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Suppression of Antigen Presentation by IL-10

Sharad K. Mittal and Paul A. Roche^{1,*}

¹Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD

Abstract

Regulated antigen presentation to immune cells determines the effectiveness of an immune response. IL-10 is an immunosuppressive cytokine that regulates immune responses by inhibiting the ability of APCs to present antigens to T cells in a variety of ways. The mechanisms of IL-10-mediated immunosuppression include interference in TLR- or IFN γ -mediated dendritic cell (DC) and macrophage activation as well as direct induction of genes that suppress APC function. In this review we will discuss current studies exploring the molecular mechanisms by which IL-10 suppresses APC function.

Our immune system protects us from various invading pathogens, and successful mounting of a CD4-dependent immune response depends on presentation of foreign antigens to CD4 T cells by antigen presenting cells (APCs). DCs are APCs that initiate this process by internalizing foreign antigens, degrading them into peptides that bind to MHC class II (MHC-II) molecules, and displaying peptide-MHC-II (pMHC-II) complexes on their surface for recognition by naïve, antigen-specific CD4 T cells [1]. Macrophages also play an important role during immune responses by degrading pathogens and expressing pathogen-derived pMHC-II complexes for recognition by CD4 T cells [2], however the role of macrophages in priming immune responses has been the subject of considerable debate [3]. These processes take place while APCs undergo an activation (or “maturation”) process that enhances their immunogenicity. Although efficient APC function is required for successful antigen-specific immune responses, suppression of activated APC function is ultimately required to prevent immune-mediated host pathology. IL-10 is one of a family of immunoregulatory cytokines that suppresses APC function, thereby limiting immune response-mediated inflammation [4]. While IL-10 is primarily immunosuppressive to APCs, IL-10 also has immunostimulatory activity on B cells, mast cells, and NK cells [5]. In this review we will summarize studies describing the molecular mechanisms by which IL-10 suppresses antigen presentation by DCs, monocytes, and macrophages.

*To whom correspondence should be addressed: Paul A. Roche; National Institutes of Health; Building 10, Room 4B36, Bethesda, MD 20892. Phone: 301-594-2595; Fax: 301-435-7923, paul.roche@nih.gov.

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Overview of IL-10 Production and Signaling

IL-10 was originally classified as a Th2 cell-specific cytokine with strong ability to suppress Th1 immune responses. However, it is now well appreciated that a wide-variety of T cells (Tregs, Th1 cells, Th17 cells) and APCs (DCs, macrophages, and B cells) are capable of producing IL-10 during the process of an immune response [6]. In addition, interaction with DCs promotes IL-10 production from Tregs [7], B cells [8] and neutrophils [9], thereby suppressing the immune response.

The recognition of various pathogen-associated molecular patterns (PAMPs) by TLR on the surface of APCs activates ERK and ultimately stimulates IL-10 production by macrophages and DCs [10] and B cells [11]. IL-10 binds to a heterodimeric IL-10 receptor complex consisting of the constitutively-expressed IL-10R2 subunit and the activation-induced IL-10R1 subunit. IL-10 signaling activates the Jak-STAT pathway [12]. The immunosuppressive effects of IL-10 in APCs are primarily mediated by phosphorylation of STAT3 [13]. IL-10 signaling also leads to transient (but modest) STAT1 phosphorylation in human monocytes [14], however the role of STAT1 in IL-10-mediated immunosuppression is not clear. Phosphorylated STATs homo- or hetero-dimerize, translocate to the nucleus, and regulate the expression of a number of different genes [15]. Other studies have also revealed that IL-10 activates the PI3K-Akt-GSK signaling pathway, a process that suppresses inflammatory gene expression [16].

In theory, IL-10 signaling results in the expression of effector molecules that could suppress T cell function either indirectly (by interfering with antigen presentation by APCs) or directly (by delivering inhibitory signals directly to T cells by IL-10 signaling mediators). The data showing direct effects of IL-10 on T cells are mixed, with some studies showing suppressing by IL-10 [17] and other studies showing no direct effect of IL-10 on T cell function [18]. In this review, we will focus exclusively on data assessing effects of IL-10 on APC function, thereby indirectly regulating T cell activation.

Effects of IL-10 on TLR-Dependent APC Activation

In their quiescent (non-activated) state, DCs express low levels of pMHC-II on their surface as well as small amounts of the costimulatory molecules CD80, CD86, CD40, which together render resting DCs relatively poor stimulators of naïve T cells [1]. Upon encounter with pathogens, the recognition of PAMPs by TLR on the DC plasma membrane (or in endocytic vesicles) activates intracellular signaling pathways [19] that ultimately “activate” the DC, thereby leading to enhanced pMHC-II complex formation, up-regulation of pMHC-II surface expression, and increased expression of costimulatory molecules on the DC surface. These changes result in enhanced ability of pathogen-exposed DCs to stimulate antigen-specific CD4 T cells that are required to help combat the offending pathogen.

Many mechanisms have been proposed to account for IL-10-mediated suppression of APC function; however, which mechanism(s) is/are at play under different conditions remain to be determined. One major mechanism of immunosuppression by IL-10 lies in the ability of IL-10 to directly suppress TLR signaling [19], thereby globally suppressing APC activation (Figure 1A). There are multiple ways by which IL-10 can attenuate TLR signaling: (a)

miRNA-mediated degradation of TLR signaling components, (b) direct suppression of MyD88-dependent and MyD88-independent TLR signaling, and (C) ubiquitin-mediated degradation of TLR signaling components.

Curtale et al. have recently shown that IL-10 signaling selectively induces expression of the microRNA miR-146b that targets the MyD88-dependent TLR4 signaling component MyD88, IRAK1, and TRAF6, as well as TLR4 itself, for degradation [20]. By contrast, IL-10 signaling inhibits expression of miR-155 [21], a TLR-induced microRNA that targets the phosphatase SHIP1 [22]. SHIP1 is a known inhibitor of MyD88-independent TBK1 activation [23] and enhanced expression of SHIP1 reduces IRF3 translocation to the nucleus. A recent study has shown that this effect is mediated by STAT3-dependent suppression of Ets2, a transcription factor required for miR-155 synthesis [24]. IL-10 also directly suppresses MyD88-dependent TLR signaling by inhibiting NF κ B activation by a variety of mechanisms. IL-10-induced SHIP inhibits the TLR-induced PI3K/Akt signaling pathway, indirectly suppressing NF κ B activation [25]. IL-10 has also been reported to directly inhibit NF κ B translocation to nucleus, however other studies suggest that nuclear translocation *per se* is not affected by IL-10 but that IL-10 signaling promotes the formation of the inhibitory NF κ B p50 homodimer as opposed to the activating NF κ B p65/p50 heterodimer [26]. IL-10 signaling also induces expression of AMP kinase, a kinase known to attenuate TLR-signaling by inhibiting I κ B degradation [27]. In agreement with this, a recent study has shown that AMP kinase-deficient mice have impaired APC function [28]. IL-10 also suppresses MyD88-dependent signaling by promoting the ubiquitin-mediated degradation of IRAK1/4 and TRAF6 [29], however the E3 ubiquitin ligase(s) responsible for this have not been identified.

IL-10 can also reduce the expression of pro-inflammatory cytokines, such as TNF- α , that have direct stimulatory function on APCs. For example, IL-10 induces expression of miR-187, a microRNA that directly suppresses transcription of the pro-inflammatory cytokine TNF- α [30] (Figure 1A). IL-10 signaling leads to the expression of tristetrapoline (TTP), a protein that binds to AU-rich sequences in mRNA and promotes destabilization of TNF- α and IL-1 α mRNAs, thereby reducing inflammation and suppressing APC activation [31]. IL-10-induced DUSP1 suppresses p38 activation and inhibits IL-6 production [32]. It is interesting to note that TLR-signaling via p38 down-regulates TTP expression [31], so IL-10 can not only directly induce expression of TTP but can interfere with TLR-dependent TTP down-regulation.

Effects of IL-10 on IFN γ -Dependent Monocyte/Macrophage Activation and Antigen Processing

Unlike DCs, macrophages and monocytes do not constitutively express MHC-II. It is only after IFN γ -induced activation that these cells synthesize MHC-II and express sufficient amounts of costimulatory molecules that enable them to act as APCs. Like the effect of IL-10 on TLR signaling, IL-10 signaling interferes with IFN γ -induced activation of monocytes (Figure 1B), thereby suppressing IFN γ -induced MHC-II and costimulatory molecule surface expression, pro-inflammatory cytokine production, and monocyte APC function [33]. Treatment of monocytes with IL-10 leads to the expression of SOCS1/

SOCS3, proteins known to inhibit IFN γ -signaling by suppressing STAT1 phosphorylation by Jak2 [34] and limiting expression of IFN γ -dependent gene transcription.

In addition to suppressing the signaling pathways required for APC activation, IL-10 can directly affect molecular mechanisms of antigenic peptide formation and pMHC-II assembly. Chan et al. have also shown that either pretreatment or simultaneous treatment of IL-10 together with IFN γ reduces IFN γ -induced cathepsin S expression in macrophages, thereby suppressing the effective formation of pMHC-II complexes [35]. These authors also found that IL-10 suppresses IFN γ -dependent MHC-II transcription, compounding the inhibitory effect of IL-10 on MHC-II expression [35]. In another study, IL-10 pretreatment of macrophages allowed to phagocytose *Mycobacterium tuberculosis* inhibited the ability of *Mycobacterium tuberculosis*-peptide-MHC-II complexes generated in endo/lysosomal compartments to traffic to the plasma membrane, resulting in poor antigen-specific T cell stimulation [36]. Since the generation of antigenic peptides from phagocytosed proteins is enhanced by TLR signaling [37] and IL-10 suppresses TLR signaling, it is possible that IL-10 also affects antigen processing indirectly by suppressing phagosome maturation, however direct evidence for this has yet to be obtained.

Role of March-I in IL-10-Mediated Suppression of APC Function

Unlike the effect of suppressive effects of IL-10 on the activation process of APCs, published studies have shown that activated DCs [38] [39] and macrophages [40] are refractory to IL-10. In agreement with these findings, we have found that IL-10 does not affect the activation status of mature DCs as determined by high surface expression of MHC-II, CD86, and CD40 and IL-10 does not suppress the ability of activated DCs to express pMHC-II and activate antigen-specific naïve CD4 T (unpublished data). By contrast, IL-10 reduces expression of MHC-II on the surface in monocytes without affecting MHC-II transcription or translation [41]. Instead, IL-10 stimulates expression of March-I [42], an E3 ubiquitin ligase that selectively ubiquitinates MHC-II and CD86 and leads to their post-translational degradation [43] (Figure 2). We have recently shown that most March-I resides in early endosomes, and it is MHC-II complexes in the process of internalization from the plasma membrane that are the primary target of March-I-mediated ubiquitination and lysosomal degradation [44]. Unlike the IL-10-dependent expression of March-I in monocytes/macrophages, March-I is constitutively expressed in resting DCs, and DC activation by any of a number of stimulatory ligands leads to rapid termination of March-I expression in activated DCs [45]. IL-10-mediated down-regulation of MHC-II expression in monocytes/macrophages is wholly March-I dependent, as IL-10-mediated MHC-II down-regulation is completely abrogated in March-I-deficient monocytes [42] and macrophages [46]. Our own unpublished work has shown that IL-10-dependent MHC-II redistribution to lysosomes (observed by Koppelman et al. [41]) as well as IL-10-mediated suppression of activated macrophage APC function is also March-I-dependent, demonstrating a key role of March-I in the immunosuppressive activity of IL-10.

Recent studies have also suggested that the immunosuppressive role of IL-10 in suppressing DC function is mediated by March-I. Tze et al. have shown that CD83 inhibits March-I binding to MHC-II and suppresses IL-10-mediated MHC-II down-regulation in activating

DC cultures in a March-I-dependent manner [47]. Moreover, a more recent study has reported that induced Tregs secrete IL-10 that induces March-I expression in DC, thereby down-regulating MHC-II and CD86 on the DC surface [48]. However, given the known suppressive effects of IL-10 on DC activation, it is possible that the effects observed in these studies were a consequence of impaired DC activation by IL-10, a process that would limit “normal” down-regulation of March-I that accompanies complete DC activation. In agreement with this idea, our unpublished data shows that IL-10 suppresses TLR-dependent DC activation (and March-I down-regulation) and that IL-10-mediated suppression of DC APC function is independent of March-I expression.

Conclusions

In this review we have summarized the mechanisms by which IL-10 can suppress the antigen presentation ability of DCs, monocytes, and macrophages. It is likely that the immunosuppressive effects of IL-10 on APCs are dependent on when the cells are exposed to IL-10. When DCs or monocytes/macrophages are exposed to IL-10 either prior to or concomitant with APC-activation stimuli, IL-10 is able to suppress the activation process in a number of ways (as discussed above) that limit APC function. As mentioned previously, activated DCs appear to be refractory to the effects of IL-10 (at least as far as antigen presenting function is concerned). By contrast, when activated monocytes/macrophages are exposed to IL-10, induced March-I targets MHC-II and CD86 for lysosomal degradation and suppresses APC function not by affecting APC activation *per se* but by limiting expression of key molecules that are important for APC function. Thus IL-10 is a cytokine capable of suppressing immune responses by multiple mechanisms that act differently in distinct APC subsets at specific stages of activation, thereby limiting inflammation-induced host pathology.

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Highlights

IL-10 inhibits T cell activation by suppressing APC function

IL-10 suppresses TLR-signaling in dendritic cells and macrophages

IL-10 suppresses IFN γ -signaling in monocytes and macrophages

IL-10 stimulates expression of the E3 ligase March-I in monocytes/macrophages

Figure 1a

Figure 1b

Figure 1. Suppression of TLR and IFN γ -signaling by IL-10-stimulated gene products

IL-10 signaling leads to the production of numerous gene products (indicated in red) that are capable of suppressing TLR- and IFN γ -signaling in a variety of ways. **A.** The binding of PAMPs to cell-surface (or intracellular) TLR initiates both MyD88-dependent and independent signaling cascades. Activation of the MyD88-dependent pathway leads to the activation of TRAF6 which in turn activates the MAPK pathway (p38, JNK and ERK MAP kinases), the PI3K/Akt pathway, and the NF κ B pathway that lead to nuclear translocation of transcription factors and expression of TLR-stimulated genes. IL-10 signaling leads to STAT3-dependent transcription of miR146b, SOCS1/3, SHIP1, DUSP1, and AMP kinase that interfere with TLR signaling in various ways. miR-146b suppresses expression of TLR4, MyD88, IRAK1/4, and TRAF6 proteins while SOCS1, DUSP1, and SHIP1 suppress

the activities of IRAK1/4, p38, and PI3K, respectively. AMP kinase interferes with NF κ B signaling by inhibiting the degradation of the NF κ B inhibitor I κ B. SHIP1 also suppresses MyD88-independent TLR activation by suppressing TBK1 phosphorylation. IL-10 dependent expression of miR-187 and TTP can affect APC function by suppressing expression of inflammatory cytokines including TNF- α . **B.** The binding of IFN γ to IFN γ -receptor on monocytes/macrophages leads to STAT1-phosphorylation, nuclear translocation of active STAT1 homodimers, and STAT1 binding to gamma-activated sequences (GAS) on IFN γ -target genes. IL-10-signaling leads to the production of SOCS3 that suppresses Jak2-dependent activation of STAT1 in IFN γ -signaled APCs.

Figure 2. IL-10 signaling leads to March-I-dependent down-regulation of MHC-II and CD86 expression in monocytes/macrophages

IFN γ -signaling leads to the activation of monocytes/macrophages, leading to surface expression of MHC-II, CD86, and other molecules important for APC function. IL-10-stimulation induces the expression of the E3 ubiquitin ligase March-I that is ultimately located on early endosomes and possibly on the plasma membrane. Cell surface MHC-II and CD86 encounter March-I during constitutive recycling from the plasma membrane. Oligo-ubiquitination of MHC-II and CD86 by March-I prevents these proteins from recycling back to the plasma membrane and leads to their transport to lysosomes where they are proteolytically degraded.