The Journal of Immunology

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J Immunol 2002; 169:3574-3580; ;
doi: 10.4049/jimmunol.169.7.3574
http://www.jimmunol.org/content/169/7/3574

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Differential Functions of IL-4 Receptor Types I and II for Dendritic Cell Maturation and IL-12 Production and Their Dependency on GM-CSF[1]

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Little is known about the distinct roles of the two types of IL-4R on DC. Here we report that IL-4 and IL-13 are able to promote DC maturation, as evaluated by up-regulation of MHC class II and costimulatory molecules, when the concentration of GM-CSF is relatively lower than the dose of IL-4 or IL-13. In addition, under these conditions both cytokines enable DC to respond to maturation stimuli such as bacterial products or proinflammatory cytokines. Both IL-4 and IL-13 act synergistically with weak maturation stimuli such as TNF-α or CD40. The IL-4R signaling for DC maturation requires the IL-4R α-chain and STAT6, but not Janus kinase 3, indicating that IL-4R type II signaling is preferentially responsible for these effects. In contrast, the production of IL-12 p70, but not IL-10 and TNF, induced by microbial products was enhanced only by IL-4, not by IL-13 or Y119D, a selective type II IL-4R agonist, in vitro and in vivo. This enhancement was dependent on the presence of Janus kinase 3, indicating that this function is exclusively mediated by the type I IL-4R. In short, we discerned the individual roles of the two IL-4R types on DC function, showing that IL-4R type I promotes IL-12 secretion independently of GM-CSF concentration, while IL-4R type II promotes the up-regulation of MHC class II and costimulatory surface markers in a GM-CSF concentration-dependent manner. *The Journal of Immunology,* **2002, 169: 3574–3580.**

Dendritic cells (DC)[3] are the major cell type for Ag-specific priming of T cells in vivo. The generation of DC from hemopoietic progenitor cells played a critical role in promoting research in the field of DC (1, 2). The major experimental source for DC of myeloid origin in the human system is peripheral blood monocytes (3–5), whereas in the mouse the culture from bone marrow (BM) precursors is most accessible and results in large cell numbers (6, 7). In all cases GM-CSF is the major cytokine for the generation of myeloid DC. For human DC maturation, notably from monocyte precursors, the additional use of IL-4 (2, 3) or IL-13 (8) is obligatory. In the mouse conflicting data have been published regarding the need for IL-4 for DC maturation, and little is known about the effects of IL-13 on DC maturation. In some experimental systems a high dose of GM-CSF alone was sufficient to generate mature DC (6, 7, 9), while IL-4 effects were observed only at low doses of GM-CSF (10).

IL-4 exerts its actions on immune cells using two types of receptors. The type I heterodimer consists of the IL-4R α-chain (IL-4Rα) in association with the common cytokine receptor γ-chain (γ_c), while the type II receptor is comprised of the IL-4Rα in association with the IL-13Rα1. While B cells, macrophages, and mast cells express both IL-4R types I and II, T cells express only IL-4R type I. IL-4 is able to bind both receptors, while IL-13 signaling is restricted to IL-4R type II (11–14).

Little information is available on the expression of the two types of IL-4R on DC and which DC functions might be affected by IL-4 or IL-13. Mature murine DC express γ_c and functionally use it for IL-4R type I signaling, as anti-γ_c mAb could affect IL-4 signaling (15). In contrast, IL-4 and IL-13 resulted in strong activation of STAT6 via the IL-4Rα of both type I and II receptors in mature BM-DC generated with high doses of GM-CSF (16). Recently, the role of IL-4 as a Th1-directing cytokine was strengthened by the observation that IL-12 p70 production by DC is increased by IL-4 (17).

Here the expression and function of IL-4R types I and II and their associated molecules STAT-6 and Janus kinase 3 (JAK3) were addressed in murine BM-DC generated with different doses of GM-CSF and using the respective knockout mice. We show that IL-4- and IL-13-mediated phenotypical maturation of DC occurs mainly via IL-4R type II and is dependent on IL-4Rα/STAT-6 signaling. In contrast, enhancement of IL-12 production by DC occurs exclusively via IL-4R type I and is dependent on JAK3.

Materials and Methods

Generation of murine BM-DC

The preparation and culture of BM cells from C57BL/6 and BALB/c mice to generate DC has been previously described (7). GM-CSF (PeproTech/Tebu, Frankfurt, Germany) was used at low (5 U/ml) or standard (200 U/ml) concentrations. IL-4R$\alpha^{-/-}$ mice were provided by F. Brombacher (University of Cape Town, Cape Town, South Africa), and STAT4$^{-/-}$, STAT6$^{-/-}$, and JAK3$^{-/-}$ mice were purchased from J. Ihle (St. Jude Children's Research Hospital, Memphis, TN).

Cytokines and IL-4R agonists/antagonists

rIL-4 and rIL-13 (purchased from PeproTech) were used at standard doses (100 U/ml) or high doses (500 U/ml). Mutated mouse IL-4 (IL-4.Y119D)

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Received for publication December 26, 2001. Accepted for publication July 3, 2002.

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[1] This work was supported by the Sonderforschungsbereich 263 (C13, A6) of the Deutsche Forschungsgemeinschaft and the ELAN program of University of Erlangen (to M.B.L., S.R., and A.G.).

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[3] Abbreviations used in this paper: DC, dendritic cell; BM, bone marrow; γ_c, common cytokine receptor γ-chain; JAK3, Janus kinase 3; SAC, *Staphylococcus aureus* Cowan I strain; MHC II, MHC class II.

0022-1767/02/$02.00

was generated as described recently (18) by expression in transfected human embryonic kidney cells (293 EBNA; Invitrogen, Groningen, The Netherlands) and subsequent purification by affinity chromatography.

RNA isolation and RT-PCR

After RNA extraction from DC populations with acidic guanidinium thiocyanate, cDNA was synthesized for each time point in 20-μl reactions containing 1 μg total RNA, 50 mM Tris-HCl (pH 8.3), 5 mM $MgCl_2$, 1 mM of each dNTP, 2.5 mM oligo(dT), 32 U RNAguard, and 17 U avian myeloblastosis virus reverse transcriptase (all from Amersham Pharmacia Biotech, Freiburg, Germany) at 42°C for 90 min. The cDNA was amplified in a 40-μl reaction volume containing 50 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, 10 mM of each dNTP, 1 U *Taq* polymerase (Pharmacia Biotech, Piscataway, NJ), and 100 nM primers during 35 cycles (1 min denaturation at 94°C, 1 min annealing at 58–63°C, 1 min extension at 72°C). Samples were analyzed on 2% agarose gels containing 0.2 μg/ml ethidium bromide. The primers used were as follows: IL-4R sense primer, 5′-GGCCTG GCAGTGGCATGGGAGGCC-3′; IL-4R antisense primer, 5′-TTATCGT GCCCGCTGGGGCCCTGC-3′ (amplified fragment of 273 bp); IL-13R sense primer, 5′-ACAGAAGTTCAGCCACCTGTGACG-3′; IL-13R antisense primer, 5′-ACACTTCATATAGCTCAGCTTATGCCA-3′ (amplified fragment of 348 bp); β-actin sense primer, 5′-CACCCGCCACCAGT TCGCCA-3′; β-actin antisense primer, 5′-CAGGTCCCGGCCAG CCAGGT-3′ (amplified fragment of 574 bp); γ_c sense primer, 5′-CCCAGAGAAAGAAGAGCAAGCACC-3′; and γ_c antisense primer, 5′-GGGGTCCTGGAGCTGGACAACAAA-3′ (amplified fragment of 429 bp).

FACS analysis

FACS analysis was performed as previously described (7). One to 5×10^5 BM-DC were stained directly with PE-conjugated mAb directed against MHC class II (M5/114), or FITC-conjugated B7-2 (GL1), CD14, and CD40 (3/23; all from BD PharMingen, Hamburg, Germany), TNFR1, TNFR2 (Serotec, Oxford, U.K.), or the appropriate fluorochrome-conjugated isotype control mAb at 2–5 μg/ml in PBS containing 0.1% sodium azide and 5% FCS for 30 min on ice in the dark. Samples were washed once in staining buffer and subsequently measured and analyzed with a FACScan (BD Biosciences, Heidelberg, Germany).

ELISA

BM-DC were taken on day 8 of culture, and 1×10^6 cells/well were transferred to a 24-well plate (Falcon; BD Biosciences) with 10 μg/ml fixed *Staphylococcus aureus* Cowan I strain (SAC; Pansorbin, Calbiochem, Bad Soden, Germany) with or without 100 U/ml IFN-γ (BD PharMingen). After 24 h cell supernatants were collected and tested for IL-12 p70, IL-12 p40, TNF-α, and IL-10 production with BD OptEIA Kits (BD PharMingen) according to the manufacturer's instructions.

In vivo cytokine induction

We used the protocol as previously published by Hochrein et al. (17). BALB/c mice or STAT6-deficient BALB/c mice were administered LPS (10 μg) with or without the addition of IL-4, IL-4Y119D, or IL-13 (1 μg) via i.p. injection. These reagents were administered in PBS containing 1% FCS. Control mice received i.p. injections of PBS/FCS alone. Mice were killed after 4 h, blood was taken, and serum was collected for IL-12 assay by ELISA.

Results

Standard doses of IL-4 do not alter the DC phenotype or Ag-presenting function when cells are cultured with standard doses of GM-CSF

We have shown previously that the generation of large numbers of mature BM-DC can be achieved by GM-CSF alone (7). Here we show that the generation of mature BM-DC using standard doses of GM-CSF (200 U/ml) cannot be further improved phenotypically or functionally by adding standard doses of IL-4 (100 U/ml) to the cultures. The proportions of mature DC represented by high surface expression of MHC class II (MHC II) and CD86 (Fig. 1*a*) as well as CD80, CD40, and CD54 (data not shown) (7) remained unaltered in cultures with or without standard doses of IL-4. This was observed throughout with different inbred mouse strains (C57BL/6, BALB/c, FVB), and the mean percentages of mature DC (MHC II^{high} and $CD86^+$) within the cultures of 16 independent experiments after 8–10 days are shown (Fig. 1*b*). These similar surface phenotypes correlated with their capacity to stimulate allogeneic MLR in vitro and were not influenced by further DC prestimulation with TNF-α (Fig. 1*c*).

IL-4R type II signaling is sufficient to promote DC maturation at low doses of GM-CSF

When low doses of GM-CSF (5 U/ml) were used for BM-DC generation (LowGM-DC), only immature DC develop, characterized by the low expression of MHC II molecules, with few or no costimulatory molecules (Fig. 2, *a* and *b*) (10). Similarly, DC generated from $STAT6^{-/-}$ mice did not respond to IL-4 and remained immature (Fig. 2*c*). In contrast, control BM-DC generated from unrelated $STAT4^{-/-}$ or wild-type mice readily matured.

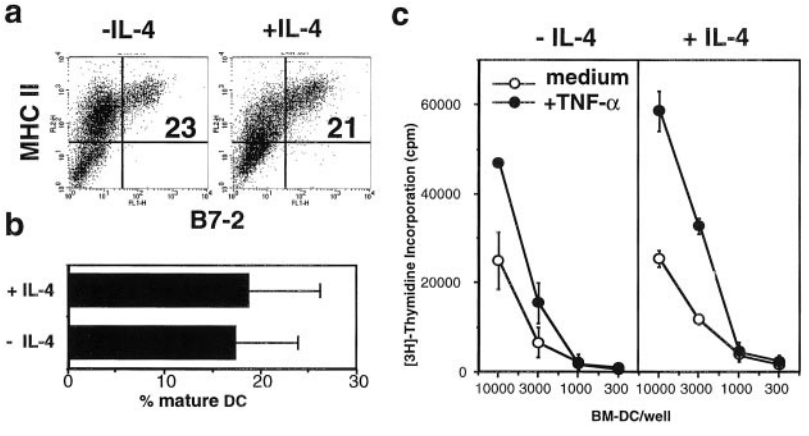

FIGURE 1. No influence of IL-4 on DC maturation was observed at standard doses of GM-CSF. *a*, DC cultures were grown with standard concentrations of GM-CSF (200 U/ml) with or without IL-4 (100 U/ml) until day 8. Then double surface staining for MHC II and B7-2 was performed. FACS analysis revealed the three typical populations within BM-DC cultures: MHC II^-/$B7\text{-}2^-$ myeloid precursors and granulocytes, MHC II^{low}/$B7\text{-}2^-$ immature DC, and MHC II^{high}/$B7\text{-}2^+$ mature DC. Numbers within the profiles represent the percentage of cells within the quadrant. *b*, The average number of mature DC and the SD of similar experiments as in *a* are shown for 16 independent experiments with day 8–10 BM-DC. *c*, For the allogeneic MLR, BM-DC were generated in the absence or the presence of IL-4 for 7 days, then treated with 500 U/ml TNF-α overnight, washed, and cocultured for 3 days with allogeneic purified $CD4^+$ T cells. Proliferation was assayed by [^{3}H]thymidine incorporation for an additional 16 h.

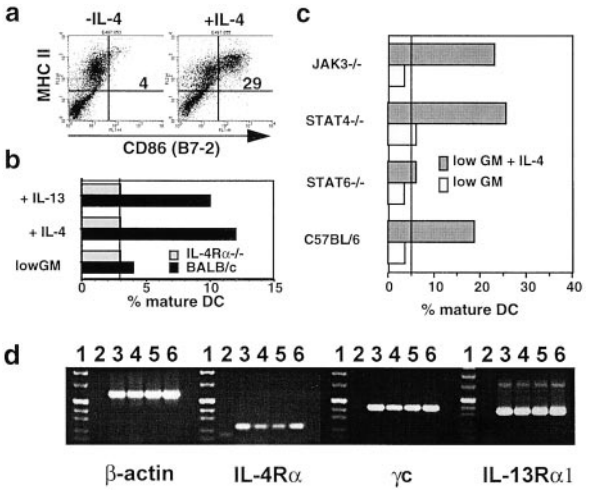

FIGURE 2. Both IL-4 and IL-13 enable DC maturation at low doses of GM-CSF in the absence of JAK3 through IL-4R type II. BM-DC were generated at low doses of GM-CSF (LowGM-DC) with or without 100 U/ml IL-4 or IL-13 from the indicated wild-type or gene-deficient mice for IL-4R$\alpha^{-/-}$, STAT6$^{-/-}$, STAT4$^{-/-}$, and JAK3$^{-/-}$. *a*, LowGM-DC were generated from C57BL/6 mice with or without 100 U/ml IL-4. Numbers within the quadrants represent percentages of mature DC. *b*, LowGM-DC form BALB/c or IL-4R$\alpha^{-/-}$ mice were cultured for 8 days with or without IL-4 or IL-13 (each 100 U/ml). Cells were analyzed for MHC II^{high} and B7-2^{+} expression by FACS as in *a*, and the percentages of mature DC are shown as bar graphs. *c*, LowGM-DC were generated from C57BL/6, STAT6$^{-/-}$, STAT4$^{-/-}$, and JAK3$^{-/-}$ mice with or without 100 U/ml IL-4 and were analyzed on day 8 by FACS. The percentages of mature DC are shown as bar graphs. *d*, BM-DC generated with low or high doses of GM-CSF with or without IL-4 do express mRNAs for all IL-4R chains. BM-DC were generated for 8 days at high or low doses of GM-CSF with or without IL-4. The total RNA of the cells was prepared, reverse transcribed, and analyzed by PCR for the expression of IL-4Rα, γ_c, IL-13Rα1, and β-actin (control). *Lane 1*, pUC-Mix standard (MBI Fermentas, Vilnius, Lithuania); *lane 2*, H_2O control; *lane 3*, high GM-CSF; *lane 4*, high GM-CSF and IL-4; *lane 5*, low GM-CSF; *lane 6*, low GM-CSF and IL-4.

IL-13 (Fig. 2*b*) and IL-4.Y119D (data not shown), a specific agonist for IL-4R type II (18), were equally potent in mediating DC maturation. Also JAK3-deficient (IL-4R type I) DC readily matured in the presence of IL-4 (Fig. 2*c*), indicating that IL-4 signaling via γ_c is not involved in DC maturation. Thus, although IL-4Rα signaling via STAT-6 is shared by both IL-4R, these data point to the type II receptor as the primary signaling pathway for this maturation enabled by IL-4 and IL-13.

To prove that all the different components of the IL-4R complexes are expressed by the different DC, we performed RT-PCR with RNA from BM-DC generated from C57BL/6 mice in the presence of low or high concentrations of GM-CSF with or without IL-4. As shown in Fig. 2*d*, the mRNAs for the IL-4Rα, γ_c, and IL-13Rα1 could be detected under all four culture conditions. The same results were achieved by analyzing BM-DC derived from γ_c-deficient (with the exception of the γ-chain) and JAK3-deficient mice (data not shown).

IL-4 signaling reverts the maturation resistance of DC at low doses of GM-CSF by combined treatment with IL-4 and TNF-α

Previous results indicated that immature LowGM-DC are resistant to maturation stimuli such as TNF-α (10). We therefore investigated whether the addition of IL-4- to TNF-α-treated LowGM-DC might be able to convert this maturation-resistant state. Indeed, the combined treatment of LowGM-DC cultures on day 8 with IL-4 and TNF-α for an additional 5 days, but not with each of the cytokines alone, resulted in DC maturation (Fig. 3). These findings raised the question of whether IL-4 and IL-13 act directly as a maturation stimulus for DC or whether they enable the responsiveness of DC to TNF-α (see below).

High doses of IL-4 enhance DC maturation at standard doses of GM-CSF alone or in cooperation with maturation stimuli

IL-4 treatment to promote BM-DC maturation at low doses of GM-CSF depends on IL-4Rα and STAT6 signaling. Likewise, GM-CSF can activate STAT6 in murine BM-DC (16) (our own observations; data not shown). However, mature DC develop normally from STAT-6-deficient BM cells with high doses of GM-CSF (data not shown), indicating clearly that in this case STAT-6 is not involved in the promotion of DC maturation.

To test whether the effects of IL-4 on DC maturation are dependent on an absolute or a relative amount of GM-CSF in relation to IL-4, BM-DC were generated with standard doses of GM-CSF (200 U/ml) until day 8, and increased concentrations of IL-4 (high

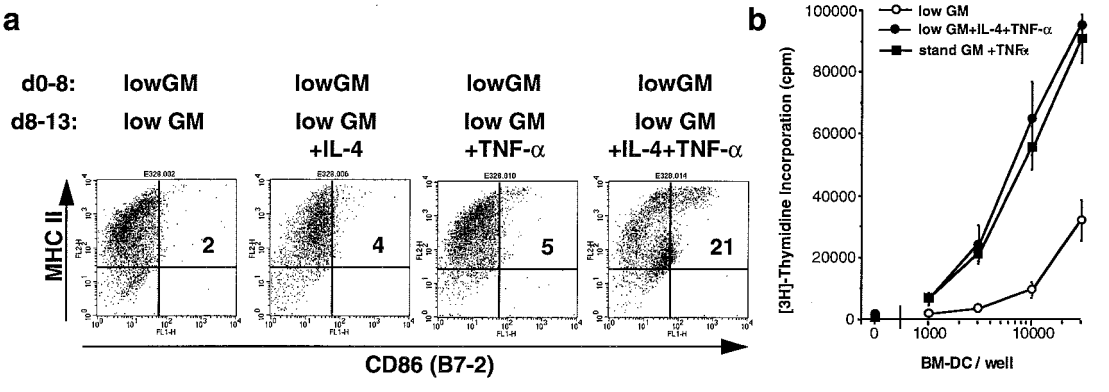

FIGURE 3. Established maturation resistance of LowGM-DC can be reversed by IL-4 plus TNF-α treatment. *a*, LowGM-DC were generated with or without 100 U/ml IL-4 until day 8. These established LowGM-DC cultures were subsequently cultured for an additional 5 days with 5 U/ml GM-CSF with or without 100 U/ml IL-4 and 500 U/ml TNF-α as indicated, stained for MHC II and CD86 (B7-2), and analyzed by FACS. Numbers within the profiles represent the percentage of mature DC within the quadrant. *b*, Cultures of different BM-DC were compared for their capacity to stimulate allogeneic T cells. BM-DC were generated for 7 days with standard doses of GM-CSF (200 U/ml) and treated for 16 h with 500 U/ml TNF-α, for 13 days with low doses of GM-CSF (like Fig. 2*a*, *left panel*), or for 8 days with low doses of GM-CSF and thereafter for 5 days with low doses of GM-CSF plus 100 U/ml IL-4 plus 500 U/ml TNF-α (like Fig. 2*a*, *right panel*). After 3 days the proliferation of purified CD4^{+} T cells was assayed by [^{3}H]thymidine incorporation for an additional 16 h.

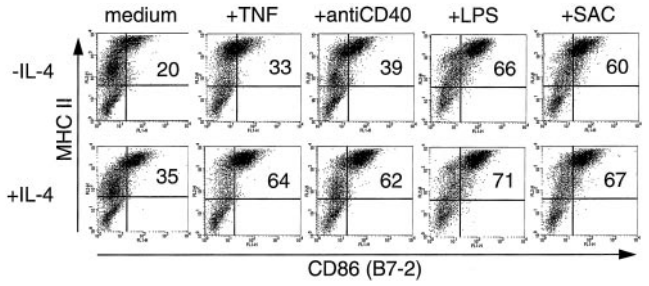

FIGURE 4. High doses of IL-4 induce DC maturation at standard doses of GM-CSF and acts synergistically with TNF-α or anti-CD40. BM-DC were generated with standard doses of GM-CSF until day 8 and then treated with or without high doses of IL-4 (500 U/ml) overnight. Simultaneously the cultures were treated with 5 μg/ml anti-CD40 mAb, 1 μg/ml LPS, or 10 μg/ml SAC. BM-DC were analyzed by FACS for MHC II and CD86 expression. Numbers within the profiles represent the percentage of mature DC within the quadrant. Similar maturation effects were observed with IL-13 and the combination of IL-4 and IL-13. No additive effects were observed with the combination (not shown).

dose; 500 U/ml) were added for 24 h alone or together with different maturation stimuli (Fig. 4). Such high doses of IL-4 were able to increase the number of mature DC in the cultures from 20 to 35% (Fig. 4). Weaker stimuli, such as TNF-α and CD40 ligation, acted synergistically together with IL-4 to further promote DC maturation up to 64%, whereas stronger stimuli, such as LPS or SAC alone, efficiently induced maximal maturation and thus showed little additional effect in combination with IL-4 (Fig. 4). These data indicate that an effect of IL-4 on DC maturation can only be observed when the doses of IL-4 are relatively higher than the doses of GM-CSF in the cultures.

Differential regulation of surface receptors mediating DC maturation by IL-4 and IL-13

Because IL-4 and IL-13 augmented TNF-α-, anti-CD40-, and LPS-induced DC maturation at relatively low doses of GM-CSF (Figs. 3 and 4), we investigated whether IL-4 and IL-13 influence the expression of the respective surface receptors used by these maturation stimuli. IL-4 only induced CD40 expression, but down-regulated TNFR2 and CD14 (Fig. 5). In contrast, IL-13 had no influence on CD40 and CD14 expression, but slightly up-regulated TNFR2 (Fig. 5). These data argue for direct effects of IL-4R-mediated signals on DC maturation, rather than indirect effects by induction of other maturation-inducing receptors. Furthermore, the different effects of IL-4 and IL-13 are indicative of distinct functions for IL-4R types I and II on DC, respectively.

Enhancement of IL-12 p70 production is mediated by IL-4, but not IL-13, through IL-4R type I and depends on JAK3 and STAT6

Besides the up-regulation of MHC II and costimulatory molecules on the surface of DC, the production of IL-12 is a major feature of DC maturation (9, 19). IL-4 was recently shown to enhance bacteria-induced IL-12 p70, but not IL-12 p40, production by human and murine DC (17). We have now extended these studies in an attempt to unravel the IL-4R signaling pathway responsible for the enhanced IL-12 production of DC by also applying IL-13 and generating BM-DC from JAK3$^{-/-}$ and STAT6$^{-/-}$ mice. BM-DC were stimulated with SAC with or without IFN-γ to produce IL-12 p70, and the additional effect of IL-4 or IL-13 on DC cytokine production was investigated. In wild-type mice IL-4 doubled the amount of IL-12 p70 produced, while IL-13 had no effect. In addition, the enhancing effect of IL-4 was abrogated in JAK3$^{-/-}$ and STAT6$^{-/-}$ mice (Fig. 6*a*). IL-4 and IL-13 did not induce IL-12 by themselves (not shown) and did not influence IL-12 p40 (Fig. 6*a*), IL-10, or TNF-α production (Fig. 6*b*). To test whether the type II receptor is functional on mature DC, we analyzed IL-13-induced activation of STAT-6. Because STAT-6 was found to be tyrosine phosphorylated in response to IL-13 to a similar extent as in IL-4-treated DC (data not shown), the type II IL-4R is functional, but not involved in IL-12 induction.

The enhancing effects of IL-4, but not IL-13, on IL-12 p70 production were further confirmed by injecting LPS into mice alone or in combination with IL-4, IL-13, or a mutated form of IL-4 (IL-4.Y119D). IL-4.Y119D has been shown previously to selectively activate the type II IL-4 receptor complex and simultaneously block type I IL-4 receptor function by competition with wild-type IL-4 (18). After 4 h serum IL-12 p70 levels were tested in differentially treated mice. Similar to the stimulation of DC in vitro, the increase in serum IL-12 p70 levels in vivo could only be induced by IL-4, not by IL-13 (Fig. 6*c*) or the type II IL-4R agonist IL-4.Y119D. These data point to IL-4R type I as the only receptor involved in the enhancement of IL-12 p70 production.

Discussion

GM-CSF and IL-4 are widely used for the generation of DC, but the decisive functions of IL-4 on DC remain unclear. In most cases IL-4 promoted DC maturation and led to increased T cell immunogenicity. Therefore, immature DC were generated in the absence of IL-4 to induce T cell anergy in vitro and in vivo (20, 21). Recently, we reported an immature tolerogenic type of DC generated with low doses of GM-CSF that was resistant to maturation by LPS, TNF-α, and CD40 ligation. To date, maturation resistance of DC has been described only for this LowGM-DC (10) and for

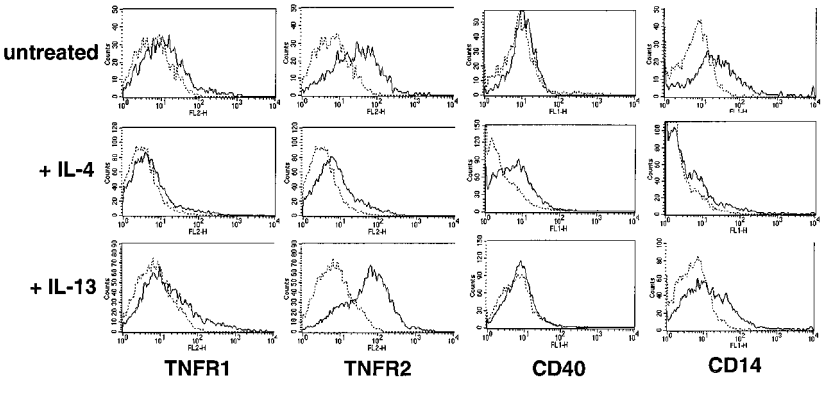

FIGURE 5. Differential effects of IL-4 and IL-13 on the expression of TNFR2, CD40, and CD14 by LowGM-DC. LowGM-DC were generated in the presence or the absence of IL-4 or IL-13. On day 8 of culture cell samples were analyzed by FACS for the surface expression of TNFR1, TNFR2, CD40, and the LPS coreceptor CD14.

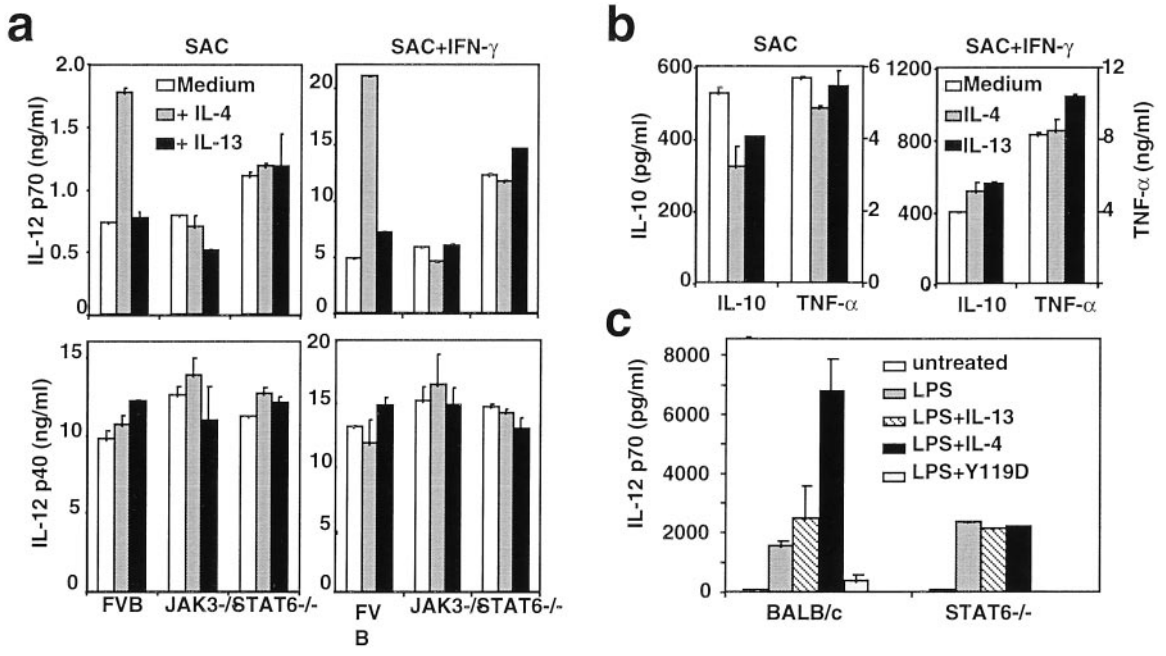

FIGURE 6. IL-4, but not IL-13, enhances IL-12 p70 production by DC via IL-4R type I. BM-DC were generated at standard doses of GM-CSF and transferred on day 8 at 1×10^6 cells/well into a 24-well plate, and the supernatant was tested by ELISA for IL-12 p70 and p40 (*a*) or IL-10 and TNF-α (*b*) production 24 h after stimulation with 10 μg/ml SAC or SAC plus 100 U/ml IFN-γ in the presence or the absence of 100 U/ml IL-4 or IL-13. *c*, BALB/c mice or STAT6-deficient BALB/c mice were administered LPS (10 μg) with or without the addition of IL-4, IL-4Y119D, or IL-13 (1 μg) via i.p. injection in PBS containing 1% FCS. Control mice received PBS/FCS alone. Mice were killed after 4 h, blood was taken, and serum was collected for IL-12 assay by ELISA.

1α,25-dihydroxyvitamin D_3-treated DC (22). Maturation resistance is advantageous for tolerance induction in vivo, as such DC remain immature after injection and are thus more effective in tolerance induction. It is consequently important to understand the mechanisms underlying such a resistance to maturation of DC.

Previously, we observed that the presence of IL-4 throughout the LowGM-DC culture could reverse the immature maturation-resistant state (10). Here we found, in addition, that the resistance to maturation via stimuli such as TNF, LPS, and anti-CD40 of already established LowGM-DC cultures can be reversed by IL-4 (Fig. 3) and IL-13 (not shown). This shows that IL-4 and IL-13 are critically involved in regulation of the maturation-resistant state of DC. However, the molecular details of maturation resistance remained unresolved.

One possibility is that IL-4 and IL-13 may up-regulate the expression of receptors for DC maturation stimuli, such as TNFR, CD40, or the LPS coreceptor CD14. It has been shown that TNF-α can be produced endogenously by adherent macrophages in BM-DC cultures (23), and IL-4/IL-13 cooperate with TNF-α on blood mononuclear cells (24), which also contain precursors for DC. Thus, IL-4 might play a role as an enhancer of the sensitivity of DC to maturation stimuli such as TNF-α in situations where GM-CSF is limited, rather than as a direct activator of DC maturation. Although IL-4 and IL-13 induced maturation of LowGM-DC equally well, their effects on TNFR were completely different. TNFR2 was down-regulated by IL-4 and slightly up-regulated by IL-13. This argues for direct effects by the IL-4 and IL-13 receptor subtypes not involving regulation of the two TNFRs. Also, CD40 and the LPS coreceptor CD14 were differentially influenced by IL-4 or IL-13, which, therefore, could not explain the similar maturation-inducing capacity of the two cytokines.

As maturation effects on DC by IL-4 and IL-13 are only detectable when the doses of GM-CSF are relatively lower than the doses of IL-4/IL-13 (i.e., 5 U/ml LowGM conditions and 100 U/ml IL-4 (Fig. 2) or standard doses of 200 U/ml GM-CSF and 500 U/ml IL-4 (Fig. 4), but not 200 U/ml GM-CSF and 100 U/ml IL-4 (Fig. 1)), these cytokines might compete for a common signaling pathway. It has been shown that GM-CSF can activate a panel of STAT molecules, including STAT6 (16) (our unpublished observations); therefore, GM-CSF and IL-4/IL-13 may cooperate in activating STAT6. When GM-CSF is limited, the insufficient STAT6 activation could be compensated for by IL-4 or IL-13. In contrast, at high doses of GM-CSF, STAT6 activation may be sufficient to sensitize for other maturation stimuli, such as TNF-α. Thus, the dual effect of low doses of GM-CSF in the absence of IL-4 might decrease STAT6 activation to amounts that render immature DC unable to respond to other maturation stimuli. This topic is currently under further investigation.

Several recent studies indicate that the effects of IL-4 on DC observed here in vitro might be of relevance in experimental and pathological situations in mice and humans. Unexpectedly, in vivo studies with IL-4-deficient mice showed that IL-4 is essential during the priming phase of Th1 or CTL immune responses (25, 26). Injections of blocking anti-IL-4 Abs prevented allogeneic T cell priming in vivo (27). Also, anti-tumor responses improve in vivo when initiated by BM-DC grown at intermediate doses of GM-CSF in the presence of IL-4 (28) or when GM-CSF is overexpressed in DC (29, 30). The Th1-inducing capacity of IL-4 observed in vivo is not likely to be mediated by direct effects on naive $CD4^+$ T cells, because IL-4 inhibits Th1 development by suppression of IL-12R expression (10–12). Thus, the Th1-inducing effect of IL-4 during the priming phase may well be dependent on DC. Recent reports indicate that the time point of IL-4 action importantly influences Th2 or Th1 outcome. When IL-4 is coadministered with an Ag at the site of DC encounter in vivo, IL-4 clearly promoted IL-12 production by DC and their Th1-driving potential (31, 32).

This indirect Th1-inducing effect of IL-4 via DC could account for the IL-4-mediated disease aggravation in some acute, but not chronic, phases of several diseases, such as atopic dermatitis (33), asthma (34), hepatitis (35), graft-vs-host disease (36), or allograft

rejection (37, 38). Furthermore, there is evidence that such IL-4-mediated sensitization in vivo is mediated via STAT6 signaling (39, 40), similar to that observed in our BM-DC cultures. In acute phases of tissue inflammation, IL-4 and IL-13 may act on the residing DC population, which renders them sensitive to TNF-α stimulation or other inflammatory cytokines. Infiltrating monocytes might be driven toward DC differentiation (41, 42), and subsequently these DC may induce Th1 immunity (17). IL-4 effects can be circumvented by increasing GM-CSF in our BM-DC cultures or possibly in vivo, when high level GM-CSF expression is found, as described for chronic atopic dermatitis (29).

To better understand the maturing and Th1-driving mechanisms of IL-4 and/or IL-13 on DC, we studied the role of their respective receptors on DC. Very little information on the expression and functional relevance of the two IL-4R complexes on DC is available to date. In vitro-generated DC have been reported to express the γ_c. However, the thymic DC compartment in mice deficient for the γ_c has been shown to be phenotypically and functionally normal (43). Analyzing the epidermal-derived DC line, XS52, which retains important functions of epidermal Langerhans cells, it has been shown that IL-4 induces a rapid up-regulation of c-Myc mRNA expression, and this IL-4-dependent signaling could be almost completely blocked by anti-γ_c mAb (15). On the surface of human monocyte-derived DC, both types I and II IL-4R are expressed and functional, as IL-4 and IL-13 both promote the generation of DC (2, 8). Analyzing the expression of IL-13Rα1 by a novel (not neutralizing) mAb in mouse lymph nodes led to the finding that follicular DC, but not interdigitating DC, express this molecule (44). Thus, depending on the type and maturation stage of DC, these cells might respond differently to IL-4 and/or IL-13.

Our data indicate that both IL-4R type I and II complexes are expressed on DC; however, their functional consequences for DC are different. In common, both IL-4 and IL-13 promote DC maturation when the doses of GM-CSF are relatively lower than the doses of IL-4 or IL-13 (see above). This effect on DC maturation requires signaling though STAT6, but not JAK3, for IL-4 and IL-13, but not GM-CSF, indicating that IL-4 and IL-13 enable DC maturation through STAT6 activation. GM-CSF might use other STATs for this process, as previously described (9). Although we could not directly exclude that the maturation signal occurs through the type II receptor due to a lack of specific type I receptor agonists, type II receptor antagonists, or type II receptor-deficient mice, we found strong evidence for the type I receptor not being involved in this process. First, IL-4 and IL-13 were equally potent in inducing DC maturation (Fig. 2), and no additive or synergistic effects could be observed by the combination of both cytokines (data not shown). Second, the DC maturation effect is maintained in JAK3$^{-/-}$ mice. Third, all other effects produced by IL-4 (Figs. 5 and 6) were not found with IL-13, although the type II receptor could have been engaged by IL-4. Fourth, mature BM-DC develop normally from STAT6$^{-/-}$ mice with high doses of GM-CSF (not shown). We therefore argue that IL-4 or IL-13 signaling responsible for DC maturation occurs exclusively through the type II receptor.

In contrast to the similar DC maturation-inducing capacities of IL-4 and IL-13, the two cytokines displayed different effects on TNFR, CD40, and CD14 expression and IL-12 p70 production by DC.

While IL-4 down-regulated the expression of TNFR2 and CD14, it up-regulated CD40 expression on DC. In contrast, IL-13 resulted in enhanced TNFR2 levels, but had no influence on CD40 and CD14 expression. This argues for distinct receptors or signaling pathways being used by each cytokine, but does not explain the reversion of maturation resistance by DC. Most likely, other mechanisms triggered similarly by both IL-4 and IL-13 through the type II receptor might be responsible for the induction of DC maturation.

The enhancing effect of IL-4 on IL-12 p70 production by DC has been described previously (17). Here we show that this effect could not be achieved through the type II receptor with IL-13 or IL-4.Y119D, a type II IL-4R-selective IL-4 mutant. Furthermore, this effect is specific for IL-12 p70, as there was no detectable effect on IL-12 p40, IL-10, or TNF-α production by DC.

The type II IL-4R is not expressed on activated or T cells, which raises the question of possible mechanisms of the previously described Th2-driving potential of IL-13 (45). As we found that IL-13 does not support IL-12 p70 production and thereby Th1 immunity, it might instead promote Th2 responses at the level of DC. The differential effects of IL-4 and IL-13 on the induction of IL-12 p70 could then explain why deficient IL-13 signaling resulted in increased Th1 immune responses (46), either by allowing default pathways or by unknown factors mediating Th2 maturation.

Taken together our data indicate that the two known types of IL-4R mediate different DC functions. While the type II IL-4R (preferentially engaged by IL-13) is responsible for the up-regulation of MHC and costimulatory molecules, the type I IL-4R (exclusively engaged by IL-4) costimulates IL-12 p70 production to support Th1 immunity. It will be of great interest to determine the expression of the different cytokine receptor molecules and their regulation at different maturation stages and in different subtypes of DC. In addition, modulation of DC functions with selective type I or type II IL-4R agonist or antagonists may be used in new therapeutic strategies of immune intervention.

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