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Regulator of Dendritic Cell Function**

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## CUTTING EDGE

Cutting Edge: NF- $\kappa$ B2 Is a Negative Regulator of Dendritic Cell Function<sup>1</sup>Kendra Speirs,\* Linda Lieberman,\* Jorge Caamano,<sup>†</sup> Christopher A. Hunter,\* and Phillip Scott<sup>2\*</sup>

*RelB, a member of the NF- $\kappa$ B family of transcription factors, is essential for dendritic cell (DC) maturation. Recent findings indicate that RelB is exclusively regulated through its interaction with cytoplasmic NF- $\kappa$ B2/p100. The studies presented in this report show that DCs lacking NF- $\kappa$ B2 have dramatically enhanced RelB activity, associated with increased MHC class II and costimulatory molecule expression and an enhanced ability to induce CD4<sup>+</sup> T cell responses. These studies identify a novel role for NF- $\kappa$ B2 in the negative regulation of RelB-induced DC maturation, with critical consequences for the regulation of adaptive immune responses. The Journal of Immunology, 2003, 171: 752–756.*

Dendritic cells (DCs)<sup>3</sup> have the unique capacity to initiate primary T cell responses and host defense against infection. Upon maturation, DCs up-regulate MHC class II and costimulatory molecule expression and produce high levels of the proinflammatory cytokines TNF- $\alpha$ , IL-1, IL-6, and IL-12. However, the intracellular signaling mechanisms that regulate DC maturation remain poorly defined. Recently, activation of the NF- $\kappa$ B family was shown to correlate with DC maturation in both mice and humans (1, 2). Indeed, DC Ag presentation could be blocked using adenoviral transfer of the endogenous inhibitor of NF- $\kappa$ B, I $\kappa$ B $\alpha$ , suggesting an absolute requirement for this transcription factor in DC function (2, 3).

There are five known NF- $\kappa$ B family members, NF- $\kappa$ B1 (p50 and its precursor p105), NF- $\kappa$ B2 (p52 and its precursor p100), RelA, RelB, and c-Rel, which are maintained in the cytoplasm as dimers bound to one of several inhibitory proteins of the I $\kappa$ B family or the precursor proteins p105 and p100 (4). Phosphorylation of the NF- $\kappa$ B inhibitor protein targets it for degradation, thus enabling NF- $\kappa$ B dimers to translocate to the nucleus and activate gene transcription (4). Analysis of mice deficient in individual NF- $\kappa$ B components has revealed unique roles for each family member in cellular function. The combined absence of NF- $\kappa$ B1 and RelA, for example, results in dramatic

defects in DC development, whereas the absence of NF- $\kappa$ B1 and c-Rel leads to impaired DC survival and IL-12 production (5). Requirements for RelB are implicated in the development of splenic CD11c<sup>+</sup>CD8<sup>-</sup> DCs, and in the maturation and Ag presentation of bone marrow-derived DCs (6, 7).

In contrast to other NF- $\kappa$ B components, whose activity is primarily regulated by association with I $\kappa$ B, a recent report finds that RelB is exclusively repressed by NF- $\kappa$ B2/p100 in HeLa cells (8). Considering the importance of RelB in DC maturation, we asked whether p100 also represses RelB activity in DCs. We show that RelB is highly active in NF- $\kappa$ B2/p100 knockout (KO) DCs and correlate dysregulated RelB activity with alterations in DC function. In the absence of NF- $\kappa$ B2, DCs are hyperactivated, showing increased MHC class II and costimulatory molecule expression both constitutively and in response to stimulation. As a consequence, NF- $\kappa$ B2 KO DCs are dramatically more efficient than wild-type (WT) DCs at inducing CD4<sup>+</sup> T cell activation. Our report provides the first demonstration of a biological requirement for NF- $\kappa$ B2/p100 in the negative regulation of DC maturation and development of adaptive immune responses.

## Materials and Methods

## Mice

C57BL/6 and C57BL/6 Thy1.1 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). OT-II mice were described previously (9). NF- $\kappa$ B2 KO mice on a C57BL/6 (eight generations) background were described previously (10).

## Bone marrow DC generation

DCs were generated as previously described (11). Briefly, bone marrow monocytes from NF- $\kappa$ B2 KO and WT B/6 mice were seeded in petri dishes at  $2 \times 10^5$  cells/ml in 10 ml of medium. At day 3, 10 ml of medium containing 20 ng/ml GM-CSF was added. At days 6 and 8, 10 ml of culture supernatant was removed and replaced with 10 ml of fresh medium containing 20 ng/ml GM-CSF. For generation of immature DCs, supernatant was removed at day 10 and replaced with only 5 ng/ml GM-CSF. Cells were harvested at day 11 and were 95% enriched for DCs (CD11c<sup>+</sup>).

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<sup>3</sup> Abbreviations used in this paper: DC, dendritic cell; KO, knockout; WT, wild type; BCL-3, B cell lymphoma 3.

### Western blot

NF- $\kappa$ B2 KO and WT DCs (day 11) were rested overnight and stimulated for 60 min with 100 ng/ml LPS (L6143; Sigma-Aldrich, St. Louis, MO). Cytoplasmic and nuclear extracts were isolated using an iso-osmotic/Nonidet P-40 lysis protocol as previously described (12). Nuclear translocation was assessed by SDS-PAGE and transferred to nitrocellulose. The membrane was blotted for RelB (C-19; Santa Cruz Biotechnology, Santa Cruz, CA), washed, and incubated with donkey anti-rabbit-HRP (Amersham Pharmacia, Arlington Heights, IL). The signal was detected using ECL chemiluminescence reagent (Amersham Pharmacia).

### EMSA

NF- $\kappa$ B2 KO and WT DCs (day 11) were incubated for 1 h with 100 ng/ml LPS. Nuclear extracts were prepared as previously described (13). The double-stranded oligodeoxynucleotides corresponding to the palindromic B site (5'-GGGAATTCCC-3') were used in this assay. EMSAs were performed as described previously (14). Supershift was conducted using anti-RelB (SC 226X) from Santa Cruz Biotechnology. Equivalent free probe was detected in all wells, confirming that probe was added in excess to each sample. Gel was exposed for 2 h without supershift and 12 h following the addition of anti-RelB.

### DC cytokine production and expression of cell surface proteins

NF- $\kappa$ B2 KO and WT DCs were primed for 4 h with 10 U/ml recombinant murine IFN- $\gamma$  and stimulated in the presence of 100 ng/ml LPS or 10  $\mu$ g/ml anti-CD40 (clone 323; BD Pharmingen, San Diego, CA). Supernatants were harvested after 72 h, and IL-12 p40, IL-6, TNF- $\alpha$ , and IL-1 $\beta$  levels were measured by ELISA, as previously described (15). Expression of DC surface molecules was quantified by flow cytometry using CD11c-FITC and MHC class II (I-A<sup>b</sup>), CD80, or CD86-PE-conjugated Abs (BD Pharmingen). Samples were analyzed using a FACSCalibur flow cytometer and CellQuest software (BD Biosciences, San Jose, CA).

### LPS injection and splenic DC analysis

LPS (L4641; Sigma-Aldrich) was i.p. injected into NF- $\kappa$ B2 KO and WT mice. After 24 h, spleens were removed and digested with 400 U/ml liberase (Roche Applied Science, Indianapolis, IN) for 30 min at 37°C. Single-cell suspensions were prepared and stained for CD11c-FITC and MHC class II (I-A<sup>b</sup>), CD80, or CD86-PE-conjugated Ab (BD Pharmingen). Samples were analyzed by flow cytometry.

### DC priming ability in vitro

NF- $\kappa$ B2 KO and WT DCs were stimulated with 1 or 10  $\mu$ g endotoxin-free OVA (Worthington, Lakewood, NJ) in the presence or absence of 100 ng/ml LPS. CD3<sup>+</sup> T cells were purified from OT-II spleens using T cell enrichment columns (R&D Systems, Minneapolis, MN). T cell proliferation was analyzed by labeling the cells with CFSE (Molecular Probes, Eugene, OR) and culturing them with the DCs at a 1:10 DC:T cell ratio (16). After 72 h, the T cells were harvested, and intracellular staining for IFN- $\gamma$  was performed, as previously described (17). Cells were washed and analyzed by flow cytometry.

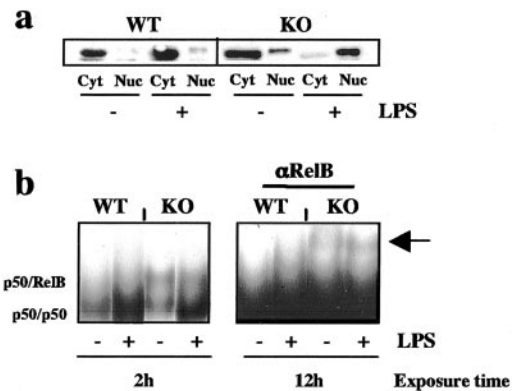
### DC priming ability in vivo

CD3<sup>+</sup> T cells from OT-II mice were purified and CFSE labeled. A total of 5  $\times$  10<sup>6</sup> cells was transferred by i.v. injection into C57BL/6 Thy 1.1 mice. Twenty-four hours later, PBS or OVA- and LPS-treated NF- $\kappa$ B2 KO or WT DCs were i.p. injected into the T cell-recipient mice. After 72 h, spleens were removed from the T cell- and DC-co-injected mice, and single-cell suspensions were prepared. Cells were surface stained with anti-CD4-CyChrome and anti-Thy1.2-PE (BD Pharmingen), and cell division was analyzed by flow cytometry.

## Results

### RelB is highly activated in NF- $\kappa$ B2 KO DCs

RelB is the primary NF- $\kappa$ B family member required for the maturation of myeloid DCs (7, 18). Because NF- $\kappa$ B2/p100 is exclusively responsible for the inhibition of RelB activity in HeLa cells, we predicted that RelB would be more active in NF- $\kappa$ B2 KO DCs (8). To test this, NF- $\kappa$ B2 KO and WT DCs were stimulated with LPS, and cytoplasmic and nuclear extracts were harvested to measure RelB activation. High levels of RelB were present in the nuclei of unstimulated NF- $\kappa$ B2 KO vs WT DCs and were further elevated in response to LPS treatment (Fig. 1a).



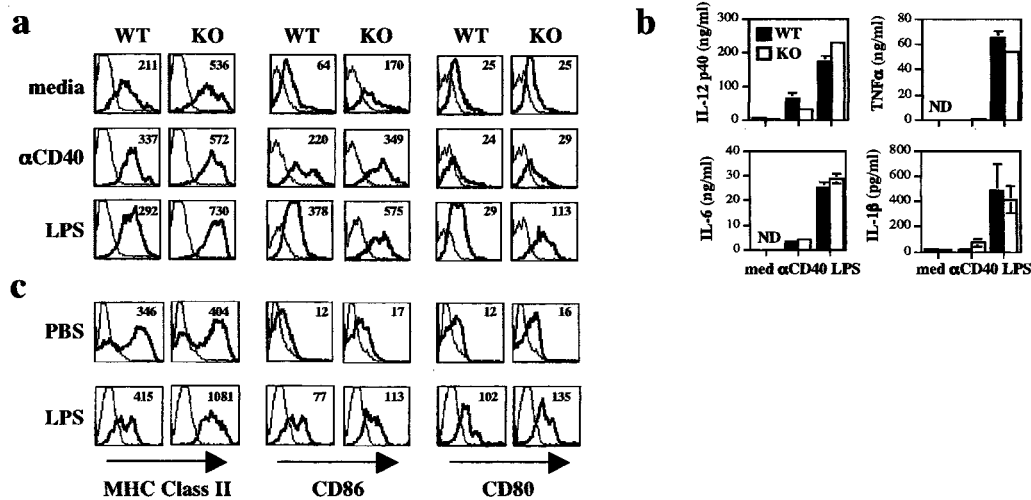
**FIGURE 1.** RelB is activated in resting and mature NF- $\kappa$ B2 KO DCs. Bone marrow DCs were activated for 1 h with LPS. *a*, Nuclear and cytoplasmic extracts were isolated, and nuclear translocation was assessed by immunoblotting with a RelB-specific mAb. *b*, Nuclear extracts were incubated with a  $\kappa$ B oligonucleotide, and the DNA binding ability of RelB was analyzed by EMSA. Gel was exposed for 2 h without supershift and 12 h following the addition of anti-RelB. Arrow indicates the RelB supershift. Data shown are representative of two experiments.

To determine whether RelB was active and able to bind DNA, nuclear extracts were incubated with a  $\kappa$ B oligonucleotide and analyzed by gel shift. As shown in Fig. 1*b*, constitutive  $\kappa$ B binding activity was enhanced following LPS treatment, with NF- $\kappa$ B2 KO and WT DCs showing a similar increase in a fast-migrating complex composed of p50/p50 homodimers (5). Nuclear extracts from NF- $\kappa$ B2 KO DCs revealed an additional slow-migrating band that was likely composed of p50/RelB heterodimers (Fig. 1*b*). Prolonged exposure of the gel and addition of specific antisera for RelB confirmed the presence of this transcription factor in NF- $\kappa$ B2 KO but not WT DCs (Fig. 1*b*, arrow). This is particularly noteworthy given that RelB is often difficult to detect by gel shift analysis (14, 19). Addition of antisera for NF- $\kappa$ B1, RelA, and c-Rel revealed no differences in the activation of these transcription factors in NF- $\kappa$ B2 KO and WT DCs (data not shown). These data illustrate that NF- $\kappa$ B2/p100 specifically inhibits RelB activation in DCs, and raise the question of how DC function may be altered in the absence of this control mechanism.

### NF- $\kappa$ B2 KO DCs show enhanced expression of activation markers but produce normal levels of cytokines

Because RelB is required for the maturation of bone marrow DCs, we asked whether dysregulated RelB activity alters this process. To test this, we compared the phenotype of NF- $\kappa$ B2 KO and WT DCs. Ten days after generation with GM-CSF, NF- $\kappa$ B2 KO and WT cultures revealed a large number of cells with characteristic DC morphology and a comparable percentage of CD11c<sup>+</sup> cells, suggesting that DCs develop normally in the absence of NF- $\kappa$ B2 (data not shown). To assess the maturation phenotype of NF- $\kappa$ B2 KO DCs, we quantitated the surface expression of MHC class II, CD86, and CD80 after stimulation with anti-CD40 or LPS. NF- $\kappa$ B2 KO DCs had a higher baseline expression of class II and CD86 than WT DCs, and were more responsive to both stimuli (Fig. 2*a*). In contrast, NF- $\kappa$ B2 KO DCs produced normal levels of IL-12 p40, IL-6, TNF- $\alpha$ , and IL-1 $\beta$ , suggesting that NF- $\kappa$ B2 is dispensable for DC cytokine production (Fig. 2*b*). These observations suggest





**FIGURE 2.** NF- $\kappa$ B2 regulates bone marrow and splenic DC maturation. *a*, Bone marrow DCs were stimulated with anti-CD40 or LPS. After 72 h, DCs were surface stained for CD11c, MHC class II, CD86, and CD80, and analyzed by flow cytometry. Plots are gated on CD11c<sup>+</sup> cells, and values represent the mean fluorescence intensity of the experimental groups (bold). Also shown are the isotype controls for each surface marker (fine). *b*, Supernatants were collected and IL-12 p40, IL-6, TNF- $\alpha$ , and IL-1 $\beta$  production was quantitated by ELISA. Data are means  $\pm$  SD of three mice and are representative from one of five experiments. *c*, Splenocytes were harvested 24 h following *in vivo* stimulation with LPS. Cells were surface stained for CD11c, MHC class II, CD86, and CD80, and analyzed by flow cytometry. Plots are gated on CD11c<sup>+</sup> cells, and values represent the mean fluorescence intensity of the experimental groups (bold). Also shown are the isotype controls for each surface marker (fine). Data are representative of three experiments.

that NF- $\kappa$ B2/p100 represses the expression of class II and costimulatory molecules, and that the two measures of DC maturation, activation marker up-regulation and proinflammatory cytokine production, are differentially controlled by NF- $\kappa$ B signaling.

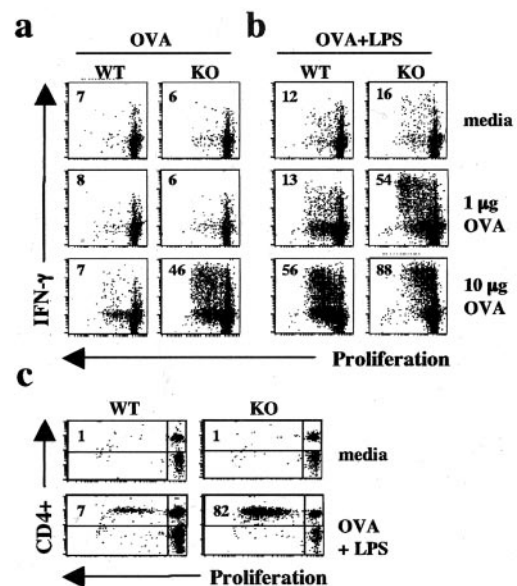
To determine whether NF- $\kappa$ B2 performs a similar function in endogenous DC populations, we investigated the *in vivo* effects of LPS on splenic DCs from NF- $\kappa$ B2 KO and WT mice. Indeed, NF- $\kappa$ B2 KO splenic DCs were more sensitive to LPS than WT DCs, as evidenced by enhanced activation marker expression (Fig. 2c). Our data show that NF- $\kappa$ B2 acts as a negative regulator of DC maturation in two distinct DC populations, and support an important role for this transcription factor in mediating immune responses.

*OVA-pulsed NF- $\kappa$ B2 KO DCs exhibit an increased capacity to stimulate CD4<sup>+</sup> T cells *in vitro**

To determine whether the enhanced maturation status of NF- $\kappa$ B2 KO DCs has functional relevance, we analyzed how effectively these cells activate Ag-specific T cell responses. NF- $\kappa$ B2 KO and WT DCs were pulsed with OVA (1 or 10  $\mu$ g) in the presence or absence of LPS, and cultured with CFSE-labeled OT-II T cells *in vitro*. After 72 h, the T cells were harvested and analyzed for their ability to proliferate and produce IFN- $\gamma$ . WT DCs pulsed with 10  $\mu$ g of OVA failed to promote T cell activation; however, OVA-pulsed NF- $\kappa$ B2 KO DCs induced strong T cell proliferation and IFN- $\gamma$  production (Fig. 3a). These data correlate with the increased baseline expression levels of class II and CD86 and suggest that NF- $\kappa$ B2 KO DCs can present Ag even in the absence of microbial stimulation. OVA- and LPS-primed NF- $\kappa$ B2 KO DCs also revealed an enhanced capacity to activate T cell responses, as illustrated by the high levels of IFN- $\gamma$  produced by the responding T cells (Fig. 3b). No differences were observed in the survival of NF- $\kappa$ B2 KO and WT DCs (data not shown). These results demonstrate that DCs induce a stronger Ag-specific T cell response in the absence of NF- $\kappa$ B2.

*OVA-pulsed NF- $\kappa$ B2 KO DCs exhibit an increased capacity to stimulate CD4<sup>+</sup> T cells *in vivo**

To determine whether NF- $\kappa$ B2 KO DCs can also promote enhanced T cell responses *in vivo*, we transferred fluorescently labeled OT-II T cells into congenic Thy1.1 mice. Twenty-four



**FIGURE 3.** NF- $\kappa$ B2 KO DCs reveal an enhanced capacity to prime T cells. *a* and *b*, Bone marrow DCs were pulsed overnight with OVA alone (1 or 10  $\mu$ g) (*a*) or OVA plus LPS (*b*), and cultured with CFSE-labeled OT-II T cells. After 72 h, T cells were stained for CD4 and intracellular IFN- $\gamma$  and analyzed by flow cytometry. Shown are the percentages of proliferating CD4<sup>+</sup> T cells producing IFN- $\gamma$ . Data are representative of three experiments. *c*, CFSE-labeled OT-II T cells were transferred *i.v.* into Thy 1.1 congenic mice. After 24 h, the same animals were injected *i.p.* with OVA- and LPS-pulsed DCs. After 72 h, spleens were removed from the recipient mice, stained for Thy1.2 and CD4, and analyzed by flow cytometry. Shown is the percentage of Thy1.2<sup>+</sup>CD4<sup>+</sup> T cells that proliferated *in vivo*. Data are representative of three experiments.

hours later, these mice were injected with unactivated or OVA- and LPS-activated NF- $\kappa$ B2 KO or WT DCs. The splenocytes were recovered after 72 h and surface stained for Thy1.2 and CD4 expression. T cells from mice receiving untreated DCs were unable to promote T cell proliferation *in vivo* (Fig. 3*c*). Importantly, T cells from mice receiving activated NF- $\kappa$ B2 KO DCs proliferated 10 times more effectively than T cells from mice receiving activated WT DCs (Fig. 3*c*). Together, these experiments provide functional relevance for the enhanced maturation status of NF- $\kappa$ B2 KO DCs and define a role for NF- $\kappa$ B2/p100 in the control of RelB activation and Ag presentation.

## Discussion

In this report, we define a novel role for NF- $\kappa$ B2 in the negative regulation of DC maturation. Our results show for the first time that NF- $\kappa$ B2/p100 represses RelB in DCs, and suggest that this regulatory pathway may function to prevent DC hyperactivation. Indeed, nuclear RelB levels are elevated in both resting and activated NF- $\kappa$ B2 KO DCs, a result that correlates with increased expression of the activation markers, MHC class II, CD86, and CD80. We show that the enhanced maturation status of NF- $\kappa$ B2 KO DCs increases their ability to induce Ag specific CD4<sup>+</sup> T cell responses. This work illustrates that NF- $\kappa$ B2 is essential for maintaining DCs in an immature state, a finding that is critical to our understanding of DC biology.

Despite the close association between RelB activation and DC maturation, there may be alternative explanations for the enhanced maturation status of NF- $\kappa$ B2 KO DCs. In addition to RelB, p100 was recently shown to associate with the death-inducing signaling complex and aid in promoting Fas-induced apoptosis (20). Although these findings are yet to be extended *in vivo*, it might be predicted that immature DCs lacking p100 are less sensitive to apoptosis and readily available to mature. However, this possibility is unlikely, because NF- $\kappa$ B2 and WT DCs have similar rates of survival (data not shown). Another factor known to interact with NF- $\kappa$ B2 is B cell lymphoma 3 (BCL-3). Although it is a member of the I $\kappa$ B family, BCL-3 has the unique ability to form a transactivating complex with p52/p52 homodimers (21). The defects in splenic microarchitecture observed in BCL-3-deficient mice are similar to those associated with the absence of NF- $\kappa$ B2 and suggest that the relationship between these factors has *in vivo* relevance (22, 23). BCL-3 is highly expressed in maturing DCs, although the specific genes induced by p52/p52/BCL-3 complexes, and their potential role in preventing DC maturation, remain to be defined (1).

We hypothesize that the hyperactivation of NF- $\kappa$ B2 KO DCs is accounted for by dysregulated RelB activity. Elevated RelB levels are similarly associated with the enhanced maturation status of DCs found in nonobese diabetic mice (24). In addition, hyperactive DCs may contribute to cutaneous T cell lymphomas that are shown to result from mutations in NF- $\kappa$ B2/p100 (25, 26). Indeed, mice deficient in the p100 portion of NF- $\kappa$ B2 fail to regulate lymphocyte activation, a phenotype that manifests itself as splenomegaly and gastric hyperplasia, and results in early postnatal death (27). The lymphoproliferation observed is associated with an increase in p52, p50, and RelB-containing complexes in nuclear extracts from sev-

eral tissues. The maturation status of p100-deficient DCs and their contribution to T cell hyperactivation and lymphoma formation remains to be determined. However, there is evidence that the clonal proliferation of T cells in cutaneous lymphomas occurs in response to specific antigenic stimulation from DCs (28).

Finally, our finding that NF- $\kappa$ B2 is a critical negative regulator of DC function may have important clinical implications. On the one hand, inhibitors of NF- $\kappa$ B2 could enhance DC Ag-presenting function in vaccination therapies designed to boost T cell responses to cancer or infection (29). Alternatively, drugs might be designed to prevent the degradation of p100 and subsequent activation of RelB, thus promoting tolerance and preventing the hyperactive DC phenotypes associated with certain autoimmune diseases.

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## References

- Neumann, M., H. Fries, C. Scheicher, P. Keikavoussi, A. Kolb-Maurer, E. Brocker, E. Serfling, and E. Kampgen. 2000. Differential expression of Rel/NF- $\kappa$ B and octamer factors is a hallmark of the generation and maturation of dendritic cells. *Blood* 95:277.
- Yoshimura, S., J. Bondeson, B. M. Foxwell, F. M. Brennan, and M. Feldmann. 2001. Effective antigen presentation by dendritic cells is NF- $\kappa$ B dependent: coordinate regulation of MHC, co-stimulatory molecules and cytokines. *Int. Immunol.* 13:675.
- Rescigno, M., M. Martino, C. L. Sutherland, M. R. Gold, and P. Ricciardi-Castagnoli. 1998. Dendritic cell survival and maturation are regulated by different signaling pathways. *J. Exp. Med.* 188:2175.
- Ghosh, S., and M. Karin. 2002. Missing pieces in the NF- $\kappa$ B puzzle. *Cell* 109(Suppl.):S81.
- Ouaaz, F., J. Arron, Y. Zheng, Y. Choi, and A. A. Beg. 2002. Dendritic cell development and survival require distinct NF- $\kappa$ B subunits. *Immunity* 16:257.
- Wu, L., A. D'Amico, K. D. Winkel, M. Suter, D. Lo, and K. Shortman. 1998. RelB is essential for the development of myeloid-related CD8 $\alpha^+$  dendritic cells but not of lymphoid-related CD8 $\alpha^+$  dendritic cells. *Immunity* 9:839.
- Zanetti, M., P. Castiglioni, S. Schoenberger, and M. Gerlioni. 2003. The role of *relB* in regulating the adaptive immune response. *Ann. NY Acad. Sci.* 987:249.
- Solan, N. J., H. Miyoshi, E. M. Carmona, G. D. Bren, and C. V. Paya. 2002. RelB cellular regulation and transcriptional activity are regulated by p100. *J. Biol. Chem.* 277:1405.
- Barnden, M. J., J. Allison, W. R. Heath, and F. R. Carbone. 1998. Defective TCR expression in transgenic mice constructed using cDNA-based  $\alpha$ - and  $\beta$ -chain genes under the control of heterologous regulatory elements. *Immunol. Cell Biol.* 76:34.
- Speirs, K., J. Caamano, M. H. Goldschmidt, C. A. Hunter, and P. Scott. 2002. NF- $\kappa$ B2 is required for optimal CD40-induced IL-12 production but dispensable for Th1 cell differentiation. *J. Immunol.* 168:4406.
- Lutz, M. B., N. Kukutsch, A. L. Ogilvie, S. Rossner, F. Koch, N. Romani, and G. Schuler. 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J. Immunol. Methods* 223:77.
- Jones, D. E., L. U. Buxbaum, and P. Scott. 2000. IL-4-independent inhibition of IL-12 responsiveness during *Leishmania amazonensis* infection. *J. Immunol.* 165:364.
- Lernbecher, T., U. Muller, and T. Wirth. 1993. Distinct NF- $\kappa$ B/Rel transcription factors are responsible for tissue-specific and inducible gene activation. *Nature* 365:767.
- Caamano, J., J. Alexander, L. Craig, R. Bravo, and C. A. Hunter. 1999. The NF- $\kappa$ B family member RelB is required for innate and adaptive immunity to *Toxoplasma gondii*. *J. Immunol.* 163:4453.
- Mosmann, T. R., and T. A. Fong. 1989. Specific assays for cytokine production by T cells. *J. Immunol. Methods* 116:151.
- Lyons, A. B., and C. R. Parish. 1994. Determination of lymphocyte division by flow cytometry. *J. Immunol. Methods* 171:131.
- Openshaw, P., E. E. Murphy, N. A. Hosken, V. Maino, K. Davis, K. Murphy, and A. O'Garra. 1995. Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 populations. *J. Exp. Med.* 182:1357.
- Koski, G. K., L. A. Lyakh, P. A. Cohen, and N. R. Rice. 2001. CD14<sup>+</sup> monocytes as dendritic cell precursors: diverse maturation-inducing pathways lead to common activation of NF- $\kappa$ B/RelB. *Crit. Rev. Immunol.* 21:179.
- Pettit, A. R., C. Quinn, K. P. MacDonald, L. L. Cavanagh, G. Thomas, W. Townsend, M. Handel, and R. Thomas. 1997. Nuclear localization of RelB is associated with effective antigen-presenting cell function. *J. Immunol.* 159:3681.

20. Wang, Y., H. Cui, A. Schroering, J. L. Ding, W. S. Lane, G. McGill, D. E. Fisher, and H. F. Ding. 2002. NF- $\kappa$ B2 p100 is a pro-apoptotic protein with anti-oncogenic function. *Nat. Cell Biol.* 4:888.
21. Bours, V., G. Franzoso, V. Azarenko, S. Park, T. Kanno, K. Brown, and U. Siebenlist. 1993. The oncoprotein Bcl-3 directly transactivates through  $\kappa$ B motifs via association with DNA-binding p50B homodimers. *Cell* 72:729.
22. Franzoso, G., L. Carlson, T. Scharon-Kersten, E. W. Shores, S. Epstein, A. Grinberg, T. Tran, E. Shacter, A. Leonardi, M. Anver, et al. 1997. Critical roles for the Bcl-3 oncoprotein in T cell-mediated immunity, splenic microarchitecture, and germinal center reactions. *Immunity* 6:479.
23. Franzoso, G., L. Carlson, L. Poljak, E. W. Shores, S. Epstein, A. Leonardi, A. Grinberg, T. Tran, T. Scharon-Kersten, M. Anver, et al. 1998. Mice deficient in nuclear factor (NF)- $\kappa$ B/p52 present with defects in humoral responses, germinal center reactions, and splenic microarchitecture. *J. Exp. Med.* 187:147.
24. Poligone, B., D. J. Weaver, Jr., P. Sen, A. S. Baldwin, Jr., and R. Tisch. 2002. Elevated NF- $\kappa$ B activation in nonobese diabetic mouse dendritic cells results in enhanced APC function. *J. Immunol.* 168:188.
25. Migliazza, A., L. Lombardi, M. Rocchi, D. Trecca, C. C. Chang, R. Antonacci, N. S. Fracchiolla, P. Ciana, A. T. Maiolo, and A. Neri. 1994. Heterogeneous chromosomal aberrations generate 3' truncations of the NF- $\kappa$ B2/lyr-10 gene in lymphoid malignancies. *Blood* 84:3850.
26. Ciana, P., A. Neri, C. Cappellini, F. Cavallo, M. Pomati, C. C. Chang, A. T. Maiolo, and L. Lombardi. 1997. Constitutive expression of lymphoma-associated NF- $\kappa$ B-2/Lyt-10 proteins is tumorigenic in murine fibroblasts. *Oncogene* 14:1805.
27. Ishikawa, H., D. Carrasco, E. Claudio, R. P. Ryseck, and R. Bravo. 1997. Gastric hyperplasia and increased proliferative responses of lymphocytes in mice lacking the COOH-terminal ankyrin domain of NF- $\kappa$ B2. *J. Exp. Med.* 186:999.
28. Edelson, R. L. 2001. Cutaneous T cell lymphoma: the helping hand of dendritic cells. *Ann. NY Acad. Sci.* 941:1.
29. Dhodapkar, M. V., R. M. Steinman, M. Sapp, H. Desai, C. Fossella, J. Krasovsky, S. M. Donahoe, P. R. Dunbar, V. Cerundolo, D. F. Nixon, and N. Bhardwaj. 1999. Rapid generation of broad T-cell immunity in humans after a single injection of mature dendritic cells. *J. Clin. Invest.* 104:173.