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Positive Regulatory Role of IL-12 in Macrophages and Modulation by IFN-γ

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Similar to myeloid dendritic cells, murine macrophages and macrophage cell lines were found to express a surface receptor for IL-12. As a result, peritoneal macrophages could be primed by IL-12 to present an otherwise poorly immunogenic tumor peptide in vivo. Using binding analysis and RNase protection assay, we detected a single class of high affinity IL-12 binding sites (Kₐ of ~ 35 pM) whose number per cell was increased by IFN-γ via up-regulation of receptor subunit expression. Autocrine production of IL-12 was suggested to be a major effect of IL-12 on macrophages when the cytokine was tested alone or after priming with IFN-γ in vitro. In vivo, combined treatment of macrophages with IFN-γ and IL-12 resulted in synergistic effects on tumor peptide presentation. Therefore, our findings suggest a general and critical role of IL-12 in potentiating the accessory function of myeloid APC. The Journal of Immunology, 2001, 167: 221–227.

Materials and Methods

Mice and cell lines

DBA/2 (H-2b) were obtained from Charles River Breeding Laboratories (Calco, Milan, Italy). Mice of either sex were used at the age of 2–4 mo. The murine monocyte-macrophage cell lines RAW-34, 9784.1, and P3U-1.8 and the murine T cell lymphoma A20 were from the American Type Culture Collection (Manassas, VA).

Cytokines, Abs, and reagents

Murine rIL-12 was a generous gift from B. Hubbard (Genetics Institute, Cambridge, MA). IL-12 was 98.8% pure, as assessed by SDS-PAGE, and endotoxin contamination was < 0.9 EU/mg on Limulus amebocyte assay. The sp. act. of the purified rIL-12 preparation, measured as ability to stimulate proliferation in human PHA-activated blasts, was 3.1 × 10⁹ U/mg. Endotoxin was removed from all solutions containing IL-12 with Detoxigel (Pierce, Rockford, IL), resulting in endotoxin contamination below the detection limit (0.05 EU/ml) of the assay (Coastest Endotoxin; Chromogenix AB, Mölndal, Sweden). The cytokine was routinely used at the concentration of 100 ng/ml. Murine rIFN-γ was from Genzyme (Boston, MA) and was used in vitro at the concentration of 200 U/ml. Murine IL-6 (10⁷ U/mg) was a generous gift of C. Uyttenhove (Ludwig Institute for Cancer Research, Brussels, Belgium) and was used at 10 ng/ml. Polyclonal anti-IL-12 (C-20, rabbit IgG specific for IL-12β1) Ab was from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal IgG Ab specific for IL-12β2 was raised in our laboratory to an epitope corresponding to an amino acid sequence (CNRLDLGINLSPDLAESRF1) mapping at the amino terminus of the murine β2 chain. On Western blot analysis, a 130-kDa protein was recognized that competed with the immunizing peptide for binding to the affinity-purified Ab. On flow cytometric analysis, this Ab specifically recognized the β2 chain on Th1, but not Th2 clone cells with the same Ag specificity (13), and recognition was impaired by the presence of cognate peptide (Fig. 1). Functionally, the Ab specifically blocked the IL-12-induced proliferative response of Con A blasts. Neutralizing rat anti-mouse IFN-γ XMG1.2 mAb was used in vitro at the concentration of 10 μg/ml (11). LPS (Sigma, St. Louis, MO) was used at the concentration of 1 μg/ml.

Macrophage cultures

Macrophages were isolated from the peritoneal cavity of mice 4–5 days after the injection of 0.5 ml of aged, endotoxin-free 10% thioglycolate medium (Difco, Detroit, MI), endotoxin being depleted from media with Detoxigel (14), After adherence on a plastic surface for 2 h, nonadherent cells were removed by three washes with medium, and adherent cells were recovered by exposure to 2 mM EDTA in PBS for 20 min. Viability was > 95%, and the cells consisted principally of macrophages (> 99%), as...
with P185AB tumor peptide were stained with the β2-specific Ab or control rabbit IgG, followed by the addition of FITC-conjugated goat anti-rabbit IgG. Staining was also investigated in the presence of 5 μg/ml competitor peptide. C: Control staining.

![image](https://example.com/image1.png)

**FIGURE 1.** Ability of rabbit polyclonal IgG Ab raised to IL-12Rβ2 to recognize the β2 chain of the receptor on a Th1, but not Th2 clone. Th1 (F76) and Th2 (F2) clone cells specific for the P815AB tumor peptide were stained with FITC-conjugated goat anti-rabbit IgG, followed by the addition of FITC-conjugated goat anti-rabbit IgG. Staining was also investigated in the presence of 5 μg/ml competitor peptide. C: Control staining.

Nuclear extracts and EMSA

The assay was performed essentially as reported previously (9). Cells were stimulated for 15 min with rIL-12 or rIFN-γ, and nuclear extracts were prepared. All DNA-binding reactions were conducted for 20 min at room temperature in a final volume of 20 μl. The reactions were started by adding 10 μg nuclear protein extract to a reaction mix containing 1 μg poly(dIdC).d(dIdC), 4 μl 5% binding buffer (50 mM Tris, pH 7.5, 250 mM NaCl, 5 mM EDTA, 25% glycerol, and 5 mM DTT), and approximately 20,000 cpm (−0.1 ng) of [γ-32P]ATP-labeled dsDNA oligonucleotide (5′-TCGGACATGGCTTCAAGGATTTGAGATGTATTTCCACAGAAATGCCTCA-3′). Samples were then loaded on a 5% native polyacrylamide gel in Tris-borate-EDTA buffer. After electrophoresis, gels were dried and separated protein-DNA complexes were visualized by autoradiography using Kodak XAR5 films.

Immunoblotting

Following incubation, macrophages were removed from the culture plates, sedimented, and lysed in buffer containing 1% Nonidet P-40. After SDS-PAGE resolution, immunoblotting was performed with anti-Stat Abs. Membranes were blocked in Tris-buffered saline containing 0.05% Tween 20, 5% nonfat dried milk, and 1% BSA, and incubated sequentially with anti-phosphoStat3 (1/1000; New England Biolabs, Beverly, MA) and anti-Stat3 (1/1000; Santa Cruz Biotechnology), or anti-phosphoStat4 (1/1000; Zymed, San Francisco, CA) and anti-Stat4 (1/1000; Santa Cruz Biotechnology), followed by HRP-conjugated anti-rabbit IgG (1/5000).

Enzyme-linked immunosorbent assay

Culture supernatants were assayed for IL-12 p70 contents by ELISA using hamster anti-mouse p35 (clone Red-T) mAb and biotinylated anti-mouse p40 (C17.8) mAb (9). The sensitivity limit of this assay was approximately 15 pg/ml for IL-12 p70.

Results

IL-12 confers priming ability on macrophages pulsed with a synthetic peptide

We have previously shown that transfer of dendritic cells exposed sequentially to IL-12 and a tumor peptide, P815AB, confers T cell-mediated reactivity on prospective recipients of an intrafootpad challenge with the peptide (21–23). We therefore wanted to investigate whether similar adjuvanticity could be exerted by IL-12 on peptide presentation by macrophages. Fig. 2 shows the effects of sensitization with P815AB using freshly harvested peritoneal macrophages (>99% Mac-1−) exposed to IL-12 before peptide pulsing and transfer into hosts to be assayed for skin test

![image](https://example.com/image2.png)

**FIGURE 2.** Ability of rIL-12 to prime macrophages for induction of skin test reactivity to P815AB. Macrophages were exposed sequentially in vitro to IL-12 (100 ng/ml for 18 h) and P815AB (5 μM for 2 h) before transfer into recipient hosts. Two weeks after cell transfer, mice were assayed for skin test reactivity in vivo. Specificity controls included the use of the antigenically unrelated P91A peptide for footpad challenge. *, Significant difference (p < 0.01) in footpad weight increase between experimental and control footpads. One experiment of five.
reactivity at 2 wk. Similar to our previous results with splenic dendritic cells as a source of myeloid APC, P815AB-specific footpad reactivity was observed only in mice receiving macrophages treated with IL-12 before peptide pulsing.

**Binding of IL-12 to macrophages**

The finding that strong adjuvanticity is displayed by IL-12 upon transfer of P815AB-pulsed macrophages into recipient mice prompted us to investigate possible binding of IL-12 to macrophages. Using peritoneal macrophages and the macrophage cell line RAW.309, we performed equilibrium-binding analysis of radiolabeled IL-12 to both types of cells, either untreated or exposed to IFN-γ overnight. Binding assays were performed with different concentrations of radioligand (Fig. 3), and Scatchard plots of the specific binding data were analyzed by means of the LIGAND program. A single site model was found to fit the data, with the resulting affinities appearing to be similar for the two types of cells ($K_d = 35.7 ± 7 \text{ pM}$ and $K_d = 33.2 ± 5.1 \text{ pM}$ for macrophages and RAW.309 cells, respectively; means ± SD of five (macrophages) or three (RAW.309) independent experiments). No significant changes in $K_d$ values were observed after treatment with IFN-γ. In contrast, the number of IL-12 binding sites per cell was greatly increased by exposure to IFN-γ, rising from 359 ± 70 (macrophages) and 246 ± 63 (RAW.309) to 1116 ± 125 and 1240 ± 198, respectively, after treatment with IFN-γ. Thus, it appeared that similar to dendritic cells, macrophages express a single class of high affinity IL-12 binding sites, with $K_d$ values at least one order of magnitude lower than those of dendritic cells (9). Both the greater affinity and the higher number of IL-12 binding sites relative to dendritic cells suggested a significant biological role of the IL-12R on macrophages, which is consistent with the data in Fig. 2. Furthermore, the binding analysis data indicated a potential function of IFN-γ as a modulator of IL-12 responsiveness in these cells.

**Analysis of IL-12R subunit expression by macrophages**

IL-12Rs, primarily expressed on activated T, NK, and dendritic cells, are gp130-like cytokine receptor superfamily members. These receptors have the general makeup of β-type cytokine receptor subunits, and are thus designated as IL-12Rβ1 and IL-12Rβ2 (24). As it is known that IFN-γ regulates IL-12Rβ2 expression in T cells (25), we examined the expression of the β1 and β2 chains in macrophages and RAW.309 cells, either untreated or exposed to IFN-γ. In a first set of experiments, we used flow cytometry for detection of IL-12R subunit expression with rabbit polyclonal Abs specific for the β1 or β2 chain (Fig. 4). Both subunits were found to be expressed by macrophages and RAW.309 cells. Treatment with IFN-γ resulted in a remarkable increase in the expression of the β1 chain.

We next used RNase protection analysis for more accurate quantitative detection of the β1 and β2 transcripts, with or without IFN-γ priming (Fig. 5). We comparatively analyzed the expression of IL-12Rβ1 and β2 messages in Con A lymphoblasts (positive control), freshly harvested macrophages, the RAW.309 cell line,
and the A20 B lymphoma line, the latter cells representing an appropriate negative control (9). Although the β1 subunit was clearly more inducible than the β2, thus confirming the results in Fig. 4, the overall effect appeared to be dependent on the type of cell. By measuring the intensities of the protected β1 and β2 fragments relative to β-actin in several independent experiments, we found that the level of β1 expression in macrophages was increased ̃10-fold by IFN-γ treatment, whereas that of the β2 chain was increased by 1.5-fold. In RAW.203 cells, the respective increases in β1 and β2 expression induced by IFN-γ were approximately 20-fold and 3-fold. RT-PCR experiments using previously described β1 (nt 1073–2239) and β2 (1760–2261) primers under defined amplification conditions (9) showed that the β1 message was barely detectable in the absence of IFN-γ, whereas the extent of the β2 message was only marginally influenced by cell exposure to IFN-γ (in both fresh macrophages and different cell lines; data not shown). In addition, sequencing of a series of amplicons spanning the β1 and the β2 genes proved these sequences to be identical to the corresponding genes in T cells (26, 27).

**IL-12 does not activate Stat3 and Stat4 in macrophages**

In T cells, signaling through the IL-12R involves ligand binding to the β1 chain, heterodimerization with the β2 chain, and phosphorylation of two receptor-associated Janus kinases, Tyk2 and Jak2 (28). These phosphorylated intermediates recruit Stat3 and Stat4 to the complex (25, 29, 30), which, after phosphorylation and dimerization, are transported to the nucleus, where they regulate transcription of a number of genes. We have previously shown that IL-12 does not activate Stat3 and Stat4 for DNA binding in dendritic cells. We performed EMSA analysis and studies of STAT phosphorylation for assessing the possible involvement of STAT proteins in IL-12 signaling in macrophages. Nuclear extracts were assayed from fresh macrophages and different macrophage cell lines using a variety of probes specific for STAT factors, including FcγRI (9), IFN-γ response region, IFN-γ activation site/Ly-61/E, and IFN-γ activation site/pim-1 (31). These experiments clearly demonstrated lack of involvement of STAT proteins in IL-12 signaling in macrophages and macrophage cell lines. In particular, Fig. 6A shows the results of an experiment in which macrophages or a macrophage cell line were treated with IL-12, and nuclear extracts were incubated with a labeled FcγRI probe in EMSA analysis. Although IL-12 induced a significant band shift with extracts of control blast cells, no such effect could be observed in macrophages, which nevertheless were susceptible to STAT induction by IFN-γ. We have previously shown that Stat4 is not activated by IL-12 in dendritic cells (9), and it is known that in their basal state, human monocytes do not express Stat4, which nevertheless is induced by combined treatment with IFN-γ and LPS (32). To assess the possible expression of Stat3/Stat4 and the ability of IL-12 to

![Image](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org)
induce their phosphorylation, cell lysates from IL-12-treated macrophages were run out on SDS-PAGE, and immunoblotted with Abs specific for STAT proteins or their phosphorylated forms. Fig. 6B shows that Stat3 was expressed in control macrophage cultures and that expression was enhanced by priming with IFN-γ plus LPS. Stat3 phosphorylation was observed at 5, 10, and 20 min in cultures treated with IL-6, but not with IL-12. In contrast, no Stat4 expression could be observed in control macrophage cultures, and phosphorylation was not induced by IFN-γ/LPS, IL-12, or a combination of both. This pattern was different from that of Con A blasts, in which expression of Stat4 was clearly observed, and phosphorylation was induced by treatment with IL-12 (Fig. 6C). RT-PCR experiments using Stat4-specific primers confirmed lack of Stat4 expression in murine macrophages (data not shown).

**Induction of endogenous IL-12 production by IL-12 and potentiation by IFN-γ**

In monocyctic cells, IFN-γ enhances IL-12 production mostly by priming cells for LPS-induced transcription of the IL-12 p40 gene (33). In contrast, induction of biologically relevant amounts of endogenous IL-12 is observed upon exposure of dendritic cells to IL-12 (9). Moreover, autocrine production of IL-12 is involved in myeloid dendritic cell modulation through CD40 ligation (11). We investigated the possible production of IL-12 by macrophages exposed to rIL-12 or a combination of IFN-γ plus LPS or plus IL-12. We measured p70 production by ELISA in supernatants of macrophage cultures treated overnight with IFN-γ, followed by LPS (4 h) or IL-12 (4 h). Appropriate controls included cells treated singly with LPS, IFN-γ, or IL-12 (Fig. 7). Cells were extensively washed and then incubated in fresh medium. Culture supernatants were harvested at 1 and 24 h. No IL-12 was found in any group at 1 h (data not shown), whereas considerable amounts of the cytokine were found at 24 h in cultures exposed to IL-12 or, even more, to a combination of IFN-γ and LPS or of IFN-γ and IL-12. Lack of IL-12 detection at 1 h clearly indicated that the p70 measured at 24 h was not derived from externally added IL-12, bound and/or internalized in the cultures. In addition, marked production of IL-12 p70 was also observed in the macrophage cell lines RAW.309, J774A.1, and PU5-1.8 upon exposure to external IL-12 with or without IFN-γ priming (data not shown).

**Effect of IFN-γ and combined effects of IFN-γ and IL-12 on tumor peptide presentation in vivo by macrophages**

We have already mentioned that the expression of an IL-12R by dendritic cells is apparently unaffected by IFN-γ (9–11). In addition, in our model system of skin test reactivity to P815AB in vivo IFN-γ may exert an inhibitory effect on peptide presentation by myeloid (CD8α−) dendritic cells via induction of tolerogenic activity in lymphoid (CD8α+) dendritic cells (12, 34). We therefore became interested in studying the combined effects of IFN-γ and IL-12 on tumor peptide presentation in vivo by macrophages. In addition, we examined the possibility that IL-12-induced IFN-γ might contribute to IL-12 effects on macrophages. Macrophage cultures were exposed overnight to medium or IFN-γ. After washing, cells were treated with IL-12 for 4 h and then washed extensively before peptide pulsing and injection into recipient hosts. P815AB-specific delayed-type hypersensitivity was assessed at 2 wk. Fig. 8 shows that IFN-γ treatment alone resulted in a modest, but significant delayed-type hypersensitivity response. As expected, IL-12 resulted in a highly significant response that was further increased by preexposure of macrophage cultures to IFN-γ. The limited response induced by IFN-γ treatment alone and its synergic effect with IL-12 could be explained either by the macrophage-activating properties of the cytokine or by up-regulation of IL-12Rs to be engaged by IL-12 in vitro or endogenous IL-12 in vivo in the macrophage recipients. The latter hypothesis is substantiated by the finding that the combined effects of IFN-γ and IL-12 were the greatest when IFN-γ treatment would precede, rather than follow, exposure to IL-12 (data not shown). Fig. 8 also shows that the addition of IFN-γ-neutralizing Ab to the macrophage cultures activated by IL-12 did not affect the expression of IL-12 activity. These data demonstrate that the production of IFN-γ in vitro is not a major mechanism whereby IL-12 primes macrophages for effective presentation of the tumor peptide in vivo.

**Discussion**

Although sporadic observations in the literature have indicated that the macrophage may be another cell type responsive to IL-12 p40/...
p70 (35–38), the present data provide the first direct evidence that murine macrophages can bind IL-12 through a specific high affinity receptor. Following observations that strong adjuvanticity is displayed by IL-12 upon transfer of peptide-pulsed macrophages into recipient mice (Fig. 2), we investigated binding of IL-12 to these cells. Experiments of receptor affinity with labeled IL-12 in equilibrium-binding analysis identified a single site with an apparent equilibrium dissociation constant of 34 pM and 339 sites per macrophage (Fig. 3). The binding of IL-12 to macrophages was saturable and specific, because the binding of radiolabeled ligand was only inhibited by IL-12 (Ki = 28 pM) and not by other cytokines (data not shown). When compared with dendritic cells (9), the affinity of the IL-12R on macrophages appeared to be even higher, thus accounting for the adjuvanticity exerted by IL-12 on both dendritic cells and macrophages in vivo.

Early in an immune response, complex bidirectional influences take place between IL-12 and IFN-γ, two major cytokines involved in the initiation of cell-mediated immunity (1, 3, 39, 40). Activation of myeloid APC, including dendritic cells and macrophages, leads to secretion of IL-12, which subsequently induces IFN-γ production by NK cells (41) and directs Th1 development (7, 8, 42). IFN-γ, in turn, acts on monocytes to initiate or augment IL-12 secretion (43, 44). Thus, IL-12 and IFN-γ comprise a positive feedback loop that is probably required for optimal production of IL-12 in vivo (45). Two observations in our study may be relevant in this regard. First, IFN-γ will enhance the number of IL-12 binding sites expressed on individual macrophages (Fig. 3). Second, IL-12 may possess an autoregulatory role in these cells and such a role may be reinforced by IFN-γ (Fig. 7).

The induction of a greater number of IL-12 binding sites by IFN-γ was confirmed by cytofluorometric analysis of the two major IL-12R subunits, IL-12Rβ1 and IL-12Rβ2, whose coexpression leads to the formation of high affinity IL-12 binding sites (27). Regulation of receptor subunit expression by IFN-γ is known to occur in T cells, in which Th1-promoting and Th2-promoting cytokines mediate their effects via the respective increase and inhibition of β2 chain expression (24, 25). In both freshly harvested macrophages and RAW.309 cells, we found that overnight exposure to IFN-γ led to only a limited increase in IL-12Rβ2 expression and yet resulted in a considerable augmentation in the expression of the β1 subunit (Fig. 4).

RT-PCR experiments not reported in the present study and RNase protection assays provided additional evidence in this direction. Using previously described β1/β2 primers under defined amplification conditions (9), we found that the β1 message, barely detectable in the absence of IFN-γ, was strongly expressed following cell exposure to this cytokine. In contrast, the extent of the β2 message was only marginally increased by cell exposure to IFN-γ. When sequenced, all of these amplicons appeared to be identical to the corresponding published sequences of T cells (26).

We next used RNase protection experiments for quantitative analysis of β1/β2-specific transcripts in macrophages either untreated or treated with IFN-γ. On measuring the intensities of the protected β1 and β2 fragments relative to β-actin, the levels of β1 expression appeared to be increased ~10-fold by IFN-γ, and those of the β2 chain were increased 1.5-fold (Fig. 5). Similar results were obtained with the RAW.309 cells, in which, however, the increase in β1-chain expression was even greater.

In T cells, IL-12 selectively induces nuclear DNA-binding complexes that contain the Stat3 and Stat4 members of the STAT family (25, 29). In dendritic cells, we have previously shown that IL-12 does not activate Stat3 and Stat4 for DNA binding, and have instead observed activation of members of the NF-κB family (9).

In the present study, we used EMSA analysis to investigate the possible involvement of STAT or NF-κB proteins in IL-12 signaling in macrophages. While the results we obtained clearly demonstrated lack of Stat3/Stat4 activation in macrophages (Fig. 6), this approach appeared to be unsuitable for studying IL-12 effects on NF-κB because of apparent baseline activation (data not shown), presumably as a result of physical manipulation of cell cultures (46). We are currently focusing on direct observation of nuclear translocation by immunostaining of macrophages reacted with Abs to individual NF-κB family members.

It is possible that IL-12 signaling in macrophages leads to cellular responses that are associated with improved APC function. Similar to dendritic cells, macrophages will present the P815AB tumor peptide in an immunogenic fashion in vivo following sequential exposure to IL-12 and peptide in vitro. IL-12, on the one hand, is likely to affect the APC function of dendritic cells in several ways, including autocrine production and increased surface expression of fully mature class II and costimulatory molecules (9, 47). On the other hand, early production of IL-12 by APC is a key event in the initiation of an immune response (6–8). Therefore, we investigated the possible production in vitro of IL-12 by macrophages exposed to rIL-12 or a combination of IFN-γ and rIL-12 (Fig. 7). Externally added IL-12 appeared to be a strong stimulator for the endogenous production of the cytokine. Unlike IL-12, IFN-γ was unable to trigger production of IL-12 by macrophages in the absence of a secondary stimulus. Yet, priming with IFN-γ resulted in increased production of IL-12 over that induced by IL-12 alone. While autocrine production of IL-12 has been observed in dendritic cells with no apparent need for IFN-γ priming (9), the present data suggest that IFN-γ increases IL-12 secretion by macrophages and this might occur via up-regulation of the IL-12R. However, it is also possible that IFN-γ contributes to increased IL-12 production by IL-12 via several mechanisms, including direct priming of the p40 gene promoter (33).

Although priming of myeloid (CD8α−) dendritic cells with IL-12 strongly increases presentation of P815AB (10), IFN-γ may act on CD8α+ (lymphoid) dendritic cells to down-regulate tumor peptide presentation in vivo (12, 34). We thus examined the effects of combined exposure of macrophages to IFN-γ and IL-12 on tumor peptide presentation (Fig. 8). Although IFN-γ treatment alone resulted in a modest, but significant response, pretreatment of macrophages with IFN-γ before IL-12 exposure greatly increased the extent of the response over that induced by IL-12 alone. Of interest, concurrent exposure of macrophage cultures to IFN-γ-neutralizing Ab and IL-12 did not affect the expression of IL-12 adjuvanticity in vivo (Fig. 8), thus arguing against a major role of IFN-γ induction in the effects of IL-12 in vitro.

IL-12 is considered to be a key cytokine in bridging innate and acquired immunity and in the initiation of cell-mediated immunity. Until recently, its effects have been thought primarily to involve actions on NK and T cells. By revealing the existence of functional high affinity receptors for the cytokine on myeloid dendritic cells (9, 10) in which IL-12 may cause autocrine effects (11), we have pointed out the possible biological relevance of IL-12 acting as a modulator of accessory cell function. By extending these observations to another myeloid APC, the macrophage, we reinforce our previous hypothesis of an autoregulatory role of IL-12 and further suggest that IL-12 regulation of APC function may be a general mechanism in acquired immunity. By showing IFN-γ regulation of IL-12R expression in macrophages, we indicate an additional potential way of reciprocal influence between the two cytokines (20, 35, 36), with IFN-γ contributing to the full expression of IL-12.
effects on these cells. Finally, by showing lack of Stat3/Stat4 activation by IL-12 in macrophages, we confirm the possible promiscuity and signaling complexity of the IL-12R as expressed by a variety of ontogenetically distinct cell types (9, 48).

References