Interplay between Th1 and Th17 effector T-cell pathways in the pathogenesis of spontaneous colitis and colon cancer in the Gαi2-deficient mouse

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Abstract

Gαi2-deficient mice spontaneously develop colitis. Using xMAP technology and RT–PCR, we investigated cytokine/chemokine profiles during histologically defined phases of disease: (i) no/mild, (ii) moderate, (iii) severe colitis without dysplasia/cancer and (iv) severe colitis with dysplasia/cancer, compared with age-matched wild-type (WT) littermates. Colonic dysplasia was observed in 4/11 mice and cancer in 1/11 mice with severe colitis. The histology correlated with progressive increases in colon weight/cm and spleen weight, and decreased thymus weight, all more advanced in mice with dysplasia/cancer. IL-1β, IL-6, IL-12p40, IL-17, TNF-α, CCL2 and CXCL1 protein levels in colons, but not small intestines increased with colitis progression and were significantly increased in mice with moderate and severe colitis compared with WT mice, irrespective of the absence/presence of dysplasia/cancer. CCL5 did not change during colitis progression. Colonic IL-17 transcription increased 40- to 70-fold in all stages of colitis, whereas IFN-γ mRNA was gradually up-regulated 12- to 55-fold with colitis progression, and further to 62-fold in mice with dysplasia/cancer. IL-27 mRNA increased 4- to 15-fold during the course of colitis, and colonic IL-21 transcription increased 3-fold in mice with severe colitis, both irrespective of the absence/presence of dysplasia/cancer. FoxP3 transcription was significantly enhanced (3.5-fold) in mice with moderate and severe colitis, but not in mice with dysplasia/cancer, compared with WT mice. Constrained correspondence analysis demonstrated an association between increased protein levels of TNF-α, CCL2, IL-1β, IL-6 and CXCL1 and dysplasia/cancer. In conclusion, colonic responses are dominated by a mixed Th1/Th17 phenotype, with increasing Th1 cytokine transcription with progression of colitis in Gαi2−/− mice.

Keywords: chemokines, constrained correspondence analysis, dysplasia, inflammatory bowel disease, real-time RT–PCR

Introduction

Crohn's disease (CD) and ulcerative colitis (UC), referred to as inflammatory bowel disease (IBD), are gastrointestinal disorders characterized by chronic, relapsing inflammation (1, 2). The common histological changes associated with IBD include ulceration and inflammation of the intestinal mucosa with leukocyte infiltration (3, 4). Though sharing some effector pathways, CD and UC are immunologically and histopathologically different diseases. CD involves a predominant Th1 response, while UC is thought to be mediated by a mixed Th1/Th2 cytokine response (5). Animal models of intestinal inflammation are invaluable tools in the quest to understand the pathogenesis of IBD in humans. Mice deficient in the inhibitory G protein subunit Gαi2 spontaneously develop chronic, progressive colitis. Previous studies by our group have demonstrated immune changes characterized by activation of pro-inflammatory Th1 cells...
in late colitis (6); enhanced production of pro-inflammatory cytokines primarily in the large intestine, but with modestly enhanced secretion also in the small intestine; increased frequencies of activated T and B lymphocytes expressing mucosal homing receptors; and antibodies specific for normal intestinal flora and autoantigens present in intestinal lavage fluid (7, 8). The precolitic changes also include the regression of Peyer’s patches (9), and a switch from an IL-10-dominated dietary antigen T-cell response in wild-type (WT) mice to a T₁, cytokine profile in Gαi2−/− mice (10). In a previous study, we found increased serum concentrations of IL-18 and IL-1Ra in mice with established disease, and increased serum IL-1Ra levels already in the precolitic stage, suggesting IL-1Ra to be an early serum marker of disease induction (11).

We and others have previously reported that Gαi2−/− mice have a higher frequency of mature thymocytes, compared with control mice (12). We have also recently reported that the in vitro suppressive function of WT and Gαi2−/− T regulatory cells (Treg cells) was indistinguishable, but that compared with WT effector T cells, Gαi2−/− effector T cells were significantly less inhibited by either Treg population. In accordance, neither WT nor Gαi2−/− Treg cells were able to suppress colitis induced by adoptive co-transfer of Gαi2−/− effector T cells to RAG2−/− recipients (13). The lack of the Gαi2 subunit has also been associated with functional disorders in epithelial cells (14), professional antigen-presenting cells (15), B cells (9, 15, 16) and CD4+ T cells (17). Thus, the mouse model reveals multiple requirements for Gαi2 in the development of mucosal immunoregulation. However, the cellular and molecular mechanisms that require the participation of Gαi2 for regulating immune responses still remain unclear.

We have previously reported a very strong T₁,7 response in Gαi2−/− mice in late colitis (18). In this study, this observation was expanded to explore a selected panel of pro-inflammatory cytokines and chemokines involved in T₁,1 and T₁,7 pathways at different time points before and after the onset of colitis in the Gαi2−/− model.

Methods

Mice

Gαi2−/−-deficient (Gαi2−/−) mice on a pure 129SvEv background were bred and maintained under specific pathogen-free conditions in microisolator cages at the Department of Experimental Biomedicine, University of Gothenburg. The mice were bred as heterozygotes, and genotyped by PCR analysis using tail genomic DNA. Gαi2-deficient mice on this background develop a progressive, lethal colitis starting at ~5–7 weeks of age. The mice were sacrificed at the age of 4–9 weeks based on clinical appearance as precolitic, early and late colitis, and were macroscopically graded for colitis. The experiments included both male and female mice as no gender differences in clinical disease or immune parameters have been found. Both Gαi2−/− and heterozygous (Gαi2−/+ ) littermates were used as WT controls during the study.

All animal procedures were carried out under local and national ethical guidelines (Swedish Board of Agriculture) and were approved by the regional ethical committee, Gothenburg Administrative Court of Appeal, with the ethical approval IDs 79-2008 and 281-2010.

Tissue and plasma sampling

Mice were anesthetized with isoflurane (Abbot Scandinavia AB, Solna, Sweden) and immediately thereafter killed by cervical dislocation. Spleens and thymis were weighed, and the intestines were carefully removed and rinsed with cold PBS, and their length and weight were recorded. Approximately 3 mm of the most distal part of the colon and small intestine was fixed in zinc–formalin solution (pH 7.4, Histolab Products, Gothenburg, Sweden), embedded in paraffin, and used for histological analysis.

The remaining colon was divided longitudinally into two pieces, snap frozen in liquid nitrogen and stored in −80°C until RNA and protein analysis.

The small intestine was opened longitudinally and divided into duodenum, jejunum and ileum. Two centimeters from the middle of each of the sections were pooled for RNA- and multiplex analysis, respectively.

Clinical assessment and macroscopic scoring of colitis

The Gαi2−/− mice were divided into three groups based on daily clinical assessment including fecal monitoring and clinical appearance: precolitic (no diarrhea, still healthy), early colitis (initial phase of diarrhea) and late colitis (severe stage of the disease). The initial groups comprised five to six Gαi2−/− mice and the WT group contained eight mice. Gαi2−/− mice were then regrouped based on histological grading of colitis, based on constrained correspondence analysis (CCA) identifying histological scoring as superior to macroscopic scoring to explain the variations in the data (see below).

Histological grading of colitis

Colitis was graded blind using a scale originally developed for grading of the inflammation in UC patients (19), where 1 = normal mucosa, 2 = mild inflammation (enhanced glands with intraepithelial granulocytes, influx of cells and/or eosinophils in the stroma), 3 = intermediate inflammation ( goblet cell depletion, loss of tubular parallelism and reduced mucin secretion in some glands, marked influx of inflammatory cells in the stroma), 4 = severe inflammation (marked gland and mucosal atrophy, evident crypt abscesses and pus on the surface, massive influx of acute inflammatory cells and follicle formation in deeper cell layers) and 5 = fulminate inflammation (ulcerations with pus, gland and mucosal atrophy, crypt abscesses, extensive stromal inflammation and deep follicles). On the basis of this scale, mice were separated into three groups: histological scores 1–2 with no apparent or very mild disease with focal influx of lymphocytes into the lamina propria; 3, moderate disease, in which the entire lamina propria is expanded with large numbers of lymphocytes; and 4–5, severe disease, typified by increased numbers of granulocytes, not seen in score 3. In addition, the presence of colon dysplasia and/or cancer was recorded.

Protein extraction and analysis

Colons and small intestines were homogenized (Ultra Turrax T8, Tamro, Sweden) in homogenization buffer with miniproteinase inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany), centrifuged for 10 min at 18000 rpm,
and the supernatants stored at −80°C. Expression of IL-1β, TNF-α, IL-6, IL-12p40, IL-17, CXCL1 (KC/GROα), CCL2 (MCP-1) and CCL5 (RANTES) was analyzed using xMAP technology (Luminex®, Austin, TX, USA). The concentrations of cytokine proteins were determined using Bioplex Multiplex Suspension Array System kits, according to the manufacturer’s instructions (Bio-Rad Laboratories, Hercules, CA, USA).

**RNA preparation and cDNA synthesis**

Total RNA was isolated with an RNeasy mini kit (Qiagen, Hilden, Germany), eluted in 50 µl RNase-free water, assayed for concentration and quality (acceptable 28S:18S ratio >1.5) with an Agilent Bioanalyzer 2100 and RNA 6000 Nano Assay Kit (Agilent Technology, Santa Clara, USA), and stored at −80°C.

For cDNA synthesis, a high-capacity cDNA reverse transcriptase was used according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA, USA). Briefly, 2 µg of total RNA was used to produce first-strand cDNA with a random primer in a final reaction volume of 40 µl. The samples were incubated according to following schedule: 10 min at 25°C; 120 min at 37°C; 5 min at 85°C and thereafter stored at −20°C.

**Real-time RT–PCR**

Real-time RT–PCR was performed using the thermal cycler TaqMan 7500 Fast Real-Time PCR System (Applied Biosystems) with 7500 Fast Sequence Detection and Relative Quantification software packages. PCR cycling conditions were as follows: step one, 95°C for 2 min; step two, 95°C for 3 s and 60°C for 30 s. The second step was repeated 40 times. Combined primers and probes were purchased from Applied Biosystems (Sweden): Gapdh (Mm99999915), Actb (Mm01205647_g1), Gusb (Mm01197698_m1), Foxp3 (Mm01351175_g1), Il23a (Mm00518984), Il21 (Mm00517640), Il27 (Mm00461164), Ilng (Mm01168134) and Il17a (Mm00439618). PCR reactions were performed in duplicates in 15 µl using the TaqMan Universal PCR Master Mix (Applied Biosystems) and 1.5 µl cDNA. The relative values were calculated by the 2−ΔΔ Ct method, where ΔCt is the difference between the mean Ct value of duplicates of the sample and of the three endogenous controls, Gapdh, Actb and Gusb, and expressed as fold change relative to the mean of the WT control group. The results are shown as the mean of the 2−ΔΔ Ct values obtained using the three different housekeeping genes.

**Statistical analysis**

Data were not normally distributed, and are shown from individual mice with median values in the text. The Mann–Whitney’s U-test for unmatched data was used for comparison of data. Differences were considered statistically significant at P < 0.05. Correlation patterns between the relative values of cytokines and transcription factors (dependent variables) and the genotype and histological grading of colitis (independent variables) were detected by constrained or ‘canonical’ correspondence analysis (CCA (20)) and are presented as ordination plots using R package vegan. Preliminary analyses (data not shown) aiming to discern the variables (e.g. genotype, macroscopic scoring of colitis, clinical assessment, age, density of the colon and histological grading of colitis) that better explained the variation in the data, indicated that genotype and histological grading of colitis were the variables that best analyzed the data matrix in the constrained model. Therefore, in all analyses the mice were grouped based on histological scoring as described above.

**Results**

**Macroscopic changes with increasing histological scoring**

In mice with histological score 4–5, i.e. severe colitis, 4/11 Gαi2− mice displayed dysplasia, and one additional Gαi2− mouse (1/11) presented with colon cancer.

Increased histopathologically defined colitis scores were accompanied by gradual and significant increases in colon weight per centimeter in mice with moderate and severe colitis (Fig. 1A). The greatest increase in colon weight was recorded in mice with severe colitis and dysplasia/cancer which was significantly higher than all other groups of Gαi2− mice (Fig. 1A).

Thymic involution was evident already in mice with no/mild colitis compared with WT mice, and then became more pronounced as the colitis developed further, with the smallest thymi found in mice with severe colitis dysplasia/cancer, clearly smaller than in mice with severe colitis without dysplasia/cancer (P = 0.06) (Fig. 1B).

Spleen weight gradually increased with colitis progression compared with WT mice, and was significantly increased in mice with moderate and severe colitis with dysplasia/cancer, but not in mice without dysplasia/cancer (Fig. 1C).

**Induction of intestinal inflammatory cytokines and chemokines in Gαi2− mice during colitis progression**

In the colons of mice with no/mild disease compared with WT colons, protein levels of TNF-α but not IL-1β, IL-6, IL-12p40, IL-17, CXCL1, CCL2 or CCL5 were significantly changed. In contrast, in mice with moderate or severe colitis, all the above colonic cytokines were significantly enhanced compared with WT mice, with the exception of CCL5, which did not differ in any of the groups (Fig. 2). Among the Gαi2− mice, IL-12p40 was significantly enhanced from no/mild to severe colitis (P < 0.01).

In the small intestine, all cytokine levels except CCL5 were significantly lower compared with colonic levels in mice with severe colitis, and with the exception of IL-1β also in mice with moderate colitis. In contrast, IL-1β and IL-12p40 levels were significantly higher in the small intestine compared with the large intestine of WT mice.

Gαi2− mice with severe colitis were found to be significantly different from the WT mice irrespective of the absence/presence of dysplasia/cancer (all P < 0.01). However, colonic IL-6, TNF-α and CCL2 levels were significantly increased in mice with dysplasia/cancer compared with those without. IL-1β showed a strong trend toward increased levels in mice with dysplasia/cancer (P = 0.06). Albeit not statistically significant, IL-17 levels were also increased in mice with dysplasia/cancer, compared with those without.
Fig. 1. (A) colon, (B) thymus and (C) spleen weight as registered during the different stages of colitis. * denotes a statistically significant difference from WT mice, whereas # denotes significant differences in Gαi2−/− mice from Gαi2−/− mice with severe colitis and dysplasia/cancer. Each symbol represents one mouse, and the median value is indicated by a line. Diamonds represents WT mice, white circles = Gαi2−/− mice with histological score 1–2 (no/mild colitis), grey circles = Gαi2−/− mice with score 3 (moderate colitis), black circles = Gαi2−/− mice with score 4–5 (severe colitis) and white triangles = Gαi2−/− mice with score 4–5 and dysplasia/cancer. * or # = \( P \leq 0.05 \). ** or ## = \( P \leq 0.01 \). *** \( P \leq 0.001 \).
Fig. 2. Protein levels of cytokines and chemokines during the different stages of colitis in colon (black circles/open triangles) and small intestine (open circles). Each symbol represents one mouse and the median value is indicated by a line. In mice with colitis score 4–5 black circles represent $G_{\alpha i2}^{-/-}$ mice without dysplasia/cancer and triangles represent $G_{\alpha i2}^{-/-}$ mice with dysplasia/cancer. * denotes a statistically significant increase from the WT mice. # denotes a significant reduction in the small intestine compared with colon, whereas § denotes a significantly increased protein level in the small intestine compared with colon in the $G_{\alpha i2}^{-/-}$ mice. *, # or § = $P \leq 0.05$. **, ## or §§ = $P \leq 0.01$. *** or ### = $P \leq 0.001$. 

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CCL5 levels were higher in the small intestine in both WT and Gαi2−/− mice in all stages of colitis compared with the colon, with a significant increase from colonic values in Gαi2−/− mice with moderate colitis. Thus, IL-1β, IL-6, IL-12p40, IL-17, TNF-α, CCL2 and CXCL1 protein levels in colonic tissue were significantly increased in mice with moderate and severe colitis, with IL-6, TNF-α and CCL2 being further enhanced in mice with dysplasia, compared with WT mice. Colonic CCL5 did not change during colitis progression.

CCA of the data, outlining correlations between dependent and independent variables in ordination maps demonstrated significant correlations between protein levels, genotype and histological scores (P = 0.02), and a clear separation of WT mice from Gαi2−/− mice, irrespective of histological scoring (Fig. 3). High levels of IL-1β, IL-17 and IL-12p40 correlated with Gαi2−/− mice with moderate colitis, whereas high levels of IL-6 and CCL2 correlated with Gαi2−/− mice with no/mild colitis (Fig. 3).

**Fig. 3.** Canonical correspondence analysis of protein profiles (dependent variables) in colon in relation to genotype (WT versus Gαi2−/−) and histological scores (no/mild, moderate or severe colitis) (independent variables) of Gαi2 mice. Locations of the independent variables indicate relative correlations with the protein profiles. CCA1 and CCA2 axes are the two dimensions that account for the highest relative correlations. Analysis is based on 4–6 WT mice, 3–5 Gαi2−/− mice with colitis score 1–2, 3–4 Gαi2−/− mice with colitis score 3 and 9–11 Gαi2−/− mice with colitis score 4–5.

**T\textsubscript{n} and T\textsubscript{h} cytokine transcripts dominate colitis in Gαi2−/− mice**

Next, we compared mRNA expression of selected cytokine and transcription factor genes involved in T\textsubscript{n} cell differentiation in the colon and small intestine. CCA showed significant relationships between the relative levels of cytokines and transcription factors, and the genotype and histological grading of colitis (P < 0.04) (Fig. 4).

High colonic levels of IL-27 and IFN-γ typified the profile of Gαi2−/− mice with moderate and severe colitis, whereas high expression of IL-17 was associated with Gαi2−/− mice independent of colitis score. A weak correlation between increased mRNA levels for IL-21 and FoxP3 and Gαi2−/− mice with moderate and severe colitis was detected. In contrast, WT mice did not present any particular cytokine or transcription factor characterizing their profiles (Fig. 4).

Cytokine and transcription factor levels in the small intestine were not significantly correlated to either genotype or histological scoring (data not shown).

The two most highly up-regulated genes in Gαi2−/− colons were IL-17 and IFN-γ. IL-17 had a median 60- to 70-fold up-regulation compared with WT mice even in mice with no/mild colitis, with no significant differences between mice in different stages of colitis (Fig. 5). IFN-γ mRNA expression in the colon was likewise significantly up-regulated (median 12-fold) even in mice with no/mild colitis versus WT mice, but increased further to 45-fold and 55-fold in mice with moderate and severe colitis compared with WT mice (Fig. 5).

**Fig. 4.** Canonical correspondence analysis of cytokine transcripts (dependent variables) in colon in relation to genotype (WT versus Gαi2−/−) and histological scores (no/mild, moderate or severe colitis) (independent variables). Locations of the independent variables indicate relative correlations with the protein profiles. CCA1 and CCA2 axes are the two dimensions that account for the highest relative correlations. Analysis is based on 5–6 WT mice, 3–4 Gαi2−/− mice with colitis score 1–2, 3–4 Gαi2−/− mice with colitis score 3 and 9 Gαi2−/− mice with colitis score 4–5.
In contrast, in the small intestine, the only significant change was a 3- to 5-fold reduced mRNA expression of FoxP3 in Gαi2−/− mice with moderate and severe colitis, compared with WT mice (data not shown).

In contrast to protein expression, no significant changes in cytokine mRNA levels were recorded between Gαi2−/− mice with severe colitis with or without dysplasia/cancer. Importantly, when these mice were analyzed as separate groups, they both displayed significantly increased mRNA levels of all four cytokines compared with WT mice. In contrast, FoxP3 transcription was significantly increased in mice without dysplasia/cancer only.

**Dysplasia/cancer and association with cytokine expression**

CCA demonstrated significant correlations \( P = 0.02 \) between cytokine protein secretions and the presence of dysplasia/cancer in Gαi2−/− mice with severe colitis: There was a strong correlation between dysplasia/cancer and high secretions of TNF-α, CCL2, IL-1β and CXCL1 while the absence of dysplasia/cancer was correlated with CCL5 and to a lesser degree IL-17 (Fig. 6). No multivariate correlations were detected between mRNA expression levels of cytokines and dysplasia/cancer (data not shown).

**Discussion**

Excessive production of pro-inflammatory cytokines and loss of immune homeostasis plays a predominant role in the pathogenesis of IBD (21, 22). Increased production of Th1 cytokines has long been a feature of active IBD, especially CD (21, 22), but the discovery of the Th17 lineage, characterized by IL-17 production, has led to a re-evaluation of IBD pathogenesis. In this study, we demonstrate that in Gαi2−/− mice the colonic response is dominated by a mixed Th1/Th17 phenotype, where IL-17 is present at high amounts already early in colitis, whereas Th1 cytokine transcription increases with disease progression.

In humans, a significant proportion of Th17 cells also secrete IFN-γ, displaying a mixed Th17/Th1 cytokine phenotype, particularly in the gut (23). Interestingly, IL-17 is detectable in the colon of mice with T cell-independent colitis, and appears to be produced by granulocytes and monocytes in the lamina propria (24).

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The 2\(^{-\Delta\Delta CT}\) method, where each gene of interest from the Gαi2−/− mice in different stages of colitis was normalized against both the three housekeeping genes and the mean \( \Delta CT \) values of the WT control mice group. The 2-fold change against the WT control mice is indicated with a line in the different diagrams. The statistics were based on the values from each gene of interest from every mouse, including Gαi2−/− and WT control mice, being normalized against the housekeeping genes. Each symbol represents one mouse and the median value is indicated by a line. Diamonds represents WT mice, white circles = Gαi2−/− mice with histological score 1–2 (no/mild colitis), grey circles = Gαi2−/− mice with score 3 (moderate colitis), black circles = Gαi2−/− mice with score 4–5 (severe colitis) and white triangles = Gαi2−/− mice with score 4–5 and dysplasia/cancer. * \( P \leq 0.05 \), ** \( P \leq 0.01 \), *** \( P \leq 0.001 \).
In this study, we also observed high levels of IL-6 protein in colon with moderate and severe inflammation, which increases the frequency of IL-17-producing effector cells in the presence of TGF-β (25). The potent actions of IL-17 to mobilize, recruit, and activate neutrophils (26–28) contribute significantly to the development of tissue injury and inflammation in IBD (29), and are also a prominent histological finding in Gαi2−/− mice with colitis.

But IL-17 also has protective functions: It reinforces tight junction formation between epithelial cells in vitro (30), and drives microbial defenses during autoimmunity (26). It also inhibits T1 cells in both mice and humans (31, 32), in the latter through down-regulation of the IL-12Rβ2 subunit (32). In support of this, treatment of mice with anti-IL-17-aggravated dextran sodium sulfate (DSS) induced colitis (33), and transfer of IL-17−/−CD45RB−/− cells into severe combined immunodeficiency (SCID) recipients resulted in less severe colitis, associated with a dramatic increase in IFN-γ-producing cells (34). A recent clinical trial in Crohn’s patients with IL-17 neutralizing antibodies demonstrated no therapeutic effects, with acute aggravation of disease in some patients (35). By contrast, a recent study comparing the ability of T1 and T17 cells to induce colitis in mice found that the T17 cells were significantly more pathogenic than their T1 counterparts (36). However, it is important to bear in mind the distinction between T17 cells and IL-17. In addition to secreting IL-17A and the closely related IL-17F, T17 cells produce a range of other pro-inflammatory cytokines including IL-22 and IL-21 (25, 37, 38).

In this study, IL-21 transcription was, however, significantly enhanced only in mice with severe colitis compared with WT mice. In line with our findings, it has been reported to be enhanced both in human IBD and murine colitis (24, 39). IL-21−/− mice develop less colitis when challenged with DSS or 2,4,6-trinitrobenzene sulfonic acid, and DSS colitis is ameliorated following treatment with an antagonistic IL-21R/Fc (40). IL-21 also renders CD25−CD4+ cells resistant to Treg-mediated suppression (41, 42).

IL-12p40 protein was up-regulated in Gαi2−/− mice with colitis. As a subunit of both IL-23 (p19/p40) and IL-12 (p35/p40), p40 has a reciprocal position in both the T1 and T17 pathways, and IL-23 is essential for the survival of the T17 cells. Depletion of IL-23 p19 using monoclonal antibodies or by genetic ablation greatly attenuated T cell-dependent colitis in T-cell transfer models of colitis and inhibited spontaneous colitis in IL-10−/− mice (39). However, in this study, IL-23p19 in the colon was not increased above 2-fold (data not shown). This could be interpreted such that normal levels of IL-23p19 were sufficient to sustain high levels of T17 cells. Alternatively, and more likely, there are more factors involved in the complex IL-23/IL-17 axis in intestinal inflammation in Gαi2−/− mice.

IL-27 transcription was enhanced most besides IL-17 and IFN-γ in our studies. IL-27 was progressively up-regulated with increasing severity of colitis. Activation of T cells in the presence of IL-27 induces the T1 transcription factor T-bet, but IL-27 is also a negative regulator of T17 cell development (43). Increased IL-27 levels in the colon would thus support T1 involvement in Gαi2−/− colitis.

We also found significantly increased colon protein levels of CXCL1, a chemoattractant for neutrophils, and the monocyte chemoattractant CCL2, even in mice with moderate colitis. The early expression of CXCL1 corresponds with the high expression of CCL2 in the gut, together with the very low expression of CCL2 and CXCL1 in both the small and large intestines in the absence of inflammation. The high expression of CCL5 in the small intestine, together with the very low expression of CCL2 and CXCL1 in both the small and large intestines in the absence of inflammation is consistent with the study by Shang et al. (44), demonstrating very low expression of CCL2 and CXCL1 in the gut, but expression of CCL5 predominantly in the small intestine.

Dysplasia/cancer in Gαi2−/− mice is only observed in animals with severe colitis and our CCA and Mann–Whitney analysis demonstrated a correlation with high production of CCL2, IL-1β, TNF-α, CXCL1 and IL-6. Supporting our findings, CCL2 blockade reduced colitis-associated colon cancer in mice given DSS and azoxymethane (45). Using the same model, intracolonic expression of TNF-α was found to be associated with development of colon cancer, and TNF-α blockade attenuated subsequent tumor formation (46). Also, 3-methylcholanthrene-induced carcinogenesis was reduced in IL-1β-deficient mice (47). In addition, both TNF-α and IL-6 are important in development of inflammation-associated intestinal tumorigenesis by suppressing apoptosis and...
accelerating the cell cycle (48, 49). Together this corroborates the relevance of CCL2, TNF-α and IL-1β as mediators in the initiation and progression of colitis-associated colon carcinogenesis, in accordance with our findings. Whereas T1 responses aid in anti-tumor responses, T17 cells are receiving increasing attention as being involved in the pathogenesis of colon tumorigenesis (50). Although not statistically significant, we found increased IL-17 protein levels in Gxi2−/− mice with dysplasia/cancer compared with those with no dysplasia.

In conclusion, our data show that, in Gxi2−/− mice, colonic responses are skewed early in life toward a T17 phenotype, presumably in response to an inherent barrier defect due to the Gxi2 deficiency (14). This T17 response drives the in situ activation of mucosal effector T cells to commensal antigens, and, once activated, these T1 cells proliferate and, because of the Gxi2 deletion they are not regulatable, as previously demonstrated by us (13). In addition, the T17 responses drives the production of neutrophil-attractant chemokines, resulting in the influx of neutrophils, a hallmark of the histopathology in the Gxi2 deficiency as well as in UC patients. The enhanced T17 response and the resulting increased production of CCL2, IL-1β, CXCL1 and TNF-α, especially in mice with severe colitis with dysplasia/cancer, likely contribute to the inflammation-associated cancer development, in accordance with several recent reports on colon carcinogenesis (45–50).

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References


