CANCER & INFLAMMATION
Monday, July 6

M.25. Consecutive Deficiency of Intestinal Mucin2 and Mucin2-associated Carbohydrates in Chronic Inflammation Leads to Tumorigenesis in the Murine Model

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We recently demonstrated that DNA hypermethylation-mediated incomplete synthesis of the blood group Sda carbohydrate antigen substantially contributes to the malignant phenotype of human gastrointestinal cancer cells. This study was aimed to examine the consecutive changes of DNA methylation and carbohydrate expression in the normal colon, inflamed colon, and colitis-associated tumor. Combination of carcinogen azoxymethane, colitis inducer dextran sodium sulfate, and/or DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine was used to induce colitis and colitis-associated tumor in mice. Regenerative crypt, colon, and tumor samples were taken at various time points. Expression of DNA methyltransferases, glycogenes, and lectin-binding carbohydrates were examined using the combination of real-time RT-PCR and immunohistochemitry. Several key findings emerge from the data. First, DNA methyltransferase 1 plays an important role in the epithelial regeneration and tumorigenesis. Second, consecutive loss of mucin2 and mucin2-associated carbohydrates is the hallmark of inflammation and colitis-associated tumor, and this lacking is under the regulation of DNA methylation. Third, incomplete synthesis of particular glycogenes/carbohydrates, including muc2/Mucin2, is specifically present in the colitis-associated tumor, and this deficiency has occurred early and persistently in the tumor-free mucosa during inflammation. We conclude that chronic inflammation causes consecutive loss of Mucin2 that plays a major role in the colitis-associated tumorigenesis.

M.26. Persistent TLR Signaling in the Intestinal Epithelium Protects Against DSS Induced Colitis and Tumorigenesis in APCmin/+ Mice

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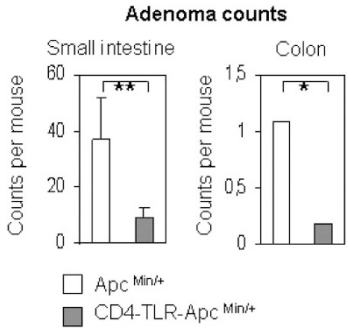

The epithelial cell transduces signals from the intestinal lumen to the mucosal immune system through toll-like receptors (TLR). However, the effect of persistent bacterial stimulation on inflammation is not fully elucidated. Furthermore, chronic inflammation predisposes to cancer. We hypothesized that long term epithelial stimulation differs from acute, possibly by up regulating signal transduction inhibitors. We developed a transgenic mouse using a constitutively-active human TLR cytoplasmic domain linked to an intestinal epithelial cell-specific promoter. Transgenic mice expressed epithelial CD4-TLR mRNA in the small and proximal large intestine, which appeared normal. The expression of tollip, an NF-κB inhibitor, was up regulated. NF-κB dependent cytokines enhance T helper 17 cell (Th17) production; we found significantly fewer circulating Th17 cells in the CD4-TLR transgenic mice than wild-type littermates. To study inflammation, colitis was induced with dextran sodium sulfate (DSS). CD4-TLR transgenic mice lost significantly less weight, with less intestinal inflammation than wild-type littermates. Finally, to investigate tumor development, CD4-TLR mice were crossed with Apc Min/+ mice, which normally develop multiple spontaneous tumors. Fewer adenomas, developed, of a smaller size, compared to Apc Min/+ mice. Thus chronic TLR stimulation of intestinal epithelial cells protects against both inflammation and tumorigenesis.

M.27. Microbiota in Inflammation-related Colon Cancer Development

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The gut microbiota plays an important role in cancer development. Its composition could have either positive or negative effect on carcinogenesis. The aim of our work was to study the effect of different microbial conditions on colon carcinogenesis in the azoxymethane/dextran sulfate sodium model of colon cancer. The colon cancer was induced in germ-free, conventional and in antibiotic-treated conventional mice and the presence of epithelial lesions was evaluated by histology and immunohistochemistry (beta-catenin and iNOS). Changes in gut microbiota were evaluated using 16S rRNA gene analysis. Expression of caveolin1 and target genes of Wnt pathway was determined by real time PCR. We found that animals with reduced microbiota burden developed fewer tumors than control animals. After chemical induction only 20% of germ-free and 55% of antibiotic-treated mice developed carcinoma in comparison with 90.5% in conventionally reared mice. Neoplastic transformation of cells was linked with accumulation of beta-catenin in nucleus and with downregulation of caveolin1. We also documented dramatic decrease in microbiota diversity in both groups of AOM/DSS-treated mice. We conclude that colon cancer risk is directly proportional to microbiota load and that the decrease in microbiota diversity could be related to colon cancer development.

M.28 Monocytes from Subjects with Celiac Disease are Inducible but Resist Intestinal Stroma-mediated Down-regulation

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We have reported that blood monocytes are the exclusive source of intestinal macrophages and that intestinal stromal products induce the differentiation of monocytes into phenotypic and functional intestinal macrophages. To elucidate the role of monocytes and intestinal macrophages in celiac disease, we investigated gliadin-induced responses in intestinal macrophages/blood monocytes from normal subjects and blood monocytes from subjects with celiac disease. Normal intestinal macrophages were unresponsive to gliadin. However, gliadin-stimulated blood monocytes produced high levels of TNF-α in an NF-κB-dependent manner and expressed increased levels of co-stimulatory molecules CD40, CD80, and CD86, predominately in the gliadin-induced CD83+ monocyte-derived cells. In sharp contrast, gliadin induced substantially lower levels of cytokine production, and co-stimulatory molecules and smaller numbers of CD83+ cells among celiac monocytes. Celiac monocytes spontaneously released TNF-α, suggesting pre-activation. Importantly, gliadin-stimulated celiac monocytes were markedly resistant to stroma-mediated down-regulation compared to the profound stromal down-regulation of gliadin-stimulated normal monocytes. In conclusion, gliadin activates both normal and celiac monocytes, but not normal intestinal macrophages. Unlike normal monocytes, gliadin-stimulated celiac monocytes were markedly resistant to stromal factor down-regulation, suggesting that intestinal macrophages may play an important role in celiac disease.

M.30. Effects of Cigarette Smoke Exposure on Changes in Gene Expression in the Uteri, Bladders and Lungs of FVB Mice

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Cigarette smoke exposure (CSE) is associated with pathologies of mucosal organs including infection, hypo- and hyper-immune responses and tumorigenesis. We tested the hypothesis that CSE would lead to gene specific changes in expression in distal organs not simply the respiratory tract. Female FVB mice had CSE for 12 weeks (4 hrs/day, 5 days/wk) at 60 mg/m3 TSP in a Teague chamber. At necropsy tissues were frozen in RNAlater. Subsequent dual hybridization expression array assays were preformed on lung, bladder and uterus comparing same organ gene expression from CSE mice with controls. Biostatistical analysis followed. Differentially expressed genes were determined significant at p=<0.01. Array analysis revealed one gene (Spon2) that was decreased in all three organ sets, 116 genes similarly affected in bladder and uterus, 15 in the uterus and lung and 12 in the lung and bladder. Several structural proteins, cytokines and their receptors, hormone receptors, and complement pathway genes were decreased. Cancer markers Tnfrsf21 and CxcL5 were upregulated in bladders of CSE mice while TLR2 was decreased. qPCR assays are underway to verify the findings in the whole genome arrays. This study reveals target genes affected by CSE that play key roles in tumorigenesis and immune protection in mucosal tissues.