INNATE IMMUNITY 1
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OR.17. NF–kB Signaling in Intestinal Epithelial Cells Affects Composition of Commensal Bacteria and Susceptibility to Intestinal Inflammation

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Bacteria living within the gastrointestinal tract have the capacity to influence immune development and function. Although recent studies have highlighted the association between alterations in commensal populations and disease, the immune components and signaling pathways that influence the composition of the intestinal microbiome are largely unknown. Mice that lack canonical NF-κB signaling in intestinal epithelial cells (IKKβΔIEC) display increased expression of pro-inflammatory cytokines, increased immune cell infiltration, and more severe intestinal damage compared to littermate controls (IKKβcontrol) following infection with the intestinal pathogen Citrobacter rodentium or when subjected to dextran sodium sulfate (DSS) colitis. Using 454 pyrosequencing of 16S ribosomal RNA genes, we examined bacterial communities in the colons of IKKβΔIEC and littermate IKKβcontrol controls. IKKβΔIEC mice exhibited significant alterations in their commensal microbiota. In particular, bacteria of the phylum Firmicutes were reduced in IKKβΔIEC mice and those of the phyla Deferribacteres and Tenericutes were increased in relative frequency compared to littermate controls. These data indicate that altered signaling in the epithelial layer of the intestine can influence intestinal bacterial composition and that these alterations correlate with increased susceptibility to infectious and chemically-induced colitis.

OR.18. Commensal Bacteria Direct Intestinal Immune Responses

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Commensal bacteria are known to influence the development and maturation of the immune system. They have also been found to be important for intestinal homeostasis. For these experiments, we depleted commensal bacteria with antibiotics before infecting mice with non-invasive *S. Typhiumuium*, which is unable to invade epithelial cells and enters the tissue through the lamina propria instead of peyer's patches. In these experiments we have found commensals to be required for limiting intestinal immune responses. Antibiotic treatment results in bacterial trafficking to the mesenteric lymph nodes (MLN), DC activation and T cell priming in the MLN. It also permits induction of systemic IgA responses. In contrast, the presence of commensal bacteria inhibits bacterial trafficking to the MLN, resulting in no DC activation or T cell priming in the MLN and lack of IgA responses. The immune regulatory effect of commensals is dependent on TLR signaling as demonstrated by the ability of non-invasive *S. Typhimurium* to traffic to the MLN in the presence of commensals in Myd88 deficient mice. Our data demonstrate that commensal-mediated inhibition of the mucosal immune system—likely mediated through inhibition of DC trafficking—facilitates the development of an appropriately modulated intestinal immune response. We hypothesize that modulation of this pathway could be used to prevent intestinal inflammatory conditions such as food allergy or colitis or enhance the efficacy for oral vaccinations.

OR.19. Intestinal Colonization Modulates B Cell Development in the Bone Marrow and Spleen

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The immune system of germ-free mice is under-developed and intestinal colonization is known to have profound physiological and immunological consequences for the host. Colonization of germ-free mice induces maturation of both the mucosal and systemic immune systems and all serum antibody titers, except IgE, increase. Here we addressed how exposure to commensal flora influences B cell development. The development of B2 cells in the bone marrow and the spleen was studied during colonization of germ free mice with an intestinal microbiota. We found that the presence of intestinal bacteria shape distinct stages of B cell development, although live bacteria never penetrate further than mesenteric lymph nodes. Proliferation of immature B cells before and after surface expression of the B cell receptor (BCR) was induced in response to intestinal colonization. Using germ-free BCR knock-in animals we determined that BCR specificity did not change the response to colonization. We hypothesized that bacterial compounds acting as Toll-like receptor (TLR) ligands that can reach systemic sites mediated the colonization-induced effects. Surprisingly, however, exposure to commensal microflora also shapes early B cell development following colonization of germ-free MyD88$^{-/-}$TRIF$^{lps2/lps2}$ mice, which lack all TLR signaling. These findings demonstrate how exposure to environmental microflora directly impact on immune development.

OR.20. Intestinal Macrophages are Non-permissive to HIV-1 Due to NF-κB Inactivation

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Macrophages play a crucial role in HIV-1 pathogenesis but the role of intestinal macrophages is poorly understood. Pro-inflammatory blood monocytes are the exclusive source of intestinal macrophages, which are non-inflammatory and do not support HIV-1 replication, as we have reported. Since TGF-β, and possibly other factors, in extra-cellular matrix (stroma) down-regulate monocyte pro-inflammatory function, we further investigated whether stromal factors also down-regulate HIV-1

permissiveness. We used lamina propria stroma (after removal of cells) and blood monocytes to recapitulate in vitro the exposure of recruited blood monocytes to the intestinal microenvironment. Pre-incubation of monocyte-derived macrophages (MDMs) with intestinal stroma-conditioned medium (S-CM) down-regulated CD4 and CCR5 expression on MDMs. Pre-incubation of MDMs with S-CM also potently down-regulated the capacity of the cells to replicate HIV-1. Moreover, simultaneous exposure of MDMs to S-CM and HIV-1, well before receptor down-regulation, impaired HIV-1 permissiveness. In addition, HIV-1 pseudotyped with vesicular stomatitis virus glycoprotein, which enters cells through a CD4/CCR5-independent mechanism, did not replicate in MDMs. Furthermore, S-CM inhibition of HIV-1 replication in MDMs correlated strongly with TGF-β-mediated down-regulation of NF-κB activation. Thus, our findings implicate NF-κB inactivation, as well as reduced HIV-1 receptor expression, in the non-permissiveness of intestinal macrophages to HIV-1.