Interleukin-4 Selectively Inhibits Interleukin-2 Secretion by Lipopolysaccharide-Activated Dendritic Cells

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Abstract

Dendritic cells (DCs) generated *in vitro* from bone marrow precursors using granulocyte-macrophage colony-stimulating factor (GM-CSF) secrete interleukin-2 (IL-2) upon activation, an event probably associated to the initiation of adaptive immune responses. Additionally, they produce IL-12, a cytokine related to T-cell polarization. To analyse the effect of IL-4 on DC differentiation and function, we assessed the capacity of murine bone marrow dendritic cells (BMDCs) differentiated with GM-CSF in the presence or absence of IL-4 to produce IL-2 and IL-12 upon lipopolysaccharide (LPS) activation. We found that although IL-4 enhanced DC IL-12p70 production, it strongly impaired IL-2 secretion by BMDCs. This inhibition, which depends on the presence of IL-4 during LPS activation, is DC specific, as IL-4 did not affect IL-2 secretion by T cells. Interestingly, inhibition of DC IL-2 production did not prevent DC priming of T lymphocytes. These results illustrate a new putative role for IL-4 on the regulation of the immune response and should help clarify the controversial reports on the effect of IL-4 on DCs.

Introduction

Dendritic cells (DCs) are antigen-presenting cells endowed with the unique capacity to initiate primary immune responses. In most peripheral tissues, DCs are present as immature cells, which are unable to stimulate T cells. However, immature DCs are extremely well equipped to capture antigens, which, if encountered in an inflammatory context, will induce their maturation and mobilization to secondary lymphoid organs [1, 2]. DC maturation is a complex process which leads to reduced endocytic and/or phagocytic capability, enhanced expression of costimulatory molecules (such as CD80, CD86 and CD40), increased antigen processing and presentation, release of cytokines and chemokines [1–4] and switch in chemokine receptor expression [5]. The ability of DCs to regulate immune responses strongly depends on their maturation state: whereas steady-state DCs are thought to maintain tolerance, mature DCs are responsible of initiating the adaptive immune response [6–9].

Cytokines are key mediators of both innate and adaptive immune responses. In particular, they can define the type of immune response developed in response to a given antigen by driving $CD4^+$ T helper cells towards T_H1 or T_H2 phenotypes. In this context, interleukin-12 (IL-12) and IL-4 appear as the principal players in this process of T-cell polarization, with IL-12 inducing T_H1 type responses and IL-4 a T_H2 type. Exactly how and when IL-4 and IL-12 exert their influence to polarize T-cell responses remains an issue of controversy [10, 11]. Recent discoveries support the hypothesis that skewing towards either type of response can be triggered by DCs. Indeed, a particular subset of DCs can induce either T_H1 or T_H2 responses depending on the dose and type of antigen and on the environmental stimuli they encounter during maturation [1, 4, 12]. In addition, there is accumulating evidence for a positive role of IL-4 in T_H1 differentiation, because it can effectively enhance DC production of IL-12p70, while reducing secretion of its antagonistic

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homodimer IL-12p40 [13–15]. Together, these data suggest that, through their ability to release and/or respond to cytokines, DCs play a central role in the process of T-cell polarization.

In addition to the known pattern of cytokines secreted by DCs, which include IL-12, it was recently observed that DCs, but not macrophages, transiently produce IL-2 at early time points after induction of maturation [16, 17]. The kinetics of IL-2 production by DCs are compatible with the first appearance of major histocompatibility complex (MHC) class II peptide complexes at their cell surface. The importance of DC-derived IL-2 in inducing T-cell activation has been confirmed by the observation that activated DCs from IL-$2^{-/-}$ mice are severely impaired in their ability to prime alloreactive T cells [16, 18]. In addition, there is evidence showing functional IL-2 signalling in DCs [19], suggesting that this cytokine may also affect DCs in an autocrine fashion.

With these facts in mind, we asked whether the cytokines involved in T-cell polarization might also regulate IL-2 secretion by DCs. To address this question, we investigated the effect of IL-4 on the ability of DCs to produce IL-2 upon maturation. Moreover, IL-4, which is known to induce T_H2-cell polarization, is often used to differentiate DCs from bone marrow progenitors *ex vivo* [6, 20]. Interestingly, we found that, even though IL-4 does not prevent DCs from priming T cells, it severely and selectively impairs the capacity of DCs to secrete IL-2. These results indicate that secretion of IL-2 by DCs is under the control of environmental cytokines and raises the question as to the contribution of DCs to T-cell activation when in the presence of IL-4.

Materials and methods

Mice and reagents. C57BL/6, B10.BR and the T-cell receptor (TCR) transgenic AND mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). F1 (B10.BRxAND) mice were obtained by in-house breeding. Recombinant IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) mouse cytokines were obtained from BD PharMingen (San Diego, CA, USA), and their specific activities were $0.25–1 \times 10^8$ U/mg and $3–9 \times 10^8$ U/ml, respectively. The following monoclonal antibodies from PharMingen were used: fluorescein isothiocyanate (FITC)-conjugated anti-I-A/I-E (2G9), FITC-conjugated anti-CD86 (GL1), phycoerythrin (PE)-conjugated anti-CD40 (3/23), PE-conjugated anti-CD11c (HL3), PE-conjugated anti-CD25 (3C7), PE-conjugated anti-CD44 (IM7), PE-conjugated anti-CD69 (H1.2F3), PE-conjugated anti-CD62L (MEL-14) and FITC-conjugated anti-Vα11.1 (RR8-1).

Generation and purification of mouse bone marrow-derived DCs. Bone marrow-derived DCs were generated as described previously [21]. Briefly, bone marrow cells were removed from femurs and tibias of 2-month-old mice. After depletion of erythrocytes by hypotonic lysis, cells were cultured at 1×10^6/ml in RPMI 1640, supplemented with 10% heat-inactivated fetal calf serum (FCS) and GM-CSF (10 ng/ml) with or without IL-4 (1 ng/ml). On day 2 and 4, 75% of the culture supernatant was carefully removed and replaced with fresh medium. To obtain highly purified DCs, cells were harvested on day 5 of culture, incubated with anti-CD16-CD32 (mouse Fc Block, BD PharMingen) and normal goat serum for 15 min at 4 °C, labelled with bead-conjugated anti-CD11c monoclonal antibody (Miltenyi Biotec, Auburn, CA) and submitted to positive selection through magnetic columns (LS columns, Miltenyi Biotec) according to the manufacturer's instructions. DC purity of 91–95% was consistently achieved.

Flow cytometry analyses. Cells were resuspended in RPMI plus 2% FCS, double-stained with the relevant monoclonal antibodies for 30 min at 4 °C, washed and analysed on a FACScan (BD Biosciences, Mountain View, CA, USA) using the Cellquest program.

Cytokine production. On day 5, purified CD11c$^+$ DCs were replated at 4×10^6 cells/ml in 250 μl of RPMI 1640 supplemented with 10% FCS and GM-CSF with or without IL-4. Where indicated, LPS was added at 100 ng/ml (Sigma, St. Louis, MO, USA). After different incubation periods, supernatants were harvested and tested for mouse IL-12p70 and IL-2 using enzyme-linked immunosorbent assay (ELISA) kits (BD PharMingen) according to the manufacturer's instructions. The detection limits for IL-12p70 and IL-2 were 32 and 1.6 pg/ml, respectively.

Bone marrow dendritic cell thymocyte cocultures. Thymocytes were obtained from 1–2-month-old and mice and depleted of IA/IE-positive cells. These cells were then cultured with F1 (B10.BRxAND) bone marrow dendritic cells (BMDCs) (2 : 1 ratio), previously differentiated with or without IL-4, in the presence of LPS (100 ng/ml) and 5 μM specific C-terminal peptide from pigeon cytochrome c (PCC_{87-104}) (KKAERADLIAYLKQATAK). After 48 h of coculture, thymocytes were double-stained for Vα11$^+$ and various activation markers and analysed by flow cytometry. Supernatants harvested after 24, 48 and 120 h of activation were tested for IL-2 production by ELISA as described above.

mRNA purification and IL-2 RT-PCR. RNA extraction was performed from purified BMDCs by using TRIzol reagent as suggested by the manufacturer (Gibco-BRL). cDNA was synthesized in 25 μl reaction containing 5 μg of total RNA, 500 ng of oligo (dT) (Gibco-BRL), 2 U of MMLV-RT (Promega, Madison, WI, USA), 15 U of RNAsin (Promega), 0.5 mM dNTP and MMLV-RT buffer (Promega) for 1 h at 42 °C. The cDNA was amplified in a 25 μl reaction volume containing 200 ng of the sense and antisense primers, 200 mM dNTP, 1.5 mM $MgCl_2$, 5 U of Taq polymerase (Gibco-BRL) and AmpliTAq buffer

(Gibco-BRL) using the following conditions: one cycle of 5 min at 94 °C; 29 cycles of 1 min at 94 °C, 1 min at 60 °C, 2 min at 72 °C and one cycle of 10 min at 72 °C. Samples were analysed on 1.4% agarose gels containing ethidium bromide. Primers used were as follow: mIL-2 sense primer, 5′-ACTTGTGCTCCTTGTCAACAGC-3′; mIL-2 antisense primer, 5′-CCATCTCCTCAGAAAGTCCACC-3′; β-actin sense primer, 5′-AAATCGTGCGTGACAT TAAGG-3′ and β-actin antisense primer, 5′-CCGATCCA CACGGAGTACTT-3′. Control experiments were performed with RNA extracted from splenocytes activated for 16 h with 1 μg/ml of ionomycin and 25 ng/ml of phorbol 12-myristate 13-acetate.

Results and discussion

IL-2 secretion by DCs is severely impaired by the presence of IL-4 during activation.

To assess the effect of IL-4 on IL-2 production by DCs, we cultured bone marrow cells with GM-CSF alone or in combination with IL-4. Although only slight differences in CD40 and CD86 surface expression were observed between purified BMDCs generated with or without IL-4, two populations of cells were distinguished by their levels of MHC class II surface expression (data not shown). Interestingly, inclusion of IL-4 in the cultures during differentiation increased the proportion of MHC-II^{high} DCs (with an average of 29.5% cells CD11c^{+}/MHC-II^{high} when differentiated with GM-CSF alone and 42% when differentiated with GM-CSF plus IL-4), suggesting, as it has been reported by others [22], that these cultures contain a mixture of DCs at different stages of maturation. As expected, enhanced maturation was achieved by treating these cells with LPS during 4 h (data not shown).

We next analysed the ability of these DCs to produce IL-2 upon LPS activation. Similar to published kinetics for *Escherichia coli*-activated DCs [16], GM-CSF-differentiated DCs showed a peak of IL-2 secretion 3 h after LPS activation (Fig. 1A). Although the amount of IL-2 secreted by DCs (30 pg/ml) apparently appears low when compared to those obtained by Granucci *et al.* [16, 17], it should be considered that, in those experiments, DCs were activated with live bacteria, one of the most potent stimulus for DC activation. As expected, no secretion of IL-2 was observed when using the CD11c-negative fraction of cells obtained after MACS purification or with LPS-treated DCs derived from C3H/HeJ mice deficient in the expression of Toll-like receptor 4 (TLR4). Moreover, no IL-2 was detected when mature DCs isolated from spleens of Flt3L-treated mice were activated with LPS for 4 h (data not shown), suggesting that fully matured DCs present in secondary lymphoid organs may have already stopped synthesizing IL-2.

Strikingly, we found that IL-2 production is severely impaired in DCs that were differentiated and activated in the presence of IL-4 ($P < 0.001$) (Fig. 1B). This inhibition of IL-2 secretion was sustained for up to 24 h of activation. In sharp contrast, IL-4 strongly enhanced the secretion of

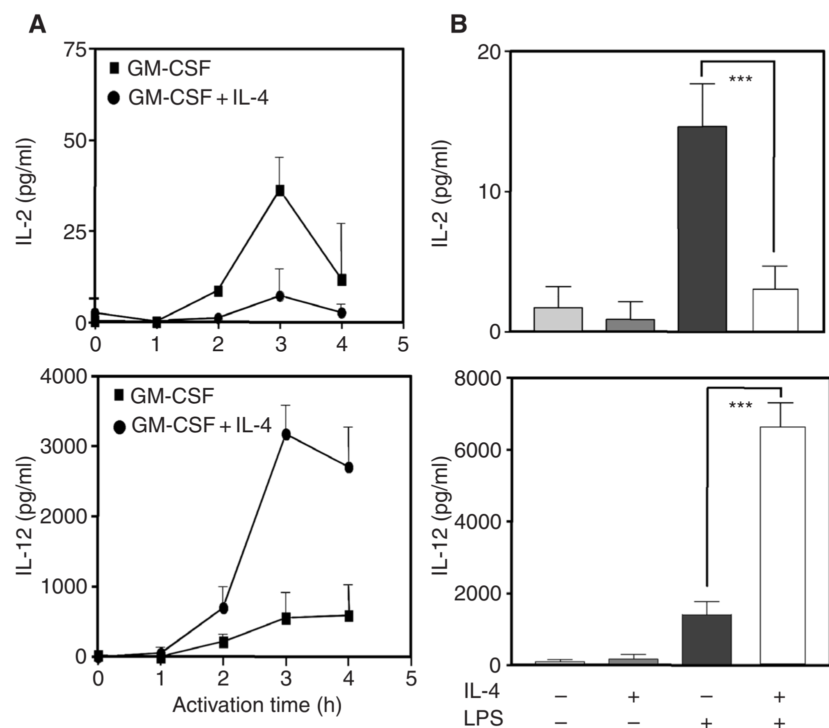

Figure 1 IL-4 inhibits the secretion of IL-2 by BMDCs. (A) BMDCs were generated with GM-CSF of GM-CSF plus IL-4, purified by immunomagnetic bead sorting and activated with LPS. Supernatants were harvested at the indicated time points and IL-2 and IL-12 production assessed using ELISA kits. Values represent the amount of cytokine secreted at each time point. (B) Total IL-2 and IL-12 secreted by DCs at 4 h are compared under each condition. Results are expressed as the mean±SD of three independent experiments. Three asterisks indicate $P < 0.001$ by one-way ANOVA.

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IL-12p70 ($P < 0.001$) (Fig. 1B), as previously reported by others [13, 14]. These data indicate that impairment of IL-2 secretion by IL-4 does not result from a broad inhibitory effect of this cytokine but is rather specific. Furthermore, the fact that IL-4 enhances the production of IL-12 by LPS-activated DCs discards the possibility that IL-4 acts by inhibiting signal transduction through the LPS receptor TLR4.

IL-4 acts at the post-transcriptional level

Next, we asked whether IL-4 acted at the transcriptional or posttranscriptional levels. To address this question, we first analysed the IL-2-secreting capacity of DCs differentiated with GM-CSF alone but activated with LPS in the presence of IL-4. Under such conditions, we found that IL-2 secretion at 4 h was considerably lower than IL-2 secretion in the absence of IL-4 (Fig. 2A). Furthermore, DCs differentiated with GM-CSF plus IL-4 but deprived of this cytokine during the LPS activation period secreted enhanced amounts of IL-2 compared to cells that were differentiated and matured in the presence of IL-4 (Fig. 2B). The opposite was true for IL-12 production, which showed a significant increase when IL-4 was included during DC activation compared to secretion when cells were differentiated and matured in the absence of IL-4 (Fig. 2C). DCs differentiated with GM-CSF plus IL-4 and activated in the absence of this cytokine secreted lower levels of IL-12 compared to DCs that were differentiated and activated in the presence of this cytokine (Fig. 2D). These results indicate that the presence of IL-4 only during the final activation step is sufficient to block IL-2 secretion, suggesting that the effect of IL-4 is post-transcriptional. This is supported by RT-PCR assays that show that the IL-2 mRNA is actually present in DCs differentiated with GM-CSF plus IL-4 and that this mRNA increased after LPS activation, in spite of the marked decrease in IL-2 secretion (Fig. 2E).

An additional issue brought up by the present work concerns the utilization of IL-4 in the different protocols

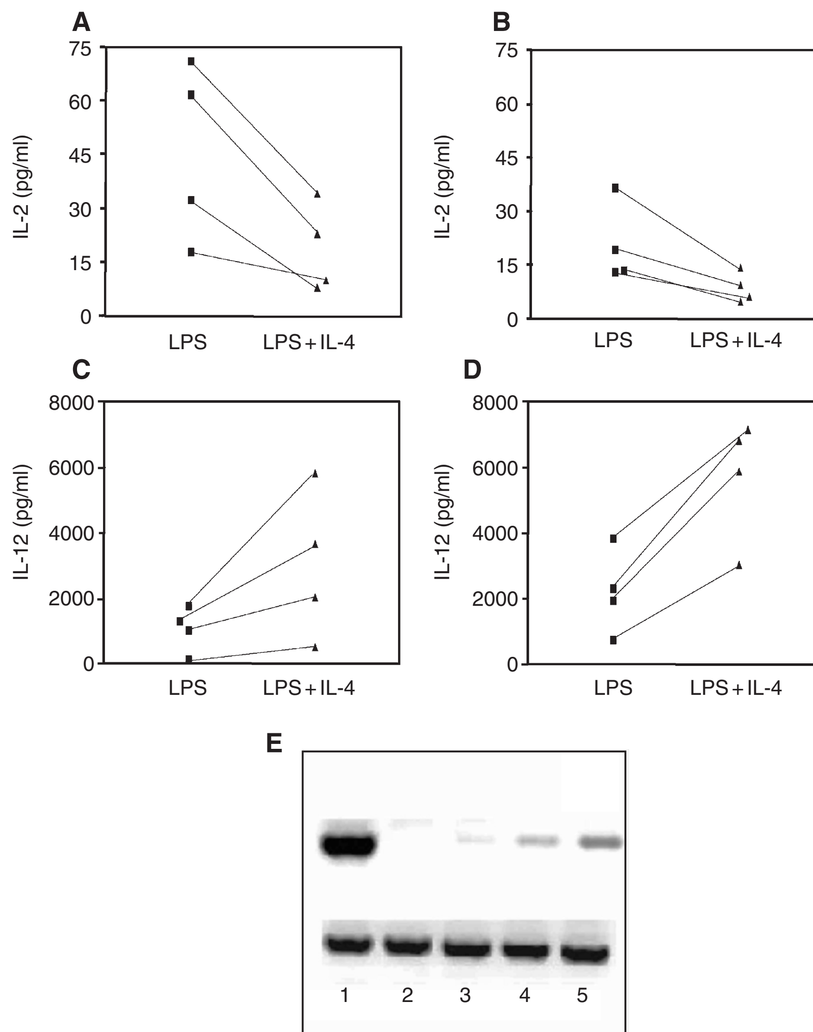

Figure 2 IL-4 effect on cytokine secretion by DCs depends on the presence of this cytokine during LPS-induced activation. BMDCs were differentiated with GM-CSF (A and C) or GM-CSF plus IL-4 (B and D), purified by immunomagnetic bead sorting and activated with LPS in the absence and presence of IL-4. After 4 hours, supernatants were harvested for IL-2 and IL-12 measurements. This figure shows the results of four independent experiments. (E) RT-PCR analysis of IL-2 gene expression in BMDCs differentiated in the presence or absence of IL-4. Lane 1: activated mouse splenocytes; lane 2: DCs differentiated in the presence of GM-CSF; lane 3: DCs differentiated in the presence of GM-CSF plus IL-4; lane 5: DCs differentiated in the presence of GM-CSF and activated with LPS.

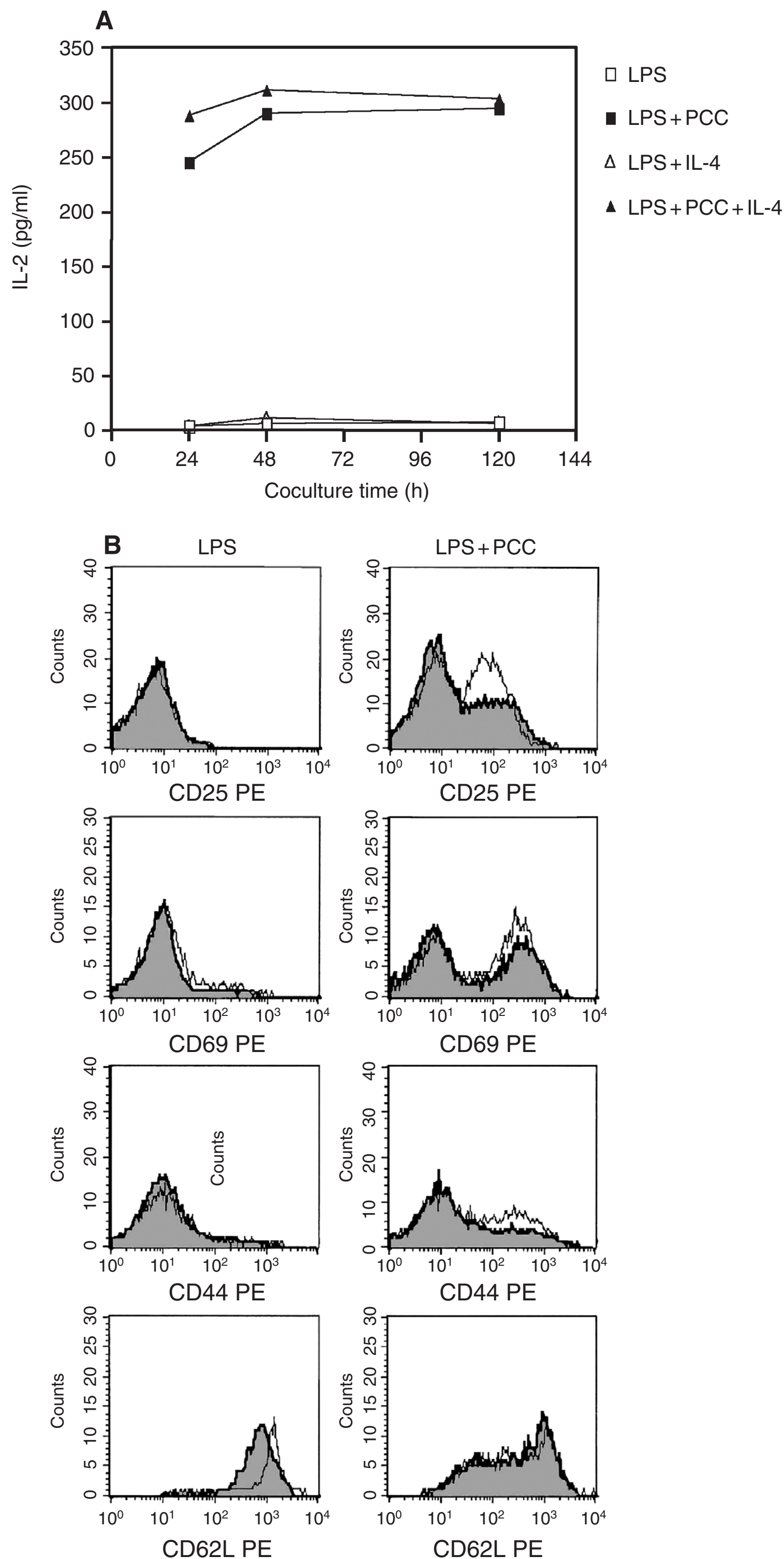

Figure 3 Effect of IL-4 on DC function. (A) BMDCs differentiated with GM-CSF alone or in combination with IL-4 were cocultured with thymocytes for 5 days in the presence of LPS or LPS plus $PCC_{87\text{-}104}$. IL-2 was measured at 24, 48 and 120 hours of coculture. (B) T lymphocyte activation markers CD25, CD44, CD69 and CD62L were analyzed in $V\alpha 11^{+}$ cells after 48 hours of coculture with BMDCs differentiated with GM-CSF (empty histograms) or GM-CSF plus IL-4 (filled histograms).

currently used for the generation of DCs *in vitro*. Our results confirm that different procedures might generate DCs with different functions. In agreement with results published by others [22], we found that DCs obtained by combining GM-CSF with IL-4 display a more mature phenotype, as evidenced by their higher MHC class II surface expression, decreased endocytosis and phagocytosis (data not shown) and enhanced IL-12 secretion. This distinctive phenotype has been associated with an increase of the *in vitro* allostimulatory capacity of IL-4-differentiated DCs as well as with enhanced protective tumour immunity *in vivo* [6, 23]. Potential differences in the ability of these various DC populations to contribute to tolerance induction *in vivo* could equally apply. The question whether IL-2 secretion contributes to these phenotypic differences of DCs awaits further investigation. During LPS activation, a variety of chemokines and cytokines are induced in DCs [4]. IL-4 inhibition of IL-2 secretion may therefore occur through an indirect effect, for example by regulating proteins critical for DC IL-2 production. This possibility is currently under study.

Inhibition of IL-2 secretion by IL-4 is specific to DCs

These results prompted us to examine whether the inhibitory effect of IL-4 on IL-2 secretion was specific to DCs or also affected T cells. To answer this question, we used T cells isolated from AND mice, which are transgenic for a TCR that recognizes a peptide from pigeon cytochrome c (PCC_{87-104}) in the context of I-E^k molecules. Thymocytes from these animals were purified and incubated with PCC_{87-104} plus LPS-activated F1 (B10.BRxAND) BMDCs, previously differentiated and maintained in the presence or absence of IL-4. The ability of the T cells to secrete IL-2 was monitored 24 h later and for various time points thereafter. As shown in Fig. 3A, we did not observe any inhibitory effect of IL-4 on IL-2 production by activated T cells. No IL-2 secretion was detected at these time points in the absence of the peptide, demonstrating that the measured IL-2 is produced by the activated T cells and not by the DCs. We therefore conclude that IL-4 specifically affects the ability of DCs to secrete IL-2. As IL-4 does not affect the capacity of T cells to secrete IL-2, these results further suggest that DCs may use an induction mechanism of IL-2 synthesis and/or secretion distinct from activated T lymphocytes.

These results also suggested that IL-4-treated DCs were still competent for activating T cells. This point was tested directly by assessing the expression of T-cell activation markers. When TCR transgenic T cells were incubated with DCs plus the specific peptide (PCC_{87-104}), there was a significant increase in CD25, CD44, CD69 expression and an important decrease in CD62L expression, regardless if the DCs were generated in the absence or presence of IL-4 (Fig. 3B). This is in agreement with the results reported by Granucci *et al.* [16], where IL-2-deficient DCs were able to upregulate the expression of the early activation marker CD69 on T cells. Our data suggest that IL-2 produced by DCs may not be required for the expression of these T-cell activation markers. In this regard, it is noteworthy that a recent report demonstrated that, in DCs, IL-2 is enriched at the sites of contact with T cells, a fact that may increase local concentration and effectiveness of this cytokine [17]. Thus, it is possible that, in our *in vitro* system, a small, localized intracellular amount of IL-2 may be delivered directly to the T cell during activation, in spite of the effect of IL-4 on bulk IL-2 secretion.

The fact that IL-2 production by LPS-activated DCs is abrogated in the presence of IL-4, a hallmark of T_H2 responses, raises new questions about DC involvement in the generation of polarized T-cell responses. Indeed, it has been suggested that there must be other mediators than IL-12 and IL-4 involved in T-lymphocyte polarization [10]. Thus, the inhibitory capacity of IL-4 on IL-2 production by LPS-activated DCs may reflect the plasticity of the immune system that can rapidly adapt to environmental changes in order to generate a more effective response against infectious agents. In view of these results, the role of DC-derived IL-2 in the process of immune induction and T_H polarization should be further investigated.

Acknowledgments

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References

1 Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245–52.
2 Banchereau J, Briere F, Caux C *et al.* Immunobiology of dendritic cells. Annu Rev Immunol 2000;18:767–811.
3 Huang Q, Liu D, Majewski P *et al.* The plasticity of dendritic cell responses to pathogens and their components. Science 2001;294: 870–5.
4 Rescigno M. Dendritic cells and the complexity of microbial infection. Trends Microbiol 2002;10:425–61.
5 Sozzani S, Allavena P, D'Amico G *et al.* Cutting edge: differential regulation of chemokine receptors during dendritic cell maturation: a model for their trafficking properties. J Immunol 1998;161:1083–6.
6 Labeur MS, Roters B, Pers B *et al.* Generation of tumor immunity by bone marrow-derived dendritic cells correlates with dendritic cell maturation stage. J Immunol 1999;162:168–75.
7 Dhodapkar MV, Steinman RM, Krasovsky J, Munz C, Bhardwaj N. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. J Exp Med 2001;193:233–8.
8 Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH. Induction of interleukin 10-producing, nonproliferating $CD4^+$ T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. J Exp Med 2000;192:1213–22.

9 Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. Annu Rev Immunol 2003;21:685–711.
10 MacDonald AS, Pearce EJ. Cutting edge: polarized Th cell response induction by transferred antigen-pulsed dendritic cells is dependent on IL-4 or IL-12 production by recipient cells. J Immunol 2002;168:3127–30.
11 De Jong EC, Vieira PL, Kalinski P *et al.* Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells *in vitro* with diverse Th cell-polarizing signals. J Immunol 2002;168:1704–9.
12 Pulendran B, Kumar P, Cutler CW, Mohamadzadeh M, Van Dyke T, Banchereau J. Lipopolysaccharides from distinct pathogens induce different classes of immune responses in vivo. J Immunol 2001;167:5067–76.
13 Hochrein H, O'Keeffe M, Luft T *et al.* Interleukin (IL)-4 is a major regulatory cytokine governing bioactive IL-12 production by mouse and human dendritic cells. J Exp Med 2000;192:823–33.
14 Bullens DM, Kasran A, Thielemans K, Bakkus M, Ceuppens JL. CD40L-induced IL-12 production is further enhanced by the Th2 cytokines IL-4 and IL-13. Scand J Immunol 2001;53:455–63.
15 Kalinski P, Smits HH, Schuitemaker JH *et al.* IL-4 is a mediator of IL-12p70 induction by human Th2 cells: reversal of polarized Th2 phenotype by dendritic cells. J Immunol 2000;165:1877–81.
16 Granucci F, Vizzardelli C, Pavelka N *et al.* Inducible IL-2 production by dendritic cells revealed by global gene expression analysis. Nat Immunol 2001;2:882–8.
17 Granucci F, Feau S, Angeli V, Trottein F, Ricciardi-Castagnoli P. Early IL-2 production by mouse dendritic cells is the result of microbial-induced priming. J Immunol 2003;170:5075–81.
18 Granucci F, Andrews DM, Degli-Esposti MA, Ricciardi-Castagnoli P. IL-2 mediates adjuvant effect of dendritic cells. Trends Immunol 2002;23:169–71.
19 Fukao T, Koyasu S. Expression of functional IL-2 receptors on mature splenic dendritic cells. Eur J Immunol 2000;30:1453–7.
20 Reid CD, Stackpoole A, Meager A, Tikerpae J. Interactions of tumor necrosis factor with granulocyte-macrophage colony-stimulating factor and other cytokines in the regulation of dendritic cell growth *in vitro* from early bipotent $CD34^{+}$ progenitors in human bone marrow. J Immunol 1992;149:2681–8.
21 Lennon-Duménil AM, Bakker AH, Maehr R *et al.* Analysis of protease activity in live antigen-presenting cells shows regulation of the phagosomal proteolytic contents during dendritic cell activation. J Exp Med 2002;196:529–40.
22 Dillon SM, Hart DN, Abernethy N, Watson JD, Baird MA. Priming to mycobacterial antigen *in vivo* using antigen-pulsed antigen presenting cells generated *in vitro* is influenced by the dose and presence of IL-4 in APC cultures. Scand J Immunol 1997;46: 1–9.
23 Basak SK, Harui A, Stolina M *et al.* Increased dendritic cell number and function following continous *in vivo* infusion of granulocyte macrophage-colony-stimulating factor and interleukin-4. Blood 2002;99:2869–79.