CD47 Engagement Inhibits Cytokine Production and Maturation of Human Dendritic Cells

C. E. Demeure, H. Tanaka, V. Mateo, M. Rubio, G. Delespesse and M. Sarfati

*J Immunol* 2000; 164:2193-2199; doi: 10.4049/jimmunol.164.4.2193

http://www.jimmunol.org/content/164/4/2193

---

**References**  This article cites 49 articles, 29 of which you can access for free at:
http://www.jimmunol.org/content/164/4/2193.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
CD47 Engagement Inhibits Cytokine Production and Maturation of Human Dendritic Cells

C. E. Demeure, H. Tanaka, V. Mateo, M. Rubio, G. Delespesse, and M. Sarfati

Upon encounter with bacterial products, immature dendritic cells (iDCs) release proinflammatory cytokines and develop into highly stimulatory mature DCs. In the present study, we show that human monocyte-derived DCs functionally express the CD47 Ag, a thrombospondin receptor. Intact or F(ab′)2 of CD47 mAb suppress bacteria-induced production of IL-12, TNF-α, GM-CSF, and IL-6 by iDCs. 4N1K, a peptide derived from the CD47-binding site of thrombospondin, also inhibits cytokine release. The inhibition of IL-12 and TNF-α is IL-10-independent inasmuch as IL-10 production is down-modulated by CD47 mAb and blocking IL-10 mAb fails to restore cytokine levels. CD47 ligation counteracts the phenotypic and functional maturation of iDCs in that it prevents the up-regulation of costimulatory molecules, the loss of endocytic activity, and the acquisition of an increased capacity to stimulate T cell proliferation and IFN-γ production. Interestingly, regardless of CD47 mAb treatment during DC maturation, mature DC restimulated by soluble CD40 ligand and IFN-γ, to mimic DC/T interaction, produce less IL-12 and more IL-18 than iDCs. Finally, CD47 ligation on iDCs does not impair their capacity to phagocytose apoptotic cells. We conclude that following exposure to microorganisms, CD47 ligation may limit the intensity and duration of the inflammatory response by preventing inflammatory cytokine production by iDCs and favoring their maintenance in an immature state. The Journal of Immunology, 2000, 164: 2193–2199.

Dendritic cells (DCs), the most potent APCs of the immune system, play an essential role at the onset of the innate and adaptive immune response against pathogens (1, 2). Under resting conditions, DCs are found in most tissues in an immature form that is characterized by a very high ability to capture and process soluble and particulate Ags (3). Upon inflammatory stimulation, they undergo maturational changes that involve down-regulation of their endocytic capacity, up-regulation of surface immunogenic MHC-peptide complexes, and increased expression of costimulatory molecules, thus becoming very efficient stimulators of naive T cells (4). As part of this process, they adapt their adhesion and chemokine receptors to migrate out of the inflamed tissue and reach secondary lymphoid organs where they meet T cells (5). Bacterial products additionally stimulate DCs to release several cytokines that participate to the inflammatory response, and chemokines that contribute to attract more DCs and other cell types (6). Among these cytokines, IL-12 plays a central role by inducing IFN-γ production by T cells and NK cells, enhancing NK cell cytotoxicity and promoting the development of cytotoxic T cells (7). Due to their capacity to produce IL-12, it is generally accepted that DCs are critically positioned to initiate the Th1 immune response required for the clearance of several pathogens (8).

Because of its potentially deleterious effects, the inflammatory response must be coordinated with mobilization of antiinflammatory mechanisms that will allow the return to the steady state. These may down-regulate IL-12 production and/or inhibit the maturation of immature DCs (iDCs) into potent T cell stimulators. Inhibitors of IL-12 include IL-4, IL-10, IL-13, TGF-β, PGE₂, glucocorticoids, as well as phagocytic receptors CR3, FcγR, and scavenger receptors (1, 9–15). IL-10, produced by various cell types including DCs themselves, may inhibit DCs maturation (16); moreover, IL-10-treated DCs were found to induce tolerance in naive T cells (17) or to bias their development into Th2 effectors (18, 19). TGF-β and glucocorticoids have also been recently found to alter the maturation of DCs in response to stimulation by bacterial products (20, 21).

CD47 Ag, also known as integrin-associated protein, is a widely expressed multispan transmembrane protein that is physically and functionally associated with αβ integrin, the vitronectin receptor (22). Indeed, CD47⁺ cell lines expressing αβ do not bind vitronectin-coated beads (23), and CD47-deficient mice rapidly die of Escherichia coli peritonitis, a phenomenon directly associated with a reduction in leukocyte activation in response to β, but not β₂, integrin ligation (24). CD47 has also been implicated in leukocyte transendothelial migration (25, 26).

In this report, we show that CD47 is functionally expressed on human DCs. Ligation of CD47 by mAb or a synthetic peptide derived from its natural ligand, i.e., thrombospondin (TSP) (27–29), inhibits cytokine production and the maturation of iDCs in response to bacterial stimulation.

Materials and Methods

Reagents

Recombinant human IL-4, soluble CD40 ligand, and GM-CSF were kindly provided by Immunex (Seattle, WA) and Dr. D. Bron (Institut Bordet, Brussels, Belgium), respectively. IL-12 was a generous gift from Dr. M. Gately (Hoffmann-LaRoche, Nutley, NJ), and IFN-γ was obtained from...
CD14 low/ 2 days in the presence of GM-CSF and IL-4 as above. FITC-labeled BSA was purchased from Sigma (St. Louis, MO).

Generation of monocyte-derived DCs.

PBMCs were isolated by density gradient centrifugation of heparinized blood from healthy volunteers using Lymphoprep (Nycomed, Olso, Norway). Enriched monocytes were prepared by cold aggregation as described (30), followed by one cycle of rosetting with S-2-aminohexylisothiouronium bromide (Aldrich, Milwau- kee, WI)-treated SRBC to deplete residual T and NK cells. Monocyte purity was shown to be >95% by flow cytometry (FACScan, Becton Dickin- son, Mountain View, CA) using PE-conjugated anti-CD14 mAb (Ansell, London, Ontario).

Human monocyte-derived iDCs were prepared exactly as described (31) except that two-thirds of culture medium was replaced by fresh medium containing GM-CSF and IL-4 every other day, and nonadherent cells were harvested at day 5. Upon microscopic analysis, >98% nonadherent cells presented cellular projections. Analysis by flow cytometry revealed that preparations consisted in a homogenous (>96%) population of CD2+, CD14low/+. CD16-, CD40-, CD54-, CD80+/low, CD86+, HLA-DR+ large cells, and <1% CD3+, CD19+, or CD56+ cells could be detected.

iDCs cultures were performed in complete serum-free HB101 medium (Irvine Scientific, Santa Ana, CA) and supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 10 mM HEPES, 100 IU penicillin, and 100 µg/ml streptomycin. iDCs were cultured at (0.5 × 10^6/ml) in 24-well flat-bottom Falcon plates (Becton Dickinson). Mature DCs (mDCs) were generated by stimulating day 5 monocyte-derived DC (0.5 × 10^6/ml) in HB101 medium with SAC (0.02% w/v) for 2 days in the presence of GM-CSF and IL-4 as above.

FIGURE 1. DCs express the CD47 Ag. Fresh monocytes, iDCs, and SAC-acti- vated DCs (mDCs) were analyzed by flow cytometry for the expression of CD47, CD14, and CD83. The results are representa- tive of four experiments.

Cytokine measurement

IL-12 p70 release was assessed by a two-site sandwich ELISA using mAb 20C2 as the capture mAb and HRP-coupled mAb 4D6 as detection probe. Both mAbs were generously provided by Dr. M. Gately (Hoffmann-La Roche). The sensitivity of the assays was 6 pg/ml. IFN-γ was measured by a sandwich solid-phase RIA using two anti-IFN-γ mAbs as previously described. TNF-α, GM-CSF, and IL-10 were measured using a sandwich ELISA or RIA as previously described (30, 32). If-13 was measured using a sandwich ELISA using rat anti-IL-13 mAb (clone 5A2, American Type Culture Collection) as capture mAb and a polyclonal rabbit anti-IL-13 Ab (Accurate Chemicals, Westbury, NY) as a detecting probe. IL-6, IL-8, IL-18, and TGF-β ELISA kits were purchased from R&D Systems (Min- neapolis, MN). All the measurements were performed in duplicate.

Ag uptake assay

Two identical aliquots of DC were washed and resuspended (10^7/ml) in complete HB101 culture medium at 37°C or 4°C (ice-cold). An equal vol- ume of BSA-FITC (50 µg/ml final concentration; Sigma) was then added and incubated for 30 min. Ag uptake was stopped by extensive washing using cold PBS containing 1% BSA and 10 mM sodium azide before analysis with a FACScan (Becton Dickinson). Negative controls were the experiments performed at 4°C. For testing the effect of CD47 engagement on endocytosis, anti-CD47 mAb or isotype-matched control (IgG1) (10 µg/ml) mAbs were added during the incubation with BSA-FITC.

Phagocytosis of apoptotic cells

B cells from chronic lymphocytic leukemia patients were isolated by density gradient centrifugation of heparinized blood followed by one cycle of rosetting with S-2-aminohexylisothiouronium bromide (Aldrich)-treated SRBC to deplete T cells. B cell purity was shown to be >98% by flow cytometry (FACSort, Becton Dickinson). Freshly purified B cells from chronic lymphocytic leukemia patients were stained with PKH26 red fluorescent cell linker (Sigma) according to the manufacturer’s instructions before induction of apoptosis by culture for 16 h in the presence of hydrocortisone (5 × 10^-4 M). More than 95% cells appeared apoptotic as determined using FITC-annexin V/PI staining. Cells were washed and were given to iDCs (2 × 10^5/ml) as a phagocytic meal (10 apoptotic cells per mDC treated DCs. All cultures were performed in complete RPMI 1640 me- dium (BioWhittaker, Walkersville, MD) containing 2 mM l-glutamine, 1 mM sodium pyruvate, 10 mM HEPES, 100 IU penicillin, 100 µg/ml strep- tomycin (BioWhittaker), and 10% FCS. Culture supernatant was collected at day 4, and cytokine content was determined.

DNA synthesis was assessed by adding 1 µCi/well of [methyl- 3 H]thy- midine (10 Ci/mmol; Amersham, Arlington Heights, IL) for the last 16 h of culture. Triplicate cultures were then harvested onto glass fiber filters, and the radioactivity was counted using liquid scintillation.

Cytokine measurement

DNA synthesis was assessed by adding 1 µCi/well of [methyl-3H]thymi- dine (10 Ci/mmol; Amersham, Arlington Heights, IL) for the last 16 h of culture. Triplicate cultures were then harvested onto glass fiber filters, and the radioactivity was counted using liquid scintillation.

B cells from chronic lymphocytic leukemia patients were isolated by density gradient centrifugation of heparinized blood followed by one cycle of rosetting with S-2-aminohexylisothiouronium bromide (Aldrich)-treated SRBC to deplete T cells. B cell purity was shown to be >98% by flow cytometry (FACSsort, Becton Dickinson). Freshly purified B cells from chronic lymphocytic leukemia patients were stained with PKH26 red fluorescent cell linker (Sigma) according to the manufacturer’s instructions before induction of apoptosis by culture for 16 h in the presence of hy- drocortisone (5 × 10^-4 M). More than 95% cells appeared apoptotic as determined using FITC-annexin V/PI staining. Cells were washed and were given to iDCs (2 × 10^5/ml) as a phagocytic meal (10 apoptotic cells per mDC treated DCs. All cultures were performed in complete RPMI 1640 me- dium (BioWhittaker, Walkersville, MD) containing 2 mM l-glutamine, 1 mM sodium pyruvate, 10 mM HEPES, 100 IU penicillin, 100 µg/ml strep- tomycin (BioWhittaker), and 10% FCS. Culture supernatant was collected at day 4, and cytokine content was determined.
one iDC for 3 h at 4°C (control) or 37°C, in the presence of anti-CD47 mAb (B6H12, 10 μg/ml) or its isotype-matched control. Endocytosis of PKH26-stained B cells was determined by FACS after gating on live DCs.

**Flow cytometry analysis**

CD47 surface expression was assessed using a two-step procedure. Briefly, cells were first incubated for 1 h at 4°C with a biotinylated mouse anti-CD47 mAb (B6H12) or class-matched negative control mAb (5 μg/ml). After washing, cells were incubated with PE-labeled streptavidin (Ancell) for 1 h at 4°C. Stained cells were analyzed using FACScan (Becton Dickinson). All other FITC- or PE-conjugated mAbs were purchased from Ancell.

**Statistical analysis**

The paired t test was used to determine statistical significance of the data.

**Results**

**CD47 expression by immature and mature DCs**

We first demonstrated CD47 expression on human monocyte-derived iDCs and bacteria-stimulated mDCs. As previously reported, all monocytes coexpressed CD14 and CD47 (33). CD14 expression was drastically reduced during the process of monocyte differentiation into iDCs in the presence of GM-CSF and IL-4, while CD83 was slightly induced (Fig. 1). As shown in Fig. 1, CD47 remained highly expressed on iDCs and was not modulated following SAC-induced maturation. Mature DCs became CD14−, CD47+, and CD83bright.

**CD47 ligation suppressed cytokine release by maturing DCs**

We next evaluated the effect of CD47 ligation by soluble mAb on cytokine production by maturing DCs. iDCs stimulated for 24 h with SAC produced large amounts of proinflammatory cytokines (Fig. 2). As seen, CD47 engagement potently suppressed IL-12 (n = 13, p < 0.0001), TNF-α (n = 13, p < 0.0001), IL-6 (n = 8, p < 0.05), and GM-CSF (n = 6, p < 0.04) production. CD47 ligation did not inhibit the production of IL-8 nor alter the viability of DCs, which was 80%, as revealed by trypan blue staining. Note that spontaneous release of TGF-β was not affected by SAC stimulation regardless of the presence of CD47 mAb.

CD47 mAb-mediated inhibition of IL-12 and TNF-α production was dose dependent, and significant suppression was seen with as little as 0.5 μg/ml of 2D3 or B6H12 mAbs directed against different CD47 epitopes (34) (Fig. 3A). Because FcγR ligation reportedly suppresses IL-12 production by monocytes (15), we excluded this mechanism by showing that F(ab')2 still inhibited cytokine release by maturing DCs (Fig. 3B). Finally, because TSP is a reported ligand of CD47, we examined the effect of 4N1K, a peptide corresponding to the CD47 binding region of TSP (27) on

---

**FIGURE 2.** CD47 ligation inhibits cytokine release by monocyte-derived DCs. Immature DCs were cultured with either medium alone or SAC (0.02%) in the presence of CD47 mAb (B6H12) or its isotype-matched control (C Ig) mAb (10 μg/ml). Cytokines were measured in culture supernatants collected after 24 h. Results are expressed as means ± SEM of n experiments. *, p < 0.05; **, p < 0.01.

**FIGURE 3.** Suppression of cytokine production by intact or F(ab')2 of CD47 Abs and synthetic peptides. Immature DCs were stimulated with SAC in the presence of graded doses of (A) B6H12 ( ), or 2D3 ( ) CD47 mAbs, (B) B6H12 F(ab')2, and (C) 4N1K (derived from the CD47-binding site of TSP) or 4NGG (mutant peptide), each at 50 μg/ml. The dotted line in A indicates SAC-induced cytokine production in the presence of a high dose (20 μg/ml) of isotype-matched control Ab (C Ig). A and B show one representative experiment of five, and C shows the means ± SD of four experiments. *, p < 0.05.
pressed by 72% (endogenous IL-10 production. We found that CD47 ligation sup-

Therefore, we determined whether CD47 mAb-mediated suppres-

Among the products released by macrophages and DCs following

Suppression of cytokine production by CD47 mAb is IL-10

IL-12 and TNF-α production. As depicted in Fig. 3C, 4N1K in-
hibited SAC-induced cytokine release by maturing DCs, whereas
the control mutant peptide 4NGG remained inactive.

Together, these results indicated that CD47 ligation by soluble
mAb or its natural ligand suppressed the production of pro-inflam-
matory cytokines by maturing DCs without inducing a generalized
dysfunction of the cells.

Inhibition of cytokine production by CD47 ligation was IL-10
independent and not overcome by IFN-γ

Among the products released by macrophages and DCs following
stimulation by bacterial products, IL-10 is known to be an auto-
crine inhibitor of proinflammatory cytokine production (9, 16).
Therefore, we determined whether CD47 mAb-mediated suppres-
sion of cytokines during DC maturation resulted from increased
endogenous IL-10 production. We found that CD47 ligation sup-
pressed by 72% (n = 7, p < 0.03) IL-10 release by SAC-stimu-
lated iDCs (Fig. 4A). To further exclude a possible role for IL-10
in CD47 mAb inhibitory effects, a neutralizing anti-IL-10 mAb
was added to SAC-stimulated cultures. In agreement with the
suppression of IL-10 release by CD47 ligation, blocking of IL-10
failed to restore IL-12 and TNF-α production in these cultures
(Fig. 4B).

IFN-γ has been reported to provide an efficient cosignal for
IL-12 p70 production by DCs stimulated by CD40 ligand or bac-
terial products such as SAC or LPS (35). Therefore, we examined
whether IFN-γ could restore cytokine release in CD47 mAb-
treated maturing DCs. As shown in Table I, CD47 engagement
significantly suppressed IL-12, TNF-α, and IL-10 in the presence
of a high dose of IFN-γ (500 U/ml), whereas IL-8 secretion still
remained unaltered.

CD47 ligation impaired DC phenotypic and functional
maturation

Upon stimulation with bacterial products, iDCs undergo several
phenotypic and functional changes, a process known as DC ma-
truration and leading to the development of fully competent Ag-
presenting and costimulatory cells (1, 2). We found that CD47
ligation during SAC stimulation of iDCs significantly altered their
maturation (Fig. 5 and 6). The SAC-induced down-regulation of
the monocyte marker CD14 and up-regulation of the DC marker
CD83 were impaired in CD47 mAb-treated cells (Fig. 5). CD47
mAb also inhibited the up-regulation of HLA-DR and of the co-
stimulatory molecules CD40, CD54, CD80, and CD86. Function-
ally, DC maturation is associated with a marked reduction of the
endocytic capacity. As seen, CD47 mAb-treated DCs retained a
higher capacity to uptake FITC-conjugated BSA. Similar results
were observed by using 4NIK peptide (C.E.D. and M.S., unpub-
lished observations).

The functional immaturity of CD47-treated DCs was further re-
vealed by examining their ability to stimulate allogeneic T cell
proliferation and cytokine production (Fig. 6). DCs that had ma-
tured in the presence of control Ab were strikingly better than
iDCs at inducing T cell proliferation (10-fold better) and IFN-γ or
IL-13 production. In contrast, DCs that had been incubated with
anti-CD47 mAb together with SAC were much less efficient to
initiate T cell proliferation (3-fold less than mDCs) and not better
than iDCs to induce IFN-γ and IL-13 production. Note that IL-4
and IL-5, two typical Th2 cytokines, could not be detected under
these experimental conditions (data not shown).

The following results suggested that the reduced capacity of
CD47 mAb-treated DCs to stimulate IFN-γ production may not
entirely result from decreased IL-12 production during DC ma-
truration. Indeed, regardless of CD47 ligation during DC maturation,
mDCs produced about 10 times less IL-12 than iDCs after restimu-
lation with soluble CD40 ligand and IFN-γ (used to mimic T cell/
DCs interaction), (Fig. 7). In contrast, we found that mDCs pro-
duced about twice more IL-18 than iDCs, and this was also not
modulated by anti-CD47 treatment.

CD47 ligation did not impair iDC phagocytic function

Immature DCs, as opposed to mDCs, are reported to be pro-
fessional phagocytes that efficiently eliminate apoptotic and necrotic
cells during early step of inflammatory process (36). Because our
data indicated that CD47 ligation during bacterial stimulation of

### Table I. IFN-γ fails to prevent inhibition of cytokine production by CD47 engagement on DCs

<table>
<thead>
<tr>
<th>Stimulation⁰</th>
<th>Additions</th>
<th>IL-12 (ng/ml)</th>
<th>TNF-α (ng/ml)</th>
<th>IL-8 (ng/ml)</th>
<th>IL-10 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAC + IFN-γ</td>
<td>Clg</td>
<td>7.69 ± 1.57</td>
<td>110.9 ± 29.7</td>
<td>27.98 ± 7.43</td>
<td>1.33 ± 0.38</td>
</tr>
<tr>
<td>SAC + IFN-γ</td>
<td>anti-CD47</td>
<td>2.64 ± 0.91</td>
<td>37.9 ± 13.7</td>
<td>27.42 ± 5.23</td>
<td>0.74 ± 0.26</td>
</tr>
</tbody>
</table>

⁰ Cytokines were measured in the supernatants taken after 24 h. Results are expressed as mean ± SEM of 6–10 experiments.

⁴ IFN-γ (500 U/ml) and anti-CD47 (B6H12) or its isotype-matched control (both at 10 μg/ml) mAbs were added together with SAC.

⁶ Values of p were calculated using the paired t test.

Downloaded from http://www.jimmunol.org/ by guest on April 1, 2017
iDCs inhibited cytokine secretion and prevented their acquisition of a mature functional phenotype, we determined whether CD47 mAb treatment would inhibit the phagocytic activity of iDCs. As shown in Fig. 8, CD47 mAb-treated iDCs phagocytosed apoptotic B cells as efficiently as untreated DCs, extending and confirming previous reports that CD47 mAb failed to interfere with the phagocytosis of aged neutrophils by macrophages (37).

Discussion
The present results indicate that CD47 is functionally expressed on human monocyte-derived DCs and that it may regulate their maturation induced by the pathogenic bacteria *Staphylococcus aureus*. Ligation of CD47 by either mAbs or a synthetic peptide corresponding to the binding region of its natural ligand, TSP, has two major effects on the response of iDCs to SAC. First, it suppresses the production of the proinflammatory cytokines IL-12, TNF-α, IL-6, and GM-CSF by maturing DCs, without affecting that of IL-8 and TGF-β. Second, it prevents the phenotypic and functional changes associated with DC maturation, including the up-regulation of MHC class II Ags, costimulatory molecules, and the acquisition of a potent T cell stimulatory activity. IL-10 and TGF-β were reported to exert similar effects on the maturation of DCs.
induced by bacterial products (16, 20). It is unlikely that anti-CD47 mAb acts by increasing the endogenous production of these inhibitory cytokines. Indeed, their production is either suppressed (IL-10) or unaffected (TGF-β), and inclusion of neutralizing Abs to IL-10 (Fig. 4) or TGF-β (data not shown) does not restore the cytokine production of anti-CD47-treated cells. Cytokine production by maturing DCs is also known to be suppressed by PGE₂ (38) or by pretreatment of iDCs with low doses of LPS (39, 40). Similar to anti-CD47 mAb, these treatments reduce IL-12 production by maturing DCs and generate mDCs that have a impaired capacity to induce IFN-γ production by allogeneic T cells; however, unlike anti-CD47 mAb, they do not prevent DCs to acquire increased T cell stimulatory activity.

In agreement with Kapsenberg and colleagues, we found that human mDCs produce 10-fold less IL-12 (and TNF-α) than iDCs (41), regardless of anti-CD47 mAb treatment. This contrasts with the increased capacity of mDCs, as compared with iDCs, to stimulate IFN-γ production by adult T cells (Ref. 6, Fig. 6), a phenomenon which is IL-12-dependent (Ref. 42 and data not shown). Moreover, mDCs are better at inducing IFN-γ production because they express higher levels of membrane-bound (CD80, CD86, CD54, and CD58) and soluble molecules (IL-18) that costimulate the effects of IL-12 (43). Inclusion of anti-CD47 mAb during DC maturation did not affect the IL-12- nor the IL-18-producing capacity of mDCs, whereas it reduced their capacity to stimulate T cell proliferation and IFN-γ production; probably by inhibiting the up-regulation of membrane bound costimulatory molecules. This interpretation is supported by two observations. First, even in the presence of exogenous IL-12 (50 pM), the production of IFN-γ by T cells stimulated with anti-CD47-treated DCs remains significantly lower than that induced by control mAb-treated DCs (44 ± 7 ng/ml of IFN-γ compared with 76 ± 11 ng/ml; mean ± SEM of four experiments, p < 0.05). Second, anti-CD47-treated DCs also display a reduced rather than enhanced capacity to induce IL-13 production by T cells (Fig. 6), a cytokine whose production is suppressed by IL-12 (44).

The inhibition of DC maturation may be biologically and clinically relevant as illustrated in a recent study showing that *Plasmodium falciparum* may use this strategy to defeat the human immune response (45). Malaria-infected human erythrocytes reportedly exert the same effects on LPS-induced maturation of iDCs as those described in the present study. Interestingly, the ability of intact malaria-infected erythrocytes to inhibit DC maturation was linked to the expression on their surface of still undefined parasite-derived proteins binding to CD54, CD36, and TSP. It is possible that TSP bound to infected erythrocytes may engage mDCs expressed not only by platelets but also by monocytes and macrophages (27). TSP is transiently expressed at high concentration in damaged and inflamed tissues (28, 29), and there is growing evidence that it has potent anti-inflammatory properties. These may involve at least two distinct mechanisms. First, TSP binds to immature TGF-β and activates it into a potent immunoregulatory cytokine (47). TSP-deficient animals display diffuse inflammatory lesions that are corrected by treatment with the active form of TGF-β and are reminiscent of those observed in TGF-β-deficient animals (48). The second mechanism involves the binding of TSP to CD47 expressed on inflammatory cells and/or APC. In addition to the present findings, engagement of CD47 by TSP reportedly inhibits IL-12 production by human monocytes stimulated via T cell-dependent mechanisms or bacterial products (49). Whether or not some pathogens exploit the CD47-mediated inhibition of DC maturation, our observations are consistent with a role for this molecule and its ligand (TSP) in regulating the early stages of the innate and adaptive immune response.

Acknowledgments

We thank Mrs. Norma DelBosco for excellent secretarial assistance and Dr. M. Gately (Hoffmann-LaRoche, Nutley, NJ) for providing anti-IL-12 Abs.

References
