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IL-4 Induces In Vivo Production of IFN-γ by NK and NKT Cells

Suzanne C. Morris,2*† Tatyana Orekhova,* Michelle J. Meadows,* Stephanie M. Heidorn,* Junqi Yang,* and Fred D. Finkelman*††

Although IL-4 and IFN-γ often have opposite effects and suppress each other’s production by T cells, IL-4 can stimulate IFN-γ production. To characterize this, we injected mice with IL-4 and quantified IFN-γ production with the in vivo cytokine capture assay. IL-4 induced Stat6-dependent IFN-γ production by NK and, to a lesser extent, NKT cells, but not conventional T cells, in 2–4 h. Increased IFN-γ production persisted at a constant rate for >24 h, but eventually declined, even with continuing IL-4 stimulation. This eventual decline in IFN-γ production was accompanied by a decrease in NK and T cell numbers. Consistent with a dominant role for NK cells in IL-4-stimulated IFN-γ secretion, IL-4 induction of IFN-γ was B and T cell-independent; suppressed by an anti-IL-2Rβ mAb that eliminates most NK and NKT cells; reduced in Stat4-deficient mice, which have decreased numbers of NK cells; and absent in Rag2/γ-double-deficient mice, which lack T, B, and NK cells. IL-4-induced IFN-γ production was not affected by neutralizing IL-12p40 and was increased by neutralizing IL-2. IL-13, which signals through the type 2 IL-4R and mimics many IL-4 effects, failed to stimulate IFN-γ production and, in most experiments, suppressed basal IFN-γ production. Thus, IL-4, acting through the type 1 IL-4R, induces Stat6-dependent IFN-γ secretion by NK and NKT cells. This explains how IL-4 can contribute to Th1 cytokine-associated immune effector functions and suggests how IL-13 can have stronger proallergic effects than IL-4. The Journal of Immunology, 2006, 176: 5299–5305.

Although the cytokines IL-4 and IFN-γ often have opposite effects and mutually suppress each other’s production by T cells (1–4), each of these cytokines can also stimulate the other’s production (5–10). IFN-γ can have a role in Th2 priming in vitro and in vivo (5), and IL-4 can contribute in vitro and in vivo to the priming of an IFN-γ response (8, 10, 11). The biological importance of IL-4 induction of IFN-γ production has been evident in infectious disease models (e.g., infection of mice with Candida albicans or Leishmania major) and transplantation models, where the absence of IL-4 leads to a defective Th1 or CTL response, and a short pulse with IL-4 at the time of immunization suppresses IL-4 production and stimulates the production of IFN-γ (9, 11, 12). Although the mechanism(s) for IL-4 enhancement of in vivo IFN-γ production is not fully known, the timing of this phenomenon and the ability of IL-4 to promote dendritic cell (DC)3 production of IL-12 have suggested that IL-4 induces IFN-γ production by first stimulating DC to make IL-12, which then acts on T cells and NK cells to promote IFN-γ production (6, 8, 11). However, because IL-4 does not promote IL-12 production in the absence of other stimuli, such as CD40 ligation (10, 13), any induction of IFN-γ production by IL-4 in immunized mice would probably result from an IL-12-independent mechanism. To determine whether IL-4 can induce IFN-γ production in vivo in the absence of purposeful immunization, we treated mice with a long-acting formulation of IL-4 (14) and evaluated in vivo IFN-γ production with the in vivo cytokine capture assay (IVCCA) (15, 16) and IFN-γ production by individual cells with a cellular cytokine capture assay (17). Our results reveal that that IL-4 acts rapidly through a Stat6-dependent, Stat4- and IL-12-independent mechanism to induce NK and NKT cells to produce IFN-γ, and that the IL-4-related cytokine, IL-13, lacks this effect.

Materials and Methods

Mice

Female BALB/c and C57BL/6 mice and male athymic nude mice were obtained from the National Cancer Institute, National Institutes of Health. Wild-type, Rag2-deficient, and Rag2/γ-double-deficient mice on a C57BL/10 background were obtained from Taconic Farms. Stat6-deficient (18) and Stat6-deficient (19) mice on a BALB/c background were a gift from Dr. M. Grusby (Harvard University, Cambridge, MA) and were bred in the animal facility of Cincinnati Veterans Administration Medical Center.

Antibodies

Goat anti-IL-12 Ab was a gift from Dr. M. Gately (provided while he was at Schering-Plough, Kenilworth, NJ). The IgG fractions of this Ab and control goat serum were purified by ammonium sulfate precipitation, followed by DE-52 ion exchange chromatography. The following rat IgG mAbs were produced as ascites in Pristane-primed (Acros Organics) athymic nude mice: R46A2 (anti-IFN-γ) (20), AN-18 (anti-IFN-γ) (21), BVD4-1D11 (anti-IL-4) (22), S4B6 (anti-IL-2) (23), TM-81 (anti-IL-2Rγ) (24), GL-117 (control rat IgG2a), and J1.2 (control rat IgGb).

Cytokines

Recombinant mouse IL-4 and IL-18 were purchased from PeproTech. Recombinant mouse IL-12 was a gift from Dr. M. Gately. Recombinant mouse IL-13 was a gift from Dr. D. Donaldson (Wyeth Research, Cambridge, MA).

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IL-4/anti-IL-4 mAb complexes (IL-4C)

A long-acting formulation of IL-4 was prepared by mixing IL-4 and BVD4–1D11 at a 2:1 molar (1/6, w/w) ratio to produce IL-4C. After 2 min at room temperature, the mixture was diluted with 1% autologous mouse serum in saline to the appropriate concentration for injection of mice with 0.2 ml of the solution. IL-4C have an in vivo half-life of ~24 h (unlike IL-4, which has an in vivo half-life of a few minutes) and slowly dissociate to maintain high IL-4 levels for 3–5 days. IL-4C have no effects in IL-4R-deficient mice, do not fix complement or bind avidly to low affinity IgG receptors, because they contain a single IgG molecule, and do not bind to IL-4Rs (BVD4–1D11 is a blocking mAb) (14).

IVCCA for IFN-γ

In vivo IFN-γ secretion was detected with the IVCCA (16). Mice were injected i.v. with 10 μg of biotin-labeled anti-IFN-γ mAb (R46A2) and bled at the appropriate time. Serum levels of biotin-anti-IFN-γ mAb/IFN-γ complexes were captured by ELISA plate wells coated with AN-18 and were detected by streptavidin-HRP, followed by a luminogenic substrate (SuperSignal ELISA femto maximum sensitivity substrate; Pierce). Wells were read immediately for luminescence using a Fluoroskan Ascent FL luminiscence-based microtiter plate reader (Labsystems). IFN-γ-biotin-anti-IFN-γ mAb complexes were prepared in vitro and used as a standard.

Detection and identification of IFN-γ-secreting cells by flow cytometry

Spleen cells were prepared from mice injected 4 h to 8 d before death. Secretion of IFN-γ by these cells was determined ex vivo without restimulation, using a detection kit designed for this purpose (Milenyi Biotec) as described by the manufacturer. Cells were also stained with fluorochrome-labeled mAbs to CD3 (2C11) (25) and NK1.1 (PK136) (26) or CD8 (53-6.7) (27) to identify CD8⁺ T cells, conventional T cells (CD3⁻NK1.1⁻), NK cells (NK1.1⁺CD8⁻), and NK cells (CD3⁺NK1.1⁺). Cells were also stained with CD1α-galactosidase-ceramide (α-gal-cer) tetramers (a gift from A. Bendelac, University of Chicago, Chicago, IL) to identify invariant NKT cells.

Statistics

Differences between groups were compared for statistical significance (p < 0.05) with a two-tailed unpaired t test (PRISM 4; GraphPad).

Results

Kinetics of the IFN-γ response to IL-4

Previous studies demonstrated that injection of mice with both goat anti-mouse δ Ab and IL-4C increases an in spleen steady-state IFN-γ mRNA levels within 24 h that is not seen in mice injected with only goat anti-mouse δ Ab (6). The IVCCA was used to determine whether this mRNA increase is matched by an increase in IFN-γ protein secretion. Injection of BALB/c mice with IL-4C induced an ~20-fold increase in IFN-γ secretion, which reached plateau levels 1–2 h after injection and remained at this elevated level for at least 24 h (Fig. 1, A and B). IFN-γ secretion returned to near baseline levels, however, by 7 days after IL-4C injection, even when IL-4C concentrations were maintained by repeated injection (Fig. 1C). The absolute quantities of IFN-γ detected in these experiments were proportional to the duration of IFN-γ secretion analyzed (note different scales in A, B, and C).

Dose requirements for IL-4 induction of IFN-γ secretion

To determine the dose of IL-4 required to induce a detectable increase in IFN-γ secretion, BALB/c mice were injected with IL-4C that contained 0.039–10 μg of IL-4. The IFN-γ response made from 1 h after injection to 2 h after injection was determined by IVCCA. This response was dose dependent; a statistically significant increase in IFN-γ production was found with as little as 0.156 μg of IL-4; even 0.039 μg of IL-4 gave a suggestion of an increase (Fig. 2).

IL-4 selectively stimulates IFN-γ production by NK and NKT cells

Most IFN-γ that is produced in vivo is made by conventional CD4⁺ T cells, NK cells, CD8⁺ T cells, and NK cells. To determine which of these cell types secretes IFN-γ in response to IL-4, we treated C57BL/6 mice with a single injection of IL-4C that contained 10 μg of IL-4 and removed their spleens 4 h later, or we treated mice with IL-4C on days 0, 3, and 6 and removed their spleens on day 8. The cellular cytokine detection assay was then used to evaluate IFN-γ secretion by T and NK cells without ex vivo restimulation (gating and raw data shown in Fig. 3). Although this technique easily detected IFN-γ secretion by each of these cell types in unstimulated mice, in vivo stimulation with IL-4 for 4 h increased IFN-γ secretion by NK and NKT cells (Fig. 4A, left lower panel); the numbers of splenic IFN-γ-secreting NK and NKT cells increased 8.9- and 3.6-fold, respectively, and the percentages of splenic NK and NKT cells secreting IFN-γ increased 14.2- and 4.5-fold, respectively. Smaller, but still significant, increases were seen when NKT cells were defined as α-gal-cer-binding cells instead of CD3⁺CD8⁻NK1.1⁺ cells (data not shown). Consistent with increased IFN-γ production at the individual cell level 4 h after IL-4C injection, the IVCCA demonstrated a nearly significant (p = 0.066) increase in IFN-γ production in vivo during the 4 h that followed IL-4C injection and a much larger increase during the subsequent 4 h (Fig. 4B). Thus, IL-4 induction of IFN-γ production in C57BL/6 mice is rapid, if not quite as rapid as that in BALB/c mice. A second similarity between C57BL/6 and BALB/c mice is that in vivo IFN-γ production decreased to baseline or near baseline levels in both strains after 8 days of in vivo IL-4 treatment (compare Fig. 1C with Fig. 4B). The decrease in IFN-γ production in C57BL/6 mice reflected declines in both the numbers of NK and NKT cells in spleen and the number of NK cells secreting IFN-γ (Fig. 4A, right panels).
Induction of IFN-γ production by IL-4 requires Stat6, but neither Stat4 nor IL-12

Most effects of IL-4 on cell differentiation require activation of the transcription factor Stat6 (28). Alternatively, because Stat6 activation is associated with multiple proallergic phenomena that are inhibited by IFN-γ (28–30), IL-4 induction of IFN-γ might involve a different signaling pathway. To distinguish between these two possibilities, wild-type and Stat6-deficient mice were treated with IL-4C. IL-4C induced IFN-γ production in wild-type, but not Stat6-deficient, mice (Fig. 5A). Furthermore, basal IFN-γ production was decreased, rather than increased, in Stat6-deficient mice, suggesting that Stat6 signaling promotes IFN-γ secretion in mice that receive only natural, environmental stimulation.

IL-4 has been reported to promote IFN-γ production by inducing DC to secrete IL-12 (8, 11, 13, 31), which acts on NK and T cells to stimulate IFN-γ (32, 33). However, the rapidity with which IL-4C stimulated IFN-γ secretion in our system suggested that a direct effect on NK cells, rather than an IL-12-mediated effect, might be responsible. To determine whether IL-12 is a necessary intermediate in IL-4-induced IFN-γ production, we first tested the ability of IL-4C to stimulate IFN-γ production in Stat4-deficient mice, which lack the signaling molecule required for IL-12 induction of IFN-γ. Although the absolute level of the IFN-γ response to IL-4C was considerably reduced in these mice, the percent increase over basal levels was at least as great (Fig. 5A). In contrast, the large LPS-induced, IL-12-dependent, IFN-γ response of LPS-stimulated wild-type mice was absent in Stat4-deficient mice (data not shown). This demonstrated that Stat4 signaling and IL-12 secretion are not absolutely required for IL-4 induction of IFN-γ production and suggested that Stat4 signaling increases NK cell number, but might not be directly responsible for IL-4 induction of NK cell IFN-γ secretion. To distinguish between these possibilities, we evaluated the ability of a neutralizing, polyclonal goat anti-IL-12 Ab to block the IL-4-induced increase in IFN-γ production. No significant suppression of this response was observed (Fig. 5B), even though the same dose of the same polyclonal Ab suppressed the much larger IFN-γ response to IL-12 and IL-18 by ~96% (Fig. 5C). Thus, IL-4 induction of IFN-γ production is IL-12 independent.

The IFN-γ response to IL-4 is inhibited by anti-IL-2Rβ mAb, but enhanced by anti-IL-2 mAb

Because NK and NKT cell survival requires IL-15 (34, 35), which signals through a receptor that contains IL-2Rβ (36), these cells can be depleted by treating mice with anti-IL-2Rβ mAb (37, 38). Thus, to confirm that NK and NKT cells contribute to the IFN-γ

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**FIGURE 2.** Relationship between IL-4C dose and IFN-γ production. BALB/c mice were injected i.v. with vehicle or IL-4C that contained 0.039–10 μg of IL-4. One hour later, the same mice were injected with 10 μg of biotin-anti-IFN-γ mAb. Mice were bled 1 h after that, and serum levels of IFN-γ complexed to biotin-anti-IFN-γ mAb were determined by ELISA.

**FIGURE 3.** IL-4 selectively stimulates IFN-γ secretion by NK and NKT cells. C57BL/6 mice (four per group) were injected i.v. with vehicle or IL-4C that contained 10 μg of IL-4 and were killed 4 h later. Cells were counted with a Coulter counter, incubated on ice with Miltenyi Biotec IFN-γ capture reagent, washed, incubated at 37°C to allow IFN-γ secretion, then stained for NK1.1, CD3, CD8, and IFN-γ and for the ability to bind CD1α/α-gal-cer tetramers. Cells were analyzed by flow cytometry for surface expression of each of these molecules. Gates used to identify NK cells, CD8+ T cells, CD8– NKT cells, invariant NKT cells (α-gal-cer/CD1 binding cells), conventional T cells, and non-T, non-NK cells are shown in the contour plots in the top row of each panel. IFN-γ staining of each spleen cell population from vehicle- and IL-4-treated mice is shown in the contour plots in the second and third rows of panels, respectively.
response to IL-4, we evaluated the ability of anti-IL-2Rβ mAb to suppress this response. Treatment with anti-IL-2Rβ mAb blocked most of the IFN-γ response in both wild-type and Stat4-deficient mice (Fig. 6A). Because IL-2 as well as IL-15 signal through a receptor that contains IL-2R β-chain (39), it was possible that IL-2, rather than IL-15, was important for the IL-4-induced IFN-γ response. To test this possibility, we evaluated the ability of a neutralizing anti-IL-2 mAb to suppress IFN-γ production. This study was performed in Stat4-deficient mice to eliminate any potential IL-2/IL-12 interactions. Instead of suppressing the IFN-γ response to IL-4, anti-IL-2 mAb substantially increased this response (Fig. 6B). Thus, the IL-2Rβ requirement reflects a need for IL-15, rather than IL-2, for IL-4-induced IFN-γ production, and IL-4 can induce IFN-γ production in the simultaneous absence of IL-12 signaling and IL-2.

IFN-γ response to IL-4 is Rag2 independent, but requires common γ-chain (γc)

If the IFN-γ response to IL-4 is derived to a large extent from NK, rather than T, cells, it should be maintained in Rag2-deficient mice, which lack B and T cells, but not in Rag2/γc-double-deficient mice, which also lack NK cells (40). These predictions were, in fact, observed; IL-4 induced similar IFN-γ responses in wild-type
and Rag2-deficient mice, but failed to induce IFN-γ production in mice deficient in both Rag2 and γc (Fig. 7A).

**IL-13 fails to induce IFN-γ production**

Because γc is a component of the type 1 IL-4R, which is activated by IL-4, but not IL-13 (41), the failure of IL-4 to induce IFN-γ in γc-deficient mice could reflect a requirement for type 1 IL-4R signaling, in addition to a requirement for NK and NKT cells. To test the former possibility, we compared the abilities of IL-4 and IL-13, which both signal through the type 2 IL-4R (41), to stimulate IFN-γ production. IFN-γ production was induced by IL-4, but not IL-13, in C57BL/10 wild-type and Rag2-deficient mice (Fig. 7A) and in BALB/c wild-type mice (Fig. 7B). In some experiments, IL-13 suppressed IFN-γ production below basal levels (Fig. 7B). The difference between the effects of IL-4 and IL-13 was not caused by our use of a complexed form of IL-4 instead of free IL-4; even free IL-4 induced an increase in IFN-γ production on NK/NKT cells is supported by our direct demonstration of selective IL-4 induction of IFN-γ production by γc-deficient mice.

**Discussion**

Our results confirm and extend previous observations by demonstrating that IL-4 stimulates NKT and NK cells in unimmunized mice to rapidly secrete IFN-γ. The dependence of IL-4-induced IFN-γ production on NK/NKT cells is supported by our direct demonstration of selective IL-4 induction of IFN-γ production by IL-4R signaling, in addition to a requirement for NK and NKT cells. To test the former possibility, we compared the abilities of IL-4 and IL-13, which both signal through the type 2 IL-4R (41), to stimulate IFN-γ production. IFN-γ production was induced by IL-4, but not IL-13, in C57BL/10 wild-type and Rag2-deficient mice (Fig. 7A) and in BALB/c wild-type mice (Fig. 7B). In some experiments, IL-13 suppressed IFN-γ production below basal levels (Fig. 7B). The difference between the effects of IL-4 and IL-13 was not caused by our use of a complexed form of IL-4 instead of free IL-4; even free IL-4 induced an increase in IFN-γ production (Fig. 7C). Thus, signaling through the type 1 IL-4R is required for IL-4 induction of IFN-γ. Consistent with this, both IL-4 and IL-13 suppressed basal IFN-γ production in Rag2/γc-double-deficient mice (Fig. 7A) and IL-13 did not inhibit IL-4 induction of IFN-γ production by wild-type mice (Fig. 7B).

**FIGURE 5.** IL-4 induction of IFN-γ secretion requires Stat6, but not Stat4 or IL-12. A, Wild-type, Stat6-deficient, or Stat4-deficient mice (four per group, all on a BALB/c background) were injected with vehicle or IL-4C (that contained 10 μg of IL-4) and with 10 μg of biotin-anti-IFN-γ mAb. Mice were bled 24 h later. B, BALB/c mice were injected i.v. with 1 μg of goat IgG or the IgG fraction of goat anti-IL-12 Ab. One hour later, the same mice were injected with vehicle or IL-4C that contained 10 μg of IL-4 and 10 μg of biotin-anti-IFN-γ mAb. Mice were bled 24 h later. C, BALB/c mice were initially left untreated or were injected i.v. with 1 μg of the IgG fraction of goat anti-IL-4. All mice were also injected with 10 μg of biotin-anti-IFN-γ mAb at the time of vehicle or IL-4C. One hour later, these mice also received vehicle or 20 ng of IL-12 plus 1 μg of IL-18. All mice were injected with 10 μg of biotin-anti-IFN-γ mAb at the same time as injection of IL-12 plus IL-18 and were bled 24 h later. For all graphs, serum levels of IFN-γ complexed to biotin-anti-IFN-γ mAb were determined by ELISA. †, Statistically significant decrease in IFN-γ production for mice treated with IL-12, IL-18, and anti-IL-12 Ab compared with mice treated only with IL-12 plus IL-18.

**FIGURE 6.** The IFN-γ response to IL-4 is inhibited by anti-IL-2Rβ mAb, but enhanced by anti-IL-2 mAb. A, BALB/c wild-type or Stat4-deficient mice (four per group) were treated with 2 μg of anti-IL-2Rβ mAb (TMB1) or an isotype-matched control mAb (J1.2) and were injected with vehicle or IL-4C that contained 10 μg of IL-4. All mice were also injected with 10 μg of biotin-anti-IFN-γ mAb at the time of injection of IL-4C and were bled 24 h later. B, Stat4-deficient mice (four per group) were injected with 2 μg of anti-IL-2 mAb (S4B6) or an isotype-matched control mAb (GL117) plus vehicle or IL-4C that contained 10 μg of IL-4. All mice were also injected with 10 μg of biotin-anti-IFN-γ mAb at the time of injection of IL-4C and were bled 24 h later. For both graphs, serum levels of IFN-γ complexed to biotin-anti-IFN-γ mAb were determined by ELISA.

**FIGURE 7.** Effects of IL-4 and IL-13 on IFN-γ production in wild-type, Rag2-deficient, and Rag2/γc-double-deficient mice. A, C57BL/10 wild-type, Rag2-deficient, or Rag2/γc-double-deficient mice were injected with 10 μg of biotin-anti-IFN-γ mAb plus vehicle or IL-4C that contained 10 μg of IL-4, or 10 μg of IL-13. Mice were bled 24 h later. B, BALB/c mice (four per group) were injected with 10 μg of biotin-anti-IFN-γ mAb plus vehicle, IL-4C that contained 10 μg of IL-4, 10 μg of IL-13, or IL-4C plus IL-13 and were bled 24 h later. C, BALB/c mice (five per group) were injected with 10 μg of biotin-anti-IFN-γ mAb plus vehicle or IL-4C that contained 10 μg of IL-4, 10 μg of uncomplexed IL-13, or 10 μg of IL-13 and were bled 4 h later. For all graphs, serum levels of IFN-γ complexed to biotin-anti-IFN-γ mAb were determined by ELISA. †, Statistically significant decrease in IFN-γ production for mice treated with IL-13 compared with mice treated with vehicle.
NK and NKT cells in vivo, by the development of an IFN-γ response to IL-4 in Rag2-deficient, but not Rag2/γc-double-deficient mice, and by suppression of this response by an anti-IL-2Rβ mAb that depletes NK and NKT cells (37). In contrast to the requirement for IL-2Rβ, which is a component of both IL-2R and IL-15R, there is no requirement for IL-2 despite in vitro studies that have suggested such a requirement (7). In fact, anti-IL-2 mAb considerably enhances the IFN-γ response to IL-4, at least in Stat4-deficient mice. The mechanism for this enhancement is not known, but could reflect IL-2 suppression of IL-4 signaling or a proapoptotic effect of IL-2, both of which have been described previously (42-44).

The rapidity with which IL-4 stimulates IFN-γ production in BALB/c mice suggests that IL-4 acts directly on NK and NKT cells to induce them to secrete IFN-γ. We cannot be certain of this, however, because attempts to demonstrate IL-4 induction of IFN-γ secretion by purified NK cells in vitro were unsuccessful, possibly because these cells were damaged by the sorting procedures used. Attempts to produce purified NK cell populations for in vitro studies of IFN-γ production by culturing cells under conditions that cause NK cells to selectively survive and proliferate were not feasible, because the culture conditions required to produce purified NK cells themselves stimulate these cells to produce IFN-γ.

In contrast to previous studies, which suggested that IL-4 promotes IFN-γ secretion indirectly by inducing DCs to secrete IL-12 (8, 10, 11, 13, 31, 45), we found that the in vivo IFN-γ response to IL-4 is IL-12 independent (Fig. 5B); it is not blocked by a polyclonal anti-IL-12 Ab that inhibits the IFN-γ response to IL-12 plus IL-18 by >95% (Fig. 5C) and is still observed, to a considerable extent, in mice that lack the critical IL-12 signaling molecule, Stat4 (Figs. 5A and 6). Although the absolute magnitude of IL-4 induction of IFN-γ is reduced in Stat4-deficient mice, the IL-4-induced percent increase in IFN-γ production is equivalent in wild-type and Stat4-deficient mice. This suggests that Stat4 primarily promotes the IFN-γ response to IL-4 by enhancing NK and/or NKT cell production and/or survival. These observations do not eliminate the possibility that IL-4 also contributes to IFN-γ production, in some circumstances by promoting DC production of IL-12. In fact, because IFN-γ primes for IL-12 production (46), our observations raise the possibility that IL-4 primes for IL-12 production at least in part by inducing IL-12-independent production of IFN-γ. We also cannot completely rule out the possibility that IL-4 induction of IFN-γ depends in part on IL-23, a second cytokine that can promote IFN-γ production by signaling through Stat4 (47). This seems unlikely, however, because IL-23-dependent effects should be at least partially blocked by the polyclonal anti-IL-12 Ab used in our experiments, which should contain Ab molecules that bind p40, a component of both IL-12 and IL-23 (47).

Although the IFN-γ response to IL-4 is Stat6 independent, it is totally Stat6 dependent. This demonstrates that the IFN-γ response is not induced by contaminants of our IL-4 or anti-IL-4 mAb preparations, such as bacterial LPS, which induce Stat6-independent, Stat6-dependent IFN-γ production. Stat6 dependence similarly rules out the possibility that IL-4C might induce IFN-γ production by fixing C and binding to C and/or FcγRs. These latter possibilities would be unlikely even if IL-4 induction of IFN-γ were Stat6 independent, because IL-4C contain only a single IgG molecule and, thus, are unable to fix C or bind more avidly than uncomplexed IgG to FcγRs (14) and because free IL-4 as well as IL-4C can stimulate IFN-γ production (Fig. 7C).

Stat6 dependence of the IFN-γ response to IL-4 also indicates that this response depends on a gene that has a Stat6 binding site in its regulatory regions. The identity of this proposed gene is not known, but it most likely is not the IFN-γ gene itself, which has no apparent Stat6 binding sites in its established enhancer or promoter regions (based on a search of mouse and human IFN-γ promoter sequences in GenBank performed by W. Chen, Cincinnati Children’s Hospital, Cincinnati, OH). Additional studies are required to determine whether Stat6 promotes IFN-γ transcription by binding to a novel, as yet undescribed, IFN-γ enhancer, which rapidly induces a second transcription factor that directly increases IFN-γ gene transcription or increases IFN-γ transcript stability.

Whatever the mechanism, NK/NKT cell production of IFN-γ in response to IL-4 may be biologically important for more than one reason. First, it may prime DC and other cell types to produce IL-12 in response to inflammatory stimuli. Second, it may provide IFN-γ that promotes Ag processing and presentation that, in turn, leads to T cell activation. Third, sufficient IFN-γ, if produced, may antagonize the effector functions of IL-4 and IL-13, promote Th1 differentiation, and act as a brake on IL-4 induction of Th2-associated inflammation. In this regard, the failure of IL-13 to induce IFN-γ makes it an unmitigated inducer of Th2-associated inflammation and may provide one explanation of why IL-13 appears to be more important than IL-4 in the effector phase of asthma and other allergic inflammatory disorders (48, 49) and induces the expression of some asthma-associated pulmonary proteins that are not induced by IL-4 (50).

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Disclosures
The authors have no financial conflict of interest.

References