

New insights into the molecular mechanism of interleukin-10-mediated immunosuppression

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Abstract: Interleukin-10 (IL-10) is an important immunomodulatory cytokine, which has attracted much attention because of its anti-inflammatory properties. It reduces antigen presentation and inhibits T cell activation. IL-10-treated myeloid cells lose their ability to respond toward the endotoxin lipopolysaccharide (LPS) with the production of several proinflammatory mediators. Thereby, IL-10 limits excessive inflammatory reactions in response to endotoxin as it occurs in colitis or endotoxin shock. Mice can be tolerized toward endotoxin shock when pretreated with a sublethal dose of LPS. This can be mimicked in vitro as LPS desensitization, resulting in a similar LPS hyporesponsiveness as observed with IL-10 pretreatment. However, an early block in LPS signaling characterizes LPS desensitization, whereas IL-10 seems to target late events. Controversial reports have been published where IL-10 would interfere with the induction of proinflammatory mediators, and little is known about the molecular mechanisms behind the anti-inflammatory activities of IL-10. Some recent publications have tried to gain more insight into the molecular mechanism of IL-10 by gene-expression profiling and functional studies in myeloid-derived cells. These results are reviewed here and compared with the progress that has been made to understand the induction of endotoxin tolerance by LPS itself. *J. Leukoc. Biol.* 77: 3–15; 2005.

Key Words: IL-10 · endotoxin tolerance · regulatory T cells · gene-expression profiling

INTRODUCTION

A proper balance between pro- and anti-inflammatory mediators is necessary for regulating an adequate immune response toward a pathogen. The proinflammatory response is essential for fighting the pathogen. Conversely, it is absolutely necessary to limit and resolve the inflammatory process to avoid damaging the host itself. An excessive inflammatory reaction can be triggered by pathogen-associated molecular patterns [PAMPS, e.g., lipopolysaccharide (LPS)], as seen in the systemic inflammatory response syndrome or septic shock. Therefore, it is somewhat surprising that there are only two major anti-inflammatory cytokines: interleukin-10 (IL-10) and tumor growth

factor- β (TGF- β), which face the challenging task to limit the induction of a vast variety of proinflammatory mediators. The physiological consequences of IL-10 or TGF- β deficiencies have been demonstrated in gene-targeting experiments in mice; for example, mice lacking IL-10 develop chronic enterocolitis [1] and are hypersensitive toward LPS shock [2]. Mice deficient in TGF- β 1, conversely, suffer from multifocal inflammatory disease [3]. An excess of both cytokines can result in immunoparalysis during septic shock [4]; however, they exploit different modes to exert their anti-inflammatory effects [5]. Peripheral tolerance is induced and maintained by regulatory T cells, which produce IL-10 and/or TGF- β upon antigen stimulation [6]. It has been suggested that IL-10 produced by regulatory T cells may contribute to allergen-specific immunotherapy [7]. Additionally, viral-encoded homologues of IL-10 are part of the immune-evading strategy of latent viruses [8, 9]. Since their discovery, these cytokines have therefore attracted attention by immunologists and clinicians. The anti-inflammatory potential of IL-10 has encouraged clinical trials with IL-10 to treat chronic inflammatory diseases including inflammatory bowel disease, rheumatoid arthritis, and psoriasis. Unfortunately, so far, these efforts have met only limited success [10]. It remains a matter of debate how IL-10 mediates its function on the molecular level. My group reviews here some recent progress toward an understanding of the molecular mechanisms of IL-10 with a focus on the inhibition of endotoxin responses in myeloid-derived cells and indirect suppression of T helper cell type 1 (TH1) cells.

IL-10: MORE THAN A CYTOKINE SYNTHESIS INHIBITORY FACTOR (CSIF)

IL-10 was originally discovered as CSIF produced by TH2 cells, which inhibited TH1 effector functions [11]. Later, it was demonstrated that it could also suppress TH2 responses [12]. More recently, the expression of IL-10 (and TGF- β) as a hallmark of different types of regulatory T cells has been described. However, the significance of IL-10 expression for the control of tolerance is not clarified yet (reviewed in ref. [6]). Most of the inhibition of T cell activation by IL-10 seems to be caused indirectly via suppressing crucial antigen-presenting

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cell (APC) functions [5, 13, 14]. However, direct effects of IL-10 on TH1 and TH2 activation have been ascribed to the suppression of IL-2 production and CD28 signaling [15, 16].

IL-10 also modulates other immune functions. It induces the proliferation of mast cells [17] and thymocytes [18]. Conversely, it suppresses the cytokine production but not preformed mediator release of mast cells upon immunoglobulin E (IgE) cross-linking [19, 20]. Along with its original description as a TH2 cytokine, IL-10 has been shown to costimulate proliferation of B cells and promote their differentiation into plasma cells [21–23]. In contrast, it seems to suppress IgE production in the presence of monocytes [24]. For natural killer (NK) cells, contradictory effects have been described depending on the cellular context. IL-10 inhibits interferon- γ (IFN- γ) production by NK cells in the presence of APCs, partially as a result of a decrease of IFN- γ -inducing cytokines [5, 25]. Conversely, IL-10 can boost IFN- γ production by NK cells when isolated NK cells are stimulated with IL-12 [26, 27].

Myeloid cells are a major source for IL-10 production, and inhibition of T cell activation is, to a great extent, rather indirect via inhibition of APCs, such as monocytes/macrophages and dendritic cells (DCs) [5, 13, 14]. PAMPs (e.g., LPS) can stimulate these APCs to produce a variety of proinflammatory mediators. These mediators, which are essential to mount a full immune response, include cytokines, chemokines, prostaglandins, and nitric oxide (NO). IL-10 antagonizes their induction by LPS to a great extent [28–30]. Thus, most cytokines are inhibited by IL-10, including crucial first-line defense cytokines [as tumor necrosis factor α (TNF- α), IL-1, and IL-6] and TH1-inducing cytokines (IL-12 and IL-18). Additionally, the production of CXC chemokines [e.g., CXC chemokine ligand 8 (CXCL8)=IL-8 and CXCL10] and CC chemokines [e.g., CC chemokine ligand 3 (CCL3) and CCL4], which recruit immune cells to the inflammatory site and activate them, is inhibited by IL-10. Furthermore, cyclooxygenase-2 induction and NO production are prohibited by IL-10 (for a comprehensive review, see ref. [31]).

Although from the above, it may appear as if IL-10 deactivates APCs globally, the situation is more complex, and in fact, IL-10 enhances certain LPS-induced events. Thus, the induction of the IL-1 receptor antagonist (IL-1Ra) is synergistically stimulated by IL-10 [32]. Furthermore, phagocytosis is increased by IL-10 in monocytes [33, 34], a function that is rather associated with inflammation. However, IL-10 reduces antigen presentation at the same time by trapping peptide-loaded major histocompatibility complex type II (MHCII) molecules in late endosomes [35]. Additionally, it reduces the expression of the costimulatory molecules CD80/CD86 [36]. Thus, the increased uptake of antigen does not lead to a stimulation of an immune response but rather serves to clear inflammatory stimuli from the site of inflammation. In fact, IL-10 induces the differentiation of monocytes into a type of macrophage prone to resolve inflammation by clearing bacterial and cellular debris without triggering release of inflammatory mediators. This effect is enhanced by inhibition of the granulocyte macrophage-colony stimulating factor (GM-CSF)/IL-4-driven differentiation of monocytes into DCs and also LPS-stimulated maturation of DCs [37–41]. However, IL-10 does not inhibit already-established, mature DCs, as they do

not express IL-10R1 anymore (ref. [42] and Robert Sabat, Charité, Berlin, Germany, personal communication).

In summary, IL-10 appears to be more than a simple cytokine synthesis-inhibitory factor but rather acts as a pleiotropic immunomodulatory cytokine with activating and deactivating properties. One of its main targets are APC cells, where it down-regulates the induction of proinflammatory mediators by PAMPs.

KNOWING YOUR ENEMIES: LPS-INDUCED SIGNALING

To investigate how IL-10 prevents induction of inflammatory mediators, it is first necessary to understand the molecular mechanism of their induction by PAMPs, e.g., LPS. It was known for a long time that LPS activates mitogen-activated protein kinases (MAPKs) and the transcription factor nuclear factor (NF)- κ B [43]. The latter is kept sequestered in the cytoplasm by its inhibitor of κ B (I κ B). Stimulation with LPS sequentially leads to phosphorylation, ubiquitination, and finally, to proteasomal degradation of I κ B. This allows NF- κ B to be translocated to the nucleus and to bind to promoter regions of genes. The discovery of Toll-like receptor 4 (TLR-4) as the crucial receptor for LPS signaling [44] has boosted a far more detailed investigation into the signaling pathways that mediate this activation (**Fig. 1**). LPS can trigger the so-called Myd88-dependent and -independent signaling cascade depending on the nature of TLR-4-associated adaptor proteins (for a comprehensive review, see ref. [45]). The adaptor proteins Myd88 and TIRAP mediate signaling via IRAK1/4 to TRAF6, which seem to be important to activate early NF- κ B and MAPKs. The adaptor proteins TRIF and TRAM, conversely, are responsible for initiating IRF-3 activation and thereby IFN- α/β secretion in the Myd88-independent pathway. Additionally, this pathway triggers late NF- κ B activation, a process not well understood so far. Myd88-dependent early and Myd88-independent late NF- κ B activation is thought to contribute to the initiation of transcription of most proinflammatory cytokines (e.g., TNF- α , IL-1, IL-6, IL-8, and IL-12). However, there are also slight but remarkable differences in the transcriptional induction of these cytokines. Whereas TNF- α or IL-12 are mainly dependent on the degradation of the NF- κ B inhibitors I κ B α/β , the production of IL-6 seems to be rather dependent on I κ B ζ activation [46] as recently shown. The IFN- α/β secretion triggered by the Myd88-independent pathway induces IFN-response genes, e.g., iNOS and IP-10, via an autocrine loop involving STAT1 activation. Additionally, Btk has been demonstrated to participate in NF- κ B activation and TNF- α production [47, 48]. For full transcriptional activity of NF- κ B, serine phosphorylations of the p65 subunit seem to be necessary. Several pathways have been implicated to mediate this phosphorylation, e.g., the PI-3K/Akt pathway. However, it is still controversial whether PI-3K/Akt has enhancing or inhibitory effects on NF- κ B activity (reviewed in ref. [49]).

Production of proinflammatory cytokines is however not only controlled by transcriptional means. In fact, post-transcriptional mechanisms play a crucial but often underestimated role in the regulation of mRNA stability, protein translation, and

tion, mice lacking the ARE in the TNF- α gene develop colitis because of permanent and uncontrolled TNF- α production [55]. Several proteins, which bind to AREs, are targets of MK2 and have since been implicated in the regulation of TNF- α mRNA turnover (reviewed in ref. [56]). The most prominent ones are members of the TTP family, which are known to destabilize TNF- α mRNA (reviewed in ref. [57]). Mice deficient in TTP show an increased TNF- α production [58]. Phosphorylation of TTP by MK2 allows binding to 14-3-3 protein, which results in stabilization of ARE-containing mRNAs, maybe by sequestering them from transcriptional-silenced stress granules (reviewed in ref. [50]).

Proteolytic conversion of proproteins into the mature cytokine provides another level of control for inflammatory cytokine production. TACE has been implicated to release membrane-bound TNF- α (reviewed in ref. [59]). For IL-1 and IL-18 secretion, a complex inflammasome containing several regulatory proteins and caspase-1 (formerly IL-1-converting enzyme) needs to be activated (reviewed in ref. [60]). This activation is controlled via exogenous ATP, which triggers K⁺-efflux when bound to the P2X7 receptor. Mice lacking the P2X7 receptor suffer from defective IL-1 and IL-18 production [61, 62].

Thus, despite the fact that most proinflammatory are transcribed after NF- κ B activation, the overall control of production for several cytokines is far more complex and includes post-transcriptional and post-translational regulation steps, which can be specific for certain cytokines.

NEGATIVE REGULATION OF LPS RESPONSE: ENDOTOXIN TOLERANCE

As an overwhelming response to LPS can lead to endotoxin shock and death, it is not surprising that nature has evolved several mechanisms to limit this proinflammatory reaction. It is long known that monocytes/macrophages react with a strongly reduced cytokine production upon reexposure to LPS *in vitro* and *in vivo*. This LPS desensitization or endotoxin tolerance seems to be mediated partially by autocrine induction of IL-10 and TGF- β . Neutralizing both cytokines during LPS priming led to a normal TNF- α response after LPS rechallenge [63]. Pretreatment of myeloid cells with IL-10 (and TGF- β) causes LPS hyporesponsiveness [28–30, 63], and injection of IL-10 can protect from endotoxin shock [64]. However, mice deficient for IL-10 can still become endotoxin-tolerant, indicating an additional, intrinsic desensitization mechanism [2]. It soon became clear that desensitizing cells with LPS impaired the signaling for subsequent LPS challenges. The first observation was a screwed NF- κ B activation toward a predominance of p50 homodimers [65]. Transcriptionally active NF- κ B consists of p65/p50 heterodimers, but NF- κ B transcription can be blocked by an excess of p50/p50 homodimers, which lack a transactivation domain but bind to the same recognition site. It is interesting that mice lacking p50 can still activate TNF- α in response to LPS but fail to become endotoxin-tolerant [66]. Later, a total loss of NF- κ B activation and MAPK signaling was observed in endotoxin-tolerant cells [67]. The most obvious explanation for this came from the observation that priming with desensitizing LPS causes a loss of TLR-4 expression on

the cell surface [68]. However, overexpression of TLR-4 and MD2 did not restore signaling events [69, 70]. Since then, several other factors have been suggested to have a negative effect on LPS signaling and cause the observed block in signaling during endotoxin tolerance (reviewed in ref. [45]). Several factors that are induced by LPS inhibit further LPS signaling by interfering with IRAK activation (Tollip, IRAK-M, and alternatively spliced Myd88s), by direct interaction or by preventing IRAK1–IRAK4 interaction. Others TIR domain-containing membrane proteins (SIGIRR and T1/ST2) seem to sequester the adaptor proteins Myd88 and TIRAP.

Very recently, two phosphatases {MAPK phosphatase 5 (MKP5) [71] and SHIP [72]} have been implicated in negative regulation of LPS responses. LPS induces MK5 and SHIP, the latter via an autocrine loop involving TGF- β . Mice lacking SHIP cannot be tolerized for endotoxin anymore [72]. However, whereas Sly et al. [72] reported a hyper-responsiveness in SHIP^{−/−} mice, Fang et al. [73] described exactly the opposite results. Apparently, these results have to be interpreted with some caution, as culture conditions (low or high cell density) seemed to have a major impact on the level of cytokine production in SHIP^{−/−} macrophages, as outlined in the discussion by Sly et al. [72]. Additionally SHIP^{−/−} cells produce far more IL-10 than wild-type cells [72].

The family of SOCS has been described originally to provide a negative-feedback system by interfering with the Janus tyrosine kinase (JAK)-STAT signaling pathway [74]. Mice lacking SOCS-1 show an increased sensitivity toward LPS and a deficient endotoxin-tolerance induction [75, 76]. SOCS-1 deficiency affects Myd88-dependent (TNF- α) and -independent genes (iNOS). The LPS hyper-responsiveness could be a result of a lack of IFN- γ inhibition, a cytokine known to prime for LPS-induced responses and to reverse endotoxin tolerance [77, 78]. However, the same phenotype has been also observed in mice lacking SOCS-1 and IFN- γ [76]. Therefore, it has been suggested that SOCS-1 would interact directly with IRAK1 [75]. Further studies need to explore the precise mechanism behind this phenotype in more detail. While this paper was in proof, the group of P. J. Murray published a study showing, in contrast to earlier reports (see above), the SOCS-1 deficiency neither leads to an alteration of TLR-4 signaling nor is involved in endotoxin tolerance [79]. Rather, as suggested here, it interferes with the autocrine IFN α/β loop and thereby the lack of SOCS-1 causes hypersensitivity to lethal LPS shock.

PROPOSED MECHANISMS FOR IL-10 IMMUNOSUPPRESSION

How does IL-10 target proinflammatory cytokine production?

As neutralization of IL-10 prevents endotoxin tolerance, and IL-10 can induce a similar state of LPS hyporesponsiveness, one would assume that IL-10 would exploit the same mechanisms as observed in LPS desensitization. In fact, the most simple way to inhibit the huge variety of LPS-induced mediators would be to block LPS signaling at an early stage. Indeed, early publications described an inhibition of p38 MAPK acti-

vation [80, 81] and also an inhibition of NF- κ B activation [82–85]. However, other investigators could not confirm these findings (refs. [86–93] and own unpublished observation). Later, it was suggested that IL-10 would rather change the composition of NF- κ B from transactivating p65/p50 heterodimers to inhibitory p50/p50 homodimers [94], as originally described for endotoxin-tolerant cells (see above).

Whereas several publications hint toward an IL-10-induced block of transcription for proinflammatory cytokines [83–86, 93, 95, 96], other observations rather favor a post-transcriptional impact, mainly by mRNA degradation [97–101]. These discrepancies might have to do with the timing of IL-10 treatment. Whereas pretreatment with IL-10 inhibits the promoter region of a reporter construct, a simultaneous incubation of IL-10 with LPS rather initiates mRNA degradation via the 3'-UTR [91]. This is in accordance with the observation that in murine DCs, only preincubation but not costimulation with IL-10 leads to a reduced IKK α / β phosphorylation and thereby NF- κ B activation [102]. Additionally, the different choices of cellular targets might contribute to the conflicting observations. Human monocytes, human DCs, mouse primary macrophages, and several cell lines (THP-1, RAW264.7, J774) have been used in the different studies.

IL-10-induced signaling

What else is known about the molecular mechanism of IL-10-induced anti-inflammatory activity? After binding to the two chains of the IL-10R (IL-10R1 and IL-10R2), activation of the JAKs (Jak1 and Tyk2) occurs, which in turn, leads to the phosphorylation and translocation of the transcription factors STAT1, -3, and -5 [103, 104]. There is growing evidence that STATs are not dimerized by tyrosine phosphorylation but do exist as homodimers already in the cytoplasm [105–109], which need to undergo conformational changes upon activation to get translocated to the nucleus [109]. Another transcription factor reported to be induced by IL-10 in prostate cancer cells is IL-10E1, which binds to the promoter of tissue inhibitor of metalloproteinase-1 [110], but there is no evidence so far that this might be relevant also for immune cells.

There are several IL-10 homologues (IL-22, IL-26, IL-28, and IL-29), which share the IL-10R2 (reviewed in refs. [111, 112]). Nevertheless, there is no evidence for a mutual influence of function among these cytokines. The respective, specific R1 chain is essential for binding each cytokine, and their distribution would suggest that IL-10 is the only cytokine enabled to signal in cells of the immune system [113]. However, the other IL-10 homologues seem to have immunological functions also. IL-22 stimulates IL-10 production in epithelial cells [114] and induces defensins to boost innate immunity in tissues [115]. IL-28 and IL-29 (also called IFN- λ), conversely, improve antiviral responses in nonimmune cells [116, 117].

IL-10 has been reported to trigger PI-3K and thereby Akt signaling without any impact on the anti-inflammatory effect of IL-10 [118]. In contrast, a very recent report suggested the opposite. Bhattacharyya et al. [102] showed that IL-10 would repress PI-3K/Akt signaling and thereby reduce IKK and NF- κ B activation. There is no doubt that STAT3 activation is absolutely essential for the anti-inflammatory effects of IL-10 [119–121]. Furthermore, the C-terminal region

of IL-10RI, which might trigger a yet-unidentified pathway, has been implicated to mediate some cytokine inhibition [122]. Several studies suggested that IL-10 signaling does not interfere directly with LPS signaling but that *de novo* protein synthesis is necessary to mount the anti-inflammatory response by IL-10 [95, 96, 98].

Gene-expression profiling

This has encouraged my group and many others to analyze genes that are regulated by IL-10 by suppression subtractive hybridization [123], serial analysis of gene expression analysis [124], and cDNA- [125, 126] or oligo-based microarrays [127–131] during the last years. The studies of IL-10-immunosuppressive effects have been performed with different cellular targets of human or murine origin: monocytes, macrophages, and DCs. Despite the different nature of these cells, IL-10 inhibits LPS responses in all of them. Genes found to be regulated in all studies should therefore be prime candidates for mediating anti-inflammatory functions. However, the methods of detection and numbers of genes screened for regulation vary between the studies, as does the threshold applied to count a gene to be regulated. Despite these differences, some genes were scored in all (or most) studies (e.g., SOCS-3, PTPN1, versican). Another set of genes seems to be shared rather between human monocytes and murine macrophages (e.g., B-ATF, growth arrest and DNA damage-inducible gene 45 β), whereas others were only detected in murine (e.g., bcl-3, arginase) or human cells [e.g., leukocyte Ig-like receptor 4 (LIR-4), SLAM, pre-B cell colony-enhancing factor]. Thus, a relatively complete picture of IL-10-regulated genes has emerged, giving a broad fundament for further studies with candidate genes for the diverse IL-10 functions.

Does IL-10 induce genes that are known to regulate LPS responses negatively?

It was obvious to look in these studies whether genes that are known to regulate LPS signaling negatively (see above) are also induced by IL-10. However, this is largely not the case. In fact, IL-10 has no effect or rather increases the expression for receptors of LPS (CD14, TLR-4, MD2) [34, 132]. It is surprising that it decreases the expression of MKP5 and SHIP [130].

The only exceptions are two members of the SOCS family: SOCS-1 and -3, which were known to be induced by IL-10 already, before the expression-profiling studies [90, 133–135]. SOCS-3 has been implicated originally in down-regulation of IFN- α and IFN- γ responses [136]. Furthermore, it was demonstrated that it could interfere with IL-4 signaling [90]. Later, it was suggested that SOCS-3 would also be responsible for the IL-10 inhibition of LPS-induced TNF- α [92]. However, viral overexpression of SOCS-3 did not inhibit LPS-induced TNF- α production (ref. [129] and own unpublished observations). Finally, analysis in mice lacking SOCS-3 showed a normal IL-10-induced inhibition of LPS responses [135, 138] regarding TNF- α and IL-12p40 induction. It still would be possible that SOCS-3 might mediate IL-10-induced inhibition of Myd88-independent genes, which largely depend on amplification via type I IFNs and therefore require a JAK-STAT signaling pathway. Unfortunately, data about IL-10-

induced inhibition of IP-10 or iNOS in SOCS-3-deficient macrophages are not available yet. However, IL-10 is a weak inhibitor of Myd88-independent genes [139, 140], and this effect seems to be indirect by blocking IFN- α/β induction by LPS [141].

It is interesting that the absence of SOCS-3 rather affects IL-6 responses [137, 138]. IL-6 has no or weak inhibitory effects on LPS-induced mediators in wild-type mice, whereas it becomes strongly immunosuppressive in the absence of SOCS-3.

As IL-10 induces not only SOCS-3 but also SOCS-1, it would be interesting to investigate whether IL-10 can still inhibit LPS responses in mice lacking SOCS-1. Conversely, IFN- γ , which primes monocytes/macrophages for an increased LPS response and reverses endotoxin tolerance [77, 78] also induces SOCS-1 and SOCS-3 [74]. Thus, what makes the difference between SOCS proteins induced by stimuli, which prime for an increased LPS response, and the ones induced by anti-inflammatory stimuli? This is an interesting question, which remains to be answered.

Another gene that was identified to be up-regulated by IL-10 in most studies is the PTPN1 (PTP1B). This tyrosine phosphatase is located at the cytoplasmic phase of the endoplasmic reticulum and has a broad spectrum of specificity (for review, see ref. [142]). It is well established that tyrosine kinase inhibitors block LPS-induced proinflammatory cytokine production, and it has been described that IL-10 can inhibit tyrosine kinase activity [80]. It is interesting that some publications suggested that the phosphatase inhibitor okadaic acid can reverse endotoxin tolerance [143–146]. It would therefore be interesting to see whether induction of PTPN1 would interfere with any of the described tyrosine kinase pathways triggered by LPS (see above). However, mice deficient for PTPN1/PTP1B are resistant to obesity and diabetes as a result of hyper-responsiveness toward leptin and insulin, but no obvious immunological defect has been reported yet [147].

IL-10-induced heme oxygenase-1 (HO-1)

In addition to LPS and anti-inflammatory cytokines, several exogenous, nonprotein factors can inhibit the production of proinflammatory mediators. In fact, many cyclic adenosine monophosphate- or cyclic guanosine monophosphate-elevating factors [e.g., prostaglandin E₂ (PGE₂), adenosine, and carbon monoxide (CO)] show anti-inflammatory effects. Some of them might be mediated partially by the autocrine induction of IL-10 (e.g., PGE₂ [148] and adenosine [149]), whereas others (CO [150]) work independently of IL-10.

HO-1 is a stress-induced heat shock protein, which generates CO. It has been implicated in an immunosuppressive function, where CO is believed to be the main mediator (reviewed in ref. [151]). Recently, IL-10 has induced HO-1, and it was suggested that this HO-1 induction would mediate the immunosuppressive effects of IL-10 [152]. Others [130, 153] could indeed confirm the induction of HO-1 by IL-10. However, the induction of detectable HO-1 protein arises late in human monocytes (own unpublished observation). In contrast to the original paper by Lee and Chau [152], my group and others could not prevent the anti-

inflammatory effects of IL-10 by the inhibition of HO-1 activity with zinc-II-protoporphyrin-IX, an irreversible inhibitor of HO-1, in human monocytes [130] nor in murine macrophages [153]. This sheds some doubt on the original observation, and the situation needs to be clarified in mice lacking HO-1.

IL-10-induced transcriptional repressors (c-maf, bcl-3, B-ATF)

It was demonstrated that at least some part of the inhibition of proinflammatory cytokines by IL-10 is caused by transcriptional repression (see above). Two important transcription factors, which regulate proinflammatory cytokines induced by LPS, are NF- κ B and AP-1.

IL-10 can induce the formation of inhibitory p50/p50 homodimers as discussed above [94]; however, all investigators do not share this observation [93]. Gene-expression profiling in murine macrophages revealed up-regulation of bcl-3 [127, 129], a gene known to associate with nuclear p50 homodimers. Bcl-3 is a member of the I κ B family but is located in the nucleus and not in the cytoplasm as other members of this family. Its association with p50 could recruit more transcriptionally inactive p50/p50 homodimers to NF- κ B sites (reviewed in ref. [154]). IL-10 or LPS rapidly induces Bcl-3, and overexpression of bcl-3 inhibits LPS-induced TNF- α . Furthermore, mice lacking bcl-3 showed a reduced (but not completely abrogated) inhibition of TNF- α production by IL-10. In contrast, the inhibition of IL-6 induction was not affected at all [129]. Thus, bcl-3 mediates some but not all of the anti-inflammatory activities of IL-10.

AP-1 consists of heterodimers of the fos/jun/ATF family (e.g., c-fos/c-jun dimers). B-ATF has been described as a negative transcriptional regulator of AP-1 [155]. Similar to p50, it does not contain a transactivation domain but has the ability to bind to AP-1-binding sites. Excess of B-ATF should therefore reduce transcription from AP-1 sites. IL-10 induces B-ATF in human monocytes [130] and also in murine macrophages [127], making it an interesting candidate for repression of AP-1-driven cytokines. My group, therefore, overexpressed B-ATF by means of adenoviral gene transfer in primary human monocytes. However, overexpression did not result in repression of LPS-induced TNF- α or IL-6 (**Fig. 3**).

The transcription factor c-maf was reported to be induced by IL-10 in human monocytes [128, 130]. Its overexpression in primary murine macrophages led to a reduced IL-12 and an increased IL-10 production. In accordance with this, mice lacking c-maf showed a reduced IL-10 production. However, IL-12 production was normal after LPS stimulation and was still inhibited by IL-10 in c-maf-deficient mice [128].

Thus, it remains to be clarified which IL-10-regulated genes mediate the transcriptional repression described for IL-10.

Candidates for post-transcriptional effects of IL-10

In addition to the transcriptional repression, IL-10 has been described to destabilize the mRNA of several proinflammatory mediators (see above). Conversely, it is able to stabilize the mRNA of IL-1Ra [156]. Turnover of mRNA is thought to be

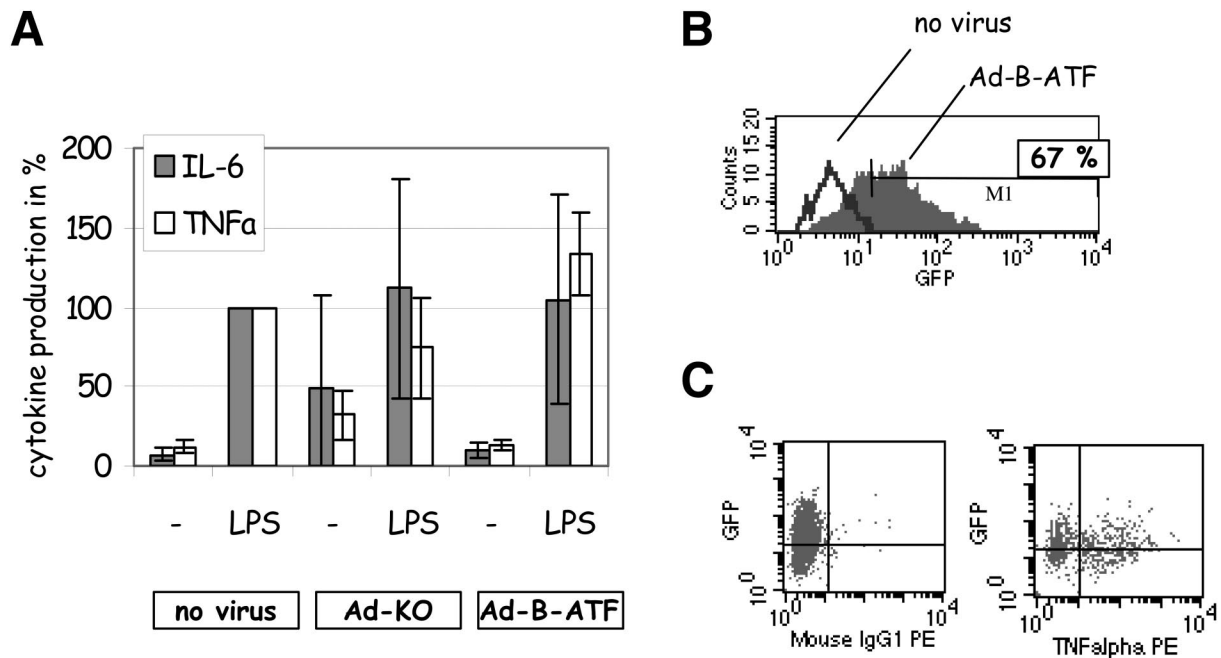


Fig. 3. Overexpression of B-ATF does not abrogate LPS-induced cytokine production. Primary human monocytes were stimulated with M-CSF for 48 h to allow adenoviral transfer and were left untreated or transduced with control virus (Ad-KO) and B-ATF-encoding virus (Ad-B-ATF), respectively. (A) Cytokine production (TNF- α and IL-6) after 6 h stimulation with 100 ng/ml LPS is shown. The response of nontransduced cells treated with LPS was set to 100% in each individual experiment because of donor-dependent differences in cytokine production. (B) Efficiency of adenoviral transduction is shown, which can be followed by green fluorescent protein (GFP) expression linked to B-ATF via an internal ribosome entry site in the adenoviral construct. (C) A representative experiment is shown where my group stained for intracellular TNF- α production (right panel) and strong B-ATF expression as judged by the GFP reporter. PE, Phycoerythrin.

regulated via AREs and the p38 MAPK pathway (reviewed in ref. [56]). Despite some earlier descriptions, it is now widely accepted that IL-10 does not inhibit activation of p38 MAPK (see above). However, it has not entirely been clarified yet whether IL-10 could interfere with the activity of the downstream kinase MK2. IL-10 might affect the binding activity or expression levels of AREBPs. The latter seems not to be the case, as judged by the published data from the gene-expression studies. However, further functional analysis of IL-10-regulated genes with unknown functions and direct binding studies of AREBPs with AREs in IL-10-treated cells are necessary to clarify this point in more detail.

In addition, IL-10 might target later events in cytokine production as proteolytic processing and secretion. In fact, IL-10 does not or only slightly inhibits LPS-induced IL-1 β mRNA levels (refs. [124, 127] and own unpublished observations). This is further evidence that LPS signaling is not inhibited by IL-10, and transcriptional activity is repressed only for some cytokines but not for all. Thus, the post-transcriptional level is important for IL-10 effects; however, clear candidate genes, which mediate this IL-10 function, still need to emerge.

Regulation of antigen presentation by IL-10

IL-10 has been shown to reduce antigen presentation by cytoplasmic retardation of peptide-loaded MHCII and down-regulation of costimulatory molecules. Paradoxically, it increases at the same time as the uptake of antigen by enhancing phagocytosis. This is known to be mediated mainly by the increase of surface expression of Fc-receptors (FcRs; CD16, CD64; re-

viewed in ref. [31]). My group has identified additional receptors for this effect, including a receptor for nonopsonized bacteria (MARCO). Additionally, several signaling molecules (e.g., rhoC), which are involved in actin reorganization, are up-regulated by IL-10 and thereby would support receptor/ligand uptake [130]. Furthermore, the study by Nolan et al. [124] noticed an increased expression of lysosomal proteins, which my group can support by our own observations. This might be a result of IL-10-induced differentiation into macrophages and would favor degradation of antigens. It could thereby lead to a reduced antigen presentation by a total digestion of peptide epitopes. Conversely, IL-10 induces genes of the ILT/LIR family (see below), which can down-regulate FcR signaling [157]. Whether this would avoid that FcR cross-linking triggers a proinflammatory response but allows phagocytic uptake needs to be clarified. Gene products that would regulate vesicular trafficking and thereby retardation of peptide-loaded MHCII in late endosomes have not emerged yet from these studies.

Indirect modulation of T and B cells by IL-10-induced genes

IL-10 inhibits TH1 cells indirectly and is known to be a B cell activator. In this context, several IL-10-induced genes are noticeable. It is interesting that IL-10 seems to induce several members of the ILT/LIR family in human monocytes and DCs [130, 158]. They comprise an extracellular Ig domain, which recognizes MHCII molecules and an intracellular domain containing ITIM motifs for recruitment of SH2-containing tyrosine phosphatase 1 (SHP-1), which down-regulates signaling from

immunoreceptor tyrosine-based activation motif-carrying receptors, e.g., FcRs (for a comprehensive review, see ref. [159]). The expression of ILT3/LIR5 and ILT4/LIR2 has been associated with tolerogenic DCs, which induce anergy upon contact with T cells [158, 160]. The contact between ILT4 and MHCII is necessary to mediate this function [160]. Therefore, an induction of these two membrane proteins in APCs by IL-10 would render them tolerogenic. This is particularly interesting, as IL-10 is produced by regulatory T cells and might explain some open questions about the role of IL-10 in peripheral tolerance. The fact that cell–cell contact is essential for regulatory T cells to render other T cells anergic raised some doubts regarding the role of soluble, anti-inflammatory cytokines in the induction of tolerance. In fact, IL-10 has been described to be essential in the maintenance rather than in the induction of tolerance [161]. It would be intriguing to assume that antigen-specific stimulation of regulatory T cells would induce IL-10 production, which in turn renders the stimulating APC itself tolerogenic by the induction of ILT3 and ILT4. Any other T cells stimulated by this tolerogenic APC would become anergic, which is additionally supported by the IL-10-induced down-regulation of costimulatory molecules. Cell–cell contact is therefore necessary for the induction of tolerogenic DCs by regulatory T cells (T cell receptor activation for production of IL-10) and also for the induction of anergy in T cells, which contact the tolerogenic DC expressing ILT3 and ILT4 (ILT3/4–MHCII contact) [162]. Other factors that have been linked with the induction of tolerogenic APCs, e.g., IFN- α , can induce ILT3 and ILT4 and enhance the effect by IL-10 [158]. The exact mechanism of ILT3- and ILT4-mediated induction of T cell anergy remains to be clarified. It is interesting that a reduced NF- κ B activation and down-regulation of CD80 have been observed in ILT3/4-overexpressing APCs [160]. ILTs can recruit phosphatases such as SHP-1, and it therefore would be intriguing to assume that IL-10-induced ILT3/4 could recruit SHIP, which has been described for negative regulation of LPS signaling (see above).

IL-10 has been reported to shift the T cell response from TH1 to TH2, a reason why it has been used for treatment of TH1-related chronic diseases [10]. My group observed that IL-10 not only down-regulates many cytokines and chemokines that activate TH1 responses but also up-regulates several chemokines that activate TH2 responses [130]. This might support (in addition to the repression of TH1-activating cytokines) the shift to a stronger TH2 response observed in psoriasis patients treated with IL-10 [163]. In addition, CXCL13 is induced by IL-10 in human DCs, which recruit and activate B cells directly [131].

Another interesting factor in this context, which has been detected to be IL-10 induced in human monocytes and DCs, is SLAM/CD150 (reviewed in ref. [164]). SLAM is expressed as a membrane receptor on mature T and B cells and was also detected recently on activated monocytes and DCs. SLAM has been demonstrated to be its own ligand by a low-affinity homophilic interaction. Splicing events lead to expression of a secreted version of SLAM, and the membrane-bound and the secreted form are induced by IL-10 [130]. Soluble and membrane-bound SLAM have been demonstrated to activate B cells

[165], and IL-10-induced SLAM could therefore mediate some of the B cell-activating effects of IL-10 [131].

Originally, it had been observed that T cells incubated with antibodies against SLAM showed an enhanced IFN- γ production and proliferation [166]. Furthermore, antibody engagement enhanced IL-12 production in APCs [167]. This suggested a positive effect by SLAM on TH1 activation. In contrast, later experiments revealed that the opposite might be the case. Patients with X-linked lymphoproliferative syndrome have a defect in the gene encoding for SLAM-associated protein (SAP), an adaptor protein that has been suggested to mediate signaling of SLAM by recruiting SHIP. It is interesting that the IFN- γ production in these patients is elevated [168]. This is supported by the observation that mice deficient for SLAM showed a strongly reduced IL-4 and an increased IFN- γ production [168]. Coexpression of SLAM and SAP leads to a defect in IFN- γ production [168]. This would rather suggest that SLAM favors a TH2 response and that the original experiments in T cells and APCs were a result of an antagonistic instead of an agonistic effect by the antibodies used. Nevertheless, SLAM-deficient mice show a reduced IL-12 and TNF- α production but an enhanced IL-6 production in response to LPS [169], which would support the original observations in APCs and question an immunosuppressive effect of SLAM in APCs. It is interesting, however, that the measles virus uses SLAM as a receptor (reviewed in ref. [170]). It has been suggested that engagement of SLAM by the virus causes immunosuppressive effects on APCs [171]. As discussed above for ILTs, this anti-inflammatory effect could be mediated by the recruitment of phosphatases such as SHIP via SAP.

CONCLUSIONS

Pretreatment with IL-10 or LPS leads to a stage of endotoxin tolerance, where cells do not react with a proper induction of proinflammatory mediators upon stimulation with LPS. However, both stimuli use different molecular pathways to mediate this anti-inflammatory effect. Whereas LPS pretreatment targets early signaling events, IL-10 inhibits rather late events in cytokine regulation. There are still conflicting results about the step of cytokine production, which is indeed inhibited by IL-10. This might be a result of differences in cell types and timing of IL-10 stimulation (pretreatment vs. costimulation). Because different cytokines are induced by slightly different signaling pathways, IL-10 might also interfere at different stages of their induction. Differential gene expression profiling has identified several putative mediators of IL-10 effects. Others have been suggested directly from functional studies. Some of them could be implicated in activating functions of IL-10. The best evidence so far for an anti-inflammatory mediator of IL-10 has been demonstrated for bcl-3. It influences the production of TNF- α partially but seems not to mediate the IL-10 effect on other cytokines. Thus, the gene-expression profiling has provided a strong fundament for understanding the molecular mechanisms of IL-10, but further functional studies are necessary to clarify, in particular, the anti-inflammatory effects of IL-10.

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