

## 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)-4R $\alpha$  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<sup>+</sup> T cells that did not express IL-4R $\alpha$  (*Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>*). We also generated mice with B cells that did not express IL-4R $\alpha$  (*mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>*) and studied induction of colitis. **RESULTS:** *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* 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 naïve, wild-type CD4<sup>+</sup> Th cells depleted of natural killer T cells to *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice restored their susceptibility to colitis. In contrast, *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice maintained their protection against colitis when IL-13–deficient CD4<sup>+</sup> 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<sup>+</sup> T helper cells. *Mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* 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<sup>+</sup> 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.<sup>1</sup> 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.<sup>2,3</sup> 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.<sup>4–6</sup>

Previous studies in T cell–, cytokine–, or cytokine receptor–deficient mouse models showed evidence for a T cell–dependent regulatory system in IBD.<sup>7–12</sup> Furthermore, lamina propria CD4<sup>+</sup> T cells reacting to enteric flora are important effector cells causing mucosal inflammation in IBD, and in vivo depletion of these cells ameliorates inflammation.<sup>13–15</sup> Together these models show that subpopulations of CD4<sup>+</sup> 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-4R $\alpha$  subunit.<sup>16</sup> Initial studies using a short-lived model of oxazolone-induced colitis showed that CD4<sup>+</sup> T cells produce large amounts of IL-4 and IL-5 and that blocking IL-4 prevented colitis.<sup>4</sup> 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.<sup>1,17,18</sup> IL-13 affecting epithelial tight junctions, apoptosis, and cell restitution is suggested to cause disease.<sup>19</sup> Because early IL-4 production by CD4<sup>+</sup> cells can initiate IL-13 production by NK T cells, it is possible that IL-4 drives the initial inflammatory response.<sup>20</sup>

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<sup>+</sup> B lymphocytes in UC, with patients showing prevalent allergic symptoms and high IgE levels.<sup>3,21,22</sup> Although IgE is known to play an important role in inflammatory reactions, little evidence is available about the role of IgE in UC.<sup>23</sup>

In this study, we investigated the role of IL-4R $\alpha$  responsiveness on T and B cells in a mouse model of UC. CD4<sup>+</sup> T cell–specific IL-4R $\alpha$ –deficient mice (*Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>*) and newly generated B cell–specific IL-4R $\alpha$ –deficient mice (*mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>*) 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<sup>+</sup> T cell- and B cell-specific IL-4R $\alpha$ -deficient mice were protected from disease. Adoptive transfer of naïve wild-type but not IL-13-deficient CD4<sup>+</sup>DX5<sup>-</sup> Th cells into *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice reverted protection back to a susceptible phenotype. Furthermore, protection correlated with impaired IgE antibody responses in *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* and *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice, and blocking IgE binding significantly protected BALB/c mice from the onset of colitis. Together, these data suggest that IL-4-promoted CD4<sup>+</sup> 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-4R $\alpha$ -Deficient Mice

CD4<sup>+</sup> T cell-specific IL-4R $\alpha$ -deficient mice (*Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>*) were previously described.<sup>24</sup> We generated novel B cell-specific IL-4R $\alpha$ -deficient mice (*mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>*) by intercrossing transgenic *mb1<sup>cre</sup>* mice with *IL-4R $\alpha$ <sup>-/-</sup>* and *IL-4R $\alpha$ <sup>lox/lox</sup>* mice.<sup>25–28</sup> Cre transgene negative littermates (*IL-4R $\alpha$ <sup>-/-lox</sup>*) expressing functional IL-4R $\alpha$  were used as controls in all experiments. *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice are described as *C.Cg-Il4ra<sup>tm1Fbb</sup>/Il4ra<sup>tm2Fbb</sup>Tg(Lck-cre)*, *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* are *Il4ra<sup>tm1Fbb</sup>/Il4ra<sup>tm2Fbb</sup>Tg(Cd79a<sup>tm1</sup>(cre)<sup>Reth</sup>)*, and *IL-4R $\alpha$ <sup>-/-</sup>* are *Il4ra<sup>tm1Fbb</sup>/Il4ra<sup>tm1Fbb</sup>*. Previously described IL-4<sup>-/-</sup> and IL-13<sup>-/-</sup> BALB/c mice were used.<sup>29,30</sup> 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 *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* and *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* 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.<sup>31</sup> Anesthetized mice were sensitized on the shaved abdomen with 3% oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one; Sigma-Aldrich, Saint Louis, MO) in 100% ethanol (150  $\mu$ L), followed 7 days later by intrarectal administration of 1% oxazolone in 50% ethanol (150  $\mu$ L). Control mice were treated with ethanol only. BALB/c mice develop rapid-onset colitis with weight loss peaking by day 3, leading to mortality or recovery within the first week.<sup>4,5</sup> Therefore, animal distress was determined 2 days postchallenge, weight loss shown for 3 days (7 days for Figure 1), and mice killed on day 3 postchallenge to measure disease parameters. Independent experiments were used to show mortality for 7 days postchallenge. IgE induction in *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice was measured using a previously described ovalbumin model.<sup>32</sup>

### Adoptive Transfer of Lymphocytes and Anti-IgE Antibody Treatment

CD4<sup>+</sup> T cells from mesenteric lymph nodes of naïve *IL-4R $\alpha$ <sup>-/-lox</sup>* littermate control (*IL-4R $\alpha$ <sup>-/-lox</sup>*), BALB/c (wild-type),

or IL-13<sup>-/-</sup> mice were enriched to >90% using negative selection with BioMag beads (Qiagen, Hilden, Germany), as previously described.<sup>33</sup> These cells were fluorescence-activated cell sorted (FACS) for CD4<sup>+</sup> (GK1.5) cells at a purity of >98%, and NK T cells were excluded using CD49b/DX5-PE (DX5) or a PBS57-loaded CD1d tetramer (provided by the National Institutes of Health Tetramer Facility, Emory University). *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* and *IL-4R $\alpha$ <sup>-/-lox</sup>* littermate control mice received  $1 \times 10^6$  sorted cells 24 hours before sensitization with oxazolone. For adoptive transfer of B cells, *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* or *IL-4R $\alpha$ <sup>-/-lox</sup>* mice received CD19<sup>+</sup> cells from wild-type or *IL-4R $\alpha$ <sup>-/-</sup>* (knockout) mice. In vivo neutralization of IgE function was performed with 2 doses of rat anti-mouse IgE antibody (84.1C), rat anti-mouse Fc $\gamma$ RII/III (2.4G2), or rat IgG isotype control (afrc-mac51) (200  $\mu$ g/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).<sup>34</sup> Mice scored on a scale of 0 to 16 were killed if the most severe score (16) was reached or  $\geq 20\%$  body weight was lost. Mortality studies (10 mice/group) followed the same 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.<sup>35</sup> 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  $1 \times 10^6$  cells/mL with or without plate-bound anti-CD3 antibody (clone 145-2C11, 20  $\mu$ g/mL). Supernatants were collected and stored at  $-80^\circ\text{C}$ , 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 described<sup>27,32</sup> with 2 anti-IgE coating antibodies (clone 84.1C and 23G3). Mouse mast cell protease (MMCP)-1 levels were measured in sera by ELISA, according to the manufacturer's protocol (Moredun Scientific, Penicuik, Midlothian, United Kingdom).

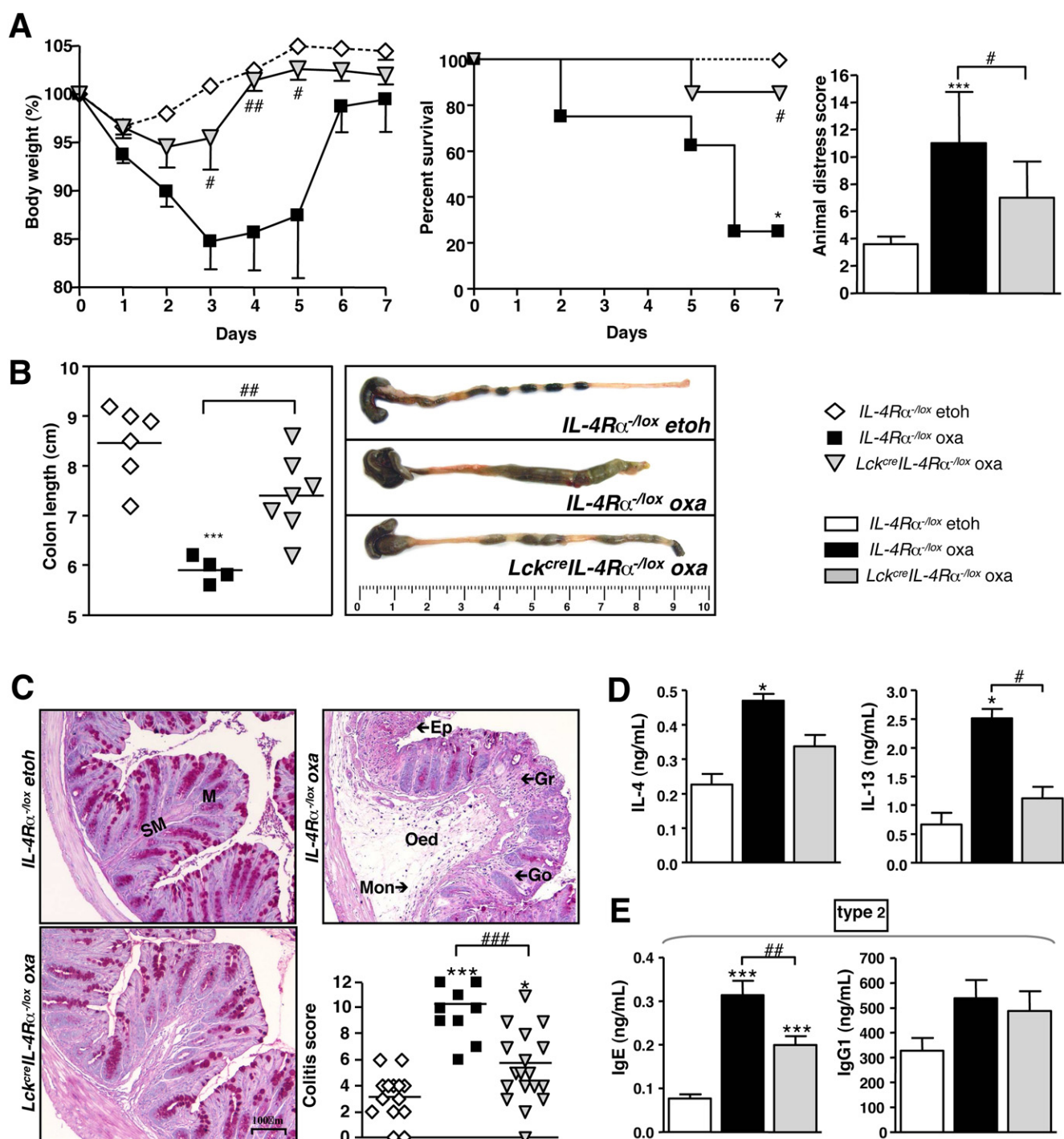
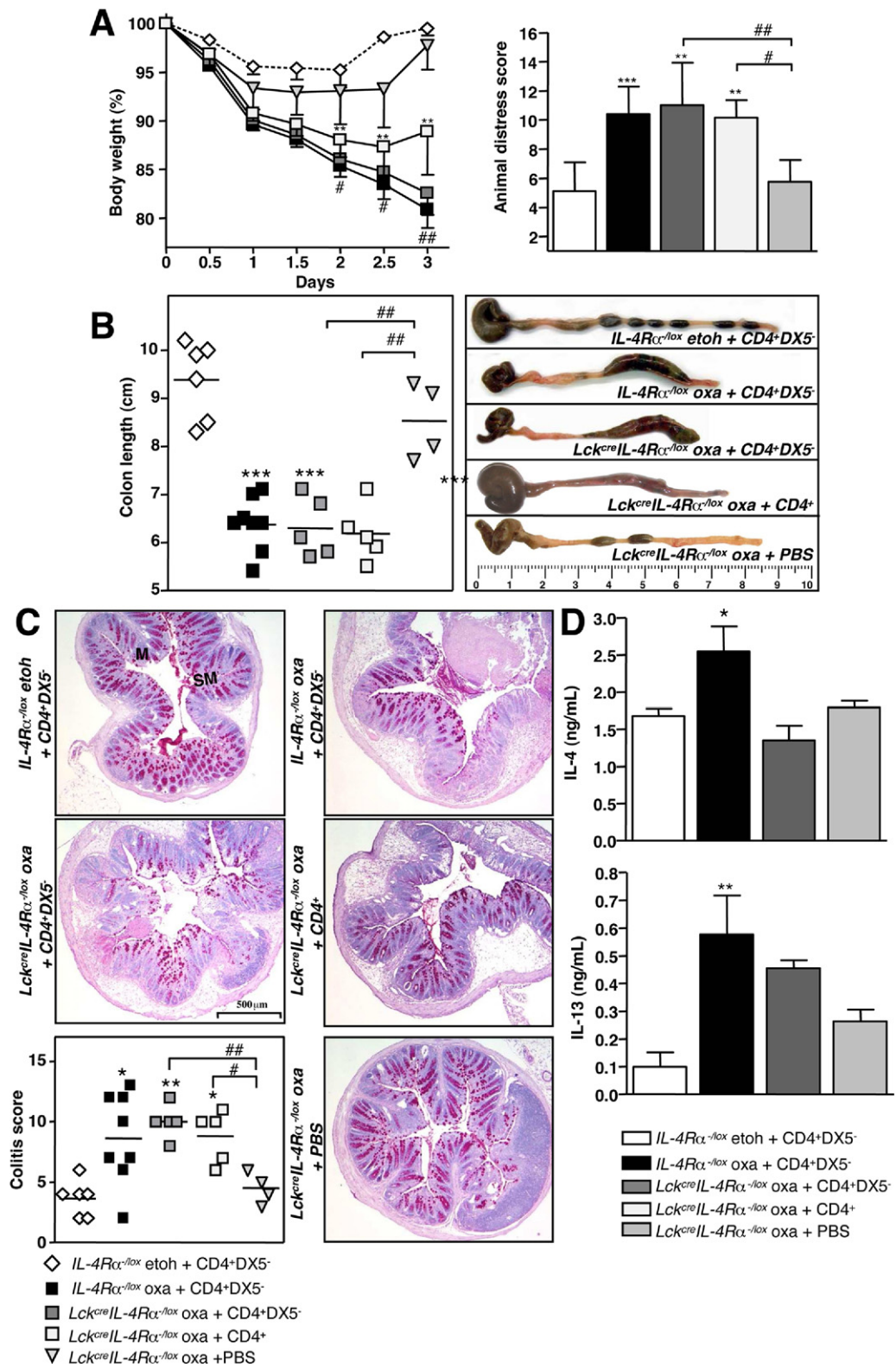


**Figure 1.** *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-</sup>* mice are protected from oxazolone-induced colitis. *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-</sup>* mice were protected from oxazolone-induced colitis compared with littermate control mice, shown by (A) significantly reduced weight loss as 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-4R $\alpha$ <sup>-/-</sup>* 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  $\pm$  SEM), mean values are  $\pm$  SD. \* $P < .05$  and \*\*\* $P < .001$  vs *IL-4R $\alpha$ <sup>-/-</sup>* EtOH controls; # $P < .05$ , ## $P < .01$ , and ### $P < .001$  *IL-4R $\alpha$ <sup>-/-</sup>* vs *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-</sup>* oxazolone.



### Efficiency of IL-4R $\alpha$ Deletion by Real-Time PCR

Genomic DNA was prepared from CD3<sup>+</sup>CD19<sup>-</sup> and CD3<sup>-</sup>CD19<sup>+</sup> sorted lymph node cells (>99% purity) from *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>*, *IL-4R $\alpha$ <sup>-/-lox</sup>*, and *IL-4R $\alpha$ <sup>-/-</sup>* mice using a FACS Vantage flow cytometer (BD Biosciences, Erembodegem, Belgium). A standard curve was prepared of serial 10-fold dilu-

tions from DNA product using primers specific for IL-4R $\alpha$  exon 5 (control) and exon 8 (deleted by hCre expression; see Supplementary Table 1).

### Flow Cytometry

IL-4R $\alpha$  surface expression was detected on spleen, lymph node, or bone marrow cells by phycoerythrin (PE) or biotinyl-

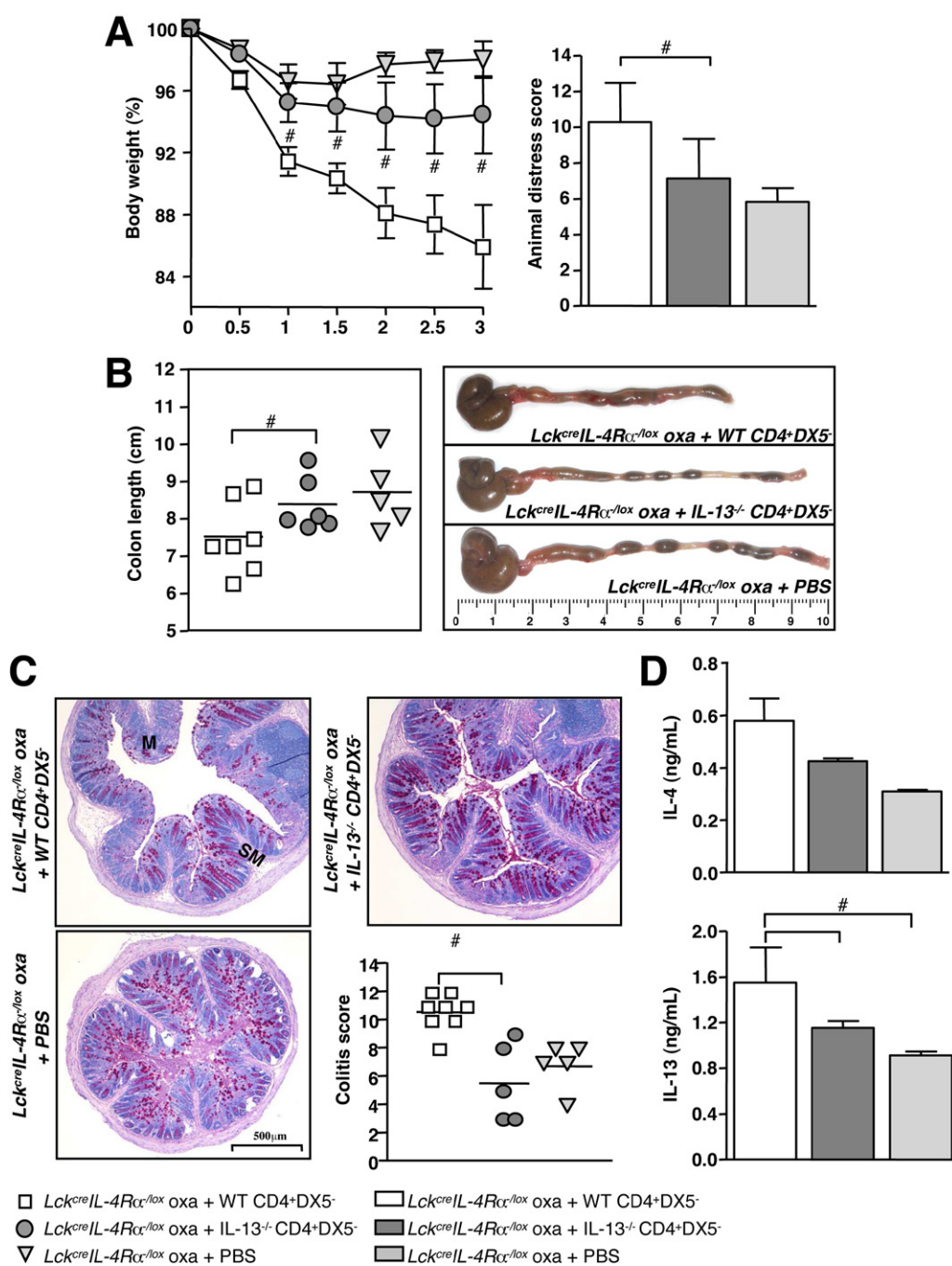


**Figure 3.** IL-13 production by CD4<sup>+</sup>/DX5<sup>-</sup> T cells causes oxazolone colitis. *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-lox</sup>* mice receiving CD4<sup>+</sup>/DX5<sup>-</sup> T cells from IL-13<sup>-/-</sup> BALB/c mice were protected from colitis, indicated by (A) significantly reduced weight loss (mean  $\pm$  SEM) and distress, (B) reduced colon shortening (cm), and (C) colitis score. (D) IL-4 and IL-13 levels were detected in triplicate from the distal colon tissue of pooled mice. Data represent 1 to 2 individual experiments (n = 5–10 mice) with mean values  $\pm$  SD (unless stated). #P < .05 for *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-lox</sup>* oxazolone + CD4<sup>+</sup>/DX5<sup>-</sup> vs *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-lox</sup>* oxazolone + IL-13<sup>-/-</sup> CD4<sup>+</sup>/DX5<sup>-</sup>.

ated anti-CD124 (IL-4R $\alpha$ , M-1) as previously described.<sup>24</sup> 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, Serotec), Fc  $\epsilon$  receptor I (Fc $\epsilon$ R $\alpha$ 1-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-

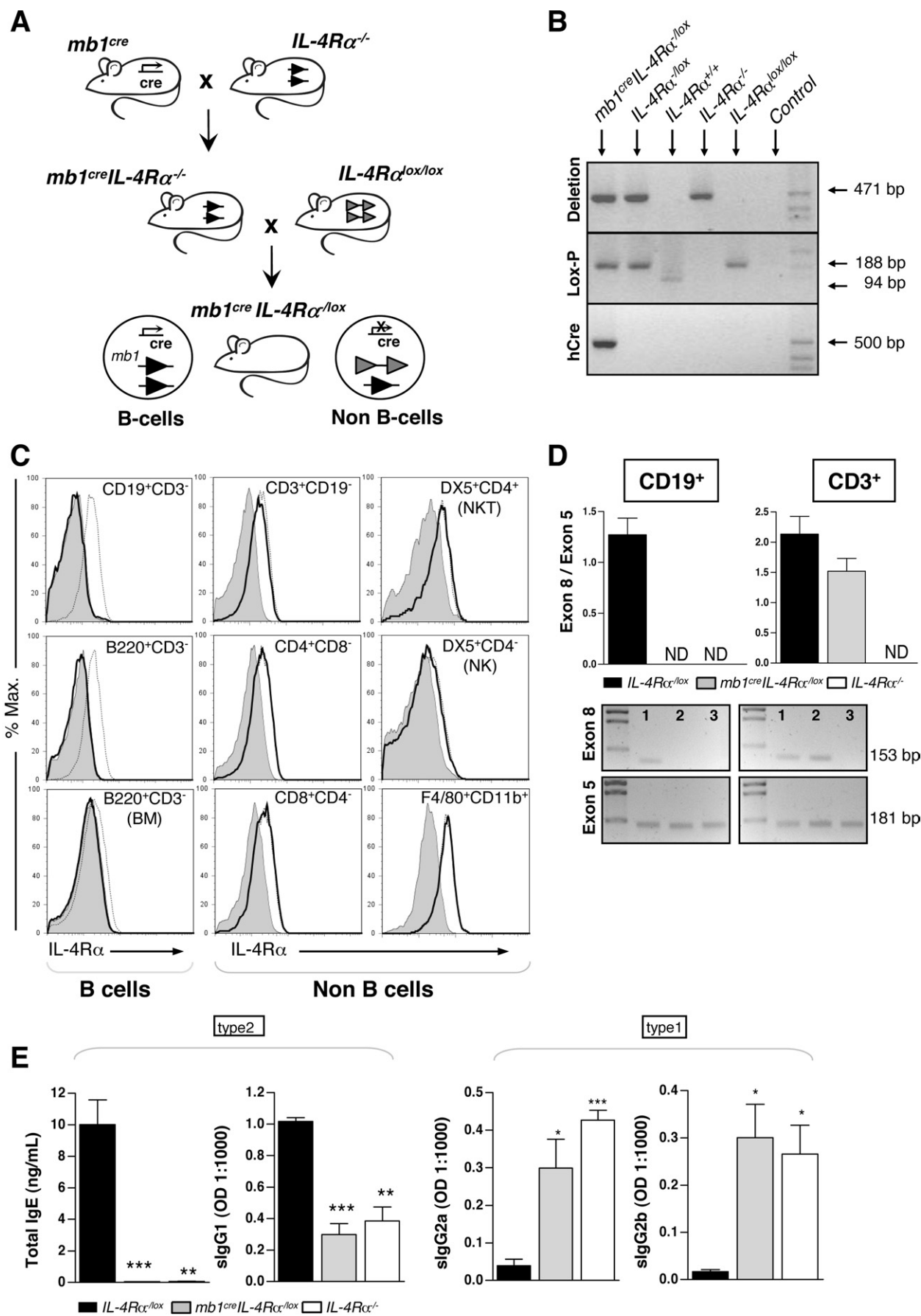
cold acetone and stained with IgE-PE (23G3) and Hoechst 33342 (Invitrogen, Carlsbad, CA). Images were captured on a Zeiss LSM 510 Meta confocal microscope (Jena, Germany).

### T-Cell Proliferation

CD4<sup>+</sup> 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. [<sup>3</sup>H] incorporation was measured as previously described.<sup>24</sup>

### In Vitro Th2 Differentiation

CD4<sup>+</sup>T cells were used for in vitro Th1/Th2 differentiation as previously described.<sup>36</sup>



### Statistical Analysis

Values are given as mean  $\pm$  SD or SEM, and significant differences were determined using Student *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<sup>+</sup> T Cell-Specific IL-4R $\alpha$ -Deficient Mice Are Protected From Oxazolone-Induced Colitis

The role of IL-4-promoted CD4<sup>+</sup> T cells was investigated in oxazolone-induced colitis using CD4<sup>+</sup> T cell-specific IL-4R $\alpha$ -deficient BALB/c mice (*Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>*). As with BALB/c (data not shown), *IL-4R $\alpha$ <sup>-/-lox</sup>* 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<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* 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<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice presented with significantly reduced type 2 cytokine and antibody responses compared with BALB/c *IL-4R $\alpha$ <sup>-/-lox</sup>* littermate control mice, as shown by reduced IL-4, IL-13, and IgE levels. Adoptive transfer of IL-4R $\alpha$ <sup>+</sup> CD4<sup>+</sup> T cells from wild-type BALB/c mice 24 hours before skin sensitization restored the disease phenotype in *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice (Figure 2A-C). Together, these results show that impairment of IL-4R $\alpha$  signaling on CD4<sup>+</sup> 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.<sup>17,37</sup> Because CD4 is expressed on a subpopulation of NK T

cells, we sorted CD4<sup>+</sup>DX5<sup>-</sup> 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<sup>+</sup>DX5<sup>-</sup> T cells, which excludes NK T cells. *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice, receiving wild-type CD4<sup>+</sup> T cells depleted of DX5<sup>+</sup> T cells, did develop oxazolone-induced colitis. This was shown by significant weight loss and distress comparable to that of *IL-4R $\alpha$ <sup>-/-lox</sup>* 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  $\alpha$ -galactosylceramide-loaded CD1d tetramer.<sup>38,39</sup> *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice receiving CD4<sup>+</sup>/DX5<sup>-</sup>/CD1d-tetramer<sup>-</sup> T cells also developed colitis (Supplementary Figure 1). Together, these data strongly suggest that IL-4-promoted CD4<sup>+</sup>/DX5<sup>-</sup>/CD1d-tetramer<sup>-</sup> T cells are involved in mediating oxazolone-induced colitis.

To investigate if these CD4<sup>+</sup>DX5<sup>-</sup> T cells produced disease-causing IL-13, CD4<sup>+</sup>DX5<sup>-</sup> T cells from IL-13<sup>-/-</sup> or wild-type BALB/c mice were transferred into *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice. Recipient mice of IL-13<sup>-/-</sup> 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<sup>-/-</sup> but not wild-type CD4<sup>+</sup> cells. Protection was specific to IL-13<sup>-/-</sup> CD4<sup>+</sup> cell transfer because mice receiving wild-type or IL-4<sup>-/-</sup> cells showed similar disease symptoms (data not shown). Together these data suggest that CD4<sup>+</sup> non-NK T cells mediate oxazolone-induced colitis by IL-13 production.

### B Cell-Specific Deletion of IL-4R $\alpha$ in *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* BALB/c Mice

Because disease protection in *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice correlated with lower IgE responses, we investigated the role of IL-4R $\alpha$ -responsive B lymphocytes in oxazolone-induced colitis. B cell-specific IL-4R $\alpha$ -deficient BALB/c mice were established by intercrossing floxed IL-4R $\alpha$  mice

**Figure 4.** Generation and characterization of *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice. (A) *IL-4R $\alpha$ <sup>-/-</sup>* BALB/c mice intercrossed with *mb1<sup>cre</sup>* expressing and *IL-4R $\alpha$ <sup>lox/lox</sup>* mice to generate *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice. "Floxed" IL-4R $\alpha$  allele is indicated by gray arrowheads, and deleted allele is indicated by black arrowheads. (B) The deleted IL-4R $\alpha$  PCR is 471 base pairs, *loxP* is 188 base pairs (floxed) or 94 base pairs (wild-type), and *hCre*-specific is 500 base pairs. (C) IL-4R $\alpha$  surface expression was analyzed by FACS on spleen, bone marrow (BM), or peritoneal cells from naive *IL-4R $\alpha$ <sup>-/-lox</sup>* (dotted line), *IL-4R $\alpha$ <sup>-/-</sup>* (gray scale), and *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* (bold line) mice. B cells were CD19<sup>+</sup>/CD3<sup>-</sup> or B220<sup>+</sup>/CD3<sup>-</sup>, T cells were CD4<sup>+</sup>/CD8<sup>-</sup> or CD8<sup>+</sup>/CD4<sup>-</sup>, NK T cells were DX5<sup>+</sup>/CD3<sup>+</sup>, NK cells were DX5<sup>+</sup>/CD3<sup>-</sup>, and macrophages were F4/80<sup>+</sup>/CD11b<sup>+</sup>. Data are representative of 3 to 5 independent experiments. (D) The ratio of IL-4R $\alpha$  exon 8 and exon 5 alleles was determined by real-time PCR from genomic DNA purified from CD19<sup>+</sup> or CD3<sup>+</sup> cells. ND, not detected. 1, *IL-4R $\alpha$ <sup>-/-lox</sup>*; 2, *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>*; 3, *IL-4R $\alpha$ <sup>-/-</sup>*. (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  $\pm$  SEM. \**P* < .05, \*\**P* < .01, \*\*\**P* < .001 vs *IL-4R $\alpha$ <sup>-/-lox</sup>* ovalbumin controls.

(exon 6 to 8 flanked by *loxP*; Figure 4A) with mice expressing Cre-recombinase (Cre) under control of the *mb1* promoter (*mb1<sup>cre</sup>* mice).<sup>25,27</sup> Transgene-bearing hemizygous mice (*mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>*) were identified by PCR genotyping (Figure 4B).

IL-4R $\alpha$  cell surface expression was analyzed in B cells and non-B cells from various organs (Figure 4C). In the *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mouse strain, efficient abrogation of IL-4R $\alpha$  surface expression was observed in B cells only. As shown in Figure 4C, splenic T cells (CD3<sup>+</sup>CD19<sup>-</sup>, CD4<sup>+</sup>CD8<sup>-</sup>, CD8<sup>+</sup>CD4<sup>+</sup>, or DX5<sup>+</sup>NK T cells), NK cells (CD3<sup>+</sup>DX5<sup>+</sup>), and peritoneal macrophages (F4/80<sup>+</sup>CD11b<sup>+</sup>) maintained IL-4R $\alpha$  expression. Cre-mediated IL-4R $\alpha$  deletion in B cells was determined at the genomic level by quantifying exon 8 (absent in Cre<sup>+</sup> cells) relative to exon 5 (present in all cells) by quantitative real-time PCR (Figure 4D). Confirming IL-4R $\alpha$  surface expression analysis, efficient deletion of *loxP*-flanked IL-4R $\alpha$  exons was observed in CD19<sup>+</sup> B cells from *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice. In contrast, CD3<sup>+</sup> cells showed a similar ratio as cells from control *IL-4R $\alpha$ <sup>-/-lox</sup>* mice (Figure 4D), demonstrating the presence of exon 8. As a consequence of impaired IL-4R $\alpha$  responsiveness, ovalbumin immunization resulted in abrogated B-cell type 2 antibody responses (IgG1 and IgE) in *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice compared with control *IL-4R $\alpha$ <sup>-/-lox</sup>* mice, with similar low levels as found in global *IL-4R $\alpha$ <sup>-/-</sup>* 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-4R $\alpha$  disruption had no effect on T-helper function, shown by normal T-helper differentiation and proliferation of CD4<sup>+</sup> T cells from *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice in response to IL-4 (Supplementary Figure 2). Together, these data show efficient B cell-specific IL-4R $\alpha$  deletion and unresponsiveness in *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice.

### ***B Cell-Specific IL-4R $\alpha$ -Deficient Mice Are Protected From Oxazolone-Induced Colitis***

*Mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* 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 *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* 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-4R $\alpha$ <sup>-/-lox</sup>* mice (Figure 5D). This suggests that Th2 cytokine responses are not sufficient for oxazolone-induced colitis but that IL-4R $\alpha$ -dependent B-cell responses are needed. This was confirmed by the induction of colitis in *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice receiving adoptive transfer of wild-type B cells but not IL-4R $\alpha$ -deficient B cells corresponding with detectable levels of IgE (Supplementary Figure 3). Together, these data show that IL-4R $\alpha$ -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<sup>+</sup> cells in anti-IgE-treated mice compared with oxazolone-treated BALB/c mice (Figure 6A). Furthermore, antibody treatment prevented IgE from binding basophils (CD49<sup>+</sup>, IgE<sup>+</sup>) in the blood without depleting them (CD49<sup>+</sup>, Fc $\epsilon$ R $\alpha$ 1<sup>+</sup>) (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 Fc $\epsilon$ R $\alpha$ I 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 MMCP-1 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-4R $\alpha$  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<sup>+</sup>T cell- or B cell-specific IL-4R $\alpha$ -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<sup>+</sup>T cell-specific IL-4R $\alpha$ -deficient mice were protected from colitis, and transfer of naïve wild-type CD4<sup>+</sup>T cells was sufficient to reverse the disease phenotype. Although IL-4R $\alpha$  is only partially deleted from CD8<sup>+</sup> T cells of *Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice, it is completely deleted from CD4<sup>+</sup> T cells.<sup>24</sup> Considering the role of transferred wild-type CD4<sup>+</sup> T cells and the fact that CD4<sup>+</sup>T cells are known to play a role in oxazolone-induced colitis, we conclude that IL-4R $\alpha$  responsiveness by CD4<sup>+</sup> T cells is responsible for the disease onset. Because C57BL/6 mice depleted of NK1.1<sup>+</sup> cells or mice deficient in CD1 were protected from experimental colitis, we speculated that transferred IL-4R $\alpha$ <sup>+</sup>NK T cells were responsible for dis-

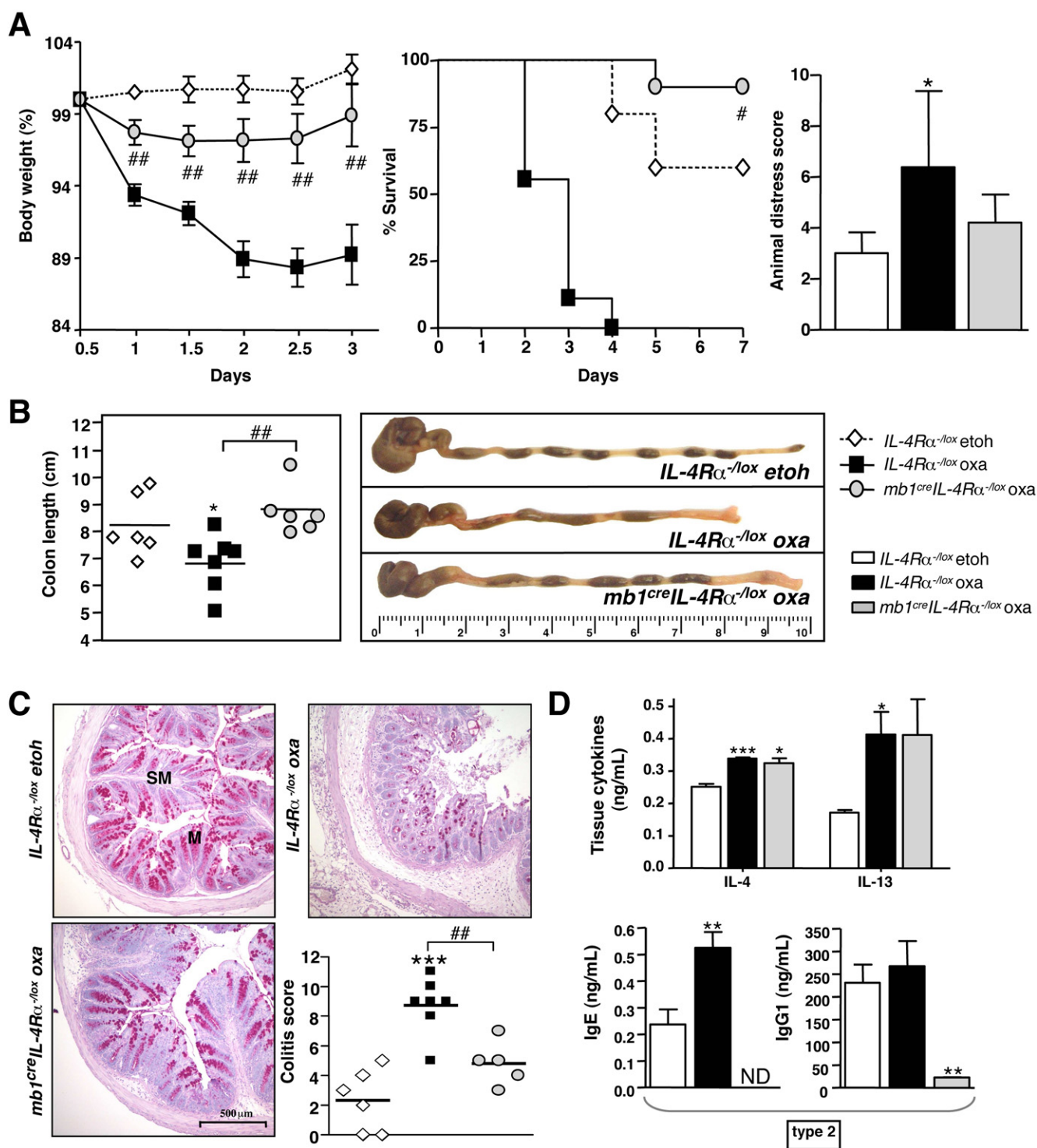


**Figure 5.** *Mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice are protected from oxazolone-induced colitis. *Mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* mice were protected from colitis, shown by (A) significantly reduced weight loss, prolonged survival, and reduced animal distress compared with *IL-4R $\alpha$ <sup>-/-lox</sup>* 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  $\pm$  SD or  $\pm$  SEM. \* $P < .05$ , \*\* $P < .01$ , and \*\*\* $P < .001$  vs ethanol-treated *IL-4R $\alpha$ <sup>-/-lox</sup>* mice. *IL-4R $\alpha$ <sup>-/-lox</sup>* vs *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* was  $##P < .01$ . ND, not detected.

ease in these mice.<sup>17</sup> However, removing DX5<sup>+</sup>CD1d-tetramer<sup>+</sup> NK T cells from the transferred CD4<sup>+</sup>T cells still restored the disease phenotype in CD4<sup>+</sup>T cell-specific *IL-4R $\alpha$* -deficient mice, strongly suggesting that *IL-4R $\alpha$* -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<sup>+</sup>T cells were not able to reverse protection in CD4<sup>+</sup>T cell-specific *IL-4R $\alpha$* -deficient mice. It has been shown that NK T cells and *IL-13* are required for disease induction in oxazolone-

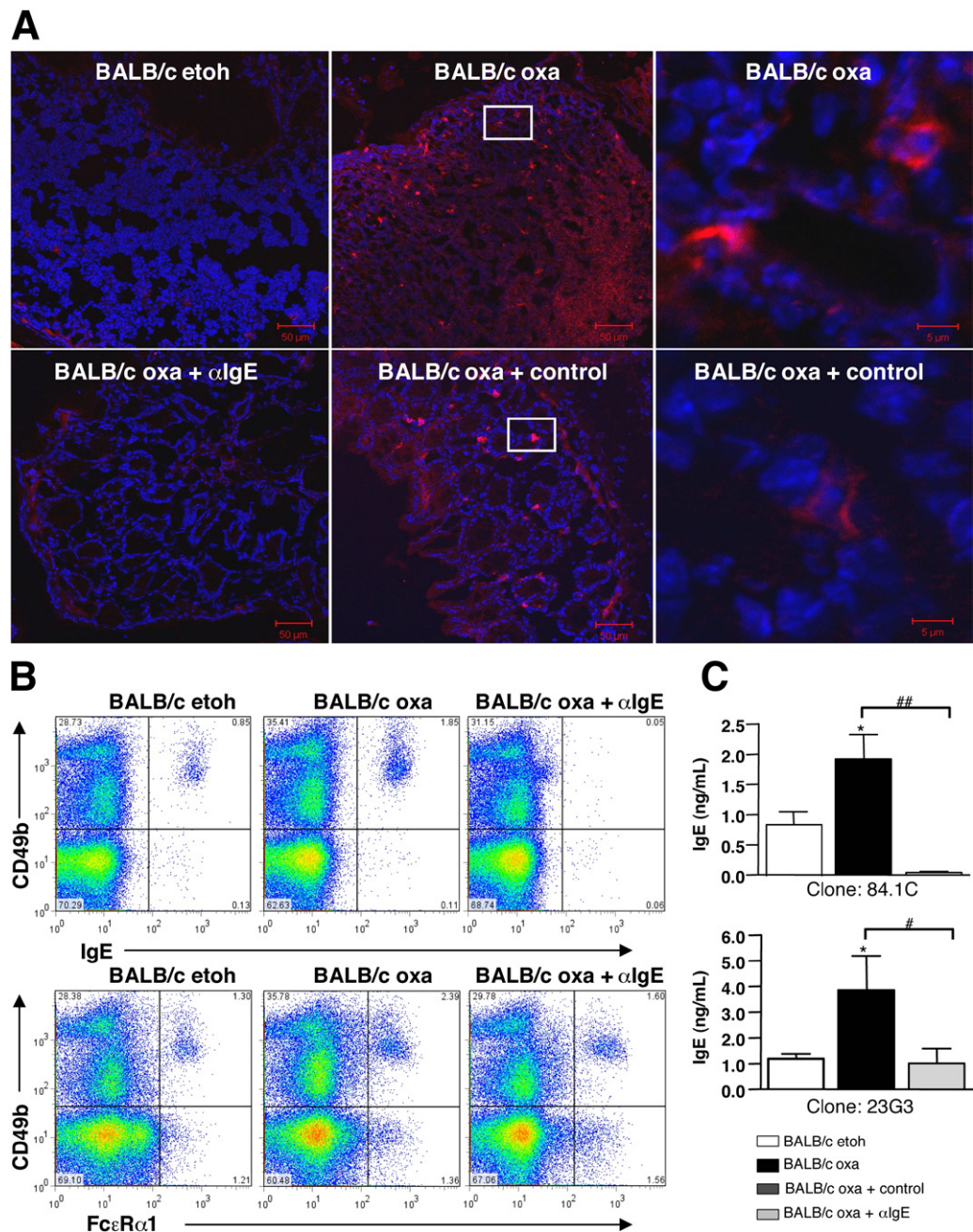


**Figure 6.** Anti-IgE antibody sufficiently blocks IgE in oxazolone-treated BALB/c mice. To determine the effectiveness of blocking IgE in BALB/c mice, PE-labeled anti-IgE antibody (23G3) was used to detect IgE-positive cells in (A) frozen distal colon sections with a confocal microscope with Hoechst (blue) for nuclei and (B) blood cells with FACS. (C) ELISA using different clones for IgE coating antibody. Data shown represent 2 independent experiments with mean values  $\pm$  SEM. \* $P < .05$  vs EtOH control or # $P < .05$  and ## $P < .01$  control vs BALB/c anti-IgE.

treated C57BL/6 mice, with NK T cells producing large amounts of IL-13 after in vitro restimulation with  $\alpha$ GalCer (see model; Supplementary Figure 6).<sup>17</sup> 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.<sup>19</sup> The precise role of CD4<sup>+</sup> 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<sup>+</sup> Th2 cells is also required for disease pathology in oxazolone-induced colitis.

IL-4 promotes Th2 differentiation and is a crucial switch factor for IgE isotype antibody production by B cells.<sup>40–42</sup> As a consequence of reduced IL-4 in protected

*Lck<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* 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-4R $\alpha$ -deficient BALB/c mice (*mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>*), allowing normal Th2 responses but impaired type 2 effector B-cell function, including impaired IgE responses. The protection of *mb1<sup>cre</sup>IL-4R $\alpha$ <sup>-/-lox</sup>* 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.<sup>43</sup> Mechanistically, IgE binds to the Fc $\epsilon$ R $\alpha$ 1 on the surface of mast cells, and second exposure to antigen results in

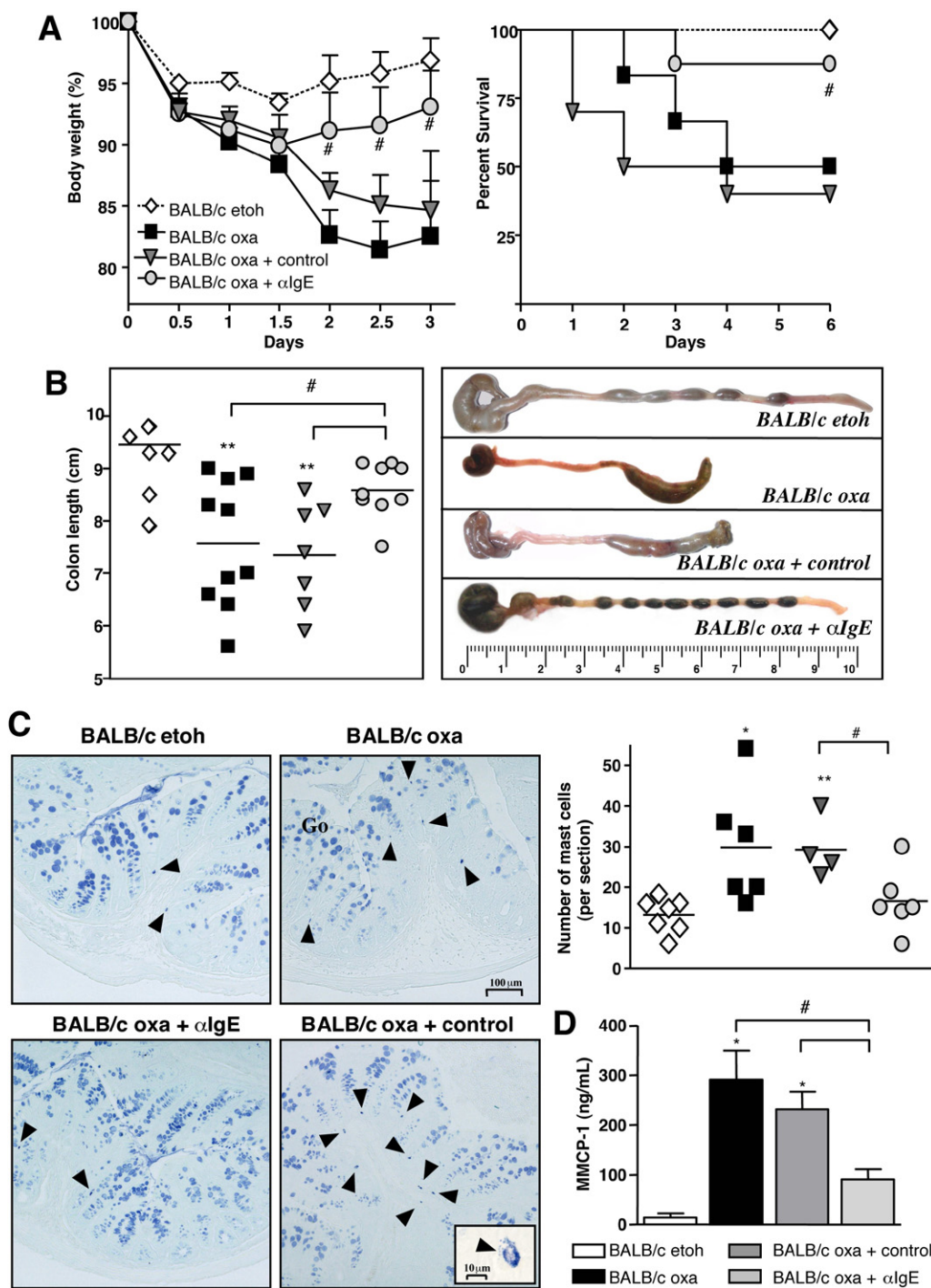


**Figure 7.** Blocking IgE protects BALB/c mice from oxazolone-induced colitis. BALB/c mice were treated with anti-IgE or control antibody. (A) Reduced weight loss and mortality are shown with (B) reduced colon shortening (cm). (C) Mast cells (arrowheads) were stained with toluidine blue and quantified 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  $\pm$  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.

cross-linking of bound IgE and mast cell degranulation with release of potent inflammatory mediators.<sup>44,45</sup> Previous studies have shown that the anti-IgE monoclonal antibody, which binds IgE at the Fc $\epsilon$ R $\alpha$ 1 binding site, prevents IgE from binding to the receptor on mast cells.<sup>46,47</sup> 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-in-

duced colitis (see Supplementary Figure 6) and may have caused intestinal anaphylaxis, similar to that shown in IBD.<sup>48–50</sup>

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.<sup>1,51,52</sup> 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-4R $\alpha$ -defi-

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-4R $\alpha$  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<sup>+</sup> 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](http://www.gastrojournal.org), and at doi: 10.1053/j.gastro.2011.09.044.

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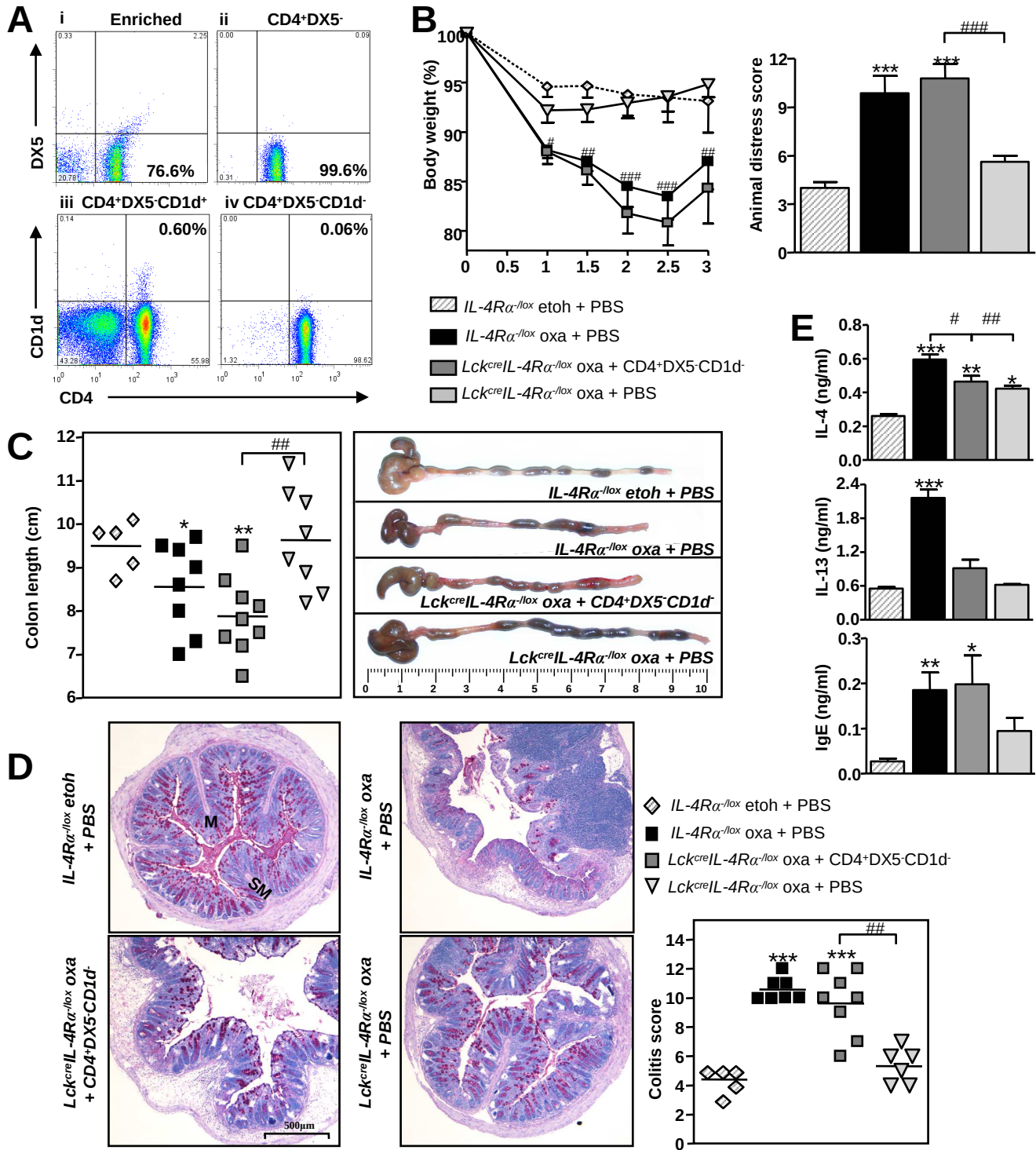
#### Conflicts of interest

The authors disclose no conflicts.

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Supplementary Figure S1

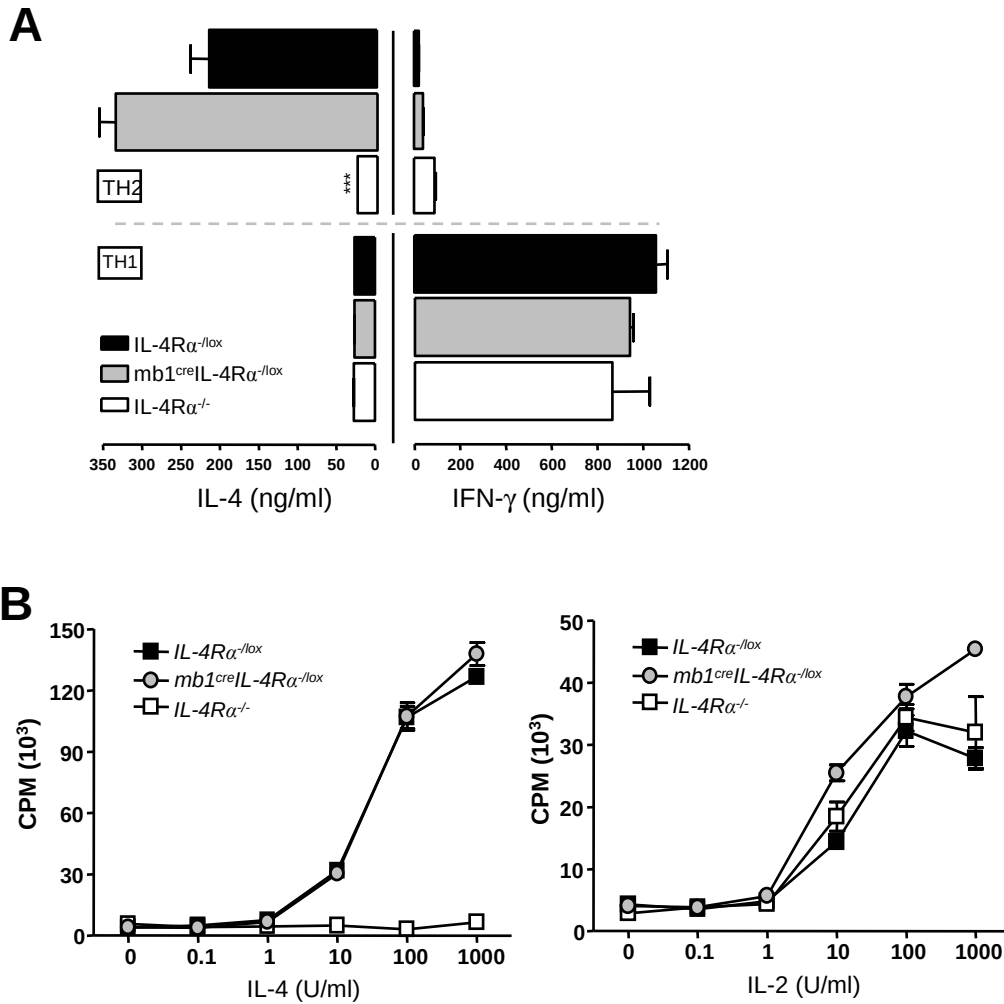


Supplementary Figure S1

### IL-4 responsive CD4<sup>+</sup>/DX5<sup>-</sup>/CD1d-tetramer<sup>-</sup> T cells induce oxazolone colitis.

DX5 is challenged as a suitable marker to identify all NK T cells. Therefore we depleted CD1d-dependent NK T cells. CD4<sup>+</sup>/DX5<sup>-</sup>/CD1d-tetramer<sup>-</sup> T cells were sorted (>98% purity) to exclude NK T cells and adoptively transferred into *Lck<sup>cre</sup>IL-4Rα<sup>-/-</sup>* mice with control mice receiving PBS only (A). Lymph node CD4<sup>+</sup> cells were enriched (i), sorted for CD4<sup>+</sup>/DX5<sup>-</sup> (ii) and CD4<sup>+</sup>/DX5<sup>-</sup>/CD1d<sup>+</sup> (iv). CD4<sup>+</sup>/DX5<sup>-</sup>/CD1d-tetramer<sup>-</sup> T cells were able to mediate the onset of oxazolone colitis in *Lck<sup>cre</sup>IL-4Rα<sup>-/-</sup>* mice compared to mice receiving PBS only shown by: (B) significant weight loss and distress (day 2), (C) colon shortening and (D) increased colitis score. (E) Cytokines measured from pooled colon tissue showed a trend towards increased IL-13 production and increased total IgE from serum. Data represents 1 or 2 experiments (n=6-9 mice) with mean values ± SEM and \* = p < 0.05, \*\* = p < 0.01 and \*\*\* = p < 0.001 vs. *IL-4Rα<sup>-/-</sup>* etoh controls while # = p < 0.05, ## = p < 0.01 and ### = p < 0.001 for *Lck<sup>cre</sup>IL-4Rα<sup>-/-</sup>* oxa + CD4<sup>+</sup>/DX5<sup>-</sup>/CD1d<sup>+</sup> cells vs. *Lck<sup>cre</sup>IL-4Rα<sup>-/-</sup>* oxa + PBS.

## Supplementary Figure S2

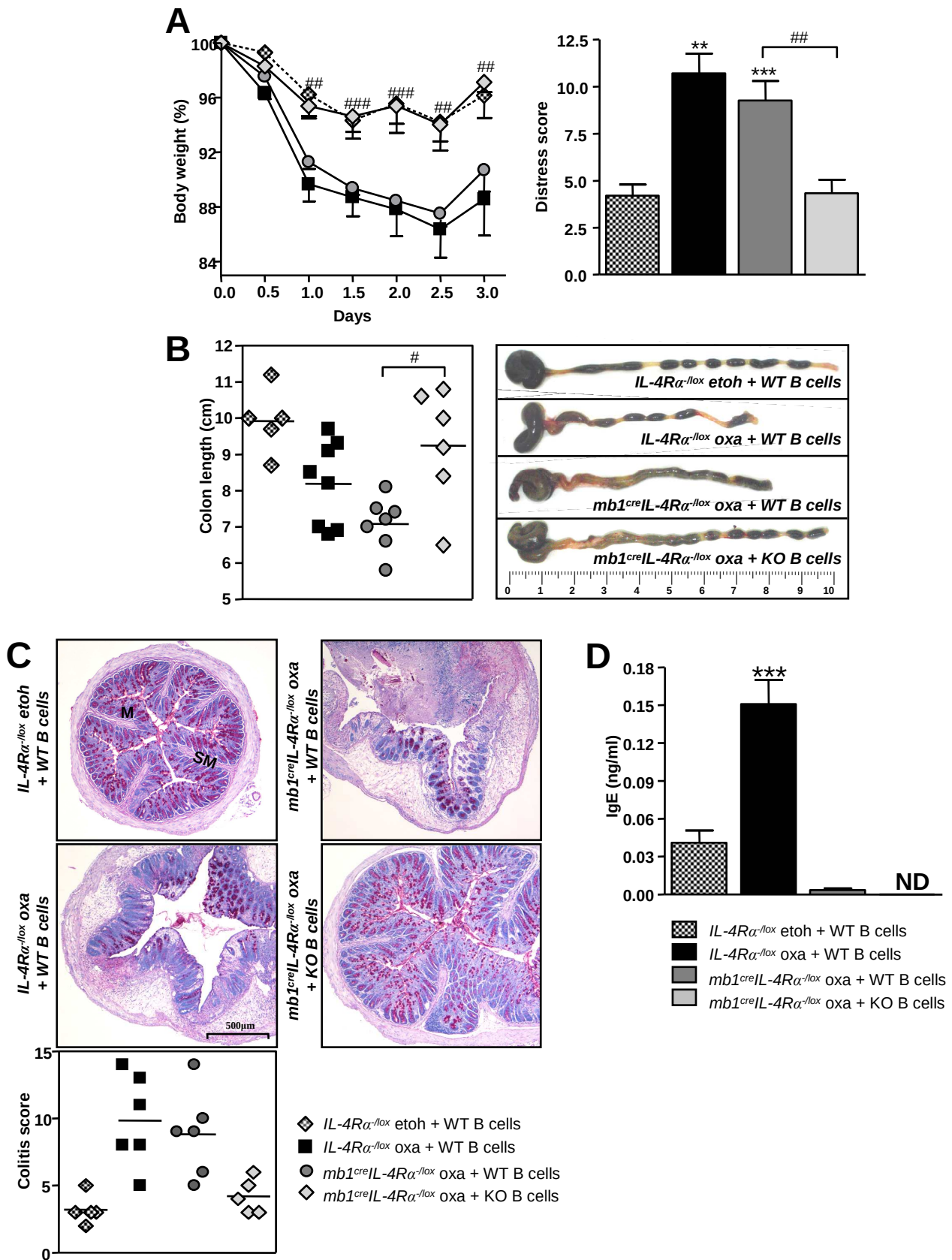


### Supplementary Figure S2

#### Normal T cell function in $mb1^{cre}IL-4R\alpha^{-/-lox}$ mice.

(A) Normal Th2 differentiation. Th1 versus Th2 differentiation was promoted from naïve  $CD4^+$  T cells with IL-4/anti-IFN- $\gamma$  or IL-12/anti-IL-4 mAbs. IFN- $\gamma$  or IL-4 production was measured by ELISA. (B) Normal T cell proliferation.  $CD4^+$  lymph node cells from naïve  $IL-4R\alpha^{-/-lox}$ ,  $mb1^{cre}IL-4R\alpha^{-/-lox}$  and  $IL-4R\alpha^{-/-}$  mice were stimulated with serial dilutions of IL-4 or IL-2 (control). DNA synthesis was measured by [ $^3H$ ] thymidine incorporation. Values represent mean  $\pm$  SEM from triplicate cultures and data represents 2 or 3 independent experiments with \* =  $p < 0.05$ , \*\* =  $p < 0.01$  and \*\*\* =  $p < 0.001$  vs.  $IL-4R\alpha^{-/-lox}$  mice.

Supplementary Figure S3

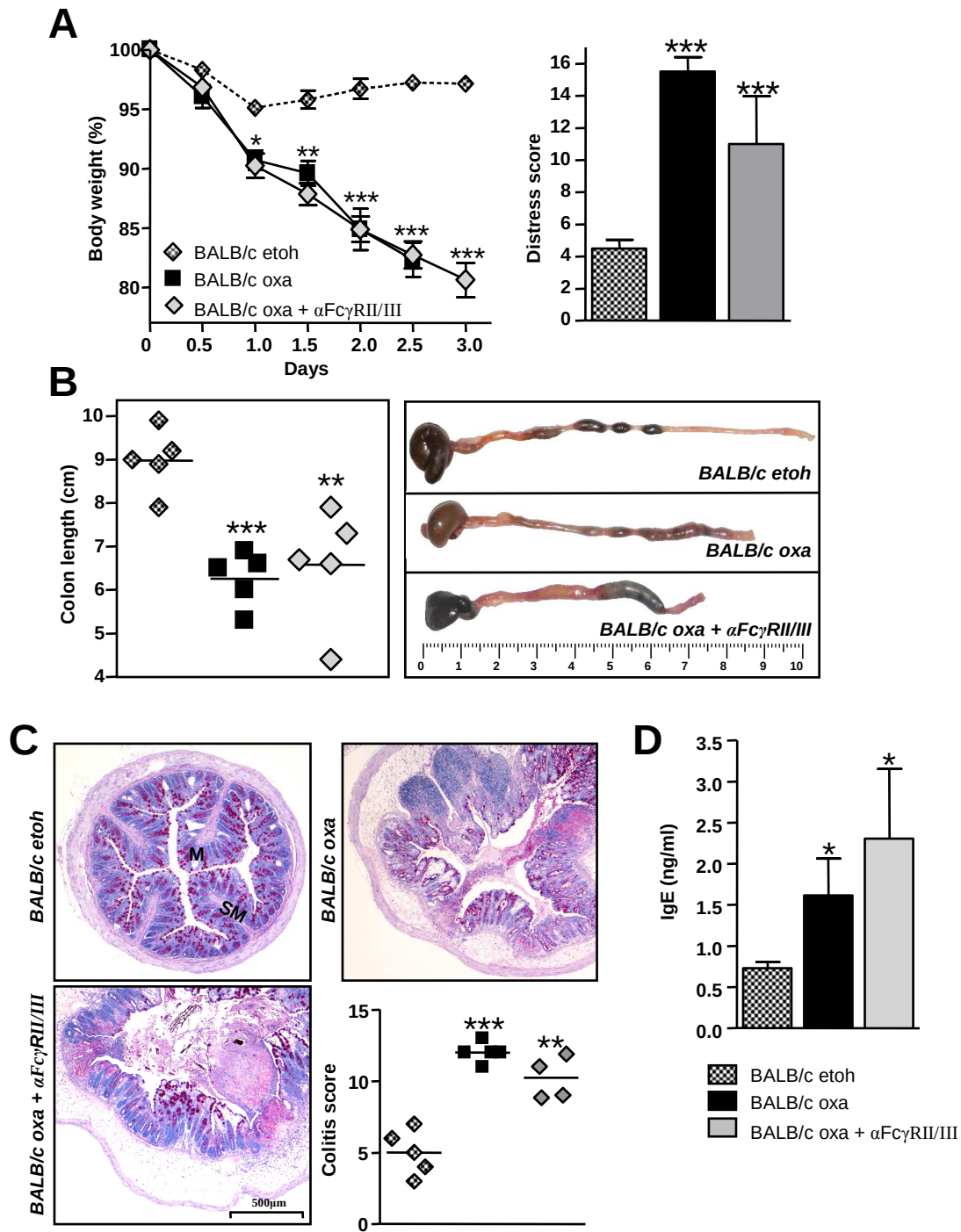


Supplementary Figure S3

**Wild type B cells induce colitis in *mb1<sup>cre</sup>IL-4Rα<sup>-/-</sup>* mice.**

Adoptive transfer of WT but not *IL-4Rα<sup>-/-</sup>* (KO) B cells back into *mb1<sup>cre</sup>IL-4Rα<sup>-/-</sup>* mice resulted in colitis shown by weight loss and distress (A), colon shortening (B) and an increased colitis score (C) with detectable IgE (mean values  $\pm$  SEM) (G). Data represents 2 individual experiments with mean values  $\pm$  SD (unless stated) and \* =  $p < 0.05$ , \*\* =  $p < 0.01$  and \*\*\* =  $p < 0.001$  vs. ethanol treated *IL-4Rα<sup>-/-</sup>* mice. *IL-4Rα<sup>-/-</sup>* vs. *mb1<sup>cre</sup>IL-4Rα<sup>-/-</sup>* or *mb1<sup>cre</sup>IL-4Rα<sup>-/-</sup>* + WT B cells vs. *mb1<sup>cre</sup>IL-4Rα<sup>-/-</sup>* + KO B cells was ### =  $p < 0.01$ . ND = not detected.

Supplementary Figure S4

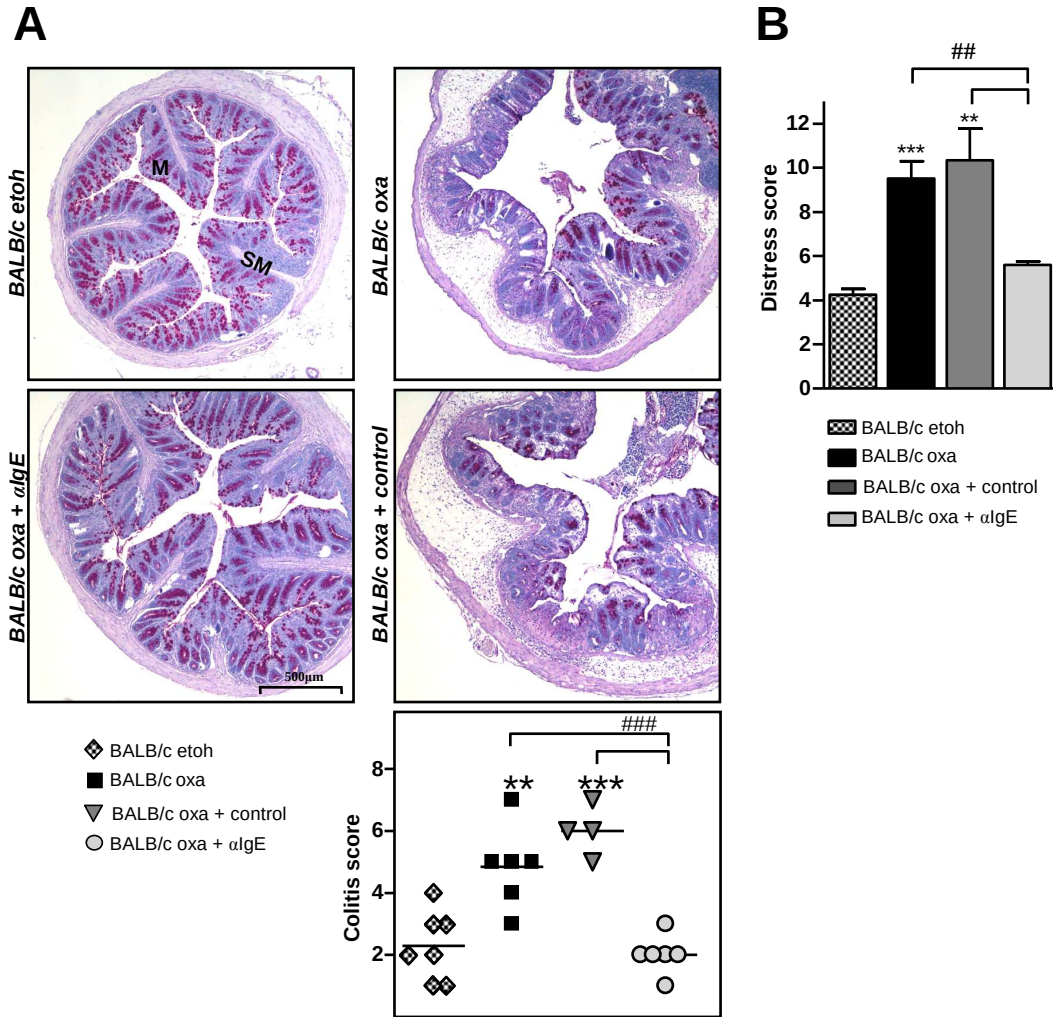


Supplementary Figure S4

**Blocking Fc $\gamma$ RII/III has no effect on colitis outcome in BALB/c mice.**

BALB/c mice were treated with anti-Fc $\gamma$ RII/III antibody before skin-sensitization and intrarectal challenge with oxazolone or ethanol-only. Treatment had no effect on disease outcome shown by weight loss, distress colon shortening, colitis score and IgE levels similar to oxazolone treated control mice. Data shown represents 2 independent experiments from mice killed at day 3 with mean values  $\pm$  SEM or SD and \* =  $p < 0.05$ , \*\* =  $p < 0.01$  and \*\*\* =  $p < 0.001$  vs. etoh control.

Supplementary Figure S5

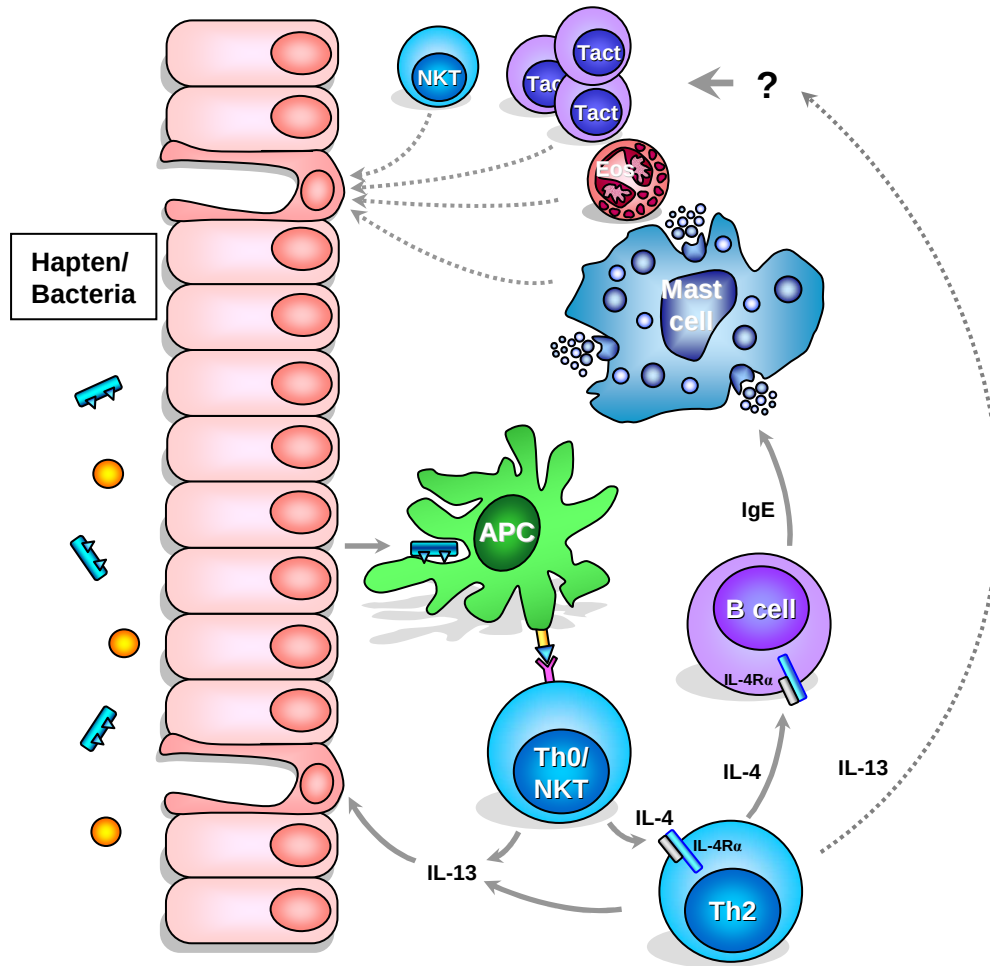


Supplementary Figure S5

**Blocking IgE protects BALB/c mice from oxazolone-induced colitis.**

BALB/c mice were treated with anti-IgE or control antibody before skin-sensitization and intrarectal challenge with oxazolone or ethanol-only. Mice treated with anti-IgE were protected from colitis shown in Figure 6 and supported by (A) decreased colitis score and (B) reduced distress. Data shown represents 3 independent experiments from mice killed at day 3 with mean values  $\pm$  SEM and \*\* =  $p < 0.01$  and \*\*\* =  $p < 0.001$  vs. etoh control or ## =  $p < 0.01$  and ### =  $p < 0.001$  was BALB/c oxa or anti-rat IgG control vs. BALB/c anti-IgE.

## Supplementary Figure S6



### Supplementary Figure S6

#### A hypothetical model of the pathogenesis of oxazolone colitis.

Commensal bacteria products or oxazolone stimulate the mucosal immune system by penetration of the mucosal barrier. Antigen presenting cells (APCs) or direct stimulation from epithelial cells activates NK T cells to drive a Th2/type 2 response. Alternatively, activated Th0 cells can drive a Th2 response, subsequently activating NK T cells. NK T and Th2 cells produce IL-13 which is toxic to epithelial cells. Furthermore, Th2 cells produce IL-4 which stimulates B cells to drive inflammation in an IgE-dependent manner, increasing mast cell numbers.

## SUPPLEMENTARY MATERIALS AND METHODS

**Table I: Primer pairs for genotyping and deletion efficiency.**

| <b>Primer</b>                   | <b>Forward</b>                 | <b>Reverse</b>                 |
|---------------------------------|--------------------------------|--------------------------------|
| <b>IL-4R<math>\alpha</math></b> | 5'-TGACCTACAAGGAACCCAGGC-3'    | 5'-CTCGGCGCACTGACCCATCT-3'     |
| <b>Deletion</b>                 | 5'-GGCTGCTGACCTGGAATAACC-3'    | 5'-CCTTTGAGAACTGCGGGCT-3'      |
| <b>loxP</b>                     | 5'-CCCTTCCTGGCCCTGAATTT-3'     | 5'-GTTTCCTCCTACCGCTGATT-3'     |
| <b>Lck Cre</b>                  | 5'-GAGGGTGGAATGAACTCTCGGT -3'  | 5'-CAGGTATGCTCAGAAAACGCCTGG-3' |
| <b>mb1Cre</b>                   | 5'-ACCTCTGATGAAGTCAGGAAGAAC-3' | 5'-GGAGATGTCCTTCACTCTGATTCT-3' |
| <b>exon 5</b>                   | 5'-AACCTGGGAAGTTGTG-3'         | 5'-CACAGTCCATCTGGTAT -3'       |
| <b>exon 8</b>                   | 5'-GTACAGCGCACATTGTTTTT-3'     | 5'-CTCGGCGCACTGACCCATCT-3'     |

**Table II: Disease activity index scoring sheet.**

| <b>Appearance</b>                         |   | <b>Natural Behavior</b>                  |   |
|-------------------------------------------|---|------------------------------------------|---|
| Normal                                    | 0 | Normal                                   | 0 |
| General lack of grooming                  | 1 | Minor changes                            | 1 |
| Coat staring. Ocular and nasal discharges | 2 | Less mobile and alert, isolated          | 2 |
| Piloerection, hunched up                  | 3 | Vocalization, restlessness, very still   | 3 |
| <b>Clinical signs</b>                     |   | <b>Provoked behavior</b>                 |   |
| Normal color & movement                   | 0 | Normal                                   | 0 |
| Slight changes in activity                | 1 | Minor depression or exaggerated response | 1 |
| Moderated Changes: weight loss, diarrhea  | 2 | Moderate change in expected behavior     | 2 |
| Severe changes: immobility, lameness      | 3 | Reacts violently or very weak            | 3 |

\* If 3 was scored more than once, an extra point was scored for every 3. **Total = 16**