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M.67. TWEAK/Fn14 Pathway Promotes Epithelial Cell Apoptosis after γ-irradiation

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TWEAK (TNF-like weak inducer of apoptosis) is a TNF family cytokine that mediates pleiotropic effects, including proinflammatory and pro-angiogenetic roles, through its receptor, FGF-inducible molecule 14 (Fn14). Fn14 expression is highly upregulated in contexts of tissue injury and regeneration, and chronic inflammatory disease. We previously investigated colitis in mouse models and found that TWEAK deficiency, Fn14 deficiency and treatment with TWEAK blocking monoclonal antibody significantly ameliorated TNBS colitis, with less deformity and branching in the regenerating crypt. This result prompted us to investigate role of TWEAK/Fn14 in epithelial cell regeneration. Whole body γ-irradiation induced upregulation of Fn14 mRNA in the both small and large intestine. Messenger RNA level of TWEAK was also elevated 24 hours after γ-irradiation, at the time point when many of intestinal epithelial cells underwent apoptosis. TWEAK or Fn14 deficient mice in both BALB/c and C57BL/6 background obviously had less numbers of apoptotic cells in the small and large intestine after γ-irradiation when compared with wild type mice. Treatment of WT mice with anti-TWEAK antibody also increased the number of regenerating crypts. Thus, TWEAK/Fn14 pathway enhances epithelial cell death, and blocking TWEAK may prevent epithelial cell damage and mucositis in irradiation therapy.

M.68. HIV-1 Increases Mucosal Epithelial Barrier Permeability to Viral Bacterial Translocation

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Sexual transmission across intestinal and genital mucosa accounts for the vast majority of HIV-1 infection. While recent studies indicate that following exposure to genital or intestinal mucosa, the main targets for HIV-1 infection are Langerhans and T cells, how HIV-1 crosses the intact epithelial barrier at mucosal sites is still not clearly understood. Here we show that exposure to HIV-1 can breach the integrity of mucosal epithelial barrier, allowing translocation of virus and bacteria. Primary epithelial cells isolated from female genital tract and T84 intestinal cell line were grown in transwells until they formed polarized, confluent monolayers. The integrity of monolayers and formation of tight junctions was measured by high transepithelial resistance (TER) values. Exposure of genital and intestinal epithelial monolayers for 24 hours to HIV-1 significantly reduced the TER by 40–50%, without affecting viability of cells measured by MTT assay. Examination of epithelial monolayers by Real-time PCR and confocal imaginf demonstrated significant reduction in mRNA levels of Claudin 1, 2, 4, occludin and ZO-1. Similar results were observed in mucosal epithelial cells following exposure to X4, R5 and clinical HIV-1 strains, but not after HSV-2 infection. The decrease in TER and tight junction proteins started 2 hours following HIV-1 exposure and reached the maximum level after 12–16 hours. This was accompanied by increased permeability seen by dextran blue dye leakage and enhanced viral and bacterial translocation across the epithelial monolayers. This permeability breach could be responsible for small but significant crossing of mucosal epithelium by virus and bacteria present in the lumen of mucosa. This is clinically relevant for the unexplained immune activation seen in HIV-1 infected individuals.

M.69. Lack of Vitamin A Imparied Innate Immunity and Exacerbated Dss-induced Colitis

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Vitamin A and its biologically active derivatives, retinoids participate in a variety of biological processes including maintenance of normal tissues. To identify the role of vitamin A in acute intestinal inflammation, we examined the effect of vitamin A-deficiency (VAD) on dextran sulfate sodium (DSS)-induced colitis. Vitamin A-sufficient (VAS) and VAD female C57BL/6 (B6) mice were fed 4% DSS for 4 days, and after termination of this treatment by changing DSS water to normal water, were followed up until day 10. All of VAD B6 mice died within 10 days, while all of VAS B6 mice survived for 10 days. Similar results were observed when DSS-treated SCID mice were used. To test the functional role of vitamin A for mucosal barrier function, we examined the effect of VAD on *S. typhimurium* infection induced by oral gavage in SCID mice. VAD SCID mice showed significantly higher bacterial loads in the spleen, and liver compared with VAS SCID mice. These results indicate that vitamin A has an important role in regulation of mucosal barrier function to conserve the homeostasis of the gastrointestinal tract.

M.70. Retinoic Acid-inducible Gene-I is Constitutively Expressed and Involved in IFN-γ-stimulated CXCL9-11 Production in Intestinal Epithelial Cells

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Retinoic acid-inducible gene-I (RIG-I) is a member of the DExH/D family proteins, and plays an important role in antiviral response via interferon-inducible genes (ISGs) and type 1 IFN. In this study, the roles of RIG-I in the epithelial cells in the cross-talk between type 2 IFN and inducible chemokines production are high-lighted. The results showed that RIG-I was constitutively expressed in normal surface epithelia lining the colonic mucosa. RIG-I was constitutively expressed in the epithelial cell lines HT-29, and IFN-γ and TNF-α enhanced the RIG-I expression in a dose-dependent manner. IFN-γ was shown

to stimulate CXCL9, CXCL10, and CXCL11 production, and RNA interference against RIG-I resulted in significant decrease of IFN-γ-induced CXCL9, 10 and 11 productions. Furthermore, IFN-γ and TNF-α have been shown to have synergistic effects on the induction of RIG-I. These results suggest that RIG-I play an important role in the crosstalk between inflammatory cytokines and immune cell trafficking. In conclusion, RIG-I might regulate the gut barrier function in homeostatic, and might be involved in the Th-1 skewed inflammatory conditions.

M.71. Role of Butyrate in Peptidoglycan-mediated Mucosal Immune Response: Regulation of Nucleotide-binding and Oligomerization Domain 2 (NOD2)

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It is well recognized that interactions between food and digestive tract microbiological flora have profound influence on human health. In particular, the interactions have an impact on the immune response. Butyrate, produced during fermentation of dietary fibers by intestinal bacteria, plays an important role in the regulation of mucosal immunity. Defense mechanisms against foreign substances such as peptidoglycan (PGN), a mesh-like layer outside the plasma membrane of bacteria are mediated by pathogen-recognition receptors, including nucleotide-binding and oligomerization domain (NOD) proteins and toll-like receptors (TLRs) of the digestive tract. Through this work, we aimed to study the role of butyrate in regulating the behaviors of NOD2 and TLR2, as well as PGN-mediated chemokine production in the polarized Caco-2 colon model. It was found that butyrate up-regulated NOD2, but not NOD1 and TLR2, through the increase of histone acetylation at the NOD2 promoter region and hence promoted PGN-induced IL8 and GRO-α secretion. Knockdown of NOD2 and TLR2 by siRNA significantly compromised PGN-mediated chemokine production, suggesting that both NOD2 and TLR2 were required for the maximum response. Our findings offer a better understanding of the mechanism by which butyrate regulates the mucosal immunity for normal intestinal function. It can also be inferred from the results of this study that dietary fibers have an impact on inflammatory bowel diseases.

M.72. Tumor Necrosis Factor Induced Mucin Release and Goblet Cell Depletion; A Potential Early Histiologic Change in Necrotizing Enterocolitis

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Necrotizing enterocolitis (NEC) affects 3% of infants admitted to an ICU and has a mortality of up to 50%. Intestinal tract immaturity and initiation of inflammation by tumor necrosis factor (TNF) are felt to be two of the major factors predisposing infants to develop NEC. Our hypothesis is that the earliest response of the newborn to intestinal damage is mucin release, leaving a subsequent mucin deficiency which may predispose the mucosa to development of NEC. Ileal samples were obtained from premature infants who developed either spontaneous intestinal perforation (SIP) or NEC, stained with PAS, and examined microscopically (40X) in a treatment-blinded manner by a single investigator. To determine the role of TNF, newborn C57BL/6J mice were given intraperitoneal injections of TNF (2.5 μg/gbw) or saline. Pups were sacrificed eight hours after injection, and the ileum was isolated and harvested for histopathologic evaluation as above. Infants with NEC have significantly greater loss of ileal mucous in the epithelial goblet cells than infants of the same gestation with SIP. SIP samples had an average goblet cell count of 114±61 compared to NEC 10±13 ($p<0.0002$). Newborn mice treated with TNF also show a significant loss of ileal epithelial goblet cell mucous when compared to mice injected with saline. Saline samples had an average goblet cell count of 550±195 compared to TNF 243±69 ($p<0.0001$). These studies demonstrate a significant decrease in mucous-laden goblet cell numbers in infants with NEC compared to aged matched controls with SIP. This loss is also seen in newborn mice treated with TNF demonstrating a TNF-dependent loss of mucous in the epithelial cells of the ileum. As intestinal mucins promote barrier defense and innate immunity, TNF-induced loss may play a key role in the pathogenesis of NEC.

M.73. Lipoxygenases in Human Lung Epithelium: A Possible Role in Bacterial Induced Neutrophilic Trans-epithelial Migration

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Pneumonia, cystic fibrosis and asthma are lung diseases afflicting many people in both developing and western nations over a wide socio-economic range. One aspect these human diseases share is a strong neutrophilic component. Our group is interested in understanding the inflammatory mechanisms that draw neutrophils into the tissues. We have observed that neutrophils migrate across human lung epithelial barriers in response to the pathogenic bacteria strains *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, but not in response to non-pathogenic *E. coli*. Lipoxygenases are enzymes involved in catalyzing the conversion of arachidonic acid into certain eicosanoids including hepoxilin A3. Hepoxilin A3 is a known neutrophil chemoattractant that we have shown to be responsible for mediating bacterial-induced migration across lung epithelial monolayers. We found that both 12-lipoxygenase and 15-lipoxygenase are expressed in the human lung cell lines H292 and BEAS-2B irrespective of stimulation with *K. pneumonia*, *P. aeruginosa or E. coli*. Furthermore, infection with pathogenic bacteria results in an increase in the epithelial cell production of the lipoxygenase product 12-s-HETE. Our future directions include generating lung cell lines with the 12- and 15-lipoxygenase genes silenced by RNAi to dissect the role of these enzymes in neutrophil migration across the lung epithelial barrier.

M.74. Innate Immune Responses by Different Epithelia to Fungal Agonists and *Candida Albicans*

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Responsiveness to microbes depends on innate recognition molecules known as pattern recognition receptors (PRRs), triggered by conserved pathogen-associated molecular patterns (PAMPs) expressed by microbes. PRRs that respond to fungi in myeloid cells are TLR4, TLR2, and dectin-1/hβ-GR, recognising mannans, phospholipomannans, and β-glucans, respectively. We set out to investigate the effects of *C. albicans* on expression of PRRs in epithelium and their importance in epithelial immune responses. Using epithelial monolayers and reconstituted human epithelium (RHE) we examined the responsiveness of epithelia to TLR1-9 agonists and *C. albicans*. All epithelia were unresponsive to TLR agonists, with the exception of TLR3 and TLR2 agonists, which stimulated pro-inflammatory cytokine release. There were differences in the pattern of cytoines/chemokines produced by different epithelial cells as well as between RHE and monolayers, demonstrating the importance of location and structure. *C. albicans* induced a strong cytokine and chemotactic response and the dose of *C. albicans* appears crucial. A concentration typically found during infection was optimal for inducing cytokine release. In conclusion, epithelial cells are unresponsive to most PAMPs, the exceptions being viral PAMPs, TLR2 PAMPs and occasionally flagellin. There are, however, cell-specific differences. *C. albicans* induces pro-inflammatory cytokine responses in epithelial cells and demonstrates cell-specific cytokine/chemo-kine profiles.

M.75. O-antigen Influences Lipopolysaccharide Recognition by Intestinal Epithelial Cells

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Similar to professional immune cells, intestinal epithelial cells express receptors of the innate immune system such as toll-like receptors (TLRs). TLRs have been shown to play an important role in the host defence against enteropathogenic bacteria such as *Salmonella Typhimurium*. Toll like receptor 4 (TLR4), the receptor for lipopolysaccharide (LPS) is expressed in intestinal epithelial (m-ICcl2) cells. In contrast to myeloid cells, that exhibit surface expression of the receptor, TLR4 expression by intestinal epithelial cells is restricted to an intracellular compartment, the Golgi apparatus and receptor activation depends on ligand internalization and intact cell traffic. Here we show that TLR4-mediated recognition of wild-type but not WaaL-deficient Salmonella lacking the O-antigen of the LPS is significantly delayed in intestinal epithelial cells but not myeloid cells. Impaired recognition is independent of the invasive phenotype of Salmonella but relies on delayed LPS internalization of smooth, O-antigen-positive LPS as compared to rough O-antigen-negative LPS by intestinal epithelial cells. Early recognition of rough LPS significantly contributes to the control of viable intracellular bacteria. The O-antigen modification of LPS might therefore represent an innate immune evasion strategy to facilitate intracellular survival of Salmonella during intestinal infection.

M.76. TNFR and LTβR Agonists Induce M Cell Specific Genes in Rat and Human Intestinal Epithelial Cells

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M cells play a role in mucosal immune surveillance by transcytosis of particles, but the mechanisms of M cell differentiation are poorly understood; moreover, there are few molecular markers of differentiation. Previously, *in vitro* M cell models used cocultures of Caco-2 cells with lymphocytes, so to refine this model, we tested the effects of lymphotoxin beta receptor (LTβR) and TNF receptor (TNFR) agonists. We found that cytokine treatment can induce FAE specific genes (CCL20 and laminin-3) in Caco2-BBe cells and IEC-6 cells as well as rodent M cell specific genes such as Sgne-1 and GP2. The cytokines have distinct but complementary effects; TNFα appeared to be more important in inducing FAE specific genes, while the LTβR agonist induced more M cell specific genes. The combination of cytokines showed synergy in the induction of CCL20, and also in the surprising expression of CD137. Functionally, cytokine treatment led to the reorganization of microvilli, Claudin 4 redistribution and enhanced endocytosis of Yersinia, though complete particle transcytosis was not enhanced. Our results will be helpful in establishing a new M cell *in vitro* model, and providing useful information for M cell differentiation *in vivo*.

M.77. Hormone Responsive Vaginal Tissue Model

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The vaginal mucosa is hormone sensitive and its proliferation and maturation are known to be influenced by reproductive hormones. Here, we report development of an endocrine hormone responsive organotypic full-thickness human vaginal tissue (VEC-FT) model. The tissue is cultured from primary fibroblasts and epithelial cells derived from non-diseased ectocervical tissue using serum free medium. The tissue model has phenotypic and architectural similarity to the *in vivo* counterpart. Immunohistochemical analysis and RT-PCR showed expression of estrogen receptors (ER-alpha and ER-beta) and progesterone receptors. Treatment with estradiol resulted in cornification of the epithelial layer, increased transepithelial electrical resistance (TEER), and upregulation of progesterone receptors. Progesterone treatment enhanced mucin-4 expression but did not produce significant changes in tissue structure or hormone receptor expression levels. The highly differentiated tissue model expresses Toll-like receptors (TLRs)

-1, 2, 3, 5, and 6 in a manner similar to that observed in vaginaectocervical tissue explants. The TLRs are responsive to ligand stimulation which resulted in release of cytokines such as IL-8 and IL-6 and other chemokines and anti microbial molecules involved in mucosal immune responses including SLPI, MIF, MCP-1, SDF-1alpha, and GRO-alpha. In conclusion, the model can serve as a valuable tool in the study of hormonal effects of: 1) vaginal mucosal immunology, 2) susceptibility to infection, and 3) preclinical assessment of toxicity and proinflammatory effects of therapeutics, microbicides, and feminine care products.

M.78. Serum IgG Protects the Intestinal Epithelium from Toxin-induced Damage, Independent of FcRn

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Shiga-like enterotoxins are extremely potent ribosome inactivating proteins associated with emerging foodborne *E.coli* infections and, in the case of ricin, are potential bioterrorism agents. A hallmark of this family of toxins is their ability to induce widespread villous atrophy, inter-epithelial swelling, and sloughing of mature absorptive enterocytes from the tips of villi. We recently developed a mouse model of intestinal ricin intoxication to better understand the effects of shiga-like toxins on epithelial physiology and barrier function *in vivo*, and to determine the role of secretory IgA (SIgA) and serum IgG in protecting the epithelium from toxin-induced damage. Intestinal immunity to ricin was achieved by immunizing mice intragastrically with ricin toxoid (RT), and protection correlated with elevated levels of anti-toxin SIgA. However, mice lacking the polymeric immunoglobulin receptor were also immune to ricin, suggesting a role for serum IgG in epithelial defense. To examine this in more detail, we performed passive protection studies in which monoclonal anti-ricin IgA and IgG antibodies were provided systemically to mice prior to ricin challenge. IgG was effective as IgA in conferring immunity to ricin. Moreover, protection was observed in β2 microglobulin knock-out mice, demonstrating that immunity was independent of FcRn. Leakage of serum components into the intestinal lumen is unlikely to explain the observed effects of IgG in this model, as ricin had a negligible impact on epithelial barrier function at early time points. These data challenge our fundamental assumptions about the mechanisms of IgG-mediated immunity in the intestinal mucosa, and highlight the potential of parenteral-based vaccines to confer protection against the shiga-like family of toxins.

M.81. The Role of TLR2 and TLR4 in Postnatal Intestinal Development

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Background: We have shown mice lacking TLR2 have increased epithelial injury in a model of necrotizing enterocolitis (NEC). Clinical trials have also demonstrated that infants receiving probiotic therapy have a decreased incidence of NEC. We hypothesize that early postnatal bacterial signaling through TLR2 and TLR4 is necessary for intestinal epithelial maturation. Methods: Two-week-old C57BL/6 wild-type (WT), B6.TLR2$^{-/-}$, B6.TLR 4$^{-/-}$, B6.TLR2/4$^{-/-}$, and antibiotic treated WT mice (microbial-reduced, MR) were injected with bromodeoxyuridine (BrdU) 90 minutes prior to sacrifice. Sections of intestine were fixed for light and electron microscopy (EM). Proliferating cells were detected with an antibody to BrdU. Slides counterstained with hematoxylin and eosin were used for structural analyses. Activated caspase-3 mediated apoptosis was detected with immunofluorescence. Flow cytometry was performed on mesenteric lymph nodes to detect T-cell activation. Results: The median numbers of BrdU positive crypt cells in the WT mice were significantly greater than the TLR deficient and MR mice. Conversely, there were less apoptotic cells and increased crypt depth in the WT mice. Subjectively, the EM images showed greater number of desmosomes in the WT mice. The TLR 2/4$^{-/-}$ mice had greater numbers of activated mesenteric CD4+ T cells (CD44+, CD69+, and CD25+). Conclusions: Mice lacking TLR 2 and TLR4 or are devoid of bacteria have impaired early postnatal intestinal epithelial cell proliferation. Those devoid of TLR 2/4$^{-/-}$ have a relative pro-inflammatory state, reflected by increased T cell activation. In addition, these mice have abnormal villus and ultra-structural development. These data may help indentify the specific bacteria or their respective molecules that are necessary in probiotic-mediated NEC protection.

M.82. Transferred CD4+ T Cells Induce IEC Differentiation and Decrease Permeability in the Distal Colon of RAG1-/- Deficient Mice

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Gut lympho-epithelial interactions occur in the epithelial and sub-epithelial space. Normal LPL induced IEC differentiation (promoting intestinal alkaline phosphatase (IAP) activity) in T84 cells, and this effect was enhanced when T84 cells were co-cultured with CD LPL. This finding was corroborated *in vivo* as crypt IECs in CD mucosa stained positively for IAP. AIM: To determine whether IEC differentiation is regulated by LPLs *in vivo*. CD4+CD45RbHi T cells and/or CD4+CD45Rblow T cells were isolated from C57BL/6 spleens and adoptively transferred into RAG1-/- mice for 3 weeks. Colonic tissues were stained for IAP, cleaved Notch, and villin or mounted into Ussing chambers to assess permeability. IAP, cleaved Notch, and villin staining were absent in RAG1-/- colonocytes, suggesting that the presence of LPL is needed for colonic IEC. Upon transfer of CD45RbHi and Lo T cells, expression of IAP, cleaved Notch, and villin was induced in colonocytes within 3 weeks of transfer. This effect was not related to the presence of inflammation as no inflammation was seen with the co-transfer. Upon transfer of Rbhi and lo cells there was a significant decrease in the permeability of the distal colon of mice correlating with the

upregulation of differentiation markers. The presence of LPLs promote IEC differentiation in the absence of inflammation. The absence of LPLs such as that seen in RAG1-/- is correlated with a defect in colonic epithelial differentiation and colonic permeability. This defect can be overcome by transferring naive +/- regulatory CD4+ T cells, suggesting a cell-dependent mechanism rather than one that is inflammation related. Thus the crosstalk occurring in the lamina propria promotes the acceleration of IEC differentiation.

M.83. Reconstruction of a New Human Endocervical Tissue Model

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The endocervical epithelium is a gate-keeper guarding against pathogen invasion and providing barriers to sperm entry into the uterus. Here, we report the reconstruction of the first human organotypic endocervical tissue model cultured using primary endocervical epithelial cells in a serum free medium. Histological and ultrastructural analysis of the tissue revealed the presence of columnar epithelial cells with tight junctions. Immunohistochemical staining showed expression of: a) mucins 1 and 4, b) cytokeratins 5, 7, and 17, c) estrogen receptor, and d) the progenitor cell marker p63 in a manner similar to that expressed in native endocervical tissue. To examine the utility of the tissue for preclinical testing of chemicals, different concentrations of Nonoxynol-9 (N-9) and Benzalkonium chloride (BZK) were topically applied to the tissue model. After a 24-hour exposure, N-9 at concentrations > 0.002% or BZK at concentrations > 0.125% were toxic to the tissue as measured by the MTT assay (n=3 lots) and elevated levels of proinflammatory cytokines including IL-1 alpha, IL-1 beta, IL-6, and IL-8 were observed. In conclusion, the new endocervical tissue model will likely serve as a valuable tool to study mucosl immunology, microbial infection mechanisms, and effects of topically applied formulations and microbicides in the mucosal microenvironment.

M.85. Expression of Intracellular Antiviral Genes in Human Uterine Primary Epithelial Cells

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Introduction: We have shown previously that estradiol enhances the secretion of antimicrobials by uterine epithelial cells (Muc. Immunol. 2008;1:317-325). The intracellular antiviral genes MxA, OAS1-3, PKR, APOBEC3G and ISG15 inhibit different stages of the viral life-cycle and may potentially inhibit HIV-1 replication. However, their expression in uterine epithelial cells and response to hormonal influence is unknown. Methods: Uterine primary epithelial cells were isolated from hysterectomy samples and grown to confluence on cell inserts. Following 5–7 days in culture, cells were treated with Toll-like receptor (TLR) agonists, estradiol (5×10^{-8}M) and/or progesterone (1×10^{-7}M) before analyzing mRNA expression by RT-PCR. Results: Epithelial cells strongly upregulate mRNA levels of all five antiviral genes only in response to the TLR 3 agonist (PolyI:C), with maximal upregulation after 12 hours. Estradiol alone, progesterone alone, or with PolyI:C, had no effect on mRNA levels of intracellular genes measured between 6–24 hours post-treatment. Conclusions: Uterine primary epithelial cells are capable of responding to viral stimuli by upregulating intracellular antiviral genes. The lack of hormone response suggests that unlike secreted antimicrobials, intracellular antiviral protection is not modulated by hormonal influence, and is therefore maintained throughout the menstrual cycle. Supported by NIH grant AI51877, AI-071761 (CRW).

M.85.5 Gut-specific Deletion of Neural Wiskott-Aldrich Syndrome Protein (N-WASP) Leads to Alteration of Intestinal Homeostasis *in vivo*

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Deficiencies in Wiskott-Aldrich Syndrome protein (WASP) are associated with human IBD and spontaneous colitis in mice. We examined the role of ubiquitously expressed N-WASP, a key regulator of the actin cytoskeleton, in the intestinal epithelium. To generate mice with gut-restricted deletion of N-WASP (intestineNWASP KO, iNWKO), mice expressing Cre recombinase under the villin promoter were mated to mice homozygous for a floxed N-WASP allele (N-WASP$^{L2L/L2L}$). In all experiments, iNWKO mice were compared with N-WASP$^{L2L/L2L}$ mice lacking the villin-Cre transgene. Intestinal epithelial cells were isolated by EDTA dissociation and centrifugation, and tissue was examined with H&E, Alcian blue, immunofluorescence and electron microscopy (EM). To examine proliferation and migration of N-WASP deficient enterocytes, mice were injected with BrdU and sacrificed at 2 and 24 hours. iNWKO mice were viable and fertile, but failed to appropriately gain weight; adults weighed on average 30% less than their littermate controls (n=25, p=0.007). There was no spontaneous development of enterocolitis. Absence N-WASP protein was confirmed by Western blot on intestinal epithelial lysates. Numbers of Paneth, goblet and BrdU-incorporating crypt progenitor cells were similar. Notably, iNWKO enterocytes contained uncondensed nuclei and exhibited markedly increased migration at 24 hours. EM revealed disorganized and clustered colonic microvilli and the absence of a terminal web. The distribution of E-cadherin was similar between iNWKO mice and WT controls. Deletion of intestinal N-WASP leads to a phenotype of wasting, increased intestinal cell turnover and microvillus structural abnormalities, linking the actin cytoskeleton to the maintenance of gut homeostasis.