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Dendritic Cell-Derived IL-12 Is Not Required for the Generation of Cytotoxic, IFN-γ-Secreting, CD8+ CTL In Vivo

Yonghong Wan, Lingmin Lu, Jonathan L. Bramson, Stefan Baral, Qing Zhu, Andrew Pilon, and Kelley Dayball

By using adoptive transfer of Ag-loaded bone marrow-derived dendritic cells (BMDC), we have established an in vivo model of CTL priming. Activation of CTL in these experiments required both CD4+ T cells and CD154, demonstrating that this model reflects CD4+ T cell-dependent dendritic cell (DC) licensing. Because IL-12 has been suggested to play an important role in CTL activation by DC, we examined the ability of BMDC to prime CTL in the complete absence of IL-12 using p40-deficient mice. We observed that the absence of IL-12 does not affect the phenotype or allostimulatory function of BMDC after in vitro maturation. Moreover, there was no difference in the ability of Ag-loaded DC to elicit CTL cytotoxicity, whether the Ag was delivered by virus infection or peptide pulsing. Equal frequencies of Ag-specific, IFN-γ-secreting CD8+ T cells developed in both wild-type and IL-12-deficient backgrounds. Finally, CTL generated in the IL-12-deficient environment were capable of protecting immunized mice against tumor challenge, demonstrating that these CTL were fully functional, despite the absence of IL-12 during the maturation process in vivo. These results indicate that IL-12 is not critical for the development of IFN-γ secreting, CD8+ T cells and that another mechanism must be used by licensed DC to prime and activate CTL. The Journal of Immunology, 2001, 167: 5027–5033.

Effective protection from virus infection and tumor challenge requires CD8+ CTL. Thus, the potency of a vaccine is measured, in part, by its ability to elicit a robust CTL response. To gain full effector function, CTL must be primed by “professional” APCs, such as dendritic cells (DCs)1 (1). Primed CTL can then recognize antigenic peptides presented by MHC class I on the surface of virus-infected cells and tumor cells, resulting in cytolysis of the target cell. However, before a DC is capable of priming a CTL, it must first undergo a functional change, through a process known as “licensing” (2). DC can be licensed by either virus infection or through cognate interaction with CD4+ T helper cells (3, 4). Originally, CD4+ T cells were thought to provide help to maturing CTL through paracrine release of cytokines like IL-2 and IFN-γ (5). More recent studies have demonstrated that, in fact, CD4+ T cells provide help through direct interaction with the DC, not the CTL (6). Ligation of CD40 on the DC by CD154 expressed on activated CD4+ T cells promotes a functional change in the DC, licensing the DC to prime CTL (7, 8). Further evidence that CD4+ T cells provide help through direct interaction with the DC comes from studies demonstrating that the requirement for CD4+ T cell help during CTL priming can be bypassed by the presence of a CD40 agonist (9).

Understanding the signals used by a licensed DC to prime CTL will have a tremendous impact on vaccine design. Although the molecular changes that take place in the DC after CD40 ligation are still being elucidated, one well-described effect is the induction of IL-12 (10, 11). IL-12 was originally identified as cytotoxic lymphocyte maturation factor, and increased IL-12 production by DCs is suggested to be a critical step in the process of CTL priming (12). Furthermore, IL-12 is needed for maximal development of IFN-γ-secreting CTL (Tc1 cells) in vitro (13, 14). Thus, incorporation of IL-12 or IL-12-inducing agents into vaccines would be expected to enhance CTL generation, and indeed, several reports have provided evidence of the adjuvant effects of IL-12 (15–18). We have recently demonstrated that in the absence of IL-12, DCs loaded with the melanoma Ag gp100 can elicit potent antitumor immunity, associated with a robust type 1 immune response, suggesting that IL-12 expression by DC may not be absolutely required for the development of cell-mediated immunity. However, the major effector cell in the gp100 model is a CD4+ T cell, and because CD4+ T cell activation does not appear to be CD40 dependent, it is quite possible that the activation mechanism is IL-12 independent as well (19).

IL-12 is used routinely as an indicator of DC activation and a surrogate maker for type 1 T cell responses, yet the importance of IL-12 in CTL priming in vivo is still unclear (20). To investigate the requirement of IL-12 in CTL maturation and activation, we have conducted a series of studies directed at evaluating CD8+ CTL priming and activation in vivo. Immunization of C57BL/6 mice with bone marrow-derived DCs (BMDCs) loaded with an immunogenic Ag, chicken egg OVA, elicited a strong CTL response that was CD4+ T cell and CD154 dependent, demonstrating that this model is representative of CD4+ T cell-mediated DC licensing. In this scenario, both wild-type and IL-12-deficient DCs can equally prime CD8+ T cell effector function, as measured by cytotoxic activity and Ag-specific IFN-γ production. Furthermore,
both classes of CTL (those generated in the presence of IL-12 and those generated in the absence of IL-12) were able to protect immunized mice from tumor challenge. These results indicate that although IL-12 may be involved in the process of CTL maturation, its role is secondary to the effect of DC licensing.

Materials and Methods

Animals and cell culture
Female C57BL/6 (H-2b) mice aged 6–8 weeks were obtained from Charles River Laboratories (Wilmington, MA). IL-12 p40 gene knockout mice, IL-12 p40+/−, which do not produce bioactive IL-12, were kindly provided by Dr. J. Magram (Hoffmann-La Roche, Nutley, NJ; Ref 21). B6,129S-H2H2bFlk1−/− (MHC class II−/−), and C57BL/6-Tnfsf4−/− (CD40L−/−) mice (initially purchased from The Jackson Laboratory, Bar Harbor, ME) were bred in our pathogen-free animal facility. The tumor cell lines used were EL-4 (C57BL/6 thymoma) and EG7 (EL-4 transfected with the cDNA of chicken OVA). Cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. EG7 cells were maintained in medium containing 400 μg/ml G418 (Life Technologies, Grand Island, NY).

Peptides
The Kb-binding peptide of OVA (OVA257-264) and Dd-binding peptide of the lymphocytic choriomeningitis virus glycoprotein (p33; KAVYFNATM) were purchased from American Peptide Company (Sunnyvale, CA) and William K. Warren Medical Research Institute (Oklahoma City, OK), respectively. Peptides were dissolved in distilled water and stored at −20°C.

Adenoviral vectors
A recombinant adenoviral vector expressing OVA (SIINFEKL) linked to the β2-microglobulin signal sequence (AdssOVA) was provided by Dr. M. J. Bevan (University of Washington, Seattle, WA; Ref 22). AdLacZ contains the gene for the Escherichia coli LacZ under control of the CMV immediate early promoter and has been described previously (23). Viruses were propagated on 293 cells and purified by centrifugation as described previously (24).

Preparation of BMDC
In vitro expansion of BMDC has been described previously (19). Briefly, murine BM cells from C57BL/6 mice (IL-12+/+ DC) or IL-12−/− mice (IL-12−/− DC) were cultured in 24-well plates at 1 × 10⁶ cells/well with RPMI 1640 medium containing 20 ng/ml recombinant murine GM-CSF and 10 ng/ml recombinant murine IL-4 (Schering-Plough Research Institute, Kenilworth, NJ). Two days after initial culture, cells were replated at 5 × 10⁶ cells/well in 6-well plates with 50% of the fresh medium, and fresh cytokines were added. On day 5, DCs were harvested and purified on a 15% metrazimade column (Sigma) before immunization or phenotypic analysis.

FACS analysis
The phenotypic analysis of the DCs from C57BL/6 or IL-12−/− mice was performed on a FACSScan flow cytometer (BD Biosciences, Mountain View, CA). Cells were stained with the following Abs (obtained from BD Pharmingen, San Diego, CA): FITC-conjugated anti-mouse CD40 (clone HM40–3E3; PE-conjugated anti-mouse CD86 (clone GL1); PE-conjugated anti-IAb (clone KH74); FITC-conjugated anti-mouse CD80 (clone 16–10A1); and FITC-conjugated anti-mouse CD11c (clone HL-3).

Immunization
Day 5 DCs were infected with adenovirus (Ad) vectors (multiplicity of infection of 50) or incubated with peptides (5 μg/ml) in culture medium for 2 h. Cells then were washed three times with PBS before immunization. To measure CTL priming, C57BL/6 and IL-12−/− mice were injected in each hind footpad with 5 × 10⁶ Ag-loaded (Ad-infected or peptide-pulsed) IL-12+/+ or IL-12−/− DCs in 50 μl of PBS. Five days after immunization, lymphocytes were obtained from draining popliteal lymph nodes for cytotoxicity assay. In some experiments, MHC class II-deficient and CD154-deficient mice were immunized to determine the role of CD4+ T cells and CD40 interaction in the priming of CD8+ CTL. To measure antitumor immunity, C57BL/6 and IL-12−/− mice were immunized with 1 × 10⁶ Ag-loaded wild-type or IL-12−/− DCs in 200 μl of PBS injected s.c. in the right hind flank. Fourteen days later, immunized animals were challenged by s.c. injection with 5 × 10⁶ tumor cells (EL-4 or EG7). Tumor size in each group was monitored daily and measured twice a week.

Cytotoxicity assays
CTL lysis was assessed by a 4-h chromium release assay using 51Cr-labeled EL-4 cells in the presence or absence of 5 μg/ml OVAp or p33 peptide. To demonstrate effector phenotype, cytotoxic activity was blocked using hybridoma ascites fluid containing mAbs 53-6.72 (anti-CD8), GK1.5 (anti-CD4), or PK136 (anti-NK1.1). Percentage of specific 51Cr release was calculated as: (cpm experimental − cpm background/cpm maximum − cpm background) × 100%.

Intracellular cytokine staining
For staining of intracellular IFN-γ, popliteal lymphocytes were harvested from mice 5 days after immunization with the DC/peptide vaccines. Lymphocytes were incubated for 5 h in 24-well plate at a concentration of 4 × 10⁶ cells/well in 2 ml of complete medium with 2.5 μg/ml indicated peptides and 0.5 mg/ml of brefeldin A (Sigma). Cells were stained for intracellular IFN-γ using the Cytotox/Cyo-perm kit (BD Pharmingen) as specified by the manufacturer. Briefly, stimulated cells were washed twice, Fc receptors were blocked by incubation with rat anti-mouse CD16/CD32 (Fc Block; Pharmingen) for 20 min, and the cells were stained with FITC-labeled rat anti-mouse CD8 (clone 53-6.7; BD Pharmingen) for 30 min. After fixation and permeabilization for 20 min, the cells were stained with PE-labeled rat anti-mouse IFN-γ Ab (clone XMG1.2; BD Pharmingen) or an isotype control Ab (rat IgG1) for 30 min and then analyzed by flow cytometry as described above. Numbers in FACS plots refer to IFN-γ+ cells as percentage of the total CD3+ population.

ELISA cytokine assay
To determine the cytokine profile in T cell cultures from primed animals, splenocytes harvested from C57BL/6 or IL-12−/− mice 14 days after s.c. immunization and stimulated with irradiated EG7 cells at a 50:1 ratio in complete medium for 72 h. Supernatants then were harvested and analyzed for IFN-γ and IL-4 using ELISA kits from R&D Systems (Minneapolis, MN).

Results

Priming of CTL by Ag-loaded BMDC requires both CD4+ T cell help and CD40-ligation
To establish an in vivo model of CTL priming, we have used adoptive transfer of DC infected with an Ad expressing the SIINFEKL peptide of OVA (AdssOVA) to provide a source of endogenous Ag for class I presentation. As shown in Fig. 1A, footpad injection of DC infected with AdssOVA induced a strong CTL response in the popliteal lymph nodes without the need for restimulation in vitro. The cytolytic activity could be fully inhibited with anti-CD8, but not anti-CD4 or anti-NK mAb, confirming that the effectors were CD8+ T cells (Fig. 1B). Administration of wild-type DC infected with AdssOVA failed to prime CTL in class II or CD154-deficient mice (Fig. 1C) demonstrating that: 1) this model reflects a CD4+ T cell/CD40-dependent mechanism of CTL priming; and 2) infection with Ad is not sufficient to license DC for CTL activation.

Deficiency of IL-12 does not affect DC differentiation or T cell stimulatory function
To determine the requirement of IL-12 in the priming of CTL after DC licensing by CD4+ T cells, we have chosen to compare wild-type DC to IL-12-deficient DC in the priming model described in the previous paragraph. Because it has been reported that DCs may respond to IL-12 in an autocrine/paracrine manner (25), the phenotypes of wild-type and IL-12-deficient BMDC were analyzed using flow cytometry to verify that any difference in CTL priming is a consequence of the effects of IL-12 on T cells and not the DC. Both IL-12−/− and IL-12−/− DCs appeared phenotypically similar, displaying equivalent levels of MHC class II, CD40, CD80, and CD86 (Fig. 2). As expected, no IL-12 production was detected in the cultures of IL-12-deficient DCs, whereas 100–300 pg IL-12/10⁶ cells/24 h was measured in wild-type cultures (data not
These results demonstrate that endogenous IL-12 is not critical for the differentiation of BMDCs. Furthermore, both IL-12+/+ and IL-12−/− DCs stimulate equivalent levels of allogenic T cell proliferation in mixed lymphocyte reaction assays, indicating that deficiency of IL-12 in DCs does not impair T cell stimulatory capacity (data not shown).

**Both IL-12+/+ and IL-12−/− DCs genetically modified to express OVA peptide effectively prime cytolytic activity and IFN-γ production**

The production of IL-12 by activated DC has been suggested to represent a critical early physiologic event in the development of cellular immunity, especially with respect to optimal maturation of CTL and the generation of Th1 cells. To determine the role of IL-12 in the process of CTL priming, IL-12-deficient DCs were transduced with AdsS.OVA and adoptively transferred into IL-12-deficient mice. The results of CTL priming in the absence of IL-12 was compared with identically prepared DC from wild-type mice that were adoptively transferred into wild-type hosts (Fig. 1C). The absence of IL-12 had no obvious effect on the cytotoxic activity of freshly primed CTL (Fig. 1A). The absence of IL-12 had no obvious effect on the cytotoxic activity of OVA-pulsed EL-4 cells (Fig. 1A). Lymphocytes from immunized wild-type mice were tested for cytolytic activity against OVA-pulsed EL-4 cells (A) and EL-4 cells (B). Target cells were mixed with effectors at a ratio of 90:1. Abs against CD4, CD8, and NK1.1 were added to the wells 30 min before the addition of the targets. C. Lymphocytes from immunized wild-type (●), class II-deficient (■), and CD40L-deficient (▲) mice were tested for cytolytic activity against OVA-pulsed EL-4 cells.

**FIGURE 1.** CTL activation after adoptive transfer of Ag-loaded DC is dependent on CD4+ T cell help and CD40 ligation. Wild-type DCs were infected with AdsS.OVA and transferred into wild-type, MHC class II-deficient, and CD154-deficient mice by footpad injection. Five days later, draining lymph nodes were harvested and assayed for the presence of cytolytic activity against OVA-pulsed EL-4 cells. A, Lymphocytes from immunized wild-type mice were tested for cytolytic activity against OVA-pulsed EL-4 cells (●) and EL-4 cells (▲). B, Target cells were mixed with effectors at a ratio of 90:1. Abs against CD4, CD8, and NK1.1 were added to the wells 30 min before the addition of the targets. C, Lymphocytes from immunized wild-type (●), class II-deficient (■), and CD40L-deficient (▲) mice were tested for cytolytic activity against OVA-pulsed EL-4 cells.

**FIGURE 2.** Surface marker expression on wild-type and IL-12-deficient BMDC. DC were stained with Abs against CD11c, MHC class II (I-A^b^), CD40, CD86, and CD80 and analyzed by FACS. Representative histograms are shown in this figure.
the frequency of IFN-γ-producing CD8+ T cells (Fig. 3B). Moreover, in a restimulation assay, splenocytes from animals immunized with either IL-12+/+ and IL-12−/− DCs secreted large amounts of IFN-γ upon in vitro stimulation (Fig. 3C), whereas no IL-4 could be detected in both cases (data not shown), indicating that the responsive T cells were primarily of the T1 phenotype. Thus, IL-12 appears to be dispensable during the CTL priming by virus-infected DC.

Lack of IL-12 dependence during CTL priming is not attributable to virus infection and is not restricted to OVA

Because Ad infection has been shown to impact on DC function/maturation, it remains possible that Ad-infected DC may have an altered phenotype, despite remaining dependent on CD4+ T cells for licensing (26). Therefore, IL-12+/+ or IL-12−/− DCs were pulsed with OVAp and adoptively transferred into wild-type and IL-12-deficient mice, respectively. As with virally infected DC, the absence of IL-12 had no impact on either the magnitude of cytolytic activity in freshly primed CTL (Fig. 4A) or the frequency of IFN-γ-producing CD8+ T cells (Fig. 4B). These results indicate that the lack of IL-12 dependence during CTL priming by BMDC is not a result of the functional changes of the DC due to Ad infection.

Because IL-12-independent CTL priming may be a unique property of the OVA peptide or the Kb allele, DC were pulsed with a D9-binding peptide from the lymphocytic choriomeningitis virus glycoprotein, p33. Again, the induction of cytotoxic activity (Fig. 5A) and IFN-γ-producing CD8+ cells (Fig. 5B) was unaffected by the absence of IL-12. These results suggest that although the DC are fully dependent on CD4+ T cells and CD40 ligation for licensing, even after virus infection, the CD4+ T cell/DC interaction must invoke changes in the DC, other than IL-12 production, that are critical for CTL priming.

Both IL-12+/+ and IL-12−/− DCs loaded with OVA can produce effective antitumor immunity against OVA-expressing tumor cell challenge

To evaluate the efficacy of CTL generated in the absence of IL-12 in a challenge model, mice immunized by our DC infected with AdssOVA (DC/AdssOVA) were challenged with EG7 tumor cells, which express OVA as a surrogate tumor Ag. Tumors grew in all unimmunized animals or animals immunized with DC infected with an Ad expressing an irrelevant Ag, such as E. coli β-galactosidase (data not shown). Wild-type mice vaccinated with wild-type DC/AdssOVA and IL-12−/− mice immunized with IL-12−/− DC/AdssOVA were both completely protected from EG7 challenge. (Fig. 6A) Protection was Ag specific because all animals challenged with EL-4 developed tumors. Similarly, DC pulsed with OVAp were fully capable of protecting animals from tumor challenge in the absence of IL-12 (Fig. 6B). In all cases, tumor protection was CD8+ T cell dependent (data not shown). These data demonstrate that CTL generated in the absence of IL-12 are not only phenotypically equivalent to CTL from wild-type animals, but they also are fully capable of protecting animals from challenge.

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**FIGURE 4.** IL-12-independent activation of CTL and IFN-γ production is not dependent on virus infection. Wild-type and IL-12-deficient DCs were pulsed with OVAp and adoptively transferred into mice by footpad injection. Five days later, draining lymph nodes were harvested and assayed for the presence of cytolytic activity against OVAp-pulsed EL-4 cells. A, Lymphocytes from immunized wild-type mice were tested for cytolytic activity against OVAp-pulsed EL-4 cells (filled symbols) and EL-4 cells (open symbols). B, Lymphocytes were stimulated with either OVAp (top) or p33 (bottom) and assayed for IFN-γ production by using the ICS method.

**FIGURE 5.** IL-12-independent activation of CTL and IFN-γ production is not limited to Kb-binding peptides. Wild-type and IL-12-deficient DCs were pulsed with p33 and adoptively transferred into mice by footpad injection. Five days later, draining lymph nodes were harvested and assayed for the presence of cytolytic activity against p33-pulsed EL-4 cells. A, Lymphocytes from immunized wild-type mice were tested for cytolytic activity against p33-pulsed EL-4 cells (filled symbols) and EL-4 cells (open symbols). B, Lymphocytes were stimulated with either OVAp (top) or p33 (bottom) and assayed for IFN-γ production using the ICS method.
Discussion

Cellular immunity, in particular the CTL, plays a central role in host defense. Thus, understanding the mechanisms of cellular immunity is expected to have a major impact on human health and vaccine development. Previous studies have implicated IL-12 as a central component of the cellular immune response, pointing to a critical role of this cytokine in bridging innate and specific immune responses by activating NK cells, promoting CTL maturation, and biasing CD4+ T cells toward Th1 differentiation (27–29). Because DCs are a principal source of IL-12 after Ag exposure, IL-12 has been suggested to be a key determinant in the outcome of immune response during Ag presentation by the DC (30). However, direct evidence for the requirement of DC-derived IL-12 during the development of cellular immunity is lacking. Indeed, our recent studies of melanoma vaccination using genetically modified DCs have demonstrated that DC-derived IL-12 was not required for the generation of protective immunity against melanoma tumor challenge. This result may be interpreted as an exception to the rule, as tumor rejection was mediated by autoreactive CD4+ T cells without involvement of CD40 signaling and CD8+ T cells (19). In this study, we have extended our previous studies to evaluate the role of DC-derived IL-12 in CTL priming and activation in vivo.

We have based our hypothesis for a central link between IL-12 production and CTL activation on the following data: 1) CD40 ligation on DC is critical for the induction of CTL, and CD40 ligation induces high-level IL-12 production in DC (11, 31–33); 2) IL-12 plays an important role in the development of type 1 T cell responses, which are characterized by strong IFN-γ secretion and CTL activity (12–14); 3) IL-12 treatment can enhance the development of CTL in vitro/in vivo (34–37); and 4) treatment of DC with IL-12 improves their ability to activate CTL (38).

By using adoptive transfer of BMDC, we have used an in vivo CTL priming model to study the molecular requirements of CTL activation. In this model, the DC require CD4+ T cells and CD154 to successfully prime CTL, indicative of the CD4+ T cell-dependent mechanism of DC licensing. Using p40-deficient mice, the requirement of IL-12 could be evaluated in a background where IL-12 is fully absent. DCs were loaded with Ag either by virus infection or peptide loading, and we evaluated both Kb- and Db-restricted CTL activation. Interestingly, the DC pulsed with the class I binding peptides OVAp and p33 still required CD4+ T cell help, even though these cells were not pulsed with class II epitopes. Under these conditions, the FBS additives used during the DC culture likely provide a source of MHC class II peptides to engage CD4+ T cell help. In all cases, the absence of IL-12 had no qualitative or quantitative impact on the development of cytotoxic, IFN-γ-secreting CD8+ CTL. IL-12 independence does not seem to be related to the maturation state of the DCs because we observed equal enhancement of the CTL response in this model using LPS or CD154 trimer to mature either IL-12+/+ or IL-12−/− DCs (Y. Wan, unpublished observations). Our results support the observation that the CD4+ T cell and CD40 ligation pathways are critically involved in the induction of CD8+ CTL, but IL-12 production after CD40 ligation does not appear to be necessary in the early phase of CD4 activation and subsequent CTL priming. Thus, either IL-12 is not required for the differentiation and maturation of T1 cells or secondary pathways exist that can compensate for the absence of IL-12.

In support of a compensatory pathway, a recent report suggests that, in fact, IL-15, not IL-12, is responsible for enhanced CTL activation by CD154 trimer-treated DC using an in vitro system (39). By contrast, when the CD154 trimer was used to enhance DNA vaccination, the effect was IL-12 dependent (40). Likewise, Guffman and Karre found that p33-pulsed DC were unable to elicit CTL after adoptive transfer into IL-12-deficient animals (41). Thus, we had expected to observe a weakened CTL response in the IL-12-deficient mice. Because we observed no loss in CTL function, we interpret these results to indicate that the effects of IL-12 in the previous models may be secondary to the process of CTL priming. The study by Guffman and Karre (41) measured splenic CTL activity after restimulation in vitro, whereas our investigation focused on freshly primed CTL response within the draining lymph node. We currently are investigating the impact of IL-12 deficiency on CTL persistence and memory. One possible explanation for the discrepancy between the reports demonstrating a critical role for IL-12 and those indicating a secondary role may stem from the type of model used. In our model and the in vitro model, the early stages of the CTL response are being investigated, whereas the other models measured recall responses. Thus, perhaps IL-12 exerts its influence in the postpriming phase of the immune response.

The literature has mixed reports regarding the importance of IL-12 in the development of CTL. Certainly, T1 cells can be generated in vitro in the presence of IL-2 alone, suggesting that IFN-γ secretion may be a default pathway during CTL priming. Addition of IL-12 during in vitro differentiation greatly increases IFN-γ production and cytolytic activity of T1 cells (13, 14). Likewise, the addition of IL-12 can dramatically improve the development of antiviral CTL in vitro using influenza-infected human DCs (37). In all of these cases, IL-12 is added exogenously to the cultures, and therefore these observations are not truly reflective of the impact of DC-derived IL-12 on CTL function. Interestingly, Bianchi et al.
have recently reported that adoptive transfer of DCs pulsed with a poorly immunogenic, class I-binding tumor peptide (P815AB) was unable to elicit a T cell response unless the DC were treated with a CD40 agonist before adoptive transfer. Remarkably, they demonstrate that neutralization of IL-12 during CD40 cross-linking in vitro abrogates the adjuvant effect, and the addition of exogenous IL-12 can overcome the requirement for a CD40 agonist, providing direct evidence that the adjuvant effect of CD40 ligation can be due to increased autocrine IL-12 (25). Similarly, Zitvogel et al. (42) observed that neutralization of IL-12 at the time of immunization with tumor peptide-pulsed DC could abrogate the protective response. However, a fundamental difference between this model and other DC immunization models outlined above is the nature of the Ag. To address this possibility, we currently are investigating the influence of endogenous or exogenous IL-12 on the immune outcome after adoptive transfer of DCs loaded with a series of poorly immunogenic, MHC class I-restricted peptides from melanoma compared with OVAp and p33.

There is increasing interest in developing vaccines using DCs as a physiological adjuvant to enhance an Ag-specific immune response, especially a cell-mediated protective immunity. In this scenario, treatment of DC with IL-12 before inoculation, provision of exogenous IL-12 after immunization, and modification of the DC to express elevated levels of IL-12 have all been shown to enhance immune induction, particularly with weak Ags (16–18, 25). Although these results do not support a role for IL-12 in the process of CTL priming, they do demonstrate that the presence of IL-12 at the time of immune induction can strongly influence the outcome of the immune response and support the inclusion of IL-12, or IL-12-inducing agents, in vaccine formulations. However, from a biological point of view, the cellular target of the IL-12 remains to be determined, although it is tempting to speculate that IL-12 exerts its influence directly on the DC.

The results of these experiments provoke reconsideration of the relationship between IL-12 production and DC phenotype. Accumulating data have indicated that DCs are not only critical for the initiation of T cell immunity, but these cells also determine the course of the subsequent immune response (i.e., tolerance vs immunity; Ref. 30). However, the mechanisms by which DC can influence the final outcome of a given immune response remain to be understood. Distinct DC subsets have been identified, leading to the hypothesis that the ability of DC to direct immunity is related to their hemopoietic lineage. Although the correlation between DC subset and the type of immune response remains controversial, the current belief is that CD8α+ DC, but not CD8α- DC, are responsible for the induction of cellular immunity in mice, based on the observations that IL-12 production is restricted to the CD8α+ subset (43–46). Our BMDCs are largely CD8α+ and tolerogenic DCs requires further studies.


