ANTIMICROBIAL DEFENSES
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OR.37. NLRC1/Nod1 Stimulation Exerts a Profound and Immediate Effect on CXCL13 Production and B Cell Homeostasis: Implications for the Host Response to Pathogens

Jorg Fritz, Dana Philpott, Jennifer Gommerman
University of Toronto, Toronto, ON, Canada

Innate immune recognition of microbe-associated molecular patterns (MAMPs) by multiple families of pattern-recognition molecules (PRMs) is key for the initiation of the first-line host and adaptive immune system to cope with pathogen intrusion. Recent observations have demonstrated that innate immune sensing of peptidoglycan (PGN) by the Nod-like receptor (NLR) family member NLRC1/Nod1 contributes to the priming of pathogen-specific T and B cell immunity as well as to the intestinal lymphoid tissue genesis induced by commensals. To further elucidate the underlying cellular and molecular mechanisms, we studied the first-line host immune response upon peripheral NLRC1/Nod1 stimulation and analyzed if NLRC1/Nod1-mediated PGN recognition of commensals impacts on the immune cell composition of the lamina propria. Peripheral stimulation by its specific agonist revealed a differential role of NLRC1/Nod1 in stromal and hematopoietic cells for the regulation of the first-line host responses. In contrast, wild type and NLRC1/Nod1-deficient animals harbor equal ratios of lamina propria resident CD4+ and CD8+ T cells, B1 and B2 cells, IgA+ plasma cells, suggesting a NLRC1 alone is not solely responsible for commensal driven intestinal homeostasis.

OR.38. STAT3 in Intestinal Epithelial Cells Regulate Proper Barrier Function and Antimicrobial Peptide Induction

Jongdae Lee, Li-Li Hu, Eyal Raz
University of California, San Diego, La Jolla, CA

Inflammatory Bowel Disease (IBD) is composed of ulcerative colitis (UC) and Crohn's disease (CD). Both genetic and environmental factors contribute to this pathogenesis which has a common feature of compromise intestinal epithelial barrier in small and large intestines. We investigate the role of STAT3 in intestinal epithelial cells (STAT3IEC) and identify the novel function of STAT3 in maintaining intestinal homeostasis. STAT3IEC deletion in mice results in compromised barrier function and the loss of IEC polarity due to low tight junction protein levels (i.e. claudin-1, -3, and -5) *in vitro* and *in vivo*. The novel function of STAT3 maintains tight junction protein level *in vitro* and *vivo by* inducing ubiquitin-mediated degradation of SNAI, a transcriptional suppressor of claudins. STAT3 binds with GSK3b, which phosphorylates SNAI for ubiquination and results in degradation. Moreover, STAT3 is essential for host defense against enteropathogenic bacteria (i.e. *Citrobacter rodentium* and enteropathogenic *E. coli*). STAT3IEC knock out mice are highly susceptible to *Citrobactor rodentium* infection due to impaired IEC barrier and no antimicrobial peptide (i.e. regIIIg) induction, which is necessary for the clearance of bacteria. Collectively, STAT3 along with GSK3b forms a SNAI destruction complex in IECs to maintain claudin levels and thus maintain proper barrier function and antibacterial defense.

OR.39. The Role of Specific IgG and Complement in Combating a Primary Mucosal Infection of the Gut Epithelium

Clara Belzer, Qing Liu, Lynn Bry
Brigham and Women's Hospital/ Harvard Medical School, Boston, MA

Citrobacter rodentium is the mouse homolog of the enteropathogenic *E. coli* (EPEC), and causes attaching and effacing (A/E) lesions on the colonic epithelium. In studying host responses that defend epithelial surfaces, we have previously shown that pathogen-specific and CD4+ T cell-dependent IgG, but not serum IgM or secretory IgA, are required for survival and clearance of this epithelial infection. The primary IgG isotypes produced during infection include systemic IgG2c (Th1-dependent antibody) and mucosal IgG2b (Th-3 dependent antibody), both of which are complement-fixing isotypes. We have subsequently evaluated the effector functions of IgG at the mucosal surface in this infection, including constructive interactions with complement. Immunostaining of infected colon has demonstrated deposition of C3 and IgG on adherent *C. rodentium*. Furthermore, C3 and IgG2b can be detected on bacteria shed in the feces. *In vitro* studies with a monoclonal IgG2b against *Citrobacter* LPS O-antigen demonstrated a lytic effect in the presence of complement. Infection of C3-deficient mice demonstrated a survival defect as compared with wild-type controls. Ongoing studies are evaluating the role of complement in the development of protective immunity and functions in controlling early events in infections as well as effecting clearance of pathogens from the mucosal surface.

OR.40. Muc2 Plays a Critical Role in Innate Host Defense Against *Citrobacter Rodentium* by Limiting Mucosal Colonization and Colonic Barrier Dysfunction

Kirk Bergstrom[1], Deanna Gibson[1], Marinieve Montero[1], Caixia Ma[1], Andy Sham[1], Jingtian Huang[1], Bruce Vallance[1], Anna Velcich[2], Kris Chadee[3], Vanessa Kissoon-Singh[3]
[1]*Child and Family Research Insititute, Vancouver, BC, Canada;* [2]*Montefiore Medical Center, Bronx, NY;* [3]*University of Calgary, Alberta, Canada*

MUC2 (mouse, Muc2) is the major mucin comprising the colonic mucus layer, and is secreted by intestinal epithelial cells. Although mucus is thought to act as an innate defense barrier, we have previously shown that Muc2 expression is reduced in the mouse colon in a T and/or B cell-dependent manner during infection by *Citrobacter rodentium* (*Cr*), an *E. coli*-related bacterial pathogen of mice that infects the colonic epithelium. We therefore wanted to determine the role of Muc2 in host defense against *Cr* by infecting Muc2-deficient ($Muc2^{-/-}$) mice. Results: $Muc2^{-/-}$ mice exhibited rapid weight loss after day(D)2 p.i., began

succumbing to infection by D6 p.i., with only 20% surviving by D11 p.i. (WT mice, 80% survived). Plating of stool revealed significantly greater luminal *Cr* burdens in *Muc2*$^{-/-}$ vs WT mice starting at D2 p.i. (P<0.05). Immunostaining for *Cr* LPS showed bacterial accumulation at the surface epithelium of *Muc2*$^{-/-}$ vs WT mice, suggesting the mucosa of *Muc2*$^{-/-}$ mice is more easily colonized. This finding was confirmed using a ligated cecal loop model, where after 9.5 hrs post injection of *Cr*, there were significantly more (P<0.05) bacteria adherent to cecal tissues in *Muc2*$^{-/-}$ vs WT mice. Histology at D6 p.i. revealed dense neutrophilic infiltrate and ulceration in the colon amid focal bacterial overgrowths. This mucosal damage suggested defects in gut barrier function, which was confirmed by a significant increase (P<0.005) of orally administered FITC-Dextran (4 kDa) probe in the serum, and greater systemic *Cr* burdens (e.g. in spleen, mLNs, and liver), in *Muc2*$^{-/-}$ vs WT mice. Conclusion: Muc2 prevents pathogenic *Cr* overgrowths at the mucosal surface, most likely by binding mucosal-associated bacteria for clearance with luminal contents, thereby preventing damage-associated defects in colonic barrier function.