Macrophages and Myeloid Dendritic Cells, but Not Plasmacytoid Dendritic Cells, Produce IL-10 in Response to MyD88- and TRIF-Dependent TLR Signals, and TLR-Independent Signals

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Microbial products activate the innate immune response and stimulate the production of cytokines, thereby determining the class of adaptive response mounted against a particular pathogen (1–4). Dendritic cells (DC) originate in the bone marrow (BM) and migrate through blood to secondary lymphoid organs and tissue. DC continuously sense pathogens and take up, process, and present Ag to T lymphocytes, thereby initiating the adaptive immune response (1–4). Macrophages play a role in the early phases of the immune response by producing cytokines and killing pathogens (5).

Th1 cell responses can be promoted through the activation of different DC subsets by a wide range of pathogens or their products, largely through the secretion of proinflammatory molecules such as bioactive IL-12p70 (consisting of the IL-12p40 and IL-12p35 subunits) and in some cases type I IFN (6–16). Activation of DC and macrophages by pathogens involves the specific interaction between pattern recognition receptors, such as the members of the TLR family, and pathogen-derived products (17, 18). Upon TLR ligation, signaling cascades are activated via Toll/IL-1 receptor domain-containing adaptors, such as MyD88 and Toll/IL-1 receptor domain-containing adaptor (TRIF), leading to cytokine production. All TLR, except TLR3, use MyD88, whereas TRIF is involved only in TLR3 and TLR4 signaling, resulting after binding of their specific ligands, dsRNA (or poly(I:C)) and LPS, respectively (17). The TRIF adaptor has been shown to be responsible for a MyD88-independent signaling pathway giving rise to IFN-β (19–21). Distinct DC subpopulations in both mice and humans (reviewed in Ref. 2) have been shown to express different TLR, and consequently to respond to distinct microbial products (22–26). For example, TLR4 is expressed by macrophages, human monocyte-derived DC, and mouse myeloid DC, but not by plasmacytoid DC (22, 23). In contrast, TLR9, specific for unmethylated DNA containing CpG motifs, abundantly present in microbial genomes, is expressed by both human and mouse plasmacytoid DC (22, 23). In contrast to TLR9 mRNA by human plasmacytoid DC and human B cells, in the mouse TLR9 mRNA is expressed not only by these cells but also by mouse macrophages, myeloid DC, and splenic CD8α+ and CD8α− DC (23). Collectively, these findings explain 1) the ability of mouse myeloid DC, macrophages, and splenic DC, but not plasmacytoid DC, to drive Th1 cell development in response to LPS (23, 28), and 2) the ability of plasmacytoid DC, and to a lesser extent myeloid DC, to respond to CpG and drive Th1 development, as we have previously shown (23). Surprisingly, although splenic CD8α+ DC, CD8α− DC, and myeloid DC express similar levels of TLR9 mRNA, CpG stimulation induced only low levels of IL-12p70 (23). Thus, in addition
to differential TLR expression, other factors may play a role in the production of bioactive IL-12p70 (29). For example, T cells have been shown to enhance the production of IL-12p70 by APCs through cell-cell contact involving at least the interaction of CD40L on activated T cells with CD40 on the APC (30–34). In contrast, negative regulation exerted by anti-inflammatory cytokines such as IL-10 has been shown to suppress IL-12p70 production by DC and macrophages, resulting in the inhibition of Th1 cell development (12, 35–37).

It has been suggested that the induction of the anti-inflammatory cytokine IL-10 after stimulation with lectins derived from pathogens can be mediated by a TLR-independent signaling pathway (38, 39). Furthermore, it has been suggested that TLR2 agonists are specialized to induce IL-10, as shown by stimulation with lipopeptides or the LcrV Ag of Yersinia pestis (40, 41).

In this study, we show that significant levels of IL-10 are secreted by macrophages and myeloid DC upon stimulation with MyD88- and TRIF-dependent TLR ligands, as well as non-TLR signals. Endogenous IL-10 suppressed IL-12p70 production by myeloid DC, macrophages, and splenic DC. In contrast, plasmacytoid DC did not produce IL-10 upon stimulation and showed unrestrained IL-12p70 production. Nevertheless, plasmacytoid DC were responsive to the inhibitory effects of exogenous IL-10.

Materials and Methods

**Mice**

BALB/c, C57BL/6, MyD88-deficient, and TRIF-deficient mice were used to provide macrophages and DC. Breeding pairs of MyD88-deficient and TRIF-deficient mice were provided by S. Akira (Osaka University, Osaka, Japan) (21, 42). All mice were bred at the National Institute for Medical Research (London, U.K.) and housed under specific pathogen-free conditions.

**Reagents and cell lines**

Culture medium was RPMI 1640 with 5% heat-inactivated FCS, 0.05 mM 2-ME, 10 mM HEPES buffer, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM l-glutamine, and 1 mM sodium pyruvate. DC were stimulated with *Salmonella minnesota* LPS (Alexis), poly(I:C) (Invivogen Life Technologies), or phosphorothioate CpG DNA (CpG1668; TCCATGACGTTCC TGATGCT; Invitrogen Life Technologies). Mouse Flt3 ligand was produced by MyD88- and TRIF-dependent TLR ligands, as well as non-TLR signals. Endogenous IL-10 suppressed IL-12p70 production by myeloid DC, macrophages, and splenic DC. In contrast, plasmacytoid DC did not produce IL-10 upon stimulation and showed unrestrained IL-12p70 production. Nevertheless, plasmacytoid DC were responsive to the inhibitory effects of exogenous IL-10.

**Preparation of splenic DC subsets**

For the purification of splenic DC, spleens were treated for 30 min at 37°C with 0.4 mg/ml Liberase Cl (Boehringer Mannheim), followed by RBC lysis as above. Cells were maintained throughout the procedure in cold PBS, 5% FCS, and 0.5 mM EDTA. Spleen cell suspensions were enriched for DC using anti-CD11c microbeads using an AutoMACS (Miltenyi Biotec) according to the manufacturer’s instructions. The enriched DC were stained with CD11c-PE, CD80-allophycocyanin, and 120G8-Alexa647 and purified using a MoFlo cytometer (DakoCytomation) as the CD11c+CD8α−, CD11c+CD8α+, and the CD11c+CD8α+ plasmacytoid DC (43). The purity was consistently ≥98%.

**In vitro stimulation of DC and macrophages, and quantitation of cytokine production**

For in vitro stimulations, 10⁵ sorted DC or macrophages were cultured in 200 μl in 96-well flat-bottom culture plates (Nunc) and stimulated with medium alone, LPS (100 ng/ml), poly(I:C) (50 μg/ml), or CpG1668 DNA (1 μM) either alone, or on a monolayer of CD40L-transfected 3T3 cells or control empty vector-transfected cells. To some cultures anti-IFN-α mAb (1B1.3; 10 μg/ml) or IL-10 (10 ng/ml) was added. After culture for 24 h, supernatants were collected, and the cytokine concentration was determined by immunoassay. Commercially available ELISA kits were used for the detection of IL-12p70, TNF, IL-10 (eBioscience; Ready-Set-Go), and IFN-β (PBL supplier). IFN-α was measured by a sandwich ELISA with an anti-IFN-α capture mAb (F18; Hyclut), and a rabbit anti-IFN-α polyclonal Ab (PBL supplier) followed by goat anti-rabbit HRP (Sigma-Aldrich). IL-12p40 was detected using C15.6.7 as capture mAb and biotinylated C17.8 as detection mAb.

**Statistical analysis**

Data from multiple experiments were analyzed by comparison to a defined control value using Dunnett’s test. Analysis was performed using GraphPad Prism software (GraphPad). In defined cases, pairwise comparison was by Student’s paired t test. Values of p < 0.05 were considered significant.

**Results**

**Cpg induces IL-10 production from macrophages and myeloid DC, but not from plasmacytoid DC**

Although macrophages, myeloid DC, splenic DC, and plasmacytoid DC all express TLR9 (23, 25) and respond to Cpg to produce IL-12p40 and TNF, we show in this study that they differ substantially with respect to the production of IL-10 and IL-12p70 (Fig. 1). We postulated that this differential IL-10 production may account for reduced production of IL-12p70 by splenic DC, myeloid DC, and macrophages.

Macrophages produced high levels of IL-10 (2260 pg/ml) upon stimulation via TLR9, whereas no IL-12p70 was detected (Fig. 1). Myeloid DC also produced IL-10 upon Cpg stimulation, albeit at lower levels than by macrophages, and only low levels of IL-12p70 were detected (~200 pg/ml). In contrast, plasmacytoid DC produced no detectable IL-10 upon TLR9 ligation with Cpg, whereas high levels of IL-12p70 were produced in response to Cpg (1080 and 1610 pg/ml for BM-derived and splenic plasmacytoid DC, respectively). The production of IL-12p40 and TNF did not mirror that of IL-10 or IL-12p70. The ratio of IL-12p40/IL-12p70 was not identical in macrophages and the different DC populations. TNF was produced at relatively high levels by macrophages, myeloid DC, and plasmacytoid DC, and at low levels by splenic CD11c⁺ DC (Fig. 1). A similar cell type-specific pattern of

**Generation of BM-derived macrophages**

BM-derived macrophages were generated in the presence of L cell-conditioned medium containing M-CSF as described by Warren et al. (44). Briefly, BM cells were isolated by flushing femurs and tibia with culture medium, and RBC lysed. Cells were plated at 0.5 × 10⁶ cells/ml in petri dishes (Bowlworld Scientific; volume, 8 ml). At day 4, 10 ml of fresh L cell-conditioned medium were added. At day 7, adherent cells were harvested by gentle flushing. The purity was always >95% as determined by staining for F4/80⁺ cells by flow cytometry.

**Generation of BM-derived DC**

BM cells were isolated by flushing femurs and tibia with culture medium. RBC were lysed using 0.83% ammonium chloride. GM-CSF-derived myeloid DC were generated as described by Inaba et al. (45). In brief, BM cells were plated at 10⁴ cells/ml in medium supplemented with 10 ng/ml GM-CSF in 12-well plates in a volume of 2 ml. At days 2 and 4, supernatant containing nonadherent cells was removed, the wells were washed gently, and fresh medium containing GM-CSF (10 ng/ml) was added. At day 6, nonadherent cells were collected, centrifuged, resuspended in fresh medium with GM-CSF (10 ng/ml), and cultured overnight in petri dishes (Nunc). Cells were purified by flow cytometry as CD11c⁺ cells using a MoFlo cytometer (DakoCytomation). Plasmacytoid DC were generated by culturing BM cells in culture medium containing 100 ng/ml Flt3 ligand for 10 days at 10⁵ cells/ml in 12-well plates in a volume of 2 ml. At day 5, 1 ml of medium was replaced by 1 ml of fresh medium containing Flt3 ligand (46). The resulting plasmacytoid DC were purified by flow cytometry as CD11c⁺CD11b⁻B220⁺ using a MoFlo cytometer (DakoCytomation). The purity was always ≥98%.

Preparation of splenic DC subsets

For the purification of splenic DC, spleens were treated for 30 min at 37°C with 0.4 mg/ml Liberace Cl (Boehringer Mannheim), followed by RBC lysis as above. Cells were maintained throughout the procedure in cold PBS, 5% FCS, and 0.5 mM EDTA. Spleen cell suspensions were enriched for DC using anti-CD11c microbeads using an AutoMACS (Miltenyi Biotec) according to the manufacturer’s instructions. The enriched DC were stained with CD11c-PE, CD80-allophycocyanin, and 120G8-Alexa647 and purified using a MoFlo cytometer (DakoCytomation) as the CD11c⁺CD8α⁻, CD11c⁺CD8α⁺, and the CD11c⁺CD8α⁺ plasmacytoid DC (43). The purity was consistently ≥98%.
FIGURE 1. CpG induces high levels of IL-10 in macrophages, intermediate levels in myeloid DC, and no detectable IL-10 in plasmacytoid DC. Macrophages or sorted DC (10^5) obtained from BALB/c mice were stimulated in a volume of 200 µl with 1 µM CpG DNA for 24 h. The differences between IL-10 production by macrophages and myeloid DC, and between the other DC populations were significant (p < 0.05 by Dunnett’s test). Cytokine levels were determined by immunoassay. The sensitivities of the ELISA were: IL-10, 50 pg/ml; IL-12p70, 50 pg/ml; IL-12p40, 1 ng/ml; and TNF, 100 pg/ml. Data are representative of four to eight experiments for each population.

IL-10, IL-12, and TNF production was observed upon stimulation with the TLR7 ligand R-848 (data not shown). Thus, macrophages, myeloid DC, and plasmacytoid DC have distinct intrinsic properties that affect their capacity to produce IL-10, IL-12p70, IL-12p40, and TNF that are not dictated by their TLR expression.

IL-10 production is induced by TLR ligands via MyD88-dependent and TRIF-dependent TLR signals in macrophages and myeloid DC

Although it is well documented that the production of proinflammatory cytokines upon TLR ligation is MyD88 dependent (21, 47, 48), it has been suggested that the production of the suppressive cytokine IL-10 is favored by usage of pathways involving triggering via C-type lectins, such as dectin-1 and DC-SIGN (38, 39), or TLR2 (40, 41). In this study, we show clearly that the induction of IL-10 by macrophages is entirely MyD88 dependent in response to CpG and partially MyD88-dependent in response to LPS, because IL-10 production by MyD88-deficient macrophages was absent or severely impaired upon stimulation with these TLR ligands (Fig. 2). IL-10 was also induced upon stimulation of wild-type macrophages with poly(I:C). Macrophages from TRIF knockout (KO) mice showed almost complete abrogation of IL-10 production induced by poly(I:C), whereas IL-10 production by MyD88 KO macrophages stimulated with poly(I:C) was not affected (Fig. 2). Residual IL-10 production in response to poly(I:C) in TRIF KO macrophages may be due to signaling via a TRIF-independent pathway using Mda-5, recently reported as inducing other proinflammatory cytokines (49). The production of IL-10 upon stimulation with LPS depended not only on MyD88 but also on TRIF-dependent signaling pathways. This indicates that IL-10 production in macrophages can be achieved via MyD88-dependent pathway as well as TRIF-dependent TLR signals. Recently, it was shown that the production of IL-10 and IFN-β are both regulated by TNFR-associated factor 3, downstream of MyD88 and TRIF, implying coordinate expression (50). Indeed, similar to IL-10, the production of IFN-β by macrophages in response to CpG was MyD88 dependent and was completely dependent on TRIF signaling in response to poly(I:C). In contrast to IL-10 production, which is dependent on TRIF and MyD88 in response to LPS, IFN-β production in macrophages upon LPS stimulation was completely dependent on TRIF and not MyD88 (Fig. 2).

Similar to macrophages, stimulation of myeloid DC with CpG and LPS induced production of IL-10 and was entirely MyD88 dependent in case of TLR9 triggering and dependent on MyD88 and TRIF upon TLR4 triggering (Fig. 2). In contrast to macrophages, myeloid DC did not or only weakly responded to poly(I:C) to produce IL-10, IL-12p40, IL-12p70, or TNF (Fig. 2 and data not shown).

IL-10 production is induced in macrophages, myeloid DC, and splenic DC subsets, but not in plasmacytoid DC upon CD40 ligation

TLR ligation of DC induces both cytokine production and the expression of costimulators like CD40, and differential regulation by T cell-derived signals, including CD40L, determines the different levels of IL-12p70 induced in different cell populations (31–33). Macrophages and DC were stimulated via CD40 using a CD40L-transfected cell line and a control empty vector-transfected cell line as a negative control. Interestingly, high levels of IL-10 were produced upon CD40 signaling in macrophages, myeloid DC, and splenic CD8α^- and CD8α^+ DC. IL-10 again was not secreted by plasmacytoid DC upon CD40 triggering (Fig. 3). In keeping with previous studies in DC (51) upon ligation of CD40 in the absence of TLR ligands, all mouse DC subsets analyzed produced significant levels of IL-12p70. This was not the case for macrophages (Fig. 3). Similar to the findings with TLR ligands, the ratio of IL-12p40/IL-12p70 was not identical in the different DC subsets, and TNF was detected only by CD40L-stimulated macrophages (Fig. 3).

In summary, stimulation of macrophages and myeloid DC resulted in IL-10 production in response to MyD88- and TRIF-dependent TLR ligands. These cells, and additionally splenic CD8α^- and CD8α^+ DC, produced IL-10 when stimulated via CD40 (Fig. 3). In contrast, no IL-10 was produced by plasmacytoid DC upon triggering of CD40, CpG, or CpG in the context of CD40 (data not shown), whereas IL-12p70 was secreted at very high levels by plasmacytoid DC upon ligation of TLR9 and/or CD40 (Figs. 1 and 3).
IL-12 production induced by simultaneous TLR and CD40 ligation is suppressed by endogenous IL-10 in macrophages, myeloid DC, and splenic CD8α+ and CD8α− DC populations, but not in plasmacytoid DC

To address the role of endogenous IL-10 in regulating cytokine production, macrophages, myeloid DC, splenic CD8α+ and CD8α− DC, and plasmacytoid DC were stimulated with CpG and CD40L in the presence or absence of a neutralizing anti-IL-10R mAb.

IL-12p70 production upon CpG stimulation by macrophages was weakly enhanced when IL-10R signaling was blocked, but not when triggered through CD40 alone (Fig. 4A). However, upon dual stimulation via TLR9 and CD40 in the absence of IL-10R signaling, substantial amounts of IL-12p70 were secreted by macrophages (780 pg/ml). Upon CpG stimulation, TNF and IL-12p40 production by macrophages was significantly enhanced upon neutralization of IL-10 activity, whereas CD40 triggering did not further increase TNF and IL-12p40 production (Fig. 4A and data not shown).

The low level of IL-12p70 induced in myeloid DC by CpG (128 pg/ml) was only slightly enhanced in the absence of endogenous IL-10R signal.
IL-10 signaling. Dual stimulation of myeloid DC with CpG plus CD40L resulted in augmentation of IL-12p70 production (496 pg/ml), compared with CD40L alone (181 pg/ml). Most significantly, however, when myeloid DC were stimulated with CpG and CD40L, abrogation of IL-10R signaling resulted in a high level of IL-12p70 production (1 or 2 ng/ml) (Fig. 4A). Significant enhancement of IL-12p70 production by myeloid DC when IL-10R signaling was abrogated was also observed upon TLR2 and TLR4 ligation together with costimulation through CD40 (data not shown).

Similarly, splenic CD8α+ and CD8α−DC produced substantially enhanced IL-12p70 when IL-10R signaling was neutralized upon triggering of TLR9 and CD40. This was most striking for CpG-stimulated splenic CD8α+ DC, which produced up to 23.5 ng/ml IL-12p70. It was consistently found that splenic CD8α−DC produced higher levels of IL-12p70 than splenic CD8α− DC, which is in agreement with previous studies (51–53). However, CD8α− DC produce significant levels of IL-12p70 (~3000 pg/ml) when stimulated with CpG and CD40L upon neutralization of endogenous IL-10 activity. Although blocking IL-10R signaling had such a striking effect on the induction of IL-12p70 from myeloid DC and splenic CD8α+ and CD8α− DC, the effect on TNF levels was less pronounced in contrast to the effect on macrophages. Furthermore, myeloid DC did not respond to CD40 ligation to the extent that splenic CD8α+ and CD8α− DC did, suggesting that the latter may be more dependent on T cell derived signals.

Exogenous IL-10 suppresses the induction of proinflammatory cytokines by plasmacytoid DC

The absence of endogenous IL-10 production by plasmacytoid DC does not exclude that IL-10 produced by other cells can regulate IL-12p70 production by plasmacytoid DC. Addition of exogenous IL-10 to plasmacytoid DC showed suppression of CpG- and CpG/CD40L-induced production of IL-12p70, TNF, and IFN-α (Fig. 5). This indicates that although plasmacytoid DC do not produce
IL-10 and are thus not subject to autocrine regulation of proinflammatory cytokines by this suppressive cytokine, they are responsive to IL-10 produced by other cells in their microenvironment.

Discussion

The present study shows that macrophages, myeloid DC and plasmacytoid DC have a differential capacity to produce IL-10 and IL-12 in response to TLR signaling. TLR-mediated MyD88-dependent and TRIF-dependent as well as non-TLR signals induce high levels of IL-10 by macrophages and intermediate levels by myeloid DC, whereas no detectable IL-10 is produced by plasmacytoid DC. Furthermore, we show that the differential IL-10 production partly explains the superior capacity of plasmacytoid DC to produce high levels of IL-12p70 as compared with macrophages or myeloid DC.

We observed striking differences in the cytokine profile of macrophages, myeloid DC, and splenic DC. CpG stimulation of macrophages resulted in high levels of IL-10, undetectable IL-12p70, relatively low IL-12p40, and relatively high levels of TNF. Myeloid DC produced intermediate levels of IL-10, low IL-12p70, very high levels of IL-12p40, and moderate amounts of TNF. In contrast, splenic DC produced moderate amounts of IL-12p40, but undetectable or very low amounts of IL-10, IL-12p70, and TNF. The limited ability of splenic DC to produce cytokines upon CpG stimulation was overcome by additional triggering via CD40, which resulted in substantial IL-12p70, IL-12p40, and TNF production, which surprisingly also induced IL-10. Interestingly, the synergy between TLR and CD40 triggering was much stronger for splenic DC than for macrophages and myeloid DC, showing their strong dependence on T cell-derived factors to induce cytokine production. Endogenous IL-10 was a crucial factor that strongly suppressed IL-12p70 production by macrophages, myeloid DC, and splenic CD8α+ and CD8α− DC upon CpG and CD40 ligation. With respect to IL-12p70 production, splenic DC, and especially splenic CD8α+ DC, were again most highly regulated by endogenous IL-10. In good agreement with this, it was reported that neutralization of IL-10, induced by Schizosaccharomyces pombe in splenic DC, resulted in enhanced Th1 cell development in vitro (54). Moreover, we previously showed in vivo that Ag-specific Th1 responses in the context of TLR ligation are suppressed by IL-10, and strong Th1 responses in vivo, induced by TLR ligation, are observed only when IL-10 signaling is abrogated (55, 56).

IL-10 production by macrophages was induced via TLR-mediated MyD88- or TRIF-dependent pathways, as well as via non-TLR signals. Triggering through TLR9 (and TLR7; data not shown) showed that the induction of high levels of IL-10 was completely MyD88 dependent in macrophages and myeloid DC (Fig. 2); this was also the case for induction of IFN-β (data not shown). Conversely, IL-10 induction via the TLR3 ligand poly(I:C) was dependent on TRIF and not MyD88 in macrophages (Fig. 2), which was also the case for the induction IFN-β (Fig. 2). LPS induction of IL-10 via TLR4 was dependent on MyD88 and TRIF (Fig. 2), contrasting with IFN-β induction by LPS, which was dependent only on TRIF (Fig. 2). Recently, it was shown that TNFR-associated factor 3, an adaptor molecule downstream of MyD88 and TRIF, regulates the production of both IL-10 and type I IFN, which is in keeping with some of our findings (50). However, we show in this study that whereas MyD88 and TRIF regulate the production of IL-10 by macrophages in response to LPS, the production of IFN-β via TLR4 is dependent on TRIF and not MyD88, suggesting that there may be independent signaling mechanisms regulating the production of IL-10 and IFN-β. Besides stimulation via TLR, CD40 triggering also resulted in IL-10 production. This indicates that induction of IL-10 can be achieved by a broad spectrum of stimuli including multiple TLR ligands, and not, as previously implied, preferentially as a result of ligation of TLR2- and MyD88-independent signals (38–41, 57). Thus, although it has been shown that triggering with pathogens of the C-type lectin DC-SIGN can induce IL-10 (38) and that dectin-1 stimulation with zymosan induces IL-10 via syk signaling (39), we show in this study that these are not exclusive signals to induce the expression of IL-10 in macrophages or DC.

We show that plasmacytoid DC do not produce IL-10 and display unrestrained IL-12p70 production upon TLR ligation, which is in keeping with their ability to induce strong Th1 development upon CpG stimulation in vitro (23). In addition, plasmacytoid DC produce relatively high levels of TNF and IFN-α upon CpG stimulation alone. Additional triggering via CD40 further enhances their production of IL-12p70, IL-12p40, and IFN-α, but not TNF.

Mouse plasmacytoid DC, however, are responsive to the inhibitory effect of IL-10 on cytokine production, as are human IFN-producing cells or plasmacytoid DC (58, 59), indicating that other IL-10-producing cells in their microenvironment may suppress their activity if colocated. Plasmacytoid DC clusters, 6 h after CpG treatment in vivo, are mostly present in the marginal zone, separated from T cells and conventional DC (60). At later time points, plasmacytoid DC partially colocalize with T cells and conventional DC in the T cell area of the spleen. The specific localization of DC during the inflammatory response may be important for IL-10, produced by myeloid DC and/or macrophages, to act in trans on plasmacytoid DC, resulting in restrained IL-12p70 production and consequently suboptimal Th1 responses.

The differential production of IL-10 suggests that macrophages, myeloid DC, and plasmacytoid DC are intrinsically different and may possess distinct intracellular signaling mechanisms to activate different sets of cytokine genes. An alternative explanation could
be that plasmacytoid DC produce other endogenous cytokines such as IFN-α or IFN-γ, which may suppress IL-10 production by plasmacytoid DC. Interestingly, although endogenous IL-10 suppresses IL-12p70 production by macrophages, myeloid DC, and splenic DC, the suppressive effect of IL-10 on TNF production is cell type specific. In this, the induction of TNF by macrophages is highly suppressed by endogenous IL-10, but TNF production by myeloid and splenic DC does not appear to be regulated by endogenous IL-10. The underlying mechanisms for this are currently being studied.

Triggering via TLR induces not only IL-12, thereby promoting Th1 immunity, but also IL-10 that counteracts the effects of IL-12 and suppresses the development of an immune response to pathogens. By doing so, IL-10 prevents excessive activation of the immune response, which limits immune pathology, but alternatively may prevent the complete eradication of pathogens by inhibiting Th1 immunity. Our findings that macrophages and DC have cell-intrinsic properties controlling their production of IL-10, which subsequently affects their production of IL-12, provide important insight into the mechanisms underlying the complex balance of IL-10 vs IL-12 induction, which is fundamental for the understanding of the dynamics of the immune response to pathogens.

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Disclosures

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References

Differential production of IL-10 by DC and macrophages


