Differential Production of IL-23 and IL-12 by Myeloid-Derived Dendritic Cells in Response to TLR Agonists

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The recently delineated role for IL-23 in enhancing Th-17 activity suggests that regulation of its expression is distinct from that of IL-12. We hypothesized that independent TLR-mediated pathways are involved in the regulation of IL-12 and IL-23 production by myeloid-derived dendritic cells (DCs). The TLR 2 ligand, lipoteichoic acid (LTA), the TLR 4 ligand, LPS, and the TLR 7/8 ligand, resimiquod (R848), induced production of IL-23 by DCs. None of these TLR ligands alone induced significant IL-12 production, except when combined with IFN-γ or other TLR ligands. Notably, IL-23 production in response to single TLR ligands was inhibited by IL-4. DCs treated with single TLR agonists induced IL-17A production by allogeneic and Ag-specific memory CD4+ T cells, an effect that was abrogated by IL-23 neutralization. Moreover, these DCs stimulated IL-17A production by tumor peptide-specific CD8+ T cells. In contrast, DCs treated with dual signals induced naive and memory Th1 responses and enhanced the functional avidity of tumor-specific CD8+ T cells. These results indicate that distinct microbial-derived stimuli are required to drive myeloid DC commitment to IL-12 or IL-23 production, thereby differentially polarizing T cell responses. The Journal of Immunology, 2008, 181: S120–S127.

Interleukin-12 and IL-23 are members of a small family of heterodimeric cytokines. Both molecules share a common p40 subunit that, in the case of IL-12, is covalently linked to a second p35 subunit. IL-23 is comprised of p40 and p19 subunits. Macrophages and dendritic cells (DCs) are the major sources of both IL-12 and IL-23, which act on receptors primarily expressed by T cells, NK cells, and NKT cells (1, 2). The role of IL-12 in inducing IFN-γ-producing CD4+ and cytotoxic CD8+ T cells and enhancing anti-bacterial and antitumor immune responses is now well established (3–6). In contrast, the regulatory functions of IL-23 are only beginning to be elucidated. In both mice and humans, IL-23 has been ascribed a role in the regulation of Th17 cells; specifically, IL-23 is required for their expansion and survival (7–9). Experiments using murine models suggest an important role for this IL-23-IL-17 axis in anti-bacterial immune responses (10–12). Moreover, IL-23 and IL-17A have been implicated in a variety of chronic inflammatory conditions, including multiple sclerosis and rheumatoid arthritis (13–15). The role of IL-23 in cancer pathogenesis and antitumor immunity remains controversial. Several studies have suggested potent antitumor activity of IL-23 (16, 17), whereas others point to a tumorigenic role (18, 19). The latter hypothesis is consistent with the observation that IL-23 is over-expressed in some human cancers and induces matrix metalloproteases and angiogenesis in murine models (18).

These divergent observations regarding the role of IL-23 in cancer pathogenesis may reflect the interplay between IL-12 and IL-23 regulatory pathways. Numerous studies have confirmed that the activation of TLRs leads to subsequent DC maturation (20–22); however, treatment of DCs with single TLR agonists is not sufficient to induce high-level IL-12 expression. IFN-γ or the TLR 7/8 agonists, R848 (Resimiquod), may prime DCs for additional stimulation with CD40L or other TLR agonist, resulting in p35 induction (23–25). In contrast, stimulation with single TLR agonists, such as LPS, has been shown to induce IL-23 production by monocyte-derived human DCs, albeit only after additional exposure to CD40L (26). The number and frequency of DC stimuli may, therefore, determine commitment toward an IL-12-induced Th1 response vs an IL-23-supported Th17 response. Evidence that the presence of IL-12 inhibits Th17 responses (27) further reinforces the importance of evaluating IL-23 expression in conjunction with IL-12 expression.

In this study, we investigated the role of TLR signaling in the regulation of IL-12 and IL-23 production by myeloid-derived DCs. In this study, we report that several TLR agonists alone can induce IL-23 expression, whereas multiple signals are required for commitment to IL-12 production. The data further demonstrate that DC commitment to IL-12 production polarizes a predominantly Th1 response, despite continued IL-23 production by the stimulator DC population. In contrast, DCs that produce IL-23 in the absence of IL-12 polarize a Th17 response.

Materials and Methods

Reagents and Abs

Purified lipoteichoic acid (LTA) and poly(I:C) were purchased from Invivogen. Escherichia coli O26:B6 LPS was purchased from Sigma-Aldrich. Recombinant human IFN-γ, IL-4, and GM-CSF were purchased from R&D Systems. Resimiquod (R848) was a gift from 3M Pharmaceuticals. Lipofectin was obtained from Invitrogen. FITC anti-CD14
(M5E2), PE-conjugated anti-CD80 (L307.4), and allophycocyanin-conjugated anti-CD83 (HB115e) were purchased from BD Biosciences. CD1c+ DC and monocyte isolation kits were obtained from Miltenyi Biotec. Recombinant human CD40L and enhancer were obtained from Axxora. Anti-IL-12p70 neutralizing Ab, anti-IL-23p19 neutralizing Ab, goat IgG (control Ab), and recombinant human IL-12 were obtained from R&D Systems.

**Generation of monocyte-derived DCs**

Elutriated mononuclear cells were cultured (1.5 × 10^6/ml) in macrophage serum-free medium (SFМ) (Invitrogen) with 50 ng/ml GM-CSF. In some experiments, IL-4 was added to the medium at a concentration of 5 ng/ml. After overnight culture, the cells were treated with either 10 μg/ml LTA, 10 ng/ml LPS, 1 μg/ml R848, or 1.25 μg/ml poly(I:C) (transfected with the addition of lipofectin per manufacturers protocol) for 24 h. Alternatively, cells were primed with 1000 U/ml IFN-γ or 1 μg/ml R848 for 4 h and then matured with LPS for additional 24 h. In some experiments, CD40L (1 μg/ml) and enhancer (1 μg/ml) were added to cultures 6 h after the addition of other maturation signals. Immature DCs (iDCs) were prepared from monocytes (1.5 × 10^6/ml) cultured in macrophage SFM (Invitrogen) with 50 ng/ml GM-CSF for 48 h.

**Isolation of CD1c+ DCs and CD14+ monocytes**

Separations were performed in accordance with the manufacturer’s instructions. DC14+ monocytes were isolated using a monoocyte isolation kit by negative selection. CD1c+ DCs were isolated using the CD1c+ DC isolation kit. After positive selection to exclude CD19+ CD1c+ B cells, positive selection was used to isolate CD1c+ DCs. Following separation, CD14+ monocytes and CD1c+ DCs were cultured as described above for the generation of monocyte-derived DCs.

**ELISA**

The capture/biotinylated detection Abs used were as follows: human IL-12p70, clone 20C2/C8.6 (BD Biosciences); IFN-γ, clone NIB42/4S.B3 (BD Biosciences); IL-6, clone MQ2-13A5/MQ2-39C3 (BD Biosciences); IL-β, clone 2805/polyclonal goat IgG (R&D Systems); MIP-1α, clone 24006/polyclonal goat IgG (R&D Systems); MIP-1β, clone 6730/polyclonal goat IgG (R&D Systems); IL-23, clone eBio473p19/C8.6 (eBioscience); IL-17A, clone eBio64CAP17/eBio64DEIC17 (eBioscience); IL-4 (BD Biosciences); and IL-5 (BD Biosciences). Microtiter plates were coated overnight with primary anti-cytokine capture Ab. The plates were then blocked with 10% FCS in PBS. The supernatant fluids and standards were added and incubated at room temperature for 2 h. The plates were washed and secondary biotinylated anti-cytokine detection Ab was added and incubated at room temperature for 1 h. The plates were washed again and developed with avidin-peroxidase (Sigma-Aldrich) and its substrate, 2,2'-azino-di-(3-ethylbenzthiazoline sulfonyl acid) and hydrogen peroxide. The colored reaction product was measured with an enzyme immunoassay plate reader at 450 nm. The amount of cytokine in each supernatant was extrapolated from a standard curve.

**Flow cytometry**

Multicolor flow cytometry was performed with a BD Biosciences Canto cytometer, and data were processed with FlowJo8.1.1 analysis software (Tree Star). Propidium iodide staining (nonviable) cells were excluded from analysis.

**Northern blot analysis**

DCs were prepared as described above. Cells were then lysed, and RNA was analyzed on Northern blots. The probes for human IL-12p40, IL-12p70, IL-23p19 were excised from plasmids purchased from Open Biosystems. RNA was analyzed on Northern blots. The probes for human IL-12p40, IL-12p70, IL-23p19, IL-10, IL-6, IL-1β, IL-12, and IL-23 p19 were excised from plasmids purchased from Open Biosystems. The probes were synthesized using standard procedures. The probes were radiolabeled with [32P] dCTP using a random primer labeling kit (New England Biolabs). The probes were electrophoresed on a 2% agarose gel and transferred to a Hybond-N+ nylon membrane (Amersham). The membranes were hybridized with the radiolabeled probes and washed with 0.1× standard saline sodium citrate (SSC). The membranes were analyzed using a PhosphorImager (Molecular Dynamics). The exposure times were determined empirically and remained constant between experiments. The blots were then stripped of the probe and rehybridized with a different probe to ensure that equal amounts of RNA were loaded. The blots were examined using a PhosphorImager (Molecular Dynamics). The results were quantified using a densitometer (Model 1250; Molecular Dynamics).

**Migration assay**

DCs were described as described above. After overnight culture, the cells were treated with either 10 μg/ml LTA, 10 ng/ml LPS, or 10 ng/ml LPS with 1 μg/ml PGE2, for 6 h. Alternatively, cells were primed with 1000 U/ml IFN-γ or 1 μg/ml R848 for 6 h and then matured with LPS for additional 6 h. Cells were harvested and counted by trypan blue dye exclusion and resuspended in SFM with 50 ng/ml GM-CSF at a final concentration of 1 × 10^6 cells/ml. A total of 600 μl/well SFM with CCL19 (250 ng/ml) and CCL21 (250 ng/ml) was added to a 24-well plate. Then, 50 μM pore size transwell inserts were placed in the wells and 2 × 10^5 cells were added into the transwell inserts. After incubation for 3 h, the transwell inserts were removed and cells in the bottom wells were counted. Migration rates were calculated as cells counted, divided by 2 × 10^5 cells.

**Preparation of T lymphocyte subsets**

Lymphocyte-rich elutriation fractions were used to prepare either CD4+ T cells or CD8+ T cells using negative depletion columns according to the manufacturer’s instruction (R&D Systems).

**Allosensitization of CD4+ T cells**

Purified allogeneic CD4+ T cells (1 × 10^6/well) were cocultured with DCs (1 × 10^6/well) in 48-well tissue culture plates. In some wells, anti-IL-12p70 neutralizing Ab (3 μg/ml added on the first day of culture), anti-IL-23p19 neutralizing Ab (5 μg/ml added on the first and third days of culture), control goat IgG, or exogenous IL-12 (5 ng/ml) were added to the medium. Nine days later, the T cells were harvested and re-stimulated on plates coated with anti-CD3 and anti-CD28 Abs. Supernatants were harvested 24 h later and analyzed by ELISA.

**In vitro sensitization of CD4+ T cells**

CD4+ T cells were cultured with autologous DCs pulsed with Candida extract at a ratio of 10:1 in 24-well plates. T cells were harvested on day 7 and tested for their Ag specificity. CD4+ T cells (10^5 cells) were re-stimulated with Candida- or pigeon cytochrome C-pulsed DCs (10^6 cells) in a 96-well plate in triplicate. After 24-h stimulation, supernatants were harvested and IFN-γ and IL-17A production was measured by ELISA.

**In vitro sensitization of CD8+ T cells**

DCs from HLA-A2+ normal donors were pulsed with MART-1 27-35 (50 μg/ml) 2 h before harvest. Harvested cells were washed and plated in fresh RPMI 1640, 5% human AB serum, and 30 IU IL-2 with purified CD8+ T cells at a T cell to DC ratio of 10:1. After 7 days in culture, the T cells were harvested and re-stimulated with Melanoma tumors cells (Mel62A A2+) that express MART-1 or a negative control cell line (Mel 624 A2-). CD8+ T cells were also tested against HLA-A2 transporter TAP-deficient T2 cells pulsed with relevant and irrelevant peptides. Supernatants were harvested after 24 h and IFN-γ and IL-17A production was measured by ELISA.

**Statistical analysis**

When appropriate, a one-way ANOVA test was used to determine the differences between groups.

**Results**

**Single TLR agonists induce DC maturation and expression of IL-23**

We aimed to determine whether DC production of IL-23 could occur independently of IL-12 production. iDCs were generated from PBMCs in SFM as previously described (28). These iDCs were then treated with single TLR agonists (LTA, LPS, poly(I:C), or R848), dual TLR agonists (R848/LPS), or IFN-γ/LPS. DCs treated with LTA, LPS, or R848 produced significant quantities of IL-23 and little or no IL-12. DCs treated with R848/LPS or IFN-γ/LPS produced IL-23, in even greater quantities, as well as IL-12 (Fig. 1A). Consistent with these data from ELISA, Northern blot analysis demonstrated induction of IL-12 p40 and IL-23 p19 mRNA transcription after single TLR agonist treatment. In contrast, dual treatment with IFN-γ/LPS or R848/LPS was required for IL-12 p35 mRNA transcription (Fig. 1B). Notably, the TLR 3 agonist poly(I:C), did not induce detectable levels of IL-23 or IL-12 protein or transcription of p19, p35, or p40 mRNA, this despite IFN-α induction (not shown) (29). Flow cytometric analysis of TLR agonist-treated DCs revealed up-regulation of CD80 and CD83 irrespective of the treatment protocol, dissociating DC cell surface phenotype from cytokine expression (Fig. 1C). The distinct patterns of IL-12 and IL-23 production following exposure to single or dual signals were consistent in numerous experiments using different donors’ DCs (Fig. 1D). An evaluation of DC morphology revealed that, following treatment with either LPS or R848/LPS, DCs formed dendrites and clustered in culture (Fig.
Additionally, all stimulated DCs demonstrated the capacity to migrate toward CCL19 and CCL21. Although the migration rate of DCs matured with two TLR agonists was greater than that of DCs treated with LPS alone, the addition of PGE2, ubiquitously produced in inflammatory responses, markedly enhanced migration by LPS-treated DCs (Fig. 1F). Both single TLR agonist- and dual signal-treated DCs produced the cytokines IL-6 and IL-1β, and the chemokines MIP-1β and MIP-3α. Production of the cytokines in particular, was generally higher following treatment with dual signal-treated DCs compared with single TLR agonist treated DCs. Only those DCs treated with IFN-γ/LPS or R848/LPS produced IL-12 (Fig. 2).
Single TLR ligands induce production of IL-23 by monocyte-derived DCs

A recent study suggested differential capacities of APC subsets to polarize Th17 responses; monocyte-derived DCs were suggested to be particularly inefficient. Moreover, monocyte-derived DCs were shown to produce IL-23 after single TLR agonist treatment only in the presence of CD40L (26). Although the elutriated cell fractions used for our experiments are predominantly comprised of monocytes, specimens contain some circulating DCs as well. Having demonstrated that DCs matured from elutriated fractions produced ample IL-23 in response to single TLR agonists, we aimed to confirm that this cytokine production was attributable to monocyte-derived DCs in addition to circulating DCs. CD14+ monocytes and CD1c+ circulating DCs were isolated by negative and positive selection, respectively. Both populations of cells were cultured in SFM with GM-CSF for 24 h before treatment with the TLR agonist LPS or IFN-γ/LPS and tested for IL-23 and IL-12 secretion by ELISA. Notably, the CD14+ derived cells demonstrated a similar pattern of IL-12 and IL-23 production to DCs derived from elutriated fractions; specifically, they produced IL-23 alone after treatment with single TLR agonists and both IL-12 and IL-23 after treatment with IFN-γ/LPS. The higher levels of IL-12 and IL-23 production by CD14+ derived cells compared with total elutriated cells or CD1c+ cells may reflect additional stimulation or removal of an inhibitory element during negative selection. Notwithstanding, these results indicate that monocyte-derived DCs have the capacity to produce IL-12 and IL-23 in a pattern consistent with that seen in experiments using elutriated cell fractions (Fig. 3).

IL-4 inhibits IL-23 production by single TLR agonist-treated monocyte-derived DCs

The more robust IL-23 production by monocyte-derived DCs we observed compared with that described in other published reports may reflect differences in environmental signals. DCs prepared from monocytes exposed to GM-CSF and IL-4, as described in most other studies (26, 30–32), appear to behave differently than do those that mature in the absence of IL-4. Notably, IL-4 has been implicated in Th17 inhibition in some murine studies (8, 33, 34). To determine the degree to which IL-4 influences IL-23 production by human monocyte-derived DCs, we compared culture with and without IL-4 (Fig. 4). IL-4 inhibited IL-23 production by all single TLR agonist-treated DCs to varying degrees (a greater than 10-fold decrease by LPS-treated DCs was observed). Single TLR- treated DCs made little IL-12 either in the presence or absence of IL-4. DCs treated with IFN-γ/LPS produced substantial IL-23 and IL-12 despite treatment with IL-4.

CD40L enhances both IL-23 and IL-12 production by single and dual signal-treated DCs

Having demonstrated that single TLR agonist stimulation induces DC production of IL-23, whereas dual signal stimulation induces DC production of both IL-12 and IL-23, we aimed to determine whether DC interaction with activated T cells (and

FIGURE 3. IL-23 and IL-12 production by monocyte-derived and circulating DCs. CD14+ monocytes and CD1c+ circulating DCs were isolated from elutriated specimens by negative and positive selection, respectively. Separation was confirmed by flow cytometric analysis before culture. After subsequent culture for 24 h, cells were treated with LPS or IFN-γ/LPS. IL-12 and IL-23 production were measured by ELISA. Error bars represent the SE of the mean based upon multiple measurements from a single experiment. The data shown are representative of three experiments.

FIGURE 4. Cytokine production by monocyte-derived DCs cultured with or without IL-4. Monocyte-derived DCs were cultured in SFM with GM-CSF and IL-4 of GM-CSF alone for 24 h before treatment with LTA, LPS, or IFN-γ/LPS. Supernatants were collected after an additional 24 h in culture and IL-12 and IL-23 were measured by ELISA. Error bars represent the SE of the mean based upon multiple measurements from a single experiment. The data shown are representative of three experiments.
exposure to CD40L) would further enhance the production of these cytokines without altering commitment to IL-23 vs IL-12/IL-23. After culture of DCs for 24 h in SFM with GM-CSF, LPS or IFN-γ/H9253/LPS were added to duplicate cultures. Four hours later, recombinant CD40L was added to one of the two wells in each group (Fig. 5). A 3- to 4-fold increase in IL-23 production by LPS-treated DCs was observed after exposure to CD40L. Notably, these cells still produced minimal IL-12. In contrast, dual signal matured DCs produced increased levels of IL-12 and IL-23 after exposure to CD40L.

**Th1 or Th17 polarization of T cells by DCs correlates with the balance between IL-12 and IL-23**

We next evaluated the capacity of DCs that produce large quantities of IL-12 and IL-23 (i.e., a relatively high ratio of IL-12:IL-23) and those that produce IL-23 in the absence of IL-12 (i.e., a relatively low ratio of IL-12:IL-23) to polarize T cells toward a Th1 or Th17 cytokine expression pattern. DCs from seven donors were prepared, as described above, and treated with LPS, IFN-γ/ LPS, or R848/LPS. A consistent pattern of IL-12 and IL-23 production was noted, irrespective of donor. Cytokine production by LPS-treated DCs was characterized by a relatively low ratio of IL-12:IL-23, whereas cytokine production by dual signal-treated DCs was characterized by a relatively high ratio of IL-12:IL-23 ($p<0.05$) (Fig. 6A). Following coculture with DCs and restimulation with anti-CD3 and anti-CD28 Abs, allogeneic CD4 T cell production of IFN-γ and IL-17A was measured. T cells cocultured with DCs that produce a high ratio of IL-12:IL-23 secreted large quantities of IFN-γ. In contrast, T cells cocultured with DCs that produce IL-23 alone secreted less IFN-γ and comparatively larger quantities of IL-17A (Fig. 6B). These findings were confirmed in multiple experiments; indeed, pooled data from 10 similar coculture experiments demonstrated a significant shift in the ratio of IL-17A:IFN-γ produced. This ratio was higher (i.e., more IL-17A relative to IFN-γ) after coculture with LPS-treated DCs (0.12) and lower (i.e., less IL-17A relative to IFN-γ) after coculture with IFN-γ/LPS- or R848/LPS-treated DCs (0.048; $p=0.03$ and 0.043; $p=0.04$, respectively by a one-way ANOVA test). Additionally, T cell production of IL-4 and IL-5 were measured by ELISA. Notably, coculture with LPS or dual signal-treated DCs resulted in no increase in IL-4 or IL-5 production compared with coculture with iDC (data not shown).

Having demonstrated differential polarization of allogeneic T cells by IL-23- vs IL-12/IL-23-producing DCs, we next evaluated...
the capacity of these DCs to polarize autologous T cell in an Ag-specific fashion. Single TLR and dual signal-treated DCs were pulsed with candida before coculture with autologous CD4+ T cells. Upon restimulation with candida or pigeon cytochrome C (negative control Ag)-pulsed DCs, T cells initially stimulated with LPS-treated DCs specifically produced large quantities of IL-17A and relatively low levels of IFN-γ. In contrast, DCs treated with IFN-γ/LPS-treated DCs induced minimal IL-17A production and polarized memory cells toward a Th1 response (Fig. 6C). Consistent with studies implicating IL-23 in polarization of memory rather than naive Th17 cells, single TLR-treated DCs pulsed with keyhole limpet hemocyanin did not induce APCs despite production of IL-23 (data not shown).

**IL-23 enhances, and IL-12 inhibits, Th17 polarization**

To determine whether IL-23 and IL-12 play mechanistically important roles in polarizing T cells toward or away from the Th17 phenotype, respectively, we cocultured single TLR and dual signal-treated DCs with allogeneic T cells and added exogenous IL-12, IL-23 neutralizing Ab, or IL-12 neutralizing Ab to selected cultures (Fig. 7). Notably, the addition of exogenous IL-12 to cocultures with DCs that do not produce IL-12 (LPS-treated DCs) enhanced IFN-γ production and inhibited IL-17A production by allogeneic T cells. Likewise, the addition of IL-23 neutralizing Ab to cocultures with LPS-treated DCs markedly inhibited IL-17A production. In contrast, neutralization of IL-12 in T cell cocultures with DCs that produced high levels of IL-12 (IFN-γ/LPS-treated DCs) inhibited IFN-γ production and enhanced IL-17A production.

**IL-23-secreting DCs induce IL-17A production by CD8+ T cells specific for tumor-derived peptides**

Building upon reports identifying CD8+ IL-17+ cells (35, 36), we evaluated the capacity of TLR-treated DCs to induce Ag-specific IL-17A production by CD8+ T cells. DCs were generated from HLA A2+ donor monocytes and were pulsed with HLA-A2-restricted peptides derived from the melanoma-associated MART-1 Ag before coculture with syngeneic CD8+ T cells. Subsequent restimulation of T cells with MART-1 Ag-loaded T2 cells induced IL-17A production in those cells cultured with single TLR agonist-treated DCs. T cells cultured with dual signal-treated DCs produced IFN-γ and no IL-17A upon restimulation (Fig. 8A). As was previously shown, CD8+ T cells cultured with dual signal activated DCs produced IFN-γ in response to restimulation with MART-1-expressing tumor cells (24, 37). In contrast, CD8+ T cells did not respond to tumor cells lines with IL-17A production suggesting lower avidity for MART-1 Ag compared with IFN-γ producing cells (Fig. 8B).

**Discussion**

The signals involved in DC commitment to IL-23 production remain a topic of active investigation. Previous studies have demonstrated that TLR agonists induce DC production of IL-23, often...
in conjunction with IL-12 (23, 38, 39). In two recent studies, IL-23 production by DCs, without associated production of IL-12, was documented following treatment with single TLR agonists (26, 40). In both cases, however, this production was achieved only under restricted conditions. In one study, IL-23 production was induced only after treatment of DCs with PGN, a TLR2 agonist; other single TLR agonists did not induce IL-23 production (40). In the second study, LPS or PGN were shown to induce IL-23 production, but only in combination with CD40L (26). In the present study, we demonstrated that DC commitment to IL-23 is readily achieved after exposure to several TLR agonist including LTA (TLR2), LPS (TLR4), and R848 (TLR7/8). Moreover, this production is greatly enhanced by exposure to CD40L, further supporting the hypothesis that commitment to IL-23 production after exposure to single TLR agonists persists following DC-T cell interaction. Notably, signaling through all of these receptors is MyD88 dependent. Following stimulation with poly(I:C), signaling via MyD88-independent TLR3 did not induce IL-23 production by DCs, indicating that MyD88-mediated signaling may be integral to DC commitment to an IL-23-producing phenotype.

Several factors help to explain why the consistent pattern of IL-12 and IL-23 production by DCs observed in this study has not been as apparent in other studies. DCs in this study were rapidly matured for 48 h culture in GM-CSF containing SFM avoiding nonphysiologic DC differentiation that may result from the progressed culture conditions used in many other studies. Perhaps more importantly, many laboratories routinely culture DCs in IL-4 (26, 30–32). In the absence of IL-4, DCs produce IL-23 in higher quantities, as was noted in this study following exposure to single TLR agonists. This influence of IL-4 on IL-23 production recapitulates the reciprocal mechanisms regulating Th1/2 vs Th17 polarization suggested by several murine studies (8, 33, 34).

The role of IL-23 in regulating T cell production of IL-17 is now well established (7–9, 14). Not surprisingly, DCs that produced high levels of IL-23 effectively polarized T cells toward the Th17 phenotype in our study. Conversely, those DCs that produced both IL-12 and IL-23 polarized T cells toward the Th1 phenotype. The observations that: 1) IL-12 production was almost always accompanied by IL-23 production and 2) Th1 polarization of T cells was predominant in the presence of both cytokines suggests antagonism of the IL-23/Th17 axis by IL-12. The dominant effect of IL-12 in polarizing T cells toward the Th1 phenotype despite the presence of IL-23 was demonstrated most clearly in experiments in which Candida-specific Th1 or Th17 responses were elicited, and reflected the presence or absence of IL-12; minimal T cell production of IL-17A was observed after coculture with IL-12/IL-23-producing DCs. This apparent antagonism of the IL-23/Th17 axis by IL-12 was further supported by the noted enhancement of Th1 producing DCs. This apparent antagonism of the IL-23/Th17 axis by IL-12 was further supported by the noted enhancement of Th1 producing DCs. This apparent antagonism of the IL-23/Th17 axis by IL-12 was further supported by the noted enhancement of Th1 producing DCs.

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Recently, a distinction has been made between factors that contribute to Th17 polarization in naive vs memory responses (40). IL-6 and IL-18, rather than IL-23, have been identified as critical factors in naive differentiation (26). Consistent with these findings, DCs that produced large quantities of IL-23 in the absence of IL-12 induced IL-17 production by allogeneic T cells and syngeneic memory CD4 T cells in our study, but not by naive CD4 T cells. Interestingly, single TLR agonist-stimulated DCs also induced Ag-specific IL-17A production by CD8 T cells. The significance of this finding remains unclear. Several studies have identified cells with a CD8 T cell phenotype (36, 42); one identified these cells in multiple tumor microenvironments in mice and humans and implicated them in tumor pathogenesis (42). Induction of CD8 T cells that produce IL-17 in response to tumor-associated Ags, achieved after coculture with LPS-treated but not dual signal-treated DCs suggests that mechanism involved in the regulation of this cell population is shared with those that regulate Th17 cells. Notably, these cells had a lower avidity for tumor Ag than did Tc1 cells; only Tc1 cells reacted to tumor Ag-expressing cell lines with cytokine secretion.

Extensive efforts have been made to induce antitumor immune responses with DC-based vaccination strategies; however, controversy persists regarding how to best prepare DCs suited for this purpose. Building upon our growing understanding of innate immune responses and the identification of TLRs, attention has recently shifted toward the use of microbial adjuvants. Induction of robust Th1 T cell responses by TLR agonist-treated DCs has been demonstrated (20, 23), sometimes with encouraging clinical results (43). The findings of this study, and work by other groups (38, 41), suggest a delicate balance between Th1 and Th17 polarization is obtained in the presence of IL-23, such findings highlight the importance of a calculated approach to the use of TLR agonist based therapies. Disclosures The authors have no financial conflict of interest.

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