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OR.5. Uptake via Glycoprotein 2 of FimH$^+$ Bacteria by M Cells Initiates Mucosal Immune Response

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Vigorous macromolecular transcytosis via M cells delivers mucosal antigens to the underlying organized lymphoid follicles and is believed to be a prerequisite for initiating antigen-specific mucosal immune responses. However, the molecular mechanisms promoting this antigen uptake are largely unknown. We here report that glycoprotein 2 (GP2), specifically expressed on the apical plasma membrane of M cells among enterocytes, serves as a transcytotic receptor for mucosal antigens. Recombinant GP2 protein selectively bound a subset of commensal and pathogenic enterobacteria, including *Escherichia coli* and *Salmonella typhimurium*, by recognizing FimH-expressing type 1 pili on the bacterial outer membrane. Likewise, these bacteria were colocalized with endogenous GP2 on the apical plasma membrane as well as in cytoplasmic vesicles in M cells. *In vivo* 3D imaging analysis revealed that *E. coli* internalized by M cells was transcytosed to the invaginated basal domain called "M-cell pocket" and subsequently transferred to dendritic cells (DCs). Such a bacterial transcytosis by M cells was defective in GP2$^{-/-}$ mice, resulting in attenuation of antigen-specific immune responses in PPs. Thus GP2 is a novel transcytotic receptor on M cells and plays a key role in mucosal immunosurveillance for type I piliated bacteria.

OR.6. Systemic Administration of Soluble RANKL to Mice Induces Development of Villous M Cells on All Small Intestinal Villi

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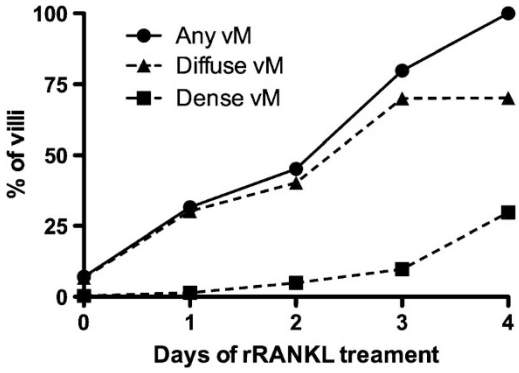

Microfold cells (M cells) are cells specialized for particulate antigen uptake in the epithelium covering Peyer's patches (PP) and other mucosal lymphoid tissues. Villous M cells (vM) have the same functions as PP M cells, but are located on small intestinal villi away from organized lymphoid tissues. Diffuse and dense patterns of distribution of vM exist. In BALB/c mice, about 7% of small intestinal villi have vM. Most of these villi have small numbers of vM in a diffuse distribution. In other studies, we showed that RANKL is essential for the development of PP M cells and that systemic administration of 100 μg/day of soluble RANKL restored a normal population of PP M cells in RANKL null mice. We asked whether RANKL treatment of BALB/c mice also induced vM. The fraction of villi with vM and the number of vM both increased, starting 24 hours after the first RANKL injection. After treatment for 4 days, essentially all small intestinal villi had vM, with 30% of villi showing a dense pattern. Induction of supraphysiologic levels of vM using exogenous RANKL is a novel strategy with the potential to improve the delivery of mucosal vaccines and/or increase efficiency of oral tolerance induction.

OR.7. Bacterial Proteases Contribute to the Development of Chronic Intestinal Inflammation by Impairing Epithelial Barrier Function

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The activation of endogenous matrix metalloproteinases (MMPs) plays an important role in the pathogenesis of chronic intestinal inflammation. In this study, we investigated the influence of bacteria-derived proteases on the development of intestinal inflammation. Monoassociation experiments of wild type (WT) and IL-10$^{-/-}$ mice (129SvEv) with *E. faecalis* strain OG1RF showed increased mRNA expression levels of the bacterial gelatinase E (GelE) under conditions of experimental colitis. To further characterize the role of this bacterial protease in the pathogenesis of chronic intestinal inflammation, we monoassociated WT and IL-10$^{-/-}$ mice for 15 weeks with *E. faecalis* strain OG1RF and isogenic mutant strains that lack GelE expression including TX5264 (gelE deletion) and TX5266 (fsrB deletion). Histopathological analysis revealed a significant reduction of colonic inflammation in the absence of bacterial GelE. Transwell experiments with concentrated conditioned media (CM) of *E. faecalis* OG1RF demonstrated a tremendous decrease of TER values in intestinal epithelial cells whereas the GelE deficient mutant strain (TX5264) did not affect the barrier integrity. Additional experiments with purified proteolytically active GelE also revealed a dramatic decrease of TER values, supporting our hypothesis that bacterial proteases lead to the impairment of epithelial barrier function and contribute to the pathogenesis of intestinal inflammation.

OR.8. IL-22 Regulates Colonic Wound Healing by Inducing Epithelial STAT3 Activity

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Inflammatory bowel diseases like Crohn's disease and ulcerative colitis are thought to result from a dysregulated intestinal immune response to bacteria present in the commensal flora. To date, it remains unclear whether a breakdown of immune tolerance is the primary cause of these diseases or occurs downstream of a primary defect of the intestinal barrier and intestinal epithelial cells. Here we demonstrate that STAT3 signalling in intestinal epithelial cells is rapidly induced in response to gut inflammation. STAT3 is a pleiotropic transcription factor with important functions in cytokine signalling and cellular homeostasis in a variety of tissues. Studies in genetically engineered mice showed that epithelial STAT3 activation in DSS-induced colitis is dependent on the cytokine IL-22 rather than IL-6. IL-22 was secreted by colonic dendritic cells in response to Toll-like receptor stimulation. Conditional knockout mice with an IEC specific deletion of STAT3 activity were highly susceptible to experimental colitis, indicating that epithelial STAT3 regulates gut homeostasis and protects mice from inflammation-related tissue destruction. STAT3IEC-KO mice, upon induction of colitis, showed a striking defect of epithelial restitution. Gene chip analysis indicated that STAT3 regulates the cellular stress response, apoptosis and pathways associated with wound healing in IEC. Consistently, epithelial STAT3 was found to be important in wound-healing experiments in STAT3IEC-KO mice *in vivo* and in cell culture experiments in vitro. In summary, our data suggest that intestinal epithelial STAT3 activation regulates immune homeostasis in the gut by promoting IL-22-dependent mucosal wound healing.