IL-12 Responsiveness and Expression of IL-12 Receptor in Human Peripheral Blood Monocyte-Derived Dendritic Cells

Hitomi Nagayama, Katsuaki Sato, Hiroshi Kawasaki, Makoto Enomoto, Chikao Morimoto, Kenji Tadokoro, Takeo Juji, Shigetaka Asano and Tsuneo A. Takahashi

*J Immunol* 2000; 165:59-66; doi: 10.4049/jimmunol.165.1.59
http://www.jimmunol.org/content/165/1/59

**References**
This article cites 45 articles, 35 of which you can access for free at:
http://www.jimmunol.org/content/165/1/59.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
IL-12 Responsiveness and Expression of IL-12 Receptor in Human Peripheral Blood Monocyte-Derived Dendritic Cells

Hitomi Nagayama,* Katsuki Sato,* Hiroshi Kawasaki, † Makoto Enomoto,* Chikao Morimoto,† Kenji Tadokoro, ‡ Takeo Juji, ‡ Shigetaka Asano,§ and Tsuneo A. Takahashi¹*

We analyzed the expression of IL-12Rβ1 and IL-12Rβ2 and the role of IL-12 in the activation of monocyte-derived dendritic cells (DCs) via IL-12Rβ1-mediated signaling events. Flow cytometric analysis revealed that IL-12Rβ1 was expressed in T cells, Con A blasts, and monocyte-derived DCs, but not in monocytes, while its transcript was detected in all of these cell types. Transcriptional expression of IL-12Rβ2 was observed in T cells, Con A blasts, and monocyte-derived DCs, but not monocytes. The ligation of DCs as well as Con A blasts by IL-12 induced the production of GM-CSF, IL-1β, IL-6, TNF-α, and IFN-γ at the transcription levels. Furthermore, stimulation of DCs with IL-12 induced IL-12p40 transcript, but not IL-12p35 transcript, whereas this stimulation caused the expressions of both transcripts in Con A blasts. Stimulation of DCs with IL-12 caused a tyrosine phosphorylation of several intracellular proteins, and the pattern of these events were distinct from those of IL-12-stimulated Con A blasts. IL-12 also induced tyrosine phosphorylation of IL-12Rβ2 as well as recruitment of several tyrosine-phosphorylated proteins to IL-12Rβ1 in DCs and Con A blasts. Receptor engagement of DCs as well as Con A blasts by IL-12 resulted in activation of Janus kinase 2 and Tyk2 kinases and Stat3 and Stat4 transcription factors and the association of these proteins to IL-12Rβ1. Stimulation with IL-12 caused a tyrosine phosphorylation and enzymatic activity of a family of mitogen-activated protein kinases, p38mapk. These results suggest that IL-12 acts directly on DCs to induce their functional activation via IL-12Rβ1-mediated signaling events. The Journal of Immunology, 2000, 165: 59–66.

Dendritic cells (DCs) are unique professional major APCs capable of stimulating resting T cells (TCs) in the primary immune response and are more potent APCs than peripheral blood monocytes/macrophages or B cells (1). DCs also play major roles in autoimmune diseases, graft rejection, HIV infection, and the generation of TC-dependent Abs (1–4). DCs capture and process Ag in nonlymphoid tissues and then migrate to TC-dependent areas of secondary lymphoid organs through afferent lymph or the blood stream to prime native TCs and initiate immune responses (5, 6).

Characterization of DCs is difficult because they represent only a small subpopulation that includes interdigitating reticulum cells in lymphoid organs, blood DCs, Langerhans cells in the epidermis of the skin, and dermal DCs (1). Previously, an in vitro culture system revealed that DCs originate from CD34+ pluripotent hematopoietic progenitor cells in the bone marrow and cord blood via myeloid lineage cells in human and murine models (7–12), and some DCs develop from thymic precursors via lymphoid lineage cells in the murine system (13). Previous studies have shown that TNF-α, IL-1β, CD40 ligand (CD40L), LPS, and ceramid can promote DC differentiation in vitro, resulting in irreversible morphological, phenotypical, and functional changes, including up-regulation of MHC products and adhesion/costimulatory molecules, down-regulation of Ag uptake, and processing capacity, which result in enhanced TC-stimulatory capacity (13–18).

IL-12, a heterodimeric cytokine (IL-12p70) composed of a 40-kDa subunit (p40) and a 35-kDa subunit (p35), is produced by activated monocytes/macrophages, DCs, neutrophils, and some B cell lines (19–21). IL-12 play an important role for induction of Th1 cells from naive Th0 cells, the generation of CTLs, as well as the their production of GM-CSF, TNF-α, and IFN-γ (19). In contrast, IL-12 is a strong inducer of activated NK cells (19–20). Studies of IL-12-deficient mice revealed that the capacity of splenocytes to produce IFN-γ was repressed following bacterial, fungal, parasitic, and viral infection, and these phenomenon were involved in their defective primary immune responses (21).

The specific effects of IL-12 on the target cell types are mediated by the IL-12R complex, which consists of IL-12Rβ1 and IL-12Rβ2 (22–27). Chua et al. have reported on the IL-12Rβ1 subunit of mice (23) and humans (24), which has strong homology to gp130, and the WSXWS motif in their extracellular domain (23, 24). Presky et al. (25) showed another IL-12Rβ2 subunit, which also has homology to gp130 and consists of a medium-affinity receptor with a β1 subunit for IL-12. A series of previous studies have shown that IL-12Rβ1 and IL-12Rβ2 are mainly expressed on activated TC or NK cells (26). Recent studies show IL-12Rβ1-deficient mice failed to produce IFN-γ in response to IL-12, and their splenocytes also failed to display IL-12-induced enhancement of NK lytic activity, suggesting that IL-12Rβ1 is an essential component for the IL-12 responsiveness in vitro and in vivo (27).

The engagement of IL-12Rβ1 by IL-12 induces an elaborate biochemical program that ultimately results in the induction of a...
variety of functions including cell proliferation and cytokine secretion. Activation signals following stimulation by IL-12 involve IL-12Rβ1-dependent calcium influx and the initiation of a protein tyrosine kinase (PTK)-dependent pathway including activation of Janus kinase (Jak) (Jak2 and Tyk2)/Stat3 and Stat4 cascades (28–36). Thierfelder et al. (31) have previously shown that IL-12-induced IFN-γ production, generation of cytolytic activity of NK cells, and induction of Th1 cells were defective in Stat4-deficient mice. Grohmann et al. (37, 38) have recently reported that IL-12Rβ1 and IL-12Rβ2 were constitutively expressed on murine splenic CD80− and CD86− DCs and their cell line at transcriptional levels (37, 38). Furthermore, stimulation of these DCs with IL-12 resulted in the increased secretion of endogenous IL-12 and the enhanced class II Ag expression via nuclear localization of NF-κB. In contrast, IL-12 reportedly induced IFN-γ production in murine splenic DCs (39, 40). However, receptor expression for IL-12 and the role of IL-12 on functional activation of human DCs remains unclear.

In this report, we examined the expression of IL-12R in human monocyte-derived DCs and the potential roles of IL-12 in the regulation of DC properties via their downstream signaling cascades.

Materials and Methods

Media and reagents

The medium used throughout was RPMI 1640 supplemented with 2 mM t-glutamine, 50 μg/ml streptomycin, 50 U/ml penicillin, and 10% heat-inactivated FCS. GM-CSF was kindly provided by Kirin Brewery (Tokyo, Japan). IL-4 and IL-12 were purchased from PeproTech (London, U.K.). HRP-conjugated anti-phosphotyrosine (pTyr) mAb (clone RC20) and Stat3 and Stat4-specific primers for IL-12Rβ1 (28) and IL-12Rβ2 as follows; 5′-GTC GAC CCT ACA ATG TGT CTG CTC TGA TTT-3′ and 5′-TCA GAG CAT GCA ATG TGT CTC TGA TTT-3′ and 5′-TCA GAG CAT GAG GTC ACA CCT CAT CTT-3′. Specific primers for β-actin and other cytokines including GM-CSF, IL-1β, IL-6, TNF-α, IFN-γ, IL-12p35, and IL-12p40 (all from Continental Laboratory Products, San Diego, CA) were also used for amplification. Thermal cycling of IL-12Rβ1 and IL-12Rβ2 primers was performed as follows: denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. Other cytokines and β-actin primers were annealed at 60°C, and all cycling was performed for 35 cycles. PCR products were analyzed by electrophoresis through 2% agarose gels and visualized under UV light after ethidium bromide staining.

Flow cytometry

TCs, Con A blasts, monocytes, or DCs, which was treated with 0.5% mouse serum (Dako, Glostrup, Denmark) for 15 min at 4°C to block the Fc receptor, were incubated with biotin conjugated anti-IL-12Rβ1 mAb for 30 min at 4°C, washed twice with cold PBS, and subsequently stained with FITC-conjugated avidin (Becton Dickinson) for 30 min at 4°C. Thereafter, the cells were washed twice and suspended in PBS containing 0.2 μg/ml propidium iodide (Sigma) to exclude dead cells. Analysis of fluorescence staining was performed with a FACSCalibur flow cytometer (Becton Dickinson) and CellQuest software.

Stimulation of Con A blasts and DCs

Con A blasts and DCs (10^7/5 ml) were unstimulated or stimulated with various concentrations of IL-12 (1–100 ng/ml) in the presence or absence of 1 μg/ml of control mouse IgG (Sigma) or anti-IL-12Rβ1 mAb (cont. IgG; Sigma) for 30 min at 4°C. For Western blotting and immunoprecipitation in the transfectants expressing IL-12Rβ1 (30), p38MAPK immunoblotting kits, their kinase assay kits, and HRP-conjugated secondary Abs were purchased from New England Biolabs (Beverly, MA).

In vitro generation and culture of human DCs

DCs were generated from PBMCs as described previously (9) with some modification (10–12). Briefly, PBMCs were obtained from 30 ml of leukocyte-enriched buffy coat from healthy donors by centrifugation with Fi-
coll-Hypaque (Pharmacia Biotech, Uppsala, Sweden), and the light density fraction from the 42.5–50% interface was recovered. The cells were re-
suspended in culture medium and allowed to adhere to six-well plates (Costar, Cambridge, MA). After 2 h at 37°C, nonadherent cells were removed and adherent cells were collected, and these cells were negatively selected with anti-CD2 mAb-conjugated immunomagnetic beads (Dynal, Oslo, Norway) and anti-CD19 mAb-conjugated immunomagnetic beads (Dynal) to deplete CD2+ cells and CD19+ cells according to the manu-
facturer’s instructions. The resulting cells (>95% CD14+ cells) were used as monocytes and cultured in 3 ml of medium supplemented with GM-CSF (50 ng/ml) and IL-4 (250 ng/ml). After 2 ha t37°C, nonadherent cells were re-
suspended in culture medium and allowed to adhere to six-well plates (Costar, Cambridge, MA). After 2 h at 37°C, nonadherent cells were removed and adherent cells were collected, and these cells were negatively selected with anti-CD2 mAb-conjugated immunomagnetic beads (Dynal, Oslo, Norway) and anti-CD19 mAb-conjugated immunomagnetic beads (Dynal) to deplete CD2+ cells and CD19+ cells according to the manu-
facturer’s instructions. The resulting cells (>95% CD14+ cells) were used as monocytes and cultured in 3 ml of medium supplemented with GM-CSF (50 ng/ml) and IL-4 (250 ng/ml). After 7 days of culture, DCs were har-

Preparation and culture of TCs and Con A blasts

TCs were isolated from monocyte-depleted cell population by E-rosetting (41), and TC preparations were typically >95% pure as indicated by anti-
CD3 mAb staining (Becton Dickinson). Con A blasts were prepared from TCs by cultivation with 5 μg/ml of Con A and 10 ng/ml of IL-2 for 48 h, followed by incubation in the presence of IL-2 alone for 5 days (41). Dead cells were removed by gradient centrifugation using Histopaque 1083 (Sig-
ma, St. Louis, MO).

Detection of IFN-γ by ELISA

The in vitro production of IFN-γ was assessed by ELISA as described previously (9). The cultured supernatants (5 ml) of unstimulated or stim-
ulated cells (10^7) were collected and assayed for IFN-γ production. IFN-γ was detected in the supernatants using a two-site sandwich ELISA (Endo-

gen, Woburn, MA). Samples were analyzed in serial 2-fold dilutions in duplicate; the sensitivity of the assay was 2 pg/ml.

Western blotting, immunoprecipitation, and immune complex kinase assay

Con A blasts and DCs were starved in serum-free medium for 16 h at 37°C and subsequently kept for 4 h on ice to reduce the basal levels of tyrosine phosphorylation of intracellular proteins (12, 41). The cells (4 × 10^5) were untreated or stimulated with IL-12 (1–100 ng/ml) for 5 min at 37°C and washed twice in cold PBS, resuspended in 100 μl of lysis buffer (1% Nonidet P-40, 20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 1% glycerol, 2 mM EDTA, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM PMSF, and 1 mM sodium orthovanadate) and total cell lysates were obtained. To pre-
pare immunoprecipitated samples, the total cell lysates (10^7 cells) were immunoprecipitated with an Ab to IL-12Rβ1, Jak2, Tyk2, Stat3, or Stat4, and the immunocomplex was collected for protein G Sepharose 4 fast flow (Pharmacia Biotech) and washed three times with lysis buffer. The total cell lysates or the immunoprecipitates sample were suspended in 2× SDS sample buffer (313 mM Tris-HCl, pH 6.8, 10% SDS, 2% 2-ME, 50 mM glycine, and 0.1% bromophenol blue) and heated for 3 min at 95°C. The samples were fractionated by 12% SDS-PAGE, transferred onto polyvi-

Semiquantitative RT-PCR

RNA from each sample (10^5) was isolated using Trizol LS reagent (Life Technologies, Gaithersburg, MD). The first-strand cDNA kit (SuperScript Preamplification System; Life Technologies) was used to make cDNA (20 μl) from 5 μg of each RNA. Amplification of each cDNA (1 μl) was performed with a SuperTaq Premix kit (Sawady Technology, Tokyo, Ja-


den) using specific primers for IL-12Rβ1 (28) and IL-12Rβ2 as follows; 5′-GTC GAC CCT ACA ATG TGT CTG CTC TGA TTT-3′ and 5′-TCA GAG CAT GAG GTC ACA CCT CAT CTT-3′. Specific primers for β-actin and other cytokines including GM-CSF, IL-1β, IL-6, TNF-α, IFN-γ, IL-12p35, and IL-12p40 (all from Continental Laboratory Products, San Diego, CA) were also used for amplification. Thermal cycling of IL-12Rβ1 and IL-12Rβ2 primers was performed as follows: denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. Other cytokines and β-actin primers were annealed at 60°C, and all cycling was performed for 35 cycles. PCR products were analyzed by electrophoresis through 2% agarose gels and visualized under UV light after ethidium bromide staining.

Flow cytometry

In this report, we examined the expression of IL-12R in human monocyte-derived DCs and the potential roles of IL-12 in the regu-

lution of DC properties via their downstream signaling cascades.
Results

Expression level of IL-12R in TCs, Con A, monocytes, and DCs

Previous studies have shown that IL-12R is detected in TCs and NK cells, and this component of IL-12R plays a crucial role for IL-12-mediated activation of these cell types (19–30). Recently, murine splenic DCs exhibited transcriptional expression of IL-12Rβ1 and IL-12Rβ2 (37, 38), and IL-12 induced activation of these cells (37–40). However, much is unknown about expression of IL-12Rβ1 and IL-12Rβ2 and the effect of IL-12 on human monocyte-derived DCs.

Flow cytometric analysis shows that IL-12Rβ1 was expressed on monocyte-derived DCs as well as TCs and Con A blasts, and the expression level of IL-12Rβ1 on monocyte-derived DCs was higher than those of TCs and Con A blasts (Fig. 1A). In contrast, little or no expression of IL-12Rβ1 were observed in monocytes (Fig. 1A).

We also examined the transcriptional expression of IL-12Rβ1 in TCs, Con A blasts, monocytes, and monocyte-derived DCs. Fig. 1B shows that IL-12Rβ1 transcript was expressed in monocytes and monocyte-derived DCs as well as TCs and Con A blasts.

We further examined the transcriptional expression of IL-12Rβ2 in TCs, Con A blasts, monocytes, and monocyte-derived DCs (Fig. 1B). Semiquantitative RT-PCR analysis revealed that the transcript of IL-12Rβ2 were expressed in TCs, Con A blasts, and DCs, and the expression level of IL-12Rβ2 in Con A blasts and DCs were higher than that of TCs. We also observed little or no transcriptional expression of IL-12Rβ2 in monocytes.

IL-12 induces production of various cytokine in DCs

To address the effect of IL-12 on the capacity of DCs to produce cytokines, monocyte-derived DCs were unstimulated or stimulated with IL-12 for 48 h, and the transcriptional levels of inducible cytokines were examined by semiquantitative RT-PCR (Fig. 2). Stimulation of Con A blasts with IL-12 caused the production of GM-CSF, IL-1β, IL-6, TNF-α, IL-12p40, and IL-12p35 at transcriptional levels. In contrast, treatment of monocyte-derived DCs with IL-12 resulted in the production of GM-CSF, IL-1β, IL-6, TNF-α, and IL-12p40. We also observed that the production level of IL-1β in IL-12-stimulated monocyte-derived DCs was higher than that of IL-12-stimulated Con A blasts, whereas the stimulation of monocyte-derived DCs with IL-12 induced lower production of IL-12p35 than that of IL-12-stimulated Con A blasts.

Stimulation of murine splenic DCs and activated macrophages with IL-12 induced IFN-γ production (39, 40). Therefore, we examined the effect of IL-12 on the production of IFN-γ in monocyte-derived DCs. The ligation by IL-12 induced the production of the transcript of IFN-γ (Fig. 2) and its product (Table I) in these cells, and this productions were comparable to those of IL-12-stimulated Con A blasts.
IL-12 induces tyrosine phosphorylation of multiple intracellular proteins in DCs via IL-12Rβ1

The engagement of IL-12Rβ1 by IL-12 increases the tyrosine phosphorylation of targeted intracellular proteins in various cell types, and these intracellular events appear to be crucial for their functional activation (32–36). However, much less is known about intracellular signaling events associated with protein tyrosine phosphorylation cascades that are responsible for IL-12-mediated activation of DCs. To address direct involvement of the PTK-dependent pathway in the responsiveness for IL-12-mediated functional activation of Con A blasts and monocyte-derived DCs, the cells were unstimulated or stimulated with IL-12 (1–100 ng/ml), and Western blots of whole-cell lysate stained with anti-pTyr mAb were performed (Fig. 3A). Under starved conditions, several intracellular proteins were weakly tyrosine phosphorylated in unstimulated monocyte-derived DCs, while elevated tyrosine phosphorylation appeared in various intracellular proteins in these cells stimulated with IL-12 in a dose-dependent manner. We also observed that IL-12 induced tyrosine phosphorylation events in Con A blasts, and the pattern of these events were distinct from those of monocyte-derived DCs.

We (30) have previously reported that a mAb to IL-12Rβ1 abolished IL-12-induced Ca2+ influx of the transfectants expressing IL-12Rβ1 and the proliferative responsiveness of Con A blasts to IL-12. In contrast, Wu et al. (27) have previously reported that Con A-activated splenocytes from IL-12Rβ1-deficient mice failed to proliferate or produce IFN-γ in response to IL-12. Therefore, we examined the role of IL-12Rβ1 in IL-12-induced tyrosine phosphorylation events in monocyte-derived DCs. As shown in Fig. 3B, anti-IL-12Rβ1 mAb, but not cont. IgG, suppressed IL-12-induced tyrosine phosphorylation of targeted intracellular proteins in monocyte-derived DCs. We also observed that IL-12-induced tyrosine phosphorylation of multiple proteins were suppressed by a mAb to IL-12Rβ1 in Con A blasts. These results indicate that IL-12-induced tyrosine phosphorylation events are mainly mediated by IL-12Rβ1 in monocyte-derived DCs and Con A blasts.

We (30) have previously reported that the ligation by IL-12-induced tyrosine phosphorylation of tyrosine residues in the cytoplasmic domain of IL-12Rβ1 and its association with various tyrosine-phosphorylated proteins in Con A blasts and IL-12Rβ1-transfected cells. To examine the tyrosine phosphorylation level of IL-12Rβ1 and its association with tyrosine-phosphorylated proteins, the total cell lysates from monocyte-derived DCs and Con A blasts unstimulated or stimulated with IL-12, were immunoprecipitated with a mAb to IL-12Rβ1, and were then subjected to Western blotting with anti-pTyr mAb (Fig. 4, A and B). We observed induction of tyrosine phosphorylation of IL-12Rβ1 in monocyte-derived DCs and Con A blasts following stimulation with IL-12. The position of IL-12Rβ1 (∼100 kDa) were determined by Western blotting with a mAb to IL-12Rβ1. Furthermore, we found significant increase in the level of tyrosine phosphorylation of various proteins associated with IL-12Rβ1 from IL-12-stimulated Con A blasts and monocyte-derived DCs, while these tyrosine-phosphorylated IL-12Rβ1-associated proteins were different between Con A blasts and monocyte-derived DCs. These results indicate that IL-12 induces tyrosine phosphorylation of IL-12Rβ1 as well as recruitment of tyrosine-phosphorylated proteins to IL-12Rβ1 in Con A blasts and monocyte-derived DCs.

Jak2/Tyk2 and Stat3/Stat4 are associated with tyrosine-phosphorylated IL-12Rβ1 in IL-12-stimulated DCs

Previous studies have shown that the engagement by IL-12 is associated with phosphorylation of two member of the Jak family (Jak2 and Tyk2) and Stat family (Stat3 and Stat4) (32–36). To address whether these proteins were directly associated with tyrosine-phosphorylated IL-12Rβ1, the total cell lysates were immunoprecipitated with IL-12Rβ1 and were subjected to Western blotting with respective Abs (Fig. 4, C–F). We found Jak2/Tyk2

Table 1. Production of IFN-γ in Con A blasts and DCs by IL-12

<table>
<thead>
<tr>
<th>Type of Cells</th>
<th>Stimulation</th>
<th>IFN-γ Production (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con A blasts</td>
<td>None</td>
<td>&lt;2 pg/ml</td>
</tr>
<tr>
<td>Con A blasts</td>
<td>IL-12</td>
<td>625 ± 173</td>
</tr>
<tr>
<td>DCs</td>
<td>None</td>
<td>&lt;2 pg/ml</td>
</tr>
<tr>
<td>DCs</td>
<td>IL-12</td>
<td>574 ± 152</td>
</tr>
</tbody>
</table>

*Con A blasts or DCs (10^7) was either unstimulated or incubated with 50 ng/ml of IL-12. After 48-h incubation, the supernatants were collected and assayed for IFN-γ production by ELISA. The results are representative of eight experiments with similar results.

FIGURE 3. IL-12-induced tyrosine phosphorylation events in DCs via IL-12Rβ1. A, IL-12-induced protein tyrosine phosphorylation events in DCs. Con A blasts or DCs (4 × 10^6) were either unstimulated (lane 1) or incubated with 1 ng/ml (lane 2), 10 ng/ml (lane 3), or 100 ng/ml (lane 4) of IL-12 for 5 min at 37°C. B, Effect of anti-IL-12Rβ1 mAb on IL-12-induced protein tyrosine phosphorylation events in DCs. The cells were either unstimulated (lane 1) or incubated with IL-12 (1 ng/ml) (lane 2), cont. IgG (1 μg/ml) (lane 3), IL-12 and cont. IgG (lane 4), anti-IL-12Rβ1 mAb (1 μg/ml) (lane 5), or IL-12 and anti-IL-12Rβ1 mAb (lane 6) for 5 min at 37°C. The total cell lysates prepared from the resulting cells were fractionated by 12% SDS-PAGE and blotted onto PVDF membranes. Tyrosine phosphorylated proteins were detected by ECL using HRP-conjugated anti-pTyr mAb. The results are representative of five experiments with similar results.
and Stat3/Stat4 proteins in the immunoprecipitates with IL-12Rβ1 in monocyte-derived DCs and Con A blasts following stimulation with IL-12, whereas little or no detection of these proteins in the immunoprecipitates with IL-12Rβ1 in unstimulated these cell types. These results indicate that ligation by IL-12 induced association of Jak2/Tyk2 and Stat3/Stat4 with tyrosine-phosphorylated IL-12Rβ1.

**IL-12 induces activation of Jak2/Tyk2 and Stat3/Stat4 in DCs**

The ligation by IL-12 initiates tyrosine phosphorylation of Jak2 and Tyk2, which leads to their enzymatical activation in TCs and NK cells (32–36). To clarify the potential involvement of Jak2 and Tyk2 in IL-12-mediated activation of monocyte-derived DCs, the cells were unstimulated or stimulated with IL-12, and the level of their phosphorylation was assessed by immunoblotting with anti-pTyr mAb (Fig. 5, A–D). Stimulation of monocyte-derived DCs increased tyrosine phosphorylation of Jak2 and Tyk2 compared with those of unstimulated cells, and similar results were observed in Con A blasts. In contrast, the total amounts of these Jaks were unchanged following stimulation in these cell types (Fig. 5, A–D).

A series of previous studies have revealed that Jak (Jak2 or Tyk2)-mediated phosphorylation of their transcription factors (Stat3 or Stat4) results in interactions with their respective enhancer elements and regulation of various gene expressions leading to activation of TCs and NK cells (32–36). To address whether IL-12-induced functional change of DCs were associated with
activation of these Jaks, the tyrosine phosphorylation forms of their transcription factors were examined (Fig. 5, A–D). Indeed, the engagement by IL-12 triggered tyrosine phosphorylation of Stat3 and Stat4 in monocyte-derived DCs as well as Con A blasts, while the total amounts of these Stat proteins were equivalent in unstimulated or IL-12-stimulated cell types. These results indicate that IL-12 induces Jak-dependent signaling events in monocyte-derived DCs and Con A blasts.

**FIGURE 5.** IL-12 induces activation of Jak2 and Tyk2 in DCs. Con A blasts or DCs (10^7) were unstimulated (lane 1) or stimulated with IL-12 (10 ng/ml) (lane 2) for 5 min at 37°C, and total cell lysates were immunoprecipitated with Abs to Jak2 (A), Tyk2 (B), Stat3 (C), or Stat4 (D). The immunoprecipitates were fractionated by 12% SDS-PAGE and blotted onto PVDF membranes. The membranes were incubated with HRP-conjugated anti-pTyr mAb, and the blots were visualized by ECL. The same membrane was subsequently stripped off, reprobed with stated Abs, and developed with HRP-conjugated secondary Abs by ECL to ensure similar amounts of their proteins in each sample. The results are representative of five experiments with similar results.

**FIGURE 6.** IL-12 induces activation of p38MAPK in DCs. Con A blasts or DCs (10^7) were unstimulated (lane 1) or stimulated with IL-12 (10 ng/ml) (lane 2) for 5 min at 37°C. The total cell lysates were analyzed by Western blotting with mAbs to p38MAPK or its tyrosine-phosphorylated form. In other experiments, immunoprecipitate with anti-tyrosine-phosphorylated p38MAPK was assayed for its kinase activity with ATF-2 used as a substrate. The results are representative of five experiments with similar results.

**IL-12 induces activation of p38MAPK in DCs**

It has been shown that a cascade involving p38MAPK is required for IFN-γ production of certain cell types (40). To test the potential involvement of p38MAPK in the IL-12-induced activation of human DCs, monocyte-derived DCs and Con A blasts were unstimulated or stimulated with IL-12, and the levels of tyrosine phosphorylation and kinase activity of p38MAPK were examined (Fig. 6). Stimulation IL-12 with monocyte-derived DCs as well as Con A blasts increased tyrosine phosphorylation and kinase activity of p38MAPK compared with unstimulated cells.

**Discussion**

IL-12 derived from DCs plays a crucial role for the initiation and the persistence of TCs activation in the process of the Ag presentation. In these study, we demonstrated that IL-12Rβ1 and IL-12Rβ2 are expressed in monocyte-derived DCs, and IL-12 activates these cells to secrete several cytokines via IL-12Rβ1-mediated signaling events.

Grohmann et al. (37, 38) have recently reported that the transcripts of IL-12Rβ1 and IL-12Rβ2 were more highly expressed in murine splenic CD8α^+ DCs than its CD8α^- counterpart. We showed that the transcripts of IL-12Rβ1 and IL-12Rβ1 and the product of IL-12Rβ1 on the cell surface were constitutively expressed in monocyte-derived CD4^+ DCs as well as Con A blasts (Fig. 1). In contrast, monocytes expressed IL-12Rβ1, but not IL-12Rβ2, at the transcriptional level, whereas cell-surface expression of IL-12Rβ1 was not observed in this cell type (Fig. 1). We also failed to detect IL-12Rβ1 products in monocytes by Western blotting with its mAb (data not shown). Although monocytes did not express IL-12Rβ1 on their cell surface, stimulation with GM-CSF plus IL-4 may regulate posttranscriptional expression of IL-12Rβ1 in the differentiation of DCs from monocytes. Further study will test this possibility.

Previous studies have shown that DCs exhibited the capacity to produce various sets of cytokines in response to several extracellular stimuli (18, 37, 39, 40, 42–45). We showed that stimulation
of monocyte-derived DCs as well as Con A blasts with IL-12 induced the production of the GM-CSF, IL-1β, IL-6, TNF-α, and IFN-γ (Fig. 2 and Table I). These results suggest that IL-12-induced DC-derived cytokine production may contribute to the activation of TCs as well as NK cells, B cells, and monocytes/macrophages to regulate immune responses.

Grohmann et al. (37) have shown that IL-12 induced endogenous production of IL-12 in splenic DCs in the murine system. We showed that exogenous stimulation of human monocyte-derived DCs with IL-12 induce the endogenous production of the IL-12p40 transcript, but not the IL-12p35 transcript, while Con A blasts produce both transcripts following exogenous stimulation with IL-12 (Fig. 2). D’Andrea et al. (20) have shown that activated PBMC preferentially produce 10- to 50-fold excess of IL-12p40 as compared with IL-12p35. Therefore, stimulation with exogenous IL-12 may be insufficient to produce IL-12p35 in Con A blasts and DCs. In contrast, Ling et al. (22) have reported that the IL-12p40 homodimer competed with the IL-12p70 heterodimer for the binding with IL-12 binding sites and suppressed the IL-12-induced proliferation of PHA blasts (22). Thus, these phenomena led us to hypothesize that IL-12-induced autocrine production of the p40 homodimer regulates IL-12-induced activation of DCs, which leads to the termination of TC activation during Ag presentation.

Previous studies have reported that the engagement by IL-12 initiates tyrosine phosphorylation of various intracellular proteins in Con A blasts and NK cells, events involved in IL-12-mediated functional activation of these cell types (19–30). We show that stimulation of monocyte-derived DCs induce tyrosine phosphorylation events, and the pattern of phosphorylated protein is distinct from those of Con A blasts (Fig. 3A). Furthermore, a mAb to IL-12Rβ1 abrogated IL-12-induced tyrosine phosphorylation events (Fig. 3B). Although IL-12Rβ1-mediated signaling events may be mediated through a protein tyrosine phosphorylation cascade in DCs and Con A blasts, distinct PTKs may be activated following stimulation of IL-12 in these cell types.

Accumulating results showed that the activation of the cascades of Jak/Stat and p38mapk are essential for IL-12-mediated activation of Con A blasts, NK cells, neutrophils, and DCs (28–36, 40). We showed that the ligation of IL-12Rβ1 by IL-12 induced tyrosine phosphorylation of the intracellular domain of IL-12Rβ1 as well as its recruitment of Jak2/Tyk2 and Stat3/Stat4 (Fig. 4). Furthermore, this stimulation caused the activation of the cascades of Jak/Stat (Fig. 5) and p38mapk (Fig. 6). These results suggest that activation of the cascades of Jak/Stat and p38mapk may be involved in IL-12-mediated functional activation of monocyte-derived DCs.

In summary, our results suggest that the endogenous secretion of IL-12 in DCs may regulate themselves as well as other cell types via production of various cytokines to control the homeostasis of the immune responses. Recently, IL-12 gene-transduced DCs have been shown to be effective for the initiation and the persistence of tumor-specific immunity (46). Defining the precise mechanisms by which DCs act on the regulation of TC activation during Ag presentation may provide further insight into the role of these cells in immune-related diseases and facilitate the use of DCs in vaccination for cancer treatment.

Acknowledgments
We thank Dr. N. Yamashita (Institute of Medical Science, University of Tokyo) for his critical comments on this manuscript.

References

Downloaded from http://www.jimmunol.org/ by guest on April 13, 2017
ROLE OF IL-12 ON THE ACTIVATION OF HUMAN DENDRITIC CELLS


