Differential Requirement for NF-κB Family Members in Control of Helminth Infection and Intestinal Inflammation

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The NF-κB family of transcription factors is critical in controlling the expression of a wide range of immune response genes. Whether individual family members perform specific roles in regulating immunity and inflammation remains unclear. We have focused on the role of NF-κB1, NF-κB2, and c-Rel in the expression of Th2 cytokine responses, development of host protective immunity, and regulation of intestinal inflammation following infection with the gut-dwelling helminth parasite Trichuris muris. While mice deficient in c-Rel mounted sufficient Th2 responses to expel infection, NF-κB1 knockout (KO) and NF-κB2 KO mice developed chronic infections associated with elevated production of Ag-specific IFN-γ. However, only infected NF-κB1 KO mice exhibited polarized IFN-γ responses associated with the loss of intestinal goblet cells and the development of destructive colitis-like pathology. Furthermore, blockade of IL-12 (previously shown to confer resistance in susceptible strains) recovered Ag-specific IL-13 responses and resistance to infection in NF-κB2 KO, but not NF-κB1 KO mice. Therefore, unique infection, immunological, and pathological outcomes were observed in different NF-κB KO strains. Taken together, these results provide direct evidence of nonoverlapping functions for NF-κB family members in the development of Th2 cytokine-mediated resistance to T. muris and the control of infection-induced intestinal inflammation.

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The NF-κB family of transcription factors is critical in controlling the expression of a wide range of immune response genes. However, whether individual family members perform specific roles in regulating immunity and inflammation remains unclear. Here we investigated the requirement for NF-κB1, NF-κB2, and c-Rel in the expression of Th2 cytokine responses, development of host protective immunity, and regulation of intestinal inflammation following infection with the gut-dwelling helminth parasite Trichuris muris. While mice deficient in c-Rel mounted sufficient Th2 responses to expel infection, NF-κB1 knockout (KO) and NF-κB2 KO mice developed chronic infections associated with elevated production of Ag-specific IFN-γ. However, only infected NF-κB1 KO mice exhibited polarized IFN-γ responses associated with the loss of intestinal goblet cells and the development of destructive colitis-like pathology. Furthermore, blockade of IL-12 (previously shown to confer resistance in susceptible strains) recovered Ag-specific IL-13 responses and resistance to infection in NF-κB2 KO, but not NF-κB1 KO mice. Therefore, unique infection, immunological, and pathological outcomes were observed in different NF-κB KO strains. Taken together, these results provide direct evidence of nonoverlapping functions for NF-κB family members in the development of Th2 cytokine-mediated resistance to T. muris and the control of infection-induced intestinal inflammation.

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T he NF-κB family of transcription factors (composed of NF-κB1 (p105/p50), NF-κB2 (p100/p52), c-Rel, RelA, and RelB) regulates the expression of a wide range of immune response genes whose products play a critical role in orchestrating inflammatory responses (1, 2). Homo- or heterodimeric forms of NF-κB are sequestered in the cytoplasm of resting cells by the IkB family of proteins. Following exposure of cells to infectious or inflammatory stimuli, IkB is phosphorylated and degraded, allowing NF-κB to translocate to the nucleus and influence the transcription of a wide range of immune response genes (3, 4).

In addition to controlling the expression of adhesion molecules and chemokines, NF-κB can regulate the expression of cytokines that promote Th1 (IL-12 and IFN-γ) and Th2 (IL-4, IL-13) responses (5–9). Therefore, NF-κB is a key transcription factor in regulating immune responses and pathogenesis associated with asthma, autoimmunity, and graft rejection (10–12). In addition, NF-κB activation is essential in initiating innate and adaptive immune responses to intracellular pathogens (13), although its role in immunity to helminth parasites has not been investigated.

NF-κB is also critical in regulating inflammation in the intestine. Activation of NF-κB and expression of inflammatory cytokines has been reported in human and murine models of inflammatory bowel disease (14, 15). Furthermore, studies in models of colitis have demonstrated that blockade NF-κB1 enhanced Helicobacter-induced intestinal inflammation, while disruption of RelA has been shown to either ameliorate or exacerbate colitis (16, 17). Together, these reports suggest NF-κB plays a complex role in regulating inflammation within the intestinal microenvironment. Infection with the gut-dwelling helminth parasite Trichuris muris is also associated with intestinal inflammation, characterized by a mild inflammatory cell infiltrate and the development of crypt hyperplasia (18, 19). T. muris is a natural pathogen of mice, inhabiting the cecum and large intestine, and provides a well-defined model of human infection and disease (20, 21). Expulsion of infection requires the initiation of Th2 cytokine responses (IL-4, IL-9, and IL-13), while susceptibility to chronic infection is promoted by type 1 cytokines (IL-12, IL-18, and IFN-γ) (22–25). In this study we have focused on the role of NF-κB in transcriptional regulation of resistance to infection and the control of helminth-induced gut pathology.

Our results identify novel differential requirements for NF-κB1, NF-κB2, and c-Rel in the regulation of Th cell responses, protective immunity, and control of intestinal inflammation following T. muris infection. Although Th2 cytokine responses were reduced in all NF-κB knockout (KO)3 strains, c-Rel-deficient mice mounted sufficient type 2 cytokine responses to clear infection. In contrast, NF-κB1 KO and NF-κB2 KO mice failed to clear infection, while only chronically infected NF-κB1 KO mice developed destructive colitis-like pathology. Moreover, blockade of endogenous IL-12 recovered Ag-specific IL-13 responses and resistance to infection in NF-κB2 KO mice, but not NF-κB1 KO mice, suggesting that NF-κB2 played an indirect role while NF-κB1 played a direct role in regulating Th2 cytokine responses and host resistance. The nonoverlapping functions of individual family members suggest that NF-κB is also critical in regulating inflammation in the intestine. Activation of NF-κB and expression of inflammatory cytokines has been reported in human and murine models of inflammatory bowel disease (14, 15). Furthermore, studies in models of colitis have demonstrated that blockade NF-κB1 enhanced Helicobacter-induced intestinal inflammation, while disruption of RelA has been shown to either ameliorate or exacerbate colitis (16, 17). Together, these reports suggest NF-κB plays a complex role in regulating inflammation within the intestinal microenvironment. Infection with the gut-dwelling helminth parasite Trichuris muris is also associated with intestinal inflammation, characterized by a mild inflammatory cell infiltrate and the development of crypt hyperplasia (18, 19). T. muris is a natural pathogen of mice, inhabiting the cecum and large intestine, and provides a well-defined model of human infection and disease (20, 21). Expulsion of infection requires the initiation of Th2 cytokine responses (IL-4, IL-9, and IL-13), while susceptibility to chronic infection is promoted by type 1 cytokines (IL-12, IL-18, and IFN-γ) (22–25). In this study we have focused on the role of NF-κB in transcriptional regulation of resistance to infection and the control of helminth-induced gut pathology.

Our results identify novel differential requirements for NF-κB1, NF-κB2, and c-Rel in the regulation of Th cell responses, protective immunity, and control of intestinal inflammation following T. muris infection. Although Th2 cytokine responses were reduced in all NF-κB knockout (KO)3 strains, c-Rel-deficient mice mounted sufficient type 2 cytokine responses to clear infection. In contrast, NF-κB1 KO and NF-κB2 KO mice failed to clear infection, while only chronically infected NF-κB1 KO mice developed destructive colitis-like pathology. Moreover, blockade of endogenous IL-12 recovered Ag-specific IL-13 responses and resistance to infection in NF-κB2 KO mice, but not NF-κB1 KO mice, suggesting that NF-κB2 played an indirect role while NF-κB1 played a direct role in regulating Th2 cytokine responses and host resistance. The nonoverlapping functions of individual family members suggest that...
targeting specific NF-κB transcription factors will be a useful therapeutic approach in manipulating cytokine responses and inflammation in infectious and other disease states.

Materials and Methods

Animals

The following genetically manipulated mice were generated as previously described and maintained in specific-pathogen free conditions at University of Pennsylvania: NF-κB1 KO (from Drs. D. Baltimore and W. Sha, Berkeley, CA) (26), NF-κB2 KO (from Dr. J. C. Caamano, University of Birmingham, Birmingham, UK.) (27), and c-Rel KO (from Dr. H. C. Liou, Cornell University Medical College, New York, NY) (28). Unless otherwise stated, all mice were backcrossed to a B6 background. In some experiments NF-κB1 KO mice maintained as random B6 × 129 hybrids were used (purchased from The Jackson Laboratory, Bar Harbor, ME). Wild-type (WT) B6 and B6 × 129 F2 mice were purchased from The Jackson Laboratory. No differences between NF-κB1 KO mice on a mixed or pure B6 background were observed in these studies. In all experiments mice were infected between 6–10 wk of age, and experimental groups contained four to six mice. Animals were maintained under specific pathogen-free conditions, and no signs of intestinal inflammation were observed in any animals before infection. All experiments were performed under the regulations of the University of Pennsylvania institutional animal care and use committee.

Parasite

The maintenance, infection, and recovery of T. muris were described previously (29). Mice were infected on day 0 with 150–200 embryonated eggs, and equivalent establishment of infection in different mouse strains was determined on day 10 postinfection (p.i.). Assessment of worm burdens and preparation of T. muris excretory-secretory Ag for use in restimulation assays were previously described (23).

EMSA

Mesenteric LN cells were isolated from naive and infected mice, and purified T cells were obtained using murine CD3 

T cell enrichment columns following the manufacturer’s instructions (R&D Systems, Minneapolis, MN). Cell purity was determined by staining with fluorochrome-labeled anti-CD3 mAb (BD PharaMingen, San Diego, CA). Cells were acquired on a FACSCalibur flow cytometer and analyzed using CellQuest software (BD Biosciences, San Jose, CA). CD3+ T cell purities between 85 and 94% were routinely obtained using this protocol. Nuclear extracts were prepared from purified CD3+ T cells as previously described (30) and used for EMSA. In brief, double-stranded oligodeoxynucleotides corresponding to the palindromic κB site (5’-GGGAAATCCC-3’) were labeled by filling the overlapping ends with the Klenow fragment of DNA polymerase I and α-[32P]dCTP. Following removal of unincorporated nucleotides, labeled oligonucleotide (50,000 cpm) was incubated with 6 μg protein extracts and 2 μg poly(dI-dC) in buffer containing 20 mM HEPES (pH 7.9), 100 mM NaCl, 5 mM MgCl2, 1 mM DTT, 0.7 mM PMSF, and 17% glycerol in a final volume of 22 μl for 15 min at 20°C. Complexes were separated on 5.5% polyacrylamide gels run on 0.25% Tris-borate-EDTA buffer, dried, and exposed to Kodak X-OMAT AR film (Rochester, NY) at −70°C.

In vivo depletions

One milligram of neutralizing anti-IL-12 mAb (C17.8; from Dr. G. Trinchieri, formerly at Wistar Institute, Philadelphia, PA) was administered i.p. on days 4, 8, 12, 16, and 20 p.i. This treatment regimen confers resistance against T. muris in mice genetically susceptible to infection (Dr. R. Gencis, University of Manchester, Manchester, U.K., personal communication). Control mice received equivalent amounts of purified rat IgG (Sigma, St. Louis, MO).

Cell culture and cytokine analysis

At necropsy, mesenteric LN were harvested, and single-cell suspensions were prepared in DMEM supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM HEPES, and 5 × 10−8 M 2-ME. Cells were plated at 5 × 105/ml in 24-well culture plates in medium alone or in the presence of T. muris ES Ag (50 μg/ml). In addition, 5 μg/ml anti-IL-4 mAb (BD PharaMingen) was added to cultures to enhance detection of IL-4.

Cell-free supernatants were harvested after 24 h, and cytokine analysis was conducted by sandwich ELISA using paired mAb to detect IL-4 (11B11 and BV6D-24G2.3; cell line from Dr. T. Mormon, University of Rochester Medical Center, Rochester, NY) and IL-13 (R&D Systems).

NF-κB IN INTESTINAL IMMUNITY AND INFLAMMATION

IFN-γ production was assessed using anti-IFN-γ mAb R46A2 (Dr. T. Mossmann) and polyclonal rabbit anti-IFN-γ, followed by peroxidase-conjugated donkey anti-rabbit polyclonal Ab (Jackson ImmunoResearch Laboratories, West Grove, PA).

Histology

One-centimeter segments of mid-cecum were removed, washed in sterile PBS, and fixed for 24 h in 10% neutral buffered formalin. Tissues were processed routinely and were paraffin-embedded using standard histological techniques. Five-micrometer sections were cut and stained with H&E or Alcian blue-periodic acid for detection of intestinal goblet cells. The numbers of intestinal goblet cells per 20 crypt units were assessed under light microscopy.

Statistical analysis

Significant differences (p < 0.05) between experimental groups were determined using the Mann-Whitney U test.

Results

NF-κB is activated following T. muris infection

A number of reports have demonstrated NF-κB activation following infection with viral, bacterial, and protozoan pathogens (reviewed in Ref. 13). To determine whether NF-κB is activated following T. muris infection, WT B6 mice were infected, and NF-κB activation was assessed by EMSA using nuclear extracts prepared from draining mesenteric LN cells at various time points. As shown in Fig. 1, NF-κB activation was detected as early as day 7 p.i. and was maintained throughout infection. The two discrete bands (Fig. 1, black arrows) suggested that at least two dominant species of NF-κB were present following infection. To our knowledge this is the first report of NF-κB activation following infection with a gut-dwelling helminth parasite.

c-Rel KO mice expel T. muris, while NF-κB1 KO and NF-κB2 KO mice develop chronic infections

To determine the roles of specific NF-κB family members in the development of Th2 cytokine-dependent resistance to T. muris infection, B6 WT mice (genetically resistant to T. muris) and mice deficient in the individual family members NF-κB1, NF-κB2, or c-Rel were infected with T. muris, and infection outcome was monitored. (The embryonic lethality of RelA KO and premature death of RelB KO mice (31, 32) precluded their inclusion in these studies.) Analysis of worm burdens on day 10 p.i. confirmed that establishment of infection was equivalent in different experimental groups (Table I). As shown in Fig. 2, WT mice cleared infection by day 35 p.i., confirming our previous results (33). Mice deficient in c-Rel were also successful in expelling T. muris, with no worms detected by day 35 p.i. In contrast, NF-κB1 KO and NF-κB2 KO mice succumbed to infection by day 35 p.i. (Fig. 2, black arrows), suggesting that the absence of NF-κB1 or NF-κB2 affected infection outcome.

FIGURE 1. NF-κB is activated following T. muris infection. Resistant B6 mice were infected with 150–200 embryonated T. muris eggs. Mesenteric lymph nodes were isolated on various days p.i., nuclear extracts were prepared, and κB binding activity was analyzed by EMSA. At least two dominant species of NF-κB were present following infection (arrows).

day post-infection 0 7 14 21

Cell culture and cytokine analysis

At necropsy, mesenteric LN were harvested, and single-cell suspensions were prepared in DMEM supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM HEPES, and 5 × 10−8 M 2-ME. Cells were plated at 5 × 105/ml in 24-well culture plates in medium alone or in the presence of T. muris ES Ag (50 μg/ml). In addition, 5 μg/ml anti-IL-4 mAb (BD PharaMingen) was added to cultures to enhance detection of IL-4.

Cell-free supernatants were harvested after 24 h, and cytokine analysis was conducted by sandwich ELISA using paired mAb to detect IL-4 (11B11 and BV6D-24G2.3; cell line from Dr. T. Mormon, University of Rochester Medical Center, Rochester, NY) and IL-13 (R&D Systems).
mice were unable to clear infection and developed chronic infections with persistent parasites beyond day 35 p.i. (Fig. 2).

**T. muris-infected NF-κB1 KO mice develop destructive colitis-like intestinal pathology**

While no differences in intestinal morphology or goblet cell numbers were observed between naive KO and WT animals (Fig. 4 and data not shown), histological evaluation of the ceca from infected mice demonstrated distinct pathological outcomes in different KO mice. WT and c-Rel KO mice showed morphology typical of mouse strains that had successfully cleared the pathogen, with little or no inflammatory infiltrate in the lamina propria or mucosa and no crypt hyperplasia (Fig. 3, A and B). Mice deficient in NF-κB2 had moderate intestinal pathology, with mild leukocytic inflammation visible in the mucosa and numerous parasites present in the intestinal lumen (Fig. 3C, red arrow). Crypt hyperplasia, a characteristic of chronic *T. muris* infection previously observed in susceptible mouse strains (19), was also evident in infected NF-κB2 KO mice (Fig. 3C, black arrow).

In contrast to NF-κB2 KO and c-Rel KO animals, the pathological outcome of infection in NF-κB1-deficient mice was notably more severe. A profound inflammatory infiltrate (composed of lymphocytes and granulocytes) was evident throughout the muscular layers, submucosa, lamina propria, and mucosa and extended to the serosal surface. Cell infiltration and edema resulted in significant thickening of these layers (Fig. 3D, black arrows). Foci of erosion and ulceration were also present in the mucosa, associated with neutrophil infiltration and fibrin deposition. Dramatic crypt hyperplasia was evident (Fig. 3D), suggesting that marked dysregulation of epithelial cell proliferation and differentiation was occurring in the absence of NF-κB1. Indeed, the luminal surface adjacent to ulceration was almost completely devoid of an epithelial layer (Fig. 3D, red arrows) and displayed pathology reminiscent of that reported in murine models of inflammatory bowel disease (34–37). Therefore, although both NF-κB1 KO and NF-κB2 KO mice developed chronic infections, these family members performed distinct roles in regulating infection-induced intestinal inflammation.

Significant differences in mucosal goblet cell responses in different KO mice were also observed following infection. Goblet cell responses are enhanced by Th2 cytokines (38, 39) and serve as useful markers of the magnitude of Th2 responses in the intestinal microenvironment. Intracellular staining of goblet cell mucins demonstrated that both c-Rel KO and WT mice had significant numbers of goblet cells in the mucosa, a characteristic of resistant mice that have developed a protective Th2 response in the intestine and expelled *T. muris* (Fig. 3, E and F, red arrows, and Fig. 4). Goblet cell hyperplasia was also observed in infected NF-κB2 KO mice, although at a significantly lower magnitude than in WT mice (Fig. 3G, red arrows, and Fig. 4). In contrast, there was a complete absence of goblet cells following infection of NF-κB1 KO mice (Figs. 3H and 4), identifying a critical role for NF-κB1 in intestinal goblet cell responses following infection.

**NF-κB1 KO, NF-κB2 KO, and c-Rel KO mice demonstrate altered Ag-specific cytokine responses following infection**

As previously reported (33), Ag-specific restimulation of mesenteric LN cells from infected B6 WT mice demonstrated enhanced IL-4 and IL-13 responses with low IFN-γ production (Fig. 5, A–C). In contrast, all NF-κB KO mice secreted low levels of IL-4 and IL-13 following restimulation (Fig. 5, A and B). While low Th2 responses were expected in NF-κB1 KO and NF-κB2 KO mice due to their susceptibility to infection, it was surprising that c-Rel KO mice (that successfully cleared infection) developed poor IL-4 and IL-13 responses. However, significant differences in production of Ag-specific IFN-γ were observed between KO mice (Fig. 5C). Mice deficient in c-Rel secreted low levels of IFN-γ (Fig. 5C). In contrast, NF-κB1 KO mice developed polarized Th1 responses, with the production of significantly higher levels of IFN-γ than observed in WT mice (Fig. 5C). Therefore, the difference in infection outcome between different KO mice was likely to reflect subtle differences in the balance of Th1 vs Th2 responses. Indeed, expression of IL-4/IFN-γ production as a ratio demonstrated that although the ratio of IL-4/IFN-γ was lower in c-Rel KO mice than in WT animals (Fig. 5D), it was higher than in other KO mice and was sufficient to mediate worm expulsion (Fig. 2). In contrast, the lower level of IL-4 and IL-13 production in the context of elevated IFN-γ responses observed in NF-κB1 KO and NF-κB2 KO mice was reflected in decreased ratio of IL-4/IFN-γ and was not sufficient to clear infection (Fig. 5D). A similar pattern in the ratio of IL-13/IFN-γ responses was observed (data not shown). T cell development is normal in the NF-κB KO strains used in these studies (26–28); in addition, no significant difference in cell recovery or the ability of cells to proliferate in response to mitogenic stimuli was observed between KO and WT mice (data not shown). Therefore, the differences in infection outcome and cytokine responses observed between different KO mice are unlikely to be due to alterations in lymphocyte subsets or defects in lymphocyte proliferation. Rather, differences in the balance of Th1 and Th2 cytokine responses between NF-κB KO mice appear to be critical in determining resistance and susceptibility to infection.

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**Table 1. Equivalent establishment of infection in NF-κB KO and WT mice at day 10 postinfection**

| Worm Burden ± SEM | KO       | WT       |
|-------------------|----------|----------|
| NF-κB1 KO         | 101 ± 13 |          |
| NF-κB2 KO         | 119.5 ± 9.5 |      |
| c-Rel KO          | 111.5 ± 11.7 |    |
| B6 WT             | 127.5 ± 15.5 |   |

* Mice deficient in NF-κB family members NF-κB1, NF-κB2, or c-Rel and WT mice were infected with 150–200 embryonated *T. muris* eggs and worm burdens assessed from three mice per group on day 10 p.i. Results are expressed as mean ± SEM.

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**FIGURE 2.** Differential requirements for NF-κB1, NF-κB2, and c-Rel in the expulsion of *T. muris* infection. Mice deficient in NF-κB family members NF-κB1, NF-κB2, or c-Rel and WT mice were infected with 150–200 embryonated *T. muris* eggs, and worm burdens were assessed in four to six mice per group on day 35 p.i. Results are expressed as the mean ± SEM. * Significant difference between KO and WT mice (p < 0.05).
and host protective immunity in NF-κB KO mice was associated with enhanced IFN-γ production in these animals, which is an important cytokine in the control of helminth infections. Absence of goblet cells in the intestine of NF-κB KO mice was associated with enhanced IFN-γ production in these animals, which is an important cytokine in the control of helminth infections.

**FIGURE 3.** NF-κB KO mice develop colitis-like destructive intestinal pathology following T. muris infection. WT (A and E) and mice deficient in NF-κB family members c-Rel (B and F), NF-κB2 (C and G), or NF-κB1 (D and H) mice were infected with 150–200 embryonated T. muris eggs. Lengths of mid-cecum were paraffin-embedded, and 5-μm sections were cut and stained with either H&E (A–D) or Alcian blue-periodic acid (E–H) for detection of intestinal goblet cells. Intestinal pathology and goblet cell hyperplasia were examined microscopically on day 35 p.i. See text for explanation of arrows. GC, goblet cells; M, muscular layer; SM, submucosa; LP, lamina propria. Bar = 200 μm (A–D) or 25 μm (E–H).

**Blockade of endogenous IL-12 recovers IL-13 responses and resistance to T. muris infection in NF-κB2 KO, but not NF-κB1 KO mice.**

Susceptibility to T. muris infection in NF-κB1 KO and NF-κB2 KO mice was associated with enhanced IFN-γ production in the absence of Th2 cytokine responses (Fig. 5). To determine whether blockade of endogenous Th1 responses would allow the expansion of protective Th2 cytokine responses and subsequent expulsion of T. muris in these animals, mice deficient in NF-κB1 or NF-κB2 were infected with T. muris and treated systemically every 4 days with either control Ig or anti-IL-12 mAb. As shown in Fig. 6, anti-IL-12 treatment of NF-κB1 KO mice significantly reduced the production of IFN-γ following Ag-specific restimulation, but failed to enhance the production of either IL-4 or IL-13. The failure of anti-IL-12 treatment to promote Th2 cytokine responses in NF-κB1 KO mice resulted in chronic infections persisting in both control and anti-IL-12-treated NF-κB1 KO mice (Fig. 7A). Increasing doses of anti-IL-12 and coadministration of anti-IL-12/anti-IFN-γ mAb also failed to unmask Ag-specific Th2 responses and host protective immunity in NF-κB1 KO mice (data not shown). In contrast, blockade of IL-12 activity in NF-κB2 KO mice led to a significant reduction in IFN-γ production and a concomitant elevation in the production of Ag-specific IL-13 (Fig. 6). However, anti-IL-12 treatment failed to enhance the production of IL-4 (Fig. 6). Critically, the enhanced IL-13 levels observed in supernatants from NF-κB2 KO mice, although still lower than levels observed in WT animals, correlated with the expulsion of T. muris (Fig. 7B). The ability of IL-13 to mediate expulsion of T. muris in the absence of IL-4 has been reported previously (23). These results demonstrate that NF-κB2 KO mice can mount functional IL-13 responses if IL-12 responses are blocked, suggesting that NF-κB2 plays an indirect role in regulating the production of Th2 cytokines. In contrast, the failure of anti-IL-12-treated NF-κB1 KO mice to develop Th2 responses suggests an absolute requirement for NF-κB1 in the induction of Th2 cytokine genes and resistance to helminth infection.

**Discussion**

A role for NF-κB in promoting proinflammatory responses has been widely reported in murine models of inflammation and immunity to intracellular pathogens (reviewed in Ref. 2). Indeed, defective IL-12- and/or IFN-γ-mediated immune responses have been reported in mice deficient in NF-κB1, NF-κB2, or c-Rel (9, 40–43). Our results provide new insights into the differential requirements for these family members in regulating Th2 cytokine-mediated resistance to helminth infection and in controlling infection-induced intestinal inflammation. Firstly, we found that in contrast to c-Rel KO animals, mice deficient in NF-κB1 or NF-κB2 developed chronic infections. Critically, blockade of IL-12 (previously shown to confer resistance to infection in susceptible mouse strains) recovered protective IL-13 responses and resistance to infection in NF-κB2 KO, but not NF-κB1 KO, mice. These results demonstrated that individual NF-κB KO mice differed in their ability to mount protective Th2 cytokine responses and control infection. Secondly, we showed that KO mice exhibited differential defects in their ability to generate intestinal goblet cell responses following infection that correlated with their ability to generate Th2 cytokine responses and control infection. Lastly, severe infection-induced intestinal pathology was only observed in infected NF-κB1 KO mice, identifying a unique role for this family member in controlling intestinal inflammation. Taken together, these results reveal nonoverlapping functions for NF-κB family members in controlling immunity and inflammation following intestinal helminth infection.

The ability of c-Rel KO, but not NF-κB1 KO or NF-κB2 KO, mice to successfully clear T. muris was reflected in the balance of Ag-specific Th1 and Th2 cytokine responses following infection. Mice deficient in NF-κB1 or NF-κB2 exhibited a low ratio of Th2:Th1 cytokine responses and developed chronic infections, while this ratio was higher in c-Rel KO mice and was sufficient to clear infection. However, the magnitude of IL-4 and IL-13 responses were significantly lower in lymph node cell cultures from c-Rel KO mice than in those from WT mice. The reduced Th2
cytokine responses in c-Rel KO mice may reflect a lower frequency of IL-4- and IL-13-producing cells or suboptimal cytokine responses in KO animals. These hypotheses are supported by in vitro studies that demonstrated a requirement for c-Rel in optimum IL-4 production by Jurkat T cells (44). Alternatively, cytokine-producing cells may have been preferentially located in the intestinal microenvironment rather than the lymph node of KO mice. The equivalent goblet cell responses, a hallmark of mucosal Th2 responses during intestinal and pulmonary inflammation (38, 39) (see below), coupled with the ability of c-Rel KO mice to clear T. muris infection support this hypothesis.

Although both NF-κB1 KO and NF-κB2 KO mice had defective Th2 cytokine responses and developed chronic infections, anti-IL-12 treatment recovered protective IL-13 responses and resistance to infection in NF-κB2 KO, but not NF-κB1 KO, mice. These results demonstrated that if endogenous Th1 responses were blocked, NF-κB2 was also dispensable for sufficient Th2 responses to control helminth infection. The ability to recover IL-13 production in the absence of NF-κB2 is supported by in vitro studies demonstrating that Th2 cell differentiation is intact following polyclonal stimulation of NF-κB2 KO T cells under Th2-polarizing conditions (43). Therefore, in vitro and in vivo studies have shown that NF-κB2 KO T cells can become Th2 cells. Taken together, these results suggested an indirect role for NF-κB2 in regulating Th2 cytokine responses. For instance, NF-κB2 may be important in accessory cell function, a suggestion supported by the predominant expression of this family member in the myeloid cell lineage (45) and reports of overproduction of proinflammatory cytokines by NF-κB2 KO accessory cells following stimulation (46).

In contrast, blockade of endogenous Th1 responses failed to recover resistance to T. muris in the absence of NF-κB1, demonstrating that NF-κB1 (unlike NF-κB2 or c-Rel) is essential in regulating the expression of Th2 cytokine genes and host protective

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** Differential requirements for NF-κB1, NF-κB2, and c-Rel in the development of Ag-specific Th cell cytokine responses following T. muris infection. Mice deficient in NF-κB family members NF-κB1, NF-κB2, or c-Rel and WT mice were infected with 150–200 embryonated T. muris eggs. On day 21 p.i. mesenteric LN cells were isolated and cultured in medium or T. muris Ag for 24 h. Supernatants were assayed for the production of IL-4 (A), IL-13 (B), and IFN-γ (C) by ELISA. The ratio of IL-4 to IFN-γ responses is expressed in arbitrary units (D). Results are expressed as the mean ± SEM for four to six mice per group. *, Significant difference between KO and WT mice (p < 0.05).

![Figure 6](http://www.jimmunol.org/)

**FIGURE 6.** Anti-IL-12 treatment recovers Ag-specific IL-13 responses in T. muris-infected NF-κB2 KO, but not NF-κB1 KO, mice. Mice deficient in NF-κB1 or NF-κB2 and WT mice were infected with 150–200 T. muris eggs. KO mice received 1 mg anti-IL-12 mAb of control Ig every 4 days between days 0 and 20 p.i. Mesenteric lymph node cells were isolated on day 21 p.i. and cultured in medium or T. muris Ag for 24 h. Supernatants were assayed for the production of IL-4, IL-13, and IFN-γ by ELISA. Results are expressed as the mean ± SEM for four or five mice per group. *, Significant difference between control and anti-IL-12-treated KO mice (p < 0.05).

![Figure 7](http://www.jimmunol.org/)

**FIGURE 7.** Anti-IL-12 treatment mediates expulsion of T. muris in NF-κB2 KO, but not NF-κB1 KO, mice. Mice deficient in NF-κB1 or NF-κB2 and WT mice were infected with 150–200 T. muris eggs. KO mice received 1 mg anti-IL-12 mAb of control Ig every 4 days between days 0 and 20 p.i. Worm burdens were assessed on day 35 p.i. Results are expressed as the mean ± SEM for four or five mice per group. *, Significant difference between control and anti-IL-12-treated KO mice (p < 0.05).
immunity following *T. muris* infection. Evidence of a direct requirement for NF-κB1 in the expression of Th2 cytokine responses is supported by in vitro polarization studies demonstrating that even under conditions favoring Th2 cell polarization, NF-κB1 KO T cells are unable to produce Th2 cytokines (our unpublished results and Ref. 8). Furthermore, a recent report by Das et al. (8) demonstrated that NF-κB1 directly influences the expression of GATA-3, a transcription factor known to control Th2 cell differentiation (47, 48). Although the in vivo expression of GATA-3 following *T. muris* infection has not been investigated, it is likely that the absence of NF-κB1 prevents up-regulation of GATA-3, resulting in the failure of KO mice to develop protective Th2 responses.

The generation of intestinal goblet cell responses following infection also differed between mice deficient in individual NF-κB family members. Intestinal goblet cells, whose differentiation is controlled by Th2 cytokines (38, 39), perform a number of functions, including secretion of mucins and other anti-microbial peptides. Indeed, goblet cells have been proposed as effector cells following infection with intestinal helminth parasites (38, 49). Consistent with their ability to mount sufficient Th2 responses to clear *T. muris* infection, mice deficient in c-Rel had goblet cell responses equivalent to those of WT mice following infection. However, while the magnitude of goblet cell responses was reduced in chronically infected NF-κB2 KO compared with WT mice, there was a complete absence of intestinal goblet cell responses in infected NF-κB1 KO mice. The loss of goblet cell responses following infection of NF-κB1 KO mice correlated with the inability of these KO mice to develop any Th2 cytokine responses and their susceptibility to infection. Goblet cell-derived mucins and other secreted products have also been implicated in regulating intestinal epithelial cell function and mucosal integrity following insult (50), and their absence in infected NF-κB1 KO mice may contribute to the colitis-like pathology observed.

The severe destructive intestinal inflammation in chronically infected NF-κB1 KO mice was not observed in other NF-κB KO strains and was accompanied by the secretion of significantly higher levels of Ag-specific IFN-γ than those in WT mice. This was a surprising result, as NF-κB1 KO mice have been reported to have defects in IFN-γ production in response to other stimuli (40). However, enhanced production of proinflammatory cytokines, including IL-12, TNF, and IFN-γ, has been reported in the intestine of NF-κB1-deficient mice following enteric bacterial infection (17). This observation suggested that NF-κB1 (either p105 or p50) might also be operating as a transcriptional repressor in the intestinal microenvironment, inhibiting the production of proinflammatory cytokines in response to enteric bacteria. Given that *Trichuris* infection can promote the invasion of enteric pathogenic bacteria into the intestinal mucosa (51), it is possible that in the absence of NF-κB1, levels of proinflammatory cytokines such as IL-12 and TNF are elevated in the intestine of KO mice. Enhanced production of these proinflammatory factors may, in turn, augment the production of Ag-specific IFN-γ following *T. muris* infection in NF-κB1 KO mice. The production of proinflammatory mediators in response to bacterial products coupled with elevated *T. muris*-specific IFN-γ production are likely to contribute to the intestinal inflammation observed.

There may also be a role for IL-10 in controlling intestinal inflammation in NF-κB1 KO mice. A recent report by Schopf et al. (52) described the development of intestinal inflammation associated with an inflammatory cell infiltrate and loss of goblet cells following *T. muris* infection of IL-10 KO mice. Although parallels exist between the pathology found in infected NF-κB1 KO and IL-10 KO mice, mice deficient in IL-10 developed weight loss and premature death around day 20 postinfection, an outcome we have never observed in NF-κB1 KO mice. Therefore, while it is possible that reduced IL-10 levels in infected NF-κB1 KO mice contribute to the development of intestinal inflammation, this is clearly not the only reason for the pathology they exhibit. Rather, defects in IL-10 coupled with the imbalance in Th1 and Th2 cytokine responses we report here are likely to result in susceptibility to infection and the severe pathological outcome observed. An interesting hypothesis we are currently investigating is that in addition to regulating protective Th2 cytokine responses, NF-κB1 is required for the development of IL-10-producing regulatory B and T cells, two populations that have been shown to inhibit intestinal inflammation in other systems (53–56).

In summary, it is clear that nonredundant functions for NF-κB family members exist following intestinal helmith infection, with NF-κB1 playing a unique and critical role in regulating protective Th2 cytokine responses and infection-induced inflammation. Key questions now include defining the cell types and receptor-ligand interactions that are required for NF-κB1 activation and the subsequent control of inflammation and induction of protective Th2 cytokines. An attractive candidate is TNF-TNFα signaling, a potent activator of NF-κB (2) and a signaling pathway we have previously shown to be required for optimum Th2 cytokine production and efficient clearance of *T. muris* (33). Indeed, TNF production and sustained NF-κB activation have also been reported in animal models of asthma (57). Identifying pathways of NF-κB1 activation and how this family member interacts with other transcription factors will provide new insights into the molecular regulation of Th2 responses and identify novel targets to specifically manipulate Th cell responses and inflammation in vivo.

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