medizin** das Lipidom der humanen Lunge.

The Division of Bioanalytical Chemistry aims to reveal signalling cascades and biosynthetic pathways on the molecular level, which are altered due to inflammation and infection. For that, we develop OMICs workflows and methods for structural characterization with a focus on lipids, glyco-conjugates, and bacterial cell wall components. In our RG, we unite competences in mass spectrometry (MS) and nuclear magnetic resonance (NMR) approaches enabling in-depth studies of metabolic processes in biological model system and for clinical application. Within the **priority area infection** we study the role of cell wall components for bacterial infection with a focus on *M. tuberculosis* and *S. pneumoniae*. During infection, lipid metabolic interaction between pathogen and host occur, which we explore for potential therapeutic applications. For a better understanding of the development of asthma and COPD we study in cooperation with groups of the **priority area allergy and asthma** and the department of **medicine** the lipidome of the human lung.

Most important findings

Insights from the human lung lipidome

Little is known about the human lung lipidome and its variability in different physiological states and its alterations during carcinogenesis and development of emphysema. We investigated how the individual health status might be mirrored in the lung lipidome. In this regard, we characterized lung cancer tissues and corresponding tumour-free alveolar tissues using a lipidomics screening approach.

Highlights

Initiation of the Lipidomics Forum conference series in cooperation with Robert Ahrends (ISAS Dortmund): 1st meeting 15-17.11.2015 at the RCB; 2nd conference 13-15.11.2016 at the ISAS.

„Scientific Award Weihenstephan of the city Freising" to N. Gisch (01.07.2015)

Funding for the consortium „Lipidomics Informatics for Life-Science"- LIFS within the German Network for Bioinformatics Infrastructure (de.NBI)

Selected publications

Marella C, Torda AE, Schwudke D. The LUX Score: A Metric for Lipidome Homology. PLOS COMPUT BIOL 2015; 11: e1004511.

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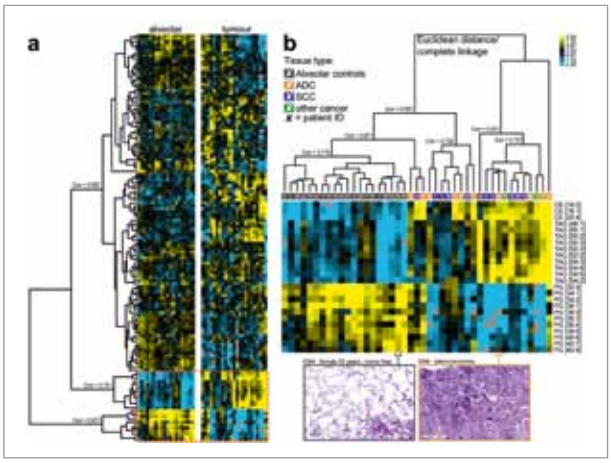

Figure 1. Tumour and alveolar control tissues can be distinguished by their lipidomes. **(a)** Heat-map of 141 lipid quantities identified in 43 lung tissues. The corresponding hierarchical clustered tree is shown panel b. **(b)** Individual lipidomes are colour-coded according to the tissue type. Scans of histology slices of tumour and alveolar control tissue of patient ID64 are shown (Eggers *et al*, submitted).

Tumour tissues exhibited elevated levels of triacylglycerols and cholesterol esters and significantly lower abundance of phosphatidylglycerols, typical lung surfactant components (Fig 1). Adenocarcinomas and squamous-cell carcinomas could be differentiated with high specificity on basis of lipid panels. Tumours exhibited distinct lipidome alterations that were strongly influenced by the histology, most noteworthy, the proportion of metabolically active tumour cells, stroma and necrosis. Alveolar tumour-free tissues showed systematic changes in its lipid profiles depending on degree of emphysema, inflammation, and age of patients that were uncovered by a partial least squares regression based model. With this study, we provide a resource for the human lung lipidome and describe associations to clinical parameters and histology that pave the way for studying lipid metabolic alterations due to infection and inflammation.

Mycobacterial phospholipids have a diagnostic potential

The number of patients diagnosed with multi-drug resistant (MDR) strains of *M. tuberculosis (M.tb)*, the causative agent of tuberculosis, is increasing globally. The emergence of MDR strains is a result of the lack of rapid and affordable diagnostic tests, monitoring bacterial drug resistance and treatment outcome.

To develop novel diagnostic tests, readily accessible biomolecules are required, which can be used to access the *M.tb* infection status. In this respect, mycobacterial lipids are ideal candidates because of their structural uniqueness. In order to identify potential diagnostic markers, we determined the lipidomes of several clinical isolates. We searched for lipids that were (1) detected in all clinical strains, (2) easy to isolate and quantify and (3) were not present in any sample material from our disease models or clinical material. In this way we identified saturated phosphatidylinositols comprising tuberculostearic acid (TSA) as most promising target molecules. We systematically studied the lipidome of blood plasma and PBMCs during antimycobacterial treatment in order to evaluate if these lipids are indicative for the outcome. For that, lipidomes were characterized during standard therapy for sensitive TB compared to MDR cases as well as to a cohort of healthy individuals. Distinct lipid species comprising

Priority Research Area **Infections**

Bioanalytical Chemistry

TSA were elevated at clinically relevant time points such as sputum conversion and culture conversion. Individual treatment profiles for such lipids suggest that TSA can help to estimate the bacterial load and the efficiency of a chosen drug regimen.

Identification of the lipoteichoic acid ligase in *Streptococcus pneumoniae*

Teichoic acids (TA) are important structures to maintain cell integrity and cell morphology of Gram-positive bacteria (Fig 2a). Pneumococcal TAs bind an important class of cell surface proteins, the choline-binding proteins (CBPs), which are involved in e.g. peptidoglycan remodeling or interactions with host factors. Together with the group of Prof. Dr. S. Hammerschmidt (University of Greifswald) we recently identified the so far unknown lipoteichoic acid ligase TacL in pneumococci (e.g. SPD_1672 in strain D39). By analysis of isogenic KO-mutant and complemented strains, we could prove that TacL is responsible for the ligation of pnTA precursor chains onto the glycolipid anchor to form the lipoteichoic acid (LTA, Fig 2b). With our results, we correct the previously hypothesized role of SPD_1672 and its homologs (formerly named RafX) in the biosynthesis of wall teichoic acid (WTA). Absence of TacL, concomitant with a total loss of LTAs but remaining presence of pnWTAs, did not alter the pneumococcal growth behaviour, cell shape and overall cell wall phosphorylcholine content, but led to strikingly decreased virulence in murine infection models. It is important to mention that this is the first LTA-deficient mutant of a Gram-positive bacterium without severe growth defects under laboratory conditions. Furthermore, the distinctly reduced virulence of *tacL*-mutants suggests TacL as a potential novel target for antimicrobial drug development.

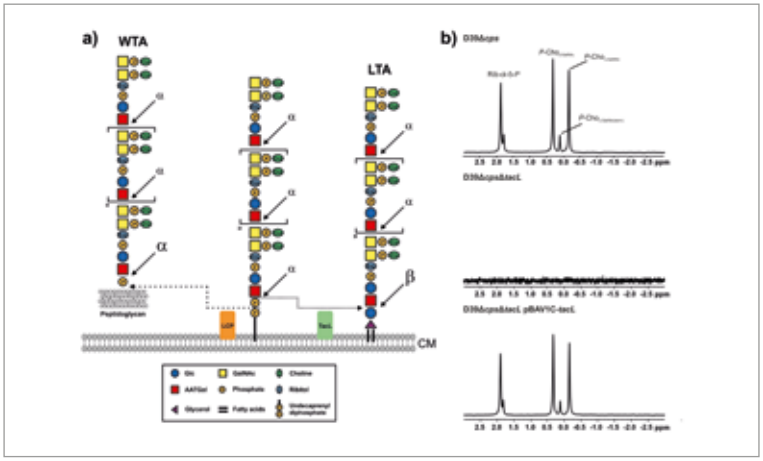

Figure 2. TacL is responsible for ligation of teichoic acid precursor chains onto the glycolipid (analog zu Fig 1). **(a)** Last steps in the TA biosynthesis pathway focusing on the incorporation of TA precursor into the cell wall in *S. pneumoniae*. **(b)** Sections (δ_p 3-(−3)) of ^{31}P NMR spectra from hydrazine-treated LTA isolated from pneumococcal strains D39Δ*cps* (top) and D39Δ*cps*Δ*tacL* pBAV1C-*tacL* (below). For the knockout mutant D39Δ*cps*Δ*tacL* (middle panel) the typical LTA containing fractions from the HIC purifications were measured without prior hydrazine treatment. (Heß, Waldow, Kohler *et al.*, in preparation).

Internal and external collaboration

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Grant support

Exzellenzcluster Inflammation at Interfaces (CL X)

DZIF TTU Tuberculosis: ClinTB, Personalised Medicine

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

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

LIPID-PEPTIDE INTERACTIONS

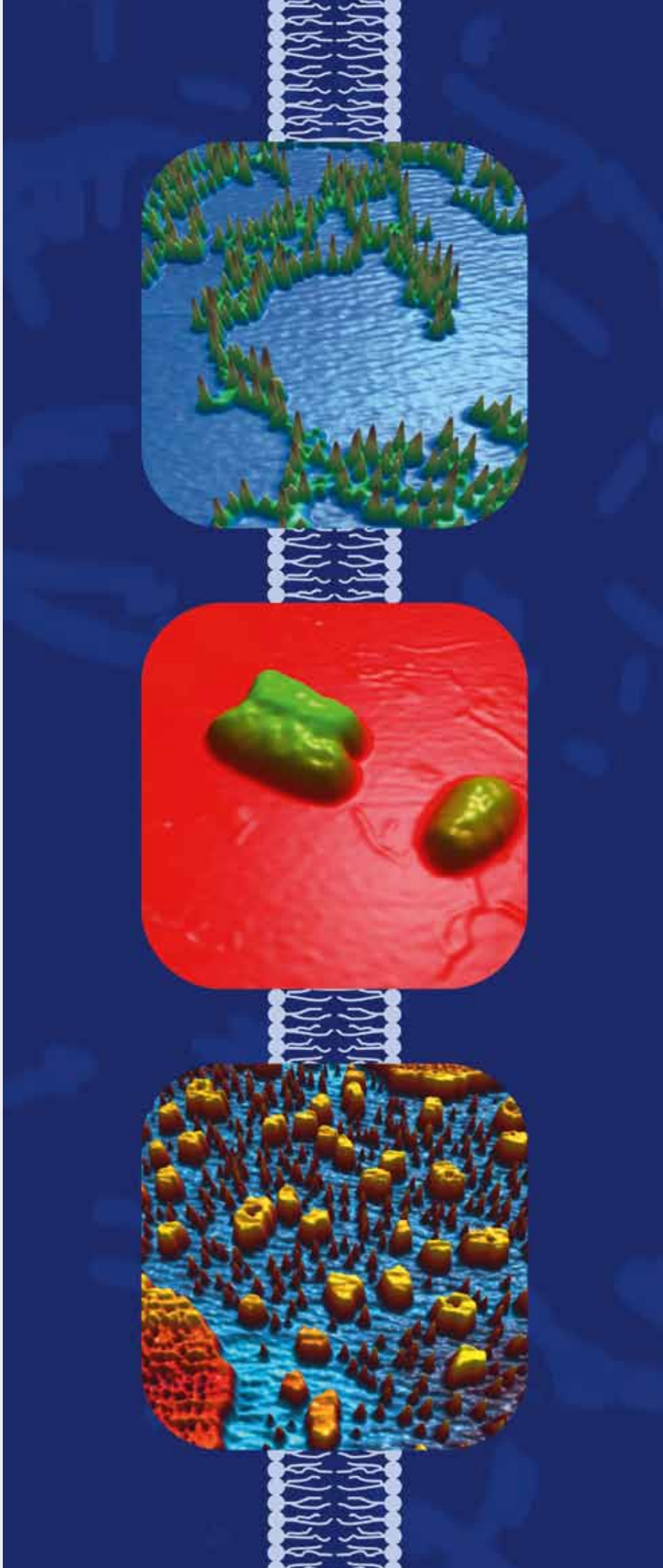
SIGNAL TRANSDUCTION

MEMBRANES

HOST DEFENSE PEPTIDES

DOMAINS

GLYCOLIPIDS



Priority Research Area **Infections**

Biophysics

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Priority Research Area **Infections**

Biophysics

Mission

Im Focus unseres Interesses stehen die Funktion von Lipidmembranen und deren Interaktion mit Peptiden und Proteinen im Kontext von Lungeninfektionen. Um biomedizinische Fragenstellungen zu beantworten und neue Therapien zu entwickeln, versuchen wir zuerst die molekularen Funktionen und dynamischen Prozesse zu verstehen. Im biomedizinischen Fokus stehen: (a) Organisation und Eigenschaften von Membranen der Mykobakterien, Gram-negativen Bakterien und humanen Zellen; (b) Funktion von Poren-formenden Peptiden, wie z.B. Host-defense peptides (HDPs) des Immunsystems in der Lunge; (c) weitere Aktivitäten membranaktiver Peptide, wie z.B. die Neutralisierung von Endotoxin und Lipoproteinen, und deren Einfluss auf Membran-assoziierte Signaltransduktionswege.

The function of lipids and lipid membranes and their interaction with peptides and proteins in the context of lung infections is in our focus. To answer biomedical questions and to develop new therapeutics we first aim at an understanding of the underlying molecular functions and dynamic processes. The biomedical focus is directed towards: (a) the organization and properties of membranes of mycobacteria, Gram-negative bacteria and human cells; (b) the function of pore-forming peptides, e.g. host defense peptides (HDPs) of the innate immune system in the lung; (c) further activities of membrane active peptides, e.g. neutralization of endotoxins and lipoproteins and involvement in membrane-associated signal transduction pathways.

Most important findings

Pore-formation by Host-defense peptides (HDPs)

Membrane-active HDPs comprise one of the most ancient substance family, that build up the first defense barrier in virtually all organisms. To understand the interaction process of HDPs on the membrane level, their current known anti bactericidal action is studied in-depth along with selected reference substances for small and large pores. A set of structurally diverse substances was chosen, covering LL-32, arenicin-1 and hBD-3-l as AMP representatives together with the ionophore nonactin and the toxin α-hemolysin. The combination of five different biophysical techniques facilitates capturing variances in membrane permeabilization and the analysis of this versatile process ranging from single pore characterization to the statistical analysis of either large membrane areas or a huge number of single membranes. Understanding the detailed mechanism of the bacterial killing strategy of HDPs thereby promotes the detection of new points of interference that are indispensable for optimizations in peptide design.

Highlights

Pore-formation by Candidalysin is the toxic principle of *C. albicans*

Host-defense peptides modify cholesterol-rich domains

Improvements of translational aspects of Aspidasept

Selected publications

Sommer A, Kordowski F, Büch J, Maretzky T, Evers A, Andrä J, Düsterhöft S, Michalek M, Lorenzen I, Somasundaram P, Tholey A, Sönnichsen FD, Kunzelmann K, Heinbockel L, Nehls C, Gutsmann T, Grötzinger J, Bhakdi S, Reiss K. Phosphatidylserine exposure is required for ADAM17 sheddase function. Nat Commun. 2016 May 10;7:11523.

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Heinbockel L, Marwitz S, Barcena Varela S, Ferrer-Espada R, Reiling N, Goldmann T, Gutsmann T, Mier W, Schürholz T, Drömann D, Brandenburg K, Martinez de Tejada G. Therapeutical Administration of Peptide Pep19-2.5 and Ibuprofen Reduces Inflammation and Prevents Lethal Sepsis. PLoS One. 2015 Jul 21;10(7):e0133291.

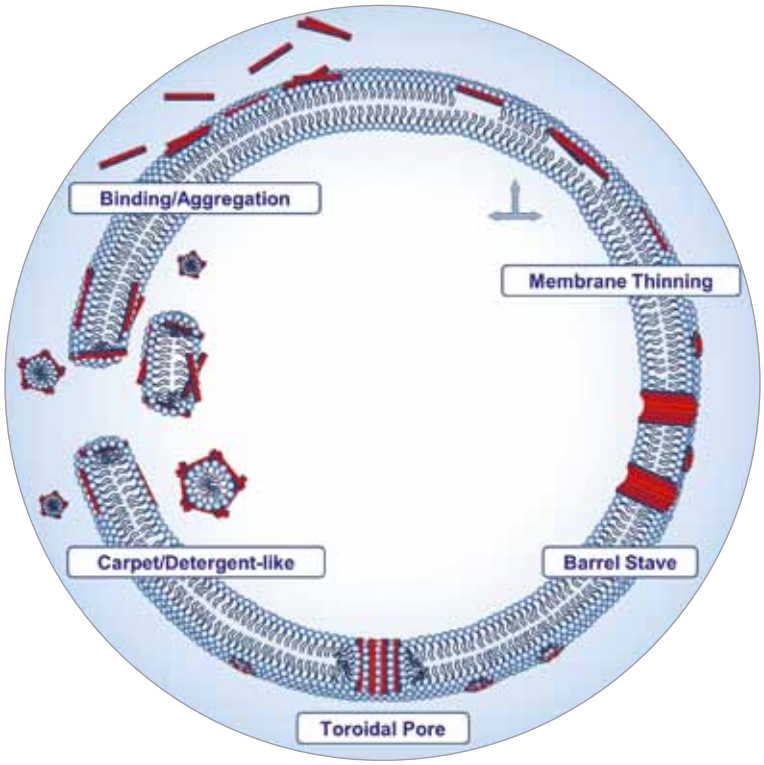

Figure 1. Possible interaction mechanisms between lipid membranes and membrane-active host defense peptides (HDPs).

The Aspidasept peptides as general anti-sepsis and anti-inflammatory drugs

The translational aspects of membrane-active peptides were further developed, in particular for the peptide Aspidasept® (Pep19-2.5), and variants in various *in vitro* and *in vivo* systems. The peptide was tested in an animal system (mouse) by oral application and exhibited also under this condition significant anti-inflammatory action. It was additionally shown that the inflammation not only by bacterial toxins (PAMPs), but also by endogenous DAMPs such as the heparanase/heparansulfate system could be inhibited. A further line of evidence for the successful application of the Aspidasept peptides as antiinfectious agents was obtained in non-systemic dermal application: The lead structure Pep19-2.5 as well as the variant Pep19-4LF were shown to have excellent activity against the inflammatory action of LPS as well as against the Gram-positive and mycoplasmic lipopeptides. This was found to hold true for keratinocytes, monocyte-derived dendritic, and Langerhans cells. In two approaches, the ability of the Aspidasept peptides to act diagnostically was monitored.

Priority Research Area **Infections**

Biophysics

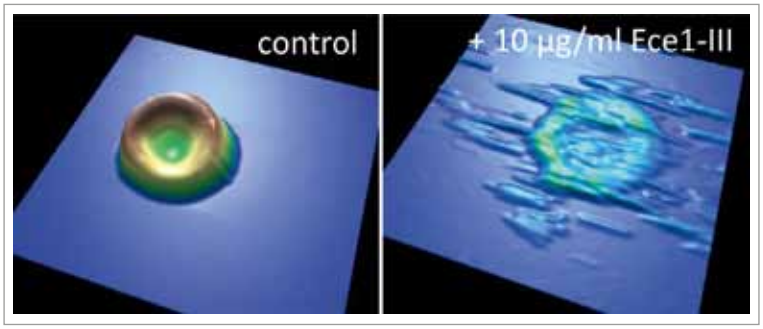

Figure 2. Atomic force micrograph of the permeablization and destruction of erythrocytes by Candidalysin (Ece1-III).

Activity of the fungal toxin candidalysin

For decades, researchers have been trying to elucidate critical determinants of the pathogenicity of human fungi. Only recently, has an international consortium of scientists succeeded in identifying such a factor – a peptide toxin secreted by the clinically important fungus *Candida albicans*. This peptide Ece1-III plays a crucial role during fungal infections of human mucosae. The peptide's virulence manifests in the direct damage of epithelial membranes, in the stimulation of a danger response signalling pathway and in the activation of epithelial immunity. We provided the first insights into the direct interaction between Ece1-III and lipid membranes. The peptide's amphiphilic alpha-helical structure is described as a fundamental prerequisite for its binding to lipid membranes. In consequence of the initialised binding, the peptide inserts between the lipid head groups and aligns parallel to the bilayer surface. Upon increasing surface accumulation, however, the helix starts to penetrate the bilayer with oblique inclination. As a result, the bilayer is destabilised and transient local collapses occur. This carpet-like disintegration eventually leads to the disruption of the entire membrane.

Internal and external collaboration

Englisch U, University of Applied Science Lübeck, Germany;
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BMWI: ZIM-Project *„Calciumhaltige Implantatoberfläche mit antibakteriellen, antiinflammatorischen Wirkstoffdepots zur Vermeidung periprothetischer Infektionen"*

DFG: Cluster of Excellence „Inflammation at Interfaces"
Project: „Anti-inflammatory regulation of immune cells by membrane active host defense peptides"

Leibniz Research Alliance: Infections´21

DRUG-NANOCARRIER

MICROBIOTA

MACROPHAGE

MYCOBACTERIUM TUBERCULOSIS

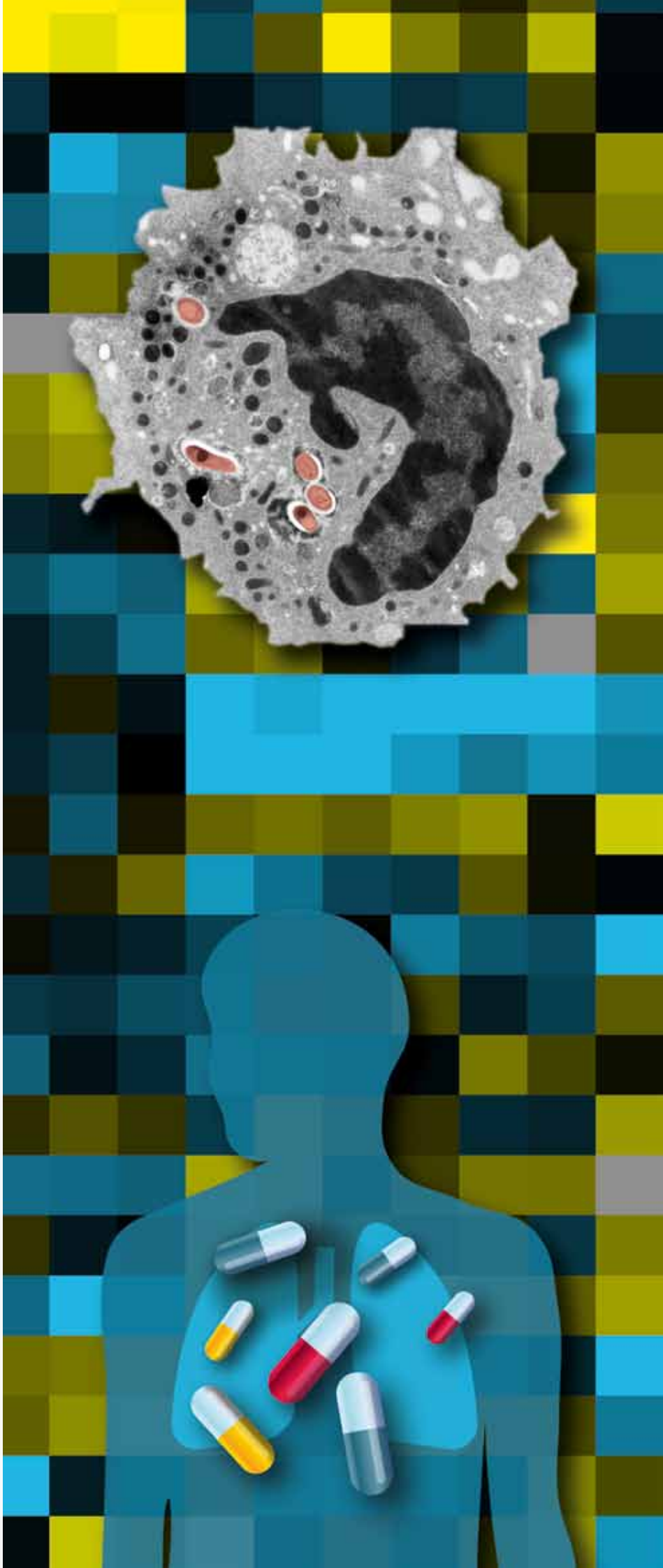
HOST-DIRECTED THERAPY

NEUTROPHIL

TUBERCULOSIS

PHAGOSOME

LIPIDS



Priority Research Area **Infections**

Cellular Microbiology

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Priority Research Area **Infections**

Cellular Microbiology

Mission

Die Forschungsgruppe Zelluläre Mikrobiologie untersucht Immunantworten und Wirts-Erreger-Interaktionen in der Tuberkulose. Der Tuberkuloserreger, *Mycobacterium tuberculosis*, ist ein fakultativ intrazellulärer Keim, der sich in Makrophagen vermehren kann. Unsere Studien basieren auf der Frage inwieweit die intrazelluläre Nische des Erregers sein Überleben, seine Vermehrung und Übertragung, aber auch angeborene und erworbene Immunreaktionen, die Pathogenese und Effizienz anti-mykobakterieller Therapien beeinflusst. Dieses Wissen nutzen wir um neue zielgerichtete Therapiestrategien gegen die Tuberkulose zu entwickeln.

The research group Cellular Microbiology studies pulmonary host defenses and host-pathogen interactions in tuberculosis. Its agent, *Mycobacterium tuberculosis*, is a facultative intracellular pathogen able to proliferate inside macrophages. Our studies are based on the question how the intracellular niche of *Mycobacterium tuberculosis* influences the pathogens survival, growth and transmission, as well as innate and acquired immunity, pathogenesis and anti-mycobacterial drug efficacy. The gained knowledge provides the basis to develop novel targeted therapeutic strategies against tuberculosis.

Most important findings

Analysis of host-pathogen interactions in tuberculosis can identify host immune properties as targets for therapeutic measures including the host microbiota in order to better control the pathogen by host-directed therapies (HDT) accompanying classical antibiotic therapy[1]. Characterizing mycobacterial niches in the host can instruct the development of targeted nanocarrier to specifically deliver antibiotics to the pathogen.

The tuberculosis (TB) agent, *Mycobacterium (M.) tuberculosis,* is able to survive and grow in resting macrophages[2]. This virulence property is in part facilitated by the pathogens capability to interfere with phagosome maturation. *M. tuberculosis*-host cell interactome analysis revealed actin cytoskeleton associated proteins selectively enriched on mycobacterial phagosomes[3]. Disruption of the actin rim around mycobacterial phagosomes by knocking down individual actin-associated proteins promoted phagosome maturation and interfered with intracellular growth of *M. tuberculosis*. These findings indicate that the actin rim around mycobacterial phagosomes is associated with failed maturation to phago-lysosomes. Interestingly, the mycobacterial cell wall lipid, trehalose dimycolate (TDM) is solely able to block phagosome maturation when coated to beads, and phagosomes containing TDM beads also associate temporarily with actin. TDM is also able to interfere with phagosome maturation when TDM-coated beads are opsonized with specific antibodies promoting FcR-mediated phagocytosis (Patin et al., under revision). In addition to blocking

Highlights

Novel host-directed therapies in tuberculosis targeting neutrophils
(Dallenga, Schaible, 2016; Dallenga et al. submitted)

Innovative iron holospheres to target antibiotics to mycobacteria
(Leidinger et al., 2015)

Selected publications

1. Dallenga T, Schaible UE 2016 Neutrophils in tuberculosis - first line of defence or booster of disease and targets for host directed therapy? *FEMS Pathogens and Disease*: 74, ftw012

2. Weiss G, Schaible UE. 2015 Macrophage defense mechanisms against intracellular bacteria *Immunological Reviews* 264(1):182-203

3. Herweg JA, Hansmeier N, Otto A, Geffken AC, Subbarayal P, Prusty BK, Becher D, Hensel M, Schaible UE, Rudel T, Hilbi H. 2015 Purification and proteomics of pathogen-modified vacuoles and membranes. *Front Cell Infect Microbiol.* 2015 Jun 2; 5: 48

4. Patin EC, Willcocks S, Orr, S Ward TH, Lang R, Schaible UE 2016 Mincle-mediated anti-inflammatory IL-10 response counter-regulates IL-12 in vitro. *Innate Immun*: 22(3):181-5

5. Schneider BE, Behrends J, Hagens K, Harmel N, Shayman JA, Schaible UE 2014 Lysosomal phospholipase A2: A novel player in host immunity to *Mycobacterium tuberculosis*. *Eur J Immunol.* 44(8): 2394

6. Leidinger, P, Treptow, J, Hagens, K, Eich, J, Zehethofer, N, Schwudke, D, Oehlmann, W, Lünsdorf, H, Goldmann, O, Schaible, UE, Dittmar, KE, Feldmann, C. 2015 Isoniazid@Fe2 O3 Nanocontainers and Their Antibacterial Effect on Tuberculosis Mycobacteria. *Angew Chem Int Ed Engl* 54(43): 12597-601

7. Yun Y, Srinivas G, Kuenzel S, Linnenbrink M, Alnahas S, Bruce KD, Steinhoff U, Baines JF, Schaible UE. 2014 Environmentally determined differences in the murine lung microbiota and their relation to alveolar architecture. *PlosOne* 9(12): e113466.

8. Hauptmann M, Schaible UE 2016 Linking microbiota and respiratory disease. *FEB S Letters* 590(21):3721-3738

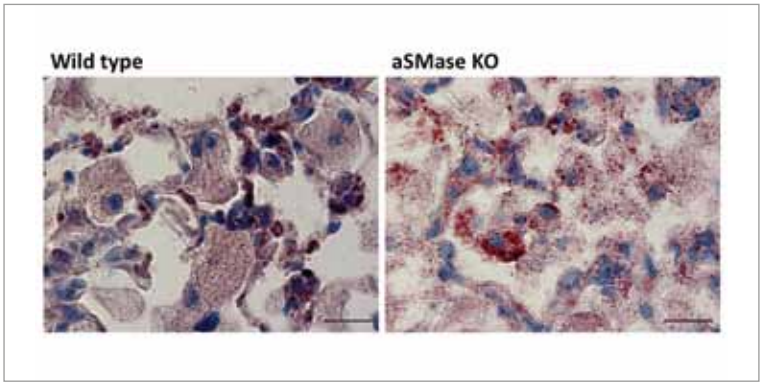

Figure 1. Oil Red staining reveals lipid storage (red labeled vesicles) in macrophages within inflammatory lung granulomas from aSMase KO but not wildtype mice upon aerosol infection with *M. tuberculosis.*

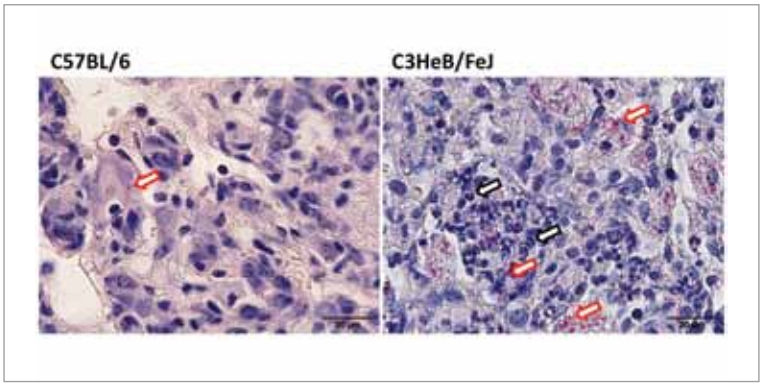

Figure 2. Acid-fast stain demonstrates association of large numbers of *M. tuberculosis* bacteria (red arrows) with the presence of neutrophils (black arrows) in inflammatory lung granulomas of susceptible C3HeB/FeJ mice, which is not observed in resistant C57BL/6 mice.

Priority Research Area **Infections**

Cellular Microbiology

phagosome maturation, TDM-beads deviate innate immune responses against mycobacteria by inducing IL-10, which subsequently inhibits IL-12 production, a prerequisite for proper pro-inflammatory immunity against mycobacteria[4]. Interestingly, both, induction of IL-10 as well as interference with FcR-mediated phagosome maturation by TDM requires the TDM receptor, Mincle. Therefore, a HDT interfering with Mincle signaling could improve phagosomal elimination of and immunity to mycobacteria.

Intracellular mycobacteria use host lipids as carbon source. Consequently, lipid metabolisms of both, host and mycobacteria are communicating but competitive systems during host-pathogen interaction. Our studies on the function of lipid degrading enzymes located in macrophage lysosomes identified the lysosomal phospholipase A_2 ($LPLA_2$)[5] as well as the acid sphingomyelinase (aSMase) as important for proper host responses to *M. tuberculosis*. Mice lacking the aSMase develop sphingomyelin storage disease, which renders them more susceptible to experimental TB (Burmeister et al., in prep). Elevated mycobacterial loads in aSMase KO mice were associated with enhanced lipid storage in macrophages, exacerbated pro-inflammatory immune responses and histopathological alterations including the presence of high numbers of multinucleated giant cells (Fig. 1). We were able to correct these outcomes of *M. tuberculosis* infection by enzyme replacement therapy using recombinant aSMase. These findings indicate that - despite the generation of proper pro-inflammatory immunity - sphingomyelin accumulation benefits *M. tuberculosis* growth, which could be targeted a HDT removing sphingomyelin from infected tissues.

A promising HDT strategy became evident by studying *M. tuberculosis* infection of neutrophil-macrophage cocultures. Neutrophils, the main infected cell population in active TB patients, quickly succumb to necrotic cell death upon M. tuberculosis infection, which requires the RD1 encoded mycobacterial virulence factor, ESAT-6, in combination with the neutrophils' own respiratory burst. Removal of infected necrotic neutrophils by macrophages promotes growth of *M. tuberculosis* in these efferocytic cells. Interestingly, using a myeloperoxidase inhibitor to block neutrophil respiratory burst prohibited mycobacterial growth in these macrophages (Dallenga et al., submitted). Another HDT targeting the neutrophil recruiting small lipid mediator, leukotriene B4, was able to reduce mycobacterial loads in susceptible mice, which develop neutrophil-associated granulomas (Paudyal et al., in prep; Fig. 2).

The alarming rise of antibiotic resistant *M. tuberculosis* strains prompted us together with national (KIT, RBT) and international partners (NAREB) to develop nanocarriers to target drugs to infection sites. We identified antibiotic loaded iron holospheres as promising nanocarriers against *M. tuberculosis*[6]. Our studies on lung immunity to *M. tuberculosis* also consider the respiratory tract microbiota as determinant for disease development. Identification of the murine lung microbiota[7] provided us with the unique opportunity to explore the link between microbiota diversity, lung immunity and tuberculosis disease[8].

Internal and external collaboration

Internal collaborations

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Aerosol Transmission: Gülsah Gabriel, HPI; Kerstin Schepanski, TROPOS; von Woedtke, INP

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DFG: SPP 1580 Intracellular compartments as places of pathogen-host-interactions; EXC Inflammation @ Interfaces (1. Lysosomal disorders and bacteria-induced inflammation; 2. Cluster Lab 9); IRTG 1911 Immuno-regulation of Inflammation in Allergy and Infection

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

Danish Research Council: Center of Nano-vaccines, Imaging nanocarrier vaccine efficacy

EU FP7: NAREB, Nanotherapeutics to treat bacterial infectious diseases

Industry: CLP

BIOMARKER
CHRONIC PULMONARY ASPERGILLOSIS
CLINTB
MDR-TB
IGRA
M/XDR-TB-CONSILIUM
NTM PRECISION MEDICINE
NEW DRUGS
XDR-TB
TB NET
TUBERCULOSIS
TB INFO

Priority Research Area **Infections**

Clinical Infectious Diseases

Head

- Prof. Dr. med. Dr. h.c. Christoph Lange

Members as of 01.12.2016

- Olga Berezovskij
- Prof. Dumitru Chesov MD PhD (Visiting Professor)
- Franziska Daduna
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- Dr. med. Ulf Greinert
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- Dr. med. Jan Heyckendorf
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- Dagmar Schaub
- Dr. med. Elena Terhalle
- Dr. med. Nasstasja Wassilew
- & nursing staff of the Division of Infectious Diseases

Priority Research Area **Infections**

Clinical Infectious Diseases

Mission

Wir möchten die Prävention, Diagnostik und Therapie von chronischen Lungenerkrankungen (Tuberkulose - besonders der M/XDR-TB, pulmonale Erkankungen durch nicht-tuberkulöse Mykobakterien - NTM-PD und chronische pulmonale Aspergillose - CPA) substantiell verbessern und wissenschaftliche Fortschritte in die klinische Praxis integrieren.

We aim to substantially improve the prevention, diagnosis and treatment of tuberculosis (especially M/XDR-TB), respiratory diseases caused by non-tuberculous mycobacteria (NTM-PD) and chronic pulmonary aspergillosis (CPA) and to integrate scientific advances into clinical practice.

Patient care (upper left), training (lower left) clinical research (lower right), and capacity building (upper right) are in the focus of the Clinical Infectious Diseases research group activities.

Most important findings

High chances for treatment success in M/XDR-TB:

We evaluated whether treatment outcomes for patients with multidrug-resistant and extensively drug-resistant tuberculosis (M/XDR-TB) can be substantially improved, when sufficient resources for personalizing medical care are available. The records of patients with MDR-TB were reviewed for epidemiological, clinical, laboratory, treatment and outcome data at a single European centre.

Highlights

Documenting high treatment success rate in M/XDR-TB with individualized therapy

Proposal of simplified treatment outcome definitions for M/XDR-TB

Identification of bactericidal activity of a ß-lactam antibiotic against TB

Selected publications

Günther G, Lange C, Alexandru S, Altet N, Avsar K, Bang D, Barbuta R, Bothamley G, Ciobanu A, Crudu V, Danilovits M, Dedicoat M, Duarte R, Gualano G, Kunst H, de Lange W, Leimane V, Magis-Escurra C, McLaughlin AM, Muylle I, Polcová V, Popa C, Rudolf Rumetshofer, Skrahina A, Solodovnikova V, Spinu V, Tiberi S, Viiklepp P, van Leth F for the TBNET. Treatment outcomes in multidrug resistant tuberculosis. N Engl J Med 2016 Sept 15;375(11):1103-5.

Lange C, Günther G, van Leth F for the TBNET. More on treatment outcomes in multidrug resistant tuberculosis (authors reply). N Engl J Med 2016 Dec 29; 375(26):2611

Diacon AH, van der Merwe L, Barnard M, von Groote-Bidlingmaier F, Lange C, García-Basteiro A, Sevene E, Ballell L, Barros-Aguirre D. ß-lactams against TB: New trick for an old dog. N Engl J Med 2016 Jul 28;375(4):393-4.

Horsburgh CR, Barry C, Lange C. Treatment of tuberculosis. N Engl J Med 2015; 373 (22): 2149-60.

Olaru ID, Lange C, Indra A, Meidlinger L, Huhulescu S, Rumetshofer R. High rates of treatment success in pulmonary multidrug-resistant tuberculosis by individually tailored treatment regimens. Ann Am Thorac Soc. 2016 Aug;13(8):1271-8.

Zellweger JP, Sotgiu G, Block M, Dore S, Altet N, Blunschi R, Bogyi M, Bothamley G, Bothe C, Codecasa L, Costa P, Dominguez J, Duarte R, Fløe A, Fresard I, García-García JM, Goletti D, Halm P, Hellwig D, Henninger E, Heykes-Uden H, Horn L, Kruczak K, Latorre I, Pache G, Rath H, Ringshausen FC, Seminario Ruiz A, Solovic I, de Souza-Galvão ML, Widmer U, Witte P, Lange C for the TBNET Risk assessment of tuberculosis in contacts by interferon-γ release assays (IGRAs). A TBNET study. Am J Respir Crit Care Med 2015 May 15;191(10):1176-84.

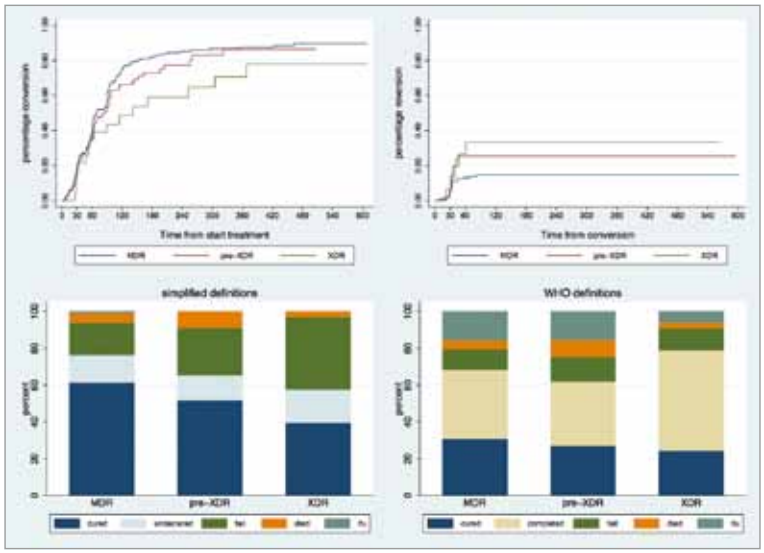

Figure 1. Early and final tuberculosis treatment outcomes. Culture conversion to negative cultures in all patients with a positive culture at the start of second-line TB treatment (upper left); culture reversion in patients who achieved culture conversion during the study (upper right); final outcome by proposed simplified definitions (lower left); final outcome by WHO definitions (lower right). MDR-TB: multidrug-resistant TB: bacillary resistance to at least isoniazid and rifampin but excluding pre-XDR and XDR-TB; pre-XDR: pre-extensively drug resistant TB: bacillary resistance to isoniazid and rifampicin, and either any fluoroquinolone or any second-line injectable drug; XDR: extensively drug-resistant TB: bacillary resistance to isoniazid and rifampicin, any fluoroquinolone, and any second-line injectable drug (amikacin, capreomycin and/or kanamycin) (Günther G. et al. NEJM 2016)

Ninety patients with pulmonary M/XDR-TB were identified. Median age was 30 years. All patients were foreign-born and 70 (78%) came from the former Soviet Union States. Thirty-nine (43%) patients had MDR-TB, 28 (31%) had additional bacillary resistance to at least one second-line injectable drug, 9 (10%) to a fluoroquinolone, 14 (16%) patients had XDR-TB. In 97.8% (n=88) of patients different drug combinations were used for treatment. Sixty-five (72.2%) patients had a successful treatment outcome, 8 (8.9%) defaulted, 3 (3.3%) died, 8 (8.9%) continued treatment in another country and their outcome was unknown, and 6 (6.7%) were still on therapy. None of the patients experienced treatment failure. Treatment outcome for XDR-TB was similar to that of MDR-TB. High rates of treatment success can be achieved in patients with M/XDR-TB when individualized tailored treatment regimen can be provided.

Outcome definitions for M/XDR-TB revised:

Despite longstanding treatment durations with costly second-line drug-regimens cure from MDR-TB remains a challenge. The World Health Organization (WHO) defines „cure“ as „treatment completion“ with at least three negative cultures after the intensive phase of therapy in the absence of „treatment failure“. Definition of „treatment failure“ requires early treatment termination or need for permanent regimen change of at least two anti-TB drugs. „Treatment success“ is defined as sum of „cure” and „treatment completed”. We evaluated treatment outcomes by WHO definitions in the TBNET cohort of 380 patients with MDR-TB at 23 European centers, including 89 pre-extensive drug-resistant (XDR)-TB and 33 XDR-TB and compared them to simplified MDR-TB treatment outcome definitions

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Clinical Infectious Diseases

„Cure": Negative culture status six months after treatment initiation, no positive culture thereafter and being relapse-free one year after treatment completion; *„Failure"*: Positive culture status six months after treatment initiation or thereafter or relapse within one year after treatment completion; *Undeclared*: Outcome not assessed (transferred out, no culture status at six months while being in care or no post-treatment assessment); *„Died"*: Death during observation; and *Lost-to-follow-up*: Not being in care six months after treatment initiation.

Fifty (57%) patients with treatment failure were not identified by the WHO definition. Assessment of WHO-defined cure was only possible for 13%, 58%, and 52% of patients in low, intermediate, and high TB-incidence countries, respectively, due to lack of sputum cultures taken after the intensive treatment phase. This could reflect limited healthcare access of mostly foreign-born patients, or the inability of patients to produce sputum in the latter stages of therapy. WHO-defined treatment success in MDR-TB is predominantly driven by completing treatment rather than by a biological endpoint, and fails to address relapse-free survival as a clinically more relevant assessment of treatment efficacy. Relapse-free cure was achieved in 61%, 52%, and 39% of patients with MDR-TB, pre-XDR-TB and XDR-TB, respectively when simplified definitions were used, in contrast to WHO-defined cure in 31%, 27%, and 24% of patients, respectively. Of patients with a negative culture status at six months, 35(11%) reverted in the continuation phase, while 9(3%) had a post-treatment relapse, suggesting culture status at six months may be a reliable predictor for relapse-free cure in patients with MDR-TB. Current WHO definitions may underestimate cure in patients with MDR-TB, and can be simplified while incorporating the assessment of post-treatment relapse.

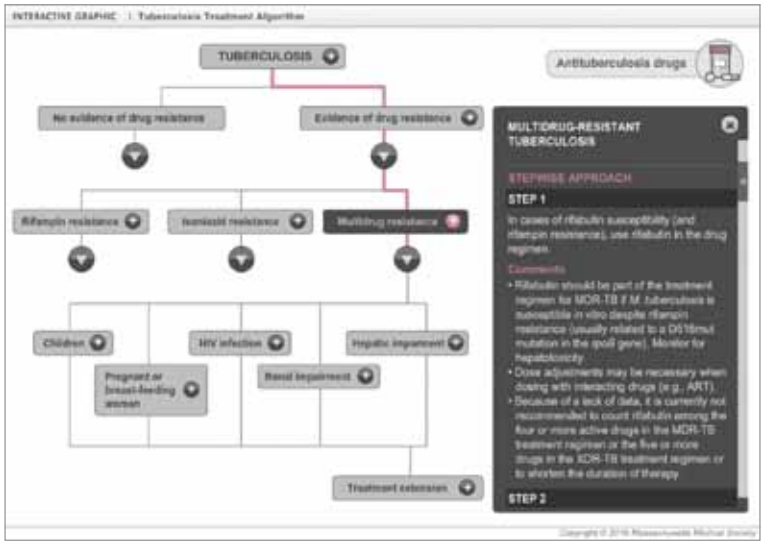


Figure 2. Interactive standardized tuberculosis treatment algorithm (Horsburgh C.R. et al. NEJM 2015)

Internal and external collaboration

Local: Prof. Dr. M. Addo, Dr. S. Andres, Prof. Dr. T. Goldmann, Dr. D. Hillemann, Dr. C. Hoelscher, Prof. Dr. A. Katalinic, Prof. Dr. K-F. Klotz, Dr. H. Karaköse, Dr. K. Kranzer, Dr. M. Merker, Prof. Dr. S. Niemann, Prof. Dr. J Rupp, Dr. S. Schmiedel, Dr. D. Schwudke, Prof. Dr. U. Schaible, Center for Infectious Diseases Borstel-Lübeck (DGI). Excellence Cluster I@I

National: Dr. K. Avsar (Gauting) Prof. Dr. M. Hoelscher (Munich), Dr. H. Hoffmann (Gauting), Dr. M. Müller (Frankfurt), Dr. A. Rachow (München), Dr. C. Schacht, (Berlin), Prof. Dr. M. Sester (Homburg), Prof. Dr. S. Stenger (Ulm), DZIF M/XDR-TB network; TBNET, TB or not TB network.

International: Prof. Dr. C. Barry (Bethesda, USA/Cape Town, South Africa), Prof. Dr. D. Chesov (Chisinau, Moldova), N. Ciobanu (Chisinau, Moldova), Dr. V. Crudu (Chisinau, Moldova), Prof. Dr. K. Dheda, (Cape Town, South Africa), Prof. Dr. A. Diacon (Stellenbosch, South Africa), Dr. A. García-Basteiro (Maputo, Mozambique), 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), M. Olegovna (Montreal, Canada), E. Schurr (Montreal, Canada), Dr. A. Skrahina (Minsk, Belarus), Prof. Dr. G. Sotgiu (Sassari, Italy), Dr. J.-P. Zellweger (Berne, Switzerland); Collaborative Group for Meta-Analysis of Individual Patient Data in MDR-TB; Medicines sans frontiers (MSF), Tuberculosis Network European Trialsgroup (TBNET), RESIST-TB.

C. Lange is Professor in International Health / Infectious Diseases at the University Lübeck, Foreign Adjunct Professor at the Karolinska Institute (Stockholm, Sweden), Associate Professor at the University of Namibia School of Medicine (Windhoek, Namibia) and Associate Professor at the State University of Medicine and Pharmacy, Chisinau (Moldova).

Grant support

AID, BMBF, DAAD, DZIF, EDCTP, EU-FP7, EU-H2020, S.-H. TB Society, TBNET

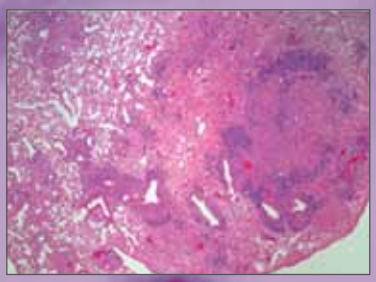
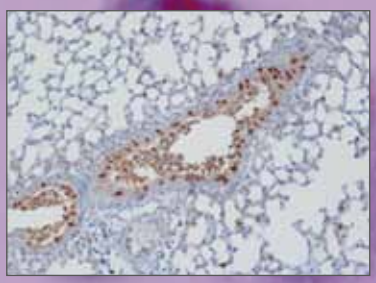
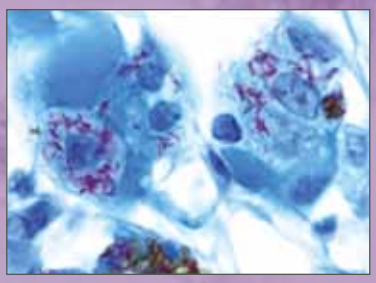
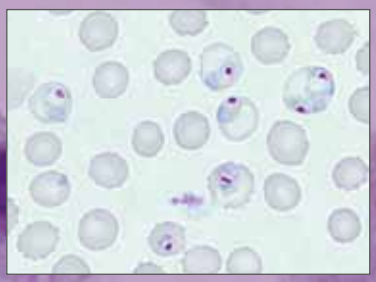
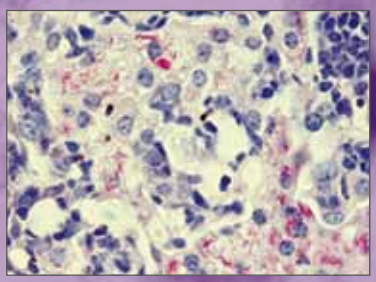
COINFECTION

MOUSE MODELS

TUBERCULOSIS

INFLAMMATION
SEX DIFFERENCES

INFLUENZA
MALARIA

Head

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- Dr. Jannike Dibbern (Blank)
- Lars Eggers
- Anna Erazo
- Sarah Ring
- Lindsay Tucker
- Julia Witschkowski

Trainees

- Vivien Sparr
- Jana Holm

Priority Research Area **Infections**

Coinfection

Mission

Eine Infektion mit *Mycobacteruim tuberculosis (Mtb)* führt in der Regel zu einer latenten Infektion, da das Immunsystem der meisten Menschen den Erreger nicht vollständig eliminieren kann. Latente Infektionen müssen über Jahrzehnte vom Immunsystem des Wirts in Schach gehalten werden, ohne dass die schützende pro-inflammatorische Immunantwort eine übermäßige Entzündung und Pathologie verursacht. Die Immunantworten einer Person werden von Faktoren wie Alter, Geschlecht, genetischer Ausstattung, Komorbiditäten und Koinfektionen beeinflusst. Die Forschungsgruppe Koinfektion untersucht mit Hilfe experimenteller Mausmodelle, wie parasitäre oder virale Koinfektionen das empfindliche immunologische Gleichgewicht beeinflussen, das eine langfristige Kontrolle einer *Mtb* Infektion ermöglicht.

Mycobacterium tuberculosis (Mtb) causes persistent infection in most people because of the inability of an otherwise effective immune system to completely eliminate the pathogen. The challenge of controlling persistent infections is to avoid overt inflammation and tissue damage in the presence of a certain pathogen burden. The inflammatory responses generated by the host are shaped by multiple factors such as age, sex, genetic makeup or comorbidities and coinfections. The Coinfection research group investigates how concurrent parasitic or viral infections impact on the delicate balance of the immune responses that facilitate long-term control of *Mtb* infection in experimental mouse models.

Most important findings

Tb and malaria

We previously showed that coinfection with *Plasmodium berghei*, a strain that causes pulmonary pathology, exacerbated chronic *Mtb* infection in C57BL/6 mice. We wondered whether a parasite which *per se* does not affect the lung also influences Tb and found that coinfection with non-lethal *P. yoelii (Py)* caused increased pulmonary pathology and cellular infiltration with a significant increase of a $CD11c^+$ population in lungs and spleens. $CD11c^+$ cells, when isolated from spleens of *Py*-infected mice, promoted survival and growth of *Mtb ex vivo* in contrast to $CD11c^+$ cells from naïve mice. We hypothesize that coinfection with *Plasmodium* parasites induce *Mtb* permissive cells which promote *Mtb* survival and propagation. Currently, we examine which mechanisms make these cells more permissive to *Mtb*.

Highlights

C57BL/6 mouse model mimics the epidemiological observations of increased male susceptibility to Tb.

Influenza coinfection tips the scale towards inflammation and rapidly exacerbates Tb.

Plasmodium infection renders $CD11c^+$ cells permissive for *Mtb* survival and replication.

Selected publications

Blank J, Eggers L, Behrends J, Jacobs T, Schneider BE (2016) One episode of self-resolving Plasmodium yoelii infection transiently exacerbates chronic *Mycobacterium tuberculosis* infection. Frontiers in Microbiology, Bd 7, S. 152., 10.3389/fmicb.2016.00152

Klionsky DJ, Abdelmohsen K, ..., Schaible, UE, , ..., Schneider BE, et al. (2016) ‚Guidelines for the use and interpretation of assays for monitoring autophagy' (3rd edition). Autophagy, Bd 12, S. 1-222.

Blank J, Behrends J, Jacobs T, Schneider BE (2015) *Mycobacterium tuberculosis* coinfection has no impact on *Plasmodium berghei* ANKA-induced experimental cerebral malaria in C57BL/6 mice. Infect Immun 84:502–510. doi:10.1128/IAI.01290-15.

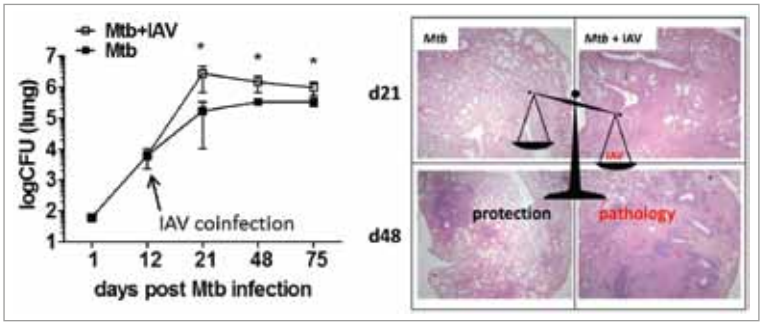

Figure 1. IAV coinfection rapidly exacerbates *Mtb* infection. **A)** Lung CFU and **B)** H&E stained lung sections of mice infected with *Mtb* alone or *Mtb* and IAV.

Tb and influenza

Epidemiological data show that people with underlying respiratory conditions including Tb are at high risk of developing influenza-related complications. Influenza viruses are potent inducers of type I IFNs which are associated with Tb disease progression in mouse and man. We sought to elucidate the consequences of influenza infection on *Mtb*-infected mice and observed a significant increase in bacterial burden as early as 9 days after influenza A virus (IAV) challenge (Fig. 1A). To our surprise, type I IFN levels were low during acute IAV infection and not detectable at later time points when bacterial numbers remained significantly elevated. Instead, we found a sustained increase in several pro-inflammatory cytokines and chemokines while IL-10 was absent indicating that counter-regulation of an overwhelming pro-inflammatory immune response is impaired. Consequently, preliminary histopathological analysis revealed exacerbated tissue pathology in co-infected lungs (Fig. 1B). Strikingly, IL1 and MCP1 were highly elevated during coinfection. IL1 is required for control of *Mtb* infection but unrestrained production can promote tissue pathology and increase Tb susceptibility. Likewise, MCP1 is associated with severe Tb and was proposed as a marker of disease severity. We propose that influenza tips the scale towards pathology and future experiments shall reveal the relevance of IL1 and MCP1 in Tb disease exacerbation after IAV coinfection. The identification of conditions and the underlying pathways that promote lung inflammation in Tb will help to identify potential host targets for adjunct therapy.

Priority Research Area **Infections**

Coinfection

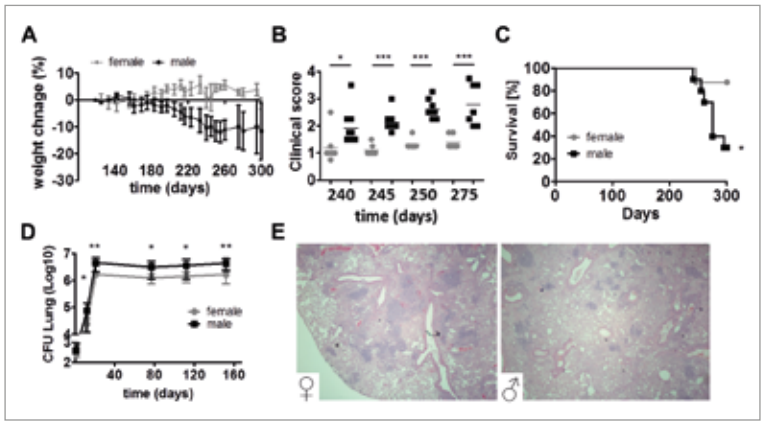

Figure 2. Increased susceptibility to *Mtb* infection in male C57BL/6 mice. Low-dose aerosol infection with *Mtb* results in accelerated disease progression and premature death in males.

Sex differences in Tb

Globally, Tb notification data show a male-to-female ratio of 2:1 and higher. While socioeconomic and cultural factors have been regarded as responsible for the bias biological factors may contribute significantly to the difference in susceptibility. Studies in our lab confirmed the increased susceptibility of males towards *Mtb* in the C57BL/6 mouse model. Accelerated disease progression in males resulted in their premature death compared to females (Fig. 2A-C). While the *Mtb* burden in male lungs was significantly elevated but controlled at a steady level (Fig. 2D), lung pathology developed differently in males and females (Fig. 2E) which might account for the accelerated disease progression in males. Mouse models that reflect the epidemiological findings present a unique opportunity for analyzing the molecular basis of sex-dependency in Tb disease outcome. In collaboration with Hanna Lotter from the BNITM we will identify inherent differences between the sexes in early immune reactions in the *Mtb* infected lung and determine the potential role of sex hormones. Targeting host processes to improve protective immunity, disease resolution, and treatment outcomes requires in-depth knowledge about the fundamental differences in disease pathogenesis and inflammatory pathways between males and females.

Internal and external collaboration

Internal collaborations:
Jochen Behrends, Christian Herzmann, Susanne Homolka, Sven Malm, Ulrich Schaible, Dominik Schwudke, Christian Utpatel, Adam Wutkowski

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 Hamburg)

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* (2012-2015 „Malaria - Tuberculosis Coinfection: Dissecting the immunological interactions during concurrent *Plasmodium* and *Mycobacterium* infection"; 2015-2018 „Impact of an influenza virus infection on the outcome of experimental tuberculosis")

PYRAZINAMIDE RESISTANCE

MDR CASES

WHO

M. CHIMAERA

SUPRANATIONAL REFERENCE LAB

Priority Research Area **Infections**

Diagnostic Mycobacteriology (NRC)

Head

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- Sönke Andres, PhD
- Dr. rer. nat. Doris Hillemann
- Julia Zallet
- Anne-Kathrin Witt
- Birgitt Voss
- Kerstin Klein
- Anne Landgraf
- Dennis Krüger
- Daniela Sievert
- Margit Kernbach
- Birte Schlüter
- Ilse Radzio
- Kirsten Ott
- Kristine Beuck
- Petra Vock
- Sylvia Höllger

Priority Research Area **Infections**

Diagnostic Mycobacteriology (NRC)

Mission

Unser Ziel ist die deutschlandweite und internationale Bereitstellung hoch qualitativer Mykobakteriendiagnostik. Dies wird durch die Entwicklung, Validierung und Verifizierung neuer diagnostischer Methoden sowie die Entwicklung und Implementierung von Qualitätsmanagement, Bereitstellung externer Qualitätskontrollen, Kapazitätsentwicklung und Schulungen gewährleistet.

We aim to provide high quality specialised diagnostic services for mycobacterial disease nationally and internationally through development, validation and verification of new diagnostics, implementation of quality control and management and capacity building and training.

Most important findings

Global *Mycobacterium chimaera* outbreak associated with cardiac surgery

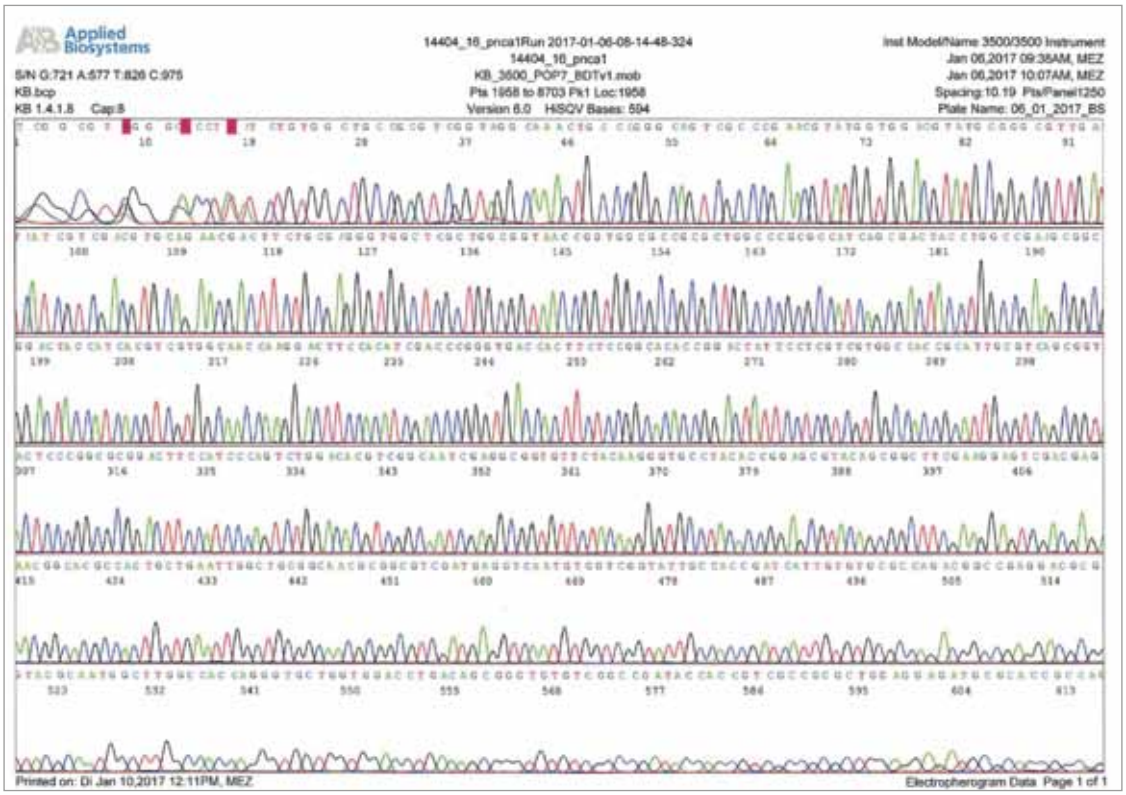
Recently a global outbreak of *M. chimaera* infection associated with open chest surgery has been reported. Cardiopulmonary bypass heater-cooler units from a certain manufacturer were suspected as source. A retrospective outbreak investigation was performed to identify patients with cardiopulmonary bypass-associated *M. chimaera* infection. Microbiological and aerobiological investigations of heater-coolers *in situ* and under controlled laboratory conditions and whole-genome sequencing of clinical and environmental isolates were performed. A total of 18 associated cases, most of those presenting as endocarditis were identified with a mortality of 50%. Investigations identified aerosol release through breaches in heater-cooler tanks. *M. chimaera* and other pathogens were recovered from water and air samples. Phylogenetic analysis found close clustering of strains from probable cases with strains from heater-cooler water. This investigations strengthened etiological evidence for the role of heater-coolers in transmission and raise the possibility of an ongoing, international point-source outbreak.

Selected publications

Chand M, Lamagni T, Kranzer K, Hedge J, Moore G, Parks S, Collins S, Del Ojo Elias C, Ahmed N, Brown T, Smith EG, Hoffman P, Kirwan P, Mason B, Smith-Palmer A, Veal P, Lalor MK, Bennett A, Walker J, Yeap A, Martin AI, Dolan G, Bhatt S, Skingsley A, Charlett A, Pearce D, Russell K, Kendall S, Klein AA, Robins S, Schelenz S, Newsholme W, Thomas S, Collyns T, Davies E, McMenamin J, Doherty L, Peto TE, Crook D, Zambon M, Phin N. Insidious Risk of Severe Mycobacterium chimaera Infection in Cardiac Surgery Patients. Clin Infect Dis. 2017 Feb 1;64(3):335-342.

Stagg HR, Harris RJ, Hatherell HA, Obach D, Zhao H, Tsuchiya N, Kranzer K, Nikolayevskyy V, Kim J, Lipman MC, Abubakar I. What are the most efficacious treatment regimens for isoniazid-resistant tuberculosis? A systematic review and network meta-analysis. Thorax. 2016 Oct;71(10):940-9

Zignol M, Dean AS, Alikhanova N, Andres S, Cabibbe AM, Cirillo DM, Dadu A, Dreyer A, Driesen M, Gilpin C, Hasan R, Hasan Z, Hoffner S, Husain A, Hussain A, Ismail N, Kamal M, Mansjö M, Mvusi L, Niemann S, Omar SV, Qadeer E, Rigouts L, Ruesch-Gerdes S, Schito M, Seyfaddinova M, Skrahina A, Tahseen S, Wells WA, Mukadi YD, Kimerling M, Floyd K, Weyer K, Raviglione MC. Population-based resistance of *Mycobacterium tuberculosis* isolates to pyrazinamide and fluoroquinolones: results from a multicountry surveillance project. Lancet Infect Dis. 2016 Oct;16(10):1185-92


Applied Biosystems
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Electropherogram Data Page 1 of 1

Priority Research Area **Infections**

Diagnostic Mycobacteriology (NRC)

High proportion of pyrazinamide resistance among MDR-TB isolates identified in drug resistance survey from five countries

Pyrazinamide is an essential antituberculosis drug in the new short course regimen (9 months) also called „Bangladesh regimen" for treatment of multi-drug resistant TB (MDR-TB). In a molecular epidemiology analysis population-based surveys from Azerbaijan, Bangladesh, Belarus, Pakistan, and South Africa were used to investigate resistance to pyrazinamide. Resistance to pyrazinamide was assessed by gene sequencing with the detection of resistance-conferring mutations in the *pncA* gene, and susceptibility testing to fluoroquinolones was conducted using the MGIT system. Pyrazinamide resistance was assessed in 4972 patients. In all settings, pyrazinamide resistance was significantly associated with rifampicin resistance. Despite this association pyrazinamide may still be effective in 19-63% of patients with rifampicin-resistant tuberculosis. This finding will inform policy makers when deciding when and where the short course regimen should be rolled-out.

Internal and external collaboration

Local:
Prof. Dr. S. Niemann, Prof. Dr. C. Lange, Dr. C. Herzmann, Dr. C. Hoelscher, Dr. D. Schwud ke, Dr. N. Reiling

National:
Dr. H. Hoffmann, Dr. S. Hofmann-Thiel, Dr. N. Heinrich, Dr. F. Maurer, Dr. B. Lange, Prof. Dr. T. Bauer, Prof. Dr. D. Wagner, Dr. F Brinkmann, DZK, RKI, INSTAND

International:
Prof. PhD L. Corbett (Blantyre, Malawi), PhD R. Ferrand (Harare, Zimabwe), Prof PhD A. Grant (London, United Kingdom), Prof PhD J. Glynn (London, United, Kingdom), H. Sohn (Baltimore, United States), Prof PhD LG. Bekker (Cape Town, South Africa), PhD K Lonnroth (Geneva, Switzerland), PhD C. Denkinger (Geneva Switzerland), PhD A. Khan (Karachi, Pakistan), PhD J. van Ingen (Nijmegen, Netherlands), WHO, ECDC

Grant support

BMBF, DZIF, Tuberkulose Verein Niedersachsen, Global Fund, Find, ECDC, WHO

X-RAY ANALYSIS OF 3D LIPID STRUCTURES

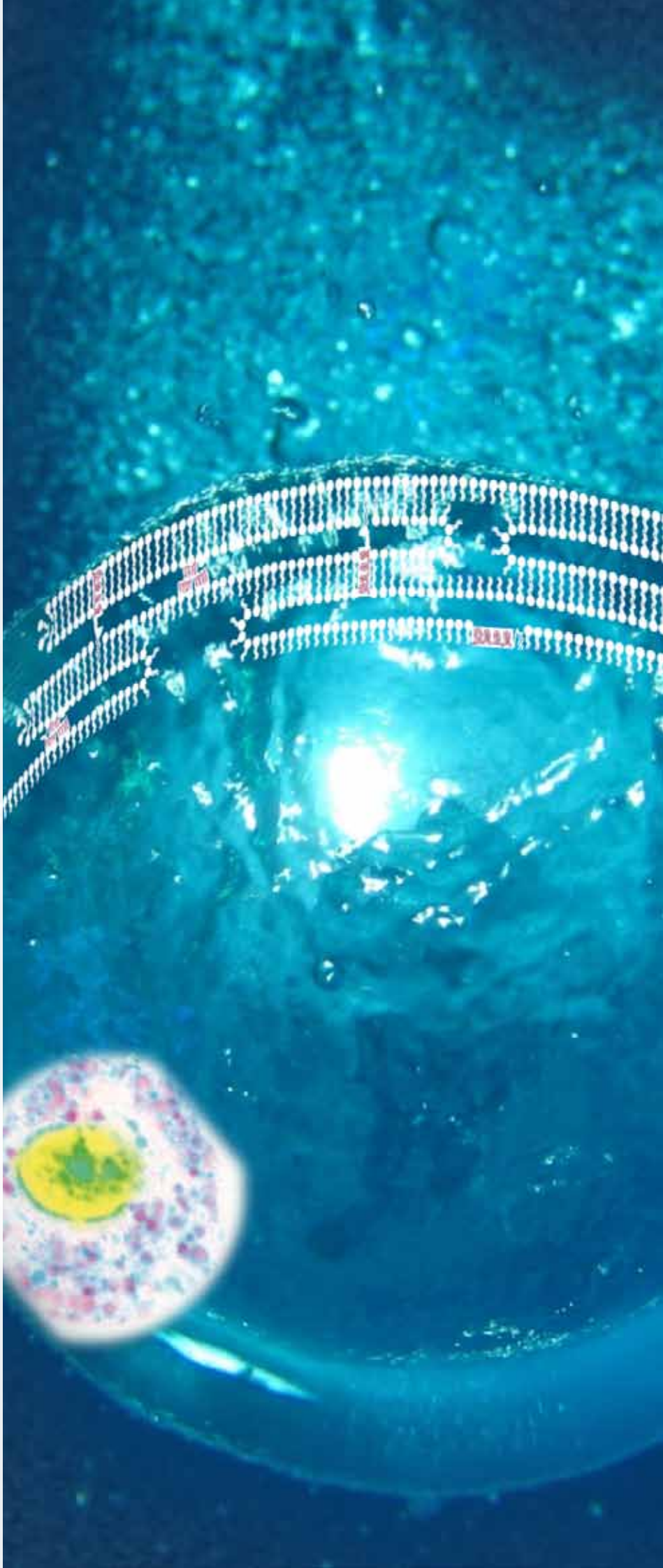
INFLAMMATION CONTROL

BACTERIAL LIPIDS

MEMBRANE BIOPHYSICS

MACROPHAGE BIOLOGY

LUNG SURFACTANT



Priority Research Area **Infections**

Immunobiophysics

Head

- PD Dr. rer. nat. Andra B. Schromm

Members

- Irina von Cube
- Dipl. Biol. Sarah Kupsch
- Anna Lehmann
- Dipl. Chem. Laura Paulowski
- Chee Keong Tan

Priority Research Area **Infections**

Immunobiophysics

Mission

Wir befassen uns mit der **Aufklärung molekularer Prinzipien der Aktivierung der angeborenen Immunreaktion durch bakterielle Lipide**. In unseren Forschungsprojekten untersuchen wir die Mechanismen der Initiierung und Regulation dieser Entzündungsreaktionen durch körpereigene anti-inflammatorische Peptide, Proteine und Lipide mit dem Ziel Strategien zu deren Nutzung in anti-entzündlichen Therapien zu entwickeln.

We investigate the **molecular principles of the innate immune response to lipids**. In our projects we analyze the mechanisms of initiation and regulation of inflammation with a special focus on the investigation of host defense peptides, proteins and lipids involved in anti-inflammatory immune regulation. The aim of these studies it to evaluate endogenous immune-response-modifiers as candidates for host-directed therapy.

Most important findings

Surfactant Lipids in anti-inflammatory Immune Therapy

Immune regulation in the lung is specifically adapted to maintain immune homeostasis to cope with the constant challenge by inhaled microbes. It is recently recognized that specific lipids of the pulmonary surfactant contribute to this anti-inflammatory status. In a collaborative project with the **Pediatric Clinic, Kiel**, the **Institute of Immunology, Kiel**, and the **Division of Experimental Pneumology, RCB**, we are investigating the application of anionic phospholipids in clinical surfactant therapy to i) modulate the immune response and ii) to reduce surfactant degradation. We could demonstrate that PG and PI lipid species are potent inhibitors of inflammasome and NF-kB dependent activation of monocyte-derived and alveolar macrophages (Fig. 1). In line with this, addition of these phospholipids to surfactant led to amendment of lung function in vivo in a piglet model of neonatal ARDS. Together with the **Division of Bioanalytical Chemistry**, we performed in depth lipidome analysis of 84 surfactant samples from piglets of five treatment groups. More than 300 lipid species could be identified. Our data provide evidence for a stabilization of surfactant levels and did not indicate selective degradation of single lipid species. This study gives insights into the mechanisms of pulmonary immune modulation and shows an advantage of phospholipid fortification of surfactant preparations in anti-inflammatory therapy of nARDS.

Highlights

Phosphatidylglycerol 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.

We have identified that the LPS-binding protein can serve as endogenous transporter to deliver microbial LPS molecules to intracellular caspases and activates caspase-dependent pyroptosis. Our findings add a new function to this central immune regulator of immune responses to microbial membrane lipids.

Selected publications

Kopp F, Kupsch S, and Schromm AB. Lipopolysaccharide-binding protein is bound and internalized by host cells and colocalizes with LPS in the cytoplasm: Implications for a role of LBP in intracellular LPS-signalling. BBA – Mol Cell Research 2016, 1863(4):660-72.

Jäger J, Keese SP, Roessle M, Steinert M, and Schromm AB. Fusion of Legionella pneumophila outer membrane vesicles with eukaryotic membrane systems is a mechanism to deliver pathogen factors to host cell membranes. Cellular Microbiology 2015, May;17(5):607-20.

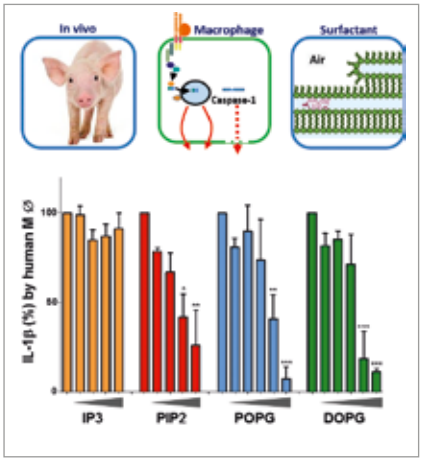


Figure 1. Evaluation of the anti-inflammatory potential of anionic phospholipids and lipid-derivatives *in vivo* (piglet model of nARDS) and *in vitro* (alveolar and monocyte-derived macrophages from piglets and human). Attenuation of inflammasome-dependent IL-1β production in LPS-stimulated human macrophages by anionic phospholipids and lipid derivatives.

Role of Lipid-transport in Inflammasome Activation

The LPS-binding protein is a central regulator of innate immune responses to bacterial infections. Carrier of a frequent SNP recently identified in humans and LBP-deficient mice have a higher risk of mortality in pneumonia. The function of LBP in pulmonary disease however is not well understood. Since LBP is the transporter for microbial lipids in serum, we set up to explore the function of LBP in the transport of LPS to intracellular receptors. We could demonstrate binding of LBP to human monocyte and macrophage cell membranes. The transport function of LBP was analyzed in eukaryotic model membranes and in host cells, providing evidence for membrane dependent transport of LPS by LBP (Fig. 2). We could show spatio-temporal correlation of intracellular LBP, LPS, and activated caspases. A natural pathway to trigger cytoplasmic caspase activation by extracellular LPS, as shown in in vivo mouse models, has so far not been identified. Our data provide evidence that LBP is involved in cytoplasmic inflammasome activation by microbial lipids, adding a new function to the repertoire of immune modulation by this host defense protein that we are currently investigating in much more detail to elucidate the molecular mechanisms.

Priority Research Area **Infections**

Immunobiophysics

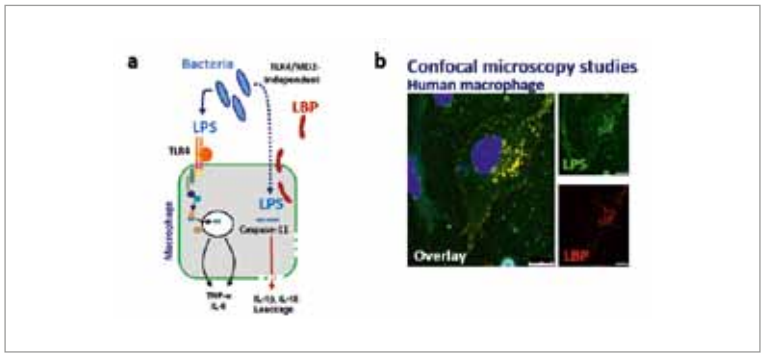


Figure 2. (a) LBP is an endogenous transporter for bacterial lipids enabling sensitive activation of the TLR4/MD-2 cell surface receptor and transport of LPS to intracellular caspase receptor systems leading to pyroptosis. **(b)** Confocal microscopy studies on human macrophages show co-transport of LPS (green) and LBP (red) into intracellular compartments in a human macrophage. Scale bar = 10 µm.

Reprogramming of Macrophage Biology by Host Defense Peptides (HDP)

Host defense peptides (HDP) represent a pool of endogenous antimicrobial effector molecules providing immune defense against infections. Recent evidence have highlighted that HDPs can also act as potent host response modifiers, a function that could be highly useful in developing HDP into therapeutics for disease triggered by hyper-inflammatory immune responses such as in COPD, pneumonia or sepsis. To gain insights into the basis of anti-inflammatory immune modulation by HDPs we have systematically analyzed a set of peptides with respect to their capacity to interfere with different steps of macrophage activation by bacterial LPS. We identified individual peptides with strong host cell-directed anti-inflammatory function. In a collaborative approach with the **Division of Biophysics at the RCB, colleagues for the Institutes of physics in Lübeck, Vienna, and Graz**, high resolution structural analysis of peptide - membrane interaction was performed by **synchrotron radiation experiments at PETRA III@DESY**, Hamburg and at the Swiss light source SLS. These investigations showed specific differences and revealed a new mode of action of HDP that strongly contributes to the anti-inflammatory effect of specific peptides. These findings will be important for further development of this class of immune response modifiers.

Internal and external collaboration

Internal collaborations:
Flow Cytometry Unit, RCB; Heinz Fehrenbach, Division of Experimental Allergology, RCB; Nicolas Gisch, Division of Bioanalytical Chemistry, RCB; Torsten Goldmann, Division of Clinical and Experimental Pathology, RCB; Thomas Gutsmann, Division of Biophysics, RCB; Christian Herzmann, Clinical Trial Center, RCB; Uwe Mamat, Division of Structural Biochemistry, RCB; Dominik Schwudke, Bioanalytical Chemistry, RCB.

External collaborations:
Koichi Fukase, Department of Chemistry, University of Osaka; Christian Hübner, Institute of Physics, University of Lübeck; Martin Krause, Clinic of Pediatrics, University Clinic Kiel; Jörg Labahn, Center for Applied Systems Biology, c/o DESY, Hamburg; Francesco Peri, Department of Biotechnology and Biosciences, University of Milano; Manfred Roessle, University of Applied Sciences, Lübeck and EMBL c/o DESY, Hamburg; Gerhard Schütz, Institute of Applied Physics, Technical University Wien; Stefan Schütze, Institute of Immunology, University of Kiel; Michael Steinert, Institute of Microbiology, Technical University Braunschweig.

Grant support

DFG/EXC 306 O TP4 Exzellenzcluster „Inflammation at Interfaces “

Project „Surfactant therapy in neonatal acute respiratory distress syndrome”

DFG/EXC 306 RA3 Exzellenzcluster „Inflammation at Interfaces“

Project „Anti-inflammatory regulation of immune cells by membrane active host defense peptides”

Beamtime grants SAXS-456 and SAXS-583 for measurements at the German Electron Synchrotron DESY (EMBL beamline P12@ PETRA III, c/o DESY)

MICE

GRANULA NECROSIS

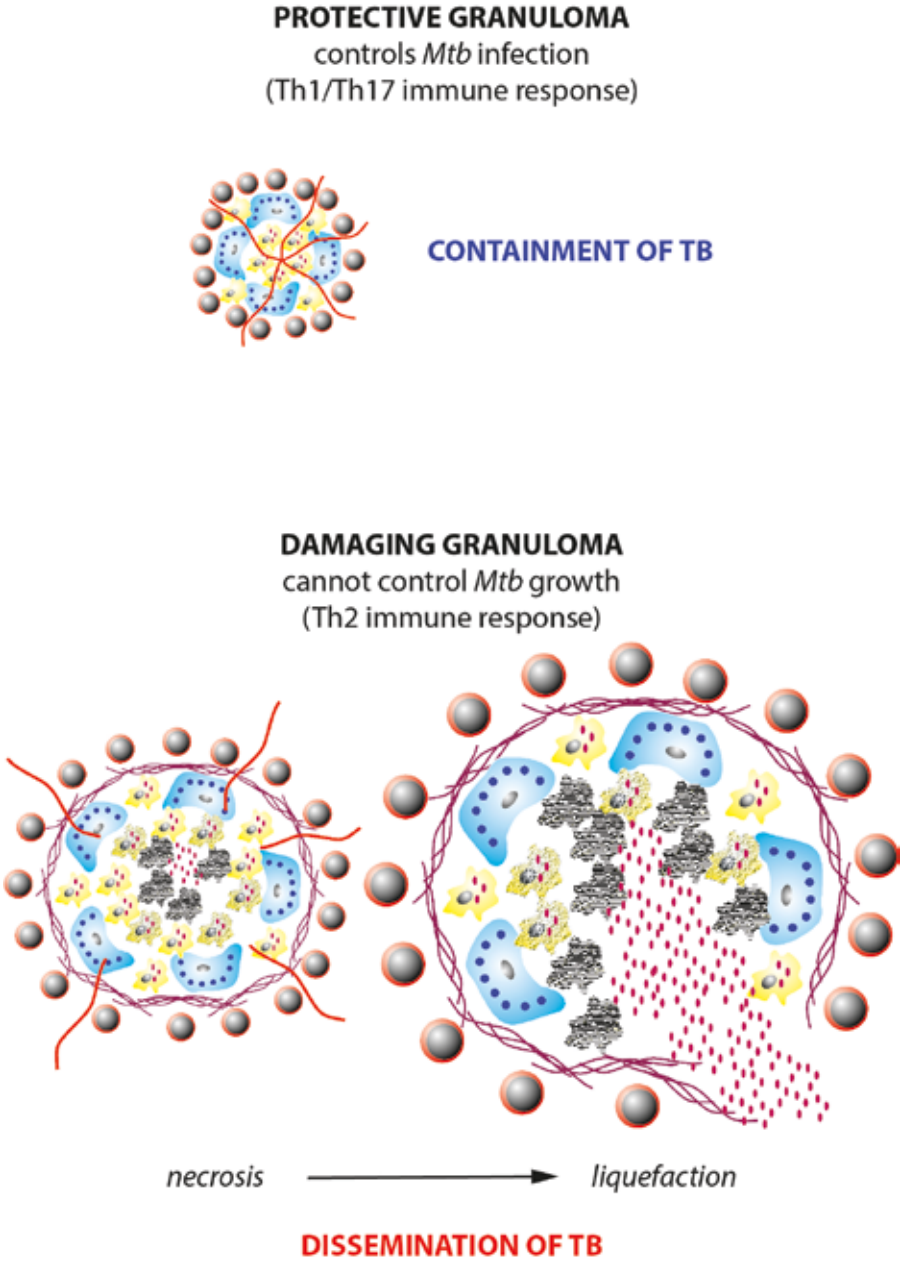
TUBERCULOSIS

INTERLEUKIN-13

INTERLEUKIN-17

INTERLEUKIN-23

MULTIFUNCTIONAL T CELLS



Priority Research Area **Infections**

Infection Immunology

Head

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Members

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- Fengyuan Deng
- Dr. Hanna Erdmann
- Alexandra Hölscher
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- Ann-Kathrin Stoltenberg
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- Filipa Varela
- Johanna Volz
- Dr. Kerstin Walter
- Melanie Wannick

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.

Most important findings

(i) Currently, the most effective vaccine against human TB is the live vaccine *Mycobacterium bovis* (BCG). However, its protective effect is limited and the development of new vaccination strategies are of great importance. The novel glycolipid adjuvant trehalose-dibehenate (TDB) potently promotes protective immune responses against *Mtb* infection in mice after immunization with the subunit *Mtb* vaccine H1. However, in experimental models vaccination with H1-TDB does not correlate with increased numbers of interferon-gamma (IFNγ)-producing T helper (Th)1 cells. The protective immune effect is rather associated with an expansion of interleukin (IL)-17A-producing Th17 cells and an augmented frequency of tumor necrosis factor (TNF)+ IL-2+ and INFγ+ TNF+ IL-2+ multifunctional T cells displaying high proliferative capacity and superior protective functions. To investigate the impact of the IL-23-IL-17A axis on the recruitment of these protective multifunctional CD4 T cells and subsequent

Highlights

A mutation of the IL-13 / IL-4 Rα axis is associated with the degree of pathology in human TB patients.

The differentiation of multinuclear macrophages during TB occurs by modified cell divisions and mitotic defects and does not involve cell-to-cell fusion.

Cathepsin G is not directly involved in the immune defence against *Mtb*.

Whereas IL-22 is dispensable for protective immune responses against *Trypanosoma cruzi* and *Mtb* it is required for the control of viral infection.

Selected publications

Walter K, Steinwede K, Aly S, Reinheckel T, Bohling J, Maus U2, Ehlers S. Cathepsin G in Experimental Tuberculosis: Relevance for Antibacterial Protection and Potential for Immunotherapy. J IMMUNOL 2015; 195: 3325.

Hernández PP, Mahlakoiv T, Yang I, Schwierzeck V, Nguyen N, Guendel F, Gronke K, Ryffel B, Hölscher C, Dumoutier L, Renauld J-C, Suerbaum S, Staeheli P, Diefenbach A. Interferon-λ and interleukin-22 cooperate for the induction of interferon-stimulated genes and control of rotavirus infection. NAT IMMUNOL 2015; 16: 698.

Böhme J, Roßnagel C, Jacobs T, Behrends J, Hölscher C, Erdmann H. Epstein-Barr virus-induced gene 3 suppresses T helper type 1, type 17 and type 2 immune responses after *Trypanosoma cruzi* infection and inhibits parasite replication by interfering with alternative macrophage activation. IMMUNOL 2016; 147: 338.

Hölscher C, Heitmann L, Owusu-Dabo E, Horstmann RD, Meyer CG, Ehlers S, Thye T. A Mutation in IL4RA Is Associated with the Degree of Pathology in Human TB Patients. MEDIATORS INFLAMM 2016; 2016: 4245028.

Erdmann H, Behrends J, Hölscher C. During acute experimental infection with the reticulotropic *Trypanosoma cruzi* strain Tulahuen IL-22 is induced IL-23-dependently but is dispensable for protection. SCI REP 2016; 6:32927.

Herrtwich L, Nanda I, Evangelou K, Nikolova T, Horn V, Erny D, Stefanowski J, Rogell L, Klein C, Gharun K, Follo M, Kremer B, Seidl M, Münke N, Sanger J, Fliegauf M, Aschman T, Pfeifer D, Sarrazin S, Sieweke M, Wagner D, Dierks C, Haaf T, Ness T, Zaiss MM, Vol R, Deshmukh S, Prinz M, Goldmann T, Hölscher C, Hauser A, Lopez-Contreras AJ, Grün D, Gorgoulis V, Diefenbach A, Henneke P, Triantafyllopoulou A. DNA damage signaling instructs polyploid macrophage fate in granulomas. CELL 2016; 167: 1264.

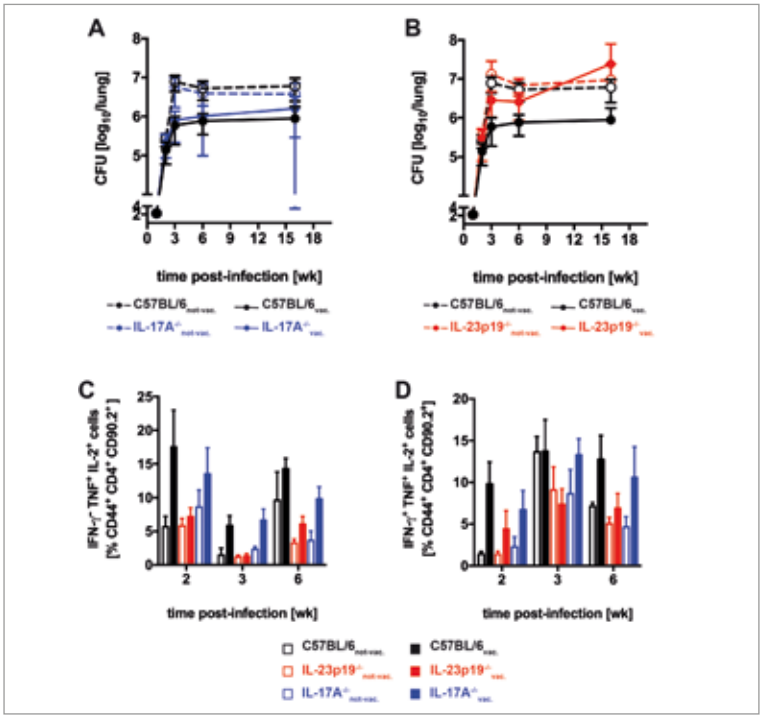


Figure 1. Protection after subunit *Mtb* vaccination depend on IL-23 but not on IL-17A. C57BL/6, IL-17A-/-, and IL-23p19-/- mice were immunized with H1-DDA/TDB or were injected with PBS 8, 6, and 4 weeks before aerosol infection with 100 CFU *Mtb* H37Rv. **(A, B)** During the course of infection, the bacterial load in the lungs were determined. **(C, D)** At the indicated time points, single cells suspensions of perfused lungs were restimulated with anti-CD3/CD28 and the expression of IFNγ, TNF and IL-2 in CD44+ CD4+ CD90.2+ cells was analysed by flowcytometry. Intracellular expression of **(C)** IFNγ- TNF+ IL-2+ and **(D)** IFNγ+ TNF+ IL-2+ multifunctional T cells.

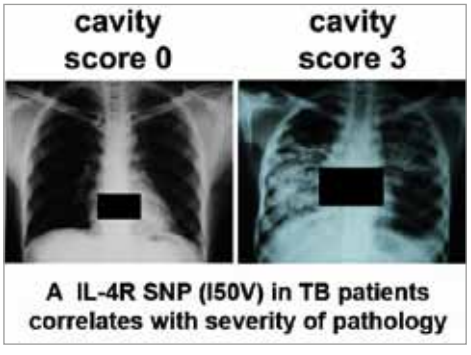


Figure 2. Posterior-anterior chest X-ray of TB cases. Exemplary cavity score 0 (no cavities), cavity score 3 (extended, large cavities) scored blinded by an experienced radiologist, cross-checked by an experienced physician.

Infection Immunology

protection after vaccination we immunized wildtype, IL-23p19-deficient (-/-) and IL-17A-/- mice with H1-TDB, and analysed the outcome of *Mtb* infection. Whereas the inability of H1-TDB-immunized IL-23p19-/- mice to reduce bacterial loads (Figure 1A) was associated with an impaired expansion of multifunctional CD4 T cells (Figure 1C, D), increased frequencies of TNF+IL-2+ and IFNγ+TNF+IL-2+ multifunctional T cells in vaccinated IL-17A-/- and wildtype animals (Figure 1C, D) were accompanied by an improved control of mycobacterial growth (Figure 1B).

These data reveal, that IL-23 but not IL-17A appears to be required for the recruitment of protective multifunctional CD4 T cells after vaccination with H1-TDB. Thus, IL-23 promotes the multifunctional T cell-associated protective effect after vaccination against *Mtb* independently of IL-17A.

(ii). We have recently shown IL-13 overexpression in mice (IL-13tg mice) to cause recrudescent *Mtb* replicaton and centrally necrotizing granulomas in experimental TB strongly resembling the pathology in human TB thus implicating IL-4/IL-13-IL-4 receptor-alpha (Rα)-mediated mechanisms to be involved in reactivation and pathology. To validate our results in human TB patients, we here determined the association of distinct variants of the IL4, IL13, IL4RA, IL13RA1 and IL13RA2 genes with cavity formation in a large Ghanaian cohort of HIV-negative individuals with newly diagnosed pulmonary TB. In fact, the structural variant of the IL4RA I50V, previously shown to result in enhanced signal transduction, was significantly associated with greater cavity size (Figure 2), and a variant of IL13Ra2 was associated with disease in females.

These data support our conclusion that the IL-4Rα is directly involved in mediating the development of central granuloma necrosis in human TB. Further analysis in human TB and in the IL-13tg mouse model will unravel IL-4Rα-mediated functions involved in the pathogenesis of post-primary TB. More important, targeting the IL-4Rα or downstream mechanisms 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; Maus U, Hannover Medical School; 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 02.702 „MycoMouse" and TTU 02.802 „Preclinical Test Station"

BMBF; DZIF TTU-TB 02.705 „Myco Drug and Trials" and TTU 02.806 „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 MD5 MD Stipend "A molecular bacterial load (MBL) assay, for measuring viable *Mycobacterium tuberculosis* should be established in mouse models of tuberculosis as an alternative to solid agar and liquid culture systems."

DFG; IRTG1911: „Immunoregulation of Inflammation in Allergy and Infection": TPB6 „Regulatory T cell modulated vaccination against tuberculosis"

DFG; GRK 1727: „Modulation von Autoimmunität": Projekt A4 „Modulation of experimental autoimmune encephalitis by the regulatory T cell-derived cytokine interleukin-35"

Universität zu Lübeck; FSP „Modulation von Infektion und Allergie": Projekt B2 „Die Rolle von Komplement bei der Infektion mit *Mycobacterium tuberculosis*" und Projekt B3 „Untersuchung der optimalen Zytokin-vermittelten T und B Zell-Antworten nach der Impfung gegen *Mycobacterium tuberculosis*"

MYCOBACTERIUM TUBERCULOSIS

PATHOGEN VARIABILITY

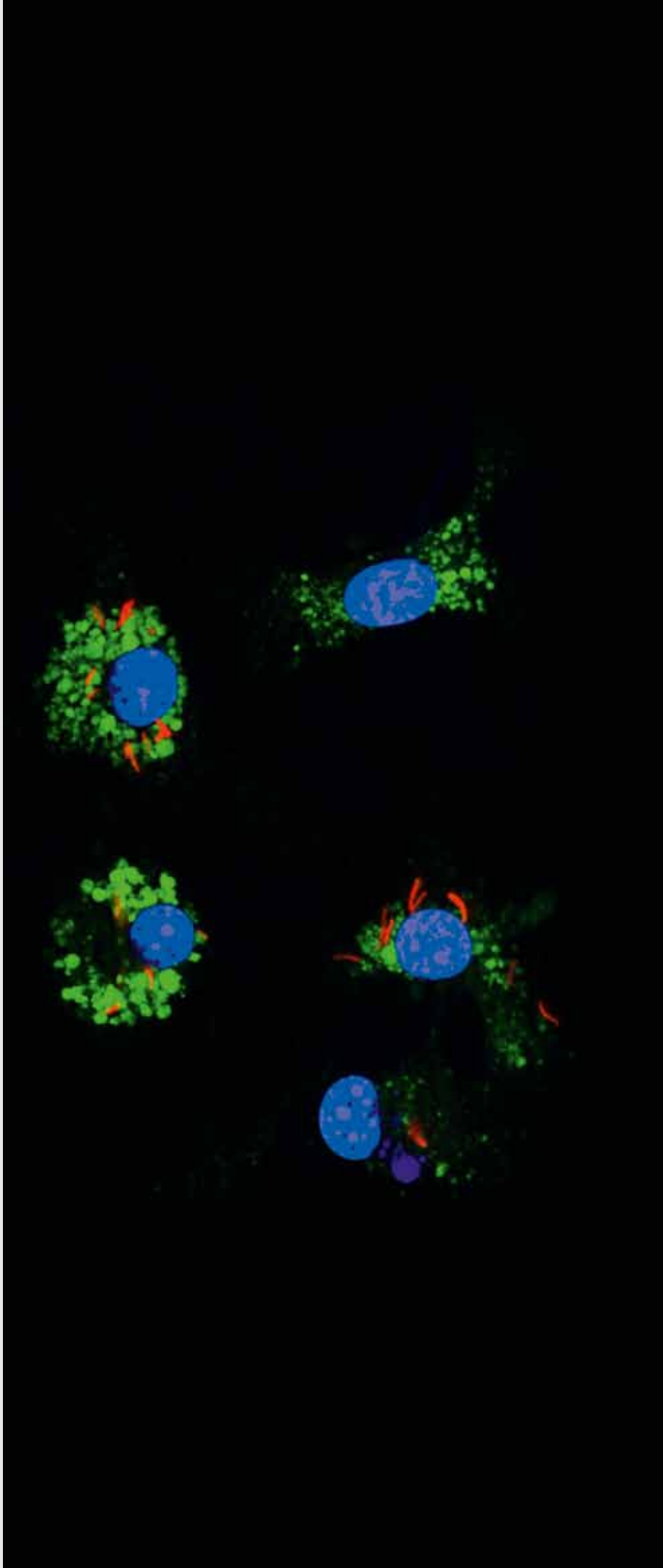
PHAGOSOME ISOLATION

MACROPHAGE

WNT SIGNALING

IN VITRO DRUG TESTING

LIPID METABOLISM



Priority Research Area **Infections**

Microbial Interface Biology

Head

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Members

- Dr. Katharina Kolbe
- Dr. Julius Brandenburg
- Victoria Sohst
- Miriam Hiller
- Hauke Baumann
- Julia Hansen
- Sebastian Mark
- Svenja Goldenbaum
- Lisa Niwinski
- Carolin Golin

Priority Research Area **Infections**

Microbial Interface Biology

Mission

Im Fokus der FG Mikrobielle Grenzflächenbiologie steht die detaillierte Charakterisierung der Interaktion von pathogenen Mykobakterien mit ihren Zielzellen den Makrophagen. Neben grundlegenden Untersuchungen zur Pathogenvariabilität von Stämmen des *M. tuberculosis* Komplex und zu antimikrobiellen Effektormechanismen der Wirtszelle, werden die Bedeutung wichtiger Parameter wie der Sauerstoffpartialdruck, die Änderungen in der Verfügbarkeit verschiedener Substrate, sowie die Bedeutung neuer immunmodulatorischen Faktoren für den Infektionsverlauf analysiert.

The Division of Microbial Interface Biology focuses on the detailed molecular characterization of the interaction between pathogenic mycobacteria and their target cells, the macrophages. In addition to basic mechanistic studies addressing *M. tuberculosis* (*Mtb*) complex pathogen variability and antimicrobial effector mechanisms of the host, we concentrate on the impact of local key parameters including oxygen pressure (hypoxia), microenviromental- and intracellular changes in metabolic substrate availability as well as local immunomodulatory factors during the infection process.

Most important findings

Hypoxia induced metabolic changes during *Mtb* infection

Direct measurement of lesional oxygen tension in rabbits, and indirect measurements in non-human primates and humans using hypoxia-sensitive probes demonstrate that many TB lesions in vivo are hypoxic (Figure 1). Oxygen tension affects both pathogen and host cell function. Infection of macrophages with *Mtb* leads to a wide array of cellular responses, most of which have been studied under normoxia. Virulent mycobacteria have developed mechanisms operative in infected cells, which allow bacillary replication and persistence by fine-tuning pro- and anti-inflammatory activity. Hypoxic conditions lead to a significant increase of antimycobacterial effector functions. However, hypoxia-mediated control of *Mtb* replication is at the same time associated with a significant metabolic reprogramming of its host cell characterized by a shift from oxidative toward glycolytic metabolism. This metabolic shift promotes foamy macrophage formation, which is characterized by the presence of triacylglyceride-rich cytoplasmic structures termed lipid droplets. The appearance of lipid droplet-rich foam cells is associated with tissue pathology and favors *Mtb* survival and persistence by providing metabolic substrates to the pathogen. Access to host lipids, as well as exposure to hypoxic conditions, promotes the adaptation of the pathogen towards an intracellular lifestyle of non-replicating persistence (NRP) in which *Mtb* is largely resistant to known bactericidal mechanisms of macrophages and many antimicrobials. Accordingly, novel therapeutic approaches targeting these processes are needed to improve tuberculosis treatment.

Highlights

Identification of human lysosomal acid lipase inhibitor Lalistat and N-phenyl 1,4-dihydro-pyridines as novel anti-mycobacterial agents

Azido pentoses as a new tool to metabolically label *Mycobacterium tuberculosis* clinical isolates

VEIAP Master Award 2015 to M. Sc. Miriam Hiller for her thesis entitled *„Induction and Presence of Factors of Type I Interferon Signaling in M. tuberculosis-infected Macrophages“*

Award for Best Dissertation 2015 to Dr. Julius Brandenburg for his dissertation entitled *„Wnt6: A novel mediator in M. tuberculosis infection linking inflammation and lipid metabolism.“*

Selected publications

Lehmann J, Vomacka J, Esser K, Nodwell M, Kolbe K, Rämer P, Protzer U, Reiling N* & Sieber SA*. Human lysosomal acid lipase inhibitor lalistat impairs *Mycobacterium tuberculosis* growth by targeting bacterial hydrolases. MedChemComm. 2016, 7, 1797-1801, 2016 (* corresponding authors)

Lentz F, Hemmer M, Reiling N, Hilgeroth A. Discovery of novel N-phenyl 1,4-dihydropyridines with a dual mode of antimycobacterial activity. Bioorg Med Chem Lett. 2016; 26(24):5896-5898.

Prosser G*, Brandenburg J*, Reiling N, Barry CE 3rd, Wilkinson RJ, Wilkinson KA. The bacillary and macrophage response to hypoxia in tuberculosis and the consequences for T cell antigen recognition. Microbes Infect. 2016 pii: S1286-4579(16)30146-0. (*equal contribution)

Brandenburg, J & Reiling, N, The Wnt blows: On the functional role of Wnt signaling in *Mycobacterium tuberculosis* infection and beyond. Frontiers in Immunology 2016, Bd 7, S. 635.

Kolbe K, Möckl L, Sohst S, Brandenburg J, Engel R, Malm S, Bräuchle C, Holst O, Lindhorst TK*, Reiling N*, Azido pentoses: A New Tool to Efficiently Label *Mycobacterium tuberculosis* Clinical Isolates. ChemBio Chem (in press). (*, equal contribution)

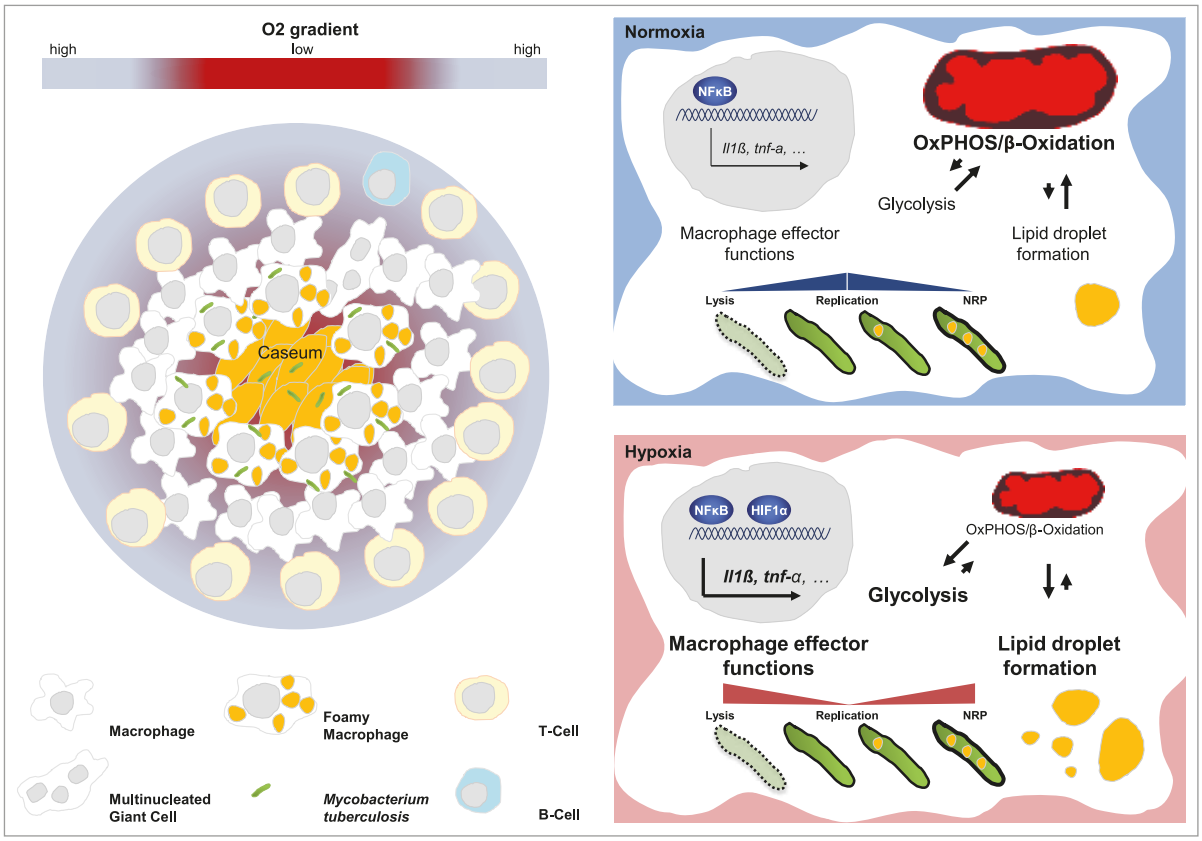

Figure 1. The cellular response to hypoxia during *M. tuberculosis* infection (Prosser G*, Brandenburg J* et al., *Microbes and Infection* 2016) (* equal contribution)

Human lysosomal acid lipase inhibitor Lalistat impairs *Mtb* growth by targeting bacterial hydrolases

Lipases are essential metabolic enzymes for many prokaryotic and eukaryotic organisms. Lalistat is a thiadiazole carbamate developed as a specific inhibitor of the human lysosomal acid lipase (LAL). This enzyme is located in cellular late endosomes hydrolysing cholesterol esters and triglycerides from incoming lipoproteins. Given the structural properties of Lalistat we addressed whether Lalistat impairs *Mtb* growth characteristics. We showed that Lalistat inhibits *Mtb* growth in bacterial culture as well as in infected macrophages. Target identification by quantitative proteomics revealed a cluster of 20 hydrolytic proteins (Figure 2). The specificity of Lalistat for a suite of mycobacterial lipases is intriguing. Given the importance of these enzymes for *Mtb* during infection they may represent promising drug targets. Future studies need to further dissect and characterize the exact function and mechanism of these enzymes in order to design customized inhibitors suited to interfere with essential metabolic processes specifically in the bacteria. The development of more specific drugs targeting mycobacterial lipid metabolism and their use in combination with known anti-mycobacterial agents may offer an urgently needed opportunity to improve TB therapy.

Priority Research Area **Infections**

Microbial Interface Biology

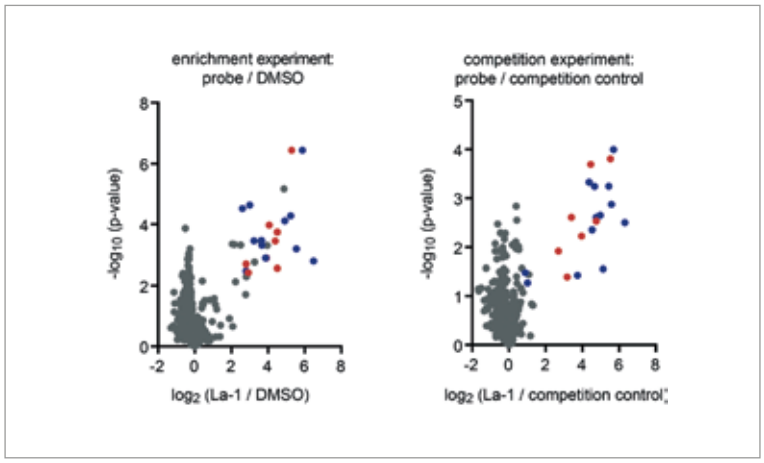

Figure 2. *In vivo* target identification of Lalistat in *M. tuberculosis* H37Rv via chemical proteomics. Enrichment and competition target identification (ABPP) volcano plot representations and corresponding list of common hits (blue and red). Proteins of the Lip family are marked in red, all others in blue. (Lehmann J, et al. *Med.Chem.Comm* 2016).

Azido pentoses: A new tool to efficiently label *Mycobacterium tuberculosis* clinical isolates

Mtb has a complex cell envelope which forms an efficient barrier to antibiotic stress, contributing to the obstacles of anti-tuberculosis therapy. However, the uniqueness of the *Mtb* cell wall can be considered an advantage and be utilized to selectively label *Mtb* bacteria. We have identified three azido pentoses, 3 azido arabinose (3AraAz), 3-azido ribose (3RiboAz) and 5-azido arabinofuranose (5AraAz), as new compounds for metabolic labeling of *Mtb*. 5AraAz demonstrated the highest level of *Mtb* labeling where it is efficiently incorporated into the *Mtb* cell wall. All new azido pentoses can be utilized to easily label a variety of *Mtb* clinical isolates (Figure 3) without influencing *Mtb*-dependent phagosomal maturation arrest in infection studies with human macrophages. Thus, this metabolic labeling method offers the opportunity to attach desired molecules to the surface of *Mtb* bacteria in order to facilitate investigation of the varying virulence characteristics of different *Mtb* clinical isolates.

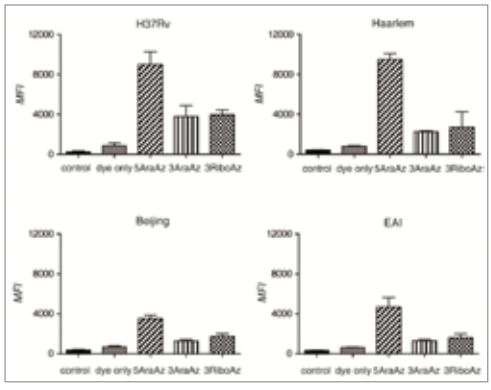

Figure 3. Labeling of *Mtb* clinical isolates with azido pentoses. H37Rv and three clinical isolates of the Haarlem (2336/02), Beijing (1934/03) and East African Indian (EAI, 1797/03) lineages were compared for their relative labeling efficiency by 5AraAz, 3AraAz, and 3RiboAz and analyzed by flow cytometry. MFI: Median fluorescence intensity (Kolbe K et. al., *Chem.Bio.Chem. (in press).*

Internal and external collaboration

Internal

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Grant support

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MOLECULAR EPIDEMIOLOGY

WHOLE GENOME SEQUENCING

HOST-PATHOGEN INTERACTION

EVOLUTION

VIRULENCE

PERSONALIZED MEDICINE

TUBERCULOSIS

PATHOBIOLOGY

POPULATION STRUCTURE

DRUG RESISTANCE

RESISTANCE MECHANISMS

Priority Research Area **Infections**

Molecular and Experimental Mycobacteriology

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Ecology of tuberculosis
Fusarium resistant wheat
Human fertility and reproduction

Priority Research Area **Infections**

Molecular and Experimental Mycobacteriology

Mission

Verbesserte Präventions-, Behandlungs-, und Kontrollstrategien sind für eine erfolgreichere Bekämpfung der Tuberkulose (TB) dringend erforderlich; vor allem, um die Ausbreitung von multidrug resistenten (MDR) *M. tuberculosis* Komplex (*Mtb*k) Stämmen einzudämmen. Hierfür ist ein besseres Verständnis der pathogenetischen Erregereigenschaften, welche Virulenz, Übertragbarkeit, Resistenzentwicklung, Wirt-Pathogen Interaktion und Epidemiologie bestimmen, von entscheidender Bedeutung. Um diese faszinierende Fragestellung zu addressieren, haben wir eine international kompetitive translationale Forschungsagenda etabliert, die (1) genomweite Analyseverfahren für molekulare Resistenzvorhersage und Transmissionsanalyse, (2) prospektive genombasierte Surveillance für ein besseres Verständnis der aktuellen Epidemiologie der TB in Niedrig- und Hochinzidenzländern mit Fokus auf MDR-TB Übertragung, (3) Analyse von Resistenzmarkern und kompensatorische (Fitness erhöhende) Veränderungen im Genom, (4) Studien zur globalen Populationsstruktur, Genomdiversität, Virulenz und Pathobiologie klinischen *Mtb*k Isolate, (5) Ansätze für individualisierte Therapie, und (6) Evolutionäre Medizin umfasst.
Die Verbindung von der faszinierenden genetischen Diversität der Erreger mit Resistenz, Wirt-Pathogen Interaktion, Virulenz, und globaler Verbreitung stellt ein hochrelevantes Forschungsthema dar.

Improved prevention, treatment and control strategies are urgently needed to fight tuberculosis (TB) more successfully esp. for stopping the spread of multidrug resistant (MDR) *M. tuberculosis* complex (*Mtbc*) strains. To achieve this, a better understanding of inherent pathogen traits determining virulence, transmissibility, drug resistance development and the outcome of host-pathogen interaction as well as of its epidemiology is needed. To target this challenging goal, we have developed a comprehensive translational research agenda that facilitates cutting edge research on (1) whole genome sequencing (WGS) based approaches for molecular resistance diagnostics and transmission analysis (2) prospective genome-based surveillance for a better understanding of TB epidemiology in low and high incidence settings esp. with regard to the epidemic of multidrug resistant (MDR) strains (3) acquisition of resistance and compensatory mechanisms, (4) global population structure/ genomic diversity, virulence and pathobiology of clinical *MTBC* isolates, (5) individualized therapy and (6) evolutionary medicine.
The link between the intriguing genomic variability of the pathogen and its association with antibiotic resistance, host-pathogen interaction, virulence and global distribution represents a highly relevant research topic.

Selected publications

Walker TM, Kohl TA, et al., Niemann S (equal contribution), Peto TE; Modernizing Medical Microbiology (MMM) Informatics Group. Whole-genome sequencing for prediction of *Mycobacterium tuberculosis* drug susceptibility and resistance: a retrospective cohort study. Lancet Infect 2015 Dis 15 (10):1193-202.

Merker M, et al., Supply P, Niemann S (equal contribution), Wirth T. Evolutionary history and global spread of the *Mycobacterium tuberculosis* Beijing lineage. Nat Genet 2015 47(3):242-9.

Sanchez-Padilla E, Merker M, et al., Niemann S. Detection of drug-resistant tuberculosis by Xpert *MTB*/RIF in Swaziland. N Engl J Med 2015 19;372(12):1181-2.

Stucki D, Brites D, Jeljeli L (equal contribution), et al. Niemann S, Gagneux S. *Mycobacterium tuberculosis* lineage 4 comprises globally distributed and geographically restricted sublineages. Nat Genet. 2016 Dec;48(12):1535-1543.

Hoffmann H, Kohl TA, Hofmann-Thiel S, et al. Niemann S. Delamanid and Bedaquiline Resistance in *Mycobacterium tuberculosis* Ancestral Beijing Genotype Causing Extensively Drug-Resistant Tuberculosis in a Tibetan Refugee. Am J Respir Crit Care Med 2016 1;193(3):337-40.

Bjorn-Mortensen K, Soborg B, Koch A, et al. Niemann S, Kohl TA. Tracing *Mycobacterium tuberculosis* transmission by whole genome sequencing in a high incidence setting: a retrospective population-based study in East Greenland. Sci Rep 2016 12;6:33180.

Awards

Prof. Dr. Stefan Niemann. Main Prize of the German Society for Hygiene and Microbiology.

Prof. Dr. Stefan Niemann. Schleswig-Holstein-Excellence-Chair.

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

Dr. Matthias Merker. DZIF PhD prize 2016.

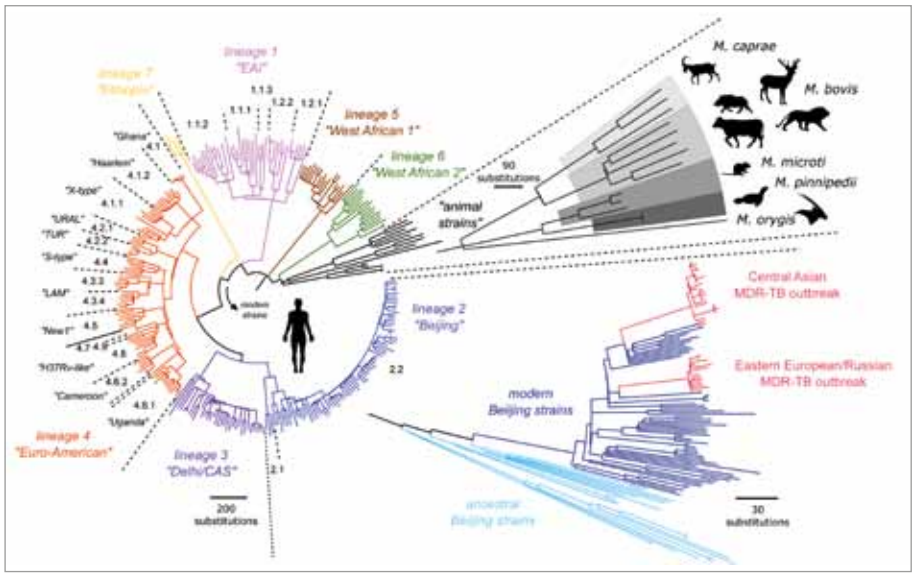

Figure 1. Global phylogenetic structure of *Mycobacterium tuberculosis* complex (*MTBC*) strains presented in a neighbor joining tree with 1,000 bootstrap replicates based on 36,963 variable sites. *MTBC* isolates can be classified into seven major lineages which are often composed of further geographically confined subgroups. So called „modern" *MTBC* lineages (lineage 2, 3, 4) are worldwide distributed, whereas infections with „ancestral" *MTBC* strains are mainly restricted to Western and Eastern Africa. (Sequence data compiled from Comas et al 2013 and Feuerriegel et al 2014).

Most important findings

The work performed has significantly contributed to the current understanding of TB transmission dynamics in low and high incidence settings by applying and developing molecular genotyping techniques. We published key studies based on next generation sequencing (NGS) and using whole genome analysis (WGS) to study transmission/evolution of the pathogen, thus opening completely new avenues for TB research and surveillance. This research has also led to a first understanding of the evolution of the pathogen in the human host, and has delivered key parameters for the interpretation of genome based data for tracing of *Mtbc* transmission (Merker et al. Nat Genet 2015, Bjorn-Mortensen et al. SciRep 2016, Hoffmann et al. AJRCCM 2016).

To allow the standardized use of NGS based molecular epidemiology of the *MTBC*, we have developed approaches for standardized NGS genotyping, e.g. the so called „core genome MLST", with the potential to lay the basis for web based *MTBC* surveillance at the genome basis and nomenclature systems for global strain tracking. The key position in this area is further exemplified by the coordination of the EU FP7-funded Patho-NGen-Trace project including 6 academic and industrial teams aiming at development of new, NGS based tools for improved molecular surveillance and diagnostics of microbial pathogens.

Another key research result has been the finding that MDR/extensively drug (XDR) strains can be effectively transmitted, thus, completely changing a long prevailing paradigm and advertising TB control measurements. Our work has led to the detection of highly transmissible MDR outbreak clones in several areas of the world and to the definition of compensatory mechanisms enabling their successful spread. Currently, we are applying NGS to understand the transmission dynamics and evolution of MDR *Mtbc* strains in different areas of the world such as Eastern Europe and Africa, and to define the global impact of the spread of highly successful MDR strains (Merker et al. Nat Genet 2015, Sanchez-Padilla E et al. NEJM 2015).

Moreover, we pioneered the use of NGS genome analysis to define resistance determinants and predict resistance in individual patients by defining the „Resistome". This paves the ground for comprehensive molecular TB resistance diagnostics in MDR TB patients with complex resistance patterns, enabling

Priority Research Area **Infections**

Molecular and Experimental Mycobacteriology

genome based individualized treatment regimens (Merker et al. Nat Genet 2015, Walker et al. Lancet ID 2015). This research is currently continued in large collaborative networks such as the Gates funded Cryptic and ReSEQTB consortia as well as the German Center for Infection Research (DZIF).

We also conducted key research on the pathogens' global population structure and the link between pathogen diversity, virulence properties and host-pathogen interaction. This work has laid the basis for an understanding of historic evolution and global population structure of the tubercle pathogens (Stucki et al Nat Gen 2016). Even more importantly, it has linked this finding with *Mtb* pathobiological diversity and the outcome of infection, thus pioneered investigations of the co-evolution and interaction of the pathogen and the host. In a complementary approach ranging from basic mycobacteriology, macrophage infection experiments to micro-array based transcriptome profiling and host genetic studies, we aim to define mycobacterial virulence factors and their correlation with host genetics. Presently, we are exploring NGS based RNAseq strategies to get a deeper understanding of virulence mechanism of the pathogen, e.g. in stress models (dormancy, persister). These studies are critical for understanding the diverse outcomes of infection, individualized treatment efficacy, and the development of new personalized diagnostics and therapeutics.

Finally, we translated these findings to world-wide used products/tools, e.g. by setting up the MIRU-VNTRplus and PhyResSe webpages (Feuerriegel et al. JCM 2015), and by developing a resistance variant encyclopedia that will pave the way for application of NGS as first line tool for resistance diagnostics. These studies had a direct impact on patient care and facilitated global standardized global strain tracking and web based analysis of typing data for the worldwide community.

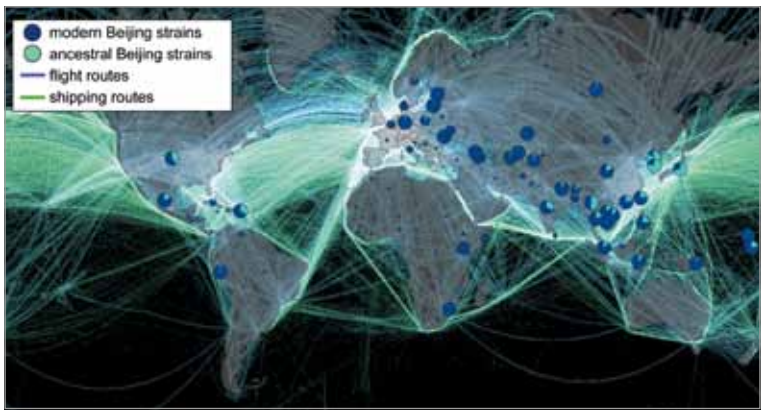

Figure 2. Geographical distribution of nearly 5,000 clinical Beijing (i.e. lineage 2) isolates (data from Merker et al 2015). Evolutionary ancestral Beijing strains are mainly dominating in Eastern Asia, the likely origin of this *MTBC* lineage, whereas modern Beijing strains are globally distributed suggesting a more virulent phenotype. In addition the effects of Globalization also shape the diversity of *MTBC* strains in different settings, yet with unknown consequences on host-pathogen interactions and tuberculosis progression (worldmap from flickr.com).

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

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Networks: Excellence Cluster I@I: 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, PathoNgenTrace Consortium: 7 partner institutions in Europe; Cryptic: Global; WHO SRL Network: Global.

Grant support

Molecular mechanisms of cell wall homeostasis in *M. tuberculosis*, DFG (PI).

Resuscitation mechanisms as virulence factors of clinical *M. tuberculosis* strains, DFG (PI).

Epidemiology of PZA-Resistance in clinical *M. tuberculosis* strains from Africa, EU-FP7 Project (PI).

PathoNgenTrace Next generation genome based high resolution tracing of pathogens, EU-FP7 (Coordinator).

Excellence Cluster "Inflammation at Interfaces" (PI).

CRyPTIC: Comprehensive Resistance Prediction for Tuberculosis: an International Consortium, Gates Foundation, Welcome Trust (PI).

National Reference Center for Mycobacteria, BMG (Co-Head).

German Center for Infection Research, BMBF. Coordinator of the Translational Thematic Unit „Tuberculosis".

Leibniz Science Campus Evolutionary Medicine of the Lung, Leibniz Association (Speaker).

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 Infektion und die Abteilung Medizin.

Grant support

DFG-Projekt: „Untersuchungen zum Einfluss dezentraler Strukturen im Bereich von Biomaterialbanken auf die Qualität von Biomaterialproben“

Head

- PD Dr. Karoline I. Gaede

Members

- Birgit Kullmann
- Romina Pritzkow
- Eva Wittmer

© Thomas Pieruschek, German Biobank Node

Medicine

BioMaterialBank Nord

Mission

Ziel der BioMaterialBank-Nord (BMB-Nord) ist die Bereitstellung von qualitativ hochwertigen Biomaterialproben wie Blut, Serum, Plasma, Spülflüssigkeiten, Urin, Gewebe- sowie Zellproben und Derivaten von detailliert phänotypisierten Probenspendern für medizinische Forschungsprojekte. Die Biomaterialproben werden nach Standardverfahren (SOPs) gesammelt, aufgearbeitet/ prozessiert, charakterisiert und langfristig archiviert, um sie für spätere Forschungsprojekte zur Verfügung stellen zu können. Die BMB-Nord ist aber auch direkt in Forschungsprojekte eingebunden und trägt hierbei die Verantwortung für die Bereiche Biobanking, Projekt- und Datamanagement.
Der Schwerpunkt der BMB-Nord liegt bei Lungenerkrankungen wie z. B. Lungenkrebs, chronisch-obstruktive Lungenerkrankungen (COPD) sowie Asthma und Allergien.
Die BMB Nord ist aktives Mitglied in verschiedenen akademischen Netzwerken so dem Deutschen Zentrum für Lungenforschung (BMBF), dem Deutschen Zentrum für Infektionsforschung (BMBF), dem popGen 2.0 Netzwerk (BMBF) und der Norddeutschen Biobanken Allianz.
Die BMB-Nord wird gemeinsam vom Forschungszentrum Borstel, der LungenClinic Grosshansdorf, der Medizinischen Klinik III/ Pneumologie und Infektiologie sowie der Pädiatrischen Pneumologie & Allergologie des Universitätsklinikums S-H, Campus Lübeck, auf der Grundlage eines Konsortialvertrages betrieben. Zentrale und administrative Strukturen sind am Forschungszentrum Borstel angesiedelt.

The aim of the BioMaterialBank-Nord (BMB-Nord) is to provide high-quality biomaterial samples such as blood, serum, plasma, rinsing fluids, urine, tissue, cell samples and derivatives of detailed phenotyped sample donors for medical research projects. The biomaterial samples are collected according to standard procedures (SOPs), processed, characterized and archived in order to make sammples and associated data available for later research projects. The BMB-Nord is also directly involved in research projects and is responsible for biobanking, project management and data management. The focus of the BMB-Nord is on pulmonary diseases such as lung cancer, chronic obstructive pulmonary disease (COPD) as well as asthma and allergies.
The BMB-Nord is an active member of the BMBF-funded German Center for Lung Research (DZL) German Center for Infection Research (DZIF; BMBF), the popGen 2.0 network (P2N, BMBF) and the North German Biobank Alliance (NBA).
The BMB-Nord is operated jointly by the Research Center Borstel, the Lung Clinic Grosshansdorf, the Medical Clinic III / Pneumology and Infectiology as well as the Pediatric Pneumology & Allergology of the University Hospital S-H, Campus Lübeck, on the basis of a consortium contract. Central and administrative structures are located at the Research Center Borstel.

Selected publications

Krause T, Röckendorf N, Gaede KI, Ramaker K, Sinnecker H, Frey A. Validation of antibody reagents for mucin analysis in chronic inflammatory airway diseases. MAbs. 2016 Dec 2:1-9. doi: 10.1080/19420862.2016.1264551.

Schnerch J, Prasse A, Vlachakis D, Schuchardt KL, Pechkovsky DV, Goldmann T, Gaede KI, Müller-Quernheim J, Zissel G. Functional Toll-Like Receptor 9 Expression and CXCR3 Ligand Release in Pulmonary Sarcoidosis. Am J Respir Cell Mol Biol. 2016 Nov; 55(5):749-757.

Ganbat D, Seehase S, Richter E, Vollmer E, Reiling N, Fellenberg K, Gaede KI, Kugler C, Goldmann T. Mycobacteria infect different cell types in the human lung and cause species dependent cellular changes in infected cells. BMC Pulm Med. 2016 Jan 23;16:19. doi: 10.1186/s12890-016-0185-5.

Internal and external collaboration

Internal collaboration
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Prof. Dr. Andreas Günther, Universitätsklinikum Giessen

TRANSLATIONAL RESEARCH

NTM
COPD

CLINICAL TRIALS

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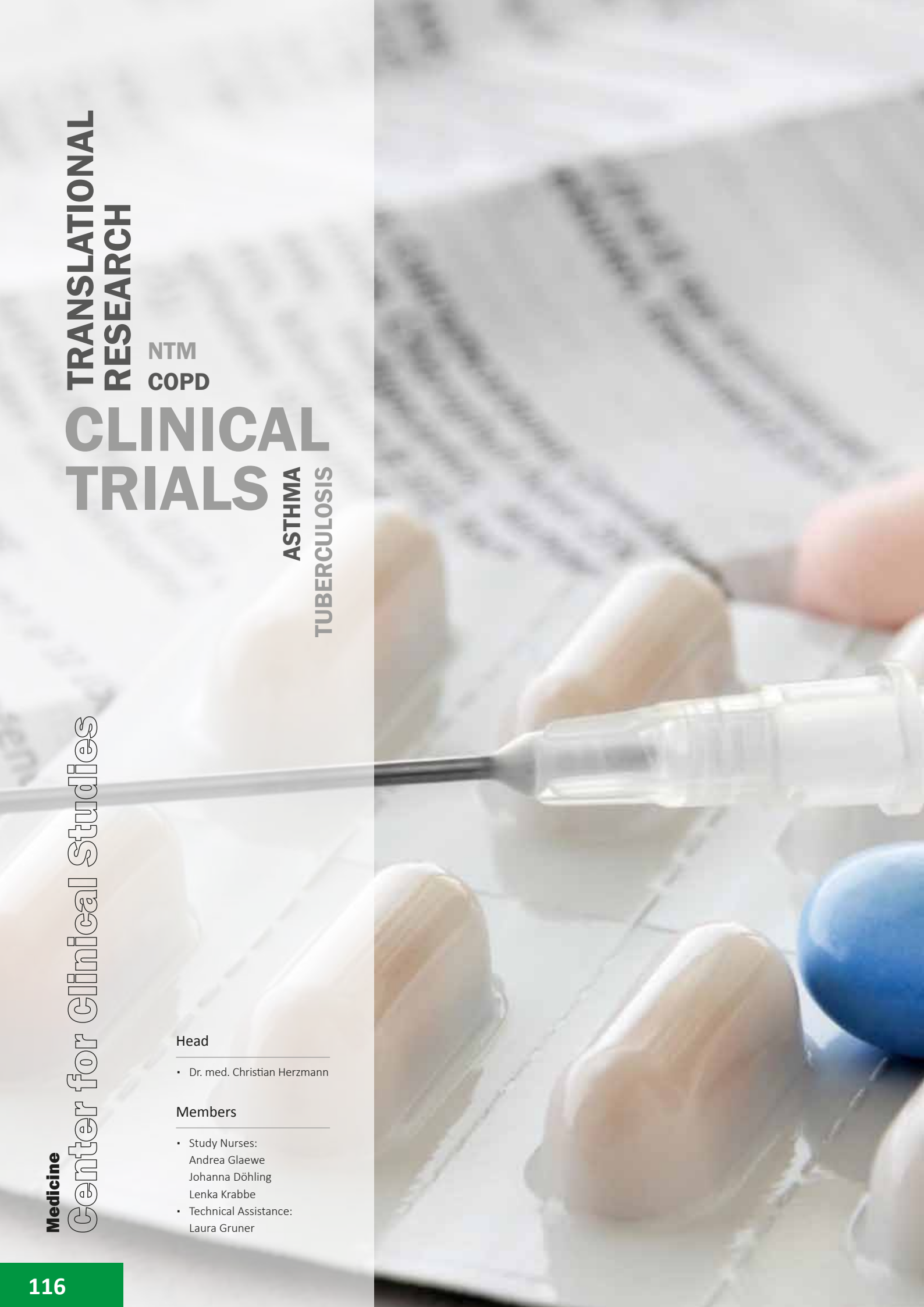

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  Johanna Döhling
  Lenka Krabbe
- Technical Assistance:
  Laura Gruner

Medicine

Center for Clinical Studies

Mission

Das Klinische Studienzentrum des Forschungszentrums Borstel ist die Schnittstelle zwischen Grundlagenwissenschaft im Forschungszentrum und Patientenversorgung. Wir gehören zur Medizinischen Klinik Borstel mit angegliedertem Medizinischen Versorgungszentrum. Unsere Expertise liegt in der Planung, Organisation und Durchführung klinischer Studien (Phasen I, II, III). Unser Spektrum umfasst translationale Forschungsprojekte in enger Kooperation mit Grundlagenwissenschaftlern, Verbundstudien pneumologischer und infektiologischer Fachverbände und Arzneimittelstudien der pharmazeutischen Industrie. Unser Fokus sind übertragbare und nicht-übertragbare Erkrankungen der Atemwege. Die Studien betreffen Asthma, Allergie, COPD, Lungenkrebs, Pneumonie, Tuberkulose, nicht-tuberkulöse Mykobakterien, Weaning und andere pneumologische Erkrankungen.

The Center for Clinical Studies Borstel is a central part of the Research Center Borstel. Our unit is located at the interface between basic molecular life sciences and clinical care for medical patients. Focused on translational research, we promote close co-operations between basic scientists, physicians, scientific and medical associations, and the pharmaceutical industry. Our expertise includes the conceptual design, organisation and conduct of clinical studies (phase I, II, III) on patients, healthy study participants and biomaterials. In accordance with the mission of the Research Center Borstel we investigate transmissible and non-transmissible respiratory diseases. The spectrum of diseases we investigate includes asthma, allergy, bronchiectasis, COPD, pneumonia, tuberculosis, non-tuberculous mycobacteria, weaning, bronchial carcinoma among other illnesses affecting the airways.

Most important findings

Risk for latent and active tuberculosis in Germany

In collaboration with the Division of Clinical Infectious Diseases, we analysed data from the TB or not TB cohorts funded by the BMBF. Healthy household contacts (HHC), health care workers (HCWs) exposed to *M. tuberculosis* and patients with pulmonary TB were recruited at 18 German centres. Interferon-γ release assay testing was performed. LTBI risk factors were evaluated by comparing IGRA-positive with IGRA-negative contacts. Risk factors for tuberculosis were evaluated by comparing PTB patients with HHCs. 603 HHCs, 295 HCWs and 856 PTBs were recruited. LTBI was found in 34.5% of HHCs and in 38.9% of HCWs. In HCWs, care for coughing patients and longstanding nursing occupation were associated with LTBI. In HHCs, predictors for LTBI were a diseased partner, sexual contact to a diseased partner and substance dependency. PTB was associated with male sex, low body weight, alcoholism, glucocorticoid therapy and diabetes. No contact developed active tuberculosis within 2 years follow-up (Herzmann et al., Infection. 2016 Nov 19).

Highlights

LAM-Responsive Polycytotoxic T Cells Are Associated with Protection in Human TB.

Within the Tb or not Tb consortium, bronchoalveolar lavage cells from donors with latent (LTBI) and active tuberculosis infection were obtained. Subjects with LTBI limited the growth of virulent *Mycobacterium tuberculosis* more efficiently than those in patients who developed disease. Unconventional, glycolipid-responsive T cells contributed to reduced mycobacterial growth because antibodies to CD1b inhibited this effect by 55%. Lipoarabinomannan was the most potent mycobacterial lipid antigen (activation of 1.3% T lymphocytes) and activated CD1b-restricted T cells that limited bacterial growth. A subset of IFN-γ-producing lipoarabinomannan-responsive T cells coexpressed the cytotoxic molecules perforin, granulysin, and granzyme B, which we termed polycytotoxic T cells. Taking advantage of two well-defined cohorts of subjects latently infected with *Mycobacterium tuberculosis* or patients who developed active disease after infection, we found a correlation between the frequency of polycytotoxic T cells and the ability to control infection (latent tuberculosis infection, 62%; posttuberculosis patients, 26%). The experiments performed by Prof. Steffen Stenger, Ulm, defined an unconventional CD8(+) T-cell subset (polycytotoxic T cells) that is based on antigen recognition and function. The results link clinical and mechanistic evidence that glycolipid-responsive, polycytotoxic T cells contribute to protection against tuberculosis.

Trial on inhaled Arykace® for pulmonary *M. avium* infections

The Center for Clinical Studies participated in a phase III trial (INS-212) sponsored by Insmed pharmaceuticals. Inhaled application of liposomal amikacin for the treatment of therapy refractory *M. avium complex* was evaluated. We enrolled patients from all over Germany and ranked on 5th position among all European study centres in terms of recruitment numbers. The study is ongoing. First reports are expected in 2017.

Pursed Lip Breathing Ventilation Trial gets funded

In 2016, the German Center for Lung Research (DZL) 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 patented in the department of sleep medicine of the Medical Clinic Borstel (Dr. Rüller). Four German DZL centers will participate in this clinical study co-ordinated by the Center for Clinical Studies and the BioMaterialBank North.

Figure 1. Mass chest X-ray screening in migrants coming from countries with a low incidence TB may not be efficient.

Figure 2. Caring for coughing TB patients and longstanding professional exposure were associated with latent tuberculosis infection in healthcare professionals.

Medicine

Center for Clinical Studies

Radiological screening of refugees coming to Germany

In collaboration with the National Reference Center for Mycobacteria, reports of chest X-rays of 38,001 migrants from 76 countries across four reception centres in Germany were obtained and reviewed. A quarter of refugees were from Syria (25%) followed by Iraq (14%) and Afghanistan (11%). CXRs were suggestive of TB in 127 migrants. Detailed clinical record review revealed: 37 individuals were lost to follow-up, TB was excluded in 38 and 52 were diagnosed with TB and treatment was initiated. Of those 42 (81%) were microbiologically confirmed by culture and/or nucleic acid amplification. Overall TB prevalence was 140/100,000. TB prevalence was 0.05%, 0.09%, 0.23% and 0.26% in migrants from countries with TB incidence of <20, 20-50, 50-100 and >100/100,000, respectively. Prevalence was lowest among Syrian (31/100,000) and Iraqi (37/100,000) migrants. Highest rates were found among migrants from Somalia and Eritrea with prevalence rates that were 4 to 10 times higher than WHO incidence estimates. (ERJ- accepted for publication)

Physical activity, airway resistance and small airway dysfunction in severe asthma

Within the Adult Register for Asthma (ERA) of the German Center for Lung Research we studied in collaboration with the LungenClinic Großhansdorf physical activity in 146 patients with asthma (severe asthma, n=63; mild-to-moderate asthma, n=83) and 29 healthy controls. Patients with severe asthma performed 6174 steps per day (SPD) on average, compared to 7831 SPD in patients with mild-to-moderate asthma and 8912 in healthy controls. Average minutes of at least moderate activity (MMA) per day were 125 in severe asthma, 151 in mild-to-moderate asthma and 163 in healthy controls, respectively. In patients with severe asthma, SPD and MMA were significantly reduced by 21% and 17% compared to mild-to-moderate asthma, and by 31% and 23% compared to healthy controls, respectively. Multivariate regression analyses adjusting for age, sex, obesity and smoking as potential confounders of PA revealed that SPD still were significantly reduced in severe asthma compared to mild-to-moderate asthma and healthy controls, while differences in MMA were no longer significant between groups. The main findings of our study are that PA is reduced in severe asthma and that reduced PA in asthma is associated with impulse oscillometric airway resistance and small airway dysfunction, but not with airflow limitation. (Eur Respir J. 2017 Jan 4;49(1))

Selected publications

Herzmann C, Sotgiu G, Bellinger O, Diel R, Gerdes S, Goetsch U, Heykes-Uden H, Schaberg T, Lange C; TB or not TB consortium. Risk for latent and active tuberculosis in Germany. Infection. 2016 Nov 19.

Labugger I, Heyckendorf J, Dees S, Häussinger E, Herzmann C, Kohl TA, Richter E, Rivera-Milla E, Lange C. Detection of transrenal DNA for the diagnosis of pulmonary tuberculosis and treatment monitoring. Infection. 2016 Oct 31.

Busch M, Herzmann C, Kallert S, Zimmermann A, Höfer C, Mayer D, Zenk SF, Muche R, Lange C, Bloom BR, Modlin RL, Stenger S; TBornotTB Network. Lipoarabinomannan-Responsive Polycytotoxic T Cells Are Associated with Protection in Human Tuberculosis. Am J Respir Crit Care Med. 2016 Aug 1;194(3):345-55. doi: 10.1164/rccm.201509-1746OC.

Salzer HJ, Wassilew N, Köhler N, Olaru ID, Günther G, Herzmann C, Kalsdorf B, Sanchez-Carballo P, Terhalle E, Rolling T, Lange C, Heyckendorf J. Personalized Medicine for Chronic Respiratory Infectious Diseases: Tuberculosis, Nontuberculous Mycobacterial Pulmonary Diseases, and Chronic Pulmonary Aspergillosis. Respiration. 2016;92(4):199-214.

Internal and external collaboration

Internal collaborations

Experimental Asthma Research (S. Krauss-Etschmann); Experimental Pneumology (G. Schramm); Mouse Models Asthma (M. Wegmann); Mucosal Immunology (A. Frey, T. Krause); 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); National Reference Center for Mycobacteria (K. Kranzer)

External collaborations

Medical Laser Center (Lübeck); Hypertech Laser (Lübeck); Insmed (Bridgewater, NJ, USA); Aradigm Corp (Hayward, CA, USA); Astra-Zeneca (Sweden); several Municipal Health Authorities (Karlsruhe, Göttingen, Augsburg, Braunschweig); University Hospital Marburg / Gießen; German Center for Infection Research (DZIF); German Center for Lung Research (DZL); LungenClinic Großhansdorf, Max Planck Institute for Infection Biology (Berlin), Mologen AG (Berlin), Novartis Pharma GmbH (Nürnberg), University Hospital Ulm

PRIMARY HUMAN CELLS

MOLECULAR DIAGNOSTICS

BAMBI

EX VIVO

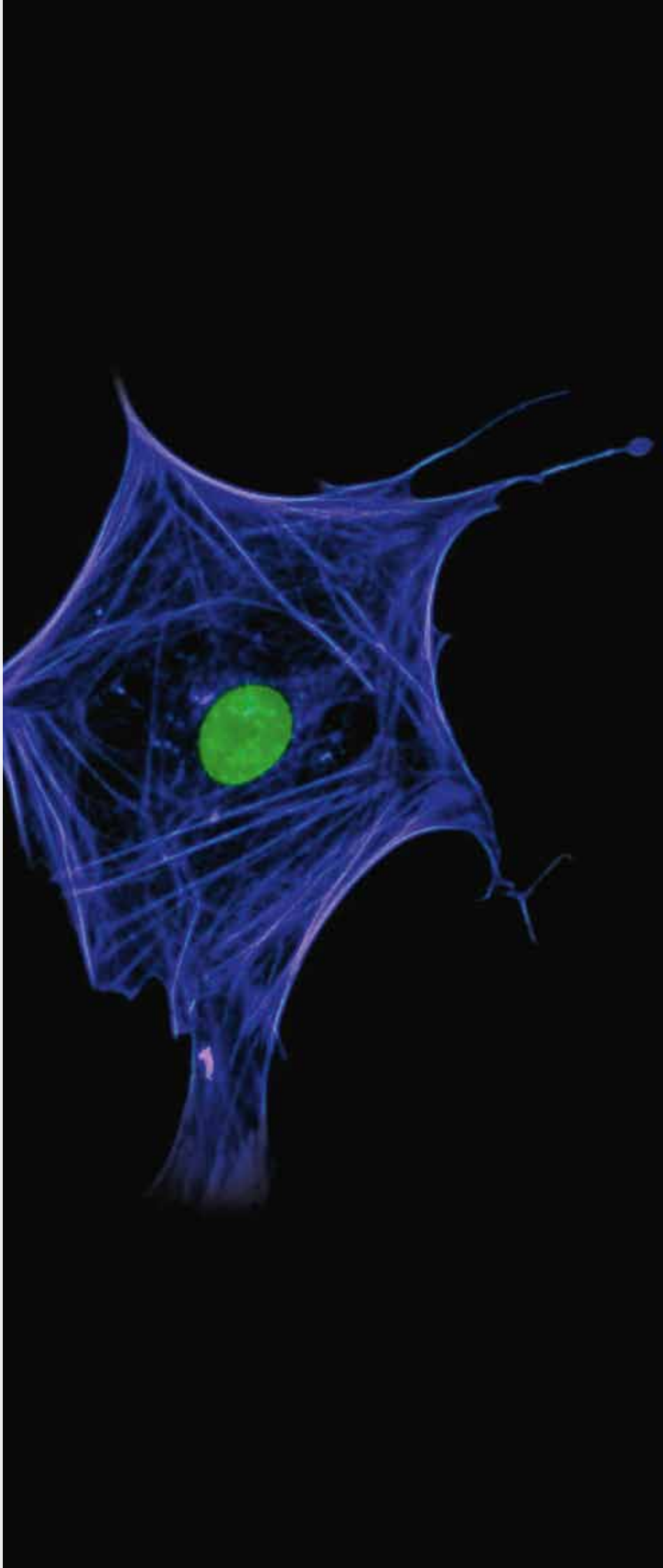
TGF-SIGNALING

IMMUNE CHECKPOINT INHIBITORS

TRANSCRIPTOME

Medicine

Clinical and Experimental Pathology



Head

- Prof. Dr. med. Sven Perner

Members

- Gabriele Cornehls
- Stefanie Fox
- Prof. Dr. rer. nat. T. Goldmann
- Frauke Groth
- Dr. rer. nat. Lena Heinbockel
- Iris Jonas
- Alina Kelp
- Angela Kelp
- Ghanima Alexa Korf
- Dr. med. Rosemarie Krupar
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- Bettina Lühr
- Dr. rer. nat. Sebastian Marwitz
- Dr. med. Julia Müller (maternal leave)
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- Silke Perner
- Dr. med. Florian Stellmacher
- Jasmin Tiebach
- Wenzel Vogel
- Rolf Warneke
- Kristin Wiczkowski

Medicine

Clinical and Experimental Pathology

Mission

Exzellente Diagnostik und Forschung in der Pneumologie.
Zentrales Werkzeug sind funktionelle Studien im humanen System wobei verschiedene Gewebekulturmodelle zum Einsatz kommen, die auf der HOPE-Technik basieren. Experimentelle Arbeiten an humanen Primärzellen und Gewebe sind ein weiteres Element. Screening-Untersuchungen auf Transkriptomebene 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 for different diseases of the lung, mostly based on the HOPE-technique. Experimental work on primary human lung cells and lung tissues are further important tools for translational research. 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

BAMBI

TGF-ß-signaling is an ancient yet not fully enlightened pathway influencing Epithelial-Mesenchymal-Transition (EMT). Our group worked on the TGF-ß-pseudoreceptor BAMBI (BMP and Membrane Bound Inhibitor), which had been discovered in the human lung by applying transcriptome analyses to ex vivo cultivated human lung tissues that had been infected with *Haemophilus influenzae*. In a cooperation project of the German Center for Lung Diseases (DZL) together with Partners in Grosshansdorf (M. Reck, Ch. Kugler, K.F. Rabe), Kiel (O. Ammerpohl) and Heidelberg (U. Klingmüller), we demonstrated that BAMBI is downregulated in Non Small Cell Lung Cancer (NSCLC) by epigenetic silencing. In a large scaled study analyzing the major nine pathway members in 156 human tissues by immunohistochemistry using HOPE-fixed materials, we show that the downregulation of BAMBI results in a steady-state-activation of TGF-signaling in NSCLC. Transcriptome and Methylome-analyses as well as Real time PCR were also conducted using HOPE-fixed materials. This nicely shows the unique possibilities of the HOPE-technique: Comprehensive molecular read outs from a single starting material.

Re-expression of BAMBI in NSCLC using a retroviral TET-on-system led to reduced invasion *in vitro* and *in vivo*, indicating a possible TGF-dependency. These results draw attention towards TGF-ß-inhibition as a potential therapy for NSCLC, which is currently under investigation in the DZL 2.0 as a flagship project.

Selected publications

Marwitz S, Depner S, Dvornikov D, Merkle R, Szczygieł M, Müller-Decker K, Lucarelli P, Wäsch M, Mairbäurl H, Rabe KF, Kugler C, Vollmer E, Reck M, Scheufele S, Kröger M, Ammerpohl O, Siebert R, Goldmann T, Klingmüller U. Downregulation of the TGF-β pseudoreceptor BAMBI in non-small cell lung cancer enhances TGF-β signaling and invasion. Cancer Res. 2016 Jul 1;76(13):3785-801.

Syring I, Klümper N, Offermann A, Braun M, Deng M, Boehm D, Queisser A, von Mässenhausen A, Brägelmann J, Vogel W, Schmidt D, Majores M, Schindler A, Kristiansen G, Müller SC, Ellinger J, Shaikhibrahim Z, Perner S. Comprehensive analysis of the transcriptional profile of the Mediator complex across human cancer types. Oncotarget. 2016 Apr 26;7(17):23043-55. doi: 10.18632/oncotarget.8469.

Herrtwich L, Nanda I, Evangelou K, Nikolova T, Horn V, Sagar, Erny D, Stefanowski J, Rogell L, Klein C, Gharun K, Follo M, Seidl M, Kremer B, Münke N, Senges J, Fliegauf M, Aschman T, Pfeifer D, Sarrazin S, Sieweke MH, Wagner D, Dierks Ch, Haaf T, Ness T, Zaiss MM, Voll RE, Deshmukh SD, Prinz M, Goldmann T, Hölscher Ch, Hauser AE, Lopez-Contreras AJ, Grün D, Gorgoulis V, Diefenbach A, Henneke Ph, Triantafyllopoulo A. DNA Damage Signaling Instructs Polyploid Macrophage Fate in Granulomas. Cell 167, 1–17, November 17, 2016.

Goldmann T, Kugler Ch, Reinmuth N, Vollmer E, Reck M. PD-L1 copy number gain in Non Small Cell Lung Cancer defines a new subset of patients for anti PD-L1 therapy. Ann Oncol. 2016 Jan;27(1):206-7.

Grant support

Deutsche Forschungsgemeinschaft (DFG):
PE1179/11-1. 1. The Biological and Clinical Relevance of EVI1 Expression in Prostate Carcinogenesis. Art: Sachbeihilfe. Projektdauer: 1.1.2016 - 31.12.2018. *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. Art: Sachbeihilfe. Projektdauer: 1.5.2015 - 1.4.2018. *Sven Perner*
DR797/3-1. Die Rolle des TGF-ß Pseudorezeptors Bambi bei der Lungenfibrose. Projektdauer: 2014 - 2018. *Daniel Drömann, Torsten Goldmann*

BMBF
Deutsches Zentrum für Lungenforschung (DZL), Airway Research Center North (ARCN), Disease Area Lung Cancer (LC, COPD, Asthma/Allergy). Projektdauer: 1.1.2016- 31.12.2020. PI: *Torsten Goldmann*
Deutsches Zentrum für Lungenforschung (DZL), Airway Research Center North (ARCN), Platform Biobanking. Projektdauer: 1.1.2016 - 31.12.2020. *Karoline Gaede, Torsten Goldmann*
Deutsches Zentrum für Lungenforschung (DZL), Airway Research Center North (ARCN), DZL-Labor für humane Primärzellen und Gewebekulturmodelle. Projektdauer: 1.1.2016- 31.12.2020. *Torsten Goldmann, Heinz Fehrenbach*

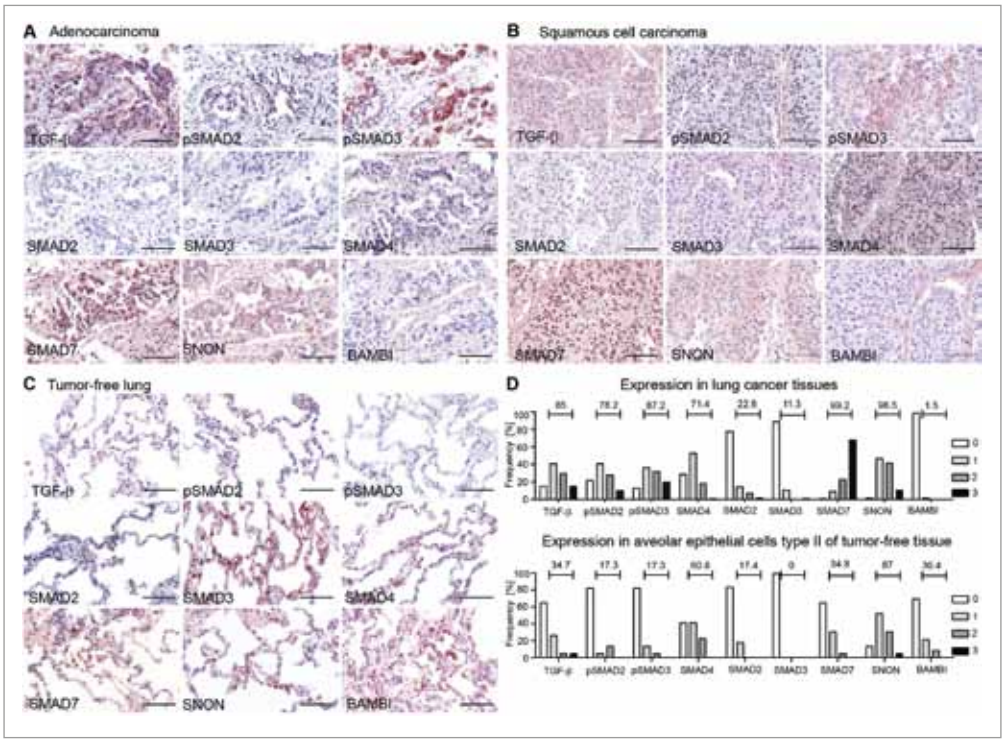

Figure 1. BAMBI is absent and the TGF-β signaling pathway is abundantly activated in lung cancer tissues.

A-C, Detection and localization of TGF-β pathway proteins via immunohistochemistry on HOPE-fixed paraffin-embedded (A) lung adenocarcinomas (*n* = 59 patients), (B) lung squamous cell carcinomas (*n* = 74 patients) and (C) tumor-free lung tissues (*n* = 23 patients). All the images are at 40x magnification with scale bar = 100 µm. Positive staining is indicated by a red color (AEC). For each antibody, a positive signal was observed for at least some of the samples. Representative images are shown for each tumor type and for tumor-free lung tissue.

D, Scoring of expression levels in lung cancer tissues (*n* = 133 patients) and in healthy type II alveolar epithelial cells (AECII) from tumor-free lung tissue (*n* = 23 patients). Bar charts indicate semi-quantitative scores based on the histological analysis of the entire specimen as follows: negative (0), focal and weak expression (1), frequent intermediate expression (2), strong expression and dominating feature of specimen (3). Numbers above the bars display the total positive cases observed overall in percent. *From: Marwitz S, Depner S, Dvornikov D, Merkle R, Szczygieł M, Müller-Decker K, Lucarelli P, Wäsch M, Mairbäurl H, Rabe KF, Kugler C, Vollmer E, Reck M, Scheufele S, Kröger M, Ammerpohl O, Siebert R, Goldmann T, Klingmüller U. Downregulation of the TGF-β pseudoreceptor BAMBI in non-small cell lung cancer enhances TGF-β signaling and invasion. Cancer Res. 2016 Jul 1;76(13):3785-80.*

Immune Checkpoint Inhibitors in therapeutic interventions

Different personalized therapies targeting Immune Checkpoint inhibitors are currently in the focus of therapeutic and diagnostic studies, regarding to lung diseases mainly in Lung Cancer. There are several medications available targeting the PD1-PDL1 axis, which show excellent results regarding to the patient's outcome. However, the selection of suitable patients by companion diagnostics remains challenging since comparably manifold different immunohistochemical approaches are promoted, some stating positivity by detection of as little as

Medicine

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1% of positive cells. In order to provide a biologically clear cut parameter, we analyzed amplifications of the PD-L1 gene in NSCLC.

By applying FISH-analysis to a cohort of 221 cases mounted on tissue microarrays, we found copy number gain of PDL1 in 5% of the cases. PD-L1 amplifications seemed to be associated with a relatively low differentiation of the tumors. All Squamous Cell Carcinomas with PD-L1 amplification were male, while all adenocarcinomas were female. Trisomy 9 was observed in three of 221 cases. Immunohistochemistry showed positivity for PD-L1 (>1% of tumor cells) in all amplified cases, and all cases with trisomy9. From the 11 amplified cases, seven exhibited an IHC score of 3 (> 10% positive tumor cells). PD-L1 amplification therefore seems to be associated with an overexpression of the protein. As gene amplifications are comparably rude genetic changes, typically accompanied by substantially altered function, we suppose that PD-L1 amplified tumors are likely to behave differently especially under PD-L1 targeting therapies. Therefore, we propose to perform FISH-analysis in studies targeting PD-L1 in order to monitor patients bearing the amplification under anti PD-L1 therapy.

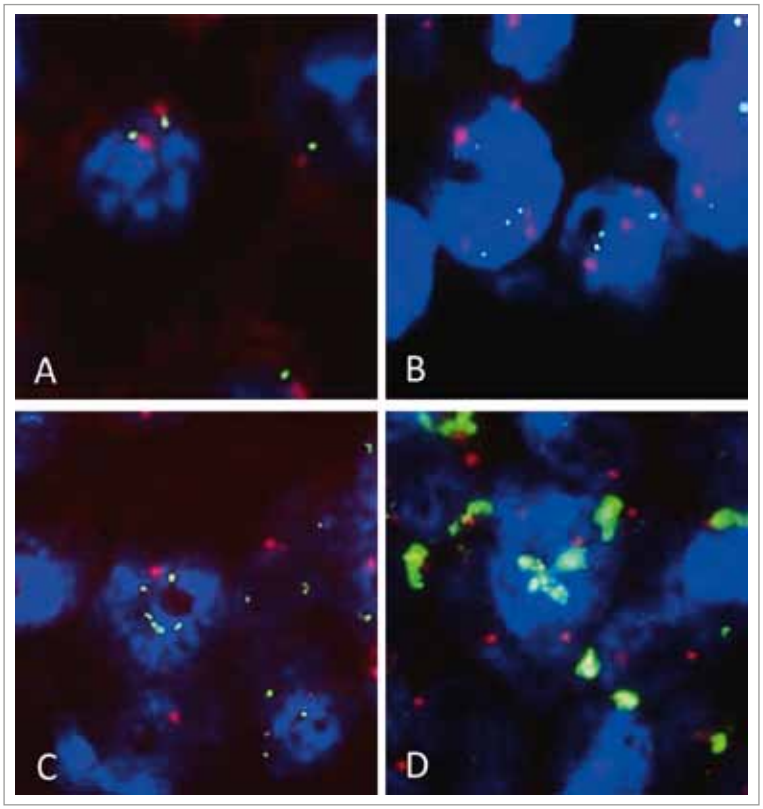

Figure 2. Results of FISH targeting PD-L1 (green) and centromere 9 (red) with DAPI-counterstain (blue) and a magnification of 630x. A: wildtype. B: trisomy 9. C: PD-L1 amplification with a PD-L1/centromere 9 ratio of 2-3. D: Strong PD-L1 amplification with a PD-L1/centromere 9 ratio of >10. *From: Goldmann T, Kugler Ch, Reinmuth N, Vollmer E, Reck M. PD-L1 copy number gain in Non Small Cell Lung Cancer defines a new subset of patients for anti PD-L1 therapy. Ann Oncol. 2016 Jan;27(1):206-7.*

Internal and external collaboration

Inhouse

Clin. Infectious Diseases (Prof. Dr. Ch. Lange), Molecular Mycobacteriology (Prof. Dr. S. Niemann), Biophysics (Prof. Dr. T. Gutsmann), Immunbiophysics (Prof. Dr. A. Schromm), Bioanalytical Chemistry (Dr. D. Schwudke), Microbial Interface Biology (Prof. Dr. N. Reiling), Cellular Microbiology (Prof. Dr. U. Schaible), Biochemical Immunology (Prof. Dr. F. Petersen), Experimental Pneumology (Prof. Dr. H. Fehrenbach), Clinical and Molecular Allergology (Prof. Dr. U. Jappe), Mucosal Immunology and Diagnostic (PD Dr. A. Frey), Medicine (Prof. Dr. P. Zabel), Core Facility Flow Cytometry (Dr. J. Behrends)

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Thoraxclinic Heidelberg, Dr. M. Meister, Dr. T. Muley, Prof. Dr. M. Thomas
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University Ulaanbaatar, Dr. D. Ganbat
University of Dohuk, Iraq, Dr. M. Abdullah
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University of Colorado, Denver,Prof. L. Heasley, Prof. F. Hirsch
Weill Cornell Medical College, New York, Prof. M. A. Rubin, Prof. D. S. Rickman,Prof. J. M. Mosquera
Institut für Pathologie, Universitätsspital Zürich, Prof. A. Soltermann, Prof. H. Moch

FLOW CYTOMETRY
LIVE CELL IMAGING

JOINT RESEARCH PROJECTS

SUPPORT

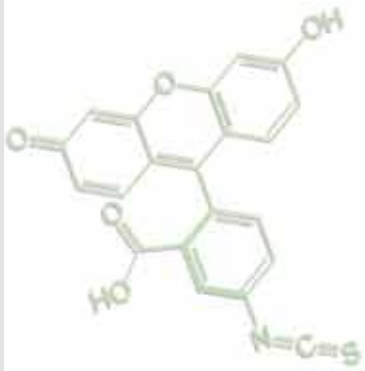
MULTIPARAMETER ANALYSIS

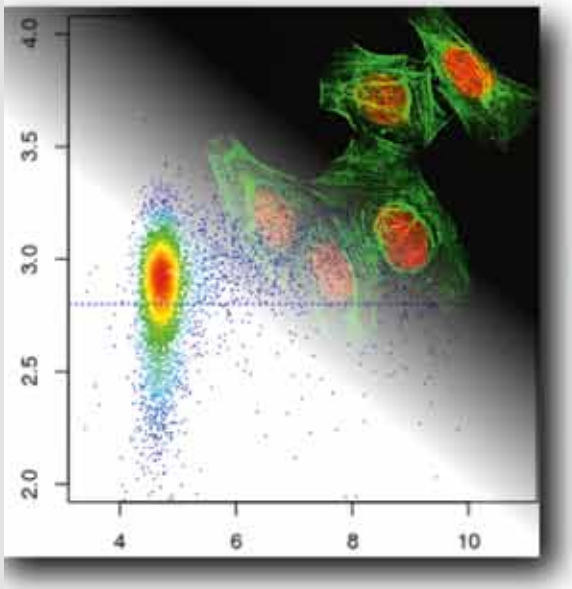
FRET/FRAP

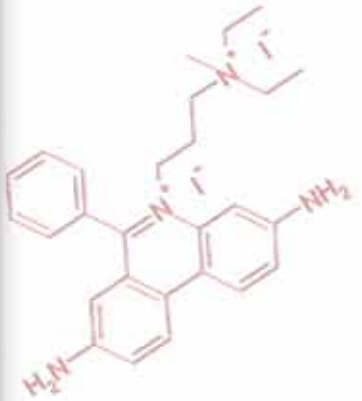
CONFOCAL MICROSCOPY

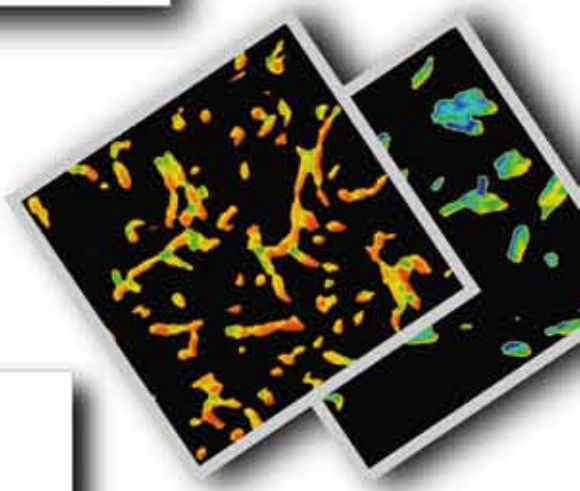
CELL SORTING, BSL3

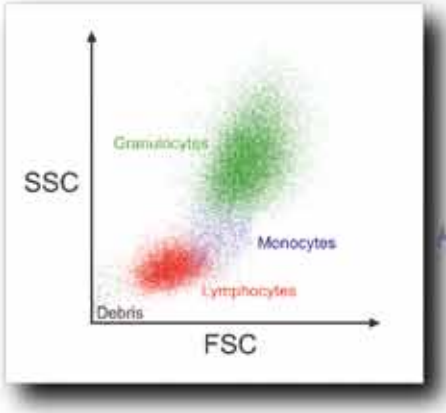
LECTURES
CONSULTATION
PRACTICAL TRAINING

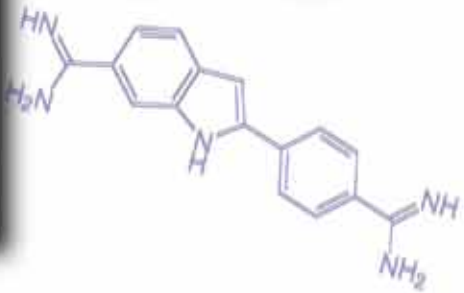












Core Facility
Fluorescence Cytometry

Head

- Dr. Jochen Behrends
- Dr. Thomas Scholzen

Members

- Martina Hein

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 Facility.

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.

Service

The core facility Fluorescence Cytometry offers service for scientists to carry out high speed cell sorting on our BD FACS ARIA IIu 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

Wang S, Hüttmann G, Scholzen T, Zhang Z, Vogel A, Hasan T, Rahmanzadeh R. A light-controlled switch after dual targeting of proliferating tumor cells via the membrane receptor EGFR and the nuclear protein Ki-67. Scientific Reports 2016; 6: 27032.

Erdmann H, Behrends J, Hölscher C. During acute experimental infection with the reticulotropic *Trypanosoma cruzi* strain Tulahuen IL-22 is induced IL-23-dependently but is dispensable for protection. Scientific Reports 2016; 6: 32927.

Blank J, Eggers L, Behrends J, Jacobs T, Schneider BE. One Episode of Self-Resolving *Plasmodium yoelii* Infection Transiently Exacerbates Chronic *Mycobacterium tuberculosis* Infection. Front Microbiol. 2016; 7: 152.

Lewis MD, Behrends J, Sá E Cunha C, Mendes AM, Lasitschka F, Sattler JM, Heiss K, Kooij TW, Prudêncio M, Bringmann G, Frischknecht F, Mueller AK. Chemical attenuation of Plasmodium in the liver modulates severe malaria disease progression. J Immunol. 2015; 194(10): 4860-70.

Lunding LP, Webering S, Vock C, Behrends J, Wagner C, Hölscher C, Fehrenbach H, Wegmann M. Poly(inosinic-cytidylic) Acid-Triggered Exacerbation of Experimental Asthma Depends on IL-17A Produced by NK Cells. J Immunol. 2015; 194(12): 5615-25.

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.

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 fall 2014. With its new 405 nm laser and the corresponding octagon detector, it can now analyze up to 14 fluorescence channels in parallel. If fewer channels are needed, analysis can also be performed using a BD FACSCalibur cytometer (four fluorescence channels) and a newly acquired MACSQuant Analyzer 10 (eight fluorescence channels). Additionally, within the BSL3 facility a BD FACSCanto II cytometer (eight fluorescence channels) and a BD FACSArray bioanalyzer (four channels) are 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 repair and maintenance is carried out by members of the Fluorescence Cytometry staff. The in-depth technical knowledge also leads to improvements of the equipment (see figure 1).

Figure 1. MACSQuant Analyzer 10 (3 lasers, 8 fluorescence channels). Part of the repair and maintenance is carried out by members of the Fluorescence Cytometry staff. The in-depth technical knowledge also leads to improvements of the equipment (e.g. the custom-made attachment of high capacity fluid containers for usage with 96-well plates).

Core Facility

Fluorescence Cytometry

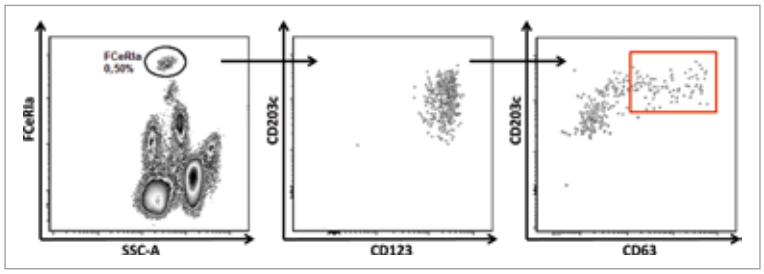


Figure 2. Using a flow cytometric basophil activation test (BAT) for an improved diagnosis of peanut-allergic patients (the CD63⁺ percentage of CD203⁺ cells is calculated; joint research project with the research group Clinical and Molecular Allergology, Prof. U. Jappe).

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.

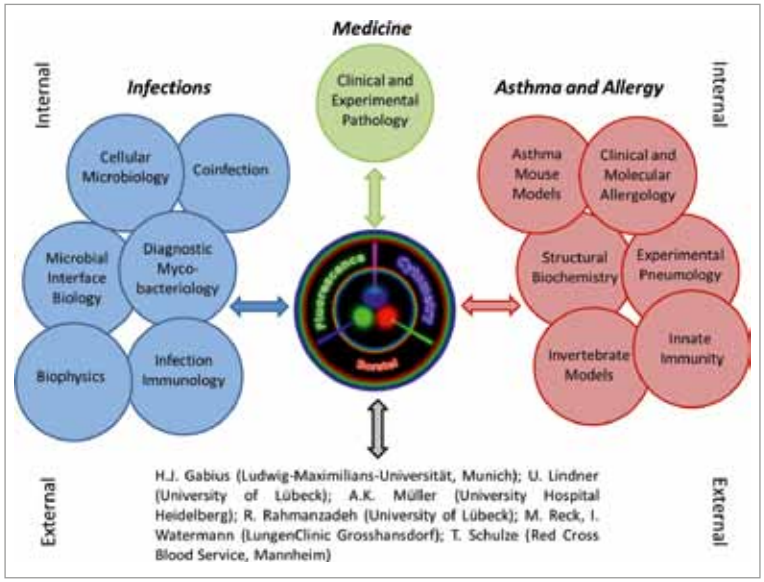


Figure 3. Joint research projects (internal and external collaboration).

Internal and external collaboration

Internal: Priority area Asthma and Allergy (Asthma Mouse Models, Experimental Pneumology, Innate Immunity, Invertebrate Models, Structural Biochemistry, Clinical and Molecular Allergology); Priority area Infections (Biophysics, Cellular Microbiology, Coinfection, Infection Immunology, Microbial Interface Biology, Diagnostic Mycobacteriology); Medicine (Clinical and Experimental Pathology).

External: H. J. Gabius (Ludwig-Maximilians-Universität, Munich); U. Lindner (University of Lübeck); A.K. Müller (University Hospital Heidelberg); R. Rahmanzadeh (University of Lübeck); M. Reck, I. Watermann (LungenClinic Grosshansdorf); T. Schulze (Red Cross Blood Service, Mannheim).

Funding (Mio. Euro)

total budget 2015		
institutional funding (federal state / federal government)		20,34
third-party funding		6,86
of which	DFG	1,28
	federal state / federal government	3,53
	EU project funding	0,21
	industry	1,11
	Leibniz competition	0,07
	foundations	0,06
	miscellanous	0,60

total budget 2016		
institutional funding (federal state / federal government)		20,60
third-party funding		6,26
of which	DFG	1,28
	federal state / federal government	3,60
	EU project funding	0,14
	industry	0,99
	Leibniz competition	0,27
	foundations	0,03
	miscellanous	0,09

Patents and Licenses

	2015	**2016**
Assignation of patents	2	3
Stock of patents	18	21
Stock of licensing agreements	24	0
Income of royalties (T Euro)	60	0

Academic Degree / Professional Qualifications

	2015	**2016**
Dissertation	7	10
Master of Science	7	7
Bachelor of Science	3	1
Technicians	11	9

Facts & Figures

Guest Scientists

	2015	2016
national	11	7
international	13	23

Conferences / Workshops

2015	2016
23	34

Peer Reviewed Publications

	2015	2016
	141	191
Ø IF	6,61	6,4

Books and Articles in Books

2015	2016
6	1

National Networks 2015/2016

DFG
- Exzellenzcluster 306 ‚Entzündung an Grenzflächen'
- SPP 1580 ‚Intracellular compartments as places of pathogen-host-interactions'
- IRTG 1911 ‚Immunregulation der Entzündung bei Allergien und Infektionen'
- GRK 1727 ‚Modulation der Autoimmunität'

BMBF
- Deutsches Zentrum für Lungenforschung
- Deutsche Zentrum für Infektionsforschung
- GLUTEVIS
  Fluoreseszenz-optisches Schnellsystem für den Nachweis von Gluten
- P2N
  PopGen 2.0 Network advancing PopGen towards a centralized biobanking infrastructure
- EDCTP
  European and Developing Countries Clinical Trials Partnership
- nanoCOLT
  Langzeitwirkung modifizierter Carbon Black Nanopartikel auf gesunde und geschädigte Lungen
- EXASENS
  vor-Ort basierte Exazerbations-Diagnostik bei Asthma & COPD
- Research Networks on Health Innovation in Subsahara-Africa- TB Sequel

Leibniz Gemeinschaft
- Leibniz Forschungsverbund (LFV) ‚INFECTIONS'21'
- LFV ‚Gesundheitstechnologien'
- LFV ‚Wirkstoffe und Biotechnologie'
- EvoLUNG - Leibniz-WissenschaftsCampus Kiel
- Leibniz Forschungsnetzwerk
  ‚Lung microbiota at the interface between airway epithelia and environment'

International Networks 2015/2016

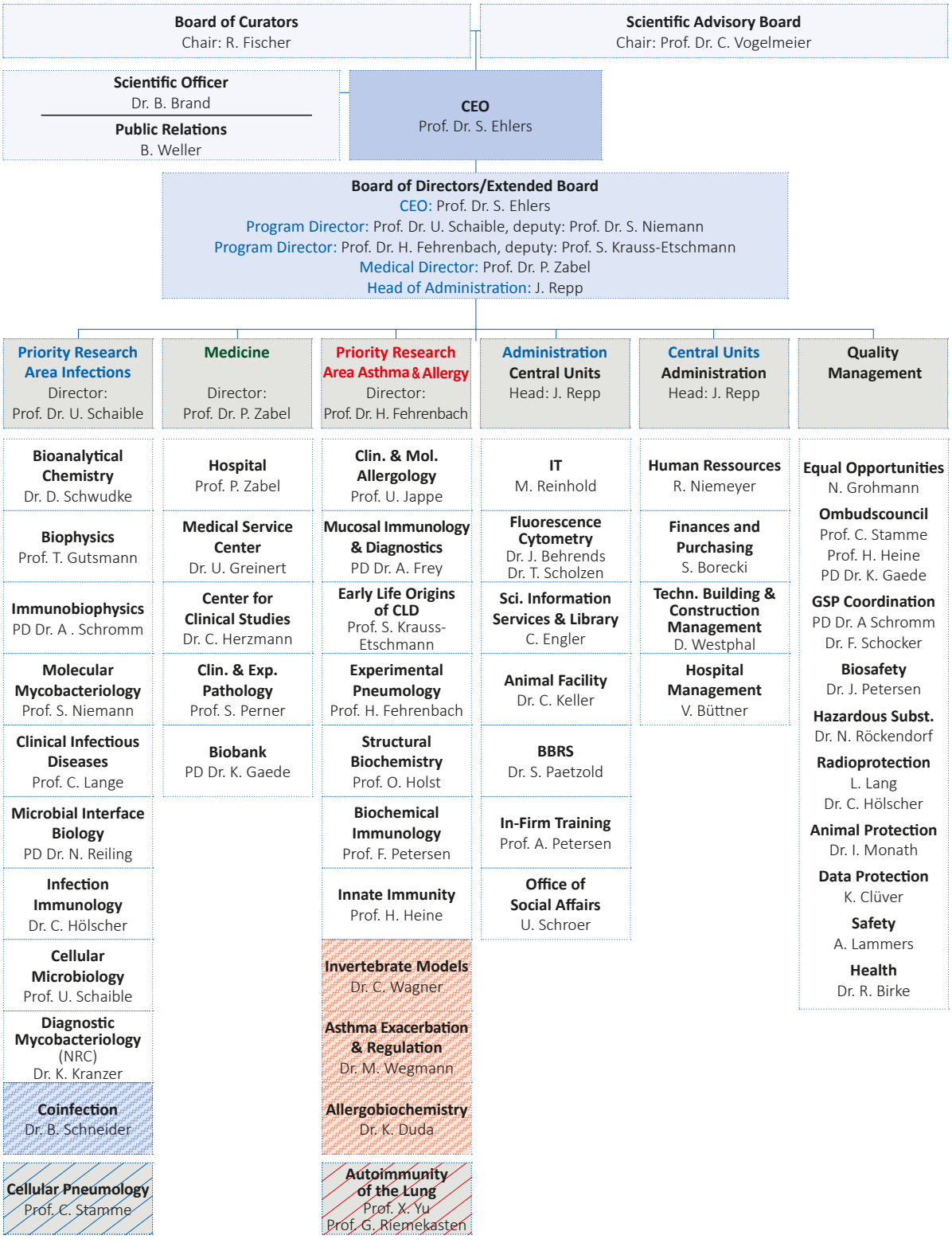
Gates Foundation
- CRyPTIC
  comprehensive resistance prediction of tuberculosis: an international consortium

WHO
- EXPAND-TB
  technical assistance and support by Supranational Reference Laboratory in Borstel

Danish Agency for Science
- Nanovaccines – imaging vaccine efficacy

7th EU-Frame Program
- Patho-NGen-Trace
  next generation genome based high resolution tracing of pathogens
- NAREB
  nanotherapeutics for antibiotic resistant emerging bacterial pathogens
- COST BM1201
  developmental origins of chronic lung disease

Organization Chart



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