B Cells That Produce Immunoglobulin E Mediate Colitis in BALB/c Mice

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BACKGROUND & AIMS: Induction of colitis in mice by administration of oxazolone is mediated by T-helper (Th) 2 cells and has features of human ulcerative colitis. We investigated whether activation of interleukin (IL)-4Rα on T and B cells determines their effector functions and mediates oxazolone-induced colitis. METHODS: We studied induction of colitis with oxazolone in wild-type mice and those with CD4+ T cells that did not express IL-4Rα (LckcreIL-4Rαlox/lox). We also generated mice with B cells that did not express IL-4Rα (mb1creIL-4Rαlox/lox) and studied induction of colitis.

RESULTS: LckcreIL-4Rαlox/lox mice did not develop colitis in response to oxazolone, and their levels of IL-4, IL-13, and immunoglobulin (Ig) E were reduced. Adoptive transfer of naive, wild-type CD4+ Th cells depleted of natural killer T cells to LckcreIL-4Rαlox/lox mice restored their susceptibility to colitis. In contrast, LckcreIL-4Rαlox/lox mice maintained their protection against colitis when IL-13-deficient CD4+ T cells were transferred. These findings indicate that development of colitis involves not only natural killer T-cell functions, but also requires IL-13 production by CD4+ T helper cells. Mb1creIL-4Rαlox/lox mice, which cannot produce IgE, were also protected against oxazolone-induced colitis. Blocking IgE binding significantly reduced mast cell numbers in colons and protected wild-type BALB/c mice from the onset of colitis.

CONCLUSIONS: IL-4 appears to induce CD4+ Th2 cells to produce IL-13 and B cells to produce IgE, which together mediate oxazolone-induced colitis in mice.

Keywords: Immune Regulation; IBD; Inflammatory Bowel Disease; T-Cell Signaling.

Ulcerative colitis (UC) is a human inflammatory bowel disease (IBD) associated with chronic inflammation of the gastrointestinal tract leading to severe diarrhea, blood loss, and progressive defects in peristaltic function.1 While T cells have been linked to UC by the effectiveness of T cell–depletion therapy and cyclosporine treatment, an increase in immunoglobulin (Ig) G1 antibody production in patients with UC suggests a role for B cells.2,3 Mouse models have played an important role in understanding the underlying immune mechanisms and the role of lymphocytes in driving the pathogenesis of UC. Treatment with the haptenating agent oxazolone induces a T-helper (Th) type 2 colitis limited to the distal half of the colon with histologic features resembling human UC, including epithelial cell loss, depletion of goblet cells, and inflammatory cell infiltration.4–6

Previous studies in T cell–, cytokine–, or cytokine receptor–deficient mouse models showed evidence for a T cell–dependent regulatory system in IBD.7–12 Furthermore, lamina propria CD4+ T cells reacting to enteric flora are important effector cells causing mucosal inflammation in IBD, and in vivo depletion of these cells ameliorates inflammation.13–15 Together these models show that subpopulations of CD4+ T cells play a crucial role in the development of IBD. Interleukin (IL)-4 and IL-13 are closely related cytokines involved in the pathogenesis of oxazolone-induced colitis and have pleiotropic immune functions that are mediated through a shared IL-4Rα subunit.16 Initial studies using a short-lived model of oxazolone-induced colitis showed that CD4+ T cells produce large amounts of IL-4 and IL-5 and that blocking IL-4 prevented colitis.4 Prolonging the oxazolone model leads to increasing levels of IL-13, and blocking IL-13 signaling protects mice from colitis. IL-13–producing natural killer (NK) T cells were identified as mediators of disease, possibly activated by antigen presentation through CD1d by professional antigen-presenting cells or epithelial cells directly.1,17,18 IL-13 affecting epithelial tight junctions, apoptosis, and cell restitution is suggested to cause disease.19 Because early IL-4 production by CD4+ cells can initiate IL-13 production by NK T cells, it is possible that IL-4 drives the initial inflammatory response.20

Many aspects of the initial events leading to B cell–mediated immunopathology and the mechanisms involving IL-4, IL-13, and type 2 antibody responses remain undefined. Distinguishing different B-cell subpopulations by surface expression of activation markers identified a unique population of activated CD19+ B lymphocytes in UC, with patients showing prevalent allergic symptoms and high IgE levels.3,21,22 Although IgE is known to play an important role in inflammatory reactions, little evidence is available about the role of IgE in UC.23

In this study, we investigated the role of IL-4Rα responsiveness on T and B cells in a mouse model of UC. CD4+ T cell–specific IL-4Rα–deficient mice (LckcreIL-4Rαlox/lox) and newly generated B cell–specific IL-4Rα–deficient mice (mb1creIL-4Rαlox/lox) were treated with oxazolone and mon-

Abbreviations used in this paper: ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; IL, interleukin; MMCP, mouse mast cell protease; NK, natural killer; PE, phycoerythrin; PCR, polymerase chain reaction; Th, T helper.

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itored for disease symptoms. CD4+ T cell- and B cell-specific IL-4Rα-deficient mice were protected from disease. Adoptive transfer of naïve wild-type but not IL-13-deficient CD4+DX5− Th cells into LckcreIL-4Rα−/lox mice reverted protection back to a susceptible phenotype. Furthermore, protection correlated with impaired IgE antibody responses in LckcreIL-4Rα−/lox and mb1creIL-4Rα−/lox mice, and blocking IgE binding significantly protected BALB/c mice from the onset of colitis. Together, these data suggest that IL-4–promoted CD4+ Th2 cells producing IL-13 and IgE-producing B cells are crucial in mediating oxazolone-induced colitis.

Materials and Methods

Generation and Genotyping of T Cell–Specific and B Cell–Specific IL-4Rα-Deficient Mice

CD4+ T cell-specific IL-4Rα-deficient mice (LckcreIL-4Rα−/lox) were previously described.24 We generated novel B cell–specific IL-4Rα-deficient mice (mb1creIL-4Rα−/lox) by intercrossing transgenic mb1cre mice with IL-4Rα−/− and IL-4Rαlox/lox mice.25–28 Cre transgene negative littermates (IL-4Rα−/−lox) expressing functional IL-4Rα were used as controls in all experiments. LckcreIL-4Rα−/lox mice are described as C57BL/6J-Lckcre/−Il4ratm1Fbb/Il4ratm1Fbb, mb1creIL-4Rα−/lox are Il4ratm1Fbb/Il4ratm1FbbTg(Lck-cre), and IL-4Rα−/− are Il4ratm1Fbb/Il4ratm1FbbTg(Cd79αtm1(cre)R2b), and IL-4Rα−/− and IL-13−/− BALB/c mice were used.29,30 Male BALB/c mice (6–10 weeks old) were used for oxazolone experiments, and sex-matched mice were used for characterization and allergy experiments. All animal experiments were approved by the Ethics Committee of the University of Cape Town (approval no. 009/056).

Genotyping

Polymerase chain reaction (PCR) was used to confirm the genotype of LckcreIL-4Rα−/lox and mb1creIL-4Rα−/lox mice (see Supplementary Table 1). PCR conditions were 94°C for 1 minute, 94°C for 30 seconds, 55°C to 60°C for 20 seconds, and 72°C for 1 minute for 40 cycles on an MJ thermocycler (Biozym, Heissisch Oldendorf, Germany).

Sensitization and Challenge Protocols

Oxazolone colitis was induced in BALB/c mice with a modified method.31 Anesthetized mice were sensitized on the shaved abdomen with 3% oxazolone (4-ethoxymethylene-2-phenylazolone; Sigma-Aldrich, Saint Louis, MO) in 100% ethanol (150 µL), followed 7 days later by intrarectal administration of 1% oxazolone in 50% ethanol (150 µL). Control mice were treated with ethanol only. BALB/c mice develop rapid-onset disease. Adoptive transfer of naïve wild-type but not IL-13–promoted CD4+Th2 cells produced IL-13 and IgE-producing B cells are crucial in mediating oxazolone-induced colitis.

Adoptive Transfer of Lymphocytes and Anti-IgE Antibody Treatment

CD4+ T cells from mesenteric lymph nodes of naïve IL-4Rα−/lox littermate control (IL-4Rα−/lox), BALB/c (wild-type), or IL-13−/− mice were enriched to >90% using negative selection with BioMag beads (Qiagen, Hilden, Germany), as previously described.33 These cells were fluorescence-activated cell sorted (FACS) for CD4+ (GK1.5) cells at a purity of >98%, and NK T cells were excluded using CD49b/DX5-PE (DX5) or a PBS7-loaded CD1d tetramer (provided by the National Institutes of Health Tetramer Facility, Emory University). LckcreIL-4Rα−/lox and IL-4Rα−/lox littermate control mice received 1 × 106 sorted cells 24 hours before sensitization with oxazolone. For adoptive transfer of B cells, mb1creIL-4Rα−/lox or IL-4Rα−/lox mice received CD19+ cells from wild-type or IL-4Rα−/− (knockout) mice. In vivo neutralization of IgE function was performed with 2 doses of rat anti-mouse IgE antibody (84.1C), rat anti-mouse FcRI/II III (2.4G2), or rat IgG isotype control (afrc-mac51) (200 µg/mouse) before sensitization and challenge with oxazolone.

Disease Activity Index

To determine disease progression, mice were weighed and monitored for distress and gastrointestinal symptoms 1 to 2 times daily. Weight loss was measured as a percentage starting weight and distress scored according to appearance, clinical signs, and natural or provoked behavior (see Supplementary Table 2).34 Mice scored on a scale of 0 to 16 were killed if the most severe score (16) was reached or ≥20% body weight was lost. Mortality studies (10 mice/group) followed the criteria as previously described.

Histologic Analysis

Distal colon sections were stained with periodic acid–Schiff reagent for inflammatory or mucus-producing goblet cells and toluidine blue for mast cells. Histopathologic grading adjusted from a previous study was (1) presence of mononuclear cells, (2) reduced goblet cells, (3) epithelial injury, (4) granulocyte infiltration, and (5) edema.35 Each criterion was scored from 0 to 3, resulting in a total additive score between 0 (no colitis) and 15 (maximal colitis activity).

Cell Preparation and Ex Vivo Restimulation of Lymphocytes

Single-cell suspensions were prepared from pooled mediastinal lymph nodes or spleen and cultured at 1 × 106 cells/mL with or without plate-bound anti-CD3 antibody (clone 145-2C11, 20 µg/mL). Supernatants were collected and stored at −80°C, and pooled samples were measured in triplicate.

Cytokine Detection in Colon Tissue Homogenates

Colon sections were pooled, snap frozen in liquid nitrogen, and homogenized in phosphate-buffered saline supplemented with protease inhibitors (Sigma, Saint Louis, MO). Homogenates were normalized to 10 mg/mL using a Bicinchoninic Acid Protein Estimation Kit (Pierce, Rockford, IL), and cytokines were detected by enzyme-linked immunosorbent assay (ELISA) from pooled samples measured in triplicate.

Antibody, Cytokine, and Mouse Mast Cell Protease 1 Detection by ELISA

Cytokines in supernatants and total or ovalbumin-specific antibody isotypes in the sera were determined as previously described27,32 with 2 anti-IgE coating antibodies (clone 84.1C and 23G3). Mouse mast cell protease (MMP)-1 levels were measured in sera by ELISA, according to the manufacturer’s protocol (Moredun Scientific, Penicuik, Midlothian, United Kingdom).
Figure 1. LckcreIL-4Rα−/lox mice are protected from oxazolone-induced colitis. LckcreIL-4Rα−/lox mice were protected from oxazolone-induced colitis compared with littermate control mice, shown by (A) significantly reduced weight loss as percentage of starting weight, prolonged survival (day 7), and reduced distress on day 2. (B) Less colon shortening (cm), indicating reduced inflammation with normal colon appearance in macroscopic pictures. (C) Reduced inflammation shown in periodic acid–Schiff–stained sections of the distal colon, while IL-4Rα−/lox oxazolone mice showed edema, reduced mucus production, and inflammatory infiltrates. SM, submucosa; M, mucosa. Semi-quantitative histopathologic scoring of colitis was based on the presence of mononuclear cells (Mon), edema (Oed), epithelial damage (Ep), loss of goblet cells (Go), and granulocyte infiltration (Gr). (D) Protection corresponded with impaired Th2 cytokine production by anti-CD3 restimulated pooled spleen cells measured in triplicate by ELISA and (E) impaired total IgE but not IgG1 concentrations measured in the serum from individual mice. All data represent mice killed 3 days postchallenge unless indicated. Data represent 2 or 3 individual experiments or pooled experiments for colitis score and IgE (n = 4–10 mice). Except for body weight and antibodies (mean ± SEM), mean values are ± SD. *P < .05 and ***P < .001 vs IL-4Rα−/lox EtOH controls; #P < .05, ##P < .01, and ###P < .001 IL-4Rα−/lox vs LckcreIL-4Rα−/lox oxazolone.
Genomic DNA was prepared from CD3+CD19- and CD3+CD19+ sorted lymph node cells (>99% purity) from mb1creIL-4Rαlox/lox, IL-4Rα−/−, and IL-4Rα+/− mice using a FACSVantage flow cytometer (BD Biosciences, Erembodegem, Belgium). A standard curve was prepared of serial 10-fold dilutions from DNA product using primers specific for IL-4Rα exon 5 (control) and exon 8 (deleted by hCre expression; see Supplementary Table 1).

**Flow Cytometry**

IL-4Rα surface expression was detected on spleen, lymph node, or bone marrow cells by phycoerythrin (PE) or biotinyl-
ated anti-CD124 (IL-4R, M-1) as previously described. Cell subpopulations were identified with fluorescein isothiocyanate, PE, or biotinylated monoclonal antibodies against CD19 (1D3), B220 (RA3-6B2), CD3 (145-2C11), CD4 (GK1.5), CD8 (53.6.72), pan-NK/CD49b-PE (DX5), CD11b (M1/70), F4/80 (A3-1, Sero-tec), Fcγ receptor I (FcγR I-mar1), or IgE (23G3). Biotin-labeled antibodies were detected by allophycocyanin, and all antibodies were from BD Pharmingen (San Diego, CA) except where noted otherwise.

Immunohistochemistry
Distal colon tissue was embedded in OCT (Tissue-Tek; Sakura, Zoeterwoude, Netherlands) using dry ice and cryosections mounted on 3-aminopropyltriethoxysilane–coated slides. After overnight dehydration at 4°C, sections were fixed in ice-cold acetone and stained with IgE-PE (23G3) and Hoechst 33342 (Invitrogen, Carlsbad, CA). Images were captured on a Zeiss LSM 510 Meta confocal microscope (Jena, Germany).

T-Cell Proliferation
CD4+ T cells from pooled lymph nodes were positively selected using anti-CD4 coupled to microbeads (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) to a purity of >95% as confirmed by FACS. [3H] incorporation was measured as previously described.

In Vitro Th2 Differentiation
CD4+ T cells were used for in vitro Th1/Th2 differentiation as previously described.
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**Statistical Analysis**

Values are given as mean ± SD or SEM, and significant differences were determined using Student’s t test or one-way analysis of variance using a Bonferroni posttest (GraphPad Prism, Irvine, CA). Values of \( P < .05 \) were considered significant.

**Results**

**CD4⁺ T Cell–Specific IL-4Rα–Deficient Mice Are Protected From Oxazolone-Induced Colitis**

The role of IL-4–promoted CD4⁺ T cells was investigated in oxazolone-induced colitis using CD4⁺ T cell-specific IL-4Rα–deficient BALB/c mice (Lck\textsuperscript{+/−}IL-4Rα\textsuperscript{−/−}). As with BALB/c (data not shown), IL-4Rα\textsuperscript{−/−} littermate control mice develop colitis with weight loss peaking by day 3, leading to mortality or recovery by day 7 accompanied by distress (Figure 1A). At day 3 postadministration, colitis becomes prominent, with striking inflammation-associated colon shortening and severe colitis limited to the distal half of the colon (Figure 1B). Superficial inflammation, characterized by epithelial cell loss, patchy ulceration, and pronounced depletion of mucus-producing goblet cells, was confirmed by semiquantitative histopathologic assessment (Figure 1C). In contrast, Lck\textsuperscript{+/−}IL-4Rα\textsuperscript{−/−} mice survived treatment without wasting disease and mild symptoms of pathology, similar to ethanol control mice (Figure 1A–C). As known, oxazolone-induced colitis induces type 2 immune responses, shown by IL-4 and IL-13 cytokine production from anti-CD3 restimulated pooled splenic cells (Figure 1D) and a significant increase in serum IgE antibody concentrations (Figure 1E). Oxazolone-treated Lck\textsuperscript{+/−}IL-4Rα\textsuperscript{−/−} mice presented with significantly reduced type 2 cytokine and antibody responses compared with BALB/c IL-4Rα\textsuperscript{−/−} littermate control mice, as shown by reduced IL-4, IL-13, and IgE levels. Adoptive transfer of IL-4Rα⁺ CD4⁺ T cells from wild-type BALB/c mice 24 hours before skin sensitization restored the disease phenotype in Lck\textsuperscript{+/−}IL-4Rα\textsuperscript{−/−} mice (Figure 2A–C). Together, these results show that impairment of IL-4Rα signaling on CD4⁺ T cells protects mice against oxazolone-induced colitis.

**IL-4–Promoted Th2 Cells Producing IL-13 Mediate Oxazolone-Induced Colitis**

Previous studies have suggested that experimental and human UC is mediated directly by NK T cells. Because CD4 is expressed on a subpopulation of NK T cells, we sorted CD4⁺ DX5⁻ T cells by FACS (>98% purity) to exclude the possibility of IL-4–responsive NK T cells mediating oxazolone-induced colitis. CD49b (detected with PE-conjugated DX5 antibodies) is a prominent NK T cell surface marker; therefore, we repeated adoptive transfer using lymph node CD4⁺/DX5⁻ T cells, which excludes NK T cells. Lck\textsuperscript{+/−}IL-4Rα\textsuperscript{−/−} mice, receiving wild-type CD4⁺ T cells depleted of DX5⁺ T cells, did develop oxazolone-induced colitis. This was shown by significant weight loss and distress comparable to that of IL-4Rα\textsuperscript{−/−} oxazolone-treated control mice (Figure 2A), colon shortening and pathology (Figure 2B), and severe colitis (Figure 2C). Although the systemic levels of IL-4 production remained low, IL-13 was increased and associated with oxazolone-induced pathology (Figure 2D). To exclude the possibility that a small number of NK T cells undetected by DX5 remained, we depleted NK T cells using an α-galactosylceramide-loaded CD1d tetramer. Lck\textsuperscript{+/−}IL-4Rα\textsuperscript{−/−} mice receiving CD4⁺/DX5⁻/CD1d-tetramer⁻ T cells also developed colitis (Supplementary Figure 1). Together, these data strongly suggest that IL-4–promoted CD4⁺/DX5⁻/CD1d-tetramer⁻ T cells are involved in mediating oxazolone-induced colitis.

To investigate if these CD4⁺DX5⁻ T cells produced disease-causing IL-13, CD4⁺ DX5⁻ T cells from IL-13⁻/⁻ or wild-type BALB/c mice were transferred into Lck\textsuperscript{+/−}IL-4Rα\textsuperscript{−/−} mice. Recipient mice of IL-13⁻/⁻ cells remained significantly protected from oxazolone-induced colitis, shown by reduced weight loss and animal distress (Figure 3A), colon shortening and pathology (Figure 3B), and inflammation (Figure 3C). Furthermore, tissue IL-13 production was reduced in mice receiving IL-13⁻/⁻ but not wild-type CD4⁺ cells. Protection was specific to IL-13⁻/⁻ CD4⁺ cell transfer because mice receiving wild-type or IL-4⁻/⁻ cells showed similar disease symptoms (data not shown). Together these data suggest that CD4⁺ non-NK T cells mediate oxazolone-induced colitis by IL-13 production.

**B Cell–Specific Deletion of IL-4Rα in mb1\textsuperscript{cre}IL-4Rα\textsuperscript{−/−} BALB/c Mice**

Because disease protection in Lck\textsuperscript{+/−}IL-4Rα\textsuperscript{−/−} mice correlated with lower IgE responses, we investigated the role of IL-4Rα–responsive B lymphocytes in oxazolone-induced colitis. B cell–specific IL-4Rα–deficient BALB/c mice were established by intercrossing floxed IL-4Rα mice with mb1\textsuperscript{cre} mice. Recipient mice of IL-13⁺/+(CD4⁺/CD8⁺) or CD4⁻/CD8⁻ B cells showed similar disease symptoms (data not shown). Together these data suggest that B cell–mediated oxazolone-induced colitis by IL-13 production.

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Figure 4. Generation and characterization of mb1\textsuperscript{cre}IL-4Rα\textsuperscript{−/−} mice. (A) IL-4Rα⁺ BALB/c mice intercrossed with mb1\textsuperscript{cre} expressing and IL-4Rα\textsuperscript{−/−} mice to generate mb1\textsuperscript{cre}IL-4Rα\textsuperscript{−/−} mice. “Floxed” IL-4Rα allele is indicated by gray arrowheads, and deleted allele is indicated by black arrowheads. (B) The deleted IL-4Rα PCR is 471 base pairs, loxP is 188 base pairs (flanked) or 94 base pairs (wild-type), and hCre-specific is 500 base pairs. (C) IL-4Rα surface expression was analyzed by FACS on spleen, bone marrow (BM), or peritoneal cells from naïve IL-4Rα\textsuperscript{−/−} (dotted line), IL-4Rα⁺ (gray scale), and mb1\textsuperscript{cre}IL-4Rα\textsuperscript{−/−} (solid line) mice. B cells were CD19⁺/CD3⁻ or B220⁺/CD3⁻. T cells were CD4⁺/CD8⁻ or CD8⁺/CD4⁻. NK T cells were DX5⁺/CD3⁻, NK cells were DX5⁻/CD3⁻, and macrophages were F4/80⁺/CD11b⁺. Data are representative of 3 to 5 independent experiments. (D) The ratio of IL-4Rα exon 8 and exon 5 alleles was determined by real-time PCR from genomic DNA purified from CD19⁺ or CD3⁺ cells. ND, not detected. (E) IL-4Rα\textsuperscript{−/−}; 2, mb1\textsuperscript{cre}IL-4Rα\textsuperscript{−/−}; 3, IL-4Rα\textsuperscript{−/−}. (E) Mice were sensitized with ovalbumin, and total IgE or antigen-specific IgG1, IgG2a, and IgG2b were measured in the serum of individual mice by ELISA. Data represent 2 independent experiments with triplicate values ± SEM. *\( P < .05 \), **\( P < .01 \), ***\( P < .001 \) vs IL-4Rα\textsuperscript{−/−} ovalbumin controls.
(exon 6 to 8 flanked byloxP; Figure 4A) with mice expressing Cre-recombinase (Cre) under control of the mb1 promoter (mb1cre mice).25,27 Transgene-bearing hemizygous mice (mb1creIL-4Ra−/lox) were identified by PCR genotyping (Figure 4B).

IL-4Ra cell surface expression was analyzed in B cells and non-B cells from various organs (Figure 4C). In the mb1creIL-4Ra−/lox mouse strain, efficient abrogation of IL-4Ra surface expression was observed in B cells only. As shown in Figure 4C, splenic T cells (CD3+/CD19−, CD4+/CD8−, CD8−/CD4+, or DX5+NK T cells), NK cells (CD3−DX5+), and peritoneal macrophages (F4/80+/CD11b+) maintained IL-4Ra expression. Cre-mediated IL-4Ra deletion in B cells was determined at the genomic level by quantifying exon 8 (absent in Cre+ cells) relative to exon 5 (present in all cells) by quantitative real-time PCR (Figure 4D). Confirming IL-4Ra surface expression analysis, efficient deletion ofloxP-flanked IL-4Ra exons was observed in CD19+B cells frommb1creIL-4Ra−/lox mice. In contrast, CD3+ cells showed a similar ratio as cells from control IL-4Ra−/lox mice (Figure 4D), demonstrating the presence of exon 8. As a consequence of impaired IL-4Ra responsiveness, ovalbumin immunization resulted in abrogated B-cell type 2 antibody responses (IgG1 and IgE) in mb1creIL-4Ra−/lox mice compared with control IL-4Ra−/lox mice, with similar low levels as found in global IL-4Ra−/− mice (Figure 4E). In contrast, type 1 antibody responses were up-regulated, as shown by elevated ovalbumin-specific IgG2a and IgG2b. B cell–specific IL-4Ra disruption had no effect on T-helper function, shown by normal T-helper differentiation and proliferation of CD4+ T cells frommb1creIL-4Ra−/lox mice in response to IL-4 (Supplementary Figure 2). Together, these data show efficient B cell–specific IL-4Ra deletion and unresponsiveness in mb1creIL-4Ra−/lox mice.

**B Cell–Specific IL-4Ra−Deficient Mice Are Protected From Oxazolone-Induced Colitis**

mb1creIL-4Ra−/lox mice were protected from oxazolone-induced pathology, avoiding rapid wasting disease and death (Figure 5A) with normal colon, histopathology, and reduced inflammation (Figure 5B and C). Serum concentrations of IgG1 and IgE were reduced in mb1creIL-4Ra−/lox mice compared with oxazolone- or ethanol-treated littermate controls, with tissue concentrations of IL-4 and IL-13 similar to those seen in susceptible control IL-4Ra−/lox mice (Figure 5D). This suggests that Th2 cytokine responses are not sufficient for oxazolone-induced colitis but that IL-4Ra–dependent B-cell responses are needed. This was confirmed by the induction of colitis in mb1creIL-4Ra−/lox mice receiving adoptive transfer of wild-type B cells but not IL-4Ra–deficient B cells corresponding with detectable levels of IgE (Supplementary Figure 3). Together, these data show that IL-4Ra–signaling B cells are involved in oxazolone-induced colitis.

**Anti-IgE Treatment Protects BALB/c Mice From Oxazolone-Induced Colitis**

To directly investigate the role of IgE as an effector molecule in colitis, IgE function was neutralized during oxazolone treatment of susceptible BALB/c mice using anti-IgE antibodies. Efficient IgE blocking was shown in immunohistochemistry of the colon with abrogated IgE+ cells in anti-IgE-treated mice compared with oxazolone-treated BALB/c mice (Figure 6A). Furthermore, antibody treatment prevented IgE from binding basophils (CD49d+, IgE+) in the blood without depleting them (CD49d+, FceRα+1+) (Figure 6B). To determine successful blocking of IgE binding, an independent anti-IgE antibody (23G3) was used for ELISA (Figure 6C), immunohistochemistry, and FACS. In vivo neutralization of IgE but not IgG function (Supplementary Figure 4) protected mice from oxazolone-induced colitis, shown by prevention of wasting disease, mortality (Figure 7A), and reduced colon shortening (Figure 7B). This was accompanied by reduced pathology and distress (Supplementary Figure 5) compared with isotype control mice. Cross-linking of IgE bound to FceRα activates mast cells and may contribute to the development of UC. After blocking IgE, reduced mast cell numbers were found in oxazolone-treated BALB/c mice compared with control mice (Figure 7C), which correlated with the reduced mast cell degranulation product MMC-P1 in serum (Figure 7D). Together these data show that IgE is a pathogenic factor during oxazolone-induced colitis, leading to the recruitment and activation of mast cells into the colon correlating with increased disease pathology.

**Discussion**

The role of IL-4/IL-4Ra signaling on T and B lymphocytes in oxazolone-induced colitis was investigated and their role in disease identified and dissected. We showed that both IL-4–promoted Th2 cells and B cells are important for disease induction in concert and critical to mediating the disease phenotype using CD4+ T cell– or B cell–specific IL-4Ra–deficient mice. A combination of adoptive transfer and in vivo neutralization studies showed that their effector molecules, IL-13 produced by Th2 cells and IgE produced by B cells, were the factors causing disease pathology.

CD4+ T cell–specific IL-4Ra–deficient mice were protected from colitis, and transfer of naive wild-type CD4+ T cells was sufficient to reverse the disease phenotype. Although IL-4Ra is only partially deleted from CD8+ T cells of LckcreIL-4Ra−/lox mice, it is completely deleted from CD4+ T cells.24 Considering the role of transferred wild-type CD4+ T cells and the fact that CD4+ T cells are known to play a role in oxazolone-induced colitis, we conclude that IL-4Ra responsiveness by CD4+ T cells is responsible for the disease onset. Because C57BL/6 mice depleted of NK1.1+ cells or mice deficient in CD1 were protected from experimental colitis, we speculated that transferred IL-4Ra+ NK T cells were responsible for dis-
However, removing DX5+CD1d-tetramer+ NK T cells from the transferred CD4+ T cells still restored the disease phenotype in CD4+ T cell–specific IL-4Rα–deficient mice, strongly suggesting that IL-4Rα-responsive Th2 cells caused the disease. We show that IL-13 is the important pathologic factor produced by transferred cells, because IL-13–deficient CD4+ T cells were not able to reverse protection in CD4+ T cell–specific IL-4Rα–deficient mice. It has been shown that NK T cells and IL-13 are required for disease induction in oxazolone-induced colitis. Mb1 creIL-4Rαfox/olox mice are protected from oxazolone-induced colitis, shown by (A) significantly reduced weight loss, prolonged survival, and reduced animal distress compared with IL-4Rαfox/olox oxazolone control mice. (B) Normal colon length (cm) and (C) reduced or no colitis. (D) Protection corresponded with the absence of total IgE production. Data represent 2 individual experiments with mean values ± SD or ± SEM. *P < .05, **P < .01, and ***P < .001 vs ethanol-treated IL-4Rαfox/olox mice. IL-4Rαfox vs mb1creIL-4Rαfoxoxa was ##P < .01. ND, not detected.
treated C57BL/6 mice, with NK T cells producing large amounts of IL-13 after in vitro restimulation with αGalCer (see model; Supplementary Figure 6). Furthermore, IL-13 has been suggested to directly harm the epithelial cell layer in the colon, causing apoptosis and increased expression of claudin-2 resulting in increased epithelial barrier permeability. The precise role of CD4+ Th2 cells in oxazolone-induced colitis remains elusive. Our data add to the proposed mechanism by showing that in BALB/c mice, IL-13 derived from CD4+ Th2 cells in oxazolone-induced colitis is required for disease pathology.

IL-4 promotes Th2 differentiation and is a crucial switch factor for IgE isotype antibody production by B cells. As a consequence of reduced IL-4 in protected LckcreIL-4R−/lox mice, IgE sera levels were also strongly reduced. Therefore, we investigated the possibility that IgE may play a role in oxazolone-induced colitis by establishing B cell–specific IL-4Rα-deficient BALB/c mice (mb1creIL-4R−/lox), allowing normal Th2 responses but impaired type 2 effector B-cell function, including impaired IgE responses. The protection of mb1creIL-4R−/lox mice from colitis indicated an important role for IgE in disease onset, and blocking IgE was sufficient to protect BALB/c mice from disease. A critical role for IgE has also been uncovered in a dextran sulfate sodium–induced mouse model of colitis, where blocking IgE reduced most of the clinical signs of disease. Mechanistically, IgE binds to the FcεRI on the surface of mast cells, and second exposure to antigen results in...
cross-linking of bound IgE and mast cell degranulation with release of potent inflammatory mediators.44,45 Previous studies have shown that the anti-IgE monoclonal antibody, which binds IgE at the FcεRI binding site, prevents IgE from binding to the receptor on mast cells.46,47 Furthermore, our data show that although basophils are still present they are not decorated with IgE, which confirms efficient blocking of IgE binding. Because neutralizing IgE function in oxazolone-treated BALB/c mice reduced mast cell numbers in the colon as well as mast cell protease levels in the serum, it is likely that mast cell activation is involved in oxazolone-induced colitis (see Supplementary Figure 6) and may have caused intestinal anaphylaxis, similar to that shown in IBD.48–50

Current treatment strategies for UC are based on broad anti-inflammatory drugs like aminosalicylates, which are bowel-specific anti-inflammatory drugs. This is considered a first-line therapy, with other treatments including corticosteroids, azathioprine, cyclosporine, and colectomy.1,51,52 By dissecting the role of disease-causing cells and factors, this study may provide more specific new potential therapeutic drug targets for UC. In summary, T cell– and B cell–specific IL-4Rα–defi-

Figure 7. Blocking IgE protects BALB/c mice from oxazolone-induced colitis. BALB/c mice were treated with anti-IgE or control antibody. (A) Reduced weight loss and mortality are shown with (B) reduced colon shortening (cm). (C) Mast cells (arrowheads) were stained with toluidine blue and quantified per section, and goblet cells (Go) stained lightly blue are also indicated. (D) Serum MMCP-1 level was measured in individual mice. Data shown represent 3 independent experiments (survival from single experiment) with mean values ± SEM. *P < .05 and **P < .01 vs EtOH control or #P < .05 BALB/c oxazolone or anti-rat IgG control vs BALB/c anti-IgE.
cient mice were used to investigate the function of IL-4 and IL-13 on individual cells in oxazolone-induced colitis. Our results show an important role for IL-4Rα signaling on both T and B cells in driving the pathology associated with oxazolone-induced colitis. We suggest that the immune mechanism that mediates oxazolone-induced colitis is not limited to IL-13 production by NK T cells, but rather a combination of IL-4–responsive CD4+ Th2 cells and NK T cells in combination with IgE-producing B cells as important effector cells in oxazolone-induced colitis.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of Gastroenterology at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2011.09.044.

References


