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IL-10 deficiency blocks the ability of LPS to regulate expression of tolerance-related molecules on dendritic cells

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Abstract

Interleukin-10 (IL-10) is an anti-inflammatory cytokine that plays an important role in regulating the local inflammatory immune response, but regulatory mechanisms of this cytokine have not been fully elucidated. Here, we demonstrate that IL-10 deficiency renders LPS treatment ineffective in regulating the expression of CD40, CD80, CD86, B7-H2, and B7-DC on dendritic cells (DCs) and blocks upregulation of IL-27. This inability to respond to LPS was found in both IL-10^{-/-} bone marrow derived and splenic DCs. Compared with wild-type DCs, IL-10^{-/-} DCs expressed similar levels of TLR4 and CD14, but produced less LPS-binding protein. The deficiency in LPS-binding protein production may explain the failure of IL-10^{-/-} DCs to respond normally to LPS. Moreover, lack of IL-10 modulated the proportions of CD11c+CD8+ and CD11c+B220+ DCs, which play an important role in local inflammatory responses and tolerance. IL-10 deficiency also blocked expression of galectin-1, CD205, and CD103, which are necessary for central and peripheral tolerance. While they did not respond to LPS, IL-10^{-/-} DCs produced increased levels of IL-6 and CCL4 after TNF- α treatment. Together, our results demonstrate that IL-10 deficiency affects the immune functions of DCs, which may contribute to the increased severity of autoimmune diseases seen in IL-10^{-/-} mice.

Keywords

Costimulatory molecule; DCs; IL-10; TLR4

Introduction

Dendritic cells (DCs) are professional APCs that play an important role in immune defenses and in maintaining immune tolerance to self-tissues [1]. DCs originate from bone marrow hematopoietic stem cells, which subsequently develop into myeloid precursor cells and then into immature myeloid cells. These cells migrate into peripheral lymphatic organs and, under the control of a series of transcription factors, differentiate into DCs. Although multiple subtypes have been identified, the main DC subtypes found in a steady-state mouse and in humans include type-1 interferon-producing plasmacytoid DCs and conventional DCs [2]. Functionally, DCs play an important role in the control of viral infections, development of autoimmune diseases, and induction of tolerance [1–3]. However, the mechanisms by which DCs regulate immune responses and tolerance have not been fully elucidated.

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It has been proposed that DCs activate naive T cells through four pathways, called Signals 0, 1, 2, and 3 [4, 5]. DCs secrete chemokine (C–C motif) ligands (CCL)–3, –4, and –17 to induce T-cell migration into local inflammatory areas (Signal 0) [6]. DCs then capture exogenous antigens and present epitopes on MHC class I/II (MHC I/II) molecules. The peptide/MHC complex on the surface of DCs interacts with TCRs on naive and memory T cells and activates them (Signal 1) [7]. Moreover, multiple costimulatory molecules are expressed on the surface of DCs, such as CD40 and B7 family members. The B7 family is currently reported to comprise at least seven members, including CD80, CD86, B7-H1, B7-H2, B7-H3, B7-H4, and B7-DC. B7-H1 and B7-H4 are negative regulators of T-cell activation, and the others are activating costimulatory molecules for T-cell functions (Signal 2) [8]. In addition, DCs secrete inflammatory and anti-inflammatory cytokines such as IL-6, IL-12, IL-23, and IL-27, which can facilitate or inhibit T-cell activity (Signal 3) [9–11]. IL-10, an anti-inflammatory cytokine, is generated by multiple immune cells such as T cells, B cells, DCs, macrophages, and monocytes [12]. IL-10 deficiency renders mice more susceptible to the induction of autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE), followed by augmented severity of disease course and impaired resolution [13, 14]. Most previous research has been focused on the effects of IL-10 on T-cell function [15]. For example, IL-10 produced by Treg cells can block effector T-cell trafficking to local inflammatory sites and inhibit local inflammatory responses [16]. However, the effect of IL-10 on the biological functions of DCs is poorly understood. We propose that IL-10 may affect expression of costimulatory molecules on DCs and block DC-mediated immune responses. To test this hypothesis, in the present study we have determined the role of IL-10 in DC differentiation and expression of costimulatory molecules, cytokines, and chemokines, which are necessary for normal DC functions in immune responses and tolerance.

Results

IL-10 deficiency precludes the effects of LPS on DCs

To investigate whether lack of IL-10 can affect expression of CD40, B7 family members, and MHC I/II on DCs, these cells were treated with LPS and analyzed by flow cytometry. LPS treatment increased expression of CD40, CD80, CD86, B7-DC, and MHC I/II on wild type (WT) DCs while downregulating B7-H2 and B7-H4 expression (Fig. 1). Expression of B7-H1 and B7-H3 remained unchanged (Fig. 1). In contrast, LPS treatment did not change expression of any B7 family molecules on IL-10^{–/–} DCs, and also failed to upregulate MHC I/II (Fig. 1). These results suggested a requirement for IL-10 in normal DC responses to toll-like receptor 4 (TLR4) activation with LPS.

IL-10 deficiency affects DC differentiation

To investigate whether lack of IL-10 affects DC differentiation, subpopulations of WT and IL-10^{–/–} CD11c⁺CD11b⁺, CD11c⁺CD8⁺, and CD11c⁺B220⁺ DCs were determined by flow cytometry. IL-10 deficiency did not affect the number of CD11c⁺CD11b⁺ DCs; however, decreased numbers of CD11c⁺CD8⁺ and CD11c⁺B220⁺ DCs were found (Fig. 2). LPS treatment reduced the number of WT CD11c⁺CD8⁺ DCs, but had no effect on this subset among IL-10^{–/–} DCs. In contrast, LPS treatment increased the number of WT CD11c⁺B220⁺ DCs, but reduced numbers of these cells among IL-10^{–/–} DCs (Fig. 2). These results showed that IL-10 deficiency affects the phenotype of DCs that developed in vitro, and indicated that IL-10 may be necessary for normal DC differentiation in vivo.

Deficiency of IL-10 blocks IL-27 production but upregulates IL-6 and CCL4 production of DCs

To investigate whether lack of IL-10 can affect the secretion of immunoregulatory cytokine IL-27, proinflammatory cytokine IL-6, and chemokine CCL4 by DCs, these cells were cultured in the presence or absence of LPS, and supernatants were assayed by ELISA. IL-10 deficiency completely precluded LPS-induced IL-27 production (Fig. 3A and B); on the other hand, there was enhanced IL-6 and CCL4 secretion after TNF- α treatment (Fig. 3C and D). These results suggest that IL-10 deficiency may facilitate TNF- α -mediated immune responses and block IL-27-mediated immune tolerance.

IL-10 deficiency leads to downregulation of galectin-1, CD205, and CD103 on DCs

Since galectin-1 expression by tolerogenic DCs is also an important mechanism in peripheral tolerance [17–19], intracellular production of galectin-1 by WT and IL-10 $^{-/-}$ DCs was compared. IL-10 deficiency resulted in downregulation of galectin-1 production by DCs irrespective of LPS treatment (Fig. 4A and B). It has been found that the expression of CD205 [20, 21] and CD103 [22, 23] is necessary for peripheral tolerance. We thus investigated whether IL-10 deficiency affects expression of these molecules. WT DCs expressed relatively high levels of CD205 (Fig. 4A and C) and CD103 (Fig. 4A and D) in both LPS-treated and -untreated conditions, while fairly small proportions of IL-10 $^{-/-}$ DCs expressed these molecules. On the other hand, significantly enhanced proliferation was observed in IL-10 $^{-/-}$ compared with WT DC cultures (Fig. 4E). Together, these results suggest that IL-10-deficient DCs exhibited a bias toward proinflammatory rather than tolerogenic phenotype.

IL-10 $^{-/-}$ DCs secrete reduced quantities of LPS-binding protein

We then investigated whether the failure of IL-10 $^{-/-}$ DCs to respond to LPS was due to an abnormal expression of LPS receptor by these cells, including TLR4, CD14, and LPS-binding protein (LBP), a soluble protein and a part of the Lipid A-LBP-CD14 complex involved in TLR4 activation [24]. TLR4 and CD14 were analyzed by flow cytometry, and LBP production in DC culture supernatants was determined by ELISA. For comparison, TNF receptors (TNF- α R1 and TNF- α R2) were also determined by flow cytometry. Our results showed that IL-10 deficiency does not affect expression of TLR4, CD14, TNF- α R1, and TNF- α R2 on DCs (Fig. 5A). However, LBP secretion by IL-10 $^{-/-}$ DCs was significantly lower compared with that of WT DCs (Fig. 5B). Thus, the decrease in LBP expression, resulting in impaired TLR4 signaling, may play a role in the failure of LPS to modulate expression of costimulatory molecules and MHC I/II on IL-10 $^{-/-}$ DCs.

Exogenous IL-10 does not restore response of IL-10 $^{-/-}$ DCs to LPS

To investigate whether the failure of IL-10 $^{-/-}$ DCs to respond to LPS can be reversed by exogenous IL-10, DCs generated from WT and IL-10 $^{-/-}$ mice were stimulated with LPS, in the presence or absence of recombinant IL-10. Their expression of costimulatory molecules and MHC II was analyzed by flow cytometry, and cytokine concentrations in culture supernatants measured by ELISA. IL-10 treatment downregulated expression of CD40, CD80, CD86, and MHC II on WT DCs treated with LPS (Fig. 6A), but did not alter expression of these molecules on IL-10 $^{-/-}$ DCs (Fig. 6A). LPS-induced production of IL-12 and IL-27 by WT DCs was downregulated after IL-10 treatment; however, only low levels of IL-12 and IL-27 were produced by IL-10 $^{-/-}$ DCs, with or without LPS stimulation, and adding IL-10 had no effect (Fig. 6B and C). These results suggested that exogenous IL-10 does not restore response of IL-10 $^{-/-}$ DCs to LPS.

IL-10^{-/-} splenic DCs express greater numbers of costimulatory molecules and MHC II

To investigate whether LPS can modulate expression of costimulatory molecules and MHC II in vivo, LPS was i.v. injected into WT and IL-10^{-/-} mice. Splenocytes were isolated after 24 h and expression of CD40, CD80, CD86, and MHC II on DCs was determined by flow cytometry. As shown in Fig. 7, splenic DCs of naïve WT mice expressed low levels of CD40, CD80, CD86, and MHC II, and their expression was upregulated after LPS injection. In contrast, splenic DCs of IL-10^{-/-} mice spontaneously expressed elevated levels of these molecules but injection of LPS did not alter their expression (Fig. 7). It can be concluded that LPS treatment failed to modulate expression of costimulatory molecules and MHC II on IL-10^{-/-} DCs that developed both in vitro (Fig. 1) and in vivo (Fig. 7).

IL-10 deficiency does not affect the ability of ODN 1826 to upregulate costimulatory molecule and MHC II expression

To investigate whether IL-10 deficiency also affects CpG-ODN 1826-mediated TLR9 signaling in DCs, WT and IL-10^{-/-} DCs were treated with CpG-ODN 1826, and expression of CD40, CD80, CD86, and MHC II on these cells was determined. CpG-ODN 1826 similarly upregulated expression of these molecules on IL-10^{-/-} and WT DCs (Fig. 8). The results imply that IL-10 deficiency specifically blocks LPS-mediated TLR4 signaling in DCs (Fig. 1), while it does not affect signaling of CpG-ODN 1826-mediated TLR9 signaling.

Discussion

IL-10 plays an anti-inflammatory role in several inflammatory diseases such as EAE, inflammatory bowel disease, Cohn's disease, and colitis [25, 26]. Despite over two decades of research, the regulatory mechanisms by which IL-10 suppresses inflammatory diseases have not been fully elucidated. Previous research has focused on IL-10 suppression of T-cell function [27]. For example, IL-10 produced by Treg cells negatively regulates survival of pathogenic T cells in the local microenvironment [16]. However, little is known about the effects of IL-10 on tolerance-related molecules expressed by DCs.

DCs regulate T-cell-mediated immune responses through expression of costimulatory molecules and their interaction with ligands expressed on T cells. LPS upregulates expression of CD40, CD80, CD86, and B7-DC on DCs, and IL-10 is known to inhibit CD40, CD80, and CD86 expression induced by IFN- γ and TNF- γ [28–30]. However, the role of endogenous IL-10 in LPS-mediated expression of costimulatory molecules on DCs, which is the focus of this work, is not known.

The expression of costimulatory molecules on DCs regulates initial T-cell activation and effector T-cell function. CD80 and CD86 expression is required for T-cell proliferation induced by DCs in IFN- γ -mediated immune responses; these molecules also play an important role in T-cell survival and amplification of inflammatory responses [30]. CD80 and CD86 expressed on DCs are also required for maintenance of Treg-cell populations and play an important role in the generation and survival of Treg cells [31]. While LPS is a well-known stimulus for DC activation in vitro and in vivo, our results demonstrate that LPS did not induce expression of costimulatory molecules in IL-10^{-/-} DCs. The failure to respond to LPS occurred not only in bone marrow derived DCs of IL-10^{-/-} mice in vitro, but also in splenic DCs in vivo, suggesting an essential role for endogenous IL-10 in LPS-induced DC activation. On the other hand, splenic DCs from naïve IL-10^{-/-} mice had elevated expression of costimulatory molecules and MHC II compared with WT DCs. These results indicate that peripheral IL-10^{-/-} DCs (e.g. in spleen) were abnormally activated, and thus may have more readily induced inflammatory responses and autoimmune disorders.

Although our data show that the LPS-induced TLR4 signal transduction pathway was inactivated in IL-10^{-/-} DCs, the mechanism underlying this phenomenon is unclear. It is known that TLR4 activation by LPS operates via the Lipid A-LBP-CD14 complex [24]; we therefore tested whether the lack of LPS effect on IL-10^{-/-} DCs was due to an incomplete LPS receptor complex. While there was no significant difference in expression of TLR4 and CD14, production of LBP, a soluble protein and a part of the Lipid A-LBP-CD14 complex [24], is significantly decreased in IL-10^{-/-} DCs compared with WT DCs. Although the reason for this phenomenon is not clear, a decreased level of LBP could negatively regulate TLR4-mediated response in DCs and prevent LPS-induced expression of costimulatory molecules and MHC II on these cells. Given that TLR4 signaling plays an important role in peripheral tolerance and inhibition of inflammatory responses [32–35], the potential inactivation of TLR4 signaling in IL-10^{-/-} mice may contribute to deregulation of inflammatory responses and immune tolerance, causing IL-10^{-/-} mice to develop more severe autoimmune diseases than WT mice.

Interestingly, the capacity of LPS to modulate expression of costimulatory molecules and MHC II could not be restored after IL-10^{-/-} DCs were treated with IL-10. We found that exogenous IL-10 efficiently downregulated LBP expression by WT DCs, and that LBP expression by IL-10^{-/-} DCs was significantly lower than by unstimulated WT DCs irrespective of LPS and/or IL-10 treatment. This may be one of the reasons why exogenous IL-10 treatment cannot overcome the effect of IL-10 deficiency in modulation of expression of costimulatory molecules, cytokines, and MHC II mediated by LPS on DCs.

It is known that there are multiple DC subpopulations and that they perform diverse biological functions in vivo [36]. For example, CD8⁺CD11c⁺ DCs and CD11b⁺CD11c⁺ DCs may have different functions in EAE, a mouse model of autoimmune inflammatory disease [37]. Bone marrow derived CD8⁺ DCs are regulatory DCs that play an important role in peripheral tolerance through depletion of autoreactive T cells and expansion of Treg cells [38, 39]. CD11c⁺B220⁺ DCs have tolerogenic functions as well [40]. Our results demonstrate that IL-10 regulates the numbers of CD8⁺CD11c⁺ and B220⁺CD11c⁺ DCs, suggesting that IL-10 may regulate immune responses in vivo through modulation of tolerogenic DC subpopulations. In contrast, IL-10 deficiency does not affect the number of CD11b⁺CD11c⁺ DCs, implying that regulation of this DC subset is IL-10-independent. Taken together, these findings suggest that deregulation of tolerogenic DC subpopulations may contribute to the development of more severe autoimmune disease in IL-10^{-/-} mice.

One of the biological functions of DCs is the secretion of cytokines such as IL-6, IL-12, and IL-27 that modulate T-cell responses. IL-10 deficiency completely blocked upregulation of IL-27 production by LPS, implying that IL-10 can also affect inflammatory responses indirectly, through modulation of IL-27 production. TNF- α treatment stimulates production of IL-6 by IL-10^{-/-} DCs. Given that LPS and TNF- α signal through independent pathways, this result suggests that IL-10 is necessary for activation of TLR4 by LPS, but not for TNF- α . As IL-6, IL-12, and IL-27 all regulate Th17-cell differentiation [41, 42], our results suggest that IL-10 may affect Th17-cell development and differentiation by modulating DC cytokine production. In addition, T cells are attracted to the site of inflammation by chemokines produced by DCs (Signal 0). For example, DCs secrete CCL3, CCL4, and CCL17, which can respectively bind to CCR1, CCR4, and CCR5 on T cells. Regulation of chemokines produced by DCs is therefore an important therapeutic target for anti-inflammatory therapy [43, 44]. Overactivation of TNF- α -induced CCL4 expression in IL-10^{-/-} DCs may lead to greater numbers of effector T cells attracted to a site of inflammation, contributing to the development of more severe autoimmune disease in IL-10^{-/-} mice.

Galectin-1, an evolutionarily conserved glycan-binding protein, plays an important role in DC-mediated peripheral tolerance [17–19]. It is not known whether IL-10 can modulate galectin-1 expression by DCs. In our study, the decreased production of galectin-1 by IL-10^{-/-} DCs suggests a novel mechanism underlying enhanced susceptibility to autoimmune diseases in IL-10^{-/-} mice. CD205-mediated endocytosis is also necessary for central and peripheral tolerance [45], and blockade of CD205 facilitates autoimmunity [46]. DCs engulf pieces of apoptotic cells via CD205 and present self-antigens on the surface, resulting in depletion of autoreactive T cells in the central and peripheral compartments of the immune system. Our results show that there is less CD205 on IL-10^{-/-} DCs (Fig. 4A and C), indicating that CD205-mediated endocytosis may be blocked in IL-10^{-/-} mice and that autoreactive T cells may not be efficiently depleted *in vivo*, resulting in greater severity of autoimmune disorders. Further, CD103⁺ DCs can inhibit inflammatory responses in the intestine [23, 47], and loss of CD103⁺ DCs leads to colitis [23, 47]. We found decreased numbers of CD103⁺CD11c⁺ DCs in IL-10^{-/-} mice compared with WT mice irrespective of LPS stimulation. These results suggest that IL-10 is required for CD103⁺ DC differentiation and that the decreased numbers of CD103⁺ DCs in IL-10^{-/-} mice may represent a mechanism underlying the pathogenesis of autoimmune diseases. Additionally, elevated DC proliferation in IL-10^{-/-} mice may further enhance inflammatory responses *in vivo*, resulting in increased severity of autoimmune disease in IL-10^{-/-} mice.

In summary, the ability of LPS to induce the expression of multiple tolerance-associated molecules by IL-10^{-/-} DCs is inhibited; this may block TLR4 signaling induced tolerance [28–31] and facilitate inflammatory responses in IL-10^{-/-} mice. These results imply that IL-10 may be a regulator of the LPS-induced TLR4 signal transduction pathway and endotoxin tolerance *in vivo*.

Materials and methods

Mice

C57BL/6J and C3Bir.129P2(B6)-IL10tm1Cgn/Lt (IL-10 knockout, IL-10^{-/-}) female mice (8–12 weeks old) were ordered from the Jackson Laboratory (Bar Harbor, ME, USA). All mice were bred at the Thomas Jefferson Animal Care facilities. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Thomas Jefferson University.

Generation of BMDCs

As described previously [48, 49], femurs and tibiae of mice were isolated from surrounding muscle tissue by gentle abrasion of surrounding muscle with Kleenex tissues. The intact bones were then put in 70% ethanol for 5 min for disinfection and washed with PBS. Both ends of the bones were cut with scissors and the bone marrow was flushed out with PBS by using a syringe with a 0.45-mm-diameter needle. Clusters within the bone marrow suspension were disintegrated by vigorous pipetting and then washed with PBS. Leucocytes from bone marrow were fed in bacteriological 100-mm Petri dishes (Falcon, Becton Dickinson, Heidelberg, Germany) at 2×10^6 /per dish. The cells were cultured in RPMI1640 complete medium (Gibco-BRL, Eggenstein, Germany) including penicillin (100 U/mL, Sigma, St. Louis, MO, USA), streptomycin (100 U/mL, Sigma), L-glutamine (2 mM, Sigma), 2-mercaptoethanol (2-ME, 50 μ M, Sigma), 10% heated inactivated and filtered (0.22 μ m, Millipore, Inc., Bedford, MA, USA) fetal calf serum (Sigma), and granulocyte-macrophage colony stimulating factor (GM-CSF, PetroTech, Rocky Hill, NJ, USA) at 20 ng/mL on day 0 (10 mL medium) per dish. A total of 10 mL fresh medium with GM-CSF at 20 ng/mL was added to each dish on day 3, and one-half of the medium (about 10 mL supernatant) was collected and centrifuged at 300g for 5 min at day 6. Cells were resuspended in 10 mL fresh

medium with GM-CSF (20 ng/mL). They were then refed in the original dish. Cells were collected by gentle pipetting and seeded in a fresh dish on day 8. A total of 10 mL fresh medium including GM-CSF at 20 ng/mL was added on day 8. Cells were treated with 1 µg/mL LPS (Sigma), 10 µg/mL CpG-ODN 1826 (Sigma) for 24 h, and/or with 20 ng/mL IL-10 for 72 h. DCs were collected by gentle pipetting and washed with PBS at 300g for 5 min on day 10. Cells were counted for flow cytometry, and supernatant was harvested and stored at -70 °C for ELISA.

Injection of LPS and spleen cell preparation

LPS was i.v. injected into C57 BL/6J and IL-10^{-/-} mice at 30 µg per mouse (in 100 µL PBS per mouse). Splenocytes were prepared after 24 h of injection. Expression of CD40, CD80, CD86, and MHC II on gated CD11c⁺ DCs was determined by flow cytometry as described below.

Flow cytometry

Cultured DCs were incubated with anti-mouse antibodies against B220, CD40, CD80, CD86, B7-H1, B7-H2, B7-H3, B7-H4, B7-DC, galectin-1, CD205, CD103, MHC I H-2Kb and MHC II (I-Ab), TLR4, CD14, TNF-αR1 and TNF-αR2 (BioLegend, San Diego, CA, USA) at 4°C for 1 h. Cells were then washed twice with 5% fetal calf serum in PBS at 300g for 5 min. Finally, cells were fixed with 5% formalin in PBS. Fixed cells were run on an FACS Aria (BD Biosciences, San Jose, CA, USA) and data were analyzed with FlowJo software (Treestar, Ashland, OR, USA).

ELISA

Anti-mouse ELISA kits for IL-6, IL-12, IL-27, and CCL4 were purchased from R&D Systems (Minneapolis, MN, USA). LBP ELISA kit was purchased from Abnova (Taipei, Taiwan). Assays were conducted following the manufacturer's protocol. Plates were read on Labsystems Multiskan MCC/340 (Fisher Scientific, Suwannee, GA, USA) and data were analyzed with DSJV ELISA software (Fisher Scientific).

Proliferation assay

WT and IL-10^{-/-} DCs were treated in the presence or absence of LPS for 24 h and subsequently labeled with 1 µCi 3H methylthymidine for 18 h. DCs were collected and determined using a scintillation counter (Packard Top Count, GMI, Inc, Ramsey, MN, USA).

Statistical analysis

A *t*-test was conducted to analyze ELISA and flow cytometry data. Differences were considered significant if *p*-value was less than 0.05.

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Figure 1.

IL-10^{-/-} DCs are nonresponsive to LPS. DCs were generated from bone marrow of WT C57BL/6J and IL-10^{-/-} mice. On day 8 of culture, cells were treated with (thick-line histogram) or without (thin-line histogram) LPS at 1 μ g/mL for 48 h and expression of CD40, CD80, CD86, B7-H1, B7-H2, B7-H3, B7-H4, and B7-DC was analyzed by flow cytometry (left). Isotype controls are shown as shaded histograms. Data are also presented as mean + SD of triplicate determinations of mean fluorescence intensity (MFI) (right). All experiments were repeated three times with similar results. * $p < 0.05$, t -test.

Figure 2.

IL-10 deficiency blocks the ability of LPS to modulate the proportion of CD8+CD11c+ and CD11c+B220+ DCs in vitro. DCs were generated from bone marrow of WT and IL-10^{-/-} mice. On day 8 of culture, cells were treated, or not, with LPS (1 μ g/mL) for 48 h. Expression of CD8, B220, CD11b, and CD11c was determined by flow cytometry. The percentages of CD11c+CD11b+ (top), CD8+CD11c+ (middle), and CD11c+B220+ (bottom) subpopulations among all cultured cells are also shown as mean $\pm$ SD of triplicate measurements (right). All experiments were repeated three times.

Figure 3.

IL-10 deficiency blocks the ability of LPS to induce IL-27 production, but upregulates IL-6 and CCL4 production in DCs. DCs were generated from bone marrow of WT and IL-10^{-/-} mice. (A, B) DCs were treated with LPS at 1 μ g/mL, or not, for (A) 24 h and (B) 72 h. (C, D) DCs were treated, or not, with TNF- α at 1000 U/mL for 48 h, and supernatant was collected for ELISA assay measurement. (A, B) IL-27, (C) IL-6, and (D) CCL4 levels are shown as mean $\pm$ SD of triplicate measurements. All experiments were repeated twice with similar results. * $p < 0.05$, t -test.

Figure 4.

IL-10 deficiency results in downregulation of galectin-1, CD205, and CD103 expression on DCs. DCs were generated from bone marrow of WT and IL-10^{-/-} mice and treated with LPS for 48 h. Expression of (A) galectin-1, CD205, and CD103 on DCs was determined by flow cytometry. Representative data from one of three experiments are shown. (B–D) Quantitative analysis of the data presented in (A). (E) Proliferation was determined by 3H-thymidine incorporation. Data are shown as mean $\pm$ SD of triplicate measurements. Experiments were repeated three times with similar results. * $p < 0.05$, t -test.

Figure 5.

Expression of TLR4, CD14, TNF α Rs and production of LPS binding protein (LBP) in IL-10^{-/-} and WT DCs. DCs were generated from bone marrow of WT and IL-10^{-/-} mice. (A) On day 10 of culture, cells were collected and expression of TLR4, CD14, TNF- α RI, and TNF- α RII on WT DCs (thick line histograms) and IL-10^{-/-} DCs (thin line histograms) was determined by flow cytometry. (B) The LBP level in culture supernatants was determined by ELISA. Data are shown as mean $\pm$ SD of triplicate measurements of LBP concentration. All experiments were repeated twice with similar results. * $p < 0.05$, t -test.

Figure 6.

Effect of exogenous IL-10 on LPS-induced expression of costimulatory molecules, MHC II, and cytokines in IL-10^{-/-} and WT DCs. DCs were generated from bone marrow of WT and IL-10^{-/-} mice. On day 8 of culture, DCs were treated with IL-10 (20 ng/mL, thin-line histograms) or medium only (thick-line histograms) and LPS (1 μ g/mL) was added to the culture 48 h later. DCs without treatment served as controls (shaded histograms). (A) Expression of CD40, CD80, CD86, and MHC II on gated CD11c⁺ DCs was determined by flow cytometry. Production of (B) IL-12 and (C) IL-27 by WT and IL-10^{-/-} DCs was determined by ELISA and shown as mean + SD of triplicate measurements. All experiments were repeated twice with similar results. * $p < 0.05$, t -test.

Figure 7.

IL-10 deficiency inhibits the ability of LPS to upregulate expression of costimulatory molecules and MHC II on splenic DCs in vivo. WT mice and IL-10^{-/-} mice were injected i.v. with 100 μ L PBS or LPS (30 μ g LPS in 100 μ L PBS per mouse). Spleen cells were isolated after 24 h. The expression of CD40, CD80, CD86, and MHC II on gated CD11c⁺ DCs of LPS-treated (thick-line histograms) and PBS-treated (thin-line histograms) mice is shown. Experiments were repeated twice with similar results.

Figure 8.

IL-10 deficiency does not affect upregulation of costimulatory molecule and MHC II expression on DCs induced by CpG-ODN 1826. WT and IL-10^{-/-} DCs were treated with CpG-ODN 1826 (10 μ g/mL, thick-line histograms) or not (thin-line histograms) for 24 h. The protein expression of CD40, CD80, CD86, and MHC II is shown. Experiments were repeated twice with similar results. The isotype control is shown as a shaded histogram.