Chapter 4

Therapeutic Potentials of IL-10 versus IL-12

Vishakha Bhurani and Sarat Kumar Dalai

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Abstract

Cytokines are low molecular weight proteins having roles in essential biological processes, particularly for the immune system. As they have a key role to play, an abnormality in their function can lead to wide variety of diseases (clinical consequences). Thus using the cytokines as therapeutic targets has been an area of active research. Of the entire family, we would like to shed light on two major ones IL-10 and IL-12 having an array of roles in cellular response to infection and autoimmunity. IL-12 is a pro-inflammatory cytokine that has been shown to enhance IFN-γ producing T cell responses and has been widely tested as a vaccine adjuvant. Many studies have shown that IL-12 acts as a link between innate and adaptive immunity by inducing IFN-γ production and polarizing naive CD4 T cells to become Th1 cells. It also has roles in CD8 T cell differentiation. On the other hand, IL-10 is an anti-inflammatory cytokine and has role in maturation of memory CD8 T-cell. It also plays a critical role in preventing autoimmunity and also limits tissue injury by interfering with the intensity and duration of immune response. We would thus like to discuss in details about the therapeutic use of these cytokines for infections as well as diseases such as cancer, autoimmune disorders etc.

Keywords: cytokines, therapy, IL-10, IL-12, inflammation, anti-tumor activity, autoimmunity

1. Introduction

Cytokines have an array of roles in immune system. They are involved in regulation of most immune responses. They comprise of a large family of low molecular weight proteins play essential role in biological processes. They are cellular hormones which mediate cell to cell communication. It comprises of interleukins, interferons, chemokines, monokines and lymphokines. They are basically involved in signaling pathways and regulating the downstream

events. Cytokines are being studied thoroughly from past 3 decades due to the variety of roles they play in various infections, diseases etc. They have prominent roles to play when it comes to immunity, inflammation, repair and migration [1]. The idea of using them as therapeutics is as old as more than 20 years. It evolves from two very different strategies, the first one being administration of recombinant purified versions of cytokines and the second in which therapeutics are designed against them inhibiting their harmful effects and limiting excessive upregulation. Interferons and CSF (colony stimulating factor) are the ones which form the success stories as therapeutics. Their major role seems to be in maintaining the Th1-Th2 paradigm [2, 3].

The basic classes of cytokines fall under the category of pro-inflammatory, promoting inflammation and anti-inflammatory, resolving inflammation. As the name suggest, the functions are opposing and hence a very fine line separates the two, striking a balance is thus extremely important. A slight dysregulation can lead to immunopathology, at times fatal to the host. Immune homeostasis is attributed via signaling through these small molecules. Thus they are prone to dysregulation by any microbial invasion or injury. Micro-organisms have found ways to fool the immune system and leading to imbalance in various regulatory pathways.

IL-10 and IL-12 have opposing roles. Owing to their pleotropic properties, they have been widely considered for therapy. Various viral infections, tumor models and autoimmune diseases are being targeted through them. Most prominent issue in translating all therapeutic approaches to clinic is their opposite effects. Since they are not constitutively expressed, perfect timing and cellular location are of paramount importance in designing the right class of therapeutics. Here we attempt to shed light on their roles in immune responses and the way of their use in therapy. A deeper understanding of their functions, mode of action, involvement of other cytokines, and link between cellular processes would serve as a key feature in developing successful modalities.

2. IL-10: structural features

IL-10 family of cytokines falls under the umbrella of class II cytokine family. IL-10 is known to limit the overt inflammation preventing tissue damage and acts mainly upon leucocytes. It has a major role in maintaining tissue homeostasis. IL-10 was first identified in 1989. It was described as a protein involved in inhibition of IFN-γ secretion from Th2 type cells. IL-10 is expressed by almost all immune cells, both of innate and adaptive immune system. It is expressed by dendritic cells (DCs), macrophages, NK cells, mast cells, T cells and B cells etc. [4–8]. Recent evidences suggest that it is also secreted by regulatory T cells (Tregs). Structurally it has a stretch of 160 amino acids forming non-covalently linked homodimers. It binds to R1 and R2 receptor chains. R1 is shown to have structural similarity with IFNR. IL-10 functions via activating the JAK-STAT signaling pathway, STAT3 being the downstream transcription factor involved [4, 9]. Various studies have shown that immune cells such as macrophages lacking STAT3 escape the suppressive effects of IL-10 on pro-inflammatory cytokine production.

2.1. IL-10 secretion

Besides immune cells several non-immune cells also secrete IL-10. Leucocytes, as well as epithelial cells both falling under different categories of cell types, secrete this anti-inflammatory cytokine highlighting its important and major role in homeostasis [10]. Initially, only Th2 type T cells were known to secrete IL-10, but more recently Th1, Th9, Th22, CD8 T cells and even regulatory T cells have been reported to secrete IL-10 [5, 6]. Pathogen induced IL-10 production occurs mainly in APCs such as DC's and macrophages [11, 12]. TLRs have a major role to play in pathogen induced secretion. Pathogen Associated Molecular Patterns (PAMPs) act as TLR ligands for the receptors. This in turn initiates a signaling cascade termed as the TLR signaling pathway. Signaling through TIR domain conjugated to adapter molecules, either TRIFF or MyD88 leads to IL-10 secretion and other cytokines [13]. TLR 2 ligand has been studied extensively and is considered as a major inducer of macrophage derived IL-10. Macrophages are the only cells also producing IL-10 via the TLR3 signaling pathway [14, 15]. LPS is also an inducer of IL-10 via common TRIF and MyD88 signaling via type I IFNs. Signaling through the MyD88 leads to activation of NF-κβ and MAPK activation. MAPK functions via ERK group of kinases. The differential levels of IL-10 secreted/expressed are dependent upon ERK activation strength in DC's and macrophages. Signaling via this route is often exploited for finding new targets of anti-inflammatory drugs. Regulatory T cells (Tregs) are very recent and major producers of IL-10. Tregs are characterized by expression of transcription factor Foxhead box P3 (FOXP3). Tregs must receive in-vivo signals to induce IL-10 expression. A proper understanding through these cell types however remains elusive.

2.2. Regulation of immune responses through IL-10

Microbial sensing through the innate immune system initiates a signaling cascade terminating into generation of pro-inflammatory cytokines. This creates an inflammatory environment favorable for the activation of adaptive immune cells, however higher levels of inflammation can give rise to systemic and metabolic imbalances having deleterious effects to the host. Therefore the immune system has developed anti-inflammatory mechanisms to limit the production of pro-inflammatory molecules thus limiting tissue damage and maintaining a state of homeostasis. Interleukin 10 (IL-10) is a potent anti-inflammatory cytokine that plays a crucial and essential role in averting inflammatory and autoimmune pathologies. It has also role in limiting antiviral, antibacterial responses, remodeling damaged tissues and wound healing. 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. Mice studies have shown that IL-10 deficiency leads to exacerbate immune responses to microbial or bacterial challenge. Thus, impaired IL-10 expression or signaling can enhance clearance of pathogens during an acute infection leading to exaggerated inflammatory responses, ultimately leading to immunopathology and tissue damage. Conversely, some pathogens can harness the immunosuppressive capacity of IL-10 to limit host immune response, leading to persistent infection characterized by unaffected pathogen load. In all, IL-10 plays an indispensable role in mediating host anti-inflammatory response and hence identifying the cellular sources of IL-10. The molecular mechanisms

that regulate IL-10 expression are extremely important in developing therapeutic strategies directed against pathology-associated impaired IL-10 production [16].

IL-10 is considered to have multiple roles when it comes to immune regulation. It has been shown to inhibit production of many inflammatory molecules such as IL-12, MHC and other costimulatory molecules from dendritic cells and macrophages [17]. It is also shown to have role in B cell survival, proliferation and antibody production. More recently, its role in tumor immunity has been elucidated [18].

2.3. IL-10 as therapy

Owing to its unique properties, IL-10 is considered as a potential candidate for use in therapy against inflammatory diseases, chronic infections, cancer and autoimmunity. IL-10 is involved in feedback regulation of Th1 and Th2 responses. It has been reported that the levels of secretion of Il-10 and IFN-γ through Th-1 is the deciding factor between clearance and persistence of infection. Studies in IL-10 deficient mice show that some intracellular pathogens could be cleared but is often accompanied by immunopathology. This clears the role of IL-10 in preventing host damage and maintaining a balance [19].

IL-10 is widely tested as a therapeutic for inflammatory diseases. Administering recombinant IL-10 has been tested in many clinical trials for rheumatoid arthritis, Crohn's disease and psoriasis [20]. All these clinical studies show the role of IL-10 in immune stimulatory as well as anti-inflammatory abilities. Because IL-10 has potential to prevent T cell-mediated tissue injury, it is often considered as a therapeutic for diseases involving autoimmune inflammation, the most studied model being autoimmune encephalomyelitis (EAE). The presence of IL-10 within the target organ is linked to its role in CNS inflammation and also in a null mouse model of acute CNS inflammation. The effectiveness of the therapy, however, depends upon the timing of IL-10 administration as well as target/route/localization because peripheral administration of IL-10 shows an exacerbated disease condition of EAE in mice [16, 19]. In case of various other infections, it is shown that a delicate balance of pro-inflammatory and anti-inflammatory environment is required for tackling the disease. Temporal and spatial IL-10 induction is critical for resolving any infection. Excessive IL-10 production can inhibit the pro-inflammatory response to a number of pathogens, including Leishmania spp., *T. cruzi*, Mycobacterium, Plasmodium spp., and Lymphocytic choriomeningitis virus, to the extent that pathogens can escape immune control, resulting in either persistent or chronic non-treatable infections [21–23]. In cases of viral infections such as HIV and HCV, elevated IL-10 signaling can inhibit pro-inflammatory cytokine production mainly via two processes, first is targeting of immune effector types directly, and second by indirectly modulating immune function eventually inhibiting maturation of different types of APCs such macrophage and dendritic cells, thus limiting co-stimulatory, chemokine secretion and antigen presentation capacity of the host. In the case of HIV, it has been reported that IL-10 hampers APC maturation, limiting antigen presentation from these cells, and initiates T cell-dependent suppression of anti-viral responses [10, 24, 25].

We can thus say that IL-10 therapy can only be successful only if everything is just right from signaling to secretion. Dysregulation of IL-10 can lead to autoimmunity or severe immunopathology due to extended inflammation. Also there are pathogens that have

found ways to promote chronic establishment by hijacking the IL-10 regulation pathways. Hence IL-10 works differently in different environments, thus having a deeper knowledge of the intermediates involved in its functioning is necessary. Promising and most studied candidate seem to be type I interferons, IFN-γ as reviewed by many, but IL-12 can also act as a potential player.

Work from Cheng G. lab and others have shed light on the role of type I IFNs, IL-27, and IL-10 as suppressors of neurodegenerative diseases like EAE. Such diseases are induced as a result of impaired functioning of the immune system, wherein the system generates response against its own cells. These studies were important contributions highlighting potential efficacy of IFNβ therapy against multiple sclerosis, which shares many of the clinical symptoms and features with EAE animal models. In case of viral infections, most pathology to the host is prevented via type I IFN. In case of acute respiratory influenza infection it is known that type I IFN exerts temporal control over excessive inflammation through the infiltration of IL-10-producing lymphocytes at the infection site. However, type I IFN signaling is also known to worsen the pathology in context of chronic or persistent viral infections. Prolonged host-derived IL-10 production can actively suppress pro-inflammatory T-cell responses, giving an opportunity to the virus to persist, as in case of LCMV infection. However, considerable success is achieved in re-establishment of T cell function by using molecules acting as IL-10 antibody block. Theoretically elevated IL-10 expression could be initiated as well as sustained through type I IFN host response generated during the primary viral infection or initial phase of infection. Thus, type I IFN signaling could play double roles firstly by promoting robust clearance of acute viral infection via host directed anti-viral response, and secondly by creating an immunosuppressive environment that paves way for persistent or chronic infection to establish. IL-10 also modulates this excessively and hence understanding its potential from infection point of view is equally important.

3. IL-12

IL-12 is a pro-inflammatory cytokine that has been shown to enhance IFN-γ producing T cell responses and has been widely tested as a vaccine adjuvant. It is produced by phagocytes in response to microbial stimulation and is an important early mediator in host defense. IL-12 is known as a conjugate between innate and adaptive immunity as it induces IFN-γ production and thereby helps in polarizing naive CD4 T cells to become Th1 cells. Over and above this, IL-12 has also been shown to enhance CD8 T cell homeostasis and provide a third signal that promotes full activation and survival of activated CD8 T cells. Collectively, many studies have documented IL-12 as a potent inducer of effector T cells, and this property has led to its testing as commercial vaccine adjuvant.

3.1. IL-12 signaling

IL-12 is primarily produced by all professional APC types such as DC's, monocytes and macrophages. IL-12 is composed of two chains *p*-35 and *p*-40 encoded by IL-12a and IL-12b respectively, activating NK cells and induce CD4 T cells to become IFN-γ producing Th1 type cells.

IFN-γ, in turn, acts on APCs to promote IL-12 secretion in a positive feedback loop. IL-12 signals via the IL-12 receptor (IL-12R) consisting of two subunits namely IL-12Rβ1 and IL-12Rβ2 known to be expressed on DCs, T cells and NK cells. IL-12 functions via non-receptor Janus kinase 2 (JAK2) and tyrosine kinase 2 (TYK2) activities, leading to the phosphorylation of signal transducers and activators of transcription (STATs), mainly, STAT4 homodimers. IL-12 is also involved in secretion of IL-2, TNF-α and GM-CSF apart from the main one IFN-γ.

3.2. Regulation of IL-12 production

Biologically active IL-12 is produced when both Il12a and Il12b genes are expressed coordinately in the same cells. Contradictory to the notion, mRNA of Il12a is widely expressed in many cell types, albeit at low levels in some cells, most of which do not even produce IL-12. The Il12b mRNA is restricted to cells that can produce biologically active heterodimer. A rate-limiting step for IL-12 production is synthesis of the p35 chain linking to limited availability of its transcripts in cells under homeostatic conditions. Over the past 2 decades, a huge plethora of molecular analyses have identified numerous transcription factors that bind to the promoter regions of Il12a and Il12b. The promoters of Il12a have been shown to bind transcription factors such as nuclear factor kappa B (NFκB), c-Rel (in DCs), c-Maf, and IFN regulatory factor 1 (IRF-1) in activated macrophages. TLR signaling in DCs through LPS and other ligands is also reported as contributor in IL-12 production.

3.3. IL-12 in therapy

Owing to its pleotropic properties IL-12 is widely used in therapy against tumors and infections. Most effects of IL-12 are mediated via IFN-γ. IL-12 regulates inflammation via linking the innate and adaptive arms of the immune system (**Figure 1a**). It thus emerges as an early pro-inflammatory cytokine in immune response to pathogens. However, complete functioning requires signals from IFN-γ, CD40-CD40L interactions and IL-15 [26–29]. Role of IL-12 in tumor regression and chemotherapy is most studied. Many pre-clinical studies have demonstrated the potent anti-tumor activity of IL-12. Extensive research is being carried out to deliver recombinant IL-12 directly to tumor site. The challenge here again is to understand at mechanistic level, the involvement of other pathways which could be immunosuppressive, administering IL-12 in a way which makes it less toxic, targeted tissue delivery and generating tailor made responses depending on the type of tumor.

To study the mechanism of protection of IL-12 in tumors, it is generally overexpressed in tumor cell lines. Subcutaneous inoculation of this cytokine in C26 colon carcinoma cells, B16 melanoma cells and few others have shown to induce tumor suppression. In melanoma cells protection is mediated by ILCs (Innate Lymphoid Cells) and in colon carcinomas it is independent of IFN-γ and rather dependent upon CD4 T cells and NK cells [30]. This sheds light on location dependence and also upon the role of various cell types involved in the process. Thus, the entire tumor microenvironment is affected by IL-12 in a variety of ways [31]. In B-16 melanoma, IL-12 showed to remodel the vasculature by upregulating adhesion molecules having role in leucocyte migration to the tissue and also by inhibiting angiogenesis through IFN-γ secretion.

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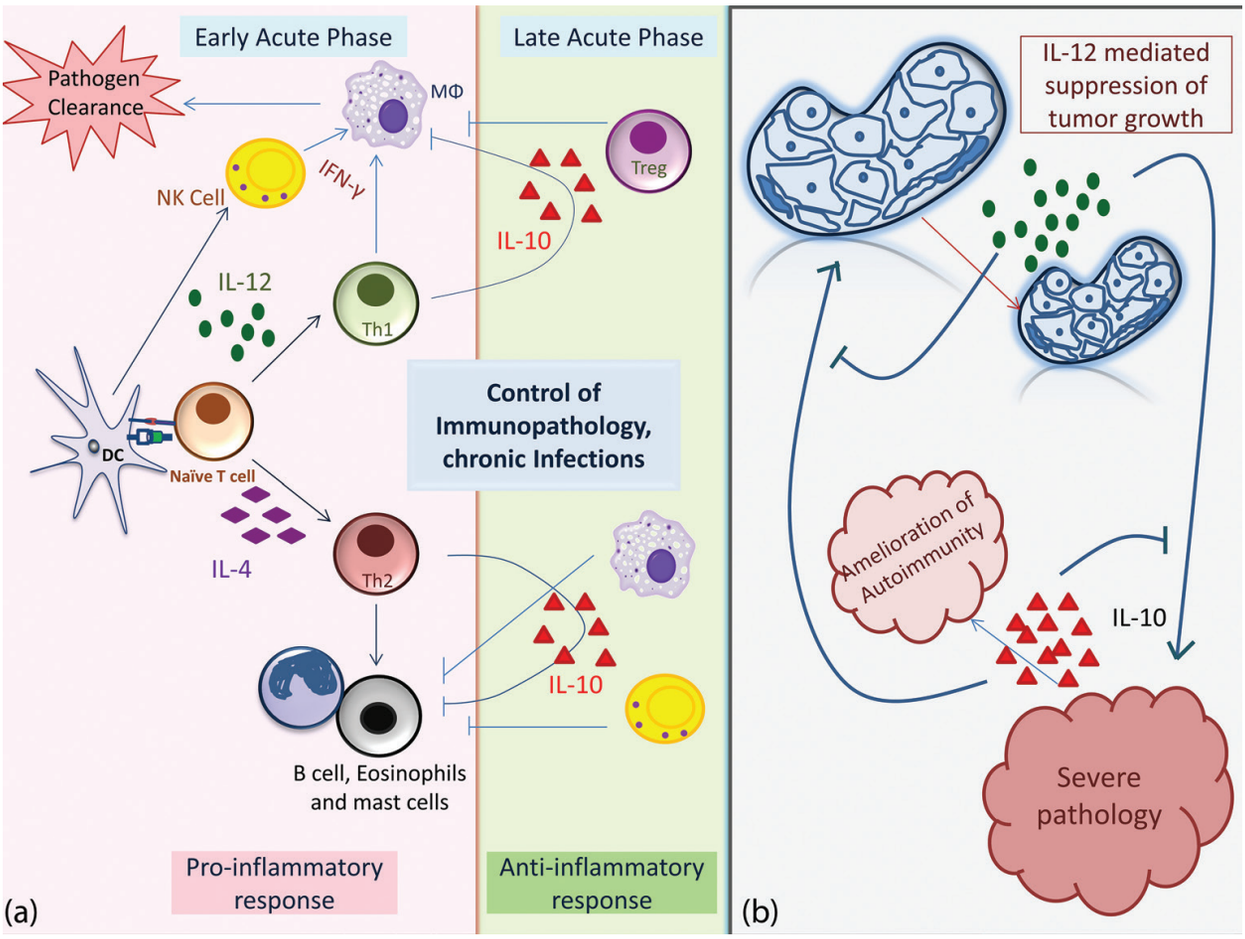

Figure 1. (a) During acute infection settings, a pro-inflammatory environment is created mainly via secretion of IL-12 through DC-T cell interaction, promoting Th1 responses which are IFN-γ promoting. APCs promote pathogen clearance and control by activating adequate effectors of adaptive immune system. On the other hand Th2 response is involved in B cell, mast cell activation under the influence of other cytokines. During late stages of infection, an anti-inflammatory environment is created by IL-10 secretion by Th1 & 2 cells, Tregs, NK cells and macrophages. (b) IL-10 and IL-12 both antagonize each other under different conditions such as tumor and auto-immunity suggesting a delicate balance between the two is necessary to prevent host from adverse effects.

Various genetically modified mice models have been used to explore the therapeutic potentials of IL-12. It is well known that IL-12 therapy is dose and context dependent; hence various routes of immunization have been tested in different localized tumor models. Delivery models used and studied include infusion of recombinant proteins, electroporation, gene therapy using non-viral and viral vectors, nanoparticles and microspheres containing IL-12 and immune cells or non-immune cells expressing IL-12. IL-12 affects a series of events involved in tumor immunity. IL-12 acts on NK cells and CD8 T cells to trigger effector functions via perforin and granzyme secretions. It also stimulates B cells to secrete anti-tumor antibodies. It acts upon CD4 T cells polarizing them to Th1 phenotype in turn secreting cytokines and IFN-γ. It in turn acts upon APCs promoting antigen and cross presentation enhancing cytotoxic activity. Owing to its activity, IL-12 is considered in combinational therapy with other cytokines, chemotherapeutic agents, peptide vaccines and monoclonal antibodies. This has also been tested in melanoma and mammary carcinoma models [32, 33].

The main issue however with these therapies is excessive systemic IFN-γ production leading to toxicity. In combination to chemotherapy modest success was observed only if IL-12 was administered at early stages. Hence the limitations lead to exploration of other modes of delivery and again based upon the type of tumor, suitable delivery methods need to be adapted. Several studies have evaluated the use of IL-12 for therapy by delivering this cytokine within the tumor site specifically. Even after most of these approaches resulted in impressive antitumor responses, the translation into the clinics was not satisfactory. There are many questions still remaining unanswered in the oncology field. Firstly, the schedule optimization for therapeutic IL-12 delivery in clinical trials has proved to be challenging. Various treatment schedules have been evaluated such as subcutaneous versus intravenous vs. intra-tumor in daily as well as consecutive injections. Even though the most successful way to administer IL-12 appeared to be in cycles of twice weekly injections, repeated administration of the cytokine leads to increased immunosuppressive properties of the tumor by the induction of IL-10 [34].

IL-12 also has major role in shaping immunological memory to viral infections. During a typical viral infection, T cells undergo via phases of T cell activation and differentiation, governed majorly by the cytokine micro-environment. IL-12 along with other cytokines such as IL-15 assists in generating appropriate T cell responses. Of the two types of T cells, CD4 and CD8, the effects of type I IFNs and IL-12 on CD8 T cell differentiation are seen to be intersecting as they both directly provide signals to the responding cells. Also they act in co-ordination with antigenic and costimulatory signals thereby promoting the development as well as expansion of short-lived effector cells [35, 36]. These cell types are eliminated from the response once there job of limiting the pathogen is complete. The T cells which survive the entire process culminate into T cell memory. IL-12 signaling induces expression of T-box expressed in T cells (T-bet), a transcription factor which determines the differentiation state of the T cells. High levels of T-bet directly correlate with the terminal differentiation of short-lived effector T cells, while lower levels of T-bet are linked to the development of memory precursor T cells which can add up to the long-lived memory pool. It has been reported that CD8 T cells lacking the IL-12 receptor, IFN receptor (IFNAR), or both inflammatory cytokine receptors, are defective in the formation of short-lived cells following infection with LCMV, VSV, or the intracellular bacteria *Listeria monocytogenes* (LM) [37]. These receptor deficient CD8 T cells are known to express lower levels of T-bet and higher levels of Eomes, the related T-box transcription factor Eomesodermin. Although these transcription factors possess overlapping roles, in terms of initiating IFN-γ, the expression of Eomes is preferentially associated with the formation of memory CD8 T cells. Eomes may operate to recruit cells into the memory pool by upregulating expression of CD122, the b-chain of the IL-2 receptor, which is also required for IL-15 signaling. Co-operation between cytokines shapes both short-term and long-lived anti-viral CD8 T cell development. Often the induction of type I IFN or IL-12 following infection boosts the expansion of highly cytolytic short-lived effector cells. Curtailing the inflammatory conditions via investigating the exact roles of IL-10 surrounding CD8 T cells might permit the formation of long-lived memory populations attributing protection against re-exposure to the infection [38].

4. Striking the IL-10/IL-12 balance for better therapeutic value

The potency of IL-12 and IL-10 in host defense makes them a target for precise regulation. Indeed, the temporal, spatial, and quantitative expression of these two cytokines during an immune response in a specific tissue microenvironment contributes to the determination of the type, extent, and resolution of the response in major ways. Disturbing the intricate control and balance frequently leads to immunologic disorders and pathologies. One of the most important and well-studied negative regulators of TLR-induced IL-12 production is IL-10. IL-10 repression of both IL12a and Il12b genes is primarily seen at the transcriptional level; however the inductions of the two genes have different requirements for de novo protein synthesis. IL-10 suppression Il12a transcription is not completely known. IL-10 acts upon the enhancer 10 kb upstream of the Il12b transcriptional start site, bound by nuclear factor, interleukin 3-regulated (NFIL3), which is a B-ZIP transcription factor. It has been reported that myeloid cells lacking NFIL3 produce excessive IL-12p40 and increased IL-12p70. This indicates that STAT3-dependent expression of NFIL3 is a key component of the negative feedback mechanism in myeloid cells that suppresses pro-inflammatory responses. Quite a bit of elegant studies have focused on the Il12b promoter transiently associated to acetylated histone H4 in WT bone marrow-derived macrophages (BMDMs), whereas association of these factors was seen to be prolonged in $Il10^{-/-}$ BMDMs. Experiments incorporating histone deacetylase (HDAC) inhibitors and HDAC3 short hairpin RNA have shown data to indicate that HDAC3 is involved in histone deacetylation of the Il12b promoter by IL-10. This means the histone deacetylation on the Il12b promoter by HDAC3 mediates the homeostatic effect of IL-10 in macrophages. More details clearly need to be worked out to understand the important homeostatic regulation of IL-12 production by IL-10, in terms of cellular pathways, different mechanisms might be taking place in a tissue specific manner etc. In this context, the IL-4-inducing transcription factor c-Maf is an interesting molecule that can directly and conversely regulate IL-12 and IL-10 gene expression in activated macrophages. Again, IRF-5 is considered as a factor leading to the "M1" polarization of macrophages thereby promoting Th1 and Th17 activities with activated transcription of inflammatory genes, including Il12a, Il12b, and Il23a, and repressed Il10 transcription [39].

A classic study by Lopez MV et al. showed the first evidence of synergistic anti-tumor effects of IL-12 and IL-10 in a novel combination cancer immunotherapy [40]. They demonstrated the eradication of established primary colon and mammary tumors by administering the Th1 and Th2 cytokine together. They observed an increased expression of IP-10, MCP-1 and TCA-3 at day 7 after administration of the combined immunotherapy. An interesting result was also the persistent expression of IFN-γ locally and the abrogation of IL-4 increase following the combined immunotherapy, indicating that infiltrating cells were expressing a Th1 phenotype. Simultaneous and timely activation of Th1 and Th2 responses is thus necessary for appropriate responses.

Autoimmunity is a condition in which host generates immune responses against its own healthy cells. Experimental allergic encephalomyelitis (EAE), a demyelinating disease of

the central nervous system, is widely used as an animal model for multiple sclerosis. Segal et al., in 1988 demonstrate that IL-12 is essential for the generation of autoreactive Th1 cells that induce EAE, in the presence as well as absence of IFN-γ. The disease-promoting effects of IL-12 are often antagonized by IL-10 having its origin from antigen nonspecific CD4 T cell which, in turn, is regulated by the endogenous production of IL-12 (**Figure 1b**). This unique immune-regulatory circuit appears to play a non-ambiguous role in controlling Th cell differentiation. It also provides a mechanism through which microbial triggers of the innate immune system can harmonize autoimmune disease [41]. IL-12 has an EAE promoting potential, it triggers formation of autoimmune effectors. Anti-IL-12 therapy helps in regression of disease through prolonged administration. The innate immune system would always trigger inflammatory responses towards autoimmune cells; a delayed IL-10 secretion by a different subset of immune cells, the adaptive ones in turn suppresses the activity of autoimmune cells. This has been established in *L. major* and *T. gondii* mice infection models. This suggests that manipulating the cytokine milieu, balancing IL-12/IL-10 ratio via the innate immune system can surmount establishment of autoimmune disorders. This may not hold true for chronic autoimmune diseases and more intervention is therefore required.

Generating CD8 T cell memory for infections due to intracellular pathogen has been an active area of research. IL-10 has been shown to promote resolution of infection, thereby promoting memory formation. The source of IL-10 is considered to be CD4 Tregs in acute LCMV model [42]. The probable reason for memory maturation of CD8 T cells could be their insulation to the pro-inflammatory effects of IL-12 driving them towards terminal effector differentiation. Pathogenic insult to the host triggers innate immune responses via NK cells and other innate immune cells. Microbial sensing occurs through PRRs (pattern recognition receptors) and the most studied of them being the TLRs (toll like receptors). TLR signaling pathway in turn leads to secretion of cytokines, pro-inflammatory ones, activating the adaptive arm. Innate immune cells together control the infection from spreading further. Upon activation of antigen specific T cells, a specific course follows which is dynamically in sync with increasing pathogen load. Upon clearing the pathogen, the antigen specific effector T cell population contracts to form a small pool of memory T cells. Several cytokines affect the phenomena of T cell maturation. The resolution phase has the role of IL-10 acting as anti-inflammatory and promoting T cell maturation which can add to the memory T cell pool. Thus this property of cytokines can be explored in vaccination strategies or designing new vaccine adjuvants. Therapeutic intervention during specific stages of T cell maturation can help in generation of protective long lived immunological memory.

5. Conclusion

Maintaining homeostasis requires an intricate interplay among various functioning systems of the body (**Figure 2**). Cytokines form an important class of such system. Understanding the system properly and deeply would allow meaningful interventions and restoring the steady state.

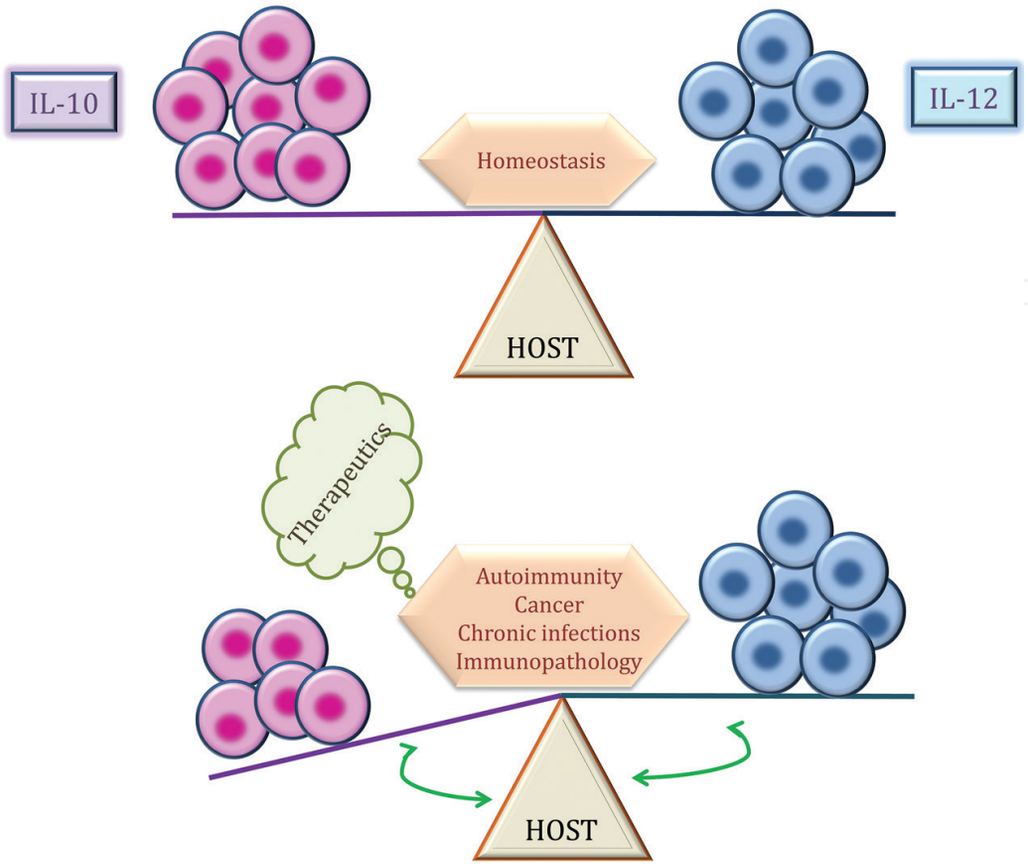

Figure 2. A balance between IL-10 and IL-12 secretion by host cells helps in maintaining homeostatic state. A slight imbalance in any of the processes of their function can lead to immunopathology, cancer, autoimmunity and chronic established infections. Targeting therapeutics towards maintain the balance could help the host restore the equilibrium.

Author details

Vishakha Bhurani and Sarat Kumar Dalai*

*Address all correspondence to: sarat.dalai@nirmauni.ac.in

Institute of Science, Nirma University, Ahmedabad, Gujarat, India

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