STAT6 Deficiency Ameliorates Severity of Oxazolone Colitis by Decreasing Expression of Claudin-2 and Th2-Inducing Cytokines


*J Immunol* 2013; 190:1849-1858; Prepublished online 9 January 2013; doi: 10.4049/jimmunol.1201373

http://www.jimmunol.org/content/190/4/1849

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Supplementary Material

http://www.jimmunol.org/content/suppl/2013/01/09/jimmunol.1201373.DC1

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Patients suffering from ulcerative colitis (UC) exhibit chronic colonic inflammation caused by a dysregulated mucosal immune response and epithelial barrier disruption. Th2 cytokines, including IL-13, have been implicated in the pathogenesis of UC. IL-13 induces phosphorylation of STAT6, a murine model of UC, by inducing colitis in STAT6-deficient (STAT6−/−) and wild type (WT) mice. We observed increased epithelial cell, T cell, macrophage, and NKT cell STAT6 phosphorylation, as well as epithelial barrier function and regulating Th2-inducing cytokine production. IL-5, IL-13, IL-6, and IFN-γ with colitis exhibited reduced secretion of IL-4, IL-5, IL-13, and IFN-γ. Patients suffering with inflammatory bowel disease, including ulcerative colitis (UC) and Crohn’s disease (CD), exhibit chronic inflammation of the intestine caused by a dysregulated mucosal immune response and epithelial barrier disruption (1). Biologic drugs targeting TNF are the mainstay of treatment for both refractory CD and UC. However, for UC, only 25% of patients achieve a sustained disease remission with anti-TNF therapy, and 20% of patients with severe UC require a total colectomy, underscoring the importance of identifying new molecular targets for UC therapy (2, 3).

The mucosal immune response in UC has been distinguished from that of CD by the involvement of Th2 inflammation (1, 4). The Th2 cytokine IL-13 has been implicated as a primary cause of epithelial barrier disruption in UC. Lamina propria lymphocytes isolated from patients with UC produce increased IL-13 compared with patients with CD and controls (5). Furthermore, in vitro, IL-13 causes increased permeability and delayed repair of model epithelial cell monolayers by stimulating apoptosis and increasing expression of the pore-forming tight junction protein Claudin-2 (6). Thus, it is plausible that interfering with IL-13 cell signaling could be an effective strategy to treat UC.

IL-13 binding to its receptor triggers a signaling cascade leading to the phosphorylation of STAT6. p-STAT6 dimerizes and translocates to the nucleus where it binds DNA promoter elements to regulate gene transcription (7). We previously showed increased activated STAT6 in the epithelium of pediatric patients at diagnosis with UC, as well as that in vitro IL-13–induced apoptosis and Claudin-2 expression are STAT6 dependent (8).

Oxazolone (OXA) colitis is a hapten-induced murine model of colitis with pathologic and immunologic features similar to UC (9, 10). IL-13 produced by Th cells or NKT cells is critical for the development of colonic inflammation in this model (10, 11). The role of STAT6 in OXA colitis has not been reported.

The Journal of Immunology, 2013, 190: 1849–1858.


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Received for publication May 15, 2012. Accepted for publication December 5, 2012.

This work was supported by National Institutes of Health Grants 5R01AT004821-04 and 3R01AT004821-02S1 (to K.T.W.); and K08HD067607 (to J.-H.W.); a Merit Review grant from the Department of Veterans Affairs (to K.T.W.); a North American Society for Pediatric Gastroenterology, Hepatology and Nutrition Foundation George Ferry Young Investigator Award (to M.I.R.); a Vanderbilt Physician Scientist Development Award (to M.I.R.); and the Vanderbilt Digestive Diseases Research Center (National Institutes of Health Grant P30DK058404), including the Center’s Pilot and Feasibility Program, Flow Cytometry, and Cellular and Animal Modeling Shared Resources.

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The online version of this article contains supplemental material.

Abbreviations used in this article: CD, Crohn’s disease; DSS, dextran sulfate sodium; ETOH, ethanol; MLN, mesenteric lymph node; NT, nontargeting; OXA, oxazolone; SAHA, suberoylanilide hydroxamic acid; shRNA, short hairpin RNA; TEC, trans-epithelial resistance; TSLP, thymic stromal lymphopoietin; UC, ulcerative colitis; WT, wild type.
In the current study, we investigated the role of STAT6 in a murine model of UC by inducing colitis with OXA in STAT6-deficient (STAT6 heterozygote) and wild type (WT) mice. Colitis was attenuated in STAT6+/− mice in association with decreased epithelial claudin-2 expression, and we show in vitro that IL-13–induced epithelial barrier dysfunction relies on STAT6 signaling. Attenuation of colitis in STAT6+/− mice was also associated with decreased colon tissue mRNA expression for the Th2-inducing cytokines IL-33 and thymic stromal lymphopoietin (TSLP), as well as decreased mesenteric lymph node (MLN) cell proinflammatory cytokine secretion. Together, these data implicate STAT6 in the pathogenesis of colitis in vivo with important roles in epithelial barrier function and regulating Th2-inducing cytokine production.

Materials and Methods

Mice

WT and STAT6+/− C57BL6 mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME) and bred in our facility under specific pathogen–free conditions. Male mice (5–7-wk-old) were used for colitis experiments. This study was carried out following recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Vanderbilt University Institutional Animal Care and Use Committee.

Colitis induction

OXA colitis was induced as previously described (12). Briefly, anesthetized mice were sensitized by topically applying 3% OXA (4-ethoxyethyl-2-phenyl-2-oxazolin-5-one; Sigma-Aldrich, St. Louis, MO) in 100% ethanol (ETOH; 150 μl) on the shaved abdomen. Seven days later, 2% OXA in 50% ETOH (150 μl) was administered intrarectally with a 5 French plastic infant feeding tube (C.R. Bard, Covington, GA). Control mice were treated with ETOH vehicle alone. Mice were sacrificed 3 d after rectal OXA administration.

Colon epithelial cell and lamina propria cell isolation

Mouse colons were washed in cold PBS and left on ice in buffer containing 0.5 mM DTT and 3 mM EDTA for 60 min. Crypts were released into the supernatant by vigorous shaking in cold PBS, filtered through a 70-μm strainer, and centrifuged at 300 × g for 10 min at 4°C. Whole-cell protein lysates were prepared from pelleted epithelial cells. The remaining colon tissue was minced and agitated in DMEM with 2 ml dispase (Roche, Indianapolis, IN) and 0.5 mg/ml collagenase (C5138; Sigma-Aldrich) at 37°C for 40 min. Released cells were filtered over a 70-μm strainer, with equal volume of DMEM containing 10% FBS, and centrifuged at 300 × g for flow cytometry. For identification of NKT cells by flow cytometry, cells were purified further using a 40–70% discontinuous Percoll gradient.

Western blot analysis

Western blot analysis on cell lysates was performed as previously described (13). Briefly, membranes were blocked with 5% nonfat dry milk in TBST for 1 h and incubated overnight at 4°C with primary Ab against total STAT6 (Millipore, Billerica, MA), p-STAT6 (Cell Signaling, Danvers, MA), claudin-2 (Abcam, Cambridge, MA), E-cadherin (BD Biosciences, San Jose, CA), or β-actin (Sigma-Aldrich). Membranes were incubated with secondary Ab, anti-rabbit or anti-mouse HRP (Cell Signaling), and protein bands were detected by chemiluminescence using Western Lightning (Perkin Elmer, Waltham, MA). Densitometry was performed using Image J software (National Institutes of Health, Bethesda, MD).

Intracellular p-STAT6 detection

p-STAT6+ lamina propria T cells, macrophages, and NKT cells were quantified by flow cytometry. A fluorescently labeled αGalCer-loaded murine CD1d tetramer was prepared from CD1d monomers (National Institutes of Health Tetramer Facility), as described previously (14). Isolated lamina propria cells were surface stained with anti-CD3 (BioLegend, San Diego, CA), anti-F4/80 (Life Technologies, Carlsbad, CA), or a combination of anti-B220 (BD Biosciences), anti–TCR-β (BioLegend), and tetramer prior to intracellular staining with anti–p-STAT6 (Cell Signaling) and/or anti–IL-13 (eBioscience, San Diego, CA). Flow cytometric analysis was performed using a BD LSRII instrument and FlowJo software (TreeStar, Ashland, OR).

High-resolution colonoscopy

The colonic mucosa was visualized in live anesthetized mice using a veterinary endoscope (KARL STORZ Imaging, Goleta, CA), according to previously described methods (15).

Histopathology

Colon sections were stained with H&E and analyzed by light microscopy. A pathologist (M.K.W.) blinded to genotype and treatment scored each mouse using a previously described 15-point scale, with a maximum of 3 points each for enterocyte loss, crypt hyperplasia, crypt inflammation, neutrophil infiltrate, and mononuclear cell infiltrate (16).

Immunohistochemistry

Sections were deparaffinized and rehydrated, and Ag was unmasked in a citrate-containing buffer (Vector Labs, Burlingame, CA). Sections were stained with mouse anti–claudin-2 Ab (Invitrogen, Carlsbad, CA) and counterstained with hematoxylin. Sections were scored in a blinded fashion by averaging the percentage of the crypt axis that was claudin-2+ for ≥50 crypts/mouse.

Cell culture

T84 cells were cultured in complete growth medium containing 1:1 DMEM/Ham’s F-12 medium, 5% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cultures were incubated at 37°C in a 95% air/5% CO2 atmosphere. Cells were exposed to IL-13 (10 ng/ml) for 24 h.

Short hairpin RNA lentiviral particle transduction

T84 cells were grown to 50% confluence and transduced with lentiviral particles carrying STAT6-specific or nontargeting (NT) short hairpin RNA (shRNA; Santa Cruz Biotechnology, Santa Cruz, CA) in medium containing 4 μg/ml Polybrene (Santa Cruz Biotechnology), with a multiplicity of infection of 0.1. Following overnight transduction, shRNA-expressing cells were expanded and selected using medium containing puromycin (8 μg/ml; Santa Cruz Biotechnology). Reduction of STAT6 expression was confirmed by Western blot analysis. Stably transduced cells were maintained with 4 μg/ml puromycin selection.

Transepithelial resistance

For measurements of transepithelial resistance (TER), T84 cell monolayers were grown on standing mixed cellulose ester membrane inserts (Millipore). TER across each monolayer was assessed using a voltmeter (WPI Sarasota, FL). Measurements were calculated in Ω·cm² and expressed as a percentage of baseline. For all experiments, baseline TER was >1000 Ω·cm².

Immunofluorescent staining

Cells cultured in chamber slides were fixed in methanol at –20°C for 5 min, permeabilized in PBS plus 0.2% Triton-X 100 (Sigma-Aldrich), and blocked in 5% goat serum (Life Technologies). Cells were stained with 2.5 μg/ml anti–claudin-2 (Abcam) overnight at 4°C, followed by secondary staining with DyLight 488–conjugated goat anti-rabbit (Jackson ImmunoResearch, West Grove, PA) and mounting with VECTASHIELD containing DAPI (Vector Labs, Burlingame, CA).

RNA expression

RNA was isolated from tissue using the RNeasy Mini Kit (QIAGEN, Valencia, CA), per the manufacturer’s instructions. RNA (1 μg) was reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). SYBR Green I Master Mix (Roche) was used for real-time PCR reactions for IL-10, IL-17, IFN-γ, the membrane-bound and soluble isoforms of ST2, and β-actin; the primers are listed in Supplemental Table I. TaqMan Gene Expression Assays (Life Technologies) were used for IL-13 (assay ID: Mm00434204_m1), IL-33 (Mm00505403_m1), TSLP (Mm00498739_m1), and GAPDH (Mm99999915_g1). All reactions were performed in duplicate with 1 μl cDNA on a LightCycler 480 II real-time PCR platform (Roche). Relative mRNA levels were determined using the 2−DDCT method with β-actin or GAPDH as the reference.

Ex vivo lymphocyte cytokine production

Single-cell suspensions were prepared from MLNs, as previously described (17). A total of 5 × 10⁶ cells was cultured in RPMI 1640 media containing...
10 mM HEPES, 10 μg/ml gentamicin, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated FBS in round-bottom 96-well plates with 5 μg/ml plate-bound anti-CD3 and 1 μg soluble anti-CD28 (BioLegend), with or without 10 ng/ml recombinant mouse IL-33 (BioLegend). Culture supernatants were stored at −80°C for cytokine quantification. Cytokine quantification was performed using a custom Milliplex

FIGURE 1. OXA colitis is associated with increased mucosal STAT6 phosphorylation. (A) Colon epithelial cells lysates from four WT mice treated with OXA and four treated with ETOH vehicle were subjected to Western blot analysis for p-STAT6, total STAT6, E-cadherin (as an epithelial marker), and β-actin loading control. (B) Densitometry of p-STAT6 corrected for both total STAT6 and β-actin. (C) Representative intracellular p-STAT6 staining from CD3⁺gated lamina propria cells isolated from WT mice treated with OXA or ETOH vehicle. (D) Summary data of total numbers of CD3⁺ and CD3⁺p-STAT6⁺ lymphocytes. (E) Representative intracellular p-STAT6 staining from F4/80⁺gated lamina propria cells. (F) Summary data of total numbers of F4/80⁺ and F4/80⁺p-STAT6⁺ macrophages. Each symbol in (D) and (F) represents an individual mouse. *p < 0.05, **p < 0.01. FSA, Forward scatter; SSA, side scatter.
MAP cytokine panel (Millipore), per the manufacturer’s protocol, on a FLEXMAP 3D (Luminox, Austin, TX).

Statistical analyses

For each set of experiments yielding continuous data in three or more groups, one-way ANOVA was applied as a global test for differences in the primary outcome variable. Log-transformation was applied if data exhibited a skewed distribution. Histopathologic scoring, as an ordinal variable, was compared globally among groups using the nonparametric Kruskal–Wallis test. Predetermined pair-wise comparisons of interest were made using the Student’s t test or Mann–Whitney U test with Bonferroni correction only when an overall effect was detected. The relationship between tissue IL-33 relative mRNA expression and histopathology score was explored using Spearman’s correlation coefficient. Repeated-measures two-way ANOVA with Bonferroni correction was used to compare measurements of mouse weight and epithelial membrane TER over time among experimental groups and to determine the effect of IL-33 treatment on activated MLN cytokine production across experimental groups. Statistical analyses were performed using Prism 5.0b (GraphPad Software, La Jolla, CA).

Results

Increased STAT6 activation in OXA colitis

We previously showed increased epithelial p-STAT6 in pediatric subjects with UC and that IL-13–induced apoptosis and claudin-2 expression in vitro is STAT6 dependent (8). We hypothesized that STAT6 would be activated in cell types acted on by increased IL-13 in OXA colitis. To determine whether epithelial p-STAT6 is induced in a murine model of UC, we assessed expression of total and p-STAT6 in colonic epithelial cells isolated from WT mice with OXA colitis by Western blot analysis. There was a 2.6-fold increase in epithelial STAT6 phosphorylation in WT mice with OXA colitis compared with WT ETOH-treated controls ($p = 0.005$), with no difference in total STAT6 expression (Fig. 1A, 1B). Important roles for STAT6 have also been established in lymphocytes and macrophages (18–21). To determine whether increased lymphocyte or macrophage STAT6 activation is involved in OXA colitis, we performed intracellular staining for p-STAT6 on isolated colonic lamina propria cells from WT mice treated with OXA or ETOH vehicle and assessed by flow cytometry. We observed increased absolute numbers of total colon CD3+ T cells and p-STAT6+CD3+ T cells in WT mice treated with OXA compared with WT control mice (Fig. 1C, 1D). Furthermore, we observed increased absolute numbers of total colon F4/80+ macrophages and p-STAT6+F4/80+ macrophages in WT mice treated with OXA compared with WT control mice (Fig. 1E, 1F). Because NKT cells have been proposed as the primary source of IL-13 in OXA colitis (10), we investigated lamina propria cells for p-STAT6+IL-13+ NKT cells (B220+TCR-β+Tetramer+) by flow cytometry. As expected, we found increased percentages of NKT cells in WT mice treated with OXA compared with WT control mice (Fig. 2A). In addition, WT colitic mice exhibited an increased percentage of p-STAT6+ and p-STAT6+IL-13+ NKT cells (Fig. 2B, 2C). These data suggest a role for STAT6 signaling in epithelial cells, T cells, macrophages, and IL-13–producing NKT cells in OXA colitis.

**OXA colitis is attenuated in STAT6-deficient mice**

To determine the role of STAT6 in vivo, we induced colitis with OXA in WT and STAT6−/− mice. STAT6−/− OXA mice recovered more quickly from colitis, as evidenced by more rapid weight gain between days 1 and 3, with complete weight recovery by day 3 (days 2 and 3, $p < 0.001$) (Fig. 3A). There were fewer deaths in STAT6−/− OXA mice compared with WT OXA mice; however, the difference in survival was not statistically significant given the overall low mortality (Fig. 3B). By high-resolution endoscopy, WT OXA mice demonstrated qualitatively obscured vascular pattern, erythematous and granular mucosa, and loose stool consistency compared with WT ETOH mice. STAT6−/− OXA mice exhibited improved vascular pattern, less erythema and granularity, and more formed stool compared with WT OXA mice (Fig. 3C). Upon dissection, there was reduced colon length, an indicator of colitis severity, in WT OXA and WT ETOH mice but not in STAT6−/− OXA mice (Fig. 3D).

As anticipated, WT OXA mice demonstrated histologic features similar to human UC, with crypt hyperplasia, crypt loss, cryptitis, and infiltrates of neutrophils and mononuclear cells into the lamina propria, which were less pronounced in STAT6−/− OXA mice (Fig. 3E). In line with these findings, histopathology scores were elevated in WT OXA mice compared with WT ETOH mice ($p < 0.001$), and STAT6−/− OXA mice scored significantly lower than did WT OXA mice ($p < 0.01$) (Fig. 3F).
IL-13–induced barrier dysfunction is mediated through STAT6

In colon epithelial cell monolayers, IL-13 impairs barrier function by inducing apoptosis and increasing expression of claudin-2 (6). We (8) and other investigators (22) previously showed that suberoylanilide hydroxamic acid (SAHA), a protein deacetylase inhibitor with STAT6-inhibitory properties, lessens IL-13–mediated reductions in TER in epithelial monolayers. Because SAHA is an established protein deacetylase inhibitor and has effects on other proteins in addition to STAT6, we developed a T84 colon epithelial cell line with stable reduction of STAT6 expression using shRNA delivered through a transduced lentiviral vector. STAT6 expression was reduced 83 ± 6% (p = 0.002) in STAT6 shRNA–transduced cells compared with cells transduced with control NT shRNA. Cells were exposed to IL-13 (10 ng/ml) for 24 h. As expected, induction of claudin-2 expression with IL-13 was reduced in STAT6 shRNA–transduced cells, as demonstrated by Western blot analysis and immunofluorescent staining (Fig. 4A, 4B). Transduced cells were plated on semipermeable Transwells and treated with IL-13 to examine whether the effect of IL-13 on TER is mediated through STAT6. Mean TER as a percentage of baseline was greater in STAT6 shRNA–transduced cells compared with NT shRNA–transduced cells after 12 and 24 h of IL-13 exposure (p < 0.05 for 12 and 24 h), confirming a role for STAT6 in IL-13–induced epithelial barrier dysfunction in vitro (Fig. 4C).

STAT6 regulates claudin-2 induction in OXA colitis

Claudin-2 expression is increased in the colonic mucosa of patients with UC, and induction of claudin-2 is an important mechanism through which IL-13 impairs colon epithelial barrier dysfunction (6, 22). We sought to determine whether epithelial claudin-2 expression is induced in OXA colitis and whether the induction is mediated through STAT6. We performed immunohistochemical staining for claudin-2 in WT and STAT6−/− mice treated with OXA or ETOH. In WT ETOH mice, claudin-2 staining was concentrated at the intercellular junctions in the lower 33.1 ± 62% of the crypt. Claudin-2 staining was increased in WT OXA mice, extending up to 45.5 ± 62% of the crypt axis (p, 0.001), but not in STAT6−/− OXA mice, which only demonstrated staining of the bottom 34.5 ± 62% of the crypt (p, 0.001, versus WT OXA) (Fig. 5).

STAT6 regulates expression of IL-33 and TSLP in OXA colitis

OXA colitis was initially described as a Th2-driven model of colitis with increased lamina propria lymphocyte production of IL-4 and IL-13 (9, 10). Because STAT6 is an important regulator of
Th2 lymphocyte differentiation (21), we hypothesized that the expression of IL-4 and IL-13 would be reduced or absent in STAT6^{−/−} OXA mice compared with WT OXA mice. Interestingly, using real-time PCR analysis of colon tissue mRNA, we observed a mixed Th lymphocyte response, with significantly increased expression of IL-13, IFN-γ, IL-17, and IL-10 (IL-4 was not detected by real-time PCR, data not shown) in WT OXA mice compared with WT ETOH mice. Furthermore, there was no detectable difference in the expression of these cytokines between WT OXA and STAT6^{−/−} OXA mice (Fig. 6A). In recent years, IL-33, TSLP, and IL-25 have emerged as important cytokines for the initiation and amplification of Th2 immune responses (23). We observed a 5.6-fold increased relative expression of IL-33 in WT OXA mice compared with WT ETOH mice, which was abrogated in STAT6^{−/−} OXA mice. We also observed a 2-fold increased relative expression of TSLP in WT OXA mice that was eliminated in STAT6^{−/−} OXA mice (Fig. 6A). We did not observe any difference in IL-25 expression among mouse groups (data not shown). IL-33 mRNA expression strongly correlated with histopathologic severity (Fig. 6B). These data suggest that STAT6 regulates mucosal expression of the Th2-inducing cytokines IL-33 and TSLP.

Deficiency of STAT6 reduces MLN cell cytokine secretion in OXA colitis

To determine the role of STAT6 in the potential for T cell cytokine production in OXA colitis, we compared cytokine secretion from MLN cells activated with anti-CD3 (5 μg/ml) and anti-CD28 (1 μg/ml) mAbs among WT ETOH, WT OXA, and STAT6^{−/−} OXA mice. We observed increased IL-4, IL-5, IL-13, IFN-γ, and IL-17 secretion from cells from WT OXA mice compared with WT ETOH mice, as well as less secretion of each of these cytokines from cells from STAT6^{−/−} OXA mice (Fig. 7). There was no difference in IL-6 secretion among groups. Although there was no significant increase in IL-10 secretion from cells from WT OXA mice compared with WT ETOH mice, there was less IL-10 secretion from cells from STAT6^{−/−} OXA mice compared with WT OXA mice. Thus, STAT6 deficiency impaired T cell secretion of multiple proinflammatory cytokines increased in OXA colitis.

IL-33 augments MLN proinflammatory cytokine secretion

Several groups have implicated IL-33 in the pathogenesis of human UC (24–27). To determine the effect of IL-33 on lymphocyte function in OXA colitis, we assessed the cytokine secretion from IL-33-exposed activated MLN cells from WT ETOH, WT OXA, and STAT6^{−/−} OXA mice. In addition to activation with anti-CD3 and anti-CD28 mAbs alone, MLN cells were similarly activated in the presence of IL-33 (10 ng/ml). IL-33 augmented the secretion of the Th2 cytokines IL-13 and IL-5 but not IL-4 (Fig. 8). IL-33 also augmented the secretion of IFN-γ and IL-6 (Fig. 8). The overall effect of IL-33 on MLN cell cytokine secretion did not differ among WT ETOH, WT OXA, and STAT6^{−/−} OXA mice. In STAT6^{−/−} OXA mice, IL-33 increased MLN cell IL-13 and IL-5 secretion to levels seen in cells from WT OXA mice activated without IL-33, indicating that these cells are still capable of Th2 cytokine production. Other investigators (25) observed increased mucosal expression of ST2, a component of the IL-33R complex, in intestinal inflammation. We assessed mRNA expression for both membrane-bound ST2 and soluble ST2 in both

![Figure 4](http://www.jimmunol.org/DownloadedFrom/1854/STAT6%20DEFICIENCY%20AMELIORATES%20SEVERITY%20OF%20OXAZOLONE%20COLITIS/1854%20STAT6%20DEFICIENCY%20AMELIORATES%20SEVERITY%20OF%20OXAZOLONE%20COLITIS)
and the Gram-negative bacterium *H. polygyrus*. In a mouse coinfection model with the helminth *O. saxatilis*, we observed exacerbation of acute dextran sulfate sodium (DSS)–induced colitis, contrast to our findings with OXA colitis, other investigators (28) reported that, in vitro, IL-13–mediated reductions in TER are lessened with SAHA, a protein deacetylase inhibitor with STAT6-inhibitory properties. In this study, we demonstrate a partial abrogation of the IL-13–mediated TER decrease in T84 cells with stable knockdown of STAT6 expression, which is in line with findings by Wu et al. (37) in CaCo2bbe cells. In contrast, other investigators (39) observed that IL-13 regulation of epithelial permeability was not STAT6 dependent, but rather was mediated by PI3K signaling. We did not observe an effect of PI3K inhibitors on IL-13–mediated reductions in TER (data not shown). These conflicting findings may be explained by the use of different cell lines, model systems (in vitro versus in vivo), or methods of interfering with STAT6 expression (transcription factor decoys versus shRNA interference). It remains plausible that, depending on the system studied, both STAT6 and PI3K signaling are involved in IL-13–induced barrier dysfunction.

We observed a mixed cytokine response in OXA colitis with regard to tissue mRNA expression and MLN cytokine secretion. Although OXA was originally described as a Th2-mediated model of colitis (9, 10), other investigators similarly reported mixed cytokine production (40). Given that STAT6 is an important transcription factor for the differentiation of Th2 cells (21, 23, 41), we anticipated markedly reduced IL-13 production in association with colitis amelioration in STAT6−/− mice. Although we did not observe any effect of STAT6 deficiency on tissue IL-13 mRNA expression in OXA colitis, there was a marked reduction in MLN cell secretion of multiple cytokines, including the Th2 cytokines IL-13, IL-4, and IL-5, in STAT6−/− mice with colitis. Therefore, lymphocytes from STAT6−/− mice have reduced capacity to produce Th2 cytokines; however, Th2 lymphocytes remain present in colitic STAT6−/− mice, and local factors (e.g., IL-33), which may be derived from other cell types, such as epithelial cells and macrophages, maintain IL-13 expression in the colon tissue. In fact, preserved Th2 responses have been observed in helminth-infected STAT6−/− mice (42, 43). Although activated STAT6 induces GATA3, the master transcription factor necessary for Th2 development (44, 45), STAT6-independent mechanisms for GATA3 induction and Th2 differentiation have been identified, including pathways involving Notch (46), TCF-1/β-catenin (47), and IL-2/STAT5A signaling (48). Because STAT6 deficiency does not prevent colon tissue IL-13 expression in OXA colitis, it likely ameliorates colitis by eliminating deleterious effects of IL-13 on cell function, such as induction of claudin-2 in epithelial cells, as shown in the current study. Other possible mechanisms could be through reduction of NKT cell cytotoxicity or B cell IgE production (5, 11).

Within the colon tissue, we also observed that STAT6 deficiency reduced the induction of mRNA expression for the Th2-inducing cytokines IL-33 and TSLP in OXA colitis. IL-33 and TSLP have emerged as key initiators and amplifiers of Th2 immune responses in acute inflammatory diseases as well as in allergic diseases (22) and inflammatory bowel disease (13). In the current study, we demonstrate an important role for STAT6 in epithelial cell induction of these Th2-inducing cytokines, thereby aggravating intestinal inflammation and Th2 cell differentiation.

In the IL-4–dependent TCRα-/- model of colitis, Okuda et al. (32) found no effect of STAT6 genetic deletion on colitis development, and colitis was not completely prevented in STAT6−/− mice. In contrast, we observed exacerbation of acute DSS–induced colitis in STAT6−/− mice, which is in line with the findings of other groups (37, 38) who demonstrated that in vivo IL-13–induced barrier dysfunction is STAT6 dependent in the small intestine. We (8) and other investigators (22) showed that, in vitro, IL-13–mediated reductions in TER are lessened with SAHA, a protein deacetylase inhibitor with STAT6-inhibitory properties. In this study, we demonstrate a partial abrogation of the IL-13–mediated TER decrease in T84 cells with stable knockdown of STAT6 expression, which is in line with findings by Wu et al. (37) in CaCo2bbe cells. In contrast, other investigators (39) observed that IL-13 regulation of epithelial permeability was not STAT6 dependent, but rather was mediated by PI3K signaling. We did not observe an effect of PI3K inhibitors on IL-13–mediated reductions in TER (data not shown). These conflicting findings may be explained by the use of different cell lines, model systems (in vitro versus in vivo), or methods of interfering with STAT6 expression (transcription factor decoys versus shRNA interference). It remains plausible that, depending on the system studied, both STAT6 and PI3K signaling are involved in IL-13–induced barrier dysfunction.

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Within the colon tissue, we also observed that STAT6 deficiency reduced the induction of mRNA expression for the Th2-inducing cytokines IL-33 and TSLP in OXA colitis. IL-33 and TSLP have emerged as key initiators and amplifiers of Th2 immune responses in acute inflammatory diseases as well as in allergic diseases (22) and inflammatory bowel disease (13). In the current study, we demonstrate an important role for STAT6 in epithelial cell induction of these Th2-inducing cytokines, thereby aggravating intestinal inflammation and Th2 cell differentiation.
responses (23). Studies showed a protective role for TSLP in chronic intestinal inflammation through induction of tolerogenic dendritic cells (49–52). However, the role of IL-33 in murine colitis remains less clear. IL-33 administration induces IL-13 and IL-5 production from Th2 cells in mice and induces goblet cell hyperplasia in the colon (53). DSS colitis is attenuated in IL-33-
deficient mice; however, recovery after DSS withdrawal is delayed (54). Mucosal IL-33 expression also correlates with disease severity in SAPPy/IfiFc mice, a spontaneous model of chronic ileitis (25). Several groups (24–27) independently observed increased IL-33 production in the inflamed mucosa of patients with UC, with most identifying intestinal epithelium as the source.

We observed that IL-33 augmented stimulated MLN cell secretion of the Th2 cytokines IL-5 and IL-13, as well as IFN-γ and IL-6. These findings are similar to the observation of a mixed cytokine response to IL-33 in SAPPy/IfiFc mice (25). Because IL-33 stimulates the production of multiple cytokines, our finding of decreased tissue IL-33 mRNA expression in colitic STAT6−/− mice may explain why these mice also demonstrated reduced MLN cytokine secretion of both Th2 and Th1 cytokines. It is also notable that IL-33 enhanced IL-13 and IL-5 production, even in MLN cells from STAT6−/− mice, which further supports a role for STAT6-independent Th2 differentiation in OXA colitis.

In conclusion, STAT6 deficiency reduces the severity of OXA colitis in mice through decreasing epithelial claudin-2 expression, reducing Th2-inducing cytokine expression, and reducing lymphocyte cytokine secretion. Thus, STAT6 may represent a promising target for future UC small molecule drug development.

Disclosures

M.J.R. has served as a consultant for Abbott Laboratories. K.T.W. has received research funding from Proctor & Gamble. The other authors have no financial conflicts of interest.

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FIGURE 8. IL-33 augments MLN cell proinflammatory cytokine production. MLN cells from WT mice treated with ETOH vehicle and WT and STAT6−/− mice treated with OXA were exposed to IL-33 (10 ng/ml) in addition to activation for 48 h with plate-bound anti-CD3 (5 μg/ml) and soluble anti-CD28 (1 μg/ml). Cytokine production was determined by Luminex analysis of culture supernatants. Each circle–square pair represents values from a single mouse: the circle represents the cytokine secretion from cells stimulated without IL-33, and the square represents cytokine secretion from cells stimulated with IL-33. Note that the data from the PBS group (circles) are presented again (from Fig. 7) for the purposes of demonstrating the effect of IL-33 on MLN cell cytokine secretion. *p < 0.05, **p < 0.01, ***p < 0.001.

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