

Division of Clinical Experimental Immunology

Head: Gerhard Zlabinger, MD, Professor of Clinical Experimental Immunology



Current Members

Johannes Hofer, PhD Student
Markus Hölzl, PhD Student
Johannes Kovarik, PhD Student
Miriam Gärtner, Diploma Student
Philip Matzinger, Diploma Student
Alessandra Mathe, Biomed. Scientist

Margarethe Merio, Biomed. Scientist

Saro König, Biomed. Scientist
Jasmin Tancos, Biomed. Scientist

Past Members

Cornelia Stöckl, Biomed. Scientist (2007)
Irene Popow, Diploma Student (2008)
Angela Trausnitz, Biomed. Scientist (2009)

It is a central concern in modern immunology to recognise how the immune system succeeds to simultaneously defeat potentially harmful invaders and to spare host tissue. Both the knowledge about triggering appropriate reactions in case of imminent threat for the host and the insight into the highly complex regulatory mechanisms operating to sustain the integrity of an individual are essential to foster the development of effective strategies for interference in case of disturbed or unwanted immune reactivity as operative in allergy, autoimmunity, malignancy or transplantation. A longstanding interest of my research group concentrates on the issue of naturally occurring ways of immunomodulation as particularly operating in local immunity and on clinical conditions in consequence of inappropriate immune reactivity as observed after transplantation or in autoimmune diseases. Identification of immunological deregulation as early as possible and the understanding of the ongoing processes are pivotal for early diagnosis and the development as well as implementation of purposeful interference strategies.

Mediators of Local Immunity. Defense against urinary tract infection has evolved to be multilayered but is far from being well-understood. A major line of defense is constituted by the steady urinary flow, causing a wash-out of bacteria from the urinary tract. To resist, bacteria express a variety of adhesive surface structures that facilitate microbial interaction with bladder or kidney cells. On the other hand soluble host proteins are able to coat pathogens and by that mechanism prevent their attachment to uroepithelial receptors so that bacteria can be eliminated by urinary flow more effectively. If pathogens overcome these physical defense mechanisms, as a first cellular reaction of the host, shedding of superficial uroepithelial cells is initiated, representing a powerful mechanism to get rid of invading microbes. In the next phase, recruitment of immunocompetent cells is essential for the clearance of bacteria. Neutrophils are attracted by chemotactic signals and may cross the uroepithelium, which manifests clinically as leukocyturia. While the innate immune system fights the establishment of UTI, recognition of pathogens by dendritic cells (DCs) induces a typical maturation program in resident DCs including upregulation of costimulatory and MHC molecules, production of cytokines and their migration to adjacent lymph nodes. At this site, naive, antigen-specific T cells are primed resulting in the induction of cellular and humoral immunity. The production of pathogen-specific antibodies leads to the establishment of protective immunity and therefore helps also to avoid recurrence of UTI.

Tamm Horsfall Protein (THP) is a heavily glycosylated protein, which is exclusively expressed in the thick ascending limb of the Henle's loop in the kidney in amounts of 30 – 50 mg/day and therefore comprises the most abundant protein in the urine. THP is normally expressed only at the luminal surface of renal tubular epithelial cells and excreted to the urine. Earlier studies demonstrated an active role of THP in the pathogenesis of interstitial nephritis, since intravenous challenge of animals with THP resulted in the induction of a tubulointerstitial inflammatory response and microscopic scarring localized to the distal nephron segments. THP binds type 1 fimbriated *Escherichia coli* and therefore constitutes a soluble receptor, which competitively inhibits bacteria to adhere to highly mannosylated uroplakin Ia and Ib receptors, present on the urothelial surface. Regarding its proinflammatory activity, it was shown that administration of THP to human monocytes induced the expression of TNF and tissue factor, while neutrophils increase chemotaxis and phagocytosis in response to THP. These findings are also in line with previous studies showing that intravenous challenge with THP or autologous urine results in rapid induction of THP specific antibodies, indicating THP might be beneficial for the host by immediately activating innate and adaptive immune responses.

Since the urinary tract is devoid of specialized barriers, local defense mechanisms may preferentially rely on soluble anti-microbial defense molecules, like THP (see Figure). Considering the properties of THP, this protein is thought to be an essential defense molecule against UTI for at least two reasons:

i) THP binds type 1 fimbriated *Escherichia coli* and therefore constitutes a soluble receptor, which competitively inhibits bacteria to adhere to highly mannosylated uroplakin Ia and Ib receptors, present on the urothelial surface. Furthermore, recent data unequivocally demonstrated that THP is pivotal to combat bacterial infection *in vivo*. THP^{-/-} mice were shown to be profoundly hampered to combat colonization of the bladder tissue when infected with uropathogenic type-1 fimbriated *E. coli*. In addition, it has been demonstrated that the absence of THP predisposes the host to severe urinary tract infection. These findings indicate that the effect of THP might be attributed to the blockade of bacterial adhesion, leading to impaired colonization.

ii) Additionally to this physical - competitive action, which takes place in the lumen of the urinary tract, we have recently identified Tamm Horsfall Protein (THP) as an endogenous molecule that potently mediates immunomodulation. Because of its restricted expression the activity of THP can be expected to exclusively take place in the genitourinary tract. We found that THP is a strong activator of human DCs and initiates the upregulation of costimulatory molecule and MHC expression, inducing de novo cytokine production and optimal T cell stimulation. Interestingly, THP was shown to induce DC maturation via activating a TLR4 dependent cell signaling machinery including activation of IRAK, Akt, p38, ERK1/2 and NF- κ B. Moreover, we have demonstrated that intravenous challenge with THP rapidly induces the production of THP-specific antibodies and systemic TNF- α release, which was completely absent in TLR4 -/- or MyD88 -/- mice, but not in TLR2 -/- or TLR9 -/- mice.

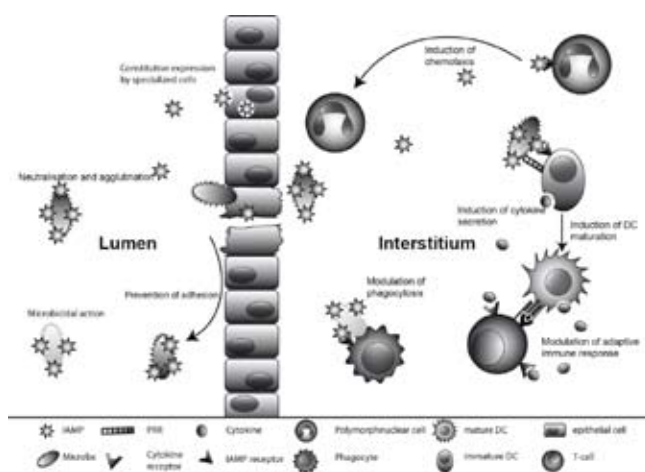


Fig. 1: THP acts as an antimicrobial protein in the lumen of the urinary tract. However, when translocated to the interstitium during urinary tract infection, it mediates immunomodulatory signal

Recent research, showed that TLR4, MD2 or CD14 are not the binding receptors for THP. Employing fluorescence activated cell sorting (FACS) we sorted THP binding BW murine lymphoma cells, expressing proteins of a DC cDNA library. Thereby we were able to identify scavenger receptors, as for example scavenger receptor on endothelial cells I (SRECI), scavenger receptor AI (SR-AI) and scavenger receptor BII (SR-BII) as binding partners for THP. Currently, we are evaluating the physiological impact of these THP binding molecules.

Hallmarks of Antibody-mediated rejection. In clinical transplantation, up to now, diagnostic and therapeutic efforts have mainly focused on T-cell-mediated immunity. However, over the last fifteen years there has been an increase in evidence for a critical role of B cell immunity and antibody-mediated effector mechanisms on allograft loss. Although in the early days of transplantation humoral immunity was recognised to be causal for hyperacute graft rejection due to preformed donor-specific antibodies, only recently the important contribution of alloantibodies to more “common” rejection types could be established, e.g. acute or chronic rejection. Pivotal for the progress in the field have been the definition of acute Antibody-Mediated Rejection (AMR) as a novel clinical entity, the description of capillary deposition of C4 complement split product C4d as marker of AMR, and the establishment of efficient “anti-humoral” treatment, most importantly apheresis.

The first two diagnostic criteria of AMR, histo-morphology and capillary C4d-deposition, are now well defined and have recently been standardised. However, despite the diagnostic value of biopsy-findings, which today are the basis for rejection therapy, histologic diagnosis in most cases comes rather late.

The indication for graft biopsy is given by a clinically manifest deterioration of organ function (e.g. rising serum creatinine concentration), which can either be a sign of an acute, potentially reversible process (e.g. acute AMR) or that of a chronic, often irreversible organ damage (e.g. chronic rejection).

For the evaluation of circulating donor specific antibodies, numerous techniques are available, including cell- and solid phase-based assays. These tests are particularly important for the assessment of the risk for acute AMR prior to transplantation. For this purpose cytotoxic crossmatch test is performed routinely before transplantation, which is suitable to detect the majority of deleterious allospecific antibodies of the recipient. Novel methods for the detection of HLA antibodies employ HLA-coated microbeads. The great advantage of these bead based methods over the use of test lymphocytes is that of employing standardised reagents which allow to perform repeated tests under the same experimental conditions, e.g. monitoring of patients post-transplantation. There are a number of hypotheses on the causes for false negative results in serologic tests, e.g. absorption of donor-specific antibodies by the graft, apheresis therapy or alloantibody concentrations simply below the detection threshold of the applied test. Since it would be of great benefit if the reactivity of donor specific B cells (i.e. alloantibody production) could be accurately assessed prior to transplantation as well as for monitoring purposes in the post transplant phase we have started to implement a test procedure for ex vivo testing of alloantibody production. Appropriate stimulation of B-cells reveals the release of particular alloantibody specificities both in healthy individuals and in sensitized transplant patients. Follow-up studies in transplant patients will demonstrate the clinical relevance of such testing.

Molecular mechanisms of butyrate action. *n*-Butyrate, a short chain fatty acid (SCFA) produced by bacterial fermentation in the intestine proved to be an important factor in maintaining mucosal homeostasis in the gastrointestinal tract. Beside its cell regulating functions, *n*-butyrate exerts potent effects on a variety of colonic mucosal functions, thereby reinforcing colonic defence barrier and decreasing oxidative stress. Furthermore, it has been shown to inhibit inflammation as well as carcinogenesis (2,3). Prominent molecular mechanisms of *n*-butyrate action are inhibition of nuclear factor kappa B transactivation and of histone deacetylation.

In previous studies our group has been engaged in investigating the effect of this SCFA on different immune cell populations and elucidating molecular pathways particularly in T-cells. With regard to the molecular way of action only limited information is available how this SCFA brings about its anti-inflammatory effects. In addition to its well-known effect as an HDAC inhibitor, which however appears not to be able to explain these effects at the molecular level the only common finding of numerous studies is inhibition of NF- κ B transactivation. Also in this regard the actual level of interference/mode of action has so far not been elucidated. A classical read-out of *n*-butyrate interference with innate immunity is maturation of dendritic cells, which we utilize in order to elucidate the molecular mechanisms in more detail. This process has been demonstrated to be efficiently inhibited by *n*-butyrate. In addition to the inhibition of transcription factor activation, modulation of cyclooxygenase and PPAR γ pathways also seem to be central mechanisms in downregulating inflammatory reactions. Here it is hypothesized that the SCFA *n*-butyrate because of its gene-regulating capacity might have an influence on that crucial pathways as well. It therefore is crucial to analyse the signalling events along the NF- κ B pathway induced by TLR2 or TLR4 ligation in great detail as well as to study the involvement of the cyclooxygenase and the PPAR γ pathway in the inhibition of DC maturation by *n*-butyrate at length. Results of this study are expected to deepen the understanding of immune regulatory capacity by this endogenous immunomodulator and could help to develop new therapeutic strategies in inflammatory conditions.

In IBD (inflammatory bowel disease) patients the mucosa characteristically shows a higher grade of local inflammation. Among the various theories implicated in the pathogenesis of IBD genetic and environmental factors as well as an imbalance of inflammatory responses including both hypo- and hyporesponsiveness to commensal bacteria have been put forward but this issue remains far from being elucidated. IBD patients seem to have an imbalance in this milieu of their mucosal environment. So it might be hypothesized that such patients do not respond appropriately to the anti-inflammatory action of such immunomodulating agents. Thus local n-butyrate concentrations might not be sufficient in IBD patients either because these patients do not have adequate n-butyrate levels or immune cells might respond in a different manner in diseased than in healthy individuals. A deficiency of n-butyrate can be excluded by findings showing that there is no significant difference in n-butyrate concentration between healthy individuals and IBD patients. It, therefore, might be hypothesized that n-butyrate cannot act appropriately in Crohn's Disease (CD) and Ulcerative Colitis (UC) patients, which should be reflected by the fact that P MNC of IBD patients were less sensitive to the inhibitory action of n-butyrate. To study potential differences between patients with IBD and healthy individuals a pilot study is under way enrolling patients with Crohn's Disease (CD) and Ulcerative Colitis (UC) as well as healthy individuals. In order to test for a feature inherent to immune cells, it is evaluated whether cytokine production by peripheral blood mononuclear cells (PBMNC) induced by TLR2 or TLR4 ligation can be inhibited with differing efficacy by n-butyrate.

Grants

FWF (Austrian Science Fund) P20508-B11 „Identification of functional receptors for the Tamm Horsfall Glycoprotein on Dendritic cells“ (2007- 2010) Else Kröner Gedächtnisstiftung A88/07 “Immunological Hallmarks of Humoral rejection“ (2008 – 2010) OeNB (Österr. Nationalbank Jubiläumsfonds) 12977 „Sensitivity to n-butyrate in patients with inflammatory bowel disease“ (2008 – 2009)
 WWTF (Vienna Science, Research and Technology Fund) – University Infrastructure Programme 2009 „Multiparameter Analysis in Immune cells“

Theses

Diploma Theses

Irene Popov: Einfluß von ATG Präparationen auf die B-Zell Funktion. (completed 2008)
 Philip Matzinger: Modulation of CD69 expression by the bacterial metabolite n-butyrate (ongoing)
 Miriam Gärtner: Flow cytometric assessment of leukocyte alpha-galactosidase A activity in Anderson Fabry patients (ongoing)

PhD Theses

Johannes Hofer: Impact of antibody-mediated effector mechanisms on the evolution of allo responsiveness (ongoing)
 Markus Hölz: Identification of functional receptors for the Tamm Horsfall glycoprotein on dendritic cells (ongoing)
 Johannes Kovarik: Molecular mechanisms of n-butyrate action (ongoing)

Diploma Theses for Biomedical Scientists

Jasmin Tancos: Nachweis der Sensibilisierung nach Therapie mit humanisierten, monoklonalen Antikörpern gegen VEGF bei Patienten mit altersbezogener Makuladegeneration (completed 2009)
 Veronika Seidl: Fluorimetrischer Nachweis der a-Galactosidase A Aktivität in Leukozyten (completed 2008)

Publications 2007-2009

1. Geyeregger R, M.Zeyda, W.Bauer, E.Kriehuber, M.D.Saemann, G.J.Zlabinger, D.Maurer, T.M.Stulnig. Liver X receptors regulate dendritic cell phenotype and function through blocked induction of the actin bundling protein fascin. *Blood* 109:4288-4295 (2007)
2. Hochegger K., P.Eller, J.M.Huber, D.Bernhard, G.Mayer, G.J.Zlabinger, A.R.Rosenkranz. Expression of granzyme A in human polymorpho-nuclear neutrophils. *Immunology* 121:166-173 (2007)
3. Mechtcheriakova D., A.Wlachs, J.Sobanov, F.Bornancin, G.Zlabinger, T.Baumruker. FTY720-phosphate is dephosphorylated by lipid phosphate phosphatase 3. *FEBS Lett.* 581(16):3063-3068 (2007)
4. Mechtcheriakova D., A.Wlachs, J.Sobanov, T.Kopp, R.Reuschel, F.Bornancin, R.Cai, B.Zemann, N.Urtz, G.Stingl, G.Zlabinger, M.Woisetschläger, T.Baumruker, A.Billich. Sphingosine 1-phosphatase phosphatase 2 is induced during inflammatory response. *Cell Signal* 19:748-760 (2007)
5. Stefanova T., N.Nikolova, A Michailova, I.Mitov, I.Iancov, G.J.Zlabinger, H.Neychev. Enhanced resistance to Salmonella enterica serovar Typhimurium infection in mice after coumarin treatment. *Microbes Infect.* 9:7-14 (2007)
6. Zeyda M., D. Farmer, J.Todoric, O.Aszman, M.Speiser, G.Gyori, G.J.Zlabinger, T.M.Stulnig. Human adipose tissue macrophages are of an anti-infl phenotype but capable of excessive pro-inflammatory mediator production. *Int.J.Obes.* 31:1420-1428 (2007)
7. Zeyda M., R.Geyeregger, M.Poglitsch, T. Weichhart, G. Zlabinger, S.Koyasu, W.H.Horl, T.M.Stulnig, B.Watschinger, M.D.Saemann. Impairment of T cell interactions with antigen-presenting cells by immunosuppressive drugs reveals involvement of calcineurin and NF-kappaB in immunological synapse formation. *J Leukoc Biol.* 81(1):319-27 (2007)
8. Hölzl, M.A., J.Hofer, P.Steinberger, K.Pfistershammer, G.J.Zlabinger. Host microbial proteins as endogenous immunomodulators. *Immunol.Letters* 119: 4-11 (2008)
9. Huber, J., F.W.Kiefer, M.Zeyda, B.Ludvik, G.R.Silberhumer, G.Prager, G.J.Zlabinger, T.M.Stulnig. CC chemokine and CC chemokine receptor profiles in visceral and subcutaneous adipose tissue are altered in human obesity. *J.Clin.Endocrin.Metab.*93:3215-3221 (2008)
10. Pfistershammer K., C.Klauser, J.Leitner, J.Stöckl, O.Majdic, T.Weichhart, Y.Sobanov, V.Bochkov, M.Saemann, G.Zlabinger, P.Steinberger. Identification of the scavenger receptors SREC-I, Cla-1 (SR-BI), and SR-AI as cellular receptors for Tamm-Horsfall protein. *J.Leukocyte Biology* 83:131-138 (2008)
11. Choremi-Papadopoulou H., G.C. Faure, B.Malenica, S.A.Misbah, G.J.Zlabinger. Position statement: Assessment strategy for implementation of the immunology curriculum of the European Board of UEMS Medical Biopathology. *Immunol Lett.* 125:59-64 (2009)
12. Funk, M., G.Schmidinger, M.-Bolz, N.Maar, T.Benesch, G.J.Zlabinger, U.Schmidt-Erfurth. Angiogenic and inflammatory markers in intraocular fluid of eye with diabetic macular edema and the influence of therapy with Bevacizumab. *Ophthalmology*, in press (2009)
13. Funk, M., K.F.Kriechbaum, F.Prager, T.Benesch, M.Georgopoulos, G.J. Zlabinger, U.Schmidt-Erfurth. Intraocular concentrations of growth factors and cytokines in retinal vein occlusion and the effect of therapy with Bevacizumab. *Invest. Ophthalmol.*50(3):1025-1032 (2009)
14. Geyeregger R., M.Shehata, M.Zeyda, F.W.Kiefer, K.M.Stuhlmeier, E.Porpaczy, G.J.Zlabinger, U.Jäger, T.M.Stulnig. Liver X receptors interfere with cytokine-induced proliferation and cell survival in normal and leukemic lymphocytes. *J.Leukoc.Biol.*, in press (2009)
15. Leitner, J., Klauser, C., Pickl, W.F., Stöckl, J., Majdic, O., Bardet A.F., Kriel, D.P., Dong, C., Yamazaki, T., Zlabinger, G., Pfistershammer, K., Steinberger, P. B7-H3 is a potent inhibitor of human T cell activation: no evidence for B7-H3 and TREML2 interaction. *Eur.J.Immunol.* 39:1754-1764 (2009)
16. Muhammad A., H.B.Schiller, F.Forster, P.Eckerstorfer, R.Geyeregger, V.Leksa, G.J.Zlabinger, M.Sibilia, A.Sonnleitner, W.Paster, H.Stockinger. Sequential cooperation of CD2 and CD48 in the buildup of the early TCR signalosome. *J.Immunol.* 182:7672-7680 (2009)
17. Regelsberger, G., R.Höftberger, W.F.Pickl, G.J.Zlabinger, U.Körmöczy, U.Salzer-Muhar, D.Luckner, O.A.Bodamer, J.A.Mayr, W.H.Muss, H.Budka, H.Bernheimer. Danon disease: Case report and detection of new mutation. *J.Inherit.Metab.Dis.* in press (2009)
18. Schrauf, C., S. Kirchberger, O. Majdic, M. Seyerl, G. Zlabinger, K. Stuhlmeier, M. Sacht, J. Seipelt, J. Stöckl. The ssRNA genome of human rhinovirus induces a type-I IFN response but fails to induce maturation in human monocyte-derived dendritic cells. *J.Immunol* in press (2009)