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# Role of Interleukin 10 Transcriptional Regulation in Inflammation and Autoimmune Disease

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## Abstract

Interleukin 10 (IL-10) is a cytokine with potent anti-inflammatory properties that plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis. Dysregulation of IL-10 is associated with enhanced immunopathology in response to infection as well as increased risk for development of many autoimmune diseases. Thus a fundamental understanding of IL-10 gene expression is critical for our comprehension of disease progression and resolution of host inflammatory response. In this review, we discuss modes of regulation of IL-10 gene expression in immune effector cell types, including signal transduction, epigenetics, promoter architecture, and post-transcriptional regulation, and how aberrant regulation contributes to immunopathology and disease progression.

### Keywords

immune response; tissue homeostasis; interferon; persistent viral infection; tolerance; interleukin 27

## I. INTRODUCTION

Immune response to pathogens involves the rapid activation of pro-inflammatory cytokines that serve to initiate host defense against microbial invasion. However, excess inflammation can give rise to systemic metabolic and hemodynamic disturbances harmful to the host. As a result, the immune system has evolved parallel anti-inflammatory mechanisms that serve to curb the production of pro-inflammatory molecules to limit tissue damage and to maintain or restore tissue homeostasis.[1,2] Interleukin 10 (IL-10) is a potent anti-inflammatory cytokine that plays a crucial, and often essential, role in preventing inflammatory and autoimmune pathologies.[3,4] Deficiency or aberrant expression of IL-10 can enhance inflammatory response to microbial challenge but also lead to development of inflammatory bowel disease and a number of autoimmune diseases.[5–7] Thus impaired IL-10 expression or signaling can enhance clearance of pathogens during an acute infection, but also exaggerate inflammatory response, resulting in exacerbated immunopathology and tissue damage.[8–10,11] Conversely, some pathogens can harness the immunosuppressive capacity of IL-10 to limit host immune response, leading to persistent infection.[12,13] All in all, Il-10 plays a largely nonredundant role in mediating host anti-inflammatory response and, therefore, identifying the cellular sources of IL-10 as well as the molecular mechanisms that regulate IL-10 expression are



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critical to developing therapeutic strategies directed against pathology-associated impaired IL-10 production.

IL-10 was initially described as a T helper 2 (32)-derived cytokine, however, it was widely accepted that IL-10 is not restricted to certain T cell subsets but instead of produced in almost all leukocytes. [4] *In vivo*, major sources of IL-10 include T helper cells, monocytes, macrophages and dendritic cells, however myriad immune effector cell types are capable of producing IL-10 in certain contexts including B cells, cytotoxic T cells, NK cells, mast cells, and granulocytes like neutrophils and eosinophils.[14–16] Additionally, non-immune effector types such as epithelial cells and keratinocytes are also capable of producing IL-10 in response to infection or tissue damage as well as tumor cells.[17]

IL-10 immunosuppressive activity is mediated by heterodimeric IL-10 receptor (IL-10R1, IL-10R2). Though the IL-10 receptor complex is expressed at varying degrees in myriad cell types, monocytes and macrophages appear to be the primary target of IL-10. Receptor ligation activates JAK/STAT signaling, leading to large changes in the expression profile of immunomodulatory genes,[18] which, in effect, serve to inhibit the release of pro-inflammatory mediators, decrease antigen presentation and phagocytosis, and concomitantly enhance the inhibitory, tolerance, and scavenger functions of these cells. Additionally, through release of anti-inflammatory molecules like interleukin-1 receptor antagonist (IL-1RA), soluble TNFα receptor, and interleukin 27 (IL-27) or via physical interactions with T lymphocytes, IL-10 can directly or indirectly inhibit the development of 31 cells,[3] suppress 32 cell and allergic responses,[19] and enhance regulatory T cell function.[20] In addition, IL-10 can positively enhance activation and proliferation of certain immune cell types, including mast cells, $CD8^+$ T cells, NK cells, and B cells, although the molecular mechanisms and functional consequences of such activity remain to be elucidated.[21–23] Finally, certain pathogens can promote a favorable environment for infection and persistence by expressing IL-10 homologs that bind the IL-10 receptor and exert immunological effects similar to that of the endogenous ligand. This is best characterized in the Epstein-Barr virus encoded IL-10 mimic, BCRF1.[24,25]

Given the powerful anti-inflammatory properties of IL-10 and the consequence of impaired IL-10 function in the development a number of experimental disease models, including chronic inflammatory bowel disease, rheumatoid arthritis, psoriasis, systemic lupus erythematosus, multiple sclerosis, transplant rejection, cancer, as well as various infectious disease models (reviewed in reference 26), there have been focused efforts to ascertain the therapeutic potential of recombinant IL-10 against these diseases. However, ectopic administration of IL-10 has had limited efficacy in clinical settings.[27]

This intimates that an understanding of how IL-10 expression is regulated in a context- and cell-specific manner are important for the development of immune intervention strategies against various pathologies. These include elucidating multiple layers of IL-10 regulation, including signaling in response to different stimuli, epigenetic regulation of chromatin structure of the *IL10* locus, identification of shared or cell-type-specific transcription factors, as well as post-transcriptional mechanisms of gene expression regulation. In this review, we discuss our current understanding of the regulation of IL-10 expression in various cell types, with an emphasis on how dysregulation of IL-10 affects immunopathology in response to infection or development of autoimmune disease.

## II. INTERLEUKIN 10: DISEASE PROGRESSION

The generation of an effective immune response to an infection while also limiting tissue damage requires a delicate balance between pro- and anti-inflammatory responses. IL-10 has potent anti-inflammatory effects and is essential for regulation of immune responses.

However, the immunosuppressive properties of IL-10 can also be exploited by pathogens to facilitate their own survival. Here, we describe the biological effects of IL-10 signaling in the response to pathogens.

## A. The Role of Interleukin 10 During Infection

Initial studies revealed that deficiencies in IL-10, through disruption of the *IL10* gene, or IL-10 signaling, via antibody blockade of the IL-10 receptor (IL-10R) that the majority of intracellular infections are controlled better or cleared faster in the absence of IL-10. Abrogation of IL-10 signaling leads to enhanced survival after infection and is associated with enhanced adaptive immune response, including CD4 T cell IFNγ production (31 response) and sustained production of the pro-inflammatory milieu. In the case of many parasitic infections, such as[1,3] *Leismania donovani*, *Yersinia pestis*, and *Yersinia enterocolitica*, IL-10 is a critical biomarker for poor disease outcome[27]. Furthermore, several pathogens have evolved mechanisms that selectively up-regulate IL-10 during the course of an infection, presumably to create a more favorable microenvironment. For example, *Toxoplasma gondii* is capable of shutting down TLR4-mediated LPS signaling in a manner that specifically blocks TNFα expression but allows for production of IL-10.[28] A similar pattern of expression is observed in patients infected with *Mycobacterium tuberculosis*.[29]

Conversely, while the absence of IL-10 is often initially beneficial to the host, prolonged IL-10 deficiency can often be detrimental in the long term. Enhanced and prolonged production of inflammatory cytokines can lead to septic shock in the context of viral, bacterial, or fungal infections.[30–32] Because inflammatory molecules can often be potent activators of cell death, increasing levels of IL-10 can moderate the extent of apoptosis that is induced in response to infection. For instance, in a *Chlamydia pneumoniae* model, where bacterial clearance is enhanced in the absence of IL-10, mice also develop severe inflammation and experience elevated levels of apoptosis.

Spatial and temporal elements of IL-10 induction are critical features of IL-10-mediated resolution of inflammation. Excessive IL-10 production can inhibit pro-inflammatory response to a number of pathogens, including *Plasmodium spp*,[33] *Leishmania spp*,[34] *T. cruzi*,[35] *Mycobacterium*,[36] and *Lymphocytic choriomeningitis* virus,[12,13] to the extent that pathogens can escape immune control, resulting in either fulminant and rapidly fatal or chronic nonhealing infections. For example, during *Mycobacterium avium* infection, early IL-10 production in Balb/c but not C57Bl/6 mice is correlated with the failure of BALB/c to control infection; ablation of IL-10 signaling led to enhanced pathogen control in BALB/c but not C57Bl/6 mice, demonstrating a causal relationship between IL-10 and the lack of pathogen control.[36] Conversely, ablation of IL-10 signaling during normally benign infections may augment pro-inflammatory responses by enhancing pathogen control at the considerable cost of more severe immunopathology.[8,30,32,37] Importantly, it is often not clear whether elevated concentrations of IL-10 during virulent infections are a cause or a consequence of high pathogen burdens. In the former case, IL-10 would directly inhibit pathogen clearance. This has been demonstrated through transgenic overexpression of IL-10 in antigen-presenting cells that lead to uncontrolled pathogen growth in *L. major*, *Listeria monocytogenes*, and *M. avium* infections.[38,39] In contrast, in scenarios where the invading pathogen is able to resist clearance, IL-10 may be produced to reduce inflammation and thereby minimize pathology. This is reflected during cases of infection by the virulent SD strain of *L. major* that drives excessive 31 response.[40] Feed-forward signaling through IL-10 promotes the development of self-limiting adaptive IL-10-producing T cells that dampen down 31 response.[41] In another case, excessive lung inflammation induced by acute influenza infections are simultaneously incurred and resolved by $CD8^+$ T cells that contribute both pro-inflammatory cytokines as well as IL-10 in measured doses in order to fine tune the extent of lung inflammation and injury associated with influenza infection.

Notably, blocking the action of the $CD8^+$ T cell–derived IL-10 results in enhanced pulmonary inflammation and lethal injury.[42,43]

Taken together, these studies indicate that resolution of infection requires a coordinated response in which initial pro-inflammatory mechanisms clear the pathogen and are subsequently limited by IL-10 before pathology occurs.

### B. Interleukin 10 and Chronic Viral Infections

While the human immune system is specialized and highly efficient in fighting microbial infections, certain pathogens have evolved strategies to escape detection or overcome immune response, rendering them capable of persisting in the host. These predominantly include viruses, such as human immunodeficiency virus (HIV), hepatitis C virus (HCV) and herpes viruses. Interestingly, in a number of these cases, an increase in systemic IL-10 production can be observed.[44–48] Elevated IL-10 signaling can inhibit pro-inflammatory cytokine production through direct targeting of immune effector types, but also indirectly modulate immune function by preventing maturation of macrophage and dendritic cells, thereby limiting co-stimulatory, antigen presentation, and chemokine secretion capacity of the host. In the case of HIV, IL-10 mediates hampering of APC maturation, reduces the efficacy of antigen presentation from these cells, and incurs T cell dependent suppression of anti-viral responses.[49,50] Interestingly, a number of viruses are capable of expressing IL-10 homologs that often bear strong sequence homology with host cellular IL-10. For instance, Epstein Barr virus expresses a viral IL-10 homolog that is 84% identical to IL-10.[51] Furthermore, single nucleotide polymorphisms in the IL-10 region have been associated with natural clearance of HCV in some populations.[52]

Thus it has been proposed that IL-10 may play a role in maintaining persistence and pathogenicity in chronic infections. Proof of this concept is best demonstrated in a model of chronic viral infection using LCMV clone 13. Here systemic IL-10 production coincides with a loss of CTL response directed against the virus.[12,13] Neutralizing IL-10 signaling either through receptor antibody blockade or specific gene deletion led to rapid resolution of persistent LCMV clone 13 infections. Thus in this case, systemic anti-inflammatory response rather than acute deficiencies in antiviral response elicited by LCMV led to chronic viral infection.

### C. Interleukin 10 and Autoimmune Disease

Interleukin 10 was first identified as a molecule that limits inflammation and supports humoral (32) immune responses.[49,53,54] IL-10-deficient animals develop lethal inflammation of the intestine, which can be relieved by ectopic administration of IL-10. [5] Interestingly, IL-10-deficient mice kept under germ free conditions do not develop enterocolitis, suggesting that the biological activity of IL-10 is primarily immunomodulatory.[55]

IL-10 has been implicated in a number of other inflammatory animal models, including experimental autoimmune encephalomyelitis,[56] pancreatitis,[57] diabetes mellitus,[58] and experimental endotoxemia.[59] IL-10 can also play a therapeutic role in various animal models of arthritis, in reducing inflammation, cellular infiltrates, and joint destruction.[60,61] The therapeutic potential of IL-10, though promising, has yet to be fully realized in animal models of autoimmune disease or in clinical trials.[4] Below, we describe a representative number of established models of chronic inflammation in which IL-10 plays a critical homeostatic role. A more comprehensive list of IL-10 associated diseases is presented in Table 1.

**1. Intestinal Bowel Disease—**Intestinal bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal track that manifests either as ulcerative colitis (UC) or Crohn's disease.[62] Though the exact etiology and cause of IBD are still unclear, an important component is a measured but dynamic balance between the host immune system and the composition and integrity of the mucosal epithelia. This requires maintenance of a mutually beneficial symbiotic interaction between the host and the resident bacterial population in two ways:[63,64] First, under normal conditions, the immune system needs to limit host response to resident microorganisms and avoid tissue damage caused by excessive inflammation by restricting luminal commensal microbes from penetrating the epithelial layer. Second, the immune system and local gastroenteric microenvironment should be able to effectively sense and control the invasion of various opportunistic pathogens in the gastrointestinal tract.

The enteric microflora in the gut are necessary for colitis development in IL-10-deficient mice. Thus IL-10-deficient mice raised in a germ-free environment do not develop colitis, and antibiotic administration can prevent onset of disease.[65] The importance of IL-10 in IBD is further corroborated in genomewide association studies that pinpoint the *IL10* gene within the locus of susceptibility for UC.[66] Elevated IL-10 has also been detected in the serum of some human UC patients.[67] Though IL-10 recombinant therapy has demonstrated efficacy in several preclinical models of IBD, in practice, recombinant IL-10 has not demonstrated strong therapeutic potential.[68–70] Multiple factors may contribute to the failure of IL-10 therapy in clinical trials. First, systemic administration may not be sufficient to deliver IL-10 to the mucosal inflammatory sites where it can exert its anti-inflammatory functions. Second, IL-10 derived from $CD4^+$ T lymphocytes, specifically T-regulatory cells but not B cells, is essential for development of colitis in IL-10-deficient mice.[71] In fact, IL-10 produced by macrophages is necessary for the T-regulatory cell–mediated prevention of colitis induced by transferred $CD4^+CD45RB^+$ T cells.[72,73] Further studies suggest that IL-10 may regulate 317 response by restricting IL-23 expression derived from DCs and macrophages.[74] Thus IL-10 therapeutic potential may be influenced by spatiotemporal contexts.

**2. Liver Protection—**IL-10 has been shown to play a protective role in two models of acquired hepatitis induced by Concavalin A or galactosamine and lipopolysaccharide.[75] Anti-IL-10 antibody treatment before administration of ConA leads to the development of a more severe hepatitis as well as elevated expression of IL-12, TNFα, and IFNγ in the serum.[75] Similar results have been observed in IL-10-deficient mice.[76]

**3. Systemic Lupus Erythematosus—**IL-10 functions as a potent B cell stimulator that enhances activation, proliferation, and differentiation of B cells. This relates to SLE, which is characterized by high autoantibody production and decreased cellular immune responses. In SLE, high levels of autoantibodies generate immune complexes that exacerbate tissue damage. Compared with healthy individuals, levels of IL-10 in SLE patients are significantly higher and there is a correlation between IL-10 levels and clinical manifestation.[77] Depletion of IL-10 by anti-IL-10 antibody *in vitro* treatment of SLE patient–derived PBMC significantly decreased autoantibody production.

**4. Allergic Asthma—**In animal models of asthma, IL-10 was shown to be capable of inhibiting allergen-induced airway inflammation and non specific responsiveness.[8] The protective activity of IL-10 remains to be clarified. Though IL-10 gene transfer to the airway abrogated cellular and physiological recall response *in vivo*, IL-10 did not prevent expansion or activation of T cell subsets.[79] A relative deficiency in IL-10 production has been observed in alveolar macrophages of atopic asthmatic patients.[80,81] Effective clinical

therapies involve shifting 32 host response towards a 31 phenotype. This modification leads to a decline in allergen- specific IgE and an increase in allergen- specific IgG production. In addition, IL-10 levels are elevated coincident with T cell anergy.[82]

## III. TRANSCRIPTIONAL REGULATION OF INTERLEUKIN 10

Interleukin 10 is an anti-inflammatory cytokine that plays a crucial role in preventing inflammatory and autoimmune pathologies.[7,83] Elevated levels of IL-10 can hinder host response to microbial pathogenesis and prevent resolution of associated tissue damage and hemodynamic disturbances. In contrast, deficient levels of IL-10 can lead to development of autoimmune disease and enhanced tumorigenicity.[3] Consequently, there remains a need for a comprehensive understanding of the cellular processes that contribute to regulation of IL-10. Importantly, the biological effects of IL-10 expression are highly contextual. Thus to decipher critical steps in IL-10 gene regulation requires consideration of the local versus systemic production, the type of stimulus, tissue specificity, and individual genetic variation.

### A. Interleukin 10 Promoter Structure

The human IL-10 gene spans about 4.7 kb on chromosome 1q21-32 and contains 5 exons and 4 introns. The murine IL-10 locus spans 5.1 kb on chromosome 1E4 and is organized in similar fashion. Importantly, the sequence identity and location of putative transcription binding sites are well conserved between the species, which suggests that the mechanisms of transcriptional regulation are similar. The proximal promoter of IL-10 in both humans and mice are characterized by a TATA box ~90 bp upstream of the translation start site (−98 bp in mice) and a CCAAT box (CCAGT in mice) located at −237 bp, upstream of the translation start site (−244 bp in mice). Primer extension studies have localized the transcription start site to approximately −57 bp upstream of the translation start site (−66 bp in mice).

A number of transcription family members, including specificity protein (Sp), signal transducers and activators of transcription (STAT), interferon regulatory factors (IRF), activator protein (AP), cAMP response element binding protein (CREB), CCATT enhancer/ binding protein (C/EBP), c-musculoaponeurotic fibrosarcoma factor (c-MAF), and nuclear factor κ-B (NF-κB), have been characterized as essential or critical factors in IL-10 regulation. The majority of these studies were carried out in macrophage cell lines and/or primary macrophage cells derived from peripheral blood, spleen, or bone marrow,[84–90] which may confound extrapolation to other cell types. Collectively, these factors are ubiquitously expressed in both immune and nonimmune effector cell types and play pleiotropic roles in regulating myriad factors in the inflammatory milieu, in addition to IL-10. Thus while these studies contribute to our general understanding of IL-10 transcriptional regulation, there remains work to be done to elucidate the contributors to tissue-specific IL-10 gene regulation in contextualized settings. In this section, we describe sets of commonly annotated transcription activators of IL-10 as well as an emerging group of tissue- or cell-specific IL-10 regulators.

**1. Specificity Factor—**Specificity factor (Sp) activators represent one of the few essential regulators of IL-10 promoter activity through binding of a G-rich region of the IL-10 promoter located −183 bp (−163 bp in mice) upstream of the translation start site.[84,85,91] A demonstrative requirement for Sp transcription factors in IL-10 activation was first shown in *Drosophila* SL2 cells that lack endogenous Sp protein expression, and mutation of this site results in complete ablation of IL-10 promoter activity.[84–86] Here, IL-10 promoter activity was ablated, but could be restored by introducing Sp1 and/or Sp3.[92] An additional Sp binding site was identified in the human IL-10 promoter but is absent in the murine IL-10 promoter −636 bp upstream of the translation start site.[93] Interestingly, this region is also the

site of a common SNP associated with a number of autoimmune diseases, including rheumatoid arthritis, systematic lupus erythamatosus, and inflammatory bowel disease[94–96] as well as changes in tumorigenesis and transplantation tolerance.[97] A SNP change from A at −627 to C renders Sp1 as a repressor potentially through interactions with E-twenty-six (Ets) family members.[93] However, the physiological manifest of this polymorphism does not coincide with any disease state.

**2. Signal Transducers and Activators of Transcription—**Signal transducers and activators of transcription (STAT) transcription factors play a critical role in IL-10 induction in both myeloid and lymphoid cell types.[84,89,98,99] In T cells, STAT1 and STAT3 are critical for IL-27-induced IL10 gene expression, whereas STAT3 is essential for IL-6-mediated IL-10 production.[98,100] Similarly, our lab has shown that IL-27 directly up-regulates IL-10 transcription through activation and recruitment of both STAT1 and STAT3 to the IL-10 promoter in bone marrow–derived macrophage.[84,101] While there exist proximal STAT binding sites at −740 (−714 mouse) and −154 (−130 mouse) relative to the translational start site, and each shows putative functional capacity in cell-based reporter assays, direct binding of STAT proteins to *cis* elements within the IL-10 promoter has yet to be observed.[84,99] In addition, the role of STAT proteins is obscured by the fact that IL-10 itself can drive its own expression in an autocrine manner in many immune effector types through activation of STAT3.[1,102] Furthermore, studies of IL-10 transcriptional regulation in naïve or activated T lymphocyte subsets derived from STAT-deficient transgenic mice lines can be compromised by dual functionality of STAT complexes in both the regulation of inflammatory molecules in addition to the differentiation process itself.

**3. c-Musculoaponeurotic Fibrosarcoma—**c-Musculoaponeurotic fibrosarcoma (c-MAF) was originally described as a 32 cell– specific factor via its ability to activate Il-4 and suppress IFNγ and subsequent 31 function.[103,104] However, more recently, c-MAF has emerged as a potentially universally required transcription factor for IL-10 transcription via its ability to transactivate IL-10 promoter activity through binding of a consensus MAF recognition element (MARE) located 233 bp upstream of the translation start site (−350 bp in mice). c-MAF has been implicated in expression of IL-10 in 32, 31, and 317 and Foxp3-regulatory T cells (Tr1) and is activated in an ERK-dependent manner.[105–107] In macrophages, c-MAF is constitutively expressed and is mobilized to the IL-10 promoter upon TLR stimulation.[84,108] Importantly, though essential, c-MAF alone is not sufficient to induce IL-10 expression.

**4. Activator Proteins—**Activator proteins (AP-1) consist of heterodimers containing c-Fos, c-Jun, activating transcription factor (ATF), and Jun dimerization partner ( JDP), and activate transcription through binding of tissue plasminogen activator (TPA)-response element (TRE). An association between AP-1 activity and IL-10 production has been shown in T cells and monocytes/macrophages.[109,110] Furthermore, AP-1 proteins may play a more definitive cell- specific role, through binding a regulatory element located 6.45 kb downstream of the IL10 translational start site to regulate IL-10 production in 32 but not 31 cells.[111,112] This complements a study showing that AP-1 activity is essential for 32-mediated IL-10 production.[113]

**5. CCAAT/Enhancer Binding Proteins—**CCAAT/enhancer binding (C/EBP) proteins function as homo- or hetero-dimers and serve to activate IL-10 transcript generation through two distinct sites −475 and −463 upstream of the translation start site.[87] Importantly, C/EBP activity serves to function in macrophages in both a TLR-dependent and -independent manner. In response to TLR ligands, like LPS, C/EBP proteins accumulate in the nucleus and appear to synergize with Sp1 to enhance IL-10 transcription. However in response to

cAMP stimulation through A2A adenosine receptor or via *E. coli* infection, C/EBP was able to activate the IL-10 promoter in a TLR4- and MyD88-independent manner.[88] These studies highlight the complexity of IL-10 transcriptional regulation and emphasize that functional studies of IL-10 regulation are context dependent.

**6. Interferon Regulatory Factors—**Interferon regulatory factors (IRFs) consist of a nine-member family of transcription factors that mediate regulation of interferon target genes in addition to genes involved in host response to pathogenesis and tissue damage, by binding specific DNA motifs such as the interferon sensitive response element (ISRE) and the gamma activation sequence (GAS). A number of putative IRF motifs have been located within the IL-10 promoter; however, the extent to which IRFs regulate IL-10 expression remains controversial. Ziegler-Heitbrock et al. demonstrated in a human B cell line that type I interferons upregulate Il-10 expression through IRF1 but not IRF2 binding to a regulatory element located 212 bp upstream of the translation start site.[89] However, in macrophage cells, in response to LPS, which leads to IL-10 production via TLR4 mediated type I interferon signaling, both the *cis* regulatory element and IRF activity are dispensable.[89] However, other innate immune receptors, such as retinoic acid inducible gene-I (RIG-I), utilize IRF3 to induce IL-10 expression in response to Epstein Barr virus–derived RNA molecules.[114] In addition, IRF4 has been posited to play an important role in 32-mediated IL-10 production.[115]

**7. cAMP Response Element Binding Protein—**Human IL-10 is responsive to cAMP stimulation in THP1, a human monocytic leukemia cell line, and macrophage cells.[87,116,117] There are at least four putative cAMP response element (CRE) targets within the human IL-10 promoter with corresponding murine homologs (−1265, −1055, 862, 411 bp upstream of translation start site) that play a role in adiponectin or zymogen stimulation of IL-10,[110,118] although no single site is absolutely essential. CREB binding is accompanied by recruitment of CBP/p300, a histone acetyl transferase, and accordingly, CREB activation is associated with hyperacetylation of histone N-terminal tails at the IL-10 promoter, though no cause and effect relationship has been demonstrated.

**8. Nuclear Factor-kappa B—**Five DNA binding members of the nuclear factor-kappa B (NF-κB) have been identified: p65/RelA, c-Rel, RelB, p50, and p52. Classically, NF-κB plays an integral role in the regulation of inflammatory cytokines. However, the role of NF-κB in IL-10 regulation remains controversial. Initial studies in human monocyte–derived macrophages demonstrated that overexpression of IκBα, which inhibits NFkB/p65 translocation into the nucleus, had little effect on TLR-induced IL-10 expression, despite inhibiting other pro-inflammatory cytokines like IL-1β and TNFα.[119] However, stimulation of murine macrophages with double-stranded RNA leads to recruitment of NF-κB to a site 925 bp upstream of the translation start site through activity of PKR.[120] An additional putative NF-κB site has been localized to 124 bp upstream of the translation start site and mediates binding of p50 homodimers implicated in basal IL-10 expression.[121] Subsequent studies have suggested that NF-κB may play a role in regulating early steps in IL-10 production through interactions with distal enhancers at the IL-10 locus. An LPS-dependent DNase I hypersensitivity site was identified 4.5 kb upstream of the translation start site, and p65 binding was verified through both EMSA and ChIP techniques.[106] Importantly, this site is conserved between both human and murine macrophage cells. However, the implications of p65 recruitment to this site in relation to temporal regulation or kinetics of IL-10 induction remain to be elucidated.

**9. GATA3—**In addition to being a master regulator of 32 differentiation, GATA3 plays a crucial role in IL-10 induction in this T helper subtype.[122,123] GATA3 is specifically

recruited to a site 86 bp upstream of the translation start site, but alone cannot transactivate the IL-10 promoter.[122] Instead, it has been proposed that GATA3 binding initiates chromatin remodeling events, and its activity is correlated with hyperacetylation of the IL10 promoter and enhanced IL10 expression. This appears to be a cell type– specific mechanism, as other T helper subtypes, such as 31, do not express GATA3 and thus initiate IL-10 via independent mechanisms.[106,124]

**10. HOX Family Transcription Factors—**Pre-B-cell leukemia transcription factors are members of the HOX family of homeodomain containing transcription factors that contribute to body patterning and development. Specifically, PBX1 in conjunction with PREP1 regulates several developmental programs, including hematopoiesis, skeletal patterning, and organogenesis.[125] Both factors are implicated in the expression of IL-10 in murine macrophages.[126] In response to apoptotic and perhaps necrotic cells, p38 signaling leads to PBX1 and PREP1 recruitment to a putative apoptotic cell response element (ACRE) in the IL10 promoter that is conserved between human and mouse (163 bp upstream of the translation start site).[126] In this context, IL-10 induction was correlated not with phagocytosis but instead scavenger function, implicating IL-10 in regulating tissue restoration and/or homeostasis.

**11. BLIMP1—**BLIMP1 was first identified as a transcriptional repressor that functions to regulate differentiation of plasma cells.[127,128] In addition, BLIMP1 activity is required for maintaining T cell homeostasis, as mice generated with specific BLIMP1 deficiencies accumulate activated T cells and develop severe immunopathologies including colitis and lung inflammation.[129,130] Blimp1 exerts influence in T cell development by inhibiting differentiation and expansion of 31 and T follicular helper cells[131,132] and promotes expansion of cytotoxic $CD8^+$ T cells and memory T cells in the context of viral infections.[133,134] Recently, BLIMP1 has been implicated in resolving inflammation by directly activating IL-10 expression in T regulatory cells and $CD8^+$ cytotoxic lymphocytes.[43,135] In T regulatory cells, BLIMP1 appears to bind to a region within intron 1 of the IL-10 locus, and binding correlates with active marks of transcription, such as loss of H3K27 trimethylation and increased histone pan-acetylation at the IL-10 promoter.[135] During influenza infections, $CD8^+$ T cells represent a primary source of IL-10 production in the respiratory tract and are required for effective viral clearance.[11] Induction of IL-10 is primarily regulated by IL-2 and IL-27 signaling derived from $CD4^+$ T helper cells and innate immune effector cell types, respectively. In this system, conditional BLIMP1 deficiency leads to decreased IL-10 production by $CD8^+$ T cells and enhanced pulmonary inflammation.

**12. Proliferator-Activated Receptor Gamma Coactivator-1 Alpha—**Studies of IL-10 transcriptional regulation have been predominantly restricted to immune cell types. However, it is well documented that IL-10 can be expressed in a wide variety of cell types, including keratinocytes, epithelial cells, and tumor cells.[136] Deficiencies in IL-10 expression or activity are associated with increased hepatic insulin resistance and sustained chronic liver disease.[136,137] Recently, PGC-1α, a transcriptional co-activator known to play a role in lipid and carbohydrate metabolism and storage in the liver[138] was shown to play a role in IL-10 up-regulation in rat hepatocytes in response to fatty acid stimulation.[139] Transcription activation was mediated via an interaction between PGC-1α, c-MAF, and NF-κB p50 subunit and subsequent recruitment to the IL-10 promoter.

### B. Interleukin 10 Epigenetic Regulation

Studies of IL-10 regulation have reinforced a view that inducible transcriptional activation requires a confluence of signaling events activated through both direct mechanisms as well

as amplified through indirect autocrine/paracrine signaling leading to coordinated recruitment of transcription factors assimilated on the IL-10 promoter platform, resulting in transcription initiation. Epigenetic modulation and chromatin remodeling events represent a critical step in initiating, sustaining, and resolving transcription at the IL-10 locus. Classically, inducible regulation of inflammatory molecules involves the presence of functional enhancers that serve to initiate, propagate, and fine tune transcript generation.[140] These elements can be located coincident to the site of transcription initiation (i.e., the promoter) but are often found at distal sites or in intronic regions within the gene locus itself. Furthermore, enhancers can facilitate tissue- specific regulation of gene expression.[141] Given the paucity of cell type– specific transcription factors (exceptions being GATA3 in 32 lymphocytes, and BLIMP1 in T regulatory and $CD8^+$ T lymphocytes) that regulate IL-10, a comprehensive survey of cell type– specific chromatin architecture supporting the IL-10 locus can significantly contribute to our understanding of the dynamics of IL-10 transcription up-regulation. For example, epigenetic mechanisms in non-dividing cells, such as macrophages and dendritic cells, may confer only transient influence over IL-10 expression, whereas in lineage committed T cells, epigenetic regulatory mechanisms can be sustained and propagated by clonal expansion to generate IL-10 at both local and systemic levels.

A number of studies have suggested that the expression of IL-10 is regulated by changes in the structure of chromatin at the *IL10* locus.[106,111,112,142] Critically, epigenetic imprinting appears to exert differential regulation in a cell type–dependent manner. For instance, enrichment of active marks of transcription such as acetylation of histone tails have been observed in high IL-10 producing 32 cells but not in low IL-10 producing 31 cells.[123] In contrast, specific phosphorylation of histone H3 appears to be an important regulatory step in IL-10 production in macrophages.[143,91] Moreover, a number of DNase I hypersensitive sites (HSS) have been identified in both T lymphoctyes and macrophage cell types.[106,112] DNase I HSS regions often correlate with depletion of nucleosomes and can indicate the presence of enhancers, locus control regions, matrix attachment regions, or insulator/ boundary elements.

**1. Epigenetic Regulation in Macrophages—**A macrophage-specific DNase I hypersensitive site was identified ~4.5 kb upstream of the translation start site that is exposed upon stimulation with different TLR ligands, including LPS, CpG, and Zymosan A.[106] This element has been shown to mediate direct NF-κB/p65 binding and is associated with hyperacetylation of histones in this region. This site has been shown to function as a *de facto* enhancer in reporter assays. Additional HSS have been localized to regions 0.12 kb and 2 kb upstream of the translational start site as well as 1.70 kb downstream. The sites at 0.12 kb and 2 kb are also found in unstimulated T lymphocytes.[111,112] However, the functional implications of these sites remain to be clarified.

In macrophages, phosphorylation of serine 10 on histone H3 (H3S10) correlates temporally with the initiation of IL-10 gene transcription.[143] H3S10 phosphorylation occurs downstream of ERK activation, and multiple ERK activators can enhance macrophage IL-10 production, including TLR ligands and FcγR cross-linking.[91] H3S10 phosphorylation is a shared mechanism for activation of inflammatory cytokines and is correlated with recruitment of NF-κB.[144] However, in the case of IL-10, H3S10 phosphorylation promotes rapid recruitment of Sp1 to the IL-10 promoter, which is followed by minor changes in histone H3 acetylation. It appears as though H3S10 phosphorylation in macrophages is the predominant epigenetic mark for transcriptional activation of the IL-10 gene because IL-10 mRNA levels are not dramatically affected by inhibitors of histone deacetylases.[143]

**2. Epigenetic Regulation in Th2 Lymphocytes—**It has been proposed that GATA3 functions as a regulator of IL-10 expression in murine 32 cells by binding to and initiating changes in the chromatin structure in the IL-10 locus.[111,122,123] This is based on studies showing that overexpression of GATA3 leads to increase in histone H3 and H4 acetylation marks at the IL-10 promoter in 32 cells. Importantly, enrichment of these marks occurred independent of IL-4 signaling, suggesting that GATA3 activation of IL-10 is independent of its regulatory role in 32 differentiation and maintenance.[122] Furthermore, in reporter assays, it was shown that though GATA3 can bind directly to the IL-10 promoter, it fails to transactivate IL-10 promoter activity in the absence of chromatin. Thus it appears GATA3 may function as a regulator of chromatin remodeling.

In addition, a 32 specific DNase I hypersensitive site was identified ~6.4 kb downstream of the translation start site in response to PMA/ionomycin stimulation.[111] Importantly, PMA/ iononmycin activity does not activate the IL-10 promoter in reporter assays, suggesting a regulatory role for chromatin in IL-10 transcript production. Moreover, this HSS region is specific to 32 cells and is not found in 31 or macrophage cell types.[111,123] PMA/ionomycin induction leads to recruitment of Jun proteins ( JunB, c-Jun) to the 6.4-kb site.[111]

**3. Epigenetic Regulation in Other Cell Types—**To date, our understanding of epigenetic regulation in most cell types remains stark. Some studies have revealed the presence of HSS regions in IL-10 producing T regulatory cells upon activation at 0.12 kb upstream and 1.65 kb and 2.98 kb downstream of the translation start site. However these regions are common to most T cell types and macrophages, and are unlikely to contribute to cell-specific regulation.

### C. Negative Regulation of Interleukin 10 Expression

Excess production of Il-10 can lead to enhanced pathogenesis. Thus critical understanding of resolving or silencing Il-10 expression may be valuable for the development of therapeutic strategies against such maladies. Alternatively, increased activity of negative regulators leading to decreased IL-10 production can lead to excessive inflammation, the development of autoimmune disease, and susceptibility to chronic infection. Thus negative feedback loops are an essential component to achieving a balance between effective immune response and immunopathology. Here, we discuss modes of negative regulation of Il-10 in innate and adaptive immune cells. From a mechanistic view it is often difficult to distinguish direct means of IL-10 inhibition from those that occur indirectly through autocrine/paracrine signaling. This is paramount when considering studies that target specific depletion of IL-10 regulators through knockout transgenic, siRNA mediated knockdown or pharmacological methods and observe enhanced IL-10 expression. For that reason, we focus mainly on transcription factors that have been shown to directly relieve IL-10 transcription.[145]

**1. Interleukin 10 Silencing Mechanisms in Innate Immune Cells—**The MHC class II transactivator (CIITA) has been shown to negatively regulate IL-10 production in bone marrow– but not spleen-derived dendritic cells.[146] Here, CIITA-deficient DCs displayed enhanced basal and LPS induced expression. Re-introduction of CIITA expression suppressed IL-10. Furthermore, CIITA was shown to directly regulate the IL-10 promoter through reporter assays. Poly (ADP) ribose polymerase 1 (PARP-1) has recently been shown to restrict IL-10 expression in macrophage/monocytes that function to engulf apoptotic cells, but not in response to LPS activation. In conditions of severe sepsis or hemorrhage, PARP-1 has emerged as a central regulator of systemic inflammation.[147] Interestingly, PARP-1 regulation of IL-10 occurs in a haplotype-dependent manner that may contribute to our understanding of IL-10 promoter polymorphism effects on IL-10 expression variability.[146] Specifically a −1082 G/A polymorphism (relative to the translational start site) represents a

highly penetrant polymorphism associated with variable IL-10 production and susceptibility to sepsis.[148] Specifically, individuals homozygote for 1082G experience elevated levels of IL-10 and increased susceptibility to sepsis and endotoxin shock than −1082 AA counterparts. PARP-1 was shown to specifically bind to the −1082 A haplotype in reporter assays and inhibit IL-10 promoter activity, thus providing an insightful model that may explain variability of IL-10 expression in the population at large.[137,146] In addition, peritoneal macrophages derived from B cell lymphoma 3 (Bcl-3)-deficient mice exhibit enhanced IL-10 production.[149] However, in this case, it is unclear whether Bcl-3 directly inhibits IL-10 transcription due to a lack of binding to the IL-10 locus.

**2. Interleukin 10 Silencing Mechanisms in Adaptive Immune Cells—**Critical insights on negative regulation of IL-10 expression may be revealed in studies of high IL-10 producing (32, Treg, Tr1) versus low IL-10 producing lymphoctyes (31, 317). One potential candidate is the ETS family of transcription factors. ETS-1 deficiency leads to elevated production of IL-10 in 31 and prevention of colitis in lymphopenic mice.[150] Currently, it is unclear whether ETS-1 directly inhibits IL-10, as no ETS-1 binding to the IL-10 locus has been observed, though a number of putative binding sites exist. Furthermore, Ets1 deficiency leads to loss of the 31 regulator T-Bet, which also has been shown to negatively regulate IL-10.[151] T-Bet deficient cells infected with *M. tuberculosis* experienced elevated IL-10 production. However, in this case, T-Bet deficiencies also led to loss of IFNγ production, which has also been shown to negatively regulate IL-10, and thus the IL-10 phenotype could reflect a blockade in the 31 developmental program.

### D. Post-Transcriptional Regulation of Interleukin 10

Modulation of inflammatory response involves a coordinated system of events that initiate, sustain, and resolve inflammation. Factors that modulate the initiation phase and the resolution phase of inflammation can determine the strength and duration of inflammatory response. These include mechanisms of transcriptional regulation as discussed above, but also require post-transcriptional mechanisms that serve to regulate transcript stability, translation, and post-translational mechanisms, including covalent modification and secretory regulation.[152,153]

Like many inflammatory transcripts, IL-10 mRNA is subject to rapid decay after synthesis. For instance, though IL-10 transcripts can be detected within a few hours after LPS stimulation in macrophages, protein accumulation occurs with severely delayed kinetics.[154] In addition, studies of LPS-mediated IL-10 gene expression in IFNαR- or IL27R-deficient macrophages have revealed defects in transcript generation but complete abrogation of protein accumulation, suggesting that these pathways also contribute to mRNA stability, translation, and decay in addition to exerting effects on transcription initiation.[84,101]

IL-10 mRNA transcripts contain clusters of adenosine- and uridine-rich elements (AREs) localized to the 3′ untranslated region (UTR) and thus belong to a class of inflammatory cytokines and chemokines subject to ARE-mediated decay mechanisms.[155–158] IL-10 mRNA contains a long segment of 3′UTR (1033 bp in humans, 702 bp in mice) that when deleted can extend the half-life of newly synthesized transcripts from 1 h to >12 h.[155] Further analysis revealed that IL-10 transcripts contain class II AREs consisting of a cluster of 6 AUUUA pentamers that promote mRNA instability in reporter assays. Transcript decay is mediated through interaction with the zinc finger binding protein tristetrapolin (TTP), which has been shown to regulate a number of inflammatory molecules, post-transcriptionally.[154] TTP bridges transcript targets to mRNA decay machinery, which includes mRNA de-capping proteins, exosome endonuclease activity, and RNA-induced silencing complex (RISC) members.[159] IL-10 mRNA decay rate is reduced in primary

macrophages obtained from TTP knockout mice relative to control littermates.[155] TTP-mediated mRNA decay can be relieved through activity of p38-MAPK-activated 2 (MK2) leading to phosphorylation of TTP at Ser 52 and Ser 178, recruitment of 14-3-3 proteins that have been proposed to block the interaction between TTP and the mRNA decay machinery.[160,161] Thus the MAPK pathway contributes to both IL-10 transcription initiation as well as post-transcriptional regulation to promote and sustain IL-10 production. Paradoxically, TTP-deficient mice develop arthritis, dermatitis, and cachexia, a syndrome caused by overexpression of pro-inflammatory cytokines, so the implications of IL-10 post-transcriptional regulation via this pathway remain uncertain.[162,163]

IL-10 transcript stability can also be positively and negatively regulated through microRNA activity. TTP binding alone to ARE is insufficient to initiate IL-10 mRNA decay but instead requires recruitment of mIR16 which contains sequence complementary to AREs.[164] Cooperative activity of this TTP/microRNA complex facilitates RNAi-mediated gene silencing. In myeloid and lymphoid cells, miR106a was shown to recognize sequences in the IL-10 3′UTR localized to 4451 to 4478 downstream of the translation site, leading to decreased mRNA stability in reporter assays.[165] Conversely, up-regulation of miR466I in response to TLR stimulation extends IL-10 transcript half-life by competitive inhibition of TTP binding sites.[166]

MicroRNAs can also indirectly regulate IL-10 protein expression. It was shown that the tumor suppressor PDCD4 targets translational machinery in myeloid cells to inhibit translation of IL-10 transcripts.[167] PDCD4-deficient mice exhibit resistance to several models of inflammatory disease, including EAE- and streptozotocin-induced type II diabetes, presumably through enhanced IL-10 expression in these animals. Recently, it was shown that this mode of regulation is relieved in LPS-stimulated macrophages through induction of miR-21, which targets PDCD4 transcripts for RNAi-mediated silencing and consequently amplifies IL-10 production in macrophage cells.[168]

The level of post-transcriptional regulation of IL-10 expression might explain why despite the existence of common pathways in IL-10 induction, distinct cell types exhibit differential IL-10 production. For instance, miR106a is not expressed in regulatory T cells, and its selective absence may contribute to elevated IL-10 expression in this cell type.[169]

## IV. POLYMORPHISMS IN THE INTERLEUKIN 10 PROMOTER

IL-10 has been shown to influence both the susceptibility and course of various diseases, and the different polymorphisms in the IL-10 gene promoter have been associated with disease prevalence and severity. In fact, some studies have inferred that 50%–70% of the observed variability of IL-10 secretion can be explained by genetic factors.[170,171] The human IL-10 gene is located on chromosome 1q31-32, a locus genetically linked to susceptibility to a number of automimmune diseases, most notably SLE.[172] A number of polymorphisms have been identified within the Il-10 locus, including 23 single nucleotide polymorphisms (SNPs) localized to the promoter alone.[146] Though most SNPs are in linkage disequilibrium, it is reasonable to assume that some may play biological roles in regulating IL-10 expression.

Three SNPs in particular have been reported to play an important causal role in regulating IL-10 promoter activity. These are SNPs situated at positions −1082, −819, and −592 relative to the translational start site.[173] Variants at −819 and −592 are in linkage disequilibrium and thus are inherited together. Thus there exist three major haplotypes at the −1082, −819, and −592 (GCC, ACC, and ATA), corresponding to high IL-10 producing, moderate producing, and low producing individuals.[174–176] To date it has been difficult to determine the exact relationship between IL-10 genotype and the corresponding cytokine

production.[177,178] However, recent work suggests that affinity of the −1082G allele for Sp1 is much stronger than the −1082A allele, which may contribute to higher levels of IL-10 production in B cells.[179] Differential expression at the −592 SNP may be mediated by greater affinity of PARP-1 for −592C versus A allele, leading to enhanced IL-10 expression.[126] Additionally, the promoter also contains two microsatellites, IL-10R and IL-10G, positioned approximately 1.1 and 4.0 kb upstream of the transcription start site.[180,181] Five IL-10R alleles spanning from 12 to 16 CA repeats and thirteen IL-10G alleles spanning from 16 to 28 CA repeats have been described. The Il-10R and IL-10G alleles are not randomly distributed between the SNPs but combine to form haplotype families.[182] Haplotype frequencies do vary between ethnic groups. For instance the frequency of GCC at positions −1082, −819, and −592 is above 50% in Caucasians but below 5% in populations of Asian descent.[173,176,183–186] Thus studies gauging a relationship between SNP frequency and disease incidence and quantifying penetrance of specific haployptes has been difficult. Most likely, combinations of different polymorphisms into haplotypes rather than single SNPs or microsatellites contribute to variability in IL-10 production and response to infection or autoimmune disease etiology.

### A. Interleukin 10 Promoter Polymorphisms and Infectious Disease

Some evidence exists that links *IL10* gene polymorphism and severity of illness.[187] In community acquired pneumonia, which is a major cause of morbidity and mortality worldwide, patients with −1082 GG haplotype experienced more severe symptoms and pathology and increased risk for septic shock than those with −1082GA or −1082AA genotypes corresponding to lower levels of IL-10 production.[187,188]

Conversely, haplotypes associated with higher Il-10 production confer increased susceptibility to viral persistence and chronic viral infection. A study comparing patients with aggressive acute EBV infection with healthy blood donors, divided into EBV-seropositive and EBV-seronegative populations, revealed that EBV-seronegative blood donors were more likely to carry the −1082G allele than other groups investigated.[189] Studies of cytomegalovirus and herpes zoster virus showed an association between ACC and ATA haplotype and seronegativity, respectively,[190,191] whereas no association between herpes virus infection and IL-10 gene polymorphisms was found. Furthermore, in chronic hepatitis B virus infection, the frequency of ATA haplotype has been reported to be significantly higher in asymptomatic carriers than in patients with chronic progressive liver disease.[192] A comprehensive study of hepatitis C patients revealed an association between the −592AA genotype and restricted infection, while the −1082GG genotype was associated with persistent infection.[193] IL-10 polymorphisms are also associated with increased susceptibility to HIV and subsequent progression to AIDS. In this case, individuals carrying the −592A allele have increased risk for HIV infection and, once infected, progress to AIDS more rapidly than those carrying the −592GG homozygotes.[194,195]

### B. Interleukin 10 Promoter Polymorphisms and Autoimmune Disease

Although myriad studies have attempted to elucidate a relationship between IL-10 SNP variability, IL-10 production, and susceptibility to and progression of autoimmune disease, no associations between these features have been robustly defined. There appears to be some degree of coincidence between polymorphisms associated with reduced levels of IL-10 production and severe forms of asthma, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), graft-versus-host disease (GVHD), and survival after bone marrow transfer.[196–199,200] A number of factors can potentially confound studies discerning this relationship, including the distribution of alleles across demographics, etiology of disease, as well as the influence of epistatic or downstream targets of IL-10 that may mask potential associations between IL-10 SNPs and disease phenotypes. In particular, autoimmune

diseases are often characterized by deficiency of IL-10 production or signaling concomitant to enhanced expression of pro-inflammatory molecules.

This is readily apparent in genetic linkage studies in SLE. The IL-10 gene is situated in a major SLE susceptibility locus;[172] however, no single SNP or IL-10 microsatellites have shown significant association with the development of SLE,[201,202] with conflicting relationships found between different ethnic groups.[203] However, SLE demonstrates strong association with specific variants localized to the *TNF*α locus.[204] When *TNF* genotypes were considered, there appeared to be strong correlations between IL-10 genotypes and the presence of auto-antibodies directed against nuclear antigen (ANA), a characteristic feature of SLE.[178] Specifically, the highest production of ANAs were found in individuals with a combined genotype "low IL10 [I-1082AA-AG]/high TNFα (-308AA-AG)." Further studies may delineate multi-locus susceptibility allele variants that strengthen the relationship between IL-10 production and autoimmune progression. Finally, with an increased understanding of IL-10 epigenetic regulation, a few studies have emerged linking chromatin marks at the IL-10 locus and potential disease risk. Here, it was shown that peripheral blood monocyte cells (PBMCs) derived from patients with rheumatoid arthritis contained characteristic hypomethylation of CpG sites within the proximal promoter of the IL-10 gene, 145 bp upstream of the transcription start site, and associated with elevated IL-10 expression.[198] When cells taken from healthy patients were treated with 5-azacytidine and CpG motifs were subsequently demethylated, allowing for recruitment of phospho-CREB, the expression levels of IL-10 transcript and protein were significantly increased in these PBMCs.

## V. CELLULAR SOURCE OF INTERLEUKIN 10

Regulation of interleukin 10 occurs in a contextualized manner. That is, the mechanism of transcript initiation depends on the stimulus, locale, and cell type. These factors also influence the strength and duration of IL-10 signaling. Though innate immune effector cell types, primarily of myeloid and lymphoid origin, represent a primary and well-studied source of IL-10, other cell types, including γδT-cells, natural killer cells, mast cells, granulocytes, as well as epithelial cells, keratinocytes, hepatocytes, and even tumor cells have shown capacity for IL-10 expression.[205] Given, the pleiotropic effects of IL-10 biological function, understanding tissue-specific mechanisms of IL-10 transcription regulation is paramount to harnessing its potential as a therapeutic agent against chronic infection and autoimmune disease. Here we describe mechanisms of IL-10 induction in major immune effector cell types.

### A. T Lymphocytes

Based on a large body of evidence, T cells are thought to be the main source of IL-10 *in vivo* and serve to deliver IL-10 at the site of inflammation to mediate tissue homeostasis and curb immune response. Although, IL-10 was initially described as a 32 cytokine, it is now widely accepted that Il-10 is expressed by subsets of all $CD4^+$ T helper populations, including 31, 32, 317, and regulatory T cells.[206,207] Importantly, animal models of infectious and autoimmune disease have demonstrated that the source of IL-10 within the T-cell subsets plays a critical role in maintaining homeostasis. For instance, Il-10 deficient mice exhibit exaggerated inflammatory responses in the intestinal mucosa and develop inflammatory bowel disease (IBD).[5] Further analysis showed that development of IBD was not a function of global IL10 deficiency but rather, conditional deletion of Il10 in $Foxp3^+$ T regulatory cells was sufficient to dysregulate inflammation in the gut, skin, and lung.[208] Models of *Toxoplasma gondii* infection implicate 31 as the predominant source of Il-10 that play a critical role in controlling collateral damage associated with the 31 response to this intracellular pathogen.[124]

IL-10 inducing signaling cascades have been studied less thoroughly in 3 cells than in macrophages and DCs. Stimulation of IL-10 expression can occur through one of three mechanisms: (1) instruction by APCs, (2) induction by IL-12 family cytokines, or (3) alternative means. T cell receptor (TCR) and endogenous Il-12 have been shown to be essential for the differentiation of IL-10 producing 31 cells as well as for maximal expression of Il-10 following re-stimulation of these cells.[106] Il-10 induction in 31 cells is STAT4 and ERK dependent. In 32 cells, Il-10 production appears to be regulated by 32 conditioning factors including IL-4, STAT6 and GATA3.[122,123,209] IL-10 expression in 317 seems to occur in a STAT3 and STAT1 dependent manner.[98,100] Unlike in myeloid cells, T-cell activation of Il-10 appears to be ERK but not p38 dependent.[210] Finally, both IL-21 and Il-27 can enhance Il-10 expression in 31, 32, and 317 cells.[98,100,211–213] Both Il-21 and IL-27 induce ERK activation, and Il-27 can further enhance Il-10 expression through STAT3 activation.[214]

### B. Regulatory T Cells

Natural T regulatory cells are constitutively produced in the thymus and express very high levels of CD25 and the transcription factor Foxp3. They require Il-2 for both their maintenance in the periphery and for the production of IL-10.[215,216] However, additional factors that induce IL-10 expression by $Foxp3^+$ Tregs including transforming growth factor β (TGFβ) have been shown to be required *in vivo.*[217] Still, IL-10-producing natural Tregs have been implicated in maintaining the balance between pathogen clearance and immunopathology against viral, bacterial, and fungal infections.[218–222]

In contrast, the emergence of antigen-driven $Foxp3^-$ Il-10-producing T cells with activity that is distinct from naturally occurring Treg cells has been implicated in preventing immune-mediated pathology. Several populations of adaptive Treg have been defined, including IL-10-producing Tr1 cells, TGFβ-producing 33 cells, and a number of populations of regulatory $CD8^+$ T cells.[38,223] In particular, Tr1 cells have been associated with clearance of *Bordella pertussis* and *Streptococcus pyogenes* infections.[224,225] These cells produce Il-10 but not IL-2, IL-4, or IFNγ, and can be generated *in vitro* using various stimuli, such as cytokines (TGFβ, IL-10, IFNα) or immunosuppressive drugs (vitamin D3, dexamethasone)[83,226,227] or *in vivo* by repeated stimulation with soluble antigen.[228]

### C. Innate Immune Cells

Antigen-presenting cells (APCs) are an important source of IL-10 that serves to provide autocrine feedback to limit or resolve pro-inflammatory molecule production, restrict antigen presentation itself, enhance scavenger and phagocytic capabilities, and influence the development of adaptive responses. Most notably, monocytes, macrophages, and myeloid but not plasmacytoid dendritic cells provide robust sources of IL-10 from the APC lineage.[2,229] A clear role for myeloid-derived IL-10 in mediating the anti-inflammatory response to endotoxin was demonstrated in mice deficient for IL-10, specifically in myeloid cells.[230] Similarly, the skin irritation response to tetradocanoyl-phorbol acetate (TPA) is enhanced in animals deficient for IL-10 or myeloid-specific IL-10-deficient mice, but not mice deficient in T cell–derived IL-10.[231] IL-10-producing APCs have distinct biological features and gene expression profiles, which are known to have a profound influence on the differentiation, maintenance, and function of various T-cell subsets.[232] The developmental activity of high IL-10 expression–low inflammatory cytokine expression macrophage subsets can lead to alteration or selective enhancement of adaptive immune responses.[233] Thus myeloid-derived IL-10 can have an important influence on adaptive immune response to bacterial infections, such as *Leishmania major*, as well as autoimmune progression, such as experimental autoimmune encephalomyelitis (EAE).[234–236] Moreover, a number of pathogens have harnessed the Il-10-producing capabilities of myeloid cells to promote

infection. For instance, $CD8^{+-}$ DCs are a potent source of IL-10 during lymphocytic choriomeningitis virus infection, directly inhibiting viral control.[12] Similarly, modulation of TLR2 signaling by *Schistosoma mansoni* lysophosphotidylserine or by *Mycobacterium tuberculosis* can lead to DC production of IL-10 and the induction of IL-10-secreting T regulatory cells that inhibit effector T-cell activity and reduce parasite control.[237,238] Finally, sustained triggering of TLR4 or TLR9 leads to TLR tolerance, and, as with repeated administration of LPS, IL-10 appears to induce the differentiation of adaptive Tregs.[239–241]

A number of distinct but overlapping signaling pathways are required for IL-10 induction. In addition, a number of post-transcriptional, translational, and secretory regulatory mechanisms exist to fine tune IL-10 production downstream of transcription initiation. When studying regulation of IL-10 expression it is important to consider both the stimulus and kinetics of induction in relation to other inflammatory cytokines. In general, productive Il-10 production in innate immune cells occurs relatively late compared to the initial pro-inflammatory response, and thus robust protein production is often detected several hours after the stimulus is added. This suggests that elaboration of IL-10 expression is subject to a number of feedback signaling modules that serve to dampen early expression and amplify later expression relative to the composite temporal inflammatory gene profile.

IL-10 expression in innate immune cells is primarily induced through the activity of pattern recognition receptors (PRRs) that specifically recognize pathogen-derived products and/or intrinsic “danger” molecules, which trigger the expression of target genes that facilitate APC, phagocytic, anti-microbial. and scavenger function as well as stimulate a cascade of signaling events leading to cellular infiltration of the inflammatory milieu to the site of infection or tissue damage (reviewed in reference 242). Importantly, both macrophages and DCs can express IL-10 following activation of specific PRRs.[224,230,243,244] These include products derived from Gram-positive and Gram-negative bacteria, viral particles, and other potential ligands that trigger activation of Toll-like receptors (TLRs) located on the cell surface or compartmentalized in endosomes, intracellular PRRs including RIG-I and Nod2-like family of receptors, C-type lectin signaling, or ligation of CD40 and Fc receptors.[233,245–247]

TLRs can initiate distinct innate immune responses through recruitment of different adaptor family members, primarily myeloid differentiation primary-response 88 (MyD88) and TIR-domain-containing adaptor protein–inducing IFNβ (TRIF).[242] Currently at least 11 TLRs have been cloned in mammals, and each receptor is involved in the recognition of a unique set of PAMPs. For example, TLRs 3, 4, and 9 recognize double-stranded RNA, lipopolysaccharide (LPS), and bacterial DNA motifs (CpG), respectively.[242] Although all TLRs share the evolutionarily conserved TIR domains, the amino acid sequences within the TIR domains of various TLRs are divergent, which provide opportunities for individual TLRs to recruit different MyD88 family members for different cellular responses. These adaptors function as platforms to organize downstream molecules into signaling complexes, leading to activation of multiple signal cascades and eventually resulting in specific cellular responses against different types of pathogen. Work from our lab and others has identified two major TLR signaling pathways: MyD88-dependent activation, utilized by all TLRs (except TLR3), which transmits signals culminating in NF-κB and MAP kinase activation, resulting in the induction of inflammatory genes such as TNFα, IL-6, and IL-1β; and the TRIF-dependent pathways, utilized by TLR3 and TLR4, involving the induction of type I interferons (IFNs) and secondary response genes activated by IFNβ in an autocrine/paracrine manner.[248–250]

Classically, type I IFNs are characterized by their potent ability to “interfere” with viral replication. However, more recently, this family of cytokines has been implicated not only

in host defense against viral infections, but also for its potential immunomodulatory effects on both innate and adaptive immune cells.[251,252] The type I IFN family mainly consists of multiple IFNα members and a single IFNβ. Induction of IFNs by both TLR and RIG-I pathways requires the TANK-binding kinase 1 (TBK1) or inducible IκB kinase (IKKi), essential for activation of IRF3 and IRF7, which control transcription of type I IFN. TLR3- and TLR4-dependent IRF3/IRF7 phosphorylation and IFN induction require the adaptor protein TRIF, whereas other TLRs, including TLR2, TLR7, TLR8, and TLR9, do not robustly induce type I IFNs in macrophage cells in most contexts. In addition to TLRs, intracellular receptors such as RIG-I and MDA-5 can also lead to IRF3/IRF7-mediated type I IFN induction through adaptor molecules like CARDIF (also known as IPS-1, MAVS, or VISA).[242,248] Binding of IFNs to the type I IFN receptor (IFNαR) results in the rapid autophosphorylation and activation of the receptor-associated JAKs TYK2 and JAK1, which in turn regulate the phosphorylation and activation of STAT transcription factors, predominantly STAT1 and STAT2 in myeloid cells.[253] Activated STATs subsequently form homodimers, heterodimers, or complexes together with IRF9 and translocate to the nucleus, where they initiate transcription by binding specific sites on the promoters of IFN-stimulated genes.

While a large subset of IFN target genes have proposed anti-viral function, type I IFNs are capable of exerting immunomodulatory effects on both innate and adaptive immune cells. In fact, systemic administration of IFNβ has been used to treat patients with a number of autoimmune diseases,[254,255,257–259] For instance, IFNβ therapy has shown efficacy against multiple sclerosis, leading to decreased inflammatory lesion formation in the CNS, prolonged remission, and lower relapse rate. In the case of MS, the interplay between inflammation and neuronal degeneration most likely contributes to the initiation and progression of tissue damage incurred within compartments of the central nervous system. Therefore, it has been proposed that type I IFNs can limit inflammation through potent induction of anti-inflammatory molecules, including IL-10. In contrast, the type II IFN, IFNγ, which abrogates IL-10 expression, appears to exacerbate MS symptoms.[236,256–260]

Although it is well established that IL-10 is induced in innate immune cells in response to TLR stimulation, the differential utilization of MyD88- and TRIF-mediated pathways have profound impact on both the kinetics and level of IL-10 expression. Presumably, this may be a reflection of tailored immunological response specific to interactions between TLRs and their respective PAMPs. Here, we present two models of IL-10 induction mediated through TLR activation.

**1. MyD88 Activation of ERK and MAPK Leads to IL-10 Induction—**Stimulation of IL-10 in macrophages or DCs stimulated with *Mycobacterium tuberculosis* or with lipopeptides and the LcrV antigen of *Yersinia pestis* primarily activate the TLR2-MYD88 cascade, leading to MAPK activation and IL-10 transcription (Fig. 1A). The MAPK cascade is composed of three major groups of kinases: extracellular signal-regulated kinases (specifically ERK1 and ERK2), Jun N-terminal kinases ( JNKs), and p38.[261] ERK activity has been shown to be essential for IL-10 production due to defects in IL-10 expression in the presence of chemical inhibitors to ERK or in ERK-deficient cells.[262–265] Interestingly, differences in IL-10 production by macrophages, myeloid DCs, and pDCs have been shown to correlate with the strength of ERK activation in each of these cell types.[265] IL-10 expression can also be compromised by inhibition of p38 signaling in LPS- or CpG-activated macrophages that recognize TLR4 and TLR9, respectively.[86,263,266] Primary cells lacking the p38 regulator dual-specific ity protein phosphatase 1 (DUSP1) have prolonged p38 activation and increased levels of IL-10 expression following TLR stimulation, which can be reversed by chemical inhibitors to p38.[267–269] Interestingly, abrogation of either ERK or p38 activation leads to a reduction, but not abrogation, of IL-10 expression, which

suggests that these two pathways may cooperate in TLR-induced IL-10 production. The production of IL-10 by macrophages and DCs is also regulated by the activation of certain inhibitory pathways. ERK- and p38-dependent Il-10 production is inhibited by IFNγ.[110] IFNγ also induces the release of glycogen 3-kinase (GSK3) by antagonizing phosphoinositide 3-kinase (PI3K)-AKT activation. This leads to inhibition of TLR-induced IL-10 production by suppressing the binding of activator protein 1 (AP1) to the IL-10 promoter.[270]

**2. TRIF-Mediated Activation of IL-10 Involves Sequential Induction of Type I Interferons and Interleukin 27—**The MyD88 pathway serves to mediate inflammatory gene induction for the majority of TLRs. TLR4 uniquely utilizes both MyD88 and TRIF to regulate induction of pro-inflammatory cytokines and type I IFNs. Interestingly, while the MyD88 pathway does contribute to regulation of IL-10 transcripts, induction of IL-10 mRNA and protein by TLR4 ligands, such as lipopolysaccharide (LPS), has an absolute requirement for the adaptor, TRIF.[84,101,236] Given the function of IL-10 as an anti-inflammatory molecule that acts to dampen initial pro-inflammatory response through auto-crine/paracrine feedback mechanisms, we proposed that TLR4-mediated IL-10 induction was subject to intrinsic temporal regulation that served to delay IL-10 induction to allow time for cells to initiate proper immune response to microbial-derived ligands like LPS. Differential utilization of adaptor molecules by a single receptor could provide such a mode of regulation. This could be explained in two ways: First, it has been shown that MyD88 signaling kinetics occurs antecedent to TRIF signaling due to differential compartmentalization of each adaptor. TLR4-MyD88 interactions initially recruit IRAKs, TRAF6, and the TAK1 complex, leading to early-phase activation of NF-κB and MAP kinases.[271] TLR4 is then endocytosed and delivered to intracellular vesicles that facilitate TRIF-mediated recruitment of TRAF3 and the protein kinases TBK1 and IKKi, which catalyze the phosphorylation of IRF3 leading to the expression of type I IFN. Alternatively, IL-10 induction by TLR4 could require *de novo* synthesis of secondary molecules required for transcription initiation, thus proving temporal restriction of gene expression.

To distinguish these two possibilities, we treated murine bone marrow–derived macrophages with cycloheximide (CHX), a molecule derived from *Streptomyces griseus*, which inhibits translation prior to LPS treatment. Thus by inhibiting *de novo* protein synthesis, we could decipher whether IL-10 induction through TLR4 occurs via direct or indirect mechanisms. Indeed, CHX treatment prior to LPS stimulation abrogates IL-10 transcription but does not inhibit expression of pro-inflammatory cytokines like TNFα and IL-1β.[84] Accordingly, we hypothesized that robust IL-10 gene expression via TLR4-TRIF signaling requires induction and signaling by type I IFNs. This was confirmed by stimulating wild-type and IFNαR-deficient BMDMs with LPS, showing that loss of the IFNαR coincided with loss of IL-10 expression.[101,236] However, to our surprise, treating BMDMs with recombinant IFNβ or IFNα in the presence of CHX led to impairment of IL-10 expression, suggesting that subsequent protein synthesis was required for robust IL-10 expression.[84]

Previous work from our lab and confirmed by others has demonstrated a requirement for IFN signaling in the induction of another anti-inflammatory molecule, interleukin 27.[236,272,273] IL-27 functions as a heterodimer composed of p28 and EBV-induced gene 3 (Ebi3), which have homologies to IL-12p35 and p40, respectively. The IL-27R complex consists of the unique subunit IL-27R (also referred to as TCCR and WSX-1) and the gp130 chain of IL-6R, which then activate transcription factors STAT1 and STAT3 via Jak-mediated phosphorylation.[98,105,213,273–278] IL-27 is produced by innate immune cells and has potent immunesuppressive effects on T-cell immunity, including the inhibition of 317 and 31 differentiation, as well as in several infection models.[98,213,236,279] In addition, IL-27R-deficient mice develop excessive tissue inflammation in the context of infection or

in autoimmune conditions.[18,23] Importantly, although the underlying molecular mechanisms of IL-27-mediated immune suppression are not well understood, a number of studies have highlighted the importance of IL-27-mediated production of IL-10 to promote an anti-inflammatory state in lymphocytes.[211,236,280]

LPS induction of IL-10 in fact is abrogated in IL-27R/TCCR/WXS-1 deficient BMDMs. In order to establish whether type I IFN and IL-27 mediated induction of IL-10 by TLR4 stimulation occurred in a linear fashion or through parallel mechanisms, LPS, recombinant IFNα, and recombinant IL-27 conditioned from wild-type BMDMs were collected and transferred to wild-type, IFNαR, and IL-27R deficient BMDMs and assessed for IL-10 induction. Here we showed that LPS- and IFNα-conditioned media were unable to generate IL-10 transcripts in IFNαR or IL-27R deficient BMDMs. In contrast, rIL-27-conditioned medium was able to induce IL-10 expression in wild-type and IFNαR deficient BMDMs, suggesting that IL-27 signaling to IL-10 transcription initiation occurs downstream of both TLR4 and IFN. In addition, through CHX experiments, we could demonstrate that IL-27 was able to induce IL-10 without the need for *de novo* protein synthesis, unlike LPS or type I IFN, which activated IL-10 through indirect methods. In this way, we propose a linear model that delineates TLR4-mediated IL-10 gene expression through sequential induction and subsequent signaling of a secondary molecule, type I IFNs (via TRIF), and a tertiary molecule, IL-27 (Fig. 1b). Further analyses suggest that IL-27 utilizes primarily STAT1, STAT3, c-MAF, and Sp1 to regulate IL-10 at the promoter.[84]

These studies are significant in two ways. First, we demonstrate that optimal IL-10 induction via TLR4 activation requires assimilation of multiple signaling platforms that leads to amplified IL-10 response coincident to transcription factor recruitment at the IL-10 promoter. Although IL-10 transcriptional regulation has been studied intensely in the past, identifying transcription factors and cis-regulatory elements that mediate up-regulation of IL-10 has been especially difficult. In the case of LPS stimulation, one potential confounding element that could obscure the relative importance of specific transcription factors or regulatory motifs I mediating IL-10 induction is the multiple gene programs and autocrine/paracrine signaling pathways induced by LPS through TLR4. That is, although a specific transcription factor or *cis* regulatory element may play an important role in LPS-mediated IL-10 induction, its relative importance may be obscured through downstream events that provide compensatory or alternative means of gene activation. In contrast, by deciphering a sequential signaling cascade via the generation of secondary and tertiary signaling intermediates, we demonstrate that IL-27-mediated expression is direct in that it does not require the synthesis of a downstream signaling molecule or transcription factor prior to generation of IL-10 transcripts. In this way we were able to identify three major IL-27 response elements located 1384, 570, and 180 bp upstream of the translation start site.[84] Importantly, these elements played functional roles in LPS, type I IFN, and IL-27 signaling consistent with a common mechanism of induction.

Secondly, these studies provide a model of IL-10 induction that may explain how IL-10 gene expression is regulated at a systems level. In particular, this model in part can clarify some of the immunosuppressive effects of type I IFN signaling. This is best demonstrated in the induction of experimental allergic encephalomyelitis (EAE), an animal model of human CNS auto-immune disease, characterized by infiltration of inflammatory cells, including macrophages and T cells, into the central nervous system (CNS) that results in the destruction of myelin sheath. Importantly, progression of EAE is more severe in mice deficient in TRIF, type I IFN receptor, IL-27 receptor, or IL-10 itself, but not MyD88.[98,213,281–286] Exacerbated EAE phenotypes are associated with increased 317 expansion, which is thought to mediate pathogenesis of EAE as well as adjuvant-induced arthritis.[17,287–300] In addition, in response to acute viral infections, innate immune effectors

generate a type I IFN response that directs anti-viral host response to obstruct various aspects of viral fidelity. However, during acute infections that induce strong inflammatory responses, IL-10 often acts beneficially to moderate excessive inflammation. For instance, during acute influenza infection, rapid but transient high-level production of IL-10 is observed in the infected respiratory tract, coincident with the onset of the adaptive immune response.[11,301,302] Recent studies have identified antiviral $CD8^+$ and $CD4^+$ T cells.[11,43] Blockade of IL-10 produced by $CD8^+$ and $CD4^+$ T cells results in enhanced lung inflammation, with elevated expression of multiple cytokines and chemokines in the infected lungs. Importantly, robust IL-10 production at the site of the infection requires the presence of IL-27, generated by innate immune effector cell types, including DCs and possibly neutrophils through the activity of the transcription factor Blimp-1.[43] This model again implicates IL-27 in the induction of IL-10 in response to infection as a means of mediating tissue repair and maintaining homeostasis. Whether IL-27 induction requires type I IFN signaling remains to be seen.

### C. Additional Cell Types that Produce Interleukin 10

In addition to macrophage, DCs, and T lymphocytes, many other cell types of the immune system are known to express IL-10. These include $CD8^+$ T cells following TCR activation or interaction with CD40 ligand.[303–305] Stimulation of B cells with autoantigens, TLR4, TLR9 ligands, or vitamin D3 can also lead to Il-10 production.[42,306–309] Recently, studies of a B-cell Il-10-deficient reporter mouse indicated a role for B-cell-derived IL-10 in limiting virus-specific $CD8^+$ T-cell responses.[310] In addition, neutrophils were reported to produce Il-10 in response to TLR and C-type lectin co-activation through MyD88 and SYK, respectively [244]. Finally, natural killer (NK) cells are another potentially important innate source of IL-10. It has been suggested that NK cells are required for antigen- specific Treg differentiation and the induction of tolerance in some mouse models.[311]

### D. Defining Interleukin 10 Producing Cell Types: Use of Reporter Transgenic Mice

The majority of studies of IL-10 regulation and function are pejorative studies using transgenic cytokine overexpression mouse lines and mice with targeted gene disruptions. However, universal gene deficiencies fail to answer more intricate questions concerning the source of IL-10 in a particular context and how Il-10 expression changes over time. Given the pluripotent effects of most cytokines and the widespread receptor expression, understanding the underlying spatiotemporal framework that dictates cell type–specific IL-10 expression is paramount to harnessing IL-10 therapeutic potential. Even cell type–specific deletions using Cre-Lox or other methods can obscure the role of IL-10 or IL-10 regulator in the differentiation of a particular cell type versus the direct effect of a specific factor on IL-10 expression itself. This becomes critical when considering that most therapeutic efforts using recombinant IL-10 have had limited success, suggesting that the source as well as the variance in the duration of IL-10 expression and signaling are important factors in understanding how IL-10 signaling facilitates tissue repair and resolution of inflammatory response in a balanced manner. To address this, a number of IL-10 reporter transgenic mice have been developed to allow the study of cytokine expression without modifying protein expression itself (Box 1). Several different approaches for the generation of cytokine reporter strains are currently in use: conventional transgenic mouse strains, targeted insertions (“knockins”), and BAC transgenes. Presumably, these strategies are able to recapitulate physiological expression of IL-10 because they contain all the necessary regulatory elements required for IL-10 expression. Though there are some concerns about variegated expression of transgenic reporters or the overall penetrance and stability of certain reporters, like GFP, these tools have begun to reveal important clues as to how IL-10 is regulated at a tissue- and cell-specific level as well as resolving potential conflicts between transcriptional and post-transcriptional regulatory mechanisms.

**Box 1**

### IL-10 Reporter Transgenic Animal Models

**Tiger Mouse**
Flavell *et al.* published an IL-10 reporter strain (*tiger*) in which IRES-GFP was introduced into the 3′untranslated region of the IL-10 gene (280). In these mice, IL-10 expression was robustly induced in intraepithelial lymphocytes and mesenteric lymph node $CD4^+$T cells after multiple rounds of anti-CD3 injection. Although the GFP signal of this transcriptions reporter was clearly associated with IL-10 protein, the authors also show a small $GFP^+$ $IL10^-$ population, possibly because such a reporter does not take into account the regulation of mRNA stability and translation as well as protein accumulation and degradation. Thus, this model may be a valuable tool for deciphering mechanisms of IL-10 post-transcriptional regulatory mechanisms.

**IL-lOeYFP mouse**
Another study has produced IL-10eYFP mice in which the first exon of the IL10 locus is disrupted by the insertion of a gene encoding enhanced yellow fluorescent protein eYFP (312). This mouse differs from the "*tiger* mouse" in that IL-10eYFP mice do not express IL-10 from the targeted allele (homozygous knockout) whereas the tiger mouse contains the endogenous *IL10* locus intact (280). Surprisingly, the reports of IL-10eYFP and *tiger* models reached contradictory conclusions concerning the allelic regulation of IL-10, where in the first model, IL-10 is expressed from a single allele, whereas in the latter model, there was bi-allelic expression of IL-10 in most cells.

**Vert-X mouse**
A 20kb fragment containing IL-10 was isolated via recombineering from a BAC clone, and re-engineered to express an IRES-GFP inserted between the endogeneous stop codon and PolyA sites. Fidelity of II-10 expression was tested in splenic leukocytes stimulated with TLR ligands (71, 310). This model has been utilized to study cell type specific IL-10 expression as well as identify major IL-10 producers in disease models. Initial studies revealed that B cells are a major source of IL-10 in response to systemic anti-IgD or TLR challenge or in the case of MCMV infection (310). Interestingly, increased polarization of B cells was correlated with higher levels of IL-10 reporter expression. Thus there appears to be an underlying gradient when observing the expression profiles of mature B cells versus plasmablasts or plasma cell precursors (310). In contrast, in studies of a murine colitis model that mimics an inflammatory bowel disease-like syndrome, the Vert-X mouse was used to reveal that myeloid derived cells serves as the predominant source of IL-10 and facilitate the sustenance of Foxp3+ T regulatory cell enrichment in the lamina propria in order to prolong immunosuppressive activity in this region (71). Given that IL-10 deficient mice spontaneously develop colitis symptoms over time and the ineffective therapeutic potential of recombinant IL-10 to rescue these mice, by identifying the major source of IL-10 in the context of intestinal inflammation, this study was able to address a critical feature of the inflammatory disease process that had eluded investigators in the past. Further studies addressing IL-10 production in the context of local viral infection as well as inflammation of the CNS have been undertaken as well with success (42).

**10BiT mouse**
Another IL-10 reporter strain (10BiT) utilizes the surface marker CD90-1 (also known as Thy1.1) transgencally under control of the IL-10 promoter (313). Here the 10BiT mice are transgenic for a bacterial artificial chromosome containing a modified gene encoding IL-10 in which the first exon is replaced with a membrane encoding the reporter CD90-1. These studies revealed that stead state IL-10 expression was limited to T cells, and in particular T regulatory cells inhabiting the gut. Interestingly, IL-10 producing regulatory T cells could be segregated into Foxp3+ and Foxp3-population based on exposure to TGFβ signaling and even within $Foxp3^+$ cells, Tregs exhibited variable II-10 expression based on whether they were lamina propria-dwelling (high IL-10 expressing) or mesenteric lymph node derived (low IL-10 expressing). Thus, microenvironment and compartmentalization plays a significant role in regulating the amplitude and duration of IL-10 expression even within a single defined cell type.

## VI. FUTURE PERSPECTIVES AND CONCLUDING REMARKS

Clearly interleukin 10 plays a critical role in regulating homeostasis at a global level. This includes resolving inflammation during acute infections or tissue injury at both a local and systemic level. Consequently, dysregulation of IL-10 can lead to more severe forms of immunopathology or development of autoimmune disease through enhanced or sustained inflammatory response. At the same time, many pathogens have harnessed the pleiotropic power of IL-10 to facilitate an inflammatory state that promotes chronic infection. Thus identifying critical intermediates that regulate IL-10 in different environments is crucial for our understanding of how IL-10 maintains physiological balance of host immune response. Attractive candidates that may contribute to this dynamic regulation are type I interferons. These molecules are rapidly induced to facilitate host anti-viral response by amplifying cellular anti-viral proteins and by immunomodulation through inflammatory signaling to mediate infiltration of inflammatory immune effectors at the site of infection. However, at

the same time, type I IFN signaling can exert powerful immunosuppressive capabilities through induction of anti-inflammatory molecules like IL-27 and IL-10. Work from our lab and others has implicated type I IFNs, IL-27, and IL-10 as suppressors of immunologically induced neurodegenerative diseases like EAE. These studies gain translational significance given the potential efficacy of IFNβ therapy against multiple sclerosis, which shares many of the clinical symptoms and features with EAE animal models. A similar mechanism may be applied by which type I IFN exerts temporal control over excessive inflammation during acute respiratory influenza infection through recruitment of IL-10-producing lymphocytes to the site of infection. However, type I IFN signaling may also serve as the culprit in the context of chronic or persistent viral infection. Prolonged host-derived IL-10 production can actively suppress T-cell responses, allowing viral persistence, as in the case of LCMV infection. Re-establishment of functional T-cell response, through IL-10 antibody blockade, can promote host control of persistent infection. Elevated IL-10 expression could in theory be initiated and/or sustained through type I IFN host response generated during the primary viral infection. In this manner, type I IFN signaling could act in dual roles—to promote robust clearance of acute viral infection by directing initial host anti-viral response, but over time engendering an immunosuppressive environment that allows for persistent or chronic infection.

Given the overall poor outcomes of systemic recombinant IL-10 therapies in treatment of autoimmune disease, it has become apparent that a critical and nuanced appreciation of IL-10 gene expression is necessary in order to understand the role IL-10 plays in mediating host immune response in a contextualized manner. From our perspective this includes defining spatial and temporal control of IL-10 expression *in vivo*. It has become clear that both the source of IL-10 and the kinetics of IL-10 production are important factors that allow coordinated host immune response and are reflective of cellular interactions between different immune effector cell types, including T cells and innate immune cells. Future studies using transgenic reporter mice will help define what immune effector cell types provide IL-10 and help us understand the cell-specific signaling, transcription factor utilization, epigenetic modification, as well as post-transcriptional regulators that govern cell type–specific IL-10 expression. This insight will help facilitate the development of treatment strategies aimed at regulating inflammation and perhaps persistent infection by manipulating IL-10.

## ABBREVIATIONS

| | |
|---|---|
| **AP** | activator protein |
| **BMDM** | bone marrow derived macrophage |
| **CREB** | cAMP response element binding protein |
| **C/EBP** | CCATT enhancer/binding protein |
| **c-MAF** | c-musculoaponeurotic fibrosarcoma factor |
| **CHX** | cycloheximide |
| **Ebi3** | EBV-induced gene 3 |
| **ERK** | extracellular signal regulated kinase |
| **GAS** | gamma activation sequence |
| **GSK3** | glycogen 3-kinase |
| **GVHD** | graft verus host disease |

| | |
|---|---|
| **IFN** | interferon |
| **IFNαR** | interferon alpha receptor |
| **IRF** | interferon regulatory factor |
| **ISRE** | interferon sensitive response element |
| **IBD** | intestinal bowel disease |
| **JAK** | Janus kinase |
| **MARE** | MAF recognition element |
| **MyD88** | myeloid differentiation primary response 88 |
| **NF-κB** | nuclear factor kappa-B |
| **PAMP** | pathogen associated molecular pattern |
| **PRR** | pattern recognition receptor |
| **PBMC** | peripheral blood mononuclear cell |
| **RIG-I** | retinoic inducible gene-I |
| **STAT** | signal transducers and activators of transcription |
| **Sp** | specificity factor |
| **SLE** | systemic lupus erythematosus |
| **TRIF** | TIR-domain-containing adaptor protein inducing IFNβ |
| **TIP** | tristetrapolin |

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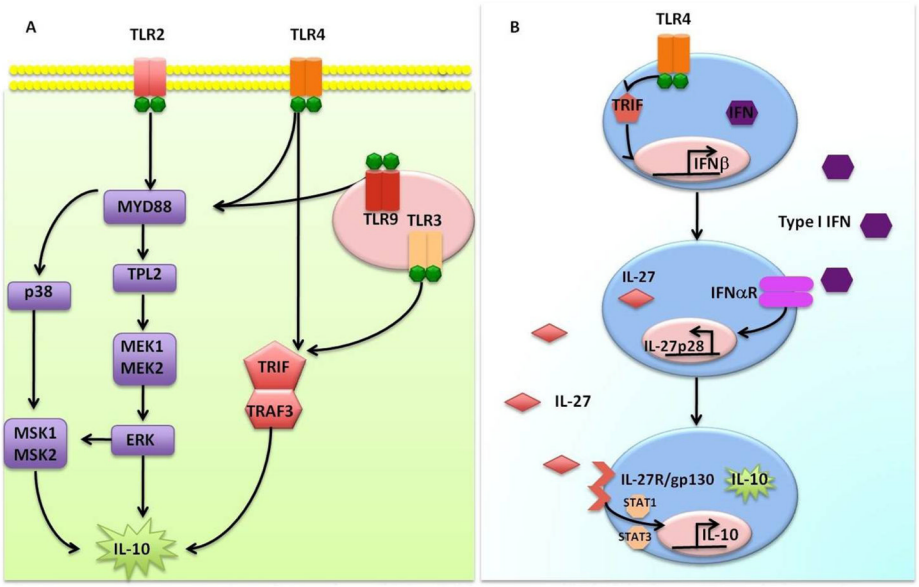


**FIGURE 1. Toll-like receptor induction of Interleukin 10**

Macrophage cells induce IL-10 in response to TLR stimulation through MYD88 and TRIF dependent mechanisms. (A) TLR ligation through MYD88 adaptor activation directs a signaling cascade via p38 and ERK stimulation leading to the induciton of pro-inflammatory cytokines and IL-10. (B) Stimulation by by lipopolysaccharide through TLR4 induces transcription of Type I IFNs (IFNb/a). Subsequent autocrine/paracrine signaling via the Type I IFN Receptor (IFNaR) leads to induction of the IL-27p28 transcript of the IL-27 hetero-dimer (p28/EBI3). Finally, signaling via the hetero-dimeric IL-27 receptor (IL-27R/gp130) leads directly to IL-10 expression by activating transcription factors STAT1 and STAT3, which are mobilized to the IL-10 locus resulting in coordinate gene transcription

## TABLE 1

IL-10-Associated Animal Models

| Model | IL-10 Deficient Phenotype | Reference |
|---|---|---|
| Inflammatory Bowel Disease | Spontaneous inflammatory bowel disease | 5, 72 |
| LPS Induced Inflammation | Elevated TNFa; Increased mortality | 10, 231, 232 |
| Experimental Autoimmune Encephalomyelitis | Increased susceptibility; more severe course of disease | 259, 242 |
| OVA-Induced Asthma | Decreased immunopathology | 201 |
| *Influenza* Infection | Enhanced viral clearance | 11 |
| *Cytomegalovirus* Infection | Enhanced viral clearance; more severe immune response | 191 |
| *Lymphcytic choriomeningitis* infection | Enhanced viral clearance | 239, 152 |
| *Mycobacterium tuberculosis* infection | Enhanced bacterial clearance; more severe immune response | 239, 152 |
| Systemic *Escherichia coli* infection | Enhanced bacterial clearance; more severe immune response; Increased mortality | 88 |
| *Toxoplasma gondii* infection | More severe immune response; Increased mortality | 8, 28, 31 |
| *Leishmani major* infection | Enhanced bacterial clearance; more severe immunopathology | 27, 40, 41, 235 |