Review

Pivotal Role of PGE_2 and IL-10 in the Cross-Regulation of Dendritic Cell-Derived Inflammatory Mediators

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Exposure to pathogens induces antigen-presenting cells (APC) such as macrophages and dendritic cells (DC) to produce various endogenous mediators, including arachidonic acid (AA)-derived eicosanoids, cytokines, and nitric oxide (NO). Many secreted products of activated APC can act by themselves in an autocrine manner and modulate their function. Moreover, the cross-interaction between endogenous bioactive molecules regulates the function of professional APC with important consequences for their ability to activate and sustain immune and inflammatory responses, and to regulate immune homeostasis. Although neglected for many years when compared to their role in cardiovascular homeostasis, cancer and inflammation, the importance of eicosanoids in immunology is becoming more defined. The role of prostaglandin (PG) E_2 (PGE_2), one of the best known and most well studied eicosanoids, is of particular interest. It modulates the activities of professional DC by acting on their differentiation, maturation and their ability to secrete cytokines. Uniquely among haematopoietic cytokines, interleukin-10 (IL-10) is a pleiotropic molecule that displays both immunostimulatory and immunoregulatory activities. IL-10 has attached much attention because of its anti-inflammatory properties. It modulates expression of cytokines, soluble mediators and cell surface molecules by cells of myeloid origin, particularly macrophages and DC. We previously reported that PGE_2 is a potent inducer of IL-10 in bone marrow-derived DC (BM-DC), and PGE_2-induced IL-10 is a key regulator of the BM-DC pro-inflammatory phenotype. BM-DC may be considered as an important model to study complex interactions between endogenous mediators, and autocrine IL-10 plays a pivotal role in the crossregulation of AA-derived lipid mediators, cytokines, and NO, with critical effects on immune and inflammatory responses. *Cellular & Molecular Immunology*. 2006;3(4):271-277.

Key Words: BM-DC, IL-10, PGE_2, feed-back regulation, cytokine, inflammation

Introduction

In the last decade, DC emerged as major APC with critical role in immunity and tolerance. They are characterized by their exceptional ability to prime naïve T cells and thereby to primarily initiate immune responses (1). After their development in the bone marrow, immature DC migrates to peripheral tissues, where they actively internalize particles and proteins in the extracellular fluids. Internalized proteins are degraded into peptides, which are captured by the major histocompatibility complex (MHC) molecules for the presentation at the plasma membrane (2). These immature APC are highly responsive to inflammatory stimuli, including lipolysaccharide (LPS), PGE_2 and TNF-α, which are able to induce DC maturation. In contrast, other factors such as IL-10 and TGF-β appear to play important roles in maintaining DC in an immature state. In response to inflammatory stimuli, DC cease their endocytic activity (3), increase their expression of MHC and co-stimulatory molecules (4), and produce large amounts of endogenous pro-inflammatory agents such as TNF-α, IL-6 (5), IL-12, and leukotriene B_4 (LTB_4) (6), and anti-inflammatory molecules such as PGE_2 and IL-10 (7, 8). It is important to note that depending on the site of encounter, endogenous inflammatory mediators may have opposite effects on professional APC phenotype and function. Among AA-derived lipid mediators, PGE_2 is one of the best characterized in terms of immuno-modulation. It is a very attractive molecule in that it by it self exhibits both pro- and anti-inflammatory effects, particularly on DC. Depending on the nature of maturation signals, PGE_2 has different and sometimes opposite effects on DC biology. We reported previously that PGE_2 exerts an inhibitory action, reducing the maturation of DC and their ability to present antigen (8). On the contrary, PGE_2 has also been shown to stimulate DC and promote IL-12 production when given in combination with TNF-α (9). PGE_2, is an environmentally

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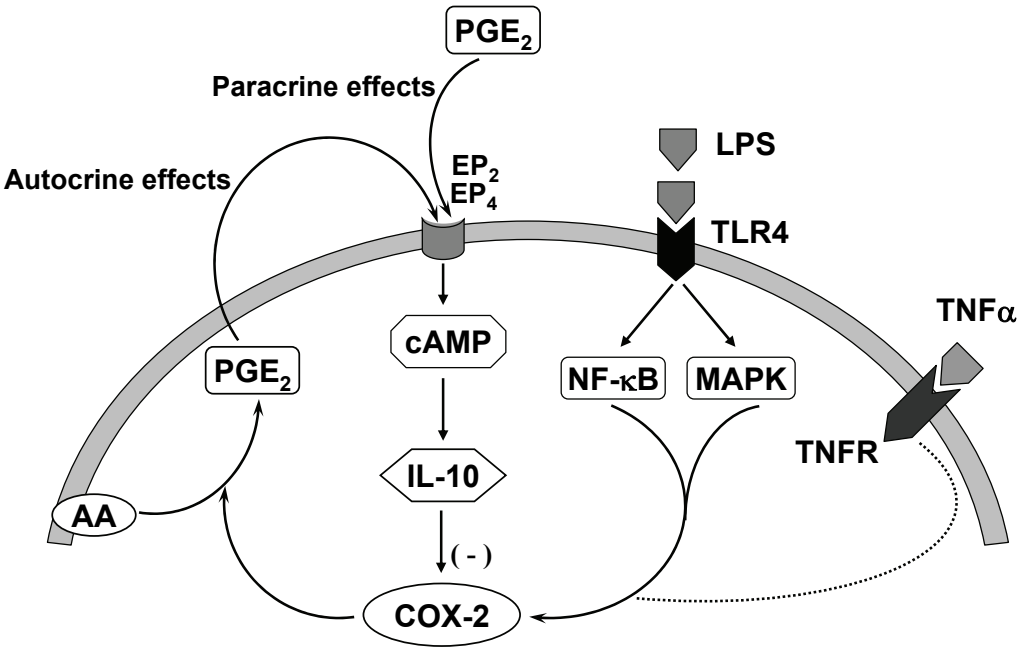

Figure 1. Autocrine and paracrine induction of IL-10 by PGE_2 in BM-DC, and its effects on COX-2 pathway.

bioactive substance. Its action is prolonged and sustained by other factors especially IL-10.

IL-10, originally described as a cytokine synthesis inhibitory factor (CSIF), is a pleiotropic molecule that displays both immunoregulatory and immunostimulatory activites (10). The anti-inflammatory effect of IL-10 is due, at least in part, to its potent biologic action on cells of myeloid origin such as macrophages and DC. It is clearly established that IL-10 potently inhibits the expression of cytokines, soluble mediators and cell surface molecules by cells of haematopoietic origin, with important consequences for their ability to activate and sustain immune and inflammatory responses (11), and leads to diminished T cell stimulation (12). IL-10 serves as potent mechanism for limiting the maturation of monocyte-derived DC and their capacity to initiate Th1 responses. Both PGE_2 and IL-10 are produced by DC and have similar immunosuppressive effects on APC. Since DC act as major initiator of the adaptive immune response and also as significant participants in the innate inflammatory response, cross-regulation of inflammatory mediators and its subsequent effect on DC is of great importance.

Receptors for PGE_2 that stimulate IL-10 release in BM-DC

IL-10 is a 35 kD protein that can be produced subpopulation of T helper cells, B cells and macrophages (13, 14). Some endogenous mediators issued from cells of the immune system can enhance the production of IL-10. Previous studies showed that PGE_2 up-regulates the production of IL-10 by macrophages (15, 16) and T cells (17). In monocytes, IL-10 synthesis was found to be enhanced after LPS exposure (11), suggesting that IL-10 may regulate the inflammatory response to LPS. We previously reported that PGE_2-primed BM-DC produce high levels of IL-10, which regulates their phenotype and function (7). The same results were obtained by other investigators (18), who demonstrated that cAMP-elevating agents, including PGE_2, affect DC function *via* an IL-10-dependent mechanism.

The effects of PGE_2 on APC are exerted by specific G protein-coupled receptors on the plasma membrane of target cells. Based on pharmacological and cDNA cloning studies, four subtypes of PGE receptors designated EP receptors (EP_1, EP_2, EP_3 and EP_4) have been identified and have been shown to differ in their signal transduction pathways. The EP_1 receptor activates phospholipase C and phosphatidylinositol turnover and stimulates the release of intracellular calcium. The EP_2 and EP_4 receptors signal by stimulating adenylate cyclase, which increases the intracellular levels of cAMP. Signaling by the EP_3 receptor is more complex because of multiple EP_3 receptor isoforms generated by alternative splicing from a single EP_3 gene. PGE_2 is known to elevate intracellular cAMP levels *via* stimulation of adenylate cyclase (19). A number of reports have started that effects of PGE_2 on cytokine production by cells of myeloid origin, are mediated by an increase in intracellular cAMP. We have demonstrated the expression of all EP receptors at the surface of BM-DC (5). Other investigators have reported that both EP2 and EP4 receptors are expressed at mRNA levels and as surface proteins on murine BM-DC (20). Among the four PGE_2 receptors, EP2 and EP4 mediate most, if not all, of the PGE_2 effects on DC (5, 20, 21). Using EP receptor-selective

synthetic agonists and dibutyryl cAMP, we demonstrated that PGE_2-EP_2 and -EP_4 signaling stimulate the production of IL-10 by BM-DC (7). Agonists of cAMP-elevating receptors, such as butaprost (EP_2 receptor), dose-dependently enhanced IL-10 production from LPS-stimulated BM-DC (22). The enhanced BM-DC release of IL-10 observed after LPS stimulation appears to be closely connected to COX-2 activity as the COX-2-selective inhibitor NS-398 significantly reduced IL-10 levels (7). In fact, microbial products such as LPS activate TLR4-derived signaling pathways including NF-κB and/or MAPK, which results in the induction of cyclooxygenase (COX)-2 and the production of PGE_2 (Figure 1).

Pro-inflammatory cytokines, such as TNF-α are also able to induce COX-2 expression and PGE_2 production by cells of immune system, particularly DC. Since BM-DC express EP receptors at their surface, they become target to autocrine and paracrine PGE_2, which by binding to EP2 and/or EP4 acts on DC and induces the production of endogenous IL-10. We also reported that exogenous PGE_2 at physiological concentrations is able to stimulate IL-10 release from BM-DC and endogenous PGE_2-induced IL-10 has an auto-regulatory effect on BM-DC phenotype and functions (23). Although, signaling through EP_2 and EP_4 receptors appears to have an important role in DC function (5, 20, 21), EP_1 and EP_1 signaling seems to have no significant roles in mediating PGE_2 effects on DC. The recent identification of different PGE synthase isoforms and the development of specific EP receptor subtype agonists and antagonists signal the emergence of novel and perhaps more specific interventions than even isoform specific COX inhibitors.

Feedback regulation of BM-DC-derived cytokines by PGE_2 and IL-10

PGE_2 and IL-10 have important regulatory roles on Th1 and Th2 immune responses. In addition to its crucial role in modulating the phenotype of APC by regulating the expression of some surface markers, PGE_2 is involved in modulating the secretion pattern of DC. One of soluble mediator secreted by DC, is IL-12. APC-derived IL-12 is necessary for efficient antigen presentation and T cell activation. It is a major inducer of differentiation of T cells towards the Th1 type, while suppressing Th2 cytokine development. Earlier works showed that IL-10 inhibited the production of IL-12 and the expression of co-stimulatory molecules by various types of DC, which correlated with its ability to inhibit primary alloantigen-specific T cell responses (24, 25). We and other have demonstrated that PGE_2 produced by DC is a potent inhibitor of human and murine IL-12 (7, 26). The inhibitory action of PGE_2 on IL-12 production is mediated by an IL-10-dependent mechanism. The fact that PGE_2 and IL-10 strongly inhibit the production of IL-12 implies a feedback mechanism at the level of the APC, and may represent an important mean controlling the differentiation of APC from bone marrow progenitors or from circulating monocytes. It is clearly established that PGE_2 and IL-12 have opposite effects on induction of Th1 versus Th2 responses. Thus the balance between the secretion of IL-12 and PGE_2 by DC will be crucial in determining whether Th1 or Th2 response will dominate.

The production of other pro-inflammatory cytokines such as IL-6 and TNF-α is differentially regulated by PGE_2 in various cell types (27). In macrophage, IL-6, a cytokine with critical role in maturation of humoral immune response, is positively regulated by PGE_2 (28, 29), whereas TNF-α release is inhibited by PGE_2 (30, 31). We have previously reported that PGE_2 inhibits the production of IL-6 and TNF-α *via* induction of endogenous IL-10 (7, 23). The suppression of TNF-α by PGE_2-induced IL-10 in BM-DC was also established by other investigators (20). We also demonstrated that leucotriene B_4 (LTB_4), a pro-inflammatory metabolite synthesized by the action of 5-Lipoxygenase (5-LO) enzyme, induces the production of IL-6 without any effect on TNF-α (6). Taken together, these results clearly demonstrate the critical role of AA-derived lipid mediators in modulating the DC ability to secrete cytokines.

IL-10, which was first identified as a factor produced by Th2 cells, inhibits cytokine production by Th1 cells, and contributes to the inhibition of accessory function of macrophages and DC (32). IL-10 is also known to enhance the production of IL-1 receptor antagonist (IL-1Ra), which has an effective anti-inflammatory property (11). The inhibitory effects of IL-10 on IL-1 and TNF production are crucial to its anti-inflammatory activities, because these cytokines often have synergistic activities on inflammatory pathways and processes, and amplify these responses by inducing secondary mediators such as chemokines, prostanoids, and platelet-activating factor (PAF). In our recent study, we have reported that endogenous IL-10 plays a crucial role in the regulation of the DC pro-inflammatory phenotype because of its potent action on the production of TNF-α, IL-6 (23). IL-10 exerts its actions through a hetero-dimeric membrane receptor formed by a binding chain (IL-10R1) and a transducing chain (IL-10R2, also known as a CFR2-4), whose mutual interaction activates a series of intracellular signaling molecules, including STAT protein (33, 34). The immunosuppressive and anti-inflammatory activities of IL-10 may be at least in part ascribed to its capability to inhibit NF-κB activity by blocking IκB kinase activity (35). The selective induction of nuclear translocation and DNA-binding of the suppressive p50/p50 homodimer is an important anti-inflammatory mechanism used by IL-10 to suppress inflammatory gene transcription (36).

PGE_2-induced IL-10 is a potent inhibitor of COX-2 pathway and other lipid mediators

Although defined as inducer of immunity by acting on Th1/Th2 balance and the secretion of various cytokines, DC are also important source of immunosuppressive AA-derived lipid mediators, which have an important role in multiple physiologic processes including blood clotting, ovulation, initiation of labor, wound healing, and kidney function.

Among AA-derived eicosanoids, PGE_2 is a potent bioactive molecule synthesized by COX enzymes. The COX enzyme exists in two isoforms: COX-1, a constitutive form that is expressed in multiple cell types and is thought to produce PGs central to physiologic homeostasis, and COX-2, an inducible form that is rapidly up-regulated in response to inflammatory stimuli and is responsible for the production of large amounts of PGs at the inflammation site. Anti-inflammatory cytokines, such as IL-4, IL-13, and IL-10 can inhibit COX-2 induction. Thus, COX-2 represents a potential target in regulating PG synthesis.

In addition to its suppressive action on pro-inflammatory cytokine synthesis, IL-10 is known to inhibit the production of PGs in monocytes/macrophages (37). Moreover, we have recently demonstrated that IL-10 suppresses COX-2 protein expression and PG production in BM-DC (23). Other investigators have reported that endogenously produced IL-10 is a key regulator of COX-2 expression and subsequent PG production (38). In contrast, the cytokine appears to exert no regulatory effect on COX-1 pathway similar to previous reports indicating that COX-1 expression is generally constitutive rather than inducible (39).

IL-10 may regulate COX-2 expression at mRNA and protein levels. COX-2 mRNA expression may be regulated by IL-10 through either direct or indirect means. Since the COX-2 promoter contains two NF-κB motifs (40) that are clearly involved in the regulation of COX-2 expression in both mouse (41) and human (42) macrophage cell lines, direct regulation of COX-2 mRNA may occur at the transcriptional level *via* modulation of NF-κB pathway. A similar mechanism has beeen described for inhibition by IL-10 of inflammatory cytokines *via* down-regulation of NF-κB function (43).

IL-10 can also regulate COX-2 gene expression at the posttranscriptional level by decreasing the half time of COX-2 mRNA. In fact, the 3' un-translated region of COX-2 mRNA contains many copies of the pentamer AUUUA (40), which have been reported to be involved in the mRNA stability and translation (44). For example, it has been reported that exogenous IL-10 may accelerate the degradation of COX-2 mRNA in human monocytes *in vitro* (45).

COX-2 pathway is known to be induced by pro-inflammatory cytokines, including TNF-α and IL-1α (46, 47). In contrast, IL-10 is an important inhibitor of pro-inflammatory cytokine synthesis in LPS-stimulated macrophages (13). Therefore, IL-10 may regulate COX-2 expression and PG production indirectly *via* modulation of the synthesis of pro-inflammatory cytokines.

Other lipid mediators derived from the metabolism of AA are also affected by the endogenously produced IL-10. We have previously reported that endogenous IL-10 inhibits the production of pro-inflammatory LTB_4, which is synthesized from AA by the action of both 5-LO and Five-Lipoxygenase-activating protein (FLAP) enzymes (6). Other investigators have reported that IL-10 may have an important regulatory role in the production of human PAF, another metabolite of AA (48). These findings suggest that autocrine IL-10 may play an important role in inflammatory process through modulation of AA-derived lipid mediators. Taken together, these anti-inflammatory and immunosuppressive effects of IL-10 on the biosynthesis of endogenous mediators by cells of myeloid origin contribute to its central role as "dampener" of inflammation.

Cross-talk between PGE_2 and NO: what do PGE_2 and IL-10 do on DC-derived NO?

PGE_2 and NO are two pleiotropic mediators produced at inflammatory sites by the inducible enzymes, COX-2 and nitric oxide synthase (iNOS), respectively. A variety of cells (e.g., endothelium, macrophages, DCs, chondrocytes) up-regulate both inducible enzyme isoforms and produce NO and PGs simultaneously in response to cytokines and other activators. Because NO synthase (NOS) and COX enzymes produce important mediators of tissue homeostasis and physiological processes, the cross-regulation of these pathways is an important focus of investigation. There are reports on the expression and the cross-regulation of both pathways in macrophages, because macrophages were considered as the major source of NO and PGs. However, little is known in DC. NO, a short lived mediator is synthesized from the amino-acid L-arginin by the enzymatic activity of NOS. There are two isoforms of constitutive NOS, the neuronal, and the endothelial, and one isoform of inducible NOS (iNOS), which is expressed in macrophage and other cell types upon stimulation (19). We previously reported that activated BM-DC produce a vast array of bioactive molecules, among them NO (7). The relevance of NO on DC function has been recently investigated. It has been clearly established that NO has an important regulatory role on monocyte-derived DC phenotype and function (49).

The interaction between COX and NOS pathways has been mainly studied in cells involved in inflammation. Inflammatory cytokines including TNF-α and IL-1 are able to enhance both COX and NOS pathways in cell of the immune system. COX and NOS pathways share a number of similarities. A variety of cells and tissues that produce PGs simultaneously release NO in response to cytokines or other activators. Both of them are potent regulators of the cell functions and mediate intracellular signal *via* cyclic nucleotides (i.e., cAMP or cGMP). In addition COX and NOS require heme as a cofactor and have constitutive and inducible isoforms. Recent published data have demonstrated closer interactions between these two signaling pathways. Although it is recognized that there is "cross-talk" between products of COX and NOS pathways, the literature is divided with respect to whether PGs activate or inhibit NO synthesis and vice versa. For example, NO is reported to activate COX activity in macrophage cell lines (50). In contrast, other investigators have shown that NO inhibits PG synthesis in LPS-stimulated macrophages (51). NO can exert divergent effects on the constitutive and inducible isoforms of COX by activating COX-1 and inactivating COX-2 in murine macrophages (52). Several lines of evidence suggest that

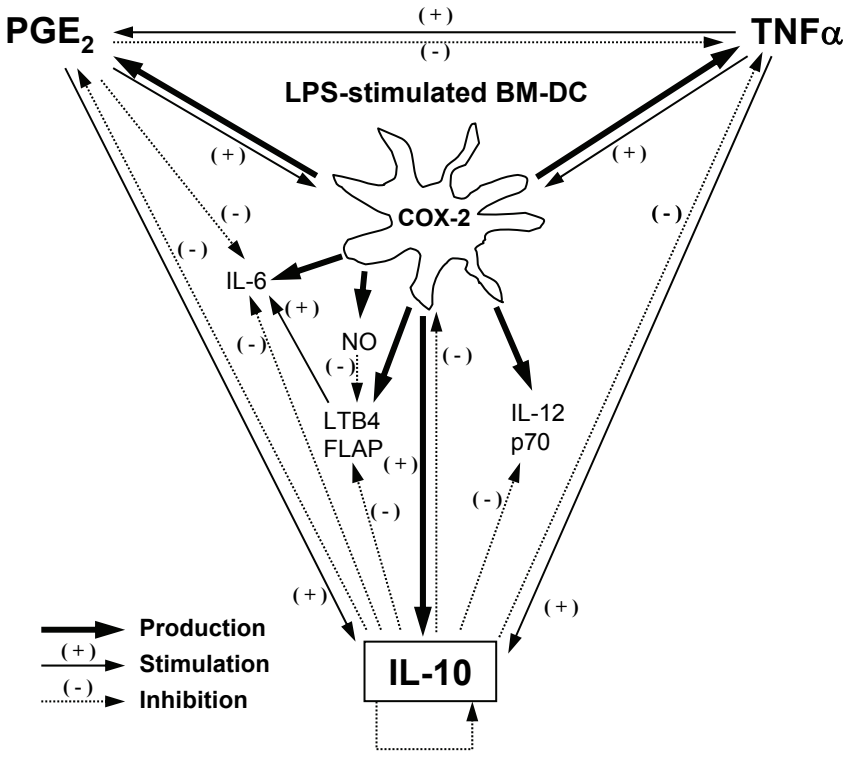

Figure 2. Negative feedback regulation of the cross-talk between eicosanoids, cytokines and NO by IL-10.

PGE_2 can regulate the immune and inflammatory responses by controlling the synthesis of some endogenous mediators such as NO (53). Previous studies reported that PGE_2 was able to inhibit the LPS-stimulated production of NO in J774 macrophages and murine peritoneal macrophages (54, 55). The mechanisms by which PGE_2 inhibits NO production are complex. It has previously demonstrated that PGE_2 potently suppresses macrophage NO production by preventing NF-κB activation (41). The molecular mechanisms by which PGE_2 possibly inhibits iNOS expression could be related to its ability to increase intracellular cAMP levels, which in turn inhibit NF-κB/DNA binding activity.

PGE_2 may regulate NO release indirectly by modulating pro-inflammatory cytokine synthesis. Since TNF-α has been reported to induce iNOS expression and PGE_2 potently suppresses TNF-α production, as we have already reported (23), the inhibitory action of PGE_2-cAMP system on NO production might be secondary to the inhibition of TNF-α generation. In BM-DC, we found that exogenous IL-10 dose-dependently inhibits iNOS protein expression and NO production following 12 h of LPS stimulation (manuscript in preparation). IL-10 represents one mean by which PGE_2 inhibits the production of NO. In contrast, there is no effect of NO donor on COX-2 pathway. Taken together, these data suggest that the "cross-talk" between COX and NOS pathways is therefore complex and will depend on the cell type, *in vitro* experimental conditions, and local environment in which PGs and NO are produced.

Conclusion

The inflammatory responses that initiate DC maturation and function involves soluble mediators, including cytokines, NO, COX products such as PGE_2 and 5-LO products such as LTB_4. Autocrine production of IL-10 is a major determinant of the inhibited release of various pro-inflammatory mediators by APC in particular DC. We have summarized the role of endogenous IL-10 in the crossregulation of eicosanoids, cytokines and NO (Figure 2). Following LPS stimulation immature BM-DC express high levels of COX-2, this is responsible for the production of large amounts of PGE_2. Activated BM-DC also produced TNF-α, IL-6 and IL-12, and other endogenous mediator including NO and LTB_4. TNF-α, may, at least in part, contribute to the induction of COX-2-derived PGE_2. Subsequently, PGE_2 not only prevents the excessive synthesis of TNF-α, but also induces the release of endogenous IL-10. TNF-α may also directly induce IL-10 synthesis by a PG-independent mechanism, and in turn, IL-10 efficiently inhibits the synthesis of TNF-α, IL-6, IL-12, NO, PGE_2, LTB_4 as well as IL-10 itself. By inhibiting various pro-inflammatory endogenous mediators, IL-10 plays central role in regulating the inflammatory responses. In addition, autocrine IL-10 and IL-10R serve as a relevant modulatory loop for the regulation of DC maturation with important consequences on the outcome of the immune response. IL-10 is important in maintaining DC in an immature state. Blocking IL-10R or

IL-10 production by DC may add to DC-based therapeutic strategies aimed at inducing or amplifying Th1 immune responses, as recently shown in murine models (56, 57). A regulatory role of autocrine IL-10 has been described previously for monocytes and macrophages (11, 58). However, it is important to note that IL-10 derived from DC can also affect DC in a paracrine fashion and alter the function of other cell types. For example, this immunosuppressive cytokine may affect the differentiation of T lymphocytes and promote the development of T regulatory cells or increase the effector functions of $CD8^+$ T cells (59). Finally, PGE_2 and IL-10 have important regulatory roles in inflammatory gene expression, and IL-10 may be crucial in controlling inflammatory responses *in vivo*.

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