B CELLS & LYMPHOID ORGANOGENESIS
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M.15. Single Cell Analysis of the Reactivity of Intestinal B Cell Compartments

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In mice, plasma cells (PCs) within the lamina propria (LP) of the gut are a major source of intestinal secretory IgA (SIgA). Luminal SIgA preparations have been shown to recognize specific antigens and AID-mediated hypermutation is required to maintain intestinal homeostasis, demonstrating the presence and relevance of antigen-specific high-affinity antibodies within SIgA. On the other hand, various studies suggest that up to 50% of LP PCs are derived from peritoneal cavity (PerCav) B1 B cells, which produce natural broadly crossreactive antibodies. It is conceivable that the LP compartment contains both polyreactive and antigen-specific PCs, however the relative contribution of these cells to the overall SIgA production has not yet been determined. To assess the antibody reactivities of LP PCs on a single cell level under homeostatic conditions, we cloned and *in vitro* expressed the antibodies of single FACS sorted $B220^{-}$ IgA^{+} LP cells from healthy C57BL/6 mice. Polyreactivity testing of more than 100 antibodies revealed that about 20% of LP PCs were polyreactive, comparable with the proportion detected in splenic PCs. In contrast, the level of polyreactivity in antibodies derived from PerCav B1 cells was over 50%. Sequence analysis of the antibodies' cDNAs showed that the vast majority of LP PCs had undergone somatic hypermutation. In summary our results show that the reactivities of LP PCs are distinct from that of PerCav B1 cells, suggesting an only minor contribution of the latter to the pool of intestinal IgA^{+} plasma cells in healthy adult mice. Our preliminary results provide the basis for further studies on the influence of the commensal flora on the repertoire and reactivity of intestinal PCs.

M.16. Lin-c-kit+Sca-1+ Cells of Mesentery Lymphoid Cluster Support IgA Production of B Cells and B1 Cells Survival by IL-5 Production

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Recently, we found a novel lymphoid cluster in mesentery of mice, which constituted a large amount of Lin-c-kit+Sca-1+ cells, T cells and B cells suggesting a new member of GALT. The lymphoid clusters were surrounded by adipose tissues, and 5 to 50 clusters were found throughout the mesentery. Lin-c-kit+Sca-1+ cells of mesentery lymphoid cluster show a high nucleus/cytoplasm ratio and scanty cytoplasm and also revealed ill-developed Golgi apparatus and endoplasmic reticulum, suggesting that these cells are of lymphoid lineage. Expression of CD45, IL-7Rα, Thy-1 and CD25 on Lin-c-kit+Sca-1+ cells recommended that these cells might be lymphoid progenitors or lymphoid tissue inducer (LTi) cells. However, Lin-c-Kit+Sca-1+ cells differentiate into neither T nor B cells and do not express RORγ, a transcription factor characterizing LTi cells. Lin-c-Kit+Sca-1+ cells proliferate in response to IL-2 and produce large amounts of Th2 cytokines such as IL-5 and IL-6. IL-5 produced by Lin-c-Kit+Sca-1+ cells supports both IgA secretion by plasma cells while suppressing IgG2b and IgG3 secretions, and the self-renewal of B1 cells. These results suggest that Lin-c-kit+Sca-1+ cells of mesentery, which we name "natural helper cells," constitute a novel innate lymphocytes population secreting Th2 cytokines and contributing to the IgA production of mucosal face or B1cells survival of peritoneal cavity.

M.17. HIV-1 Attenuates Virus-specific IgG2 and IgA Responses by Delivering Class Switch-inhibiting Signals to B Cells via Long-range Intercellular Conduits

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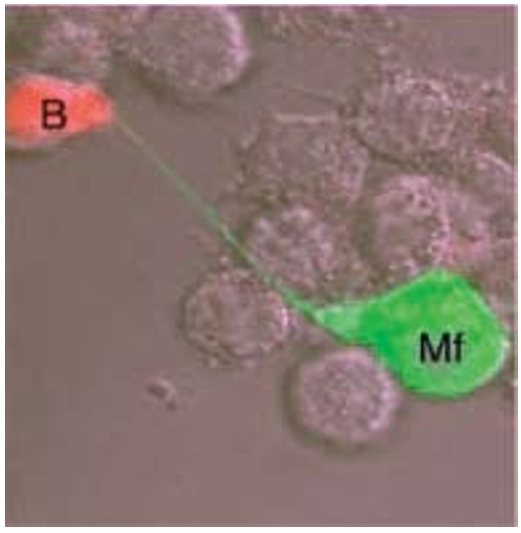

Immunoglobulin G (IgG) and IgA class switching provides immune protection against pathogens, including viruses. We found attenuated IgG2 and IgA class switching and perturbed germinal center differentiation in B cells from systemic and intestinal lymphoid follicles infected by human immunodeficiency virus type-1 (HIV-1). In these follicles, B cells were physically connected with infected macrophages through long-range membrane conduits containing negative factor (Nef), an immunosuppressive viral protein. After stimulating conduit formation, Nef trafficked from macrophages to B cells through an actin-propelled mechanism involving clathrin-dependent and clathrin-independent endocytic pathways. Invasion by Nef rendered B cells less responsive to class switch-inducing signals from CD4+ T cells, including CD40 ligand. By showing that virus-specific IgG2, IgA1 and IgA2 were more abundant in patients infected with Nef-deficient virions than in individuals harboring Nef-sufficient virions, our data suggest that HIV-1 uses Nef-trafficking intercellular conduits to evade class-switched antibody responses in systemic and intestinal districts.