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T.99. Anaphylaxis-like Shock Induced by LPS Plus Anti-neutrophil Antibodies in Mice

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Background and Aim: During the course of experiments on neutrophil depletion, we happened to find that intravenous injection of an anti-mouse monoclonal antibody (anti-Gr-1) induces anaphylaxis-like shock in mice pretreated with LPS. From this finding we speculate that there might be a phase in which neutrophils play an important role as effector cells during the development of septic shock. In the present study, we examined the mechanism underlying the shock reaction induced by LPS and anti-Gr-1 antibody. Methods: Mice were intravenously injected with *E. coli* LPS (10 μg/kg), and 2 hours later anti-Gr-1 antibody (500 μg/kg) was intravenously injected. Severities of shock were evaluated by decrease in rectal temperature or by scoring shock signs within 1 h after anti-Gr-1 injection. Results and Discussion: We obtained results suggesting that (1) neutrophils are involved in the shock reaction, (2) the reaction is primed by a very low dose of LPS (1 μg/kg), (3) the reaction depends on macrophages and TLR4, (4) complement C5 (but neither histamine, IL-1, TNF, nor active oxygen) may be involved in the shock. We expect that clarifying the detailed mechanism may contribute to understand the pathology of septic shock and to find effective strategies against the disease.

T.100. Nod1 and Nod2 Regulate the Mucosal Inflammatory Response to *Citrobacter Rodentium*

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Citrobacter rodentium is an attaching-effacing bacterium that is closely related to the human pathogen Enterepathogenic *E. coli.* Mice infected with *C. rodentium* develop a transient colitis which harbors many of the hallmarks seen in inflammatory bowel disease, these include: inflammatory cell infiltration, goblet cell depletion and epithelial layer remodeling. Innate immune pattern recognition of bacterial products occurs via the membrane-bound Toll-Like Receptors (TLRs) and/or the cytosolic Nod-like receptors (NLRs). Importantly, mutations in Nod1 and Nod2 are associated with increased susceptibility to develop, respectively, asthma and Crohn's disease. While the TLRs have been implicated in controlling the intestinal inflammatory response to *C. rodentium*, the roles played by NLRs in this model remain unknown. Here, we report that mice lacking both Nod1 and Nod2 have lower increases in colonic inflammatory scores and less enterocyte hyper-proliferation compared to wild-type mice at 7 days post-infection (p.i.). Furthermore, this early hyporesponsiveness is associated with increased bacterial translocation at day 14 p.i., which is the peak of infection. Interestingly, Nod1 and Nod2 were required in non-hematopoietic cells to prevent *C. rodentium* translocation, indicating epithelial-specific functions for these receptors in the colonic mucosa. Together, our findings bring new insights into how recognition of peptidoglycan by the innate immune system may be contributing to the pathogenesis of mucosal auto-inflammatory disorders.

T.101. TNF-α Production was Specifically Enhanced by *Streptococcus Sanguis* Cell Walls through PKR Related Pathway in Behçet's Disease

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Behçet's disease (BD) is a chronic and systemic disorder, and hypersensitivity to *Streptococci* seems to play an important role in its pathogenesis. We had previously obtained *Streptococcus sanguis* (strain BD113-20), isolated from the oral cavity of patients with BD. To compare the responses to *S. sanguis* cell walls in peripheral blood mononuclear cells (PBMC) from patients with BD and with ulcerative colitis (UC), PBMC were cultured with or without *S. sanguis* cell walls in the presence or absence of specific antagonists, and then TNF-α and IL-8 production in the supernatant was assessed by ELISA. The titers of IL-8 and TNF-α spontaneously produced by PBMC from UC patients were significantly higher than those of healthy controls. The titer of TNF-α was significantly enhanced by cell walls of *S. sanguis* in BD but not in UC. From the cDNA array based data, PKR was significantly up-regulated in BD in comparison with UC and controls. Furthermore, PKR inhibitor 2-AP, as well as p38 MAPK inhibitor SB203580 and the proteasome inhibitor MG132, were shown to significanlty suppress the cell wall-stimulated TNF-α production in BD. The paresent study revealed that an innate immune response against *S. sanguis* BD 113-20 via PKR related pathway may have a specific role in the pathogenesis of BD.

T.102. Toll-like Receptor 3 Regulates Late-Phase Reaction of Experimental Allergic Conjunctivitis

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Background: Although a relationship between viral infection and allergic inflammation has been reported, the function of Toll like receptor 3 (TLR3) in allergic inflammation remains to be defined. Objectives: We investigated whether TLR3 regulates the development of murine experimental allergic conjunctivitis (EAC). Methods: EAC was induced by immunization of mice with short ragweed pollen (RW) followed by challenge in eye drops of RW using wild type, TLR3 knock-out (KO) and TLR3 transgenic (Tg) mice, and eosinophil infiltration were examined. Quantitative RT-PCR was used to detect expression

of TLR3. Results: TLR3 KO mice demonstrated significantly decreased eosinophil infiltration in conjunctiva after RW-challenge compared to wild-type mice. Conversely, TLR3 Tg mice demonstrated significantly increased eosinophil infiltration in conjunctiva after RW-challenge compared to wild-type mice. We confirmed that TLR3 mRNA expression in eyelids of TLR3 Tg mice was larger than that of wild type mice after sensitization with challenge, and that TLR3 mRNA expression in eyelids of TLR3 KO mice was undetectable. Conclusion: These data suggest that TLR3 positively regulates late-phase reaction of EAC.

T.103. Oral Chitin Administration Ameliorates Chronic Colitis in TCRα Knockout Mice by Upregulating IFNγ-production and Downregulating Chitinase 3-like-1 (CHI3L1) Expression in Mucosal Tissues

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We have previously reported that CHI3L1 expression is significantly upregulated in colonic epithelial cells (CEC) during the development of colitis. CHI3L1 binds chitin, (a polymer of N-acetylglucosamine) an integral component of crustaceans, parasites, insects, and fungi. Chitin does not, however, exist in mammals and work in other laboratories has implicated an immunoregulatory role for mammalian chitinases in Th2-type inflammation. We have examined the effect of oral chitin administration on the development of Th2-type colitis. 1.5 mg of chitin microparticles (<10 μm) or PBS was orally administered to TCRα KO mice twice/week for six weeks, beginning at weaning. The mice were sacrificed at 5-6 months old and the effects were evaluated by histology, BrdU-index, cytokine production, CHI3L1 expression and enteric bacteria culture. As a result, disease severity in chitin-treated TCRα KO mice was suppressed compared to PBS-treated control mice when evaluated by histology. IFNγ was upregulated and IL-4 was downregulated in the colon and MLN of chitin-treated mice compared to PBS controls. B220+ cells, but not CD4+ T cells and NK1.1+ cells, isolated from the MLN of chitin-treated mice produced higher levels of IFNγ when compared to those of PBS administered group. CHI3L1 mRNA expression in the colon, spleen and MLN were significantly downregulated in chitin-treated mice compared with control mice. CHI3L1 protein expression was also significantly downregulated in CEC and lamina propria of the cecum and colon of chitin-treated mice compared to controls. In addition, enteric bacterial numbers in colon, cecum and liver were significantly reduced in chitin-treated mice. In conclusion, oral administration of chitin microparticles significantly suppresses the development of Th2-mediated chronic colitis in TCRα KO mice.

T.104. Standardizing the Assessment of Immunomodulation of Mushroom Compounds Using an Intestinal Epithelial Cell/Macrophage Co-culture System

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To reproducibly assess the immunomodulatory effects of food compounds we developed an intestinal epithelial cell/macrophage co-culture system. The incidence of allergies and autoimmune diseases might be reduced by foods that modulate immunity. Mushrooms are known for long to assert such effects. Their PAMPs, a.o. β-glucans, bind PRRs, such as dectin-1 and TLRs and influence innate and adaptive immunity. Standardized assays to validate immunomodulation by food compounds are required. Thereto, we developed a co-culture system based on intestinal epithelial Caco-2 cells and THP-1 cells differentiated into macrophages. Upon co-culturing these cells in a transwell system, mushroom extracts were added to the 'lumenal' side of the Caco-2 cells. Culture medium samples were taken at the lumenal and 'submucosal' side of the Caco-2 cells at different time points. The immunomodulatory effects were assessed by measuring the expression levels of a variety of cytokines including IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, and INF-γ. The pro- and anti-inflammatory effects of mushroom compounds, including different β-Glucans, will be presented and the possibilities to explore these compounds to prevent and ameliorate allergies and auto-immune diseases are discussed.

T.105. The Role of the γδ T Cell Receptor in Thymic γδ Cell Development

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γδ T cells are non-MHC-restricted T cells that complement and regulate the activities of αβ T cells particularly in tissues. TCRγδ$^+$ intra epithelial lymphocytes (IELs) play an important role in mucosal repair, homeostasis, and tumor surveillance. Upon activation these multi-functional T cells are able to produce IFN-γ, TNF-α and are also an important source of IL-17. The surface expression of the γδ T Cell Receptor (TCR) is essential for γδ cell development. However, the nature of the specific ligands for the TCRγδ is only just beginning to be understood. Likewise, the precise requirements for ligand binding, activation and signalling through the TCRγδ during γδ cell development in the thymus are still unclear. We aim to clarify the TCR requirements of γδ cells during their thymic development and how this relates to functional potential and location of the γδ cell in the body. We will use retroviral vectors to express in T cell-deficient E14 foetal thymocytes truncated forms of tagged TCRδ and TCRγ chains that lack various regions of the TCR extracellular domains. After 6–12 day foetal thymic organ cultures (FTOC), infected cells will be assessed for the generation of γδTCR$^+$ cells. If present, these

cells will be analysed for characteristics of both a lymphoid and epithelial γδ cell phenotype. Preliminary results show that the expression of full length TCRδ in $\delta^{-/-}$ E14 thymocytes induces γδ T cell development and also that the extracellular region of the δ-chain is essential for the generation γδ T cells.

T.106. Natural Killer Cell Heterogeneity in the Mucosae and Secondary Lymphoid Tissues of SIV-infected Rhesus Macaques

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Although natural killer (NK) cells are among the first cells to respond to acute viral infections and are critical to prevent viral spread, little is known about the nature of these cells in mucosal and secondary lymphoid tissues of primates, where transmission and replication of many viruses, including HIV and SIV, primarily takes place. To address this deficit, we undertook a comprehensive analysis of NK cells in lymph nodes, spleen, and digestive and reproductive mucosae of rhesus macaques. NK cells were defined as CD3-CD8+ NKG2A+ and further divided into four subsets based on expression of CD56 and CD16. Subset distribution was highly disparate - cytolytic CD16+ NK cells predominated in spleen and PBMC, while the majority of mucosal NK cells were CD56+, and LN included both CD16-CD56+ and CD16-CD56- subsets. Interestingly, after infection with pathogenic SIV or vaccination with the live attenuated virus SIVΔnef, NK cell numbers increased as much as 8-fold, as did intracellular Ki67, cell-surface HLA-DR, and serum levels of the NK chemoattractant, IP-10, all of which preceded SIV-specific adaptive responses. Our data indicate that both lymphoid and mucosal tissues are under surveillance by diverse NK cell subsets that mobilize and become activated in response to lentiviruses. NK cells could, therefore, not only play a major role in inhibiting initial rounds of virus replication at the mucosal interface, but also contribute to protective immunity induced by live attenuated lentiviral vaccines.

T.109. Expression of Toll-like Receptors on Subsets of Human Islet Beta Cells

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Several studies documented that inflammatory mediators such as IL-1β, TNF-α, and/or nitric oxide (NO) may play a role in the beta cell destruction associated with type 1 diabetes. Toll-Like receptors (TLR) mediate innate immune responses through NF-κB activation and induction of inflammatory mediators. In this study, we investigated expression of TLRs in human pancreata. Confocal laser scanning microscopy and double immunolabelling for insulin revealed subsets of human insulin-positive cells expressing TLR2, 3 and 4, but almost no insulin positive cells were found to co-express TLR1, 5, 6, 7, 8, 9, 10 and CD180. Interestingly, only a proportion of beta cells expressed TLR2, 3 and 4. In conclusion, TLR2, 3 and 4 are expressed on subsets of human beta cells. Because these pattern recognition receptors are expressed only on a proportion of beta cells they may be used to differentiate among various subsets - possibly highly metabolically active and/or stressed beta cells. Functional expression of TLR2, 3 and 4 on beta cells may represent a gateway for innate immune mechanisms in early stages of beta cell aggression.

T.111. Human Milk Oligosaccharides Inhibit Proinflammatory Processes in Human Intestinal Mucosa

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Breastfeeding infants have a lower risk of inflammatory enteric diseases than in infants fed artificial diets. Several human milk components have been described as anti-inflammatory. Oligosaccharides are collectively one of the largest constituents of human milk, with concentrations exceeded only by lactose and fat. We tested whether the human milk oligosaccharides were capable of inhibiting inflammatory processes in organ culture of immature human intestinal mucosa. IL-8 production, a measure of proinflammatory response, was stimulated by administration of flagelin, polyinosinic-polycytidilic double stranded RNA, and IL-1β. The pro-inflammatory responses to these ligands were attenuated by the presence of human milk oligosaccharides in the medium at concentrations typical of human milk. These results suggest that the human milk oligosaccharides could be a major milk component that contributes to the anti-inflammatory activity of human milk. These data suggest that milk oligosaccharides could be primary contributors to the ability of human milk feeding to reduce the risk of inflammatory conditions in the immature infant.