**Brief Definitive Report**

**Interleukin 12–dependent Interferon γ Production by CD8α+ Lymphoid Dendritic Cells**

By Toshiaki Ohteki,* Taro Fukao,* Kazutomo Suzue,* Chikako Maki,* Mamoru Ito,‡ Masataka Nakamura,§ and Shigeo Koyasu*+

From the *Department of Immunology, Keio University School of Medicine, Shinjuku-ku, Tokyo 160-8582, Japan; the ‡Laboratory of Immunology, Central Institute for Experimental Animals, Kawasaki 216-0001, Japan; and the §Human Gene Sciences Center, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113, Japan

**Summary**

We investigated the role of antigen-presenting cells in early interferon (IFN)-γ production in normal and recombinase activating gene 2–deficient (Rag-2<sup>−/−</sup>) mice in response to *Listeria monocytogenes* (LM) infection and interleukin (IL)-12 administration. Levels of serum IFN-γ in Rag-2<sup>−/−</sup> mice were comparable to those of normal mice upon either LM infection or IL-12 injection. Depletion of natural killer (NK) cells by administration of anti-asialoGM1 antibodies had little effect on IFN-γ levels in the sera of Rag-2<sup>−/−</sup> mice after LM infection or IL-12 injection. Incubation of splenocytes from NK cell–depleted Rag-2<sup>−/−</sup> mice with LM resulted in the production of IFN-γ that was completely blocked by addition of anti–IL-12 antibodies. Both dendritic cells (DCs) and monocytes purified from splenocytes were capable of producing IFN-γ when cultured in the presence of IL-12. Intracellular immunofluorescence analysis confirmed the IFN-γ production from DCs. It was further shown that IFN-γ was produced predominantly by CD8α<sup>+</sup> lymphoid DCs rather than CD8α<sup>−</sup> myeloid DCs. Collectively, our data indicated that DCs are potent in producing IFN-γ in response to bacterial infection and play an important role in innate immunity and subsequent T helper cell type 1 development in vivo.

Key words: recombinase-activating gene 2 knockout mouse • nonobese diabetic-severe combined immunodeficiency disease mouse • γ<sub>c</sub> knockout mouse • natural killer cells • *Listeria monocytogenes*

**Dendritic cells (DCs)** are bone marrow (BM)-derived professional APCs. Peripheral DCs are characterized by high capability for antigen capture and processing, migration to lymphoid organs, and expression of various costimulatory molecules for antigen-specific lymphocyte activation. Cytokine secretion by DCs initiates and enhances both innate and acquired immunity (1).

Activation of macrophages and DCs by infectious agents leads to secretion of IL-12, which subsequently induces IFN-γ production by NK cells and directs Th1 development. IFN-γ, in turn, acts on monocytes to augment IL-12 secretion and to produce nitric oxide that eradicates infected microbes (2, 3). Thus, IL-12 and IFN-γ comprise a positive feedback system, which is probably required for optimal production of IL-12 in vivo (4, 5). Studies using neutralizing Abs against IFN-γ and mice deficient for IL-12 or IFN-γ have confirmed the importance of these cytokines for innate immunity and Th1 development for controlling intracellular pathogens (6-11).

It was generally assumed that the only cells producing IFN-γ in response to IL-12 are NK and T cells. However, recent studies have shown that IFN-γ is also produced by peritoneal macrophages in response to IL-12 and by BM-derived macrophages in response to a combination of IL-12 and IL-18, suggesting the presence of an autocrine activation pathway (12, 13).

In the study presented here, we examined IFN-γ production pathways in NK cell-depleted recombinase activating gene 2–deficient (Rag-2<sup>−/−</sup>) mice upon *Listeria monocytogenes* (LM) infection or IL-12 administration. We found that the levels of IFN-γ produced in the sera of these mice were unaltered as compared with those of Rag-2<sup>−/−</sup> mice with NK cells, suggesting an important role for a non-T, -B, and/or -NK cell type(s) in producing IFN-γ in vivo. We show here that purified DCs were capable of producing significant amounts of IFN-γ in response to IL-12. Among DCs, CD8α<sup>+</sup> lymphoid DCs are the major producers of IFN-γ. Thus, DCs produce IFN-γ in an autocrine manner by re-
**Materials and Methods**

**Mice.** B10.D2 and C57BL/6 mice were purchased from Sankei Labo Service Co. Inc. (Japan). B10.D2–R ag-2−/− mice, generated by backcross of R ag-2+/− mice to B10.D2 (n51) for 10 generations (reference 14 and unpublished data) were obtained from Taconic Farms. C57BL/6-Rag-2−/− mice which also lack the cytokine receptor common γ chain (hereafter C57BL/6-γc−/−(Y)) were collected after 72 h and subjected to ELISA.

**Production of IFN-γ in the Sera of NK Cell-depleted Rag-2−/− Mice.** Several studies have demonstrated that IFN-γ produced by NK cells and Th1 cells is a crucial cytokine for limiting and clearing infectious intracellular agents such as protozoan and bacterial pathogens (6-8, 19-22). To examine the role of NK cells in the early production of IFN-γ, we injected polyclonal αASGM1 Abs into B10.D2 or B10.D2–R ag-2−/− mice to deplete NK cells as previously demonstrated (23). Consistent with previous studies, NK cells were absent in the spleen of both B10.D2 and B10.D2–R ag-2−/− mice 3 d after administration of 300 μg αASGM1 Ab (Fig. 1A). These mice were then injected with 2 × 10^5 LM as described above. Titers of IFN-γ in serum and culture supernatants were determined by Quantikine Kit (R&D Systems).

**Results**

Production of IFN-γ in vivo. 0.5 μg IL-12 or 2 × 10^6 LM were intraperitoneally injected into mice. In vivo experiments, cells were cultured in the presence of 1 ng/ml IL-12 for 72 h or 4 × 10^5 LM as described above. Titers of IFN-γ in the sera and culture supernatants were determined by Quantikine Kit (R&D Systems).

**Intracellular Immunofluorescence Analysis.** Immunofluorescence staining of intracellular IFN-γ was conducted as previously described (18). Sorted DCs were grown on coverslips coated with Cell-Tak (Becton Dickinson Labware) and fixed for 15 min with 3% paraformaldehyde in PBS. After surface staining with FITC-conjugated mAbs against CD11c, these cells were permeabilized with 0.5% saponin/1% BSA in PBS for 30 min. Cells were further incubated with polyclonal rabbit anti-mouse IFN-γ Ab (Pestka Biomedical Labs.), polyclonal rabbit anti-mouse IL-12 β Ab (Santa Cruz Biotechnology, Inc.), or normal rabbit serum as a negative control. Specimens were further developed with rhodamine-conjugated goat anti-rabbit IgG (ICN Pharmaceuticals, Inc.). Samples on coverslips were mounted onto glass slides with Mowiol (Calbiochem Corp.) and examined under a fluorescence microscope Axiovert 100 (Carl Zeiss, Inc.) equipped with an image analysis system (Signal Analytics Co.).
suggest that the contribution of NK cells to early IFN-γ production in response to LM infection or IL-12 administration is minimal. We further examined C57BL/6-γc−/−Rag-2−/− mice lacking T, B, and NK cells, as well as NOD/LtSz-scid/scid mice lacking T and B cells and having functional defects in NK cells and monocytes/macrophages (16). After 24 h of IL-12 administration, only a small amount of serum IFN-γ was detected in C57BL/6-γc−/−Rag-2−/− mice, whereas substantial amounts of IFN-γ were produced by NOD/LtSz-scid/scid mice. IFN-γ production in NOD/LtSz-scid/scid mice was also unaffected by pretreatment with αASGM1 Ab (Fig. 1 B, bottom, and data not shown).

To identify the IFN-γ-producing cells in αASGM1-treated Rag-2−/− mice, splenocytes were prepared from mice treated with αASGM1 and infected LM in vitro. As shown in Fig. 1 C, IFN-γ was produced by Rag-2−/− splenocytes in the absence of NK cells, and the production of IFN-γ was completely blocked by the addition of anti–IL-12 Ab, indicating that IL-12 plays a critical role in IFN-γ production upon LM infection. These results further indicate the presence of IFN-γ-producing cells other than T, B, and NK cells. In contrast, amounts of IFN-γ produced by C57BL/6-γc−/−Rag-2−/− splenocytes were 1–5% of those from NK-depleted Rag-2−/− splenocytes upon either Listeria infection or IL-12 administration, suggesting that IFN-γ production is impaired in C57BL/6-γc−/−Rag-2−/− mice.

IFN-γ Production by DCs. To further identify IFN-γ-producing cells, DCs as well as macrophages and NK cells were freshly isolated from collagenase-treated spleen cells of unprimed mice by cell sorting. Highly purified CD11c+ I-A−, M*Ac1-F4/80+, and CD3 NK1.1+ cells were used as DCs, macrophages, and NK cells, respectively (Fig. 2 A and data not shown). These cells were cultured for 3 d in the presence of 1 ng/ml IL-12 in vitro. As shown in Fig. 2 B, significant amounts of IFN-γ were detected in the culture supernatants of DCs and macrophages. The amounts of IFN-γ from DCs and macrophages were significantly higher than those from NK cells. DCs cultured in the absence of IL-12 produced IFN-γ to a certain level, probably due to the cross-linking of surface MHC class II molecules by the use of anti-I-A mAb for DC preparation (25). Consistent with this interpretation, DCs purified using anti-CD86 mAb (26) instead of anti–I-A mAb did not produce IFN-γ without IL-12 (see Fig. 3 B).

**Figure 1.** IFN-γ production in the sera of NK cell–depleted Rag-2−/− mice. (A) 300 μg αASGM1 Ab were injected into B10.D2 and B10.D2-Rag-2−/− mice. Spleen cells were collected on day 3 and stained for 145-2C11–PE (anti-CD3ε) and DX5-FITC (anti-pan NK). (B) Saline, 0.5 μg IL-12, or 2 × 106 LM were intraperitoneally injected into B10.D2 or B10.D2-Rag-2−/− mice with or without treatment with αASGM1 3 d before injection (top), or into C57BL/6-γc−/−Rag-2−/− and NOD/LtSz-scid/scid mice (bottom). Sera were collected 24 h later from IL-12-injected mice and 48 h later from LM-infected and control mice, and the amounts of IFN-γ were measured by ELISA. (C) In vitro IFN-γ production from NK cell–depleted Rag-2−/− splenocytes, LM. 106 collagenase-treated splenocytes were obtained from NK cell–depleted B10.D2, B10.D2-Rag-2−/−, and C57BL/6-γc−/−Rag-2−/− mice. The cells were infected with 4 × 105 LM and cultured for 3 d with or without either 10 μg/ml anti-IL-12 mAb (top) or 1 ng/ml IL-12 (bottom). The amounts of IFN-γ were measured by ELISA.

**Figure 2.** IFN-γ production from isolated DCs. (A) Isolation of splenic DCs CD11c+ I-A− DCs were isolated from C57BL/6 splenocytes on a FACS Vantage™, and the purity of the cells was checked. (B) IFN-γ production in culture supernatants of DCs, macrophages, and NK cells, respectively (Fig. 2 A and data not shown). These cells were cultured for 3 d in the presence of 1 ng/ml IL-12 in vitro. As shown in Fig. 2 B, significant amounts of IFN-γ were detected in the culture supernatants of DCs and macrophages. The amounts of IFN-γ from DCs and macrophages were significantly higher than those from NK cells. DCs cultured in the absence of IL-12 produced IFN-γ to a certain level, probably due to the cross-linking of surface MHC class II molecules by the use of anti-I-A mAb for DC preparation (25). Consistent with this interpretation, DCs purified using anti-CD86 mAb (26) instead of anti-I-A mAb did not produce IFN-γ without IL-12 (see Fig. 3 B).
The CD8α+ Lymphoid DC Population Is a Major Source of IFN-γ Production. There are two different types of DCs in the spleen of an adult mouse (27–30). They differ in surface phenotypes (CD8α−DEC-205−CD11b+ versus CD8α+ DEC-205+CD11b+), origin (myeloid versus lymphoid), requirement of cytokines for their development (GM-CSF versus IL-3), and biological function. To this end, we examined IFN-γ production from DC subpopulations CD8α− DCs (myeloid DCs) and CD8α+ DCs (lymphoid DCs) were isolated by cell sorting and cultured with 1 ng/ml IL-12 for 3 d. As shown in Fig. 3, CD8α+ DCs were found to produce an approximately fivefold higher level of IFN-γ than do CD8α− DCs, indicating that CD8α+ lymphoid DCs are the major IFN-γ producers in response to IL-12 stimulation.

Detection of Intracellular IFN-γ in DCs. Immunofluorescence microscopy was conducted to directly detect the expression of IFN-γ protein in DCs. Purified CD11c+ CD86+ splenic DCs were cultured in the presence of IL-12 for 3 d, fixed on coverslips, and subjected to intracellular immunofluorescence microscopic analysis. As shown in Fig. 4, IFN-γ proteins were clearly detected in the cytoplasm of CD11c+ DCs (Fig. 4, A and D), whereas staining was undetectable with the control rabbit serum (Fig. 4, C and F). Consistent with a previous study (31), expression of IL-12Rγ was readily observed on the cell surface of DCs (Fig. 4, B and E). IFN-γ was not detected in freshly isolated DCs but was detected in splenic macrophages upon IL-12 stimulation by immunofluorescence microscopy (data not shown).

Discussion

We presented here evidence that NK cells play a small role in the production of IFN-γ at early stages of LM infection or IL-12 administration, and that DCs and macrophages produce IFN-γ. Among DC subpopulations, CD8α+ lymphoid DCs are major producers of IFN-γ in response to IL-12. Recent studies have also reported the ability of macrophages to produce IFN-γ (12, 13). Amounts of IFN-γ produced by DCs and macrophages were substantially larger than the amount produced by NK cells.

It has long been assumed that IL-12 is initially produced by macrophages in response to various intracellular pathogens and later by DCs (32, 33), based on the observations that DCs produce IL-12 through ligation of CD40 on DCs by CD40L on activated T cells, or through cross-linking of MHC class II molecules by the TCR (25, 34). However, it has been shown recently that phagocytosis of microparticle-adsorbed proteins stimulates DCs to synthesize IL-12 without interacting with T cells (35), and that DCs but not macrophages produce IL-12 in vivo in microbial infection such as Toxoplasma gondii (36). Furthermore, accumulating evidence has indicated that resting macrophages are unable to produce IL-12 in response to bacteria or microbial products such as LPS without prior activation by certain cytokines such as IFN-γ (4, 37, 38).

In this paper we showed that DCs are able to produce IFN-γ upon IL-12 stimulation. Because DCs produce IL-12 upon phagocytosis and microbial infection, and IL-12 in turn augments the production of IL-12 itself from DCs (31, 35, 36), it is likely that DCs produce IL-12 and IFN-γ by an autocarmin role once they have been triggered by microbial infection. The fact that the addition of anti-IL-12 Ab completely blocked the IFN-γ production by NK cell-depleted Ag-2−/− splenocytes upon LM infection supports...
this notion. In addition to IL-12, IL-18 and IL-1β are also likely to be involved in augmenting IFN-γ production from DCs in vivo, as observed in T and NK cells (39, 40).

Our results on C57BL/6-γc−/−(γ)Rag-2−/− mice are consistent with a recent paper by Andersson et al. that reports that γc−/−(γ)Rag-2−/− mice produce minimal amount of IFN-γ (41). Since these mice lack NK cells as well as T and B cells, it was concluded that NK cells are the major producers of IFN-γ. Flow cytometric analysis showed the presence of normal numbers of DCs and macrophages in the spleens of C57BL/6-γc−/−(γ)Rag-2−/− mice (Ohteki, T., and S. Koyasu, unpublished results). It is likely that APCs in the γc−/−(γ)Rag-2−/− mice have some functional rather than developmental defects that remain to be examined.

It is likely that the IFN-γ derived from DCs plays a key role in priming and activating macrophages to produce IL-12 in response to intracellular pathogens. DC-derived IFN-γ, together with IL-12, may also be important in up-regulation of surface molecules on DCs such as MHC class II. Once IL-12 and IFN-γ are produced by DCs, a positive feedback pathway(s) would be activated between DCs and macrophages even in the absence of NK cell-derived IFN-γ. M. acrophiases then secrete IFN-γ in response to IL-12 or a combination of IL-12 and IL-18 (12, 13), which also activates macrophages in an autocrine manner to produce nitric oxide. In microbial infection such pathways would be quicker than the pathway through NK cell-derived IFN-γ, and thus important, although not sufficient, for an early stage of innate immune response.

DCs are divided into at least two subpopulations by origins, surface molecules, and the requirement of cytokines for their development (27–30). One subpopulation is myeloid DCs without CD8α expression, and the second is lymphoid DCs expressing CD8α. It has been shown that CD8α+ lymphoid DCs primarily produce IL-12 in vivo in intracellular protozoan infection (36). Given that the CD8α+ DCs produce IFN-γ in response to IL-12 (Fig. 3) and predominantly localized in the T cell area of the spleen (30), lymphoid CD8α+ DCs rather than myeloid CD8α− DCs are probably the most efficient initiators for innate immune response upon infection of intracellular microorganisms, as well as the directors of subsequent Th1 differentiation in vivo.

We thank Dr. M. Mitsuyama for providing LM, and Drs. N. Hozumi and J. Hata for providing NOD/Lts1-scid/scid mice. We also thank A. Sakurai for excellent animal care.

This work was supported by a grant from the KANAE Foundation for Life & Socio-Medical Science to T. Ohteki, a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports and Culture of Japan (10153261), a National Grant-in-Aid for the Establishment of a High-Tech Research Center in a Private University, a Keio University Special Grant-in-Aid for Innovative Collaborative Research Projects, and a grant from the Japan Society for the Promotion of Science (JSPS-R FT F 97L00701) to S. Koyasu. S. Suzue is supported by a Research Fellowship of the Japan Society for the Promotion of Science for Young Scientists.

Address correspondence to Shigeo Koyasu, Department of Immunology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. Phone: 81-3-3353-1746; Fax: 81-3-5361-7658; E-mail: koyasu@sun.microb.med.keio.ac.jp

Received for publication 1 February 1999 and in revised form 19 April 1999.

References

1. Banchereau, J., and R.M. Steinman. 1998. Dendritic cells and the control of immunity. Nature. 392:245–252.
2. Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu. Rev. Immunol. 13:251–276.
3. Maclarking, J., Q.-W. Xie, and C. Nathan. 1997. Nitric oxide and macrophage function. Annu. Rev. Immunol. 15:323–350.
4. M. A., J.M. Chow, G. Giri, F. Carra, S.F. Gerosa, R. Woffs, R. Dziallo, and G. Trinchieri. 1996. The interleukin 12 p40 gene promoter is primed by interferon-γ in monocytic cells. J. Exp. Med. 12:187–157.
5. Kubin, M., J.M. Chow, and G. Trinchieri. 1994. Differential regulation of interleukin-12 (IL-12), tumor necrosis factor-α, and IL-1β production in human myeloid leukemia cell lines and peripheral blood mononuclear cells. Blood. 83:1847–1855.
6. Buchmeier, N.A., and R.D. Schreiber. 1985. Requirement of endogenous interferon-γ production for resolution of Listeria monocytogenes infection. Proc. Natl. Acad. Sci. USA. 82:7404–7408.
7. Harty, J.T., and M.J. Bevan. 1995. Specific immunity to Listeria monocytogenes in the absence of IFN-γ. Immunity. 3:109–117.
8. Dai, W.J., W. Bartens, G. Kohler, M. Hufnagel, M. Kopf, and F. Brombacher. 1997. Impaired macrophage listericidal and cytokine activities are responsible for the rapid death of Listeria monocytogenes-infected IFN-γ receptor-deficient mice. J. Immunol. 158:5297–5304.
9. Magram, J., S.E. Connaughton, R.R. Warner, D.M. Carvajal, C.Y. Wu, J. Ferrante, C. Stewart, U. Sarmiento, D.A. Faherty, and M.K. Gately. 1996. IL-12-deficient mice are defective in IFN-γ production and type 1 cytokine responses. Immunity. 4:471–481.
10. Magram, J., J. Sfarra, S. Connaughton, D.A. Faherty, R. War-}

11. Harty, J.T., and M.J. Bevan. 1995. Specific immunity to Listeria monocytogenes in the absence of IFN-γ. Immunity. 3:109–117.
12. Dai, W.J., W. Bartens, G. Kohler, M. Hufnagel, M. Kopf, and F. Brombacher. 1997. Impaired macrophage listericidal and cytokine activities are responsible for the rapid death of Listeria monocytogenes-infected IFN-γ receptor-deficient mice. J. Immunol. 158:5297–5304.
13. Magram, J., S.E. Connaughton, R.R. Warner, D.M. Carvajal, C.Y. Wu, J. Ferrante, C. Stewart, U. Sarmiento, D.A. Faherty, and M.K. Gately. 1996. IL-12-deficient mice are defective in IFN-γ production and type 1 cytokine responses. Immunity. 4:471–481.
12. Puddu, P., L. Fantuzzi, P. Borghi, V. Barbara, G. Rinaldi, E. Guillemand, W. Malorni, P. Nicaise, S.F. Wolf, F. Bellardelli, and S. Gessani. 1997. IL-12 induces IFN-γ expression and secretion in mouse peritoneal macrophages. J. Immunol. 159:3490–3497.

13. Munder, M., M. Malo, K. Eichmann, and M. Modolell. 1998. M urine macrophages secrete interferon-γ upon combined stimulation with interleukin (IL)-12 and IL-18: a novel pathway of autocrine macrophage activation. J. Exp. Med. 187:2103–2108.

14. Shinkai, Y., G. Rathbun, K.-P. Lam, E.M. Oltz, V. Stewart, M. Endoelson, J. Charron, M. Datta, F. Young, A.M. Stall, and F.W. Alt. 1992. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. Cell 68:855–867.

15. Ohbo, K., T. Suda, I. Hasiyama, M. Azuma, H. Yagita, K. Okumura, P.S. Linsley, S. Sakuta, M. Marukawa, S. K. Ikehara, et al. 1994. The tissue distribution of the B7-2 costimulatory molecule and its function as a modulator of immune responses. J. Exp. Med. 180:1849–1860.

16. Shultz, L.D., P.A. Schweitzer, S.W. Christianson, B. Gott, L.A. Herzenberg, C. Blackwell, and S.H. Kaufmann. 1995. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. J. Immunol. 154:180–191.

17. Steenman, R.M., W.C. Van Voorhis, and D.M. Spalding. 1986. Dendritic cells. In Handbook of Experimental Immunology. D.W. Weir, L.A. Herzenberg, C. Blackwell, and S.H. Kaufmann. 1996:178–224.

18. Rovere, P., V.S. Zimmermann, F. Forquet, D. Demandolx, J. Trucy, P. Ricciardi-Castagnoli, and J. Davoust. 1998. Dendritic cell maturation and antigen presentation in the absence of MHC class II and CD40 molecules and downregulation by IL-12 production by murine dendritic cells: upregulation via pathway of autocrine macrophage activation. J. Exp. Med. 184:741–747.

19. Bancroft, G.J., R.D. Schreiber, and E.R. Unanue. 1991. Natural immunity: A T cell independent pathway of macrophage activation, defined in the scid mouse. Immunol. Rev. 124:5–24.

20. Laskay, T., R. Rollinghoff, and W. Solbach. 1993. Natura killer cell precursors in the early defense against Leishmania major infection in mice. Eur. J. Immunol. 23:2237–2241.

21. Scharton, T.M., and P. Scott. 1993. Natural killer cells are a source of interferon that drives differentiation of CD4+ T cell subsets and induces early resistance to Leishmania major in mice. J. Exp. Med. 178:567–577.

22. Denkers, E.Y., R.T. Gazzinelli, D. Martin, and A. Sher. 1993. Emergence of NK1.1+ cells as effectors of IFN-γ dependent immunity to Toxoplasma gondii in MHC class I-deficient mice. J. Exp. Med. 178:1465–1472.

23. Yu, Y.Y., V. Kummer, and M. Bennett. 1992. M urine natural killer and marrow graft rejection. Annu. Rev. Immunol. 10:189–213.

24. Yoshimoto, T., H. Okamura, Y.J. Tagawa, Y. Iwakura, and K. Nakashima. 1997. Interleukin 18 together with interleukin 12 inhibits IgE production by induction of interferon-gamma production from activated B cells. Proc. Natl. Acad. Sci. USA 94:3948–3953.

25. Koch, F., U. Stanzl, P. Jennewein, K. Janke, C. Heuffer, E. Kampgen, N. Romani, and G. Schuler. 1996. High level IL-12 production by murine dendritic cells upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. J. Exp. Med. 184:741–747.

26. Inaba, K., M. Witmer-Pack, M. Inaba, S. Hathcock, H. Sakuta, M. Azuma, H. Yagita, K. Okumura, P.S. Linsley, S. Ikehara, et al. 1994. The tissue distribution of the B7-2 costimulator in mice: abundant expression on dendritic cells in situ and during maturation in vitro. J. Exp. Med. 180:1849–1860.

27. Vremec, D., M. Zorbas, R. Scolly, D.J. Saunders C.F. Ardaivin, L. Wu, and K. Shortman. 1992. The surface phenotype of dendritic cells purified from mouse thymus and spleen: investigation of the CD8+ subset by expression of subpopulation of dendritic cells. J. Exp. Med. 176:47–58.

28. Yu, L., C.-L. Li, and K. Shortman. 1996. Thymic dendritic cell precursors relationship to the T-lymphocyte lineage and phenotype of the dendritic cell progeny. J. Exp. Med. 184:903–911.

29. Vremec, D., and K. Shortman. 1997. Dendritic cell subtypes in mouse lymphoid organs cross-correlation of surface markers, changes with incubation, and differences among thymus, spleen, and lymph nodes. J. Immunol. 159:565–573.

30. Steinman, R.M., M. Pack, and K. Inaba. 1997. Dendritic cells in the T-cell areas of lymphoid organs. Immunol. Rev. 156:25–37.

31. Grohmann, U., M. Belladonna, C. Bianchi, O. Rabona, E. Ayrola, M. Fioretti, and P. Puccetti. 1998. IL-12 acts directly on DC to promote nuclear localization of NF-κB and primes DC for IL-12 production. Immunity. 9:315–323.

32. Locksley, R.M. 1993. Interleukin 12 in host defense against microbial pathogens Proc. Natl. Acad. Sci. USA. 90:5879–5880.

33. Hseih, C.-S., T.E. Macatonia, C.S. Tripp, S.F. Wolf, A. O’Garra, and K.M. Murphy. 1993. Development of T1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages Science. 260:547–549.

34. Celli, M., D. Scheidenberger, K. Palmer-Lehmann, P. Lane, A. Lanzavecchia, and G. Alber. 1996. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-7 help via APC activation. J. Exp. Med. 184:747–752.

35. Sheisher, C., M. Mehlig, H.-P. Dielen, and K. Reske. 1995. Uptake of microparticle-adsorbed protein antigen by bone marrow-derived dendritic cells results in up-regulation of interleukin-1α and interleukin-12 p40/p35 and triggers prolonged, efficient antigen presentation. Eur. J. Immunol. 25:1566–1572.

36. Sousa, C.R., S. Hencry, T. Scharton-Kersten, D. Jankovic, H. Charest, R.N. Germann, and A. Sher. 1997. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. J. Exp. Med. 186:1819–1829.

37. Flesch, I.E., J.H. Hess, S. Huang, M. Aguet, J. Rothe, H. Bluthmann, and S.H. Kaufmann. 1995. Early interleukin 12 production by macrophages in response to mycobacterial infection depends on interferon-γ and tumor necrosis factor α. J. Exp. Med. 181:1615–1621.

38. Skeen, M.J., M.A. Miller, T.M. Shinnick, and H.K. Ziegler. 1996. Regulation of murine macrophage IL-12 production. Activation of macrophages in vivo restoration in vitro, and modulation by other cytokines. J. Immunol. 156:1196–1206.

39. Okamura, H., H. Tatsi, T. Komatsu, M. Yutsudo, A. Hakura, T. Tanimoto, K. Hattori, T. Ikehara, et al. 1995. Cloning of a new cytokine that induces IFN-γ production by T cells. Nature. 378:88–91.

40. Hunter, C.A., R. Chizzonite, and J.S. Remington. 1995. IL-1b is required for IL-12 to induce production of IL-1b by N K cells. J. Immunol. 155:4347–4354.

41. Anderson, A., W.J. Dai, J.P. Di Santo, and F. Brombacher. 1998. Early IFN-γ production and innate immunity during Listeria monocytogenes infection in the absence of N K cells. J. Immunol. 161:5600–5606.