Cyclosporine Preconditions Dendritic Cells During Differentiation and Reduces IL-2 And IL-12 Production Following Activation: A Potential Tolerogenic Effect

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ABSTRACT

The mode of action of cyclosporine (CsA) has been ascribed to its capacity to inhibit IL-2 and IFNγ production by T cells, two cytokines implicated in allograft rejection. Recently, it has been reported that upon activation, dendritic cells (DCs) exhibit transient production of IL-2, a property that appears to be related to their capacity to initiate immune responses. On the other hand, DCs can generate signals determining Th_1/Th_2 polarizing effects, an effect that can drastically influence the outcome of organ transplant. The purpose of the present study was to investigate the effect of CsA on cytokine production by immature and mature DCs. DC precursors from mouse bone marrow were induced to differentiate by incubation with GM-CSF for 5 days followed by activation with LPS for 4 hours. CsA was added at different times during this process. Our results show that when CsA is added during the differentiation period following activation with LPS, IL-2 and IL-12 secretion are significantly reduced without affecting the evolution of the DC. Conversely, CsA had no effect when added during the LPS activation period. These results show that CsA affects DCs before they receive the final activation stimulus, preconditioning them to antigen stimulation. This preconditioning of DCs by calcineurin-inhibiting drugs conceptually integrates the mode of action of CsA with the tolerogenic and T-cell polarization function ascribed to DCs. These results may be especially meaningful for the future design of immunosuppressive protocols.

STUDIES ON the role of dendritic cells (DCs) in organ transplantation represent a valuable tool to understand graft rejection versus the acquisition of peripheral tolerance. In the context of organ transplantation, donor DCs are responsible for direct antigen presentation, while recipient DCs participate indirectly in peptide presentation to T cells following intracellular MHC antigen processing.[1] As a consequence of these events, transplanted organs may suffer either acute or chronic rejection possibly according to the source of the antigen-presenting cell. Induction of T-cell proliferation by DCs has been associated with DC maturation including expression of costimulatory and accessory molecules, such as CD40, CD80, and CD86, as well as production of proinflammatory cytokines such as TNF and IL-12. Alternatively, immature DCs, which are deficient in costimulatory molecule expression, are known to suppress alloantigen-induced T-cell activation.[1] In this regard, immunosuppressive agents are presently being actively investigated for their potential to act upon DCs to induce T-cell tolerance.

DCs are involved in innate as well as adaptive immune responses. Both functions rely on a complex maturation process following antigen stimulation. Indeed, recognition of an infectious agent (or donor MHC antigen in the case of transplant patients) triggers profound changes in immature, tissue resident DCs.[2] Functional genomic studies have revealed changes in the expression level of not less than

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1330 genes after the encounter of DCs with antigen.[3] These changes include the induction of DC migration to secondary lymphoid organs as well as cytokine and chemokine secretion and costimulatory molecule expression, all of which are believed to be crucial for T-cell activation, Th_1/Th_2 polarization or downregulation of the immune response. Although the activating function of DCs has been well documented, recently a tolerizing function of these cells has also been highlighted, implicating DCs in allograft acceptance.[2] Although the mechanisms involved in the downregulation of the immune response by DCs are not presently known, this function seems to be related to their maturation state.

Recently, Granucci et al[4] demonstrated that the transition of DCs from a resting to an activated state involves early and transient production of IL-2 mRNA followed by two waves of IL-2 protein secretion, which occur coincident with the upregulation of MHC-peptide expression on the cell surface. It was proposed that this phenomenon has a critical role to trigger naïve T-cell maturation.[4] Additionally, DCs are known to regulate Th_1/Th_2 polarization through their secretion of IL-12 versus other cytokines. This property gives DCs a special influence on the outcome of organ transplantation.[1] In addition, it has recently been shown that exposure of replicating DC progenitors to CsA reduces costimulatory molecule expression[5] and allostimulatory capacity,[6] effects which may an induce peripheral tolerance and allograft acceptance.

These observations lead us to hypothesize that calcineurin-inhibiting drugs, might not also inhibit IL-2 production by T cells but also IL-2 secretion by DCs.[7] In addition, given the importance of IL-12 secretion by DCs for T-cell polarization, we also studied the effect of CsA on both IL-2 and IL-12 production by immature and mature DCs.

Our results show that, in spite of the fact that the presence of CsA does not affect DC differentiation significantly, it does inhibit IL-2 and IL-12 production by these cells when added during the differentiation period. This preconditioning of DCs by anticalcineurinic drugs may influence the way we consider anticalcineurinic drug administration to organ transplant recipients.

METHODS

Bone marrow-derived DCs were generated as described previously with minor modifications.[8] Briefly, bone marrow cells were removed from the femurs and tibias of C57BL/6 mice and depleted of erythrocytes by hypotonic lysis. The cells (1×10^6) were cultured for 5 days in 1 mL of RPMI 1640, supplemented with 10% v/v heat-inactivated FCS, recombinant murine GM-CSF (10 ng/mL) and the presence or absence of CsA (750 ng/mL) at 37 °C in 5% CO_2. On days 2 and 4, 75% of the culture supernate was replaced with fresh complete medium ± CsA. On day 5, cells were harvested, treated with Fc block (anti-CD16-CD32) in 10% v/v normal goat serum for 15 minutes at 4 °C, labeled with bead-conjugated anti-CD11c mAb (Miltenyi Biotec, Auburn, Calif, USA) followed by positive selection through magnetic columns (LS columns; Miltenyi Biotec) according to the manufacturer's instructions. DCs

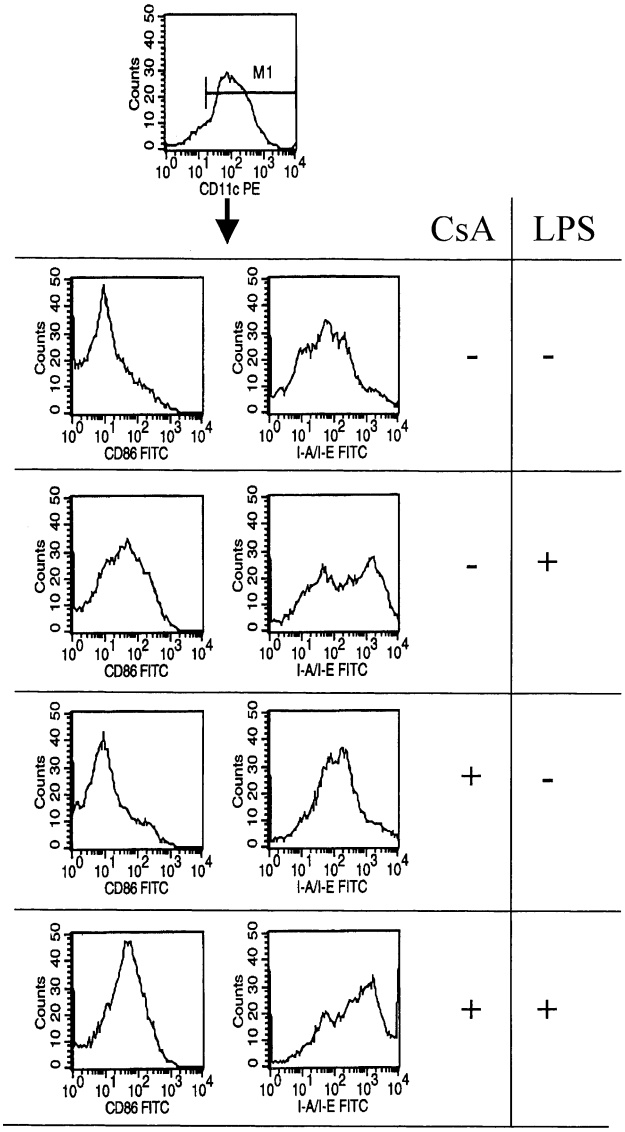

Fig 1. Cytofluorometric analysis of day 5 GM-CSF-derived bone marrow DCs cultured in the absence or presence of CsA (750 ng/mL). Cells were harvested and submitted to immunomagnetic isolation using beads coated with anti-CD11c mAb. Positively purified cells were further incubated at 37 °C with or without LPS (100 ng/mL) and CsA. After 4 hours, cells were harvested, co-stained with PE-labeled anti-CD11c and either FITC-labeled anti-CD86 or anti-I-A/E^b mAbs, and analyzed on a FACScan.

were consistently obtained with a purity of 91% to 95% (Fig 1, upper panel). Activation of these in vitro-generated DCs was achieved by incubating the $CD11c^+$ cells with 100 ng/mL of LPS in complete medium for 4 hours. After this incubation period, supernates were harvested and tested for cytokine production by ELISA while the cell fraction was removed for FACS analysis. For cytometric analysis cells were suspended in RPMI + 2% FCS,

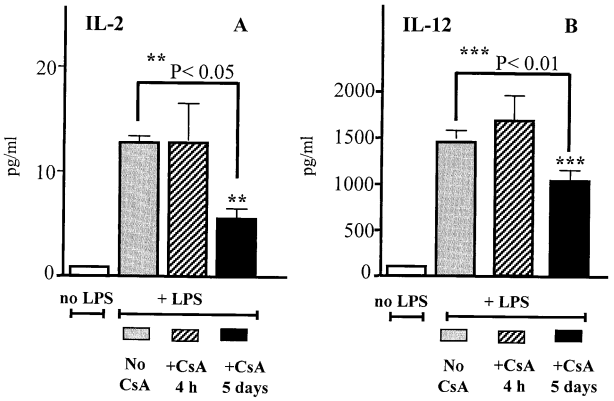

Fig 2. Effect of CsA on IL-2 and IL-12 expression by bone marrow-derived dendritic cells. DCs were generated from mouse bone marrow cells incubated with GM-CSF for 5 days. Supernatants from unstimulated DCs (no LPS, white bars) or from LPS stimulated DCs were collected for IL-2 (A) and IL-12 (B) assays. To examine the effect of drug treatment, DCs were left untreated (no CsA, gray bars) or incubated with CsA during final LPS stimulation period (+CsA 4 hours, hatched bars) or during the whole GM-CSF treatment period (+CsA 5 days, black bars). The results show the mean of duplicate experiments and are representative of 4 experiments. Two or three asterisks indicate $P < .05$ or $P < .001$, respectively, by one-way ANOVA. No statistical difference was found on cytokine secretion between GM-CSF generated DCs that were stimulated with LPS in the presence or absence of CsA.

incubated with the relevant fluorochrome-labeled mAb for 30 minutes at 4 °C, washed, and analyzed on a FACScan.

RESULTS

After 5 days of treatment with GM-CSF, most bone marrow cells consistently showed a differentiated immature DC phenotype ($CD11c^+$, $CD86^{low}$, $MHCII^{low}$; Fig 1). No IL-2 or IL-12 secretion was detected in the cultures supernates at any time during the differentiation period (data not shown). Treatment of the purified DC population with LPS for 4 hours increased the number of mature DCs ($CD11c^+$, $CD86^{low}$, MHC II^{high}; Fig 1) as well as the secretion of IL-2 (10 to 20 pg/mL) and IL-12 (1000 to 2000 pg/mL) (Fig 2). Although inclusion of CsA during the differentiation period did not significantly modify the maturation phenotype of the DCs (Fig 1), it reduced LPS generated IL-2 by approximately 57% and IL-12 by approximately 35% (Fig 2). On the other hand, adding CsA during the final LPS activation step did not significantly affect IL-2 or IL-12 secretion (Fig 2).

DISCUSSION

Since IL-2 and IL-12 secretion occurred only after activation with LPS, CsA seemed to exert its action during the differentiation step. CsA acts by preconditioning DCs before these cells receive the final activating stimulus. In addition, they suggest that inhibition of calcineurin by CsA does not fully account for the effect of CsA on IL-2 and IL-12 production by DCs. In this regard, recent reports have assigned an important role to protein kinases in IL-12 secretion by DCs,[9] a finding that may be especially relevant to the results reported here. Further studies should give insight into the mechanisms of action of CsA on DCs.

Although the mechanism involved in CsA action on DCs remains to be elucidated, our results support the hypothesis that CsA inhibits the secretion of important cytokines in DC, especially IL-2. This might affect the capacity of DCs to activate naïve T cells or change an activating into a tolerogenic signal,[6] which may lead to a T-cell suppression leading to allograft acceptance.

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