

# Dendritic Cell-Intrinsic Expression of NF- $\kappa$ B1 Is Required to Promote Optimal Th2 Cell Differentiation<sup>1</sup>

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A number of receptors and signaling pathways can influence the ability of dendritic cells (DC) to promote CD4<sup>+</sup> Th type 1 (Th1) responses. In contrast, the regulatory pathways and signaling events that govern the ability of DC to instruct Th2 cell differentiation remain poorly defined. In this report, we demonstrate that NF- $\kappa$ B1 expression within DC is required to promote optimal Th2 responses following exposure to *Schistosoma mansoni* eggs, a potent and natural Th2-inducing stimulus. Although injection of *S. mansoni* eggs induced production of IL-4, IL-5, and IL-13 in the draining lymph node of wild-type (WT) mice, NF- $\kappa$ B1<sup>-/-</sup> hosts failed to express Th2 cytokines and developed a polarized Ag-specific IFN- $\gamma$  response. In an in vivo adoptive transfer model in which NF- $\kappa$ B-sufficient OVA-specific DO11.10 TCR transgenic T cells were injected into OVA-immunized WT or NF- $\kappa$ B1<sup>-/-</sup> hosts, NF- $\kappa$ B1<sup>-/-</sup> APCs efficiently promoted CD4<sup>+</sup> T cell proliferation and IFN- $\gamma$  responses, but failed to promote Ag-specific IL-4 production. Further, bone marrow-derived DC from NF- $\kappa$ B1<sup>-/-</sup> mice failed to promote OVA-specific Th2 cell differentiation in vitro coculture studies. Last, *S. mansoni* egg Ag-pulsed NF- $\kappa$ B1<sup>-/-</sup> DC failed to prime for Th2 cytokine responses following injection into syngeneic WT hosts. Impaired Th2 priming by NF- $\kappa$ B1<sup>-/-</sup> DC was accompanied by a reduction in MAPK phosphorylation in Ag-pulsed DC. Taken together, these studies identify a novel requirement for DC-intrinsic expression of NF- $\kappa$ B1 in regulating the MAPK pathway and governing the competence of DC to instruct Th2 cell differentiation. *The Journal of Immunology*, 2005, 174: 7154–7159.

Dendritic cells are specialized APCs with the ability to promote naive T cell proliferation (1). Depending on the nature of the stimulus and the environmental context, DC can also influence the differentiation of Th cell subsets through programmed expression of costimulatory molecules and cytokines (2, 3). For instance, pathogen-derived TLR ligands, including double-stranded RNA, unmethylated CpG dinucleotide-containing bacterial DNA and LPS, can promote the ability of DC to drive Th1 cell differentiation, whereas cholera toxin, fungal, and helminth-derived Ag can induce DC populations that prime for Th2 cell differentiation (reviewed in Refs. 4 and 5).

A number of receptors and signaling pathways have been implicated in governing DC activation and their ability to promote Th1 responses. For examples, TLR-induced MyD88 recruitment, the Notch ligands Delta1 and Delta4, and T-bet expression have all been implicated in determining the ability of DC to drive Th1 cell

differentiation following exposure to proinflammatory signals (4, 6, 7). NF- $\kappa$ B proteins are also critical in regulating DC activation and cytokine responses that can influence Th1 cytokine responses (8–10). In contrast to Th1-promoting DC, the regulatory pathways and signaling events that govern the ability of DC to instruct Th2 cell differentiation remain unknown. Indeed, DC remain largely quiescent following exposure to Th2-inducing stimuli, although they do exhibit modest activation of MAPK and *c-fos* pathways (11, 12). Using a combination of in vitro and in vivo assays, studies outlined in this report identify a specific requirement for DC-intrinsic expression of NF- $\kappa$ B1 in determining the competence of DC to promote Th2 responses. Although NF- $\kappa$ B1<sup>-/-</sup> DC efficiently promote T cell proliferation and IFN- $\gamma$  production, they exhibit impaired activation of the MAPK pathway and fail to promote Ag-specific Th2 cell cytokine responses following exposure to a natural helminth-derived Th2-inducing stimuli.

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## Materials and Methods

### Animals

NF- $\kappa$ B1<sup>-/-</sup> mice were generated as previously described (13) and backcrossed to a B6 or BALB/c background for six to ten generations. Littermates or purchased age- and sex-matched wild type (WT)<sup>4</sup> C57BL/6 or BALB/c mice (The Jackson Laboratory) were used as controls. Mice transgenic for the OVA<sub>323–339</sub>-specific and I-A<sup>d</sup>-restricted DO11.10 TCR- $\alpha\beta$  on a BALB/c genetic background (14) were provided by Dr. K. Murphy (University of Washington, St. Louis, MO). All experiments were conducted following the guidelines of the University of Pennsylvania Institutional Animal Care and Use Committee.

### T cell cultures and adoptive transfers

WT and NF- $\kappa$ B1<sup>-/-</sup> mice were injected with 1000 *S. mansoni* eggs in the footpad and draining lymph node (LN) cell suspensions prepared 7 days

<sup>4</sup> Abbreviations used in this paper: DC, dendritic cell; LN, lymph node; SEA, *S. mansoni* egg Ag; WT, wild type.

later. T cell proliferation, intracellular cytokine staining, and cytokine secretion were assayed as previously described (15). In some studies, cells were labeled with CFSE (1.25  $\mu$ M for 5 min at 37°C) (Molecular Probes), plated at 1–4  $\times$  10<sup>6</sup>/ml in either 96- or 24-well plates, and cultured in medium or  $\alpha$ CD3/ $\alpha$ CD28 mAb (BD Pharmingen) (both 1  $\mu$ g/ml) for 3–5 days under neutral, Th1 (rIL-12, 10 ng/ml (a gift from Dr. S. Wolf and Dr. J. Sypek, Genetics Institute, Cambridge, MA); anti-IL-4 mAb (10  $\mu$ g/ml 11B11) or Th2 (40 ng/ml rIL-4; R&D Systems); anti-IL-12 mAb (10  $\mu$ g/ml C17.8) polarizing conditions. OVA-specific CD3<sup>+</sup> T cells were purified using T cell enrichment columns (R&D Systems), and either cocultured with OVA-pulsed DC (ratio 1:10, DC:T cells) in the presence or absence of IL-4 (40 ng/ml) or resuspended in PBS and 1  $\times$  10<sup>7</sup> cells adoptively transferred i.v. OVA-IFA was injected s.c. or i.p (5  $\mu$ g/ml) 1 day after cell transfer.

#### DC assays

Bone marrow-derived DC were cultured for 10 days in medium (RPMI 1640 supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin, and 5  $\times$  10<sup>-5</sup> M 2-ME) supplemented with rmGM-CSF (PeproTech) as previously described (16) and stained with fluorochrome-conjugated mAb against CD11c, CD8 $\alpha$ , CD40, CD80, and CD86 (BD Pharmingen). Purities of CD11c<sup>+</sup> cells were similar in WT and NF- $\kappa$ B1<sup>-/-</sup> cultures. DC were pulsed overnight with either OVA peptide (OVA<sub>323–339</sub>) (5  $\mu$ g/ml) for T cell coculture studies or *S. mansoni* egg Ag (SEA) (50  $\mu$ g/ml), washed and 5  $\times$  10<sup>5</sup> cells adoptively transferred i.p. (17).

#### Preparation of cell lysates and Western blot analysis

DC were pulsed with SEA and whole cell lysates prepared in lysis buffer (0.1% Triton X-100, 20 mM HEPES, pH 7.5, 10% glycerol, 150 mM NaCl, and 1 mM DTT). Lysates were resolved on a 10% SDS/PAGE gel and then transferred to a nitrocellulose membrane (Bio-Rad) using a Trans-Blot SemiDry cell (Bio-Rad). Membranes were blocked in 5% milk protein for 1 h and incubated with Abs against total or phosphorylated ERK1 (p-Thr202/p-Tyr204)/ERK2 (p-Thr202/p-Tyr204) (Cell Signaling Technology). HRP-conjugated anti-rabbit IgG was obtained from Pierce, and immunoreactive bands were visualized by the ECL system (Amersham Biosciences) and band intensities relative to background determined using the Bio-Rad Quantity One system (Bio-Rad).

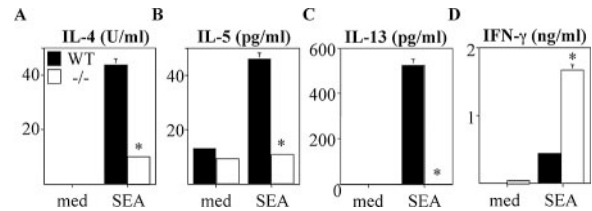
#### Statistics

Statistical differences between experimental groups were determined by Student's *t* test.

## Results

### Defective Schistosome egg-induced Th2 cytokine responses in NF- $\kappa$ B1<sup>-/-</sup> mice

We and others have previously demonstrated the requirement for NF- $\kappa$ B1 expression in the development of optimal Th2 cytokine responses in murine models of airway inflammation and gastrointestinal helminth infection (31, 32). However, the cell-intrinsic requirements for NF- $\kappa$ B1 and mechanisms that govern NF- $\kappa$ B1-dependent Th2 responses remain undefined. Schistosome eggs are a potent natural Th2 cytokine-inducing stimulus and provide a well-defined in vivo model of type 2 inflammation. Furthermore, SEA-pulsed DC can prime for Th2 cytokine responses following adoptive transfer into naive mice, providing a model to dissect cell-intrinsic regulatory pathways that influence Th2 cytokine responses (17, 20, 21). To investigate whether NF- $\kappa$ B1 regulates Th2 cytokine responses in this model, Schistosome eggs were injected s.c. into WT and NF- $\kappa$ B1<sup>-/-</sup> mice, and cytokine production in the draining popliteal LN was determined 7 days later. SEA-restimulated WT LN cells exhibited a Th2-polarized phenotype, secreting IL-4 (Fig. 1A), IL-5 (Fig. 1B), IL-13 (Fig. 1C), and low levels of IFN- $\gamma$  (Fig. 1D). In contrast, LN cells isolated from NF- $\kappa$ B1<sup>-/-</sup> mice produced minimal levels of Th2 cytokines (Fig. 1, A–C) but significantly higher levels of IFN- $\gamma$  than detected in WT LN cultures (Fig. 1D). Therefore, potent SEA-specific IFN- $\gamma$  responses can develop in the absence of NF- $\kappa$ B1, although the expression of Th2 cytokines is NF- $\kappa$ B1 dependent.



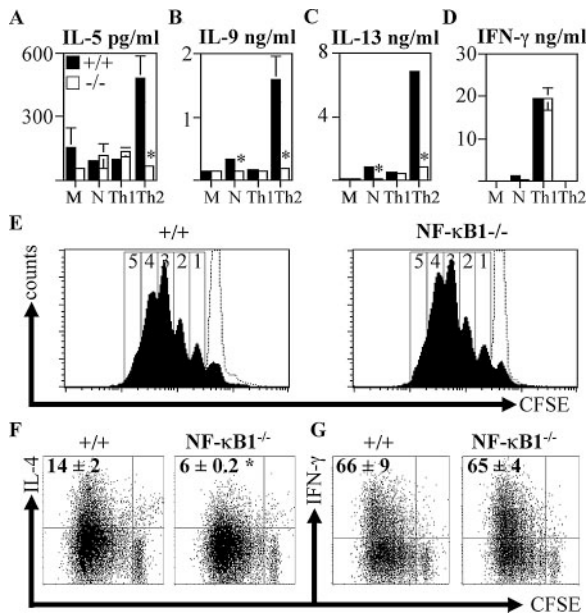
**FIGURE 1.** Defective Schistosome egg-induced Th2 cytokine responses in NF- $\kappa$ B1<sup>-/-</sup> mice. WT and NF- $\kappa$ B1<sup>-/-</sup> mice were injected subcutaneously with *S. mansoni* eggs, and 7 days later, draining LN cells were isolated and cultured in medium (med) alone or SEA (50  $\mu$ g/ml) for 48 h. Supernatants were collected and assayed for secretion of IL-4 (A), IL-5 (B), IL-13 (C), and IFN- $\gamma$  (D) by ELISA. \*, Significant difference between WT and NF- $\kappa$ B1<sup>-/-</sup> ( $p < 0.05$ ). Results are expressed as the mean  $\pm$  SEM of three animals per group and are representative of two to three independent experiments.

### Defective Th2 cell differentiation but intact proliferation and IFN- $\gamma$ production following polyclonal stimulation of NF- $\kappa$ B1<sup>-/-</sup> CD4<sup>+</sup> T cells

Th cell differentiation is closely linked to proliferation and a recent study demonstrated that Th2 cell differentiation was associated with DC-controlled proliferative arrest of CD4<sup>+</sup> T cells (21). To determine whether impaired Th2 cytokine responses in NF- $\kappa$ B1<sup>-/-</sup> mice were associated with defects in T cell activation or proliferation, an in vitro Th cell differentiation assay was adopted. Spleen cells were isolated from BALB/c WT and NF- $\kappa$ B1<sup>-/-</sup> mice and labeled with CFSE, and proliferation and acquisition of cytokines following polyclonal stimulation was determined by a combination of ELISA and flow cytometry. Analysis of secreted cytokines demonstrated that under neutral and Th2-polarizing conditions, there was a specific requirement for NF- $\kappa$ B1 expression in the induction of IL-5 (Fig. 2A), IL-9 (Fig. 2B), and IL-13 (Fig. 2C). Therefore, NF- $\kappa$ B1-dependent signals are essential for Th2 cell differentiation under neutral and Th2 polarizing conditions. In contrast, under Th1-polarizing conditions, equivalent IFN- $\gamma$  production was observed in WT and NF- $\kappa$ B1<sup>-/-</sup> cultures (Fig. 2D). Despite the defect in Th2 cell differentiation, similar proliferative profiles, with four distinct generations of proliferating CD4<sup>+</sup> T cells, were observed in WT and NF- $\kappa$ B1<sup>-/-</sup> CD4<sup>+</sup> T cell cultures (Fig. 2E), demonstrating that impaired expression of Th2 cytokines in NF- $\kappa$ B1<sup>-/-</sup> cell cultures was not the result of dysregulated CD4<sup>+</sup> T cell proliferation. Analysis of CD4<sup>+</sup> T cell proliferation vs acquisition of effector cytokines confirmed that under Th2-polarizing conditions the frequency of proliferating CD4<sup>+</sup> T cells expressing IL-4 was significantly lower than observed in WT CD4<sup>+</sup> T cells (Fig. 2F, WT, 14%; NF- $\kappa$ B1<sup>-/-</sup>, 6%) ( $p < 0.05$ ), whereas similar frequencies of proliferating WT and NF- $\kappa$ B1<sup>-/-</sup> CD4<sup>+</sup> T cells stained positive for IFN- $\gamma$  under Th1-polarizing conditions (Fig. 2G, WT, 66%; NF- $\kappa$ B1<sup>-/-</sup>, 65%). Taken together, the in vivo challenge with Schistosome eggs coupled with the in vitro T cell differentiation assay demonstrate that CD4<sup>+</sup> T cell proliferation and IFN- $\gamma$  responses are not impaired in the absence of NF- $\kappa$ B1, although there is a specific requirement for NF- $\kappa$ B1 expression in promoting optimal Th2 cell differentiation.

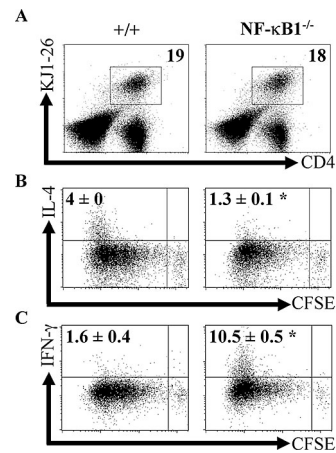
### NF- $\kappa$ B1<sup>-/-</sup> APC fail to promote Ag-specific Th2 cell differentiation

NF- $\kappa$ B1 has been reported to regulate expression of GATA-3 and Th2 cell function (18) and is likely, at least in part, to explain the defect in Th2 cell differentiation observed above. However, NF- $\kappa$ B



**FIGURE 2.** Defective Th2 cell differentiation in NF- $\kappa$ B1<sup>-/-</sup> CD4<sup>+</sup> T cells. WT and NF- $\kappa$ B1<sup>-/-</sup> spleen cells were isolated, labeled with CFSE and polyclonally stimulated under neutral (N) polarizing Th1, or Th2 conditions for 4 d (A–D). Supernatants were collected and assayed for secretion of IL-5 (A), IL-9 (B), IL-13 (C), and IFN- $\gamma$  (D) by ELISA. Dilution of CFSE was used to determine the number of generations of proliferating CD4<sup>+</sup> T cells (E) and staining for intracellular cytokines (IL-4 (F) and IFN- $\gamma$  (G)) allowed analysis of the percentage of proliferating CD4<sup>+</sup> T cells staining positive for effector cytokines under Th2 (F) or Th1 (G) polarizing conditions. \*, Significant difference between WT and NF- $\kappa$ B1<sup>-/-</sup> ( $p < 0.05$ ). Results are expressed as the mean  $\pm$  SEM of two to three animals per group and are representative of three to four independent experiments.

family members can also influence multiple DC functions, including expression of costimulatory molecules and proinflammatory cytokines (19). To test whether the requirement for NF- $\kappa$ B1 expression in Th2 cell differentiation was intrinsic to the T cell lineage or whether NF- $\kappa$ B1 can regulate APC function during a Th2 response, an adoptive transfer model in which purified NF- $\kappa$ B-sufficient OVA-specific DO11.10 TCR Tg CD3<sup>+</sup> T cells were transferred into syngeneic WT or NF- $\kappa$ B1<sup>-/-</sup> recipients was used. Following administration of OVA, in vivo T cell proliferation and commitment to IL-4 or IFN- $\gamma$  production were determined by flow cytometry. Equivalent frequencies of OVA-specific T cells (stained with the clonotypic marker KJ1-26) were observed in the draining LN of WT and NF- $\kappa$ B1<sup>-/-</sup> recipients (Fig. 3A) and analysis of OVA-specific CD4<sup>+</sup> T cell proliferation (visualized by in vivo dilution of CFSE) demonstrated that equivalent expansion of OVA-specific KJ1-26<sup>+</sup> CD4<sup>+</sup> T cells occurred in WT and NF- $\kappa$ B1<sup>-/-</sup> recipients (Fig. 3, B and C). Despite equivalent expansion, there were significant differences in the patterns of OVA-specific Th cell differentiation between WT and NF- $\kappa$ B1<sup>-/-</sup> recipients. WT APC promoted IL-4 expression in donor T cells (Fig. 3B) and minimal expression of IFN- $\gamma$  (Fig. 3C) whereas NF- $\kappa$ B1<sup>-/-</sup> APC failed to promote OVA-specific Th2 cell differentiation (Fig. 3B). Further, the frequency of OVA-specific CD4<sup>+</sup> T cells staining positive for IFN- $\gamma$  was >5-fold higher in NF- $\kappa$ B1<sup>-/-</sup> compared with WT recipients (Fig. 2C; WT, 1.6%; NF- $\kappa$ B1<sup>-/-</sup>, 10.5%). The defect in OVA-specific Th2 cell differentiation observed in NF- $\kappa$ B1<sup>-/-</sup> mice was independent of a defect in recipient T cells as

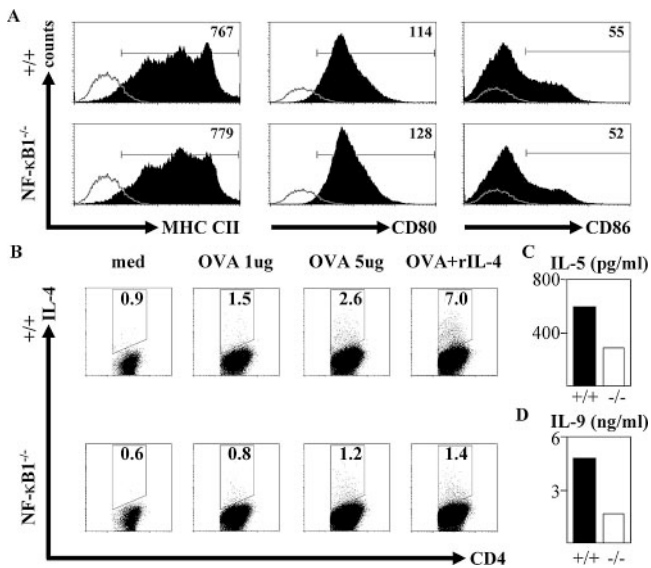


**FIGURE 3.** Defective OVA-specific CD4<sup>+</sup> Th2 cell differentiation in NF- $\kappa$ B1<sup>-/-</sup> mice. Purified OVA-specific DO11.10 TCR Tg T cells were labeled with CFSE and adoptively transferred into syngeneic WT or NF- $\kappa$ B1<sup>-/-</sup> recipients. Recipient animals received OVA peptide in IFA 1 day later. Draining LN cells were isolated 4 days after administration of OVA and surface stained for CD4 and OVA-specific TCR (KJ1-26) (A) and intracellular IL-4 (B) or IFN- $\gamma$  (C). \*, Significant difference between WT and NF- $\kappa$ B1<sup>-/-</sup> ( $p < 0.05$ ). Results are expressed as the mean  $\pm$  SEM of two to three animals per group and are representative of four independent experiments.

neither WT nor NF- $\kappa$ B1<sup>-/-</sup> recipient T cells were making significant levels of IL-4 (<1% in both genotypes), and there was no defect in OVA-specific Th2 responses observed in NF- $\kappa$ B-sufficient SCID recipients (data not shown). Therefore, NF- $\kappa$ B1<sup>-/-</sup> APC can promote efficient in vivo CD4<sup>+</sup> T cell proliferation and expansion but fail to deliver signals required to instruct Ag-specific Th2 cell differentiation.

#### NF- $\kappa$ B1<sup>-/-</sup> DC fail to induce Ag-specific Th2 cell differentiation

To test whether the APC-intrinsic requirement for NF- $\kappa$ B1 expression in promoting Th2 cell differentiation was specific to DC or involved other accessory cell populations, WT and NF- $\kappa$ B1<sup>-/-</sup> bone marrow-derived DC were generated, pulsed with OVA, and cocultured with purified NF- $\kappa$ B1-sufficient OVA-specific DO11.10 TCR Tg CD3<sup>+</sup> T cells. There was no significant difference in surface expression of MHC class II, CD40, CD80, and CD86 in resting CD11c<sup>+</sup> WT and NF- $\kappa$ B1<sup>-/-</sup> DC (Fig. 4A and data not shown). Following coculture with OVA-specific T cells, OVA-pulsed WT DC efficiently promoted Ag-specific expansion of CD4<sup>+</sup> T cells producing IL-4 in a dose-dependent manner, with maximal induction of OVA-specific Th2 cells occurring in the presence of exogenous IL-4 (Fig. 4B). In contrast, under neutral and Th2-polarizing conditions, the percentage of OVA-specific T cells producing IL-4 was up to 4-fold lower in cultures exposed to NF- $\kappa$ B1<sup>-/-</sup> DC compared with WT DC (Fig. 4B). Analysis of culture supernatants also revealed reduced levels of the other signature Th2 cytokines IL-5 (Fig. 4C) and IL-9 (Fig. 4D) in T cell cultures exposed to NF- $\kappa$ B1<sup>-/-</sup> vs WT DC. Therefore, consistent with results presented in Fig. 2, even in the presence of exogenous IL-4, DC-derived NF- $\kappa$ B1-dependent signals are required for optimal Th2 cell differentiation. Under neutral and Th2-permissive conditions, no IFN- $\gamma$  was detected in culture supernatants exposed to either NF- $\kappa$ B1<sup>-/-</sup> or WT DC (data not shown). These results



**FIGURE 4.** NF- $\kappa$ B1<sup>-/-</sup> DC fail to promote OVA-specific CD4<sup>+</sup> Th2 cell differentiation in vitro. GM-CSF-enriched WT and NF- $\kappa$ B1<sup>-/-</sup> bone marrow-derived DC were generated, and surface expression of MHC class II, CD80, and CD86 on CD11c<sup>+</sup> DC was determined by flow cytometry (A). Numbers refer to mean fluorescence intensity. DC were pulsed overnight with OVA peptide and cocultured with purified OVA-specific DO11.10 TCR Tg T cells for 4 days. Cells were stained for surface CD4, intracellular IL-4, and analyzed by flow cytometry (B). Numbers refer to percentages of CD4<sup>+</sup> T cells expressing IL-4. Supernatants were collected from OVA-pulsed (5  $\mu$ g/ml) DC:T cell cultures in the presence of IL-4 and assayed for IL-5 (C) and IL-9 (D) secretion by ELISA. Results are representative of two to three independent experiments.

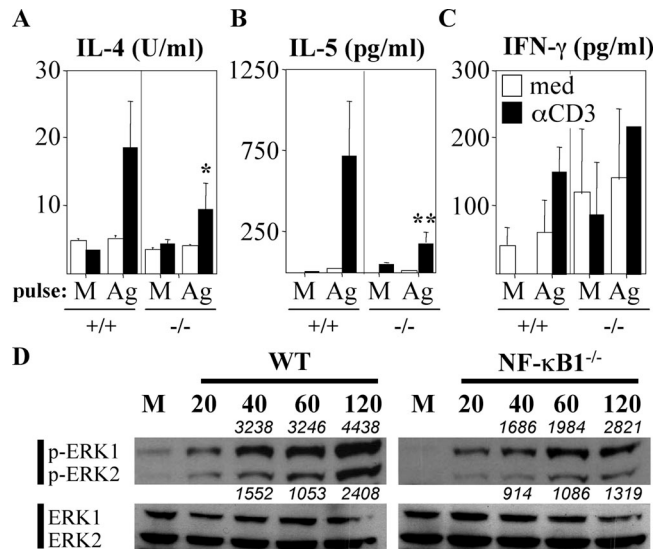
demonstrate that within the APC compartment, DC-intrinsic expression of NF- $\kappa$ B1 is required for programming of DC capable of promoting Th2 cell differentiation.

#### NF- $\kappa$ B1<sup>-/-</sup> DC fail to induce SEA-specific Th2 responses

To test whether DC-intrinsic expression of NF- $\kappa$ B1 is required in an in vivo physiologically relevant Th2 response, WT or NF- $\kappa$ B1<sup>-/-</sup> DC were pulsed with SEA, and their ability to prime for Th2 responses following adoptive transfer into syngeneic WT mice was assessed. Schistosome eggs are potent inducers of Th2 responses and in previous studies SEA-pulsed WT DC, although showing minimal up-regulation of classical activation markers were able to prime for Th2 cytokine production (17, 20, 21). Although no significant differences in expression of MHC class II, CD80, CD86, or CD40 were observed between SEA-pulsed WT and NF- $\kappa$ B1<sup>-/-</sup> DC (data not shown), the ability of NF- $\kappa$ B1<sup>-/-</sup> DC to prime for IL-4 and IL-5 production was lower than WT counterparts (Fig. 5, A and B). WT and NF- $\kappa$ B1<sup>-/-</sup> DC induced low, but similar, IFN- $\gamma$  responses (Fig. 5C). Together with the defective OVA-specific Th2 responses detailed above, these results demonstrate that DC-intrinsic expression of NF- $\kappa$ B1 is critical in governing the competence of DC to drive *S. mansoni*-specific Th2 responses in vivo.

#### DC-intrinsic expression of NF- $\kappa$ B1 regulates helminth Ag-induced MAPK phosphorylation

Previous studies have shown that conditioning of DC by Th2-inducing stimuli, including components of SEA and other helminth-derived Ag, is associated with MAPK phosphorylation (11, 12). MAPK, including ERK1 and ERK2, are effector kinases that



**FIGURE 5.** DC-intrinsic expression of NF- $\kappa$ B1 regulates helminth Ag-induced Th2 cytokine responses and MAPK phosphorylation. GM-CSF-enriched WT and NF- $\kappa$ B1<sup>-/-</sup> bone marrow-derived DC were pulsed with SEA (Ag) and adoptively transferred into syngeneic WT mice. Spleen cells were isolated 7 days after transfer, and the magnitude of in vivo priming for IL-4 (A), IL-5 (B), and IFN- $\gamma$  (C) production was determined by ELISA. Levels of phosphorylated (p-Thr/p-Tyr) and total ERK1 and ERK2 (D) in SEA-pulsed DC were determined by Western blotting. Band intensities are shown in italics. Significant difference between WT and NF- $\kappa$ B1<sup>-/-</sup> (\*,  $p < 0.07$ ; \*\*,  $p < 0.05$ ). Results are expressed as the mean  $\pm$  SEM of three to four animals per group (in vivo studies) and are representative of two to three independent experiments.

cooperatively transactivate a wide range of immune response genes (22). Given that the NF- $\kappa$ B pathway has been implicated in regulating MAPK activation (23, 24), we sought to determine whether SEA-induced MAPK phosphorylation was altered in NF- $\kappa$ B1<sup>-/-</sup> DC. Although levels of total MAPK (Fig. 5D) were equivalent in WT and NF- $\kappa$ B1<sup>-/-</sup> DC extracts following exposure to SEA, MAPK phosphorylation was defective in NF- $\kappa$ B1<sup>-/-</sup> DC. Exposure of WT DC to SEA led to a time-dependent increase in phosphorylated ERK1 and ERK2 (Fig. 5D), although phosphorylation was modest compared with LPS stimulation (data not shown). In contrast, Ag-pulsed NF- $\kappa$ B1<sup>-/-</sup> DC exhibited reduced ERK1 and ERK2 phosphorylation, particularly at 60–120 min poststimulation (Fig. 5D). Taken together, these studies implicate NF- $\kappa$ B1 in regulating DC-intrinsic MAPK activation and their ability to drive Th2 cell differentiation.

## Discussion

Multiple transcription factors have been implicated in determining the ability of DC to drive Th1 responses; however, no DC-intrinsic transcription factors are known to govern the competence of DC to instruct Th2 cell differentiation. The results presented in this report demonstrate that expression of NF- $\kappa$ B1 within DC is required to promote optimal Th2 cytokine responses. Critically, NF- $\kappa$ B1<sup>-/-</sup> DC efficiently promoted CD4<sup>+</sup> T cell proliferation and IFN- $\gamma$  production, demonstrating the specificity of DC-intrinsic NF- $\kappa$ B1 expression in controlling Th2 cell priming.

NF- $\kappa$ B1 can determine DC function directly through activation or repression of gene transcription or indirectly by acting as an

upstream regulator of other signaling cascades such as MAPK. The observation that impaired priming for Th2 cytokine responses by NF- $\kappa$ B1<sup>-/-</sup> DC following exposure to Schistosome Ags is associated with reduced activation of the MAPK provides evidence that the latter pathway is operating in DC. The ability of NF- $\kappa$ B1 to regulate MAPK activation through the sequestration of Tpl2 (an upstream kinase required for optimal MAPK activation) in a stable and inactive complex has been well characterized (23). Macrophages deficient in NF- $\kappa$ B1 exhibited defective expression of Tpl2 and reduced MAPK activation (23). Furthermore, mice deficient in Tpl2 exhibited skewed Th1 responses and defective Ag-specific IL-4 responses (25) similar to those observed in NF- $\kappa$ B1<sup>-/-</sup> mice in the present study. Therefore, we propose that following exposure to Th2-inducing stimuli, NF- $\kappa$ B1 expression is critical in regulating the stability and function of upstream components of the MAPK pathway and that NF- $\kappa$ B1-dependent MAPK activation is critical in determining the ability of DC to drive Th2 responses. Consistent with this model, possible functions of NF- $\kappa$ B-dependent MAPK activation in Schistosome Ag-conditioned DC include the induction of factors that would suppress proinflammatory signals (12, 22, 26) and allow instruction of Th2 cell differentiation. Alternatively, these factors may control proliferative arrest of CD4<sup>+</sup> T cells, another pathway that has been proposed to influence Th2 cell differentiation (21).

NF- $\kappa$ B1<sup>-/-</sup> DC were also unable to drive Th2 cell differentiation in coculture studies with OVA-specific T cells and suggest that activation of the MAPK may not be the only mechanism through which NF- $\kappa$ B1 regulates DC responses during ongoing Th2 responses. Indeed, in a recent study, expression of Jagged (a family of Notch ligands) on the surface of DC was shown to provide instructive signals for Th2 cell differentiation (7). Previous studies have shown that NF- $\kappa$ B can regulate expression of Jagged (27), therefore NF- $\kappa$ B1 may also be critical in regulating expression of Jagged or Delta family members and so influencing the ability of DC to prime for Th2 cytokine responses. Alternatively, NF- $\kappa$ B1 may be essential in transducing signals delivered to DC following Jagged-Notch interactions, although there is little known about this pathway at present.

Whether DC populations that promote Th2 responses develop as a "default" in the absence of proinflammatory stimuli or arise due to specific recognition of, and activation by, Th2-inducing stimuli remains a controversial issue (2, 28). The demonstration that conditioning of DC capable of driving Th2 responses requires NF- $\kappa$ B1-dependent events, coupled with previous studies showing that Th2 responses do not necessarily develop in the absence of proinflammatory signals (20, 29, 30), support the contention that DC-driven Th2 cytokine responses are not the product of default programming.

The demonstration that NF- $\kappa$ B1 governs the ability of DC to prime optimal Th2 cytokine responses also provides new insights into the regulation of Th2-mediated inflammation. For instance, previous observations that Th2 cytokine responses are impaired in NF- $\kappa$ B1<sup>-/-</sup> mice following exposure to helminth infection or induction of airway hyperresponsiveness (31, 32), attributed predominantly to defective GATA-3 expression in CD4 T cells (18), are also likely to be influenced by the inability of NF- $\kappa$ B1<sup>-/-</sup> DC to prime for Th2 cell differentiation at mucosal sites. In this context, the results of the present study highlight the therapeutic potential of manipulating NF- $\kappa$ B1 to regulate specific DC functions. For instance, blockade of NF- $\kappa$ B1 may prove valuable in DC-based therapies for chronic Th2 cytokine-mediated pathologies such as allergy, autoimmunity, and forms of inflammatory bowel disease that have proven refractory to conventional therapies.

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## Disclosures

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