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Gene Expression Patterns in Experimental Colitis in IL-10 Deficient Mice

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Abstract

While others have described gene expression patterns in humans with inflammatory bowel diseases and animals with chemically-induced colitis, a genome-wide comparison of gene expression in genetically susceptible animals that develop spontaneous colitis has not been reported. We used microarray technology to compare gene expression profiles in cecal specimens from specific pathogen-free IL10-deficient (IL10^{-/-}) mice with colitis and normal wild-type (WT) mice. RNA isolated from ceca of IL10^{-/-} and WT mice was subjected to microarray analysis. Results were confirmed by real-time PCR and immunofluorescence microscopy of selected molecules. Expression of the selected genes in DSS-treated mice with colitis and epithelial cell lines activated with pathophysiologic stimuli was measured by real-time PCR. Histological inflammation of the colon and IL-12/23p40 secretion from intestinal explants were greater in IL10^{-/-} and DSS-treated mice vs. WT and untreated mice. Microarray analysis demonstrated >10-fold induction of the following molecules in the ceca of IL10^{-/-} mice: Mitochondrial ribosomal protein-L33, aquaporin-4, indoleamine-pyrrole-2,3- dioxygenase, and MHC class II with 63, 25, 20, and 12-fold increases, respectively. Cytochrome-P450, pancreatic lipase-related protein-2, and transthyretin were down-regulated in IL10^{-/-} mice. MHC II was increased throughout the colon, and aquaporin-4 was increased in the basolateral aspect of cecal epithelial cells. MHC II mRNA was increased in epithelial cells treated with IFN γ , but not TNF or Toll-like receptor ligands. Although most upregulated genes in experimental colitis are immune-related, aquaporin-4 and mitochondrial ribosomal protein, which have not been previously associated with inflammation, were most highly upregulated.

Keywords

colitis; interleukin-10; DSS; microarray; animal models; inflammation

INTRODUCTION

The idiopathic inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC) are the result of dysregulated immune responses to commensal enteric flora in genetically susceptible hosts 1. These aberrant responses include up- or down-regulation of various effector and regulatory molecules on immune, epithelial, endothelial and mesenchymal cells of the gastrointestinal tract.

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Conflict of interest: None

Other investigators have characterized gene expression patterns in human IBD². In colonic mucosal biopsy specimens from patients with CD and UC, genes involved in fatty acid metabolism are similarly up- or down-regulated versus control specimens³. Langmann et al. have determined using a large human microarray that several genes involved in detoxification, such as CYP3A family members, are downregulated in the colonic mucosa of patients with CD and UC⁴. Others have shown with limited microarrays that chemokines, markers of neutrophil activation, and anti-inflammatory genes are upregulated in IBD^{5,6}.

More extensive analysis of gene expression in experimental colitis has identified chitinase 3-like-1 protein as a facilitator of bacterial invasion of colonic epithelial cells in mice treated with dextran sodium sulfate (DSS)⁷. Consistent with data from human samples, microarray analysis of lamina propria cells from colitic mice lacking the alpha chain of the T-cell receptor (TCR α $-/-$) revealed downregulation of detoxification gene expression and upregulation of genes involved in antigen presentation and immunoregulation⁸. Conversely, in C57/B16 mice treated for two days with trinitrobenzene sulfonic acid (TNBS), microarray analysis of colonic mucosal specimens demonstrated that similar antigen presenting molecules were downregulated⁹. Such disparities underscore differences in the pathogenic mechanisms involved in various models of experimental colitis during different phases (acute vs. chronic) of the immune response.

The pathogenesis and phenotype of colitis in the TCR α $-/-$ and acute TNBS models differs significantly. TCR α $-/-$ mice exhibit a slow onset, progressive, mild colitis with diarrhea, weight loss and rectal prolapse that is dependent on T-helper 2 (Th2) cells similar to human ulcerative colitis¹⁰⁻¹², while C57/B16 mice treated with a single dose of TNBS develop very transient, mild, inflammation that is not T-cell mediated¹³.

Despite the differences in these two examples of experimental colitis, a unifying feature of most models of chronic colitis is the requirement of commensal microbiota for development of intestinal inflammation. For example, germ-free (sterile) mice lacking the interleukin 10 gene (IL10 $-/-$) appear healthy and have no evidence of immunologic activation¹⁴. However, upon transfer to a specific pathogen free (SPF) environment, they rapidly develop chronic, non-lethal, Th1-mediated colitis with certain features resembling Crohn's disease¹⁴. These mice develop lethal small and large intestinal inflammation when housed in conventional (dirty) conditions¹⁵. While the pathophysiologic mechanisms of colitis in IL10 $-/-$ mice have not been completely elucidated, these animals have impaired immunoregulatory function of antigen presenting cells leading to an enhanced Th1/Th17 responses to colonic bacterial antigens¹⁶⁻²¹.

While others have reported gene expression profiles in murine models with acute, non-T-cell-mediated colitis (acute DSS, acute TNBS) and chronic Th2-mediated colitis (TCR α $-/-$), less is known about gene expression patterns in chronic Th1-mediated experimental colitis. We hypothesized that bacterial colonization of IL10 $-/-$ animals alters intestinal gene expression, which leads to intestinal inflammation. To this end, we performed microarray analysis of RNA obtained from inflamed full-thickness cecal specimens in SPF IL10 $-/-$ mice.

MATERIALS AND METHODS

Animals

Inbred 129S6/SvEv IL10 $-/-$ mice (originally obtained from DNAX laboratories, Palo Alto, CA) and wild-type 129S6/SvEv mice (Taconic Laboratories, Germantown, NY); sterilely derived by hysterectomy at the Gnotobiotic Laboratory, University of Wisconsin, Madison were maintained in germ free conditions at the National Gnotobiotic Rodent Resource

Center at the University of North Carolina, Chapel Hill. Four weeks after colonization with fecal slurries from SPF WT 129S6/SvEv mice, a time-point corresponding to the onset of severe colitis as described previously 14, 4 mice/group underwent necropsy. Tissue for frozen sections was obtained from additional animals. For time course experiments, germ-free mice and ex-germ-free mice colonized with SPF bacteria for three and fourteen days were also used. For DSS experiments, adult C57/B16 mice were given 4% DSS (w/v) (MW 44,000, TDB Consultancy AB, Uppsala, Sweden) in drinking water for eight days. Mice were weighed daily and drinking water was refreshed every 2–3 days. Some animals were given regular drinking water for three days after DSS treatment. Animal-use protocols were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committees.

Histologic Analysis and Intestinal Explant Culture

Intestinal tissue was harvested, fixed for 24 hours in 10% neutral-buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Blinded histological scoring of inflammation was performed using a validated scale (0–4) as described 14,22. Histological scoring of specimens from DSS treated animals was based on a composite score that included: degree lamina propria mononuclear cell infiltration (0–4), prevalence of crypt abscesses (0–4), and degree of epithelial cell loss (0–4). Sections of mid-colon were harvested, processed, and explants were cultured for 24 hrs. without stimulation 23,24. Supernatants from explant cultures were analyzed in duplicate for IL-12/23p40 using a validated ELISA technique 14.

RNA Isolation

Freshly-harvested duodenum, mesenteric lymph nodes, cecum, and segments of ascending, transverse, and descending colon were placed immediately in Trizol Reagent (Invitrogen, Carlsbad, CA) and snap-frozen in liquid nitrogen. RNA was isolated per the manufacturer's instructions.

Microarray Analysis

Total RNA from ceca of 4 mice/group was submitted to the Duke University Microarray Core Facility (Durham, NC) for microarray analysis using the Operon Mouse Oligo Set, Version 3.0, representing approximately 30,000 murine genes. In brief, cDNA was synthesized from 10 µg of total RNA or universal mouse reference RNA (Stratagene, La Jolla, CA) and labeled with Cy5-dCTP at 42°C for 2 hrs using oligo-dT primers and Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA). After hydrolysis of the RNA, the cDNA was purified using the Qiaquick PCR purification kit (Qiagen, Valencia, CA) and then vacuum dried. Microarray slides were pre-hybridized with 5X SSC, 0.1% SDS, 0.5% BSA at 42°C for 1 hr, washed twice with deionized water, and allowed to air-dry. Labeled cDNA was resuspended in 30 µL of hybridization buffer (50% formamide, 5X SSC, and 0.1% SDS) containing 20 µg of mouse COT-1 DNA (Invitrogen, Carlsbad, CA) and poly(A)-DNA to decrease non-specific binding. The probe solution was heated at 95°C for 2 min, placed on ice for 30 sec, and then incubated on the microarray slide at 42°C for 16–20 hrs. Slides were washed successively in 1xSSC, 0.2%SDS at 42°C for 1 minute; 0.2xSSC, 0.01%SDS at room temperature for 1 minute; 0.2xSSC at room temperature for 2 minutes; 0.05xSSC at room temperature for 1 minute; and then air-dried for 2 minutes. Arrays were scanned on an Axon Genepix 4000B Scanner (Molecular Devices, Union City, CA) and pictures processed using Axon Genepix 5.1 software. Data was analyzed using GeneSpring 7.2 software (Agilent Technologies, Palo Alto, CA). Genes from IL10^{-/-} mice with normalized and data values 5-fold above or below the values from wild-type mice were selected.

Real-time PCR

Intron-spanning real-time oligonucleotide primers were designed using Primer Express 3.0 software (Applied Biosystems, Foster City, CA). Mouse primers were as follows: CD38F 5'-TGCTCTTGCCACATTGGA-3', CD38R 5'-CCCATTGAGCATCACTTGGA-3', H2AaF (MHC II) 5'-TGGGCACCATCTTCATCATTC-3', H2AaR 5'-GGTACCCAGCACACCACTT-3', s100gF 5'-TCACCTGCTGTTCCCTGTCTGA-3', s100gR 5'-GCCTTCCTTGCTGCATATTT-3', aqp4F 5'-TTGGACCCGCAGTTATCATG-3', aqp4R 5'-GCGACGTTTGAGCTCCACAT-3', GAPDHf 5'-TCCCCTCTTCCACCTTCGA-3', GAPDHR 5'-GTCCACCACCTGTTGCTGTA-3', mrpL33F 5'-GGTGAGAATCGGTGGACCAT-3', mrpL33R 5'-CGGAGTCGGCTTCTCTTG-3', vil1F 5'-CAGGCCTCGGCAAACC-3', vil1R 5'-TCAAACCTCACCTGTTCCACCTT-3'. Human primers were as follows: hAqp4F 5'-AGCGTTAATGGCAGTTGTGTGT-3', hAqpR 5'-TTCCTAGCACCGAAGAGAATCAG-3', hMrpL33F 5'-CTCCGCGGTCTTCTTTGC-3', hMrpL33R 5'-CCAGCTTCGCTCACCTTCT-3', HLADRAF (MHC II) 5'-AGGGATTGCGCAAAGCA-3', HLADRAR 5'-TCACCTCCATGTGCCTTACAGA-3', GAPDH same as mouse primers. First strand cDNA was synthesized from 1 µg total RNA using M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA) per the manufacturer's instructions. Two µL of the reaction were added to 3.75 pmol of each primer and 2x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) in a final volume of 12.5 µL and analyzed in an ABI 7900HT thermocycler using the universal thermocycling conditions per the manufacturer's instructions (Applied Biosystems, Foster City, CA). Specificity of the PCR primers was confirmed by agarose gel electrophoresis of PCR products (data not shown). All reactions were run in duplicate or triplicate and results are expressed relative to the housekeeping gene GAPDH. To adjust for epithelial cell loss in DSS-treated animals, real-time PCR results for Aqp4 and MHC II in tissue from these animals were also normalized to Villin 1 expression.

Immunofluorescence

Frozen sections of intestinal tissue were prepared in OCT compound (Tissue-Tek, Torrance, CA) using standard techniques. To detect MHC class II, sections were fixed in acetone for 2 min at -20°C, and incubated with a 1:50 dilution of biotinylated anti-IA^b monoclonal antibody clone AF6-120.1 (BD Biosciences, San Jose, CA) or isotype control for 60 min at 25°C followed by a 1:200 dilution of streptavidin-phycoerythrin (BD Biosciences, San Jose, CA) for 60 min. To detect Aquaporin 4, sections were fixed and permeabilized in 1% paraformaldehyde/0.1% Triton X-100 for 30 min at 25°C, incubated with a 1:100 dilution of rabbit polyclonal anti-aquaporin 4 (Chemicon, Temecula, CA) overnight at 4°C and then a 1:100 dilution of anti-rabbit IgG-phycoerythrin (Santa Cruz Biotechnology, Santa Cruz, CA) for 2 hrs at 25°C. Where indicated, sections were counterstained with the Sytox Green nuclear stain (Molecular Probes, Eugene, OR) per the manufacturer's instructions. Images were acquired on a Zeiss LSM 210 confocal microscope.

In vitro Stimulation

The murine rectal carcinoma cell-line, CMT-93 (ATCC# CCL-223, Manassas, VA) and the human colorectal adenocarcinoma cell-line, CaCo2 (kind gift from Dr. Christian Jobin), were cultured in DMEM supplemented with 10% FBS and 100 IU/mL penicillin/streptomycin. 1×10^5 cells per well were plated in a 6-well tissue culture plate. Twenty-four hours later, cells were either mock-treated or treated with the following agents at the indicated concentrations: PG-PS (2 µg/mL, prepared as before²⁵), LPS (6 µg/mL, Sigma #L2018, St. Louis, MO), MDP (6 µg/mL, InvivoGen tlrl-mdp, San Diego, CA), murine tumor necrosis factor (TNF) (0.1ng/mL, R&D Systems #410-MT, Minneapolis, MN), or murine interferon-γ (IFN-γ) (2.5–20 ng/mL, R&D Systems, Minneapolis, MN). CaCo2 cells

were similarly treated with LPS (200 mcg/mL, Sigma, #L3012, St. Louis, MO), PG-PS, MDP, human TNF (10ng/mL, R&D Systems, 210-TA, Minneapolis, MN), or human IFN- γ (10 ng/mL, R&D Systems, 285-IF, Minneapolis, MN). Twenty-four hours later, cells were rinsed with PBS and RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturers instructions.

Data and Statistical Analysis

Statistical analysis of microarray results was performed using GeneSpring 7.2 software (Agilent Technologies, Palo Alto, CA). The list of highly regulated genes was narrowed to those genes with statistically significant differences when grouped by genetic characteristic (Welch t-test, p-value cutoff 0.05, multiple testing correction: Benjamini and Hochberg False Discovery Rate). All other data are presented as the mean + SEM. P values were calculated using the two-tailed student's T test.

RESULTS

The SPF IL10 $^{-/-}$ model of experimental colitis is characterized by mononuclear infiltration of the colonic lamina propria and enhanced secretion of IL-12/23p40 14·15. We assessed intestinal inflammation in four wild-type (WT) and IL10 $^{-/-}$ mice colonized with SPF flora for four weeks by blinded histological scoring of tissue sections and measurement of spontaneous IL-12/23p40 secretion from mid-colonic explants (Figure 1). Histologic inflammation was increased in all intestinal segments from IL10 $^{-/-}$ mice compared to WT mice. In addition, more IL-12/23p40 was secreted by explants from IL10 $^{-/-}$ mice than from WT. These results are consistent with our prior observations that IL10 $^{-/-}$ mice rapidly develop colonic inflammation when colonized with SPF bacteria 14.

We studied patterns of gene regulation in the inflamed intestine by microarray analysis of RNA isolated from the ceca of the same mice since inflammation was macro- and microscopically most consistent in this region. The majority of the highly upregulated genes were related to innate and adaptive immune responses (Table 1, also see supplementary Table 2). Interestingly, the two most highly upregulated genes, mitochondrial ribosomal protein L33 (MrpL33) and aquaporin 4 (Aqp4), are not known to be associated with inflammatory responses. In contrast, highly downregulated genes were associated with a variety of functions including detoxification and epithelial electrolyte transport (also see supplementary Table 2).

Because microarray data are semi-quantitative and only reflect relative mRNA levels, we validated the results for selected upregulated genes using real-time PCR and immunofluorescence. We selected five of the 50 most-highly upregulated genes identified by the microarray analysis that either were markedly upregulated (MrpL33, Aqp4), encoded cell-surface molecules (Aqp4, MHC II, CD38 – a marker on immature and activated T and B cells) or had previously been demonstrated to play a role in intestinal inflammation (MHC II, S100g – a calprotectin family member). As measured by real-time PCR, the number of transcripts of MrpL33, Aqp4, S100g, MHC II, and CD38 were respectively 8000, 23, 13, 11, and 5 fold higher in IL10 $^{-/-}$ compared to WT mice, which, except for MrpL33, is consistent with the microarray studies (Figure 2). The discrepancy between fold-change in MrpL33 as detected by real-time PCR and the microarray is most likely explained by the virtually undetectable levels in specimens from WT mice. Next, we evaluated mRNA expression patterns of MrpL33, Aqp4 and MHC II, three of the most highly upregulated molecules, in the MLN and various intestinal compartments by real-time PCR. Aqp4 is abundantly expressed in the transverse and distal colon, but virtually absent in the duodenum, cecum, and MLN of WT mice (Figure 3A). Interestingly, in IL10 $^{-/-}$ mice with pancolitis, Aqp4 mRNA is only increased in the cecum. On the other hand, MHC II

expression is upregulated along the entire colon of SPF IL10^{-/-} mice, but is unchanged from WT mice in the duodenum and MLN (Figure 3B). In separate experiments using mice colonized with SPF microbiota for eight weeks, MHC II, but not Aqp4, mRNA was increased in the jejunum and ileum of IL10^{-/-} mice compared to WT mice (data not shown). In WT mice, MHC II mRNA is detected at low levels in the duodenum and throughout the colon and at much higher levels in the MLN (Figure 3B). To further assess the kinetics of induction of Aqp4, MHC II, and MrpL33, we measured cecal mRNA levels in germ-free IL10^{-/-} mice (day 0), and ex-germ-free IL10^{-/-} mice colonized with SPF fecal microbiota for 3, 14, and 28 days. As shown in figure 3C, by 14 days post-colonization, both MHC II and Aqp4 mRNA levels were increased compared to those observed in germ-free animals. However, MrpL33 mRNA levels remained unchanged during this period (Figure 3C). Moreover, MrpL33 expression was increased throughout the entire colon, duodenum, and MLN of IL10^{-/-} vs. WT mice (data not shown). Immunofluorescent staining of frozen sections showed increased Aqp4 and MHC II protein in the cecum of IL10^{-/-} mice (Figure 4). While MHC II protein was found in both the lamina propria cells and the apical side of colonic epithelial cells, Aqp4 protein was seen exclusively in the basolateral aspect of the colonic epithelium with the highest levels observed in the surface epithelial cells.

To determine whether the increase in Aqp4, MHC II, and MrpL33 expression was specific to IL10^{-/-} mice with colitis, we also examined mRNA levels of these genes in the cecum and colon of DSS-treated mice with colitis. Mice were given either water alone (control), 4% DSS in drinking water for 8 days, or 4% DSS in drinking water for 8 days followed by water alone for 3 days prior to necropsy. Mice given DSS had significantly more weight loss, histological inflammation, and spontaneous IL12/23p40 secretion from intestinal explants compared to control animals (Figure 5A and 5B). Similar to results in the IL10^{-/-} mice, Aqp4 expression normalized to the epithelial cell marker villin-1 was increased in the cecum, but not the colon of mice with DSS-induced colitis compared to controls (Figure 6A). MHC II expression was elevated in both the cecum and colon in DSS-treated mice compared to controls comparable to that observed in the IL10^{-/-} mice (Figure 6B). Interestingly, MrpL33 expression in both the cecum and colon was not significantly affected by DSS-treatment, suggesting that the upregulation of MrpL33 noted in tissues from IL10^{-/-} animals may be due to the absence of IL10 and not the presence of colonic inflammation (Figure 6C).

Since Th1-cytokines are prevalent in both IL10^{-/-} and DSS-treated mice with colitis, we next examined whether certain Th-1 cytokines, such as TNF and IFN- γ , or microbial products important in the induction of innate immune responses, such as lipopolysaccharide, peptidoglycan-polysaccharide, and muramyl-dipeptide, regulate expression of MHC II, Aqp4, and MrpL33. Because MHC II and Aqp4 proteins were increased predominantly in intestinal epithelial cells (Figure 4), we examined the effects of the afore-mentioned Th1 cytokines and microbial products on MHC II, Aqp4, and MrpL33 mRNA levels in the CaCo2 human adenocarcinoma cell-line using real-time PCR. As expected, IFN- γ exposure increased MHC II mRNA levels; however none of the other stimuli affected MHC II expression (Figure 7B). No significant changes in expression of either Aqp4 or MrpL33 were observed in response to any of the stimuli (Figure 7A and 7C). Similar results were obtained when the experiments were repeated using the CMT-93 mouse recatal carcinoma cell-line (data not shown). Therefore, we conclude that in these epithelial cell lines, the prototypic Th1 cytokine, IFN- γ , upregulates MHC II, but not Aqp4 or MrpL33.

DISCUSSION

Human IBD represents a spectrum of diseases that are partially represented by various animal models of chronic intestinal inflammation 26. For example, oxazolone-treated mice develop an atypical Th2-mediated inflammation with elevated IL-13 similar to that of human ulcerative colitis 27, whereas the chronic TNBS and IL10^{-/-} models are characterized by a Th1-dominant cytokine profile and activated innate immune pathways that characterizes Crohn's disease 28-32. Elucidating the pathogenesis of experimental colitis will contribute to our understanding of immunopathogenic mechanisms of human IBD 1:26.

In the present study we used microarray analysis to show that the majority of upregulated genes in the inflamed ceca of IL10^{-/-} mice were related to the innate and adaptive immune systems, as expected. Interestingly, the two most highly upregulated genes, MrpL33 and Aqp4, were not known to be associated with immune activation.

MrpL33 encodes a protein constituent of the 50S subunit of the mitochondrial ribosome. It is possible that expanded populations of metabolically active neutrophils, lymphocytes, dendritic and epithelial cells in the inflamed tissue are associated with increased numbers of mitochondria and therefore increased levels of MrpL33. Alternatively, since mitochondria are phylogenetically related to prokaryotes, the increased expression of MrpL33 may represent altered numbers and/or characteristics of luminal microbiota in the inflamed colons of these animals 33:34. However, MrpL33 was similarly upregulated in non-inflamed colonic sections from IL10^{-/-} vs. WT mice housed in germ-free conditions and was not induced by exposure to enteric bacteria in kinetic studies. Furthermore, MrpL33 was not upregulated in a different model of experimental colitis, DSS-treated mice. Therefore, the increased expression of this molecule in IL10^{-/-} mice is likely due to the lack of IL-10 rather than the presence of bacteria or inflammation. The discordance between MrpL33 mRNA levels and histological inflammation was consistent with the inability of various Th1 cytokines and microbial products to increase MrpL33 expression in two colonic epithelial cell lines. Due to the lack of available antibodies, we were unable to localize expression of this protein. It is interesting that the sequence of another mitochondrial ribosomal protein, MrpS29, is identical to that of death associated protein-3, a protein implicated in apoptosis 35. Further investigations into the regulation and function of MrpL33 in IL10^{-/-} animals are warranted.

Aqp4 belongs to the aquaporin family of eleven genes that encode proteins important in water transport and is conserved across plants, bacteria, and mammals. Six isoforms (Aqp1, 3, 4, 5, 8, and 9) are expressed in the digestive system 36. Aqp4 has been detected in the basolateral membrane of parietal cells, small intestinal crypt cells, and differentiated, surface colonic epithelial cells 36:37. Outside of the digestive system, Aqp4 is found in the lung, kidney, brain, and eye 38:39. Despite the established role of Aqp4 in water transport *in vitro*, Aqp4 deficient mice had only a slightly higher colonic osmotic water permeability coefficient and had little or no difference in stool water content, indicating a possible alternative role for Aqp4 in the colonic epithelium 40:41. Indeed, different aquaporins have roles in the maturation of dendritic cells and activation of peripheral blood lymphocytes 42:43. Moreover, a different type of aquaporin, Aqp3, may promote colonocyte proliferation and protect mice from DSS-induced colitis 44. While upregulation of Aqp4 in the inflamed cecum of IL10^{-/-} mice was isolated to the basolateral aspect of differentiated, surface epithelial cells; it is conceivable that Aqp4 may be involved in the colonic inflammatory response by regulating the intracellular characteristics of epithelia that form an important barrier to the luminal microbiota and have antigen presenting function 45:46. Alternatively, similar to Aqp3, Aqp4 may play a role in epithelial cell proliferation and restitution

following injury during inflammation. Further functional studies with Aqp4 knockout mice are needed to determine whether upregulation of Aqp4 in the cecum contributes to, or is a consequence of, intestinal inflammation. Factors that regulate Aqp4 expression in colitis are also unknown. Preliminary studies reveal that Aqp4 mRNA is increased ~2.5-fold in the ceca of germ-free IL10^{-/-} vs. WT mice, albeit at very low absolute levels relative to SPF animals (data not shown). Thus, similar to MrpL33, IL10 may be important in downregulating Aqp4 expression in the cecum.

Hardin et al. describe down-regulation of Aqp7 and 8 in colonic tissue from ulcerative colitis patients and Aqp4, 7, and 8 in the proximal colon of mice treated with DSS for 7 days 47. They did not comment on Aqp expression in the cecum. However, they noted that expression was patchier after DSS treatment, consistent with toxic effects of DSS on colonic epithelial cells. In our experiments, increased expression of Aqp4 was confined to the cecum of colitic DSS-treated and IL10^{-/-} mice, with no significant differences in the transverse and/or distal colon relative to WT mice.

The third most highly upregulated gene in our microarray studies encodes indoleamine-pyrrole 2,3 dioxygenase (Indo), an enzyme that catalyzes the rate-limiting step in tryptophan degradation. Depletion of tryptophan and accumulation of its degradation products provide a signal for T-cell apoptosis, which may play a role in controlling inflammation 48-50. Interestingly, Indo was shown to be upregulated in a proteomic survey of intestinal epithelial cells from patients with Crohn's disease 51. Moreover, TNBS treatment of highly susceptible SJL/J mice upregulated colonic Indo expression and inhibition of Indo worsened the colitis 52. Since Th1 cells mediate chronic colitis in TNBS-treated and IL10^{-/-} mice 28, similarities in expression profiles in these 2 models are not unexpected.

Consistent with findings in human IBD, we observed upregulation of MHC II in lamina propria and epithelial cells throughout the colon of SPF IL10^{-/-} and DSS-treated mice 53. Moreover, MHC II expression was increased in mouse and human colonic epithelial cell lines treated with IFN γ similar to results in primary human colonocytes 54. However, while the mean number of MHC II molecules per MLN cell was slightly increased in SPF IL10^{-/-} vs. WT mice, the proportion of MHC II-positive MLN cells was similar (~33%) as determined by flow cytometry (data not shown). The lack of increase in proportion of MHC II-positive MLN cells may be due to the high constitutive expression of MHC II on activated B-cells, dendritic cells and macrophages that traffic to the MLN in SPF WT mice 55-56.

While not the primary focus of the current work, several genes were downregulated in the intestines of SPF IL10^{-/-} mice compared to WT mice. Notably, Cyp4b1, a member of the cytochrome P450 family of oxidoreductases important in detoxification was the most highly downregulated gene. Langmann et al. described downregulation of another cytochrome P450 family member, Cyp3A4, in patients with UC and CD 4. Testis expressed genes 19 (Tex19) and 15 (Tex15), that encode products with unknown functions, were also downregulated in our microarray and real-time PCR studies (data not shown). However, treatment of a mouse colonic epithelial cell line with TNF, IFN γ , or various TLR ligands did not significantly affect Tex19 or Tex15 mRNA levels by real-time PCR (data not shown).

In summary, we report a validated gene expression profile of cecal tissue from the IL10^{-/-} model of Th1-mediated colitis. While most of the upregulated genes are relevant to innate and adaptive immune responses, further study of expression patterns of genes heretofore unrelated to the immune system, such as Aqp4 and MrpL33, may provide insight into novel pathogenic and/or repair mechanisms of IBD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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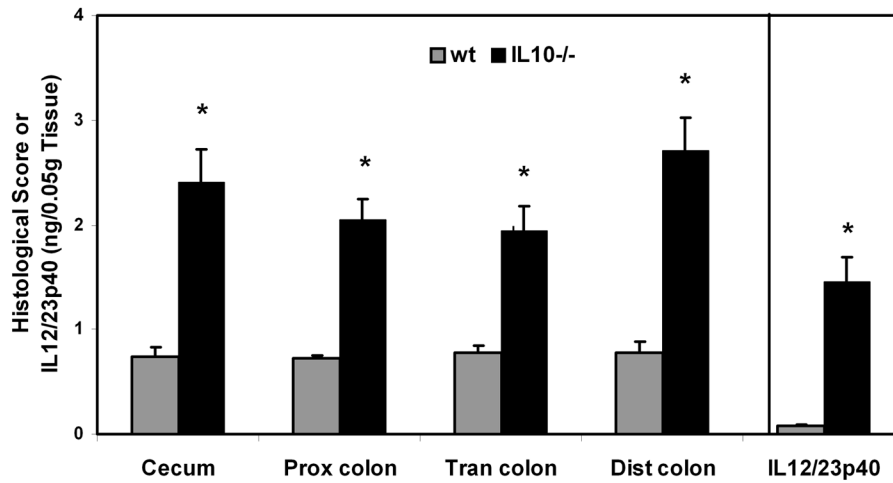


Figure 1. Pan-colonic inflammation is present in SPF IL10^{-/-} mice. Four WT and IL10^{-/-} mice born germ-free were killed 4 weeks after colonization with SPF flora. Inflammation was quantified using blinded histological scoring (left) and mid-colonic explant culture for IL12/23p40 secretion detected by ELISA (right). Data represents the mean + SEM. * p<0.005 vs. WT value.

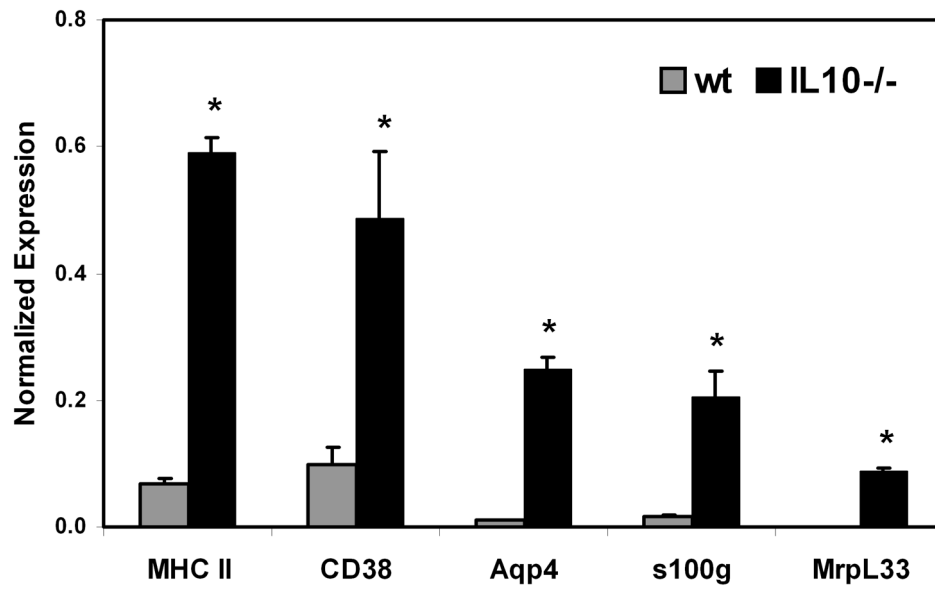


Figure 2.

Transcripts of immune and non-immune genes are increased in ceca from SPF IL10^{-/-} mice. Real-time PCR of total cecal mRNA from the same animals as above was performed using primers for selected upregulated genes. Results are normalized to GAPDH and represent the mean + SEM. S100 = s100 calcium binding protein G, MHC II = major histocompatibility complex II, Aqp4 = aquaporin 4. * p<0.0005 vs. WT value.

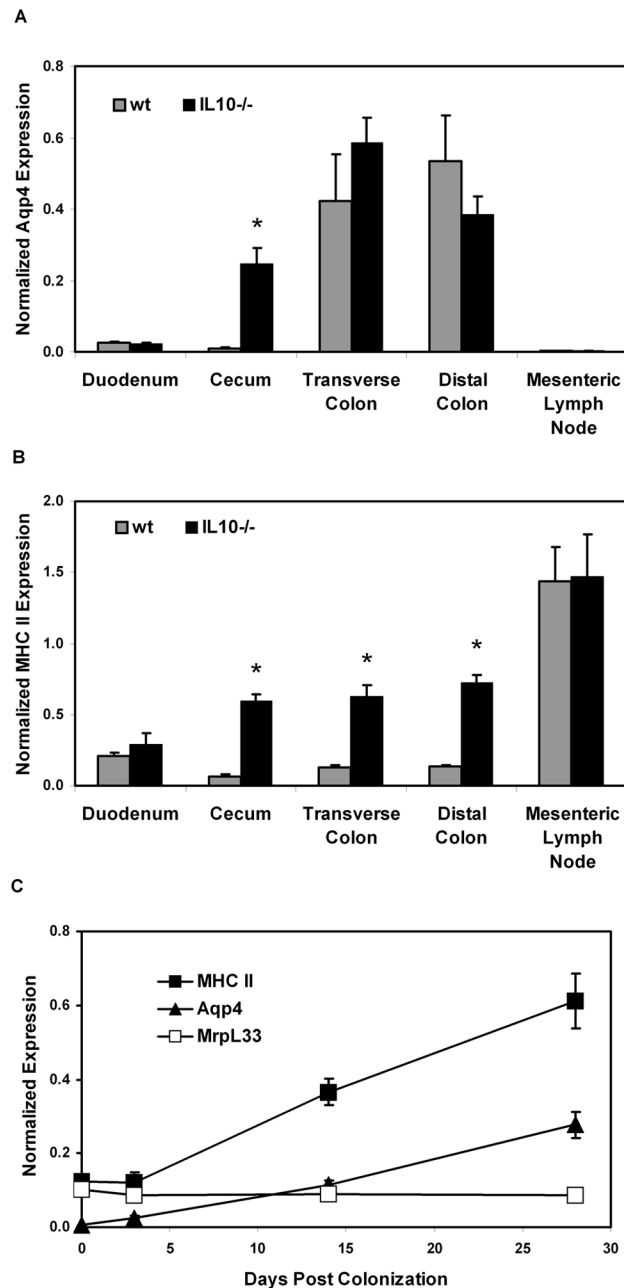


Figure 3.

Aqp 4 and MHC II are differentially upregulated in colonic segments from SPF IL10^{-/-} mice. Expression of Aqp4 (a) and MHC II (b) in the MLN and segments of intestine from the same animals as above was assessed by real-time PCR. Expression of Aqp4, MHC II, and MrpL33 in the cecum of IL10^{-/-} mice colonized for various times with SPF fecal microbiota (c). Results, normalized to GAPDH expression, are displayed as the mean + SEM. Duod = duodenum, Trans = transverse, Dist = distal. * $p < 0.005$ vs. WT value (a and b) or vs. 3 days (c).

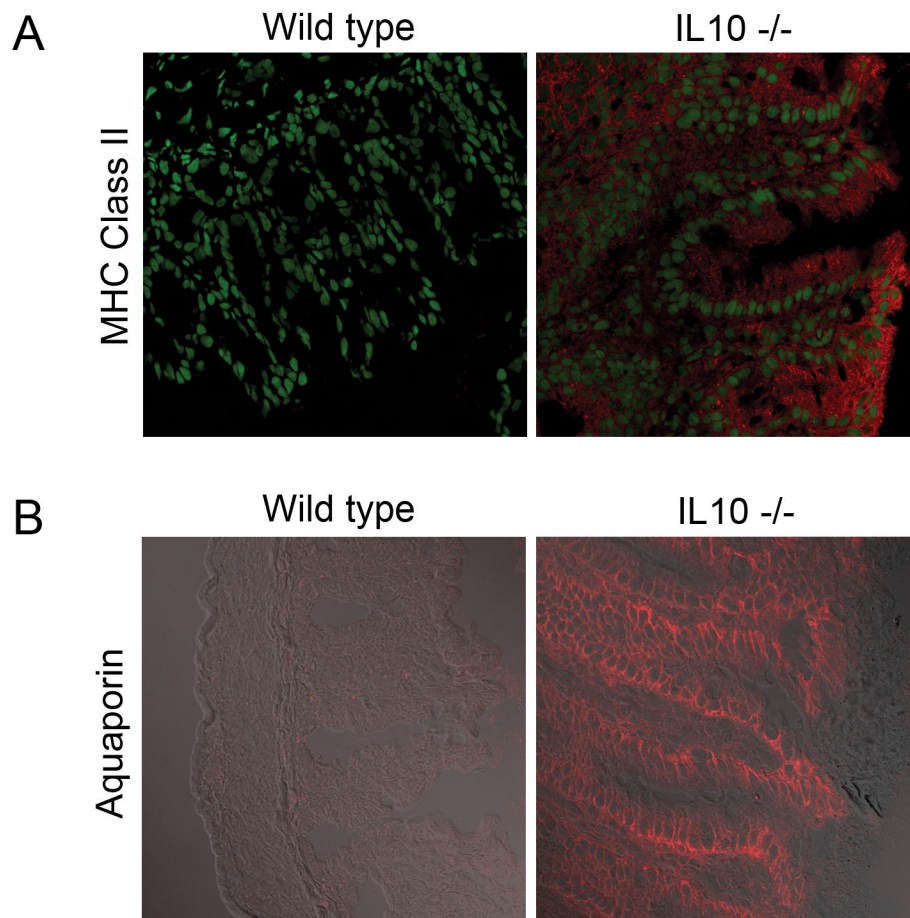


Figure 4.

Aqp 4 and MHC II are predominantly upregulated in colonic epithelial cells of SPF IL10^{-/-} mice. Frozen sections of ceca from SPF WT and IL10^{-/-} mice were incubated with either anti-MHC II (a) or anti-Aqp4 (b) antibodies and then with phycoerythrin-conjugated secondary antibodies (red). Sections were visualized by confocal microscopy and images overlaid with either a nuclear counterstained (green) image (a) or differential interference contrast image (b).

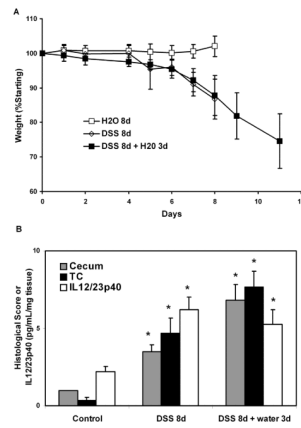


Figure 5. DSS-treated mice develop acute colitis within eight days. C57/B16 mice were given water, 4% DSS for 8 days, or 4% DSS for 8 days followed by 3 days of water. Animals were weighed daily (a). Histological inflammation and spontaneous IL12/23p40 secretion from colonic explants was assessed at time of necropsy (b). Results are presented as the mean + SEM (n=6 mice per group). *p<0.05 vs. control.

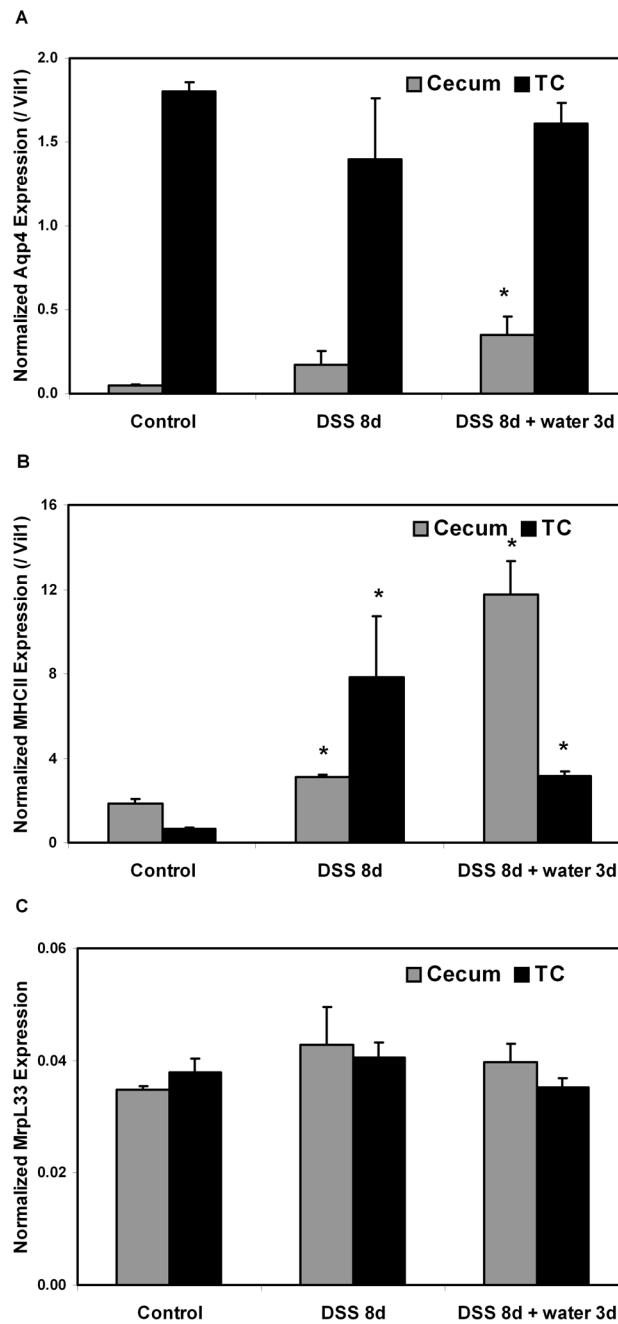


Figure 6.

Aqp 4 and MHC II are differentially upregulated in colonic segments from DSS treated mice. Expression of Aqp4 (a), MHC II (b), and MrpL33 (c) in the cecum (C) and transverse colon (TC) from the same DSS animals as above was assessed by real-time PCR. Results were normalized to GAPDH. For Aqp4 and MHC II, which are primarily upregulated in the colonic epithelium of the *IL10*^{-/-} animals, results were then normalized to Villin1 expression to adjust for increased colonic epithelial cell loss in DSS treated animals. Data are presented as the mean + SEM (n=6 mice per group). *p<0.05 vs. control.

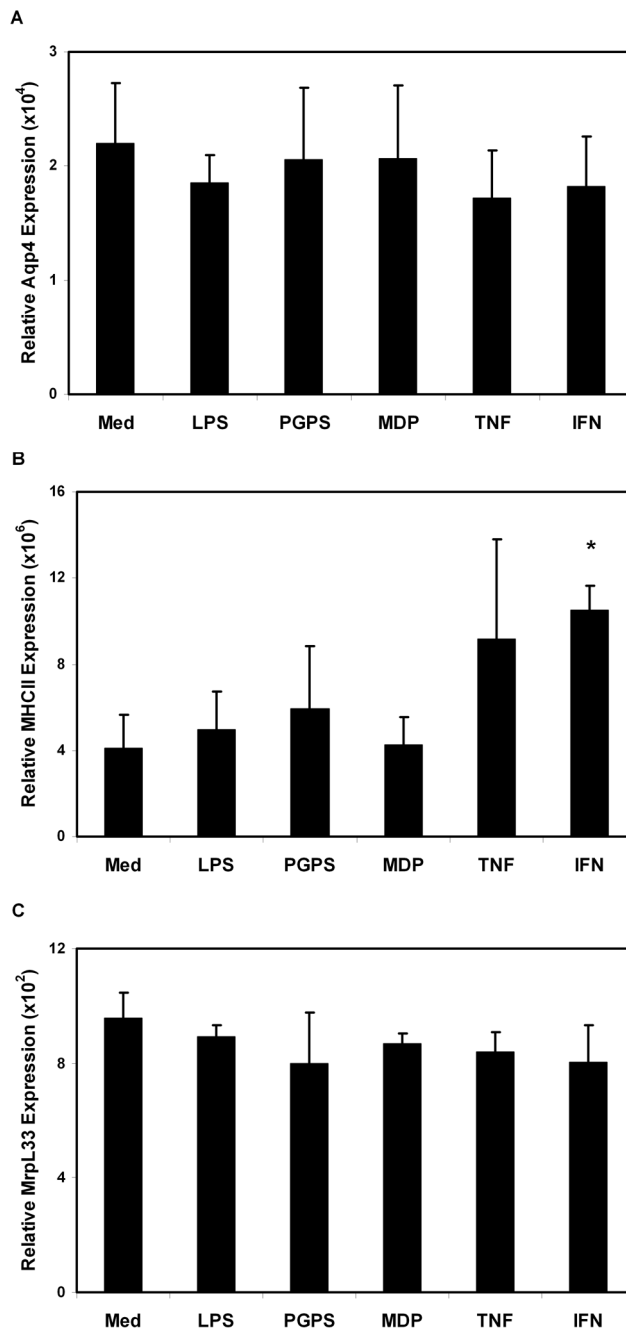


Figure 7. IFN γ upregulates MHC II, but not Aqp4 or MrpL33 in a human colonic epithelial cell line. Caco2 cells were stimulated with certain cytokines and TLR ligands for 24 hrs. Expression of Aqp4 (a), MHC II (b), and MrpL33 (c) was assessed by real-time PCR. Results are normalized to GAPDH expression and presented as the mean + SEM (n=3). *p<0.05 vs. media control.

Table 1Up- and Down-Regulated Genes in Inflamed Cecal Tissue from IL10^{-/-} vs. WT Mice

Gene Name	Genbank No.	Function	Fold Change
Mitochondrial ribosomal protein L33 (Mrpl33)	AB049651	Structural constituent of ribosome	63
Aquaporin 4 (Aqp4)	U88623	Integral membrane water channel	25
Indoleamine-pyrrole 2,3 dioxygenase (Indo)	M69109	Heme binding, oxidoreductase activity	20
T-cell specific GTPase (Tgtp)	AK088858	GTP binding	15
Granzyme A (Gzma)	M26183	Endopeptidase, T-cells	14
Mouse T-cell activating protein (Ly6a, Sca-1)	M59713	T-cell signaling, adhesion	13
24p3 (Lcn2, NGAL)	X81627	Neutrophil granule product	12
Myeloid related protein 8 (MRP8, S100a8)	X87966	Neutrophil calcium binding protein	12
MHC class II beta chain (H2-DMb1)	X62743	Antigen presentation	12
Phospholipase A2 (Pla2)	AF162713	Lipid metabolism, signaling	12
Cytochrome P450 4b (Cyp4b1)	BC008996	Monoxygenase, oxidoreductase activity	-17
Pancreatic lipase-related protein 2	M30687	Hydrolase activity	-13
Testis expressed gene 19 (Tex19)	AF285590	Unknown	-9
Transthyretin (Ttr)	D89076	Steroid, retinol binding	-7
Apolipoprotein C2 (ApoC2)	Z22217	Lipoprotein metabolism	-6
Solute carrier family 20 (Slc20a1, PiT1, Glvr1)	AF172633	Sodium/Phosphate cotransporter	-6
Phosphatase, orphan 1 (Phospho1)	AJ457190	Phosphatase, unknown	-6
Testis expressed gene 15 (Tex15)	AF285589	Unknown	-5
Chloride channel calcium activated 3 (Clca3)	AB017156	Chloride, hydrogen transport	-5