# Contents

Preface 3

Scientific Staff 5

Research Groups
1. Prof. Dr. Dieter Adam 7
2. Prof. Dr. Sabine Adam 13
3. Prof. Dr. Ottmar Janßen 17
4. PD Dr. Stefan Jenisch, Dr. E. Susanne Quabius 33
5. Dr. Christiane Kling, Dr. Matthias Marget 37
6. Prof. Dr. Stefan Schütze 43
7. PD Dr. Daniela Wesch, Prof. Dr. Dietrich Kabelitz 57

Appendix
1. Institute Seminars 2007 and 2008 - Invited Speakers 69
1a. Scientific Events 72
2. Completed MD and PhD Theses 2007 and 2008 74
3. Awards 74
4. Additional Scientific Activities 75
5. Impactfactors and Grants (Summary 2007 and 2008) 77
6. Publications 2007 and 2008 77
Contact

Institute of Immunology
Universitätsklinikum Schleswig-Holstein
Campus Kiel
Michaelisstrasse 5
D-24105 Kiel
Germany

phone: +49 (0) 431/597-3341
fax: +49 (0) 431/597-3335
email: office@immunologie.uni-kiel.de
http: www.immunologie-kiel.uk-sh.de
Preface

The fourth bi-annual Research Report of the Institute of Immunology covers the years 2007 and 2008. The major research interests of our institute continue to focus on molecular pathways of caspase-dependent and -independent cell death, intracellular signaling pathways as exemplified by death receptor signaling, and T-cell biology with special attention given to the characterization of human γδ T-cells. The Collaborative Research Center (SFB) 415 “Specificity and Pathophysiology of Signal Transduction Pathways” was successfully reviewed and recommended for a final fourth funding period which now runs until June 2010. Additional financial support for our research was provided by the Deutsche Forschungsgemeinschaft through individual grants and the Clinical Research Group KFO 170, by the Werner-and-Klara-Kreitz Foundation, and by intramural support from the Medical Faculty. Several researchers of our institute are members of the Cluster of Excellence “Inflammation-at-Interfaces” which was granted by the Deutsche Forschungsgemeinschaft in late 2007. Financial support provided by the cluster enabled us to up-grade the 2-D DIGE proteomic facility at our institute with Ettan Digester and Ettan Spotter equipment for automated sample processing.

This Research Report summarizes our scientific activities in the years 2007 and 2008. Please visit our homepage (www.immunologie-kiel.uk-sh.de) for further information, and do not hesitate to contact us if you have any further question. We always offer motivated diploma, MD and PhD students positions to work with us – just ask us. Last not least, I would like to thank Birgit Schlenga for preparing this Report.

Kiel, October 2009

Dieter Kabelitz
Scientific Staff Members 2007/2008

Scientists:

Abukishe, Ahmad, Dr. med.
Adam, Dieter, Prof. Dr. rer. nat.
Adam, Sabine, Prof. Dr. rer. nat.
Beetz, Susann, Dr. rer. nat. (until 01/08)
Bertsch, Uwe, Dr. rer. nat. (DFG)
Fritsch, Jürgen, Dr. rer. nat. (DFG)
Gesch, Gernot, Dr. med.
Heidebrecht, Hans-Jürgen, Dr. rer. nat. (associated)
Janßen, Ottmar, Prof. Dr. rer. biol. hum. – Head of Molecular Immunology (since 03/06)
Jenisch, Stefan, PD Dr. med.
Kabelitz, Dietrich, Prof. Dr. med. - Director
Kling, Christiane, Dr. med.
Lange, Arne, Dr. rer. nat. (DFG) (until 03/08)
Lengl-Janssen, Beate, Dr. rer. nat. (until 03/08
Leptin, Tatiana
Lettau, Marcus, Dr. rer. nat. (DFG)
Marget, Matthias, Dr. rer. nat.
Oberg, Hans-Heinrich, Dr. sc. hum. (DFG)
Quabius, Elgar Susanne, Dr. rer. nat. (Medical Faculty)
Schütze, Stefan, Prof. Dr. rer. nat. – Vice Director
Steinmann, Jörg, Prof. Dr. med. (until 09/07)
Tchikov, Vladimir, Dr. (DFG)
Thon, Lutz, Dr. rer. nat. (Research Commission Medical Faculty) (until 07/07)
Wesch, Daniela, PD Dr. rer. nat.
Westphal, Eckhard, Dr. med.
Winoto-Morbach, Supandi, Dr. rer. nat.
Guest Scientists:
Yan Xiaoyi, PhD, Zhejiang University, School of Medicine, Hangzhou, China
Justyna Sosna, Student, University of Silesia, Faculty of Biology and Environmental Protection
Oliver Merkel, AG Wulf Schneider Institut für Med. Mikrobiologie und Hygiene, Klinikum der Universität Regensburg, Regensburg

PhD Students:
Edelmann, Bärbel, Master of Science (DFG)
Fazio, Juliane, Dipl.-Biol. (DFG)
Lettau, Marcus, Dipl.-Biol. (DFG until 05/07)
Marischen, Lothar, Dipl.-Biochem. (DFG)
Meyer, Tim, Dipl.-Biol.(DFG & Medical Faculty)
Paulsen, Maren, Dipl.-Biochem.
Peters, Christian, Dipl.-Biol. (Medical Faculty since 07/08)
Puchert, Malte, Dipl.-Biol.
Philipp, Stephan, Master of Science (since 06/08)
Schmidt, Hendrik Dipl.-Biochem. (Medical Faculty until 06/09)
Stephan, Mario, Dipl.-Biochem. (DFG)

MD Students:
Frederiksen, Marie-Catherine
Hellmich, Isabel
Jurike, Matthias
Linkermann, Andreas
Mathew, Biny
Pietschmann, Kathrin
Reinicke, Maike
Seifert, Ulrike
Shojaei, Hamed
Welte, Stefan
Wrobel, Philine

Diploma Students:
Jung, Andrea
Koop, Anja
Latendorf, Ties
Lepenies, Inga
Voss, Matthias
Pieper, Jennifer
Sellami, Nadia
Tartanikova, Anastasia

B. Sc. Students:
Voigt, Susann
M. Sc. Students:
Philipp, Stephan (until 01/08)
1. Research Group D. Adam

A Group Leader: Prof. Dr. rer. nat. Dieter Adam

B Lab Members: Scientists: Dr. rer. nat. Lutz Thon (Research Commission of the Medical Faculty, Kiel, until 07/2007)

Ph. D. Students: Dipl.-Biol. Malte Puchert (Research Commission of the Medical Faculty, Kiel, DFG, from 06/2008)

Diploma Student: Andrea Jung (11/06 – 03/07)

B.Sc. Student: Susann Voigt (08/08 – 01/09)

M.Sc. Students: Stephan Philipp (04/07 – 01/08)
Justyna Sosna (from 04/09)

Medical Students: Ulrike Wehkamp (until 04/07)
Marie-Catherine Frederiksen (until 01/09)
Annika Hecht (from 04/09)

Technicians: Sabine Mathieu (Research Commission of the Medical Faculty, Kiel)
Kyoung-Ae Yoo-Ott (from 10/07)

C Research Report:

C. 1. Signal transduction through the 55 kDa TNF receptor: molecular and functional characterization of components of the N-SMase pathway (DFG, Research Commission of the Medical Faculty, Kiel)

Loss of function of the lipase neutral sphingomyelinase (N-SMase, Smpd3) manifests itself in severe developmental defects. The N-SMase signaling pathway, which leads to activation of N-SMase by the 55 kDa tumor necrosis factor (TNF) receptor (TNF-R55), as well as the physiological significance of N-SMase is only partially understood. A protein that we have identified as an interaction partner of N-SMase could represent the last missing link and thereby complete the signaling chain from TNF-R55 to N-SMase, simultaneously connecting the N-SMase pathway with integrins. One of our goals is therefore to further characterize this protein and its role within the N-SMase pathway. Independently, we have recently shown that another component of the N-SMase pathway, the protein FAN, has as yet unknown functions in the regulation of lysosome size. By comparative analyses of the lysosomal proteomes of wildtype and FAN-deficient cells, we will to identify candidate proteins that participate in the
size regulation by FAN. Furthermore, the functional inactivation of individual components of the N-SMase signaling pathway will provide deeper insight into the relevance of these components and the N-SMase pathway in general with regard to immunologically important processes such as regulation of lysosome size or integrin-mediated cell adhesion.

Fig. 1: Lysosome size in FAN-deficient cells and their wildtype counterparts. Lysosomes of FAN-wildtype (FAN+/+, A) and FAN-deficient (FAN-/-, B) cells were stained with LysoTracker Red and visualized by confocal laserscanning microscopy. Bar, 10 µm. (C) The average lysosome size is shown for 6 individual FAN+/+ and FAN-/- cells.

C. 2. Analyses of the in-vivo function of the proinflammatory lipid ceramide in septic and hyperacute shock (Research Commission of the Medical Faculty, Kiel)

As a consequence of a systemic inflammation reaction which is mostly caused by bacterial infection, septic shock is responsible for 40,000 fatalities in Germany alone and - being the third-most cause of death - represents a major problem for the public health care systems. As
a central mediator of inflammation, the cytokine tumor necrosis factor (TNF) is discussed as a causative factor in the pathogenesis of septic shock. We have shown in previous work that TNF mediates its organ-damaging effects through a signaling pathway which involves the 55 kDa TNF receptor (TNF-R55), the enzyme PC-PLC and the sphingolipid ceramide. As a signaling molecule of the inflammatory response, ceramide regulates the expression of inflammation-relevant genes as well as the apoptotic demise of cells. Experiments to ameliorate apoptotic damage during septic shock by inhibiting caspases astonishingly did not show an improvement but rather a dramatic acceleration of the clinical symptoms (hyperacute shock). This is attributed to the induction of alternative signaling pathways which elicit caspase-independent cell death. Our group has recently shown that ceramide is a central mediator of these signaling pathways. In first preliminary in-vivo experiments, we have correspondingly observed an increased resistance of mice with genetic deficiencies in ceramide metabolism against hyperacute shock and also against bacterially induced sepsis. In this project, we therefore want to characterize the in-vivo role of ceramide in TNF- or bacterially induced septic and hyperacute shock in the available mouse models, as well as to answer the question whether pharmacological interference with ceramide production can increase the survival of wild type mice. In the long run, the planned studies will hopefully prove beneficial for the future treatment of sepsis patients.

C. 3. Characterization of TRAIL-induced ceramide-mediated caspase-independent programmed cell death (Research Commission of the Medical Faculty, Kiel)

Death receptors such as the 55 kDa tumor necrosis factor (TNF) receptor (TNF-R55) or Fas can initiate both apoptotic (caspase-dependent) and caspase-independent routes to programmed cell death (PCD). Our group has recently demonstrated for the first time that the single murine receptor for (TNF)-related apoptosis-inducing ligand (mTRAIL-R2) can induce a caspase-independent form of PCD with necrosis-like features in addition to apoptosis. Analysis of morphological and cellular features of caspase-independent PCD in response to TRAIL and TNF suggests that mTRAIL-R2 and TNF-R55 elicit caspase-independent PCD through similar pathways, although without participation of cathepsins. Cells overexpressing acid ceramidase (AC), an enzyme that metabolizes the sphingolipid ceramide, show enhanced survival from TRAIL-induced caspase-independent PCD but not from apoptosis, implicating a function of ceramide as a key mediator in caspase-independent PCD (but not apoptosis) induced by mTRAIL-R2. In this project we characterize the corresponding signaling
pathways with regard to similarities and differences between the two death receptors as well as the exact role of ceramide in these pathways.


Programmed cell death (PCD) is essential for the elimination of unwanted or potentially dangerous cells such as tumor cells and thus vital for the survival of an organism. Parallel to “classical”, caspase-dependent apoptosis, we and others have shown the existence of “alternative,” caspase-independent forms of PCD (ciPCD). These alternative forms of cell suicide provide redundancy for incidents in which the apoptosis machinery of the cell is blocked or inactivated. Due to mutations, this is frequently the case especially in tumor cells. Therefore, at the clinical level, the induction of ciPCD could represent an additional option to eliminate such apoptosis-resistant tumor cells. In previous work, we have confirmed that ciPCD, induced by the cytokines TNF and TRAIL can eliminate established laboratory tumor cell lines and that the sphingolipid ceramide is essential for this process. In our model systems, the induction of ceramide-mediated ciPCD additionally led to a pronounced reduction of clonogenicity of these tumor cells. In this project, we will therefore extend our results to clinically more relevant cell systems (susceptibility of tumor cells and -lines), also to clarify whether - and how efficient - an elimination of these tumor cells can be achieved by inducing ceramide-mediated ciPCD. By these studies, we will furthermore identify additional potential components of the involved signaling pathways and thus potential starting points for novel therapeutic approaches.

D Publications

2007

2008

E Grants

E.1. Molekulare Charakterisierung der Rolle von WD-repeat Proteinen in der Aktivierung von Natürlichen Killerzellen durch Tumor Nekrose Faktor
Hensel-Stiftung

E.2. Signaltransduktion durch den 55 kDa TNF-Rezeptor: Molekulare und funktionelle Charakterisierung von Komponenten des N-SMase Signalwegs
Research Commission of the Medical Faculty, Kiel
53.000 € (2007)

E.3. Charakterisierung des TRAIL-induzierten Ceramid-vermittelten Caspase-unabhängigen programmierten Zelltods
Research Commission of the Medical Faculty, Kiel

Werner und Klara Kreitz-Stiftung
7.500 € (2008-2009)

E.5. Untersuchungen zur in-vivo Funktion des proinflammatorischen Lipids Ceramid im septischen und hyperakuten Schock
Research Commission of the Medical Faculty, Kiel
116.000 € (2008-2010)

E.6. Signaltransduktion durch den 55 kDa TNF-Rezeptor: Molekulare und funktionelle Charakterisierung von Komponenten des N-SMase Signalwegs
DFG
166.750 € (2008-2011)

E.7. Signaltransduktion durch den 55 kDa TNF-Rezeptor: Molekulare und funktionelle Charakterisierung von Komponenten des N-SMase Signalwegs
Research Commission of the Medical Faculty, Kiel
14.000 € (2008-2010)
2. Research Group S. Adam

A Group Leader: Prof. Dr. rer. nat. Sabine Adam

B Lab Members: Ph. D. Student: Dipl. Biol. Juliane Fazio (KFO 170, from 05/2008)

Diploma Students: Nadia Sellami (05/2007 - 03/2008)
Inga Lepenies (10/2007 - 06/2008)
Anja Koop (from 11/2008)

Technicians: Sandra Ussat (until 09/2007)
Parvin Davarnia (from 09/2007)
Gudrun Scherer

C Research Report:

C.1. The role of caspases for T cell proliferation

The activation of proapoptotic caspases is essential for activation-induced proliferation of mature T lymphocytes. It has been puzzling, however, how the T cells ensure their survival in the presence of active caspases, especially the deathly effector caspases-3 and -7. We have shown that caspase-3/7 activity is restrained by interaction with the endogenous caspase inhibitor XIAP thereby preventing apoptosis to occur (Fig. 1). This correlates with a strict cytoplasmic localization of active caspases in proliferating T cells as compared to a broad distribution in apoptotic cells. After the induction of apoptosis, the XIAP antagonist Smac/DIABLO is released from mitochondria, blocking XIAP and, thereby, enabling full activation of caspases-3/-7, pronounced substrate cleavage and cell death (Fig. 1; Paulsen et al., 2008). In future studies, we plan to identify proteins that interact with active caspase-3 and -8 and, therefore, might represent substrates of the respective caspases. This will be achieved using the biotinylated pseudo-substrates biotin-VAD, biotin-IETD (selective for caspase-8), or biotin-DEVD (selective for caspase-3/7) followed by precipitation with streptavidin sepharose.
C. 2 T cell tolerance mediated by anti-HLA-DR treated monocytes

In collaboration with Dr. Martin Kriegel (Harvard Medical School, Boston, MA, USA), we investigate T cell anergy mediated by monocytes. Anti-HLA-DR treatment generates tolerogenic monocytes that transmit T cell anergy coinciding with early and transient ERK activation and upregulation of the cell cycle inhibitors p21cip and p27kip. Caspase activation known to be essential for T cell activation, however, is non-specifically decreased in the anergic T cells merely reflecting the lack of proliferation. This work is completed and published (Kriegel et al., 2007).

C. 3 Analysis of different splice variants of the protein kinase IKKε

The expression of type I interferons is essential for an effective anti-viral immune response. The transcriptional activation of the respective genes is achieved by the transcription factors IRF3/7, which are activated by phosphorylation leading to their dimerization and translocation into the nucleus. The serine/threonine kinase IKKε directly phosphorylates IRF3 and, thus, plays an important role in the induction of anti-viral immunity. Moreover, IKKε appears to be involved in the activation of NF-κB. We have identified two novel isoforms of
IKKε that are generated by alternative splicing of the IKKε-coding mRNA leading to a truncated C-terminus. Overexpression of full-length IKKε leads to the activation of NF-κB and IRF3. While none of the isoforms activates IRF3, one splice variant still activates NF-κB, while the other isoform does not. Current studies focus on the molecular mechanism of this functional dichotomy and investigate the expression of the various isoforms in different cell types and after various stimulations.

C. 4 Characterization of in vitro generated Proteinase 3-specific effector-memory T cells

The autoimmune disease Wegener’s Granulomatosis (WG) is mainly mediated by autoreactive antibodies against proteinase 3. Although proteinase 3-specific CD4+ effector-memory T cells expand early after onset of the disease, their role in the pathogenesis of WG remains unclear. In collaboration with the group Kabelitz/Wesch, various expression constructs for proteinase 3 will be generated and transfected into dendritic cells either directly or after translation into RNA. The dendritic cells will then be used for in vitro generation of effector-memory T cells. The detailed characterization of these T cells will help to understand the functional relevance of autoreactive T cell expansion for the development of WG.

D Publications

2007

2008


E Grants

E. 1. Untersuchungen zur Funktion und Regulation des neuen Cancer/Testis Antigens CT45 Medical Faculty (Collaboration with Dr. H.-J. Heidebrecht, Pathology) 28.400 € (2007)
3. **Research Group O. Janssen (Molecular Immunology)**

A **Group Leader:** Prof. Dr. rer. biol. hum. Ottmar Janßen

B **Lab Members:**

**Scientists:**
- Dr. rer. nat. Marcus Lettau (DFG, SFB 415)
- Dr. rer. nat. Beate Lengl-Janßen (Medical Faculty)
- Dr. rer. nat. Hans-Jürgen Heidebrecht (associated)

**Ph. D. Students:**
- Dipl. Biochem. Hendrik Schmidt (Medical Faculty*)
- Dipl. Biochemist Maren Paulsen (Medical Faculty*)

**Master of Science:**
- Stephan Phillip (Medical Faculty)

**M.D. Students:**
- Andreas Linkermann (Dr. med. in 2008)
- Biny Mathew (Dr. med. in 2008)

**Diploma Students:**
- Ties Latendorf (Biochemistry)
- Jennifer Pieper (Biochemistry)
- Matthias Voss (Biochemistry)

**Technicians:**
- Alyn Beyer (DFG, SFB 415)
- Ulrike Naumann (Medical Faculty*)
- Signe Valentin (Medical Faculty*)
- Melanie Nebendahl (Medical Faculty)

*in course of the appointment of OJ as Professor for Molecular Immunology

C **Research Report**

C.1. **Regulation of expression and signal transduction of the FasL in T cells and tumor cells**

In this project, we focus on FasL (TNFSF6) as a key regulator of immune cell homeostasis. In T cells, FasL serves as an apoptosis-inducing effector protein and as a costimulatory molecule. In recent years, we identified several interactors of the cytosolic poly-proline region of FasL that turned out to be important for the storage and transport but also for reverse signal transduction of the death factor. We presently characterize individual protein-protein interactions *in vitro*, in transfectants and in primary T cells. We developed and collected a series of tools (expression constructs, fusion proteins, monoclonal antibodies, T cell clones and lines) that enable us to investigate interactions of endogenous proteins in T lymphocytes
and tumor cells. At present, we focus on a set of structurally related FasL interactors belonging to the so-called PCH protein family and on the adapter protein Nck. We know from previous studies that several PCH proteins target FasL to the lysosomal compartment in transfectants resulting in reduced FasL surface expression (Fig. 1) and cytotoxic potential.

**Fig. 1.** PCH proteins target FasL to the lysosomal compartment in transfectants resulting in reduced FasL surface expression. (Confocal image by M. Lettau)

In addition, ongoing studies by Dr. Marcus Lettau and Alyn Beyer, employing our own monoclonal antibodies, already revealed that PCH proteins (e.g. CD2BP1 and CIP4) are regulated in an activation-dependent manner in primary T cells. In the case of CIP4, we also observed an association with microvesicles that are released from T cells early during the onset of apoptosis. In order to better understand the protein networks formed around FasL binding PCH proteins or Nck, we analyzed their interactomes using pulldown approaches with individual interaction domains (see below).

In the course of our FasL studies, Dr. Marcus Lettau developed a cell-based assay to monitor stimulation-dependent FasL surface expression in activated T cells in the presence or absence of pharmacological agents. In collaboration with Prof. Dr. Paul Saftig and Dr. Karina Reiss (Biochemical Institute, CAU Kiel), he used this system to identify ADAM10 as a FasL sheddase during his PhD work. We learned that ADAM10 also cleaves FasL from activated T cells. However, the physiological relevance of FasL shedding and the fate of the resulting extra- or intracellular FasL fragments are still under investigation (Fig. 2).
Fig. 2: FasL proteolytic processing. Recent data suggest that FasL is processed by ADAM10 and RIPped by SPPL2a generating a free intracellular domain. This ICD is assumed to translocate to the nucleus to regulate gene expression, a process resembling the well characterised Notch signalling pathway. Since FasL may associate with SH3 domain proteins and since these interactions significantly affect FasL biology, including sorting, activation-induced release and reverse signaling, further studies have to determine how the processing of FasL affects interactions with SH3 domain proteins. Furthermore, it is unclear to date, which interactions regulate the trafficking of processed FasL fragments. (For more details, see Voss et al., 2009)

To address these issues, we meanwhile generated a monoclonal anti-FasL antibody that reacts with the intracellular portion of the molecule and thus enables us to discriminate between full length FasL and N-terminal fragments. From a study of our collaborator PD Dr. Marin Zörnig (GSH Frankfurt), we learned that upon shedding of FasL by ADAM 10, the N-terminal remnant is further processes by the secretase-like protease SPPL2a. Using a phage-display approach, Matthias Voss presently searches for novel FasL interactors that might selectively bind to FasL NTFs. Maren Paulsen (PhD thesis) and Biny Mathew (MD thesis), together with Ulrike Naumann and Signe Valentin, followed our previous observation that FasL and also Fas (APO-1, CD95) modulate the TCR-induced activation of resting primary human T cells. We found that FasL ligation by plate-bound but not soluble fusion proteins or monoclonal antibodies interferes with T cell activation at an early stage of signal induction (Fig. 3).
Fig. 3: FasL as a positive (A) or negative (B) costimulatory molecule in T cell activation. Studies with murine CD8+ T cells revealed a costimulatory capacity of FasL on signal transduction in TCR-stimulated cells, associated with enhanced MAPK, NFkB and PLCγ activation. In this scenario also the phosphorylation of FasL itself through the CK1 as well as the enhanced WASP activation relevant for TCR clustering seems to be important (A). In contrast, FasL has been shown to completely block T cell activation (B). In human CD4+ cells, cell cycle arrest appears to be caused by reduced WASP/Arp2/3-mediated TCR internalisation and inhibition of PLCγ and MAPK activation, possibly initiated through an interaction with the adaptor protein CD2BP1 and the tyrosine phosphatase PTP-PEST. (For more details, see Voss et al., 2009)

Interestingly, Fas costimulation results in either a block or an enhancement of T cell activation. Briefly, the simultaneous ligation of Fas by FasLFc fusion proteins abrogates primary T cell activation whereas ligation by agonistic anti-APO-1 antibodies massively augments cell cycle progression in the presence of anti-CD3 and anti-CD28 antibodies.

C.2. Networks in signal transduction: identification of interaction partners for SH3 and WW domains by proteome analyses

A longterm goal of our group is the identification of protein-protein-interactions mediated by small modular interaction domains. Here, we mainly focus on so-called Src homology 2 and 3 (SH2, SH3) and WW domain proteins, since many of those are present in the described FasL interactors. With pull-down analyses followed by standard SDS-PAGE and MALDI-TOF-based peptide mass finger printing, Andreas Linkermann and Jennifer Pieper collected information about potential protein networks around FasL interactors. From different T cell populations (including PHA blasts, Jurkat or HUT78), they precipitated SH3-binding proteins of PCH proteins (Andreas Linkermann, MD thesis), and SH2 or SH3 binding proteins of the adapter protein NCK (Jennifer Pieper, Diploma thesis, Fig. 4). The obtained results clearly
confirmed the expectation that all these proteins form complexes that are involved in the regulation of the actin cytoskeleton, of vesicular trafficking and endo- or exocytosis.

**Fig. 4: Pull-Down experiment to identify Nck interacting proteins from PHA blasts.** Proteins were visualized using Flamingo Pink (for further details including the identification of individual proteins annotated by numbers in purple, see Diploma thesis of Jennifer Pieper).

The identification and ongoing functional characterization of interaction partners of the adapter protein Nck is part of a “junior research project” of Dr. Marcus Lettau. In the case of PCH proteins, the collected data as described in the context of FasL storage and transport, the available new monoclonal antibodies and the identification of various SH3 domain interaction partners prompted us to submit a grant application to the DFG to address the expression and function of PCH proteins in T cells.

### C.3. Establishment of an in-house 2D-DIGE facility

In 2006, the 2D-DIGE facility was established as part of the research focus “Transplantation and Oncology” of the Medical Faculty of the University Hospital Schleswig-Holstein Campus Kiel. Sponsored by the Cluster of Excellence "Inflammation at Interfaces" of the Christian-Albrechts University, the platform was upgraded for automated in gel digestion spotting to MALDI grids (Fig. 5). Within the cluster, we have established a successful collaboration with
the MALDI group at the Zoological Institute of the CAU (Prof. Dr. Matthias Leippe and Dr. Christoph Gelhaus) and thus became able to identify large numbers of proteins by peptide mass fingerprinting or MS/MS analyses.

**Fig. 5:** Upper panel: Basic principle of the 2D difference gel electrophoresis (2D-DIGE). Lower panels: Ettan® equipment and sponsors of the unit. (©GE Healthcare)

Besides our own studies aimed at the characterization of secretory vesicles in T cell subsets and NK cells and at the identification of Marker proteins for T cell subsets (see below), a number of cooperations were established with various members of the Cluster. We perform analyses for at least ten unrelated projects in collaboration with groups from Hamburg, Lübeck, Borstel and Kiel (from peanut allergens to plasmodial proteins, from drug-treated tumor cells to different strains of Entamoeba, from metalloproteinase substrates to various knockout mice). Some of these analyses are listed below (see C.7.) and proved already quite successful and are now processed for publication.

**C.4. Proteome analysis of secretory lysosomes**

Cytotoxic effector cells represent the cellular end-point for an efficient elimination of virally transformed or malignant cells by the immune system. After specific recognition, cytotoxic T lymphocytes and Natural Killer (NK) cells have to ensure the rapid destruction of potentially dangerous target cells. Therefore, T and NK cells use a unique vesicular compartment that combines properties of conventional lysosomes and exocytotic vesicles. Secretory lysosomes
contain soluble and membrane-associated cytotoxic effector molecules including perforins and granzymes and the death factor FasL as an integral transmembrane component.

Fig. 6: 2D-DIGE analysis of enriched SLs from NKL and YTS cells in comparison with expanded NK cells. Fifty microgram each of enriched SL preparations was labeled with Cy5, Cy3, and Cy2, respectively. (A) Overlay of three individual channels with the following code: NK/Cy5/red, NKL/Cy3/blue, and YTS/Cy2/green. (B) Cy3 image with SL from NKL cells. (C) Cy5 image with SL from NK cells and (D) Cy2 image with SL from YTS cells. One representative experiment out of three is shown. Spots with a difference in spot volume greater three-fold were marked for NK and NKL cells (B) or for NK and YTS cells (D). Based on the expression in NK cells, increased spots in leukemic cells are displayed in blue and decreased spots in red. (For more details, see Schmidt et al., Proteomics 2008)

Based on previous experiments by Dr. Marcus Lettau, Hendrik Schmidt and Melanie Nebendahl initially enriched this lysosomal compartment from primary NK cells and NK cell lines to compare the normal and diseased vesicular proteome. Since FasL is regarded as a marker for secretory vesicles in T and NK cells, we initially checked individual fractions obtained by differential and density gradient centrifugation for the presence of FasL. Subsequently, using 2D-DIGE technology (see below), FasL-containing LAMP1-positive fractions of the individual populations were compared. With the help of Dr. Christoph Gelhaus (Zoological Institute of the CAU), we were able to identify a large number of characteristic lysosomal proteins and describe significant differences in the composition of cytotoxic versus immunoregulatory components within the isolated vesicles. Of note, the
detected alterations relate to reported defects in these cell lines. At present, Hendrik Schmidt and Melanie Nebendahl extend their analyses to various T cell subpopulations.

C.5. **Identification of molecular markers for human regulatory T cells**

Regulatory T cells (Treg) play a central role in the control of immune responses. They prevent the activation of surrounding effector T cells. At present, they are regarded a major target cell population for therapeutic immune intervention. To fight autoimmune diseases, allergy and transplant rejection, an increase in the number of Tregs would be beneficial. In contrast, a blockade of Treg function would potentially enable more efficient anti-tumor or anti-inflammatory responses. Although Tregs became a major focus of many laboratories worldwide, so far there is no specific maker for this subpopulation of CD4-positive cells. Tregs express high levels of CD25 and several other more or less characteristic surface proteins. Of note, none of these surface proteins is only present on Tregs. Therefore, there is an obvious need for the identification of a lineage-specific Treg marker protein. In the course of our project, which was performed by Dr. Beate Lengl-Janßen in collaboration with PD Dr. Daniela Wesch and Dr. Hans-Heinrich Oberg, we used purified Treg populations from peripheral blood and corresponding CD4-positive responder cells of healthy individuals. The idea to simply perform 2D difference gel electrophoresis comparing regulatory and conventional T cells was hampered by the very low cell numbers of freshly isolated Tregs, and the incompatibility of the resulting minute amounts of protein to direct CyDye labeling and standard IEF. In addition, most of the proteins that were identified in collaboration with Dr. Christoph Gelhaus turned out to be cytosolic proteins (Fig. 7). Although, Dr. Lengl-Janßen successfully established several methods to enrich for plasma membrane-associated proteins, she still faced the problem of low cell numbers. We will therefore follow the established protocols using leukapheresis material that would yield much higher cell numbers and much more protein in individual isolates.
Fig. 7: 2D-DIGE comparison of regulatory and responder T cells. Fifty microgram of whole cell lysates was labeled with Cy5 and Cy3 respectively. (Images by Dr. Beate Lengl-Janßen)

C.6. Proteome analysis of cell adherence-mediating plasma membrane proteins on *P. falciparum*-infected human erythrocytes

A fatal event during *Plasmodium falciparum*-induced Malaria is the interaction of plasmodial proteins on the surface of infected erythrocytes with different receptors on host cells or endothelia. The adhesion of infected erythrocytes induces local inflammation often followed by capillary occlusion, which eventually can result in multi organ failure. The protein composition of the interface between infected erythrocytes and host tissue cells has only been poorly analyzed at fairly low resolution. Thus, plasmodial proteins that are transported to the erythrocyte surface to serve as anchor proteins have not yet been identified or functionally characterized. New approaches for the isolation of and electrophoretic separation and sensitive detection of transmembrane or membrane-associated proteins have not been applied in this context. In our project, we want to determine and quantitate the proteome of this interphase compartment. In the context of putative targets for therapy, we are mainly interested in plasmodial proteins that appear on the erythrocyte surface. The characterization of the interface proteome is performed as a close collaboration between the Molecular Immunology group (2D-DIGE platform) and the Department of Zoophysiology (MALDI platform). Stephan Phillip meanwhile established a modified method to obviously enrich membranes and membrane-associated proteins from uninfected and infected erythrocytes.
These isolates can be separated by 2D electrophoresis (Fig. 8) and it will be tested soon whether they contain enough membrane proteins to directly identify the plasmodial proteins on erythrocyte membranes by 2D-DIGE analyses.

![Fig. 8: 2D electrophoresis of putative membrane preparations from uninfected (RBC) and Plasmodium falciparum-infected red blood cells (iRBC). (Images by Stephan Phillip)](image)

C.7. 2D-DIGE cooperative projects

As mentioned, the 2D-DIGE technology has been successfully established within the last two years as an integral part of the Molecular Immunology laboratory group. This was possible only with the enormous engagement of Hendrik Schmidt who – still being a PhD student doing his own research – eventually had to manage four different 2-D collaborations at a time. This of course would not have been possible without the expert technical assistance by Melanie Nebendahl. Of note, in most cases, subsequent MALDI analyses were performed once more in collaboration with Dr. Christoph Gelhaus and Prof. Dr. Matthias Leippe at the Zoological Institute of the Christian-Albrechts University. Besides the already mentioned projects of our group, we presently work on a number of interesting projects with partners from Kiel, Borstel, Lübeck, Hamburg and Heidelberg (only more advanced studies are listed in the following):

- Proteome analyses of peanut extracts – characterization of peanut allergens in conventional peanuts and varieties from Indonesia, especially Bali (Fig. 9, Diploma thesis of Ties Latendorf, cooperation with Dr. Wolf-Meinhard Becker, FZ Borstel)
Fig. 9: 2D-Topology of allergens in basic peanut extracts. (From Schmidt et al., Proteomics 2009)

- differential analyses of benign and malign variants of pancreas carcinoma cells and pancreas carcinoma cells exposed to therapeutic drugs (Fig. 10, cooperation with Prof. Dr. Holger Kalthoff, Molecular Oncology and Dr. Ole Ammerpohl, Human Genetics)

Fig. 10: 2D-DIGE analysis of pancreatic tumor cells. A818-6 cells, growing as monolayer or in hollow spheres, were lysed and subjected to 2D-DIGE. (Images by Holger Kalthoff and colleagues, 2D gels Hendrik Schmidt and Melanie Nebendahl)

- Comparison of two genetically related *Entamoeba histolytica* cell lines derived from the same isolate with different pathogenic properties (Fig. 11, cooperation with PD Dr. Iris Bruchhaus BNI, Hamburg)
Fig. 11: 2D-DIGE analyses of *Entamoeba histolytica* cell lines. Sublines of Amoeba displaying different virulence were lysed and subjected to 2D-DIGE. (2D gels by Laura Biller (BNI), Hendrik Schmidt and Melanie Nebendahl)

C.8. Characterization of the cancer/testis antigen CT45

The monoclonal antibody Ki-A10, generated by immunizing mice with lysates of the Hodgkin's lymphoma-derived cell line L428, detects a nuclear antigen with a unique distribution pattern in normal human tissues. The antigen is exclusively expressed in premeiotic precursors during spermatogenesis but is totally absent in normal human somatic tissues. Most human tumors also do not express this antigen. Only a proportion of germ cell–derived tumors and some malignant Hodgkin’s lymphomas are stained by Ki-A10. This distribution pattern is reminiscent of that exhibited by the so-called cancer/testis antigens. Therefore, the antigen has been termed cancer/testis antigen 45 (CT45). Cancer/testis antigens, a rapidly expanding family of tumor-associated proteins, are of broad interest because of their diagnostic and possible therapeutic implications. In recent experiments, Dr. Hans-Jürgen Heidebrecht and colleagues could inhibit or induce CT45 expression in various cell types and were able to demonstrate that CT45 expression is regulated by methylation (Heidebrecht et al., Clin Cancer Res, 2006, 16: 4804-4811). As for many other CT-antigens, the biological function of CT45 is completely unknown. These experiments offer the possibility to get first informations about the function of CT45. The highly restrictive occurrence of CT45 in normal and in neoplastic cells, such as seminoma and Hodgkin and
Reed-Sternberg cells, justifies a closer look into the biology of the antigen and the diagnostic potential of the mAb Ki-A10.

**Fig. 12: Intracellular localization of CT45.** Ki-A10 staining of 5-aza-2’-deoxycytidine treated and mitogen stimulated peripheral blood lymphocytes. (Confocal image by Dr. Marie-Luise Kruse and Dr. Hans-Jürgen Heidebrecht)

CT45 is already used as an additional marker for possibly highly malignant Lymphomas at the Department of Pathology. As for many other CT-antigens, the biological function of CT45 is completely unknown. Since it is located in association with nuclear speckles (Fig. 12), a role in the regulation of transcription or splicing is suspected. Using a variety of different biochemical and cell biological approaches, we hope to define a biological function for CT45. In addition, we will test whether CT45 might also be used as a marker for high malignancy in other tumor types.
**D Publications**

**2007**


**2008**


Friedrich K, Janssen O, Hass R. Watching molecules talking to each other - Highlights of the 11th Joint Meeting Meeting ‘Signal Transduction: Receptors, Mediators and Genes’. Science Signaling, 1 mr, 2008


E Grants and financial support

E.1. Appointment as W2 Professor for Molecular Immunology. Medical Faculty, 300.000 € (2006-2009)

E.2. Regulation of expression and signal transduction of FasL in T cells and tumor cells. DFG SFB 415, Project A9; 1x BAT II, 19.000 €/year (2007-2010)


E.5. Identification and functional characterization of interaction partners of the adapter protein Nck. Medical Faculty (M. Lettau Junior Research Grant 2008) 20.000 €

E.6. Establishment and coordination of the proteome (2D-DIGE) facility of the research platform Oncology and Transplantation, Medical Faculty, equipment 250.000 €; consumables/staff 80.000 € (2006-2007)


E.8. Upgrade of the proteome (2D-DIGE) facility, Cluster of Excellence Inflammation @ Interfaces, equipment 140.000 € (2007)
4. Research Group Jenisch / Quabius

A Group Leader: PD. Dr. med Stefan Jenisch

B Lab Members: Scientist: Dr. phil. II Elgar Susanne Quabius

Technician: Hilke Clasen

C Research Report:

C. 1. Gene expression profiling in patients with Psoriasis vulgaris

Background: Patients suffering from psoriasis show itching plaque-forming red alterations of the skin (see figure 1) on all body parts. Although the disease is not life-threatening the patients often suffer from not only physiological but also psychological symptoms. Psoriasis vulgaris is a multi-factorial disease with an incidence rate of 2-5% in the Caucasian population. To date no cure of the causes but only remedies to aid the symptoms (itching, plaque reduction) are available. Psoriasis is triggered by environmental and genetic factors, showing all signs of a T-cell mediated autoimmune disease. However, to date no autoantigen has unambiguously been identified. A multitude of pro- and anti-inflammatory cytokines are over-expressed in psoriatic skin with interferon-γ and Tumor Necrosis Factor (TNF) α as key players. Release of these cytokines is triggered by specific T-cell populations and dendritic cells (DCs), accompanied by an increase in CD4+ and CD8+ T-cells.

Lines of research:

A: Genetic analysis

Previous, research based on genetic analysis of over 300 patients suffering from psoriasis and an equal number of healthy controls confirmed the relevance of genes located in the so called PSORS1 (psoriasis susceptibility region 1) region as a genetic prerequisite for the onset of psoriasis. In addition a correlation between psoriasis and certain MHC genes could be determined. Most importantly, however, and of significant importance for the second line of research carried out in our group was the identification of MIC B but not MIC A as a possible candidate marker for psoriasis. Figure 2 shows the results of a Linkage Disequilibrium analysis based on a German and an American cohort. The data show the existence of second region containing the genes for P5-1, TNF α and MIC B, but not MIC A, which based on the colour coding of the used analytical tool (HAPMAX) shows a similar association with psoriasis as the already established PSORS1.
B: Gene expression analysis

In a second line of research we try to establish whether blood can be used as a tool to perform gene expression analysis to identify genes that are differentially expressed in patients suffering from psoriasis when compared to healthy controls. In collaboration with the Dermatology Department of the UK-SH Campus Kiel some 90 blood samples of patients suffering from psoriasis were obtained. Healthy controls were recruited within the Department of Immunology. Based on the above mentioned findings, concerning the potential role of MIC B in the pathogenesis of psoriasis, initial experiments focussed on the expression of this gene. We could, indeed, show that MIC B is overexpressed in 50% of the patients and that this expression pattern is strongly correlated with the expression of interferon $\gamma$. To obtain a more detailed picture of genes differentially regulated in the blood of patients suffering from psoriasis whole genome Microarray analysis was performed and the results also show that MIC B but not MIC A is differentially regulated in psoriatic patients, corroborating the earlier genetic findings which indicate a role of MIC B but not MIC A in the pathogenesis of psoriasis. Figure 3 shows the results of a pathway analysis based on the microarray data, indicating not only differential expression of MIC B but also of NKG2D, and parts of the c-Jun/cFos pathway resulting in up-regulation of IFN $\gamma$ and consequently altered cytokine patterns. Consequently these data support the notion that psoriasis is indeed an autoimmune disease and they show that blood has the potential to be used for marker analysis.
Figure 3: Pathway analysis of microarray data. Microarray data, analysed on an Affymetrix human whole Genome Chip HG-U-133+2.0, were obtained from blood of either healthy controls (n=6) of patients suffering from psoriasis (n=12). Gene Chip data normalized for genes showing at least a 2 fold change in their expression pattern were analysed with a Trial version of the GeneGO microarray analysis software MetaCore. Genes highlighted by a thermometer are differentially expressed.

D Publications

2007

2008

E Grants

E. 1. Intramurale Fakultätsförderung im Forschungszentrum Entzündungsmedizin 132800€ until December 2009 to cover personnel (E.S. Quabius) and consumables
6. Research Group Marget/ Kling

A Group Leaders: Dr. rer. nat. Matthias Marget / Dr.med. Christiane Kling

B Lab Members:

Medical doctors: Tatiana Leptin
   Julia Magez

Technicians: Andrea Iwersen
   Kyong Yoo-Ott
   Parvin Darvania

Data management: Kristina Dudda
   Heike Ebeling

C Research Report:

C.1. Analysis of regulatory T-cells before and after lymphocyte immunotherapy

Lymphocyte immunotherapy (LIT) has some beneficial effect in recurrent spontaneous abortion or implantation failure. In order to find reliable parameters indicating successful immunization we investigated markers linked to presence and activity of T regulatory cells (T_{reg}). For the evaluation peripheral blood was collected from women undergoing LIT before and 4 weeks after treatment. In a retrospective analysis on cryopreserved serum sIL-2R (sCD25) levels of 100 women were also tested in an automated luminescence assay. Prospectively, we characterized lymphocytes for their Treg proportion from 73 women by testing for CD4, CD8, CD25, CD45RO expression by flow cytometry. Since Treg express FOXP3 we measured transcripts of FOXP3 in CD4^{+}CD25^{+} enriched cells by quantitative RT-PCR normalized vs. RNA polymerase II and FOXP3 protein by intracellular staining. The serum level of sIL-2R decreased slightly after LIT indicating repressed T cell activation. Since sIL-2R levels declined irrespective of the outcome regarding a following pregnancy it is not an appropriate marker. In the prospective analysis, there was a significant increase of the number of CD25^{hi} CD4^{+} T cells, which are thought to represent the T_{reg} population. In this population FOXP3 expression remained unchanged on mRNA and protein level.

CONCLUSION: Whereas sIL-2R was of no diagnostic value, increasing levels of CD4^{+}25^{hi} cells after LIT might represent expansion of T_{reg} and might serve as an useful marker following appropriate correlation with pregnancy outcome.
C.2. Evaluation of couples treated with intradermal lymphocyte immunotherapy

C.2.1. Comparison of gestational characteristics in single IVF vs. spontaneous pregnancies after recurrent implantation failure

In 1999 – 2002, 513 couples received LIT for recurrent implantation failure and reported on singleton deliveries later on. The data obtained by questionnaires were evaluated concerning the course of the pregnancies and possible risk markers. Information on additional deliveries was obtained in 2006.

**RESULTS**: After IVF (in-vitro fertilisation) embryo transfer 409 infants were born, and 104 infants after spontaneous conception. The two groups did not differ statistically in the characteristics of preceding infertility treatment and maternal complication rates in the third trimester of pregnancy.

Nevertheless, the rate of preterm delivery (<37\textsuperscript{th} gestational week) was higher after embryo transfer (13\% vs. 6\%, p<0.05), and extreme prematurity (<32. week) was observed after embryo transfer only (3\% vs. 0\%). Several complications in pregnancy may lead to premature delivery. But even if no complications were noted, still 1\% of IVF children were born before the 32nd week (3/256, Fig. 1).

![Figure 1: Prematurity after uncomplicated IVF pregnancy (n=256)](image)

Birth weight after embryo transfer was slightly below the 50\% percentile in Germany for boys as well as for girls. There was no apparent difference between IVF with or without ICSI (intracytoplasmatic sperm injection), transfer of fresh or cryopreserved embryos.
After embryo transfer imminent abortion and bleeding in the first and second trimester occurred more frequently than after spontaneous conception (23.2% vs. 8.7%, p<0.001) and more frequently after IVF than after ICSI (29.8% vs. 20.1%, p<0.05). Vanishing multiples contributed to imminent abortion in 1/3 of cases after embryo transfer.

After imminent first or second trimester abortion premature delivery was twice as frequent as after pregnancies not complicated by vaginal bleeding (22.1% vs. 10.8%, p<0.005).

Approximately 4 years (1-7 years) after the first delivery 116 of 513 women (23%) had a second childbirth, and 16 women (3%) for the third time. The chance to deliver again was higher after a preceding spontaneous conception (36/104= 35%) than after an IVF conception (80/409 = 16%, p<0.002). 32 of 36 women (89%) conceived a second time spontaneously (Fig. 2).

![Figure 2: Further outcome after first delivery](image)

**CONCLUSION**: Prematurity <32th week is a specific gestational risk for singletons after embryo transfer. Imminent abortion is three times as common than after spontaneous conception and indicates a doubled risk for prematurity. Transfer of more than one embryo may contribute to this risk. Underlying maternal factors leading to sterility and prematurity cannot be excluded. One third of women who conceived spontaneously experience another spontaneous conception within 4 years.
C.2.2. Outcome and prognostic factors after lymphocyte immunotherapy for recurrent spontaneous abortions (MD thesis Julia Magez)

Couples who experience three or more recurrent abortions may benefit from lymphocyte immunotherapy (LIT) after other apparent causes of pregnancy failure (e.g. genetic, uterine, haemostaseological) have been excluded. Emotional distress can be relieved when prognostic data referring to the prospective outcome after a definite period of time are included in counselling.

In 2005, 229 couples who had been referred to our outpatients department between 1996 and 2003 were followed up in a retrospective trial. Of these, 115 were treated with LIT and 114 were not, according to the selection criteria for LIT (HLA antibody status, HLA). The women were 20 - 39 years old, had at least 3 first trimester abortions, no late pregnancy or deliveries. Evaluation was possible for another two years after referral.

RESULTS: Cumulative pregnancy and delivery rates over two years are significantly influenced by female age, number of preceding abortions and previous ultrasound evidence of fetal vital signs. The chance to deliver was 70 to 95% for women under 35 years, no more than three abortions and a fetal heart beat in at least 2 pregnancies. It was diminished to 40-70% for elder women, after four and more abortions and missing vital signs. Additionally, these cases are at risk for secondary infertility. After LIT, the chance for delivery was higher than without LIT (Fig. 3). It has to be taken into account that the setting does not represent a controlled trial on LIT.

CONCLUSION: A benefit from LIT has been reported elsewhere and may be a therapeutic option prior to another pregnancy in some cases. Nevertheless, main factors apparently derive from oocyte/embryonic development. After diagnostic evaluation, most couples can be reassured on their generally good prognosis over time. In some cases, therapeutic alternatives and evaluation for infertility have to be discussed.
C.3. Reconstitutive bone regeneration in X-ray induced osteonecrosis

The skeleton provides a structural scaffold and mechanical support that allows the body to sustain function and to move in a gravitational environment. The repair of bony defects sometimes requires substitution of irreversibly lost material. Usually, this is achieved by using autologous bone grafts. The engineering of replacement bones in the laboratory through combination of cells, bioactive factors, and supportive three-dimensional matrices may provide an alternative source of bones. Three fundamental parameters are required for the successful tissue engineering of bone: (1) soluble osteoinductive signals; (2) viable responding cells capable of differentiating into bone-forming cells; and (3) a suitable extracellular matrix. Such an osteoinductive signal is bone morphogenetic protein 2 (BMP-2); a member of the transforming growth factor (TGF-β) superfamily. We cloned BMP-2 coding cDNA into a mammalian expression vector and transfected several cell lines. BMP-2 expression was detected by Western blot analysis. Further experiments will investigate BMP-2 induced collagen formation prior to seeding these cells onto a supporting matrix which in turn can then be used as a bone graft.

This is a collaboration with: Klinik für Mund-, Kiefer- und Gesichtschirurgie; Direktor: Prof. Dr. Dr. J. Wiltfang; Priv. Doz. Dr. med. Dr. med. dent. Ingo Springer, Dr. med. Peter Niehoff, Prof. Dr. rer. nat. Yahya Açil.
D Publications

2007

2008

E Grants:
Untersuchungen zur Entwicklung regulatorischer T-Lymphozyten nach intracutaner Alloimmunisierung (Collaboration with Dr. C. Kling and Dr. M. Marget), Medical Faculty of Kiel University, 10 000,- Euro
6. **Research Group Schütze**

A **Group Leader:** Prof. Dr. rer. nat. Stefan Schütze

B **Lab Members:**

**Scientists:**
- Dr. rer. nat. Vladimir Tchikov (DFG)
- Dr. rer. nat. Uwe Bertsch (DFG)
- Dr. rer. nat Jürgen Fritsch (DFG)
- Dr. rer. nat Supandi Winoto-Morbach

**Ph.D. Students:**
- Dipl.-Biol. Bärbel Edelmann (DFG)
- Dipl.-Biol. Mario Stephan (DFG)

**Medical Students:**
- Isabel Hellmich
- Maike Reinicke

**Diploma Student:**
- Anastasia Tartanikova

**Technicians:**
- Andrea Hethke (DFG)
- Feretshteh Ebrahim (DFG)
- Casimir Malanda

C **Research Report**

C.1. **Immunomagnetic isolation of soluble proteins and subcellular organells containing receptor-activated signaling complexes** (Medical Faculty support)

We developed a magnetic system based on target-specific immunomagnetic labeling of cell surface receptors and a patented free-flow magnetic chamber with specific properties allowing selective purification of biological materials from cellular lysates, in particular morphological and functional intact endosomes containing activated receptors and receptor-associated proteins (see our research report 2005-2006). The system is currently also used to isolate intracellular phagosomes after infection of cells by bacteria (i.e mycobacteria).

After successful application of our magnetic separation device for the isolation and functional characterization of TNF-receptosomes (Schütze and Tchikov, Methods in Enzymology, 2008; Schneider-Brachert et al., Immunity, 2004; Schneider-Brachert et al., J. Clin. Invest., 2006), as well as CD95 receptosomes (Lee et al., EMBO J., 2006; Feig et al., EMBO J., 2007) we subsequently adopted this approach for the immunomagnetic isolation of soluble proteins and intact organelles from total cell lysates. Intracellular organelles are usually isolated based on their different biophysical properties by isokinetic or isopycnic density gradient centrifugation. These methods, however, do not allow the separation of membrane compartments that share similar densities but exhibit diverse functional properties like different stages of vesicular maturation and physiological functions.
By coupling antibodies specific for signature proteins of intracellular endosome trafficking and fusion events like rab5, Vti1b and Lamp-1, we could isolate subcellular organelles at various stages of vesicular maturation that contain internalized TNF receptors (TNF-R1) in early endosomes (Figure 1) and activated TNF-R1-associated signaling complexes like caspase-8 and cathepsin D in late endosomes / multivesicular organells (Figure 1).

**Figure 1  Early endosomes contain TNF-receptors**

After treatment with TNF for 10 min. U937 cells were mechanically homogenized. One aliquot of the lysate was incubated with anti-rab5 antibodies coupled to magnetic nanobeads and subjected to our free-flow magnetic chamber. Both, the lysates and magnetic fractions were analyzed by western-blotting for rab5, TNF-R1 and the early antigen EEA1. The isolated vesicles represent early endosomes, not yet fused with other membrane compartments (lack of EEA-1) and contain the TNF-R1, which is highly enriched compared to the lysates.
Figure 2  Late endosomes / multivesicular organelles contain TNF-receptors and activated caspase-8 as well as cathepsin D
U937 cells were either left untreated or incubated with TNF for 30 min. Cells were mechanically homogenized and one aliquot of untreated cells and one aliquot of TNF treated cells was incubated with anti-Vti-1b antibodies coupled to magnetic nanobeads and the labeled organelles were purified in our free-flow magnetic chamber. Both, the lysates and magnetic fractions were analyzed by western-blotting for the trans-golgi SNARE-protein Vti-1b, TNF-R1, caspase-8 and cathepsin D. TNF-R1 and elements of the TNF-R1 signalling cascade like activated caspase-8 and cathepsin D are detected.

C.4. Compartmentalization of TNF-R1 signalling decisive for NF-κB activation or induction of apoptosis. (DFG Collaborative Research Centre 415, project A11)
Our previous studies revealed that the induction of apoptotic cell death requires internalization of TNF-R1 and recruitment of the “death inducing signalling complex” (DISC) composed of the adaptor-proteins TRADD, FADD and caspase-8 at the internalized endosomal vesicles (TNF-R1 receptosomes) (Schneider-Brachert et al., Immunity, 2004; Schneider-Brachert et al., J. Clin. Invest., 2006). Since other reports claimed, that the FADD and caspase-8 complex (called complex II) is formed in the cytosol, not bound to the TNF receptor, we analyzed the intracellular localization of active caspase-8 and internalized TNF-R1 by confocal microscopy. As shown in Figure 3, a colocalization of internalized TNF-R1 (stained green) and activated caspase-8 (stained red) can be detected (indicated by yellow staining). Thus TNF-induced DISC formation clearly takes place at the TNF receptor and active caspase-8 is present in TNF receptosomes even after 45 minutes.
Figure 3  Hela cells were incubated with biotin-TNF and streptavidin-FITC for 1 h at 4°C. Internalization of the activated TNF receptor was induced by shifting the temperature to 37°C. The cells were fixed at the time points indicated and stained with a mouse monoclonal antibody directed against the cleaved (active) form of caspase-8, followed by incubation with the secondary Alexa Fluor 555 labeled goat anti-mouse antibody. Colocalization of intracellular TNF receptosomes and active caspase-8 was analyzed by confocal fluorescence laser-scan microscopy.

The results of our recent investigations on the compartmentalization of TNF-R1 signalling using the immuno-magnetic isolation approach are summarized in a model depicted in Figure 4. For details and discussion see Schütze et al., Nat. Rev. Mol. Cell Biol., 2008.
Figure 4  Compartmentalization of TNF-R1 signalling

TNF-R1 at the cell surface promotes nuclear factor (NF)-κB activation upon TNF stimulation. When internalization of the receptor is blocked by mutations within the TNF-receptor internalization domain (TRID) or by the adenovirus protein 14.7K, the recruitment of receptor interacting protein-1 (RIP-1) and TNF receptor associated protein-2 (TRAF-2) to the cytoplasmic death domain (DD) of cell surface TNF-R1 are sufficient for NF-κB signalling. TNF-R1 internalization is taking place through clathrin-dependent endocytosis (this involves formation of clathrin-coated pits (CCP) and recruitment of AP-2 and dynamin-1) within minutes following ligand binding. The “death inducing signalling complex” (DISC) proteins TRADD, Fas-associated via death domain protein (FADD), and caspase-8 are then recruited to TNF-R1 at the internalized receptosomes (clathrin-coated vesicles, CCV). Within the receptosome-bound DISC, caspase-8 is activated and can induce caspase-3 activation in type I cells. Along the RAB4-, RAB5-, RAB7-regulated endocytic pathway, TNF-receptosomes fuse with syntaxin-6, GRP78, p47a and Vti-1b positive trans-Golgi vesicles that contain pro-acid sphingomyelinase (pro-A-SMase) and pre-pro cathepsin D (pre-pro CTSD) to form multivesicular bodies (MVB). Within the MVB activated caspase-8 stimulates the A-SMase / ceramide / CTSD cascade, which is capable of mediating apoptosis through cleavage of Bid, generation of tBid and activation of caspase-9. Caspase-9 then activates caspase-3 to execute apoptosis.

How are signaling events during endocytosis of TNF receptor complexes regulated? In particular, since TRADD, RIP-1 and TRAF-2 are co-internalized with TNF-R1, NF-κB signalling has to be downregulated during TNF receptor endocytosis, allowing for full propagation of DISC-mediated pro-apoptotic signalling. Very recently, in cooperation with the group of Srinivasa Srinivasula (NIH Bethesda, USA) we could identify the RING-domain-containing ubiquitin protein ligase CARP-2 as a constitutive negative regulator of TNF-induced NF-κB activation (Liao et al., Curr. Biol., 2008). CARP-2 is localized to endocytic vesicles where it interacts with internalized TNF receptosomes, resulting in
ubiquitinylation and degradation of RIP-1 and subsequently in downregulation of NF-κB signalling.

In summary, two temporary and spatially distinct TNF-R1 signalling complexes are formed with the capacity to either signal for NF-κB activation from the cell surface or for apoptosis from internalized receptosomes, indicating that TNF-R1 compartmentalization has an important role in the diversification of TNF-mediated biological responses.

C.5. Identification of CARP-2 as an endosome-associated E3 ubiquitin protein ligase for RIP that regulates TNF-induced NF-κB activation (DFG Collaborative Research Centre 415, project A11)

While the activated TNF-R1 receptor complex at the plasma membrane or in endocytosed cargo signals IKK activation, its fusion with endocytic vesicles results in termination of NF-κB activation via an unknown mechanism, presumably mediated by factors associated with endocytic vesicles. Recent reports on other signaling pathways indicate that a number of cellular proteins that localize to intracellular compartments via binding to specific phospholipids regulate intracellular signaling. These peripheral (as opposed to integral membrane) proteins target the receptor complexes to specific organelles through lipid-protein and protein-protein interactions, and provide necessary spatial and temporal regulation for signaling.

Because internalized TNF-R1 complexes are known to associate with endocytic membrane vesicles, we hypothesized that proteins in these intracellular compartments might affect TNF-induced NF-κB activation by targeting signaling complexes. In cooperation with the group of S. Srinivasula, NIH Bethesda, USA, we could demonstrate that CARP-2, a RING domain-containing ubiquitin protein ligase (E3), is a negative regulator of TNF-induced NF-κB activation (Liao et al., Curr. Biol., 2008).
Figure 5  
**CARP-2 is recruited to TNF-R1 receptosomes.** Magnetic TNF-R1 fractions were prepared from U937 cells at various time points after incubation with biotin TNF/streptavidin-microbeads in the absence and presents of the proteasome inhibitor MG132 and immunoblotted with the antibodies indicated. Post-nuclear extracts were used as controls (lysate). Internalization and endosomal/lysosomal maturation of TNF receptosomes is indicated by rapid and transient recruitment of Rab5 and Rab7 and late recruitment of LAMP-1 to TNF-R1 magnetic fractions. Inhibition of proteasomal degradation by MG 132 pretreatment leads to stabilization of CARP-2, RIP and TNF-R1 protein levels.

Figure 6  
**Co-immunoprecipitation of CARP-2 with RIP and TNF-R1**  
TNF receptosomes were prepared from Hela cells, lysed and immunoprecipitations were performed using anti-CARP-2 antibodies and analyzed for association with RIP and TNF-R1. The results demonstrate physical interaction between CARP-2 and RIP at the TNF-receptor.
C.6. **CD95 compartmentalization is linked to pro- and non-apoptotic signalling.**

(DFG project SCHU 733/8-1)

In collaboration with Marcus E. Peter, University of Chicago, USA, we investigated the role of post-translational modifications in CD95 for CD95 signalling: Ligation of CD95 leads to rapid formation of SDS-stable microaggregates (CD95$_{hi}$), which translocate to lipid raft plasma membrane microdomains, a process that is regulated by palmitoylation (PA) and ezrin-mediated association of the receptor with the actin cytoskeleton (Feig et al., EMBO J., 2007).
Figure 8  Compartmentalization of CD95 signalling
Low amounts of FADD and caspase-8 are recruited within this very early time frame and “Signalling protein oligomerization transduction structures” (SPOTS) are then formed resulting in clustering of CD95 (“capping”) which depends on active caspase-8. This leads to formation of large lipid raft platforms. At this stage, CD95 has the potential to activate nonapoptotic pathways by inducing activation of the mitogen activated protein kinase (MAPK) and the transcription factor nuclear factor NF-κB, leading to cell proliferation and migration, but is unable to kill type I cells. Between 5-15 min after CD95 triggering, CD95 is internalized in a clathrin-dependent manner into endosomal compartments via ezrin-mediated actin filament association. During endosomal trafficking, DISC proteins (FADD and caspase-8) are massively recruited, resulting in strong caspase-8 activation within very high molecular weight structures of many mega Dalton in size (hiDISC complexes) resulting in propagation of apoptosis signalling.

C.7. Role of sphingomyelinases and sphingolipid-mediators in disease
(DFG Priority programme SPP1267, project SCHU 733/9-1)
Within our DFG project “Topology, function and regulation of ceramide production in death receptor signaling“ of the DFG Priority programme “Sphingolipids and disease (SPP1267) we investigated the molecular mechanism of TNF-induced activation of acid sphingomyelinase within TNF-R1 receptosomes. The results will be presented in our next report (2009-2010). The following results on the role of sphingomyelinases and lipid mediators in diseases were obtained in cooperation with other groups:
1.) Association of prion proteins and sphingolipid-mediated signaling.

In collaboration with the group of H.A. Kretzschmar, University Munich, we could demonstrate that cellular prion proteins (PrP<sup>c</sup>) are involved in sphingolipid-associated signalling: PrP<sup>c</sup> associates with lipid rafts, highly glycolipid-rich membrane domains containing a large variety of signaling molecules, e.g. sphingolipids. In this study, we investigated possible connections between PrP<sup>c</sup> and sphingolipid-associated signaling pathways. Using PrP<sup>c</sup>-wt and PrP<sup>c</sup>-k.o. hippocampal cell lines and mouse brains higher activity of neutral and acid sphingomyelinase (SMase) was detected in PrP<sup>c</sup>-k.o.-groups, while ceramide and sphingomyelin-levels were unchanged. Furthermore, despite lower basal expression levels of sphingosine kinase (SphK) in PrP<sup>c</sup>-k.o.-groups, the levels of its metabolite sphingosine-1-phosphate were increased, whereas S1P<sub>3</sub>-receptor expression was higher in PrP<sup>c</sup>-wt-groups again. In addition, we detected enhanced activity of phospholipase D1 (PLD1), an enzyme that seems to be suitable to act as a connector between the S1P<sub>3</sub> receptor and continuative signaling. Finally, evidence for an impact on downstream signaling cascades, especially activation of the PI-3/Akt pathway, was found. In summary, these data suggest that PrP<sup>c</sup> is involved in sphingolipid-associated signaling, modulating pathways that exert anti-apoptotic functions, hence indicating that PrP<sup>c</sup> plays a role in neuroprotection. (Schmalzbauer et al., J. Neurochem., 2008).

![Figure 9 Modulation of the sphingolipid-metabolism by prion proteins](image)

In hippocampal cell lines expressing mutated (PrP<sup>c</sup>) -k.o. proteins and in the brain from mice infected with (PrP<sup>c</sup>)-k.o.protein levels of neutral and acid sphingomyelinase activity and sphingosine-1-phosphate expression was higher than in wildtype (PrP<sup>c</sup>) expressing cells.
2.) Reduced acid sphingomyelinase activity improves lung edema and gas exchanges in a newborn piglet lavage model.

In acute inflammatory lung disease in newborn infants, exogenous surfactant only transiently improves lung function. Based on our previous observation on the function of acid sphingomyelinase in PAF-mediated pulmonary edema (Göggel et al., Nat. Med. 2004), we hypothesized that the transient nature of this protection may be in part explained by elevated A-SMase activity that may inactivate surfactant and promote proinflammatory responses. In collaboration with the group of M.F. Krause, Department of Pediatrics, University Hospital Schleswig-Holstein, Campus Kiel and S. Uhlig, University Aachen, we demonstrated the role of acid sphingomyelinase (A-SMase) in the lung function in a neonatal piglet lavage model: stabilization of exogenous surfactant by inhibiting A-SMase through imipramine-treatment improved pulmonary function in the clinically relevant piglet model (von Bismarck et al., Am. J. Resp. Crit. Care Med., 177, 2008).

Figure 10  Acid sphingomyelinase activity and ceramide levels in pulverized lung tissue (A,B) and in serum (C,D) after repeated airway lavage, intervention and 24 hours of mechanical ventilation. Piglets received an air bolus (control group), surfactant (Surf-group) or surfactant + imipramine (Surf+ Imi). Reduced levels of A-SMase activities and ceramide levels are observed in the Surf + Imi group.
3.) Surfactant "fortification" by topical inhibition of NF-κB in a newborn piglet model of airway lavage

Again in collaboration with M.F. Krause we investigated the effect of topical downregulation of the proinflammatory nuclear transcription factor NF-κB on the level of endogenous surfactant in the course of the acute respiratory distress syndrome. We could show that supplementation of exogenous surfactant with the NF-κB inhibitor IKK-NBD (IkB-kinase-NF-κB essential modulator binding domain peptide) to create a “fortified” surfactant improved lung function and pulmonary edema during 24 hours of mechanical ventilation. Inhibition of NF-κB also suppressed acid sphingomyelinase activity and ceramide generation, indicating a novel proinflammatory link of NF-κB (von Bismarck et al., Crit. Care Med. 35, 2007).

![Figure 11](image_url)  
**Figure 11 Acid sphingomyelinase (a-SMase) activity and ceramide levels in lung tissue**  
Piglets were treated with surfactant + inhibitor (S+IKK-NBD), surfactant alone (s), with air bolus as control (c), or were neither ventilated nor lavaged (cc) as a comparison. Treatment with surfactant plus NF-κB-inhibitor peptide reduced A-SMase activity and ceramide levels in the lung tissue to almost control levels of untreated animals (for details see von Bismarck et al., Crit. Care Med., 2007).
2007

Feig C, Tchikov V, Schütze S, Peter ME. Palmitoylation of CD95 facilitates formation of SDS-stable receptor aggregates that initiate apoptosis signaling. EMBO J. 26:221-231, 2007


2008


E. Grants 2007-2008

E.1. Funktionelle Charakterisierung intrazellulärer Ceramid-Bindeproteine in der Signaltransduktion von TNF
DFG - SFB 415 Teilprojekt A11 für 3 Jahre (bis 06/07)
(1 x BAT IIa; 1 x BAT IV b, Sachmittel 20.452 € / Jahr)
337.756 €

E.2. „Stellenwert der Sphingomyelinase in der Diagnose der Sepsis“ Forschungsförderung der Medizinischen Fakultät für 1 Jahr. (bis 03/08)
(1x BAT IIa/2, Sachmittel 10.000 €)
53.000 €

E.3. „Immunomagnetische Organellen-Isolierung“ Forschungsförderung der Medizinischen Fakultät für 1 Jahr. (bis 03/09)
Sachmittel
40.000 €

E.4. „Das M. tuberculosis-Phagosome: Dynamik der erregerinduzierten Modulation intrazellulärer Grenzflächen“ (mit PD Dr. Norbert Reiling, FZ Borstel).
DFG-Einzelantrag (SCHU 733/7-1) für 3 Jahre (bis 02/09)
(1x BAT IIa, Sachmittel 38.750 €)
292.650 €

E.5. „Funktionelle Bedeutung der Internalisierung des CD95 / Fas-Rezeptors für pro- und nicht-apoptotische Signalggebung in Typ I und Typ II Zellen“.
DFG Einzelantrag (SCHU 733/8-1) für 3 Jahre (bis 04/10)
(1x BAT IIa; Sachmittel 65.250 €)
215.150 €

(1 x BAT IIa; 1 x BAT IV b, Sachmittel 20.452 € / Jahr)
337.756 €

(1 x BAT IIa; 1 x BAT IV b, Sachmittel 20.452 € / Jahr)
241.650 €
7. Research Group Wesch/Kabelitz

A  **Group Leaders:**  PD Dr. rer. nat. Daniela Wesch/Prof. Dr. med. Dieter Kabelitz

B  **Lab Members:**

  **Scientists:**  Dr. sc. hum. Hans-Heinrich Oberg (DFG)
                 Dr. rer. nat. Susann Beetz (DFG; until 01/08)

  **Ph.D. Students:**  Dipl.-Biochem. Lothar Marischen
                        Dipl.-Biol. Tim Meyer (DFG; Medical Faculty)
                        Dipl.-Biol. Christian Peters (Medical Faculty)

  **Medical Students:**  Philine Wrobel (until 03/07)
                        Hamed Shojaei
                        Stefan Welte
                        Kathrin Pietschmann
                        Matthias Jurike

  **Technicians:**  Hoa Ly
                    Monika Kunz
                    Ina Martens
                    Sandra Ussat

C  **Research Report:**

C.1. **Innate Immune functions of human γδ T cells (Medical Faculty)**

The group has a long standing interest in the functional characterization of γδ T cell. We observed that γδ T cells express certain Toll like receptors (TLRs) such as TLR1, 2, 3, 5, (6) and 7 and directly respond to the corresponding ligands (Wesch et al. 2006; Beetz/Wesch et al. 2007; Pietschmann/ Welte, unpublished observations). TLRs are a family of pattern recognition receptors (PRRs) which recognize invariant pathogen associated molecular patterns (PAMPs). We have demonstrated a striking co-stimulatory effect of the TLR3 ligand polyinosinic-polycytidylic acid [poly(I:C)] on TCR-stimulated IFN-γ, TNF-α and MIP-1α production of γδ T cells (Wesch et al. 2006; Beetz/Wesch et al. 2007). Moreover, we found that the integrated signals from TLR3 and TCR together induce a stronger anti-viral effector function in Vδ2+ γδ T cells than in Vδ1+ γδ T cells (Pietschmann, unpublished data). In contrast, combined TLR5 and TCR activation induces additional IFN-γ production only in Vδ1 γδ T cells, whereas TLR2 together with TCR-stimulation induces an anti-bacterial effector function by enhanced production of various cytokines and/or chemokines in both γδ T cell subsets.
C.2. Features of γδ T cells in immune reactions

Human γδ T-cells represent only 1-5 % of peripheral blood T-cells, but contribute considerably to the T-cells present at sites of inflammation in epithelial tissues. Because of their localization and their ability to respond rapidly and in an MHC-independent fashion to antigens, γδ T cells are considered as part of a first line of defense.

Since NOD2 is an intracellular receptor for distinct fragments of the bacterial cell wall, it is considered as element of the first line of defense. Therefore we investigated the expression of NOD2 in γδ T cells. While the NOD2-mRNA was readily detectable, the NOD2-protein was very weakly if at all detectable. However, preliminary data generated in cooperation with the group of Prof. Dr. Stefan Schütze showed the presence of NOD2-protein in γδ T cells. Functional assays with PBMCs including γδ T cells revealed an increase of the IFNγ-secretion of γδ T cells when stimulated with the NOD2-ligand MDP. In line with these results, analysis...
of isolated γδ T cells showed an increased IFNγ-secretion after stimulation with MDP and co-stimulation with the TLR2-ligand PAM2CSK4 (PAM2) in about one third of the tested samples as shown in figure 2 (Marischen et al., unpublished data):

![Figure 2](image)

**Fig. 2. Measurement of IFN-γ production in freshly isolated γδ T cells.** γδ T cells of healthy donors were cultured in medium or stimulated as followed: MDPa: “original version” of MDP, recognized by NOD2, induces signal transduction, MDPi: version of MDP that is recognized by NOD2 but abolishes the signal transduction, PAM2: one ligand for the TLR2-receptor that is present on γδ T cells. Each symbol represent the data (IFN-γ production in stimulated cultures/ IFN-γ production in the medium control) of an individual donor. Means of the different stimulations are shown as bars.

Elafin, also known as SKALP or trappin-2, is a protease inhibitor known for antimicrobial effects on pathogens as well. While this effector protein is regularly produced by epithelial cells after stimulation, we investigated if γδ T cells, being present in epithelial tissues, are also able to synthesize elafin. For stimulation, we used the supernatant of adherently growing *Ps. aeruginosa*, which was described in recent papers [Bellemare et al., 2007; Meyer-Hoffert et al., 2003, Simpson et al., 1999] as inductor and/or target for elafin. The up-regulation of the elafin-mRNA in stimulated γδ T cells was readily detectable. Elafin protein expression was detected by intracellular flow cytometry and by Western Blot analysis (Marischen et al, in press).
C.3. Tumor reactivity of human γδ T cells: Involvement of NKG2D ligands and modulation by TLR agonists

There is a substantial interest in γδ T cell-based cancer immunotherapy due to their potent MHC-non-restricted cytotoxicity against various tumor cells. We recently reported that in addition to TCR-dependent recognition, the NKG2D pathway is involved in the lysis of different epithelial tumor cells by human γδ T cells (Wrobel et al. 2007). Moreover, TLR agonists are suggested as adjuvants in clinical trials for different types of cancer. We analyzed whether TLR agonists influenced γδ T cell-cytotoxicity against tumor cells. We observed that the pre-treatment of either TLR3- or TLR7-expressing pancreatic adenocarcinomas or squamous cell carcinomas of the head and neck as well as pre-treatment of γδ T cells with poly(I:C) (TLR3 ligand) or imiquimod (TLR7 ligand) resulted in an enhanced γδ T cell cytotoxicity against these tested tumor cells (Fig. 3A). Pre-treatment of tumor cells with imiquimod induced a downregulation of MHC class I molecules on tumor cells suggesting a reduced binding affinity for inhibitory receptors such as NKG2A expressed on γδ T cells and regulating cytotoxicity of these cells. In contrast, the activating NKG2D/NKG2D-ligand pathway was not affected by TLR3- or 7 ligation as demonstrated by unaffected expression of NKG2D ligands and inhibition of tumor cell lysis by anti-NKG2D antibody which was not modulated by poly(I:C) or imiquimod. Pre-treatment of tumor cells with poly(I:C) induced an up-regulation of CD54 (ICAM-1) which enhanced γδ T cell cytotoxicity. Moreover, pre-treatment of T cell-receptor (TCR)-stimulated [anti-γδ TCR mAb or phosphoantigens such as bromohydrin pyrophosphate (BrHPP)] γδ T cells of healthy donors and cancer patients with either poly(I:C) or imiquimod resulted in an enhanced production of perforin and granzymes by γδ T cells (Fig. 3B). Our results indicate that the usage of TCR-stimulation in combination with TLR3- or TLR7 agonists could be useful to expand γδ T cells of cancer patients in vitro to enhance their cytotoxic activity upon adoptive T-cell transfer (Shojaei, et al, in press).
(A.) Pre-treatment of γδ T cells with TLR ligands

(B.) Granzyme B expression of γδ T cells

Fig. 3. Enhanced γδ T cell cytotoxicity. (A.) Pre-treatment of γδ T cells with TLR3- (pink circles) or TLR7 (blue triangle) ligands did not result in an enhanced cytolytic activity against pancreatic adenocarcinoma PT45-P1 (a) in comparison to pre-treatment with medium (black square) unless γδ T cells were additionally activated via phoshoantigen BrHPP, as measured in a Cr51 release assay (b). (B.) Pre-treatment of γδ T cells with poly(I:C) followed by co-culturing these cells with PT45-P1 resulted in an enhanced granzyme B production after co-stimulation with BrHPP.

C.4. Role of TLR3 stimulation in CD4⁺ T cell activation (Innate immune functions, DFG; Medical Faculty)

We observed that the activation of T cell receptor (TCR) stimulated γδ T cells (Wesch et al. 2006) as well as CD4⁺ αβ T cells (Meyer et al., unpublished observation) is enhanced upon co-stimulation with poly(I:C). In order to investigate the underlying signal transduction pathways which transmit this co-stimulatory capacity of TLR3, we started to work with the leukemic T cell line Jurkat and with primary CD4⁺ T cells. We found that Jurkat cells as well as CD4⁺ T cells also express TLR3 and that combined stimulation via the TCR and the co-stimulatory molecule CD28 and TLR3 receptor resulted in enhanced T cell activation compared to TCR/CD28 stimulation alone. The effects ranged from increased secretion of a broad variety of cytokines and chemokines to enhanced activation of the transcription factor NFκB. Furthermore, with respect to cytokine/chemokine secretion, TLR3 stimulation was able to replace CD28 co-stimulation. Interestingly, TLR3 stimulation alone induced the transcription factor IRF3. The activity of IRF3 was further enhanced upon TCR stimulation.
The molecular mechanisms leading to IRF3 co-stimulation and the molecular basis for an observed growth inhibition upon TCR/CD28/poly(I:C) stimulation remain to be identified.

**Fig. 4. Quantitative analysis of CSF2 and IRF7 mRNA expression.** The transcription of CSF2 depends on the activity of NFκB, whereas the IRF7 transcription depends on the activity of IRF3. Freshly isolated (primary) CD4+ T cells were cultured for 4h, 6h and 8h in medium, with 50 µg/ml poly(I:C), with 2 µg/ml agonistic anti-CD3 mAb OKT3 or a combination of both (++;). All cells were co-stimulated by 1µg/ml soluble anti-CD28 mAb. The expression was analyzed by quantitative real-time PCR. CSF2 and IRF7 mRNA expression are depicted as the number of transcripts per 100 copies of the housekeeping gene human polymerase (HuPo).

C.5. **Proliferation and suppression of T cells : Role of TLR ligands and IL-6 (DFG SFB415, Project A15)/ Role of regulatory T cells in tumor diseases (Werner-Klara Kreitz-Foundation)**

Regulatory T cells (Treg) control peripheral tolerance and cellular immune responses. We investigated the impact of Toll-like receptor (TLR)2 ligands and selected cytokines on Treg activity. We observed that addition of IL-6 as well as pre-treatment of human Treg with a mixture of TLR2 ligands Pam3CSK4, FSL-1 and Pam3CSK4 reduced the Treg mediated suppression of CD4+CD25+ responder T cells in the majority of the analyzed donors. The reduction of Treg suppression by IL-6 or even more by Hyper IL-6 (sIL-6R/IL-6 fusion protein) was due to a co-stimulatory effect on CD4+CD25+ responder T cells (Oberg/Wesch et al 2006), whereas the mixture of TLR2 ligands acted directly on Treg (Fig. 5). The TLR2 ligand mediated abrogation of human Treg activity is not associated with an expansion of Treg (Fig 6) or with a down-regulation of FoxP3 transcription factor. Moreover, we excluded an effect of TLR2 ligands on granzyme A/B release by human Treg as a molecular mechanism to abolish Treg mediated suppression. The exact molecular mechanism of the observed TLR2 ligands-mediated abrogation of Treg activity is under investigation. In collaboration with the group of Prof. Dr. Ottmar Janssen, we will investigate whether Treg
versus responder T cells differentially express proteins in the presence or absence of TLR2 ligands by 2-D gel analysis and mass spectrometry.

The aim of these studies is to understand the molecular mechanism of Treg-mediated suppression and modulation by TLR ligands in more detail, a prerequisite for therapeutical manipulation of Treg, e.g. during tumor defense.

In this context and in collaboration with Dr. Sebastian Hinz (General Surgery and Thoraxsurgery) und Prof. Dr. Holger Kalthoff (Molecular Onkology), we study the induction of a regulatory phenotype (FoxP3+) and suppressive activity in human CD4+CD25− responder T cells and the role of effector molecules and cytokines of tumors during the suppression of tumor specific immune responses.

Fig. 5: Pre-treatment of Treg but not of responder T cells with lipeopeptides abolished suppression of responder T cells. Treg (a) and CD4+CD25− responder T cells (b) were pre-treated for 24 hours without (grey bars) or with 1µg/ml Pam3CSK4 (lavender bars), FSL-1 (blue bars) or Pam2CSK4 (green bars). For suppression assay, 1×10^4 CD4+CD25− responder T cells and 2,5×10^5 Treg cells were co-cultured and stimulated with T Cell Activation/Expansion Beads. ^3H-TdR-uptake was measured after 5 days of proliferation. Suppression was calculated as [(1-cpm of responder with Treg/cpm of responder without Treg) x100]. Mean ± SD of 14 different experiments with pre-treated Treg (a) and 11 with pre-treated responder T cells (b) are shown. Only the pre-treatment of Treg with one of the TLR2 ligands induced a significant reversal of the Treg mediated suppression in 9 out of 14 tested donors, whereas on 5 out of 14 donors pre-treatment had no effect (Fig.5a). In contrast, the pre-treatment of responder T cells with TLR2 ligands only moderately reduced the suppressive effect in 3 out of 11 tested donors (Fig 5b). Responder are presented on the left side of Fig.5a,b and Non-responder on the right side. Significance are represented as * (p < 0.05).

Fig. 6: Abrogation of Treg mediated suppression after pre-treatment with TLR2 ligands is not caused by Treg proliferation. 10^5 CD4+CD25− responder T cells were stained with Cell Trace^TM^ DDAO-SE and 7,5×10^5 Treg with CFSE. DDAO-SE labelled responder T cells were stimulated with T Cell Activation/Expansion Beads without (a) or with untreated (b) or TLR2 ligand mixture pre-treated (c) CFSE-labelled Treg. As a control untreated Treg alone cultured in medium are presented (d). Proliferation of untreated (e) or TLR2 ligand mixture pre-treated (f) CFSE-labelled Tregs from the co-culture with responder T cells in (b) or (c), respectively, are shown. DDAO-SE- and CFSE- fluorescence of one representative donor out of five tested donors at one time point (day 7) out of 5 tested time points (day 3 to 7) are shown.
C.6. Possible regulatory function of γδ T cells (Innate immune functions, DFG; Medical Faculty)

Preliminary results suggest that γδ T cells have regulatory capacity as already known of Tregs with an αβ TCR phenotype: We observed that the proliferation of CD4⁺CD25⁻ responder T cells in response to tetanus toxoid (TT) or low dose (<1ng/ml) staphylococcal enterotoxin superantigen is inhibited, if γδ T cells are simultaneously activated by phosphoantigens such as BrHPP. The inhibition of CD4⁺CD25⁻ responder T cell growth was dependent on cell contact with γδ T cells. We used the feeder cell-free suppression assay based on anti-CD3/anti-CD28 coated microbeads (Oberg et al. 2006) to analyze the regulatory function of γδ T cells in the absence of Antigen-presenting cells. We observed significant suppression comparable to Treg on CD4⁺CD25⁻ responder T cell proliferation three days after co-culture of these both T cell populations (Peters et al, unpublished observations). In ongoing experiments, we investigate if the suppression is mediated by only a subpopulation of phosphoantigen-reactive Vδ2⁺ γδ T cells (e.g. naïve, central– or effector memory or terminal differentiated CD45RA⁺ effector memory) and which molecular mechanisms play a role. Others have published that Vδ1⁺ γδ T cells function as regulatory T cells in liver transplanted patients and in breast-cancer patients. In this context, we study the distribution of γδ T cell subpopulations and the reactivity and possible regulatory function of Vδ2⁺ γδ T cells of the peripheral blood in liver transplanted children in collaboration with Prof. Dr. Burdelski (General pediatric clinic, Kiel) and of the peripheral blood in comparison to ascites of cancer patients in cooperation with PD Dr. C. Mundhenke (Gynecology, Kiel) and Prof. Dr. F-Gieseler (I. Medical Clinic, Kiel).

C.7. Characterization of in vitro generated Proteinase 3-specific effector memory T cells (KFO group, Project 3)

Autoreactive antibodies against proteinase 3 (PR3) and PR3-specific effector memory T cells were found already at the initial stage of the autoimmune disease Wegener’s Granulomatosis (WG). We study the specific functions of the expanded PR3-specific memory T cells during WG in collaboration with the group of Prof. Dr. Sabine Adam and Prof. Dr. Peter Lamprecht (Clinic of Rheumatology in Lübeck and Bad Bramstedt) to understand the functional basis of autoreactive T cell expansion for the development of WG and to identify possible therapeutically relevant effector molecules. To this end, the PD student Juliane Fazio generates various expression constructs for PR3 and transfects them into dendritic cells.
directly or after translation into RNA and co-cultures them with T cells to generate PR3-specific effector memory T cells which will then be analyzed with regard to e.g. their phenotype, cytokine production and specificity.

C.8. Central cell sorter facility
The high-speed FACS Aria runs as a central core unit of the Medical Faculty. The facility to purify diverse cell populations is used by several groups of our institute and from groups on the UK S-H campus and other institutions. Jan Lenke (until 07/07), Sandra Ussat (since09/07) and Heiner Oberg sorted many primary T cell subpopulations such as γδ T cells and regulatory T cells or lymphocyte precursor cells, GFP-transfected immortalized cell lines and tumor cell lines as well as GFP-tagged cells from hydra. Moreover, some applications required the usage of the automatic cloning device unit (ACDU).

D Publications
2007


2008


Beetz S1, Wesch D1, Marischen L, Welte S, Oberg HH, Kabelitz D. Innate immune functions of human γδ T cells. Immunobiol 213: 173-182, 2008 (1 equally contributing authors)


Kabelitz D. Small molecules for the activation of human γδ T cell responses against infection. Recent Patents Anti-Infect Drug Disc 3: 1-9, 2008


Grants

E. 1. Innate immune functions of human γδ T cells.
DFG Priority Program Innate Immunity SPP 1110 (Kabelitz)
2x BAT IIa, (until 2007)

E. 2. Proliferation und Suppression von T-Zellen: Rolle von gp130-abhängigen Zytokinen
DFG SFB 415, Teilprojekt A15 (Wesch/Kabelitz)
1x BAT IIa, 19.500 €/year, until 06/2007

E. 3. Proliferation and suppression of T cells: Role of TLR ligands and IL-6
DFG SFB 415, Teilprojekt (TP) A15 (Wesch/Kabelitz)
1x BAT IIa, 15.00 €/year, until 06/2010

E. 4. Rolle von regulatorischen T Zellen bei Tumorerkrankungen
Werner Klara Kreitz-Stiftung, grant to Dr. H.-H. Oberg, 2007, 9.000 €

E. 5. TLR-Liganden und γδ T-Zellen
Medical Faculty, grant to PD Dr. D. Wesch, until 04/2009, 19.000 € for staff appropriations and 26.000 € for material expenses

E. 6. Mechanismen der T-Zellaktivierung durch TLR3-Liganden
Medical Faculty, grant to Dr. S. Beetz and Dr. H.-H. Oberg, until 02/2009, 12.500 € for staff appropriations and 17.500 € for material expenses

E. 7. γδ T-Zellen nach Lebertransplantation
Medical Faculty, grant to Prof. Dr. D. Kabelitz together with Prof. Burdelski (Dept. of Pediatrics) and Prof. Fändrich (Dept. of General Surgery), until 12/2008, 30.000 € for staff appropriations and material expenses

E. 8. Memory T-Zell Dynamik bei Wegenerscher Granulomatose
KFO 170, TP3
½ BAT IIa, 12.500 €/year, until 2010
Appendix

1. Institute Seminars 2007 and 2008 - Invited Speakers
   (including SFB 415 Seminars)

18.01.2007  Prof. Dr. Erwin Wagner: Functions of AP-1 in development and inflammatory diseases - Universität Wien

25.01.2007  Prof. Dr. Alexander Enk: Dendritic cells in tolerance induction – Hautklinik, Universitätsklinikum Heidelberg

01.02.2007  Prof. Dr. Ursula Klingmüller: Systems Biology of Signal Transduction and Cancer - DKFZ Heidelberg

19.04.2007  PD Dr. Andrea Kruse: Materno-fetale Interaktion im Mausmodell - Institut für Immunologie u. Transfusionsmedizin, AG Reproduktionsimmunologie, UK-SH Campus Lübeck

03.05.2007  Prof. Dr. Hansjörg Schild: How regulatory T cells control the induction of adaptive immune responses - Universität Mainz

10.05.2007  Dr. Gerald Willinsky: A new look at the immune response in a mouse model of sporadic cancer - Institut für Immunologie, Charité Campus Benjamin Franklin

24.05.2007  PD Dr. Jürgen Ruland: Signal specific activation of NF-κB in immunity and lymphomagenesis - TU München

31.05.2007  PD Dr. Bernhard Moser: APC properties in human γδ T cells: facts and fiction - Institute of Cell Biology, University of Bern

21.06.2007  PD Dr. Brigitte Biesinger: Cellular targets of T-cell transformation by rhadinoviruses - Institut für Klinische und Molekulare Virologie, Friedrich-Alexander-Universität Erlangen-Nürnberg

28.06.2007  Prof. Dr. Ingrid Herr: Differentielle Regulation von Apoptosesignalwegen in der Onkologie und therapeutische Strategien - Universität Heidelberg

05.07.2007  PD Dr. Ulrike Kämmerer: Führen zelluläre Interaktionen zur fetalen Toleranz? - Forschungslabor der Universitäts- Frauenklinik Würzburg

25.10.2007  Prof. Dr. Jan Buer: Regulation of antigen-specific immunity in inflammatory disease - Universitätsklinikum Essen

08.11.2007  Dr. Ralf Claus: Lobeda-Sphingomyelinhydrolyse bei Entzündung - Klinikum der Friedrich-Schiller-Universität Jena, Arbeitsgruppe Experimentelle Anästhesie, Forschungszentrum

69
Dr. Marc Veldhoen: Differentiation of T helper subsets and their role in immunity - National Institute for Medical Research, London

PD Dr. Kerstin Steinbrink: Cross talk of cell cycle regulation and signal transduction in regulatory T cells - Einheit für Dermatologische Onkologie und Autoimmunerkrankungen, Hautklinik der Universität Mainz

Prof. Dr. Thomas Tüting: The role of the endocannabinoid system in the regulation of cellular immunity in a mouse model experimental contact hypersensitivity - Universität Bonn

Prof. Dr. Manfred Schartl: Oncogenic signaling in melanoma: From fish models to human cancer - Lehrstuhl Physiologische Chemie I, Biozentrum, Julius-Maximilians-Universität Würzburg

Prof. Dr. Thomas Wölfel: Target antigens of anti-tumor T cell reactions in autologous model systems - III. Medizinische Klinik und Poliklinik, Johannes Gutenberg-Universität Mainz

Prof. Dr. Gundram Jung: Improving antitumor antibodies: some new suggestions - Sektion für Experimentelle Immuntherapie, Abteilung Immunologie, Eberhard-Karls-Universität Tübingen

Prof. Dr. Thomas Brunner: News from the gut: how intestinal glucocorticoid synthesis contributes to local immune homeostasis – Universität Bern

Joel LeMaoult, PhD: Trogocytosis-based generation of suppressive NK - Service de Recherches en Hemato-Immunologie, Institut Universitaire d'Hematologie, Hopital Saint Louis Paris

Prof. Dr. Michael Neumaier: Role of CeaCam-1 in colon tumorigenesis - Universitätsklinik Mannheim

Dr. Gudrun Strauß: CD95L - a modulator of T cell response – Universitätsklinik für Kinder- und Jugendmedizin, Ulm

PD Dr. Karsten Mahnke: Regulatory crosstalk between Treg and DC in health and allergic diseases - Universität Heidelberg

PD Dr. Carsten Watzl: Integration of positive and negative signals in the regulation of human Natural Killer Cell activity - Institut für Immunologie, Universität Heidelberg

Ulrich Kalinke, PhD: The impact of IFN-α/β on virus elimination in periphery and brain - Division of Immunology, Paul Ehrlich Institute Langen

Dr. Axel Behrens: JNK signalling in cancer and stem cells - Cancer Research Institute London
10.07.2008  Prof. Dr. Thomas Boehm: Evolution of quality control in self/non-self discrimination - Department of Developmental Immunology, Max-Planck-Institute of Immunobiology Freiburg-

30.10.2008  Prof. Dr. Michael Boutros: Dissection of Wnt signaling pathways by genome-wide RNAI - DKFZ Heidelberg

13.11.2008  Prof. Dr. Gerd Walz: Polycystic kidney disease - from bench-to-bedside - Klinik für Nephrologie u. Allgemeinmedizin, Universität Freiburg

27.11.2008  Prof. Dr. Thomas Miethke: Bacterial Toll/Interleukin 1 receptor proteins modulate the function of innate immune cells – TU München

11.12.2008  Prof. Dr. Anna Starzinski-Powitz: Role of junction-associated protein shrew-1 in growth factor-mediated cell dynamics – Universität Frankfurt

04.12.2008  Prof. Dr. Christian Kaltschmidt: NF-kappaB in the nervous system - Lehrstuhl für Zellbiologie der Tiere, Fakultät für Biologie, Universität Bielefeld
Sehr geehrte Damen und Herren, liebe Kollegen und Gäste,

Zu unserer Fortbildung zum Thema Reproduktionsimmunologie begrüßen wir Sie herzlich in Kiel!

Der Beitrag des mütterlichen Immunsystems zum erfolgreichen Verlauf der Schwangerschaft ist seit langem belegt. Die Möglichkeiten der Immundiagnostik und -therapie werden jedoch noch immer kontrovers diskutiert.

In einer Bestandsaufnahme möchten wir Ihnen einen Überblick über den heutigen Stand der Forschung und die Konsequenzen für die Praxis geben.

Wir freuen uns auf eine anregende Diskussion!
Sehr geehrte Damen und Herren, liebe Kollegen und Gäste,

zu unserem Symposium anlässlich der Verabschiedung von unserem Oberarzt

Dr. Eckhard Westphal

begrüßen wir Sie herzlichst und danken Ihnen für Ihr Kommen.


Wir wünschen allen Beteiligten einen interessanten und unterhaltsamen Tag in der Kieler Kunsthalle
2. Completed MD and PhD Theses 2007 and 2008

2007

Hartmut Fischer  
Dr. med.  
Herstellung von rekombinanenten löslichen HLA-A2 Molekülen in humanen Zell-Linien

Markus Lettau  
Dr. rer. nat.  
Regulation der Expression und Freisetzung des Fas-Liganden

Jens Müller  
Dr. med.  
HLA Sequenzierung bei Knochenmarktransplantation

Nicolai Aljoscha Schurbohm  
Dr. med.  
Chrarakterisierung der IFN\(\gamma\)-sezernierenden Zellen im Blut von Patientinnen mit Ovarialkarzinom nach Stimulation mit autologen Tumorzellen.

Christiane Maria Stuhlmann-Laeisz  
Dr. med.  
Quantitative und qualitative Untersuchungen zur Biofilmbildung auf verschiedenen modifizierten Implantatoberflächen

Wehkamp, geb. Seifert, Ulrike  
Dr. med.  
Molekulare Grundlagen der Tumor-Nekrose-Faktor-alpha vermittelten Inhibition des Insulinrezeptors bei Diabetes mellitus Typ 2

Philine Wrobel  
Dr. med.  
Tumorreaktivität humaner γδ T-Zellen: T-Zell-Rezeptor – versus NKG2D-abhängige Erkennung und Modulation durch Aminobisphosphonate

2008

Andreas Linkermann  
Dr. med.  
Proteomanalyse von Interaktionspartnern der prolinreichen, zytoplasmtischen Region des Fas Liganden

Biny Mathews  
Dr. med.  
The Fas/FasL System influences human T cell activation

3. Awards

2007

Maren Paulsen - „Best Presentation Award 2007“, Meeting of the North German Immunologists, Borstel

Prof. Dr. Ottmar Janssen – „Hensel-Preis 2007“, Hensel-Stiftung, Kiel

Hendrik Schmidt – „GBM-Innovation-Award for Young Scientists“, STS Meeting, Weimar
Hendrik Schmidt—„STS Poster Award“, STS Meeting, Weimar

Dr. med. Philine Wrobel – “Best Thesis Award” of the Beigl Foundation, Kiel

Dr. med. Philine Wrobel – “Award of the Medical Faculty for outstanding MD thesis”, Kiel

2008

Dr. rer. nat. Marcus Lettau – “Otto-Westphal-Award for outstanding PhD thesis”, German Society for Immunology

Dr. med. Andreas Linkermann – “Award of the Medical Faculty for outstanding MD thesis”. Kiel

Dipl.-Biochem. Lothar Marischen – “Poster Award“, Annual Meeting of the German Society for Immunology (DGfI), Vienna

4. Additional Scientific Activities

D. Adam:

Member (Deputy) Promotionsausschuss of the Medical Faculty, Christian-Albrechts University Kiel

Fachimmunologe Deutsche Gesellschaft für Immunologie (DGfI)

Reviewer Boards: Deutsche Forschungsgemeinschaft
Deutsche Krebshilfe
Studienstiftung des Deutschen Volkes
Eberhard Karls Universität Tübingen: fortüne-Programme
University of Ulm: Habilitation-Commission

Reviewer for Scientific Journals:

O. Janssen:

Member of the Organizing Committee of the “Spring School of Immunology” of the German Society for Immunology
Organizer of the 10th and 11th Meeting of the Signal Transduction Society, Weimar
Vice-President of the Signal Transduction Society

Editor of Cell Communication and Signaling, BMC/Springer
Reviewer Boards: Deutsche Forschungsgemeinschaft (German Research Foundation)  
Fonds zur Förderung der wissenschaftlichen Forschung (FWF)  
German-Israeli Foundation for Scientific Research and Development  
Studienstiftung des Deutschen Volkes  
The Wellcome Trust

Reviewer for Scientific Journals:
Biological Chemistry, Blood, Cancer, Immunology, Immunotherapy, Cell Communication and Signaling, Cell Death and Differentiation, Clinical Cancer Research, FEBS Letters, Gastroenterology, Gut, Immunology, Immunology Letters, Infection and Immunity, International Archives of Allergy and Immunology, International Immunology, Journal of Leukocyte Biology, Leukemia, Leukemia and Lymphoma, Molecular Cancer Research, Molecular Cancer Therapeutics, Oncogene, Scandinavian Journal of Immunology, Signal Transduction, Viral Immunology

D. Kabelitz:

Editorial Boards: European Journal of Medical Research  
International Archives of Allergy and Immunology  
The Journal of Immunology  
Frontiers in Bioscience  
Deutsche Medizinische Wochenschrift  
Christiana Albertina  
Recent Patents on Anti-Infective Drug Discovery  
Scandinavian Journal of Immunology  
Infection and Immunity  
Immunotherapy

Advisory Boards: Deutsche Gesellschaft für Immunologie (DGfI)  
Member of the Council and Chairman of the Scientific Committee of the "Deutsche Gesellschaft für Autoimmun-Erkrankungen" (German Association for Autoimmune Diseases, DGfAE)

Reviewer Boards: DFG: Member of the Reviewer Board "Fachkollegium 15: Mikrobiologie, Virologie und Immunologie"  
Bundesministerium für Bildung und Forschung (BMBF/DLR)  
Austrian Academy of Sciences (APART)  
Mildred-Scheel-Stiftung (Deutsche Krebshilfe)  
National Science Foundation, USA  
Human Frontier Science Program  
Israel Science Foundation  
German-Israeli Foundation for Scientific Research and Development  
Ministero dell’ Università e delle Ricerca (MIUR), Italy  
Associazione Italiana per la Ricerca sul Cancro (AIRC), Italy  
Vienna Science and Technology Fund (WWTF), Austria  
Werner und Klara Kreitz-Stiftung

Others: Chairman of SFB 415  
Honorary Member of the Scandinavian Society for Immunology (SSI)
5. Impact factors of Publications and Grants  
(Summary 2007 and 2008)

<table>
<thead>
<tr>
<th>Year</th>
<th>Cumulated ISI Impact Factors</th>
<th>Grant Support (peer-reviewed) in € per year</th>
<th>Grant Support (non-reviewed, e.g. Industry, Medical Faculty) in € per year</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>88.2</td>
<td>868,340</td>
<td>698,700</td>
</tr>
<tr>
<td>2008</td>
<td>144.8</td>
<td>649,150</td>
<td>463,385</td>
</tr>
</tbody>
</table>


2007

Original Papers and Reviews

Complementary effects of HDAC inhibitor 4-PB on gap junction communication and cellular export mechanisms support restoration of chemosensitivity of PDAC cells. Brit J Cancer 96: 73-81, 2007

Beetz S, Diekhoff D, Steiner LA. 

Beetz S, Marischen L, Kabelitz D, Wesch D. 


Feig C, Tchikov V, Schütze S, Peter ME. 
Palmitoylation of CD95 facilitates formation of SDS-stable receptor aggregates that initiate apoptosis signalling. EMBO J 26: 221-231, 2007


Kabelitz D. 

Kabelitz D, Medzhitov R. 

Kabelitz D, Wesch D. 


2008

Original Papers and Reviews


Anti-tumor necrosis factor therapy inhibits pancreatic tumor growth and metastasis.
Cancer Res, 86: 1443-1450, 2008

Feller SM, Hass R, Janssen O, Friedrich KH
Cell Communication and Signaling is becoming the official journal of the Signal Transduction Society
Cell Commun Signal 6: 1, 2008

Humoral immune responses against the immature laminin receptor protein show prognostic significance in patients with chronic lymphocytic leukemia.

Friedrich KH, Janssen O, Hass R
Watching molecules talking to each other
Science Signaling 1: mr1, 2008

A cell-based approach to the minimization of immunosuppression in renal transplantation
Transplant Int 21: 742-752, 2008

Preoperative treatment of a presensitized kidney transplant recipient with donor-derived transplant acceptance-inducing cells.
Transplant, 21, 808-813, 2008

Kabelitz D, Geissler EK, Soria B, Schroeder IS, Fändrich F, Chatenoud L.
Toward cell-based therapy of type I diabetes.

Kabelitz D.
Small molecules for the activation of human γδ T cell responses against infection.
Recent Patents Anti-Infect Drug Disc 3: 1-9, 2008

Kling C, Schmutzler A, Wilke G, Hedderich J, Kabelitz D.
Prognose im IVF-Programm nach wiederholtem Implantationsversagen: Erfahrungen aus deutschen Zentren
Geburtsh Frauenheilk 68: 505-511, 2008

Kling C, Schmutzler A, Wilke G, Hedderich J, Kabelitz D.
Two-year outcome after recurrent implantation failure: prognostic factors and additional interventions
Arch Gynecol Obstet 278: 135-142, 2008

Anti-HLA-DR triggered monocytes mediate in vitro T cell anergy

Lettau M, Paulsen M, Kabelitz D, Janssen O
Storage, expression and function of Fas ligand, the key death factor of immune cells

Z Rheumatol 67: 151-156, 2008
Srinivasula
CARP-2 is an endosomal ubiquitin protein ligase for RIP and regulates TNF-induced NF-kappaB activation.

Nair RP, Ruether A, Stuart PE, Jenisch S, Tejasvi T, Hiremagalare R, Schreiber S, Kabelitz D, Lim HW,
Voorhees JJ, Christophers E, Elder JT, Weichenthal M.
Polymorphisms of the IL12B and IL23R genes are associated with psoriasis

HDR brachytherapy irradiation of the jaw – as experimental model of radiogenic bone damage

Pan J, Jin P, Yan J, Kabelitz D.
57: 1105-1114, 2008

Interaction with XIAP prevents full caspase-3/-7 activation in proliferating human T lymphocytes

Schütze S, Tchikov V, Schneider-Brachert W
Regulation of TNF-R1 and CD95 signalling by receptor compartmentalization

Evidence for an association of prion protein and sphingolipid-mediated signaling

Schmidt H, Gelhaus C, Nebendahl M, Lettau M, Watzl C, Leippe M, Janssen O.
2D-DIGE analyses of enriched secretory lysosomes reveal heterogeneous profiles of functionally relevant
proteins in leukemic and activated human NK cells.
Proteomics 8: 151-156, 2008

In vivo detectable autoantibodies directed against the oncofetal antigen/immature laminin receptor can recognize
and control myeloma cells – clinical implications.
Leukemia 22: 2115-2118, 2008

BMP-2 and bFGF in an irradiated bone model

Tchikov V, Schütze S.
Immunomagnetic isolation of tumor necrosis factor receptosomes
Meth in Enzymol 422:101-123, 2008

A role of membrane-bound CD147 in NOD2-mediated recognition of bacterial cytoinvasion

Tong Y, Jin J, Marget M, Humpe A, Neppert J, Flesch BK.
Variant FCG transcription and possible origins
Asian Pac J Allergy 26: 223-228, 2008

Voss M, Lettau M, Paulsen M, Janssen O
Posttranslation regulation of Fas ligand function
Cell Commun Signal 6: 11, 2008

Vaccination strategy to target lysyl oxidase-like 4 in dendritic cell based immunotherapy for head and neck cancer
