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T Cell Biasing by Activated Dendritic Cells

Charles F. Anderson, Mark Lucas, Laila Gutiérrez-Kobeh, Ann E. Field, and David M. Mosser

We have previously reported that the ligation of FcγRs on activated macrophages affected their production of cytokines and their ability to influence T cell activation. Dendritic cells (DC) are important APCs that also express FcγR. In the present work, we sought to determine whether DC responded to immune complexes in a manner similar to macrophages. We confirmed that activated murine DC produced IL-12, and, as a result, induced naive T cells to produce primarily IFN-γ upon stimulation. However, DC activated in the presence of immune complexes shut off their production of IL-12p70 and induced a Th2-like cytokine response. Thus, DC respond to immune complexes by altering their cytokine production, which, in turn, influences T cell responses. A DC transfer experiment was performed to determine the extent that APC exposure to immune complexes could influence adaptive immune responses. Vaccination of mice with Ag, along with DC that were activated in the presence of immune complexes, resulted in higher levels of Ag-specific IgG1 Ab, relative to mice that were vaccinated with activated DC and Ag alone. The mechanism by which DC altered their cytokine production in response to immune complexes was different from macrophages.

Macrophages down-regulated the transcription of both the p40 and p35 subunits of IL-12, whereas DC decreased only p35 expression. We conclude that APCs expressing FcγR on their surface can respond to immune complexes by shutting off IL-12 biosynthesis, to prevent the Th1-type T cell biasing that normally accompanies innate immune activation. The Journal of Immunology, 2004, 173: 955–961.

Naïve CD4+ lymphocytes can differentiate into a variety of different effector cell types following activation by APCs (1). Th1 cells are generally involved in cell-mediated immunity and secrete IL-2, IFN-γ, and lymphotoxin as their signature cytokines (2). Th2 effector cells are generally associated with humoral immunity and the clearance of parasitic infections, and are identified by the secretion of IL-4, IL-5, IL-10, and IL-13 (3). The factors that determine what type of effector cells develops during an immune response include Ag dose (4), genetic makeup of the host (5), and the APC involved (6). However, the dominant factor appears to be the cytokine environment where the T cells develop (7). IL-12 is the major inducer of Th1 differentiation. This cytokine is produced primarily by macrophages and dendritic cells (DC) following exposure to stimuli such as bacterial products or CD40 ligation (2, 5, 8). The biologically active form of this cytokine is a secreted 70-kDa heterodimer composed of a disulfide-linked p40 and p35 subunit, which are transcribed from separate genes (9). The prototypical Th2-inducing cytokine is IL-4, which appears to be produced by activated CD4+ cells themselves early in the immune response (7).

We have recently shown that macrophages activated in the presence of immune complexes are different from classically activated macrophages (10). Whereas classically activated macrophages produce high levels of IL-12 and low to moderate levels of IL-10, macrophages activated in the presence of immune complexes shut off IL-12 production (11) and induce IL-10 biosynthesis (12). When these two types of differentially activated macrophages were used as APCs, they induced different CD4+ T cell responses (13). Classically activated macrophages induced the development of Th1 cells by virtue of their IL-12 secretion, whereas macrophages given immune complexes induced a Th2 response due to the absence of IL-12 and an increased production of IL-10. We have termed these latter macrophages type II-activated macrophages, due to their ability to promote Th2-type immune responses (14).

DC are professional APCs specialized for Ag uptake, migration to secondary lymphoid organs, and Ag presentation to T cells. Indeed, DC are considered to be the most relevant APC in stimulating naïve CD4+ and CD8+ lymphocytes in secondary lymphoid organs (15). DC are capable of producing IL-12 following exposure to components of pathogens, including bacteria, viruses, and protozoa, or through CD40L by activated T cells (9). IL-12 production by DC has been shown to induce Th1 immunity (16, 17).

DC express FcγRs, and these receptors have been shown to play an important role in the maturation of immature DC (18, 19). They are also believed to play a role in the phenomenon of cross-presentation of Ags (20, 21). Two of the FcγRs, FcγRI and FcγRIIa, associate with and signal through a common γ subunit, which contains an ITAM motif. Recently, other receptors on DC, such as DCAR, have been shown to also signal through the γ-chain (22).

In the present work, we measured IL-10 and IL-12 production by activated DC following FcγR ligation. We sought to determine whether immune complexes could influence the outcome of an adaptive immune response when DC were used as APCs. We show that DC shut off their production of IL-12p70 following FcγR
ligation, and as a result induced a Th2-like T cell response. These results indicate that APCs that express FcγR on their surface can respond to immune complexes by reversing the Th1 biasing of innate immune stimuli.

Materials and Methods

Mice

Eight-week-old BALB/c and C57BL/6 mice were purchased from National Cancer Institute, Charles River Laboratories (Frederick, MD). Breeding pairs of DO11.10 mice transgenic for OVA<sub>323-339</sub> (23) and TCRαβ were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were maintained in HEPA-filtered caging units (Thoren Caging Systems, Hazleton, PA) at the University of Maryland (College Park, MD). Mice were used for experiments at 8–10 wk of age.

Reagents

Bacterial oligodeoxynucleotides containing CpG motifs were a kind gift from R. Seder (Vaccine Research Center, National Institutes of Health, Bethesda, MD) and had the sequences TCAAGC<sub>7</sub>TGAG(GT) (24). OVA (grade VI) was purchased from Worthington Biochemical (Lakewood, NJ). In some cases, OVA was passed over a Detoxi-Gel column (Pierce, Rockford, IL) to remove trace LPS contaminations. Rabbit IgG Ab to OVA (anti-OVA IgG) was purchased from Capel (Durham, NC). IgG-OVA immune complexes were made by mixing a 10:1 molar excess of anti-OVA:OVA at room temperature for 30 min, as previously described (13). LPS from Escherichia coli 0117:B8 was obtained from Sigma-Aldrich (St. Louis, MO).

Cells

Bone marrow-derived DC (BMDC) were prepared as described by Lutz et al, (25), with minor modifications. Briefly, bone marrow was flushed from the femurs and tibias from mice and plated in 100-mm petri dishes at a concentration of 1 × 10<sup>6</sup> cells/ml in RPMI 1640 supplemented with 10% FCS, penicillin/streptomycin, HEPES, glutamine, 50 μM 2-ME, and 5 ng/ml recombinant murine GM-CSF (R&D Systems, Minneapolis, MN). On days 3 and 6, half the medium was removed and replaced with fresh medium. On day 9, nonadherent cells were removed and replated on 100-mm tissue-culture-treated dishes overnight to further remove adherent cells. Nonadherent cells were removed from dishes and used for experiments. By flow cytometry, cells were generally 80% CD11c<sup>+</sup> pure and had a CD11c<sup>+</sup>CD11b<sup>+</sup>CD8α<sup>-</sup> phenotype, with only moderate expression of MHC class II. For in vitro maturation of DC, TNF-α (R&D Systems) was added to day-7 cultures at a concentration of 20 ng/ml. Cells remained in culture for 36 h for maturation before use in experiments. These cells were determined to be mature by analyzing expression of MHC class II, CD40, and CD86 by flow cytometry.

Splenic DC (SPDC) were prepared by digestion of spleens using liberase (Roche, Indianapolis, IN) at a concentration of 0.2 U/ml at 37°C for 30 min. Cells were then passed through a strainer to remove tissue debris and washed by centrifugation. To isolate DC, positive selection using anti-CD11c<sup>+</sup> microbeads (Miltenyi Biotec, Auburn, CA) were used according to manufacturer’s instructions. By flow cytometry, cells were generally 75–80% CD11c<sup>+</sup> and had the phenotype CD11c<sup>+</sup>CD8α<sup>-</sup>/MHC class II<sup>+</sup>.

Bone marrow-derived macrophages (BMM<sub>d</sub>) were prepared as described previously (11). Briefly, bone marrow was flushed from the femurs and tibias from mice, and cells were plated in petri dishes in DMEM supplemented with 10% FCS, penicillin/streptomycin, glutamine, and 20% conditioned medium from the supernatant of M-CSF-secreting L929 fibroblasts. Media was replaced on day 6, and cells were used at 10–12 days for experiments.

CD4<sup>+</sup> T cells were prepared from the spleens of DO11.10 mice by immunomagnetic positive selection using anti-CD4 (L3T4) microbeads (Miltenyi Biotec) as previously described (13). Cells were >95% CD4<sup>+</sup>CD45R<sup>B</sup><sup>+</sup> as determined by flow cytometry.

Cell stimulation assays

For stimulation of macrophages, 2 × 10<sup>5</sup> macrophages were plated per well in a 48-well plate in RPMI 1640 and primed for 12 h with 100 U/ml recombinant murine IFN-γ (R&D Systems). Cells were then washed, and activated with 5 μg/ml CpG DNA and either 150 μg/ml OVA or 140 μg/ml OVA plus 10 μg/ml IgG-OVA. For stimulation of DC, 2 × 10<sup>5</sup> cells/well were added to 48-well plates containing 5 μg/ml CpG and either 150 μg/ml OVA or 150 μg/ml OVA plus IgG-OVA. For T cell stimulation assays, 5 × 10<sup>5</sup> CD4<sup>+</sup> T cells were added 2 h later. One week following primary stimulation, CD4<sup>+</sup> cells were harvested, washed, and restimulated with 5 × 10<sup>6</sup> splenocytes. Cytokines were analyzed 24 h after secondary stimulation. For intracellular staining, CD4<sup>+</sup> cells were secondarily activated with PMA/ionomycin for 4 h followed by intracellular staining. Some T cells were stained in the presence of 500 pg/ml IL-12 (R&D Systems). In some cases, CD40L-expressing L929 fibroblasts were used in place of CD4<sup>+</sup> T cells for CD40 signaling. For this, 1 × 10<sup>5</sup> fibroblasts were adhered to plates the night before.

Cytokine production

Cytokines were measured by ELISA using the following Ab pairs from BD Pharmingen (San Jose, CA): IL-12p70, 9A5 and C17.8; IL-12p40, C15.6 and C17.8; IL-10, JES-2A5 and JES-16E3; IFN-γ, R4-6A2 and XMG1.2; and IL-4, 11B11 and BV426-24G2. For intracellular staining, brefeldin A was added 1 h following PMA/ionomycin addition. Cells were then fixed and permeabilized 3 h later using Cytotox/Cytoperm kit from BD Pharmingen. Following staining, cells were analyzed by a BD FACs-Calibur flow cytometer (BD Pharmingen).

Cytokine mRNA was measured by real-time PCR. BMDC and BMM<sub>d</sub> were stimulated on day 6 with LPS (10 ng/ml) ± erythropoiesis opsonized with IgG for 5 h. Cells were harvested and washed by centrifugation, and then lysed in TRIzol (Invitrogen Life Technologies, Carlsbad, CA). RNA was extracted following the manufacturer’s protocol and stored at −80°C. Real-time PCR was conducted using the ABI PRISM 7700 Sequence Detection System and SYBR Green core PCR reagents (Applied Biosystems, Foster City, CA). Murine IL-12p35 primer sequences used were sense, 5’-CCGCGTCGACCATCA; and anti-sense, 5’-CAGAGGGCTTGAAGCTTCT; and p40 sequences were sense, 5’-ACATTAGCAGAA-GTCAAATGCA; and anti-sense, 5’-GGAACACATGC CACCTGCT.

FIGURE 1. Differential production of IL-12 and IL-10 by activated DC and macrophages. BMDC (left) and BMM<sub>d</sub> (right) were added to 48-well tissue culture plates and stimulated with CpG oligonucleotides and either OVA alone ( ), or OVA plus IgG-OVA immune complexes ( ). Two hours later, CD4<sup>+</sup> cells were added to the culture. Twenty-four hours following stimulation of cells, IL-12p70 (A), IL-12p40 (B), and IL-10 (C) levels were measured by ELISA. Data are the mean ± SD from one experiment performed in triplicate, and are representative of at least three independent experiments.

+, Significant difference (p < 0.01) between OVA and IgG-OVA groups.
DC transfer experiments

BMDC were resuspended in complete medium at a concentration of \(8 \times 10^6\) cells/ml. One group of DC received 100 \(\mu\)g/ml OVA alone. The second group received the same amount of OVA plus 10 ng/ml LPS and 5 \(\mu\)g/ml CpG oligonucleotides. The third group received LPS/CpG and a total of 100 \(\mu\)g/ml OVA, of which 7 \(\mu\)g/ml was IgG-OVA. Medium was added to bring the final cell concentration to \(4 \times 10^5\) cells/ml, and stimulated DC were incubated in vitro for 8 h. Individual BALB/c mice were immunized i.p. with a total of 2 \(\times\) \(10^6\) stimulated DC and 50 \(\mu\)g OVA in a volume of 500 \(\mu\)l. No adjuvants were administered. This vaccination with OVA and stimulated DC was repeated on day 12. Serum was collected 7 days following the second vaccination and the titers of total OVA-specific IgG, IgG1, and IgG2a were determined as previously described (13). Briefly, OVA-coated plates were incubated with increasing dilutions of serum, and IgG1, and IgG2a were determined as above.

Results

Cytokine production by CpG-stimulated DC

DC were generated from murine bone marrow precursors (BMDC), and stimulated in vitro with CpG oligonucleotides and Ag (OVA). Stimulation resulted in the production of IL-12p70 (Fig. 1A, □) and IL-12p40 (Fig. 1B) from these cells. Activated BMDC made minimal IL-10 in response to CpG stimulation (Fig. 1C). BMMφ were stimulated in parallel (Fig. 1, right). When stimulated with OVA and CpG oligonucleotides, macrophages produced high levels of IL-12p70 (Fig 1A), IL-12p40 (Fig 1B), and they secreted moderate levels IL-10 (Fig. 1C). Both BMDC and BMMφ were also stimulated in the presence of IgG-opsonized OVA (Fig. 1, □). DC activated in the presence of immune complexes produced almost no detectable IL-12p70 (Fig. 1A). However, immune complexes did not reduce IL-12p40 biosynthesis by DC, nor did they affect the low amounts of IL-10 produced by DC (Fig. 1C). In macrophages, the synthesis of all three of these molecules was affected by the presence of immune complexes. Both IL-12p70 and IL-12p40 were dramatically reduced following activation in the presence of immune complexes (Fig. 1, A and B) and IL-10 biosynthesis was increased (Fig. 1C), as previously reported by us using different stimuli (13, 26). Thus, both DC and macrophage respond to immune complexes by altering their production of IL-12p70.

The alteration of cytokine production by DC in response to immune complexes was independent of the type of stimulation condition. Both unprimed and IFN-γ-primed DC, stimulated with either CpG (Fig. 2, □) or LPS (■), responded to immune complexes by down-regulating IL-12p70.

T cell biasing by immune complexes

To determine the effect that APC cytokine production had on T cell activation, differentially activated macrophages or DC were used as APCs for naive DO11.10 CD4+ T cells. We previously demonstrated that activated macrophages induced T cells to produce primarily IFN-γ in response to Ag (13). This observation is confirmed in Fig. 3A. Macrophages receiving CpG and OVA Ag induced T cells to produce relatively high levels of IFN-γ and little IL-4 in the secondary response (Fig. 3A, bottom). In contrast, macrophages receiving IgG immune complexes (IgG-OVA) induced the production of higher levels of IL-4 and reduced amounts of IFN-γ from T cells. This deviation in T cell cytokine production also occurred when DC were used as APCs (Fig. 3A, top). DC that were activated in the presence of IgG immune complexes gave rise to T cells that produced primarily IL-4, whereas DC that were activated in the presence of OVA Ag alone gave rise to T cells that secreted significantly more IFN-γ than IL-4 (Fig. 3A).

Intracellular staining of the CD4+ cells was performed to determine the proportions of T cells making IFN-γ or IL-4 (Fig. 3B).
In agreement with the ELISA data, 41% of CD4\(^+\) cells activated by BMDC receiving CpG and OVA produced IFN-\(\gamma\), whereas only 1% produced IL-4. However, BMDC that were given CpG and IgG-OVA reversed this profile. Only 5% of the CD4\(^+\) cells produced IFN-\(\gamma\), whereas 21% produced IL-4. Thus, BMDC respond to immune complexes by altering their own cytokine production, which influences T cell cytokine responses.

To determine the contribution of DC-derived IL-12 to T cell biasing, similar studies were performed with DC activated in the presence of immune complexes and rIL-12. As shown above, the addition of immune complexes to activated DC induced a Th2-type biasing of naive T cells (Fig. 4). However, the simultaneous addition of rIL-12 to these cells at the time of activation reversed this biasing and restored Th1-like responses. Thus, IL-12 is a major contributor to Th1 biasing by activated DC, and the abrogation of IL-12 biosynthesis by immune complexes reverses this biasing.

FIGURE 4. Measurement of CD4\(^+\) T cell cytokine production following stimulation by BMDC. CD4\(^+\) T cells from DO11.10 mice were added to BMDC that had been stimulated with CpG oligonucleotides in the presence of OVA alone (□) or OVA plus IgG-OVA (■). A parallel population of T cells were stimulated with DC receiving OVA plus IgG-OVA, along with 500 pg rIL-12 (▲). Cytokine production was measured by ELISA 4 days following the primary stimulation.

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FIGURE 5. Flow cytometry analysis of BMDC and SPDC. Surface expression of MHC II, CD40, CD86, and Fc\(\gamma\)R expression was determined by flow cytometry on BMDC (left panels) or SPDC (right panels). Cells were fixed and stained for surface expression of MHC class II, CD40, CD86, and Fc\(\gamma\)R II/III as described in Materials and Methods.

FIGURE 5. Flow cytometry analysis of BMDC and SPDC. Surface expression of MHC II, CD40, CD86, and Fc\(\gamma\)R expression was determined by flow cytometry on BMDC (left panels) or SPDC (right panels). Cells were fixed and stained for surface expression of MHC class II, CD40, CD86, and Fc\(\gamma\)R II/III as described in Materials and Methods.

FIGURE 6. IL-12 and IL-10 production from activated mature SPDC. Primary SPDC were cultivated overnight and then stimulated with CpG oligonucleotides and either OVA Ag alone (□) or IgG-OVA immune complexes (■). Two hours later, CD4\(^+\) cells were added to the culture. Twenty-four hours following stimulation of macrophages and DC, IL-12p70 (A), IL-12p40 (B), and IL-10 (C) levels were measured by ELISA. Data are the mean ± SD of triplicate determinations from an experiment that is representative of at least three independent experiments.

FIGURE 6. IL-12 and IL-10 production from activated mature SPDC. Primary SPDC were cultivated overnight and then stimulated with CpG oligonucleotides and either OVA Ag alone (□) or IgG-OVA immune complexes (■). Two hours later, CD4\(^+\) cells were added to the culture. Twenty-four hours following stimulation of macrophages and DC, IL-12p70 (A), IL-12p40 (B), and IL-10 (C) levels were measured by ELISA. Data are the mean ± SD of triplicate determinations from an experiment that is representative of at least three independent experiments.

FIGURE 7. Measurement of CD4\(^+\) T cell cytokine production following stimulation with activated mature SPDC. A, CD4\(^+\) T cells from DO11.10 mice were added to SPDC in the presence of OVA (□) or OVA plus IgG-OVA (■). One week later, T cells were washed and given a secondary stimulation under nonbiasing conditions, using T-depleted splenocytes and OVA. The following day T cell cytokine production was measured by ELISA (A) or by intracellular staining (B). For intracellular staining, CD4\(^+\) cells were stimulated as above and then restimulated in vitro with PMA/ionomycin for an additional 5 h. Cells were fixed and permeabilized and stained with mAbs to IFN-\(\gamma\) (abscissa) and IL-4 (ordinate). Results are from one experiment that is representative of at least three.

FIGURE 7. Measurement of CD4\(^+\) T cell cytokine production following stimulation with activated mature SPDC. A, CD4\(^+\) T cells from DO11.10 mice were added to SPDC in the presence of OVA (□) or OVA plus IgG-OVA (■). One week later, T cells were washed and given a secondary stimulation under nonbiasing conditions, using T-depleted splenocytes and OVA. The following day T cell cytokine production was measured by ELISA (A) or by intracellular staining (B). For intracellular staining, CD4\(^+\) cells were stimulated as above and then restimulated in vitro with PMA/ionomycin for an additional 5 h. Cells were fixed and permeabilized and stained with mAbs to IFN-\(\gamma\) (abscissa) and IL-4 (ordinate). Results are from one experiment that is representative of at least three.
DC maturation and T cell biasing

To correlate DC responses with FcγR expression, we performed similar types of experiments, using two populations of DC-lacking FcγR. Primary SPDC were allowed to spontaneously mature in culture, as previously described (27). The mature phenotype of these cells was confirmed by flow cytometry, showing an increase in surface CD40 and CD86 expression on these cells relative to immature BMDC (Fig. 5). Importantly, this maturation process resulted in a marked decrease in the surface FcγR expression on these cells (Fig. 5, bottom). Similar to the studies described above, these mature SPDC were activated in the presence or absence of immune complexes, and then used as APCs to present Ag to naive CD4⁺ T cells. T cell cytokine production in response to OVA or IgG-OVA was measured by ELISA 24 h after secondary stimulation.

**FIGURE 8.** Cytokine production by mature and immature BMDC. Immature BMDC were induced to mature by the addition of 20 ng/ml TNF-α (R&D Systems) to the culture, 36 h before stimulation. Immature DC were maintained in the absence of TNF. Thirty-six hours later cells were washed and stimulated with CpG oligonucleotides and either OVA or IgG-OVA. IL-12p40 (top) and IL-12p70 (bottom) production was measured by ELISA. To determine the effect of BMDC cytokine production on T cell responses, mature (inset) and immature (data not shown) BMDC were used as APCs to present Ag to naive CD4⁺ T cells. T cell cytokine production in response to OVA or IgG-OVA was measured by ELISA 24 h after secondary stimulation.

**FIGURE 9.** Quantitative real-time PCR analysis of IL-12p35 and IL-12p40. BMDC (top panels) and BMMφ (bottom panels) were stimulated with LPS and Ag for 5 h, and total RNA was extracted for real-time PCR analysis as described in Materials and Methods. □, Unstimulated cells; ■, cells stimulated in the presence of OVA; ●, cells stimulated in the presence of OVA plus erythrocytes opsonized with IgG. Relative RNA levels for p35 and p40 are expressed as fold change over unstimulated cells. Hypoxanthine phosphoribosyltransferase was used as the internal control.

BMDC differ from macrophages in their regulation of IL-12

We examined the mechanism of down-regulation of IL-12p70 by macrophages and immature DC following their exposure to immune complexes. Real-time PCR (Fig. 9) was performed to measure IL-12p40 and IL-12p35 mRNA levels in parallel populations of BMDC and macrophages following stimulation in the presence (□) or absence (■) of immune complexes. Unlike macrophages, which down-regulated both IL-12p40 and IL-12p35 in response to immune complexes (Fig. 9), BMDC show no decrease in IL-12p40 following FcγR ligation. However, they did exhibit a dramatic decrease in IL-12p35 (Fig. 9). Thus, although both macrophages and DC shut off IL-12p70 production following FcγR ligation, they do so by different mechanisms.

**In vivo biasing at the level of the APC**

Vaccination studies using BMDC that were activated in vitro in the presence or absence of immune complexes were performed to determine the extent that DC could influence Ab production to a nominal Ag. DC were stimulated in vitro in the presence of OVA Ag, and then both DC plus Ag were injected into mice. No adjuvants other than the activated DC were added. The total amount of OVA-specific IgG and the isotype of OVA-specific IgG was measured following a second boost with Ag and stimulated DC. The administration of OVA Ag plus resting DC induced a minimal immune response (Fig. 10), presumably due to the lack of innate activation by the LPS-free OVA that was used in these studies.
Vaccination with OVA plus CpG/LPS-activated DC induced a better IgG response, which resulted in the production of moderate levels of IgG1 and a modest amount of IgG2a (Fig. 10). However, DC activated in the presence of immune complexes produced a significantly higher level of total OVA-specific IgG, and the majority of this increase was due to a dramatic increase in the level of IgG1 (Fig. 10). Thus, DC exposed to immune complexes at the time of activation can preferentially induce an increased humoral immune response to Ag, resulting in higher IgG1 production.

Discussion

In this work, we show that innate activation of DC results in the production of IL-12 by these cells. This observation confirms previous reports of others (28). We extend these observations to show that DC activation in the presence of immune complexes has a profound effect on the IL-12 biosynthesis. Exposure of DC to immune complexes causes a dramatic decrease in the production of biologically active IL-12p70 (Fig. 1). The extent of this decrease is comparable to what we had previously observed in activated macrophages (13). We also show that the alterations in DC cytokine production following their exposure to immune complexes can have a direct result on T cell biasing. The IL-12 that is produced following their exposure to immune complexes can in a direct result on T cell biasing. The IL-12 that is produced following their exposure to immune complexes can have a direct result on T cell biasing. However, on closer examination there were several important differences between activated macrophages and BMDC. First, DC made only minimal amounts of IL-10, and these levels were not increased in response to immune complexes. This is clearly different from macrophages, which produce prodigious amounts of IL-10 in response to FcγR ligation (12). Second, although both populations of cells decreased the production of IL-12p70 in response to immune complexes, BMDC did not diminish the transcription of p40. Rather, they regulate IL-12 production exclusively at the level of the p35 subunit.

The alteration of cytokine production by APCs in response to immune complexes that we observe may contribute in an unexpected way to the elevated production of Ab that is produced in a secondary immune response. Although it is well appreciated that the expansion and differentiation of memory B and T cells represents the major reasons for the efficiency of the secondary immune response, the present studies suggest that APC alterations may also contribute to this process. Fundamental differences in cytokine production by macrophages and DC, caused by the presence of IgG immune complexes, may result in a cytokine environment that preferentially induces T cells to promote B cell development and differentiation. Further studies to elucidate the extent that APCs can influence or modify host immune responses during immunity and immunopathogenesis are warranted.

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