SCIENCEFORECAST
A digital science library

Review Article
Published: 28 Feb, 2018

Journal of Bacteriology and Vaccine Research

Immunomodulators as Therapeutic Option in Parasitic Infections

*Ratna A[1] and Arora SK[2]**

[1]Department of Medicine, University of Massachusetts Medical School, Massachusetts, USA, 01655

[2]Department of Immunopathology, Postgraduate Institute of Medical Education & Research, Chandigarh, India

Abstract

The immune system is an example of precise autoregulation in which we can intervene with medications and the therapeutical use of this intervention is called immunomodulation. Induction or restoration of immune effector functions using parasite antigens, drugs of natural and synthetic origin, and proteins derived from the immune system represent some of the current immunomodulators. The burden of infectious diseases caused or transmitted by parasites mostly comprising of protozoans and helminths, often rests on communities in the tropics and subtropics and responsible for substantial morbidity and mortality. Despite extensive investment and research, no vaccines are yet available for prevention of human parasitic infections. Chemotherapy is currently the only option for the management of these infections, yet the limited number of available drugs and their serious drawbacks reinforce the urgent need for innovative therapies. Of late, there have been substantive advances in our understanding of the use of various immunomodulatory agents during parasitic infections. Various antigenic determinants, cytokines and recombinant proteins have shown promising results during treatment against parasites. Despite this impressive progress, there is still much to explore in order to prevent or cure parasitic infections completely. In this review we have focused on the implications and outcomes of various immunomodulatory agents during parasitic infections.

Keywords: Parasites; Infections; Immune system; Immunomodulators

Introduction

The immune system is a complex interwoven network of biological structures and processes that defend our body against disease-causing microorganisms. Since many years, immune responses have been modulated by various agents in order to assuage the disease. Recent advances in the knowledge of intricate interactions of parasites with the immune system have led to the identification of important mechanisms germane to protection. This understanding has fostered formulation of novel strategies and current concepts. The immune system can be manipulated specifically by vaccination or non-specifically by immunomodulation [1-4]. During the last decade, immunomodulators have evolved to become a viable adjunct to established therapeutic modalities in infectious diseases. The initial work in the field of immunomodulation was the search of immunomodulatory agents for the treatment of residual cancer [5]. Cytokines and interferons have not only been tried in the immunotherapy of cancer [6] but also used as immunoadjuvants along with vaccines. Cyclosporin a potent immunosuppressant has proved to be a boon for prevention of graft rejection [5] and some autoimmune diseases.

Therefore, from a therapeutic point of view, immunomodulation refers to a process and a course of action in which an immune response is altered to a desired level. In this review, we have focused on the use of various immunomodulators during parasitic diseases.

Immunomodulator

It is a substance that alters the immune response by augmenting or reducing the ability of the immune system to produce antibodies or sensitized cells that recognize and react with the antigen which initiated their production (Mosby's Medical Dictionary, 8th edition.©2009, Elsevier). The mode of action includes augmentation of the anti-infectious immunity by the cells of the immune system including lymphocyte subsets, macrophages, and natural killer cells as depicted in Figure 1 [7]. Other mechanisms can involve induction or restoration of immune effector functions. Microbial products, drugs of natural and synthetic origin, and proteins derived from the immune

OPEN ACCESS

Correspondence:
Sunil K Arora, Department of Immunopathology, PGIMER, Chandigarh-160 012, India.
Tel: *+91-172-2755192*
E-mail: *skarorain@gmail.com*

Received Date: *29 Jan 2018*
Accepted Date: *26 Feb 2018*
Published Date: *28 Feb 2018*

Citation: *Ratna A, Arora SK. Immunomodulators as Therapeutic Option in Parasitic Infections. J Bacteriol Vaccin Res. 2018; 1(1): 1002.*

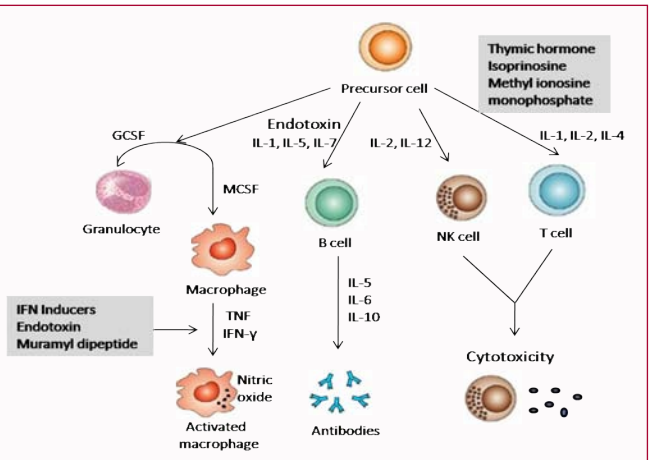

Figure 1: Enhancement of immune responses by immunomodulation [7].

system represent some of the immunomodulators currently being explored. Some immunomodulators are naturally present in the body, and some are available in pharmacologic preparations which ultimately stimulate host defense mechanisms for the prophylaxis and/or immunotherapeutic treatment of diverse infectious diseases.

The immunomodulators are classified as (i) Immunostimulants: which potentiate body's resistance against infections; enhance the basal levels of immune response and in individuals with impaired immune response as immunotherapeutic agent (ii) Immunosuppressant: which suppresses the immune response and could be used for the control of pathological immune response in autoimmune diseases, graft rejections etc. [8].

Parasitic infections and immune system

Parasites are eukaryotic pathogens, and broadly comprise protozoa, fungi, helminths and arthropods that complete a part or all of their life cycle within a host organism. Like other pathogens, parasites must survive in the face of a highly potent immune system. They succeed in this endeavor through a great diversity of strategies for avoiding immune detection, suppressing cellular immunity and deflecting immune attack mechanisms [9]. Metazoan and protozoan parasites are major causes of human and animal disease, causing extensive morbidity and mortality, particularly in tropical and sub-tropical climatic regions. While progress has been made in the last decade in the cloning and expression of protective antigens from a large number of parasites, the majority have failed to stimulate practically useful levels of protective immunity in trials [10].

As part of the primary immune response, the two important players of the innate immune system, the neutrophils and monocytes, continuously patrol the body in search of invading pathogens, and infiltrate into infected/injured tissues upon detecting microbial products [11]. Neutrophils arrive at the infection site early and in high numbers, and thus usually kill more invading bacteria than other phagocytes [12]. However, neutrophils are short-lived, with an average lifespan of 1–2 days: after engulfing and killing several bacteria, neutrophils exhaust intracellular enzymes and subsequently undergo apoptotic cell death. Upon reaching extravascular tissues, monocytes can differentiate into tissue-specific macrophages. Macrophages can ingest and eliminate larger pathogens that are not handled by the neutrophils; in addition, they remove the cell debris of apoptotic neutrophils in order to resolve an inflammatory response [13]. Phagocytes engulf pathogens or damaged cells, and eliminate them through the generation of reactive oxygen species and hydrolytic enzymes.

Current control and vaccine efficacy

Drugs remain central to alleviating the clinical disease and for larger scale disease control programmes. However, available drugs have often been in use for decades and drug resistance in the target parasites is now prevalent. The recent trend of emergence of drug resistant isolates of various pathogens has prompted us to develop an alternative strategy to surmount this problem. The search for immunomodulatory determinants as agents to enhance the efficacy of drugs or vaccine candidates is an ever expanding area of research. Vaccine development was originally focused on 'fractionate and vaccinate' approach. Antigens are purified from parasite extracts, harvested following *in vitro* culture using a variety of protein fractionation procedures [14] and then evaluated for protective efficacy in control trials in the target host or frequently in a laboratory infection model. Antigens can be selected on the basis of presumed functional importance to parasite survival such as enzymes required for feeding/migration, immunomodulatory molecules or on the basis of immune recognition by hosts leading to immunity against infection on repeated exposure. The induction of a mixed Th1/Th2 response has been observed with DNA or multivalent vaccination strategy. The efficacy achieved by vaccination using new formulations has often fallen short of what is required to control the disease, especially in case of parasitic infections.

In general, the pathogen specific host immune components play an active role in the elimination of pathogen; however their non-effectiveness in doing so ensued in full-blown infection. A number of parasite antigens have been shown to possess the capacity to modulate the immune response of the host. It is well evident that modulation of host immune system may be of great importance in containing the parasite replication inside the host cells.

Immunomodulators: arrays of possibilities in parasitic diseases

Immunomodulation acts as a major driving force during the overall host-parasite relationship. Anti-parasitic agents used during the treatment of infectious diseases avert rapid multiplication and hamper vital physiological activities of the parasite, but at the same time, the role of host immune system cannot be neglected. Basically it is the immune system that plays a major role in the complete suppression/elimination of the pathogens. In fact parasites weaken immune armory as a strategy to establish themselves in the host. It can be speculated that activation/rejuvenation of the host immune system could be an effective way to successfully combat various infectious diseases [15-18]. Use of immunomodulatory agents seems an attractive approach as an adjunct modality for control of several parasitic diseases.

Leishmaniasis

Leishmaniasis caused by several species of flagellated protozoan parasites of *Leishmania* genus is associated with significant morbidity and mortality in tropical and subtropical regions. According to the latest report, from world Health Organization (WHO, 2016), every year, 700,000–1 million new cases and 20,000 to 30,000 deaths occur annually (http://www.who.int/mediacentre/factsheets/fs375/en/). During an infection, the parasites have a remarkable adaptive capacity as they are able to survive and proliferate inside the hostile phagocytic cells. Leishmaniasis is characterized by the parasite-induced immunosuppression executed not only by active subversion but

also by immune deviation such that the resulting immune responses suppress the *Leishmania* specific immune response. Recovery from leishmaniasis is strongly correlated with the development of distinct Th1 type cellular immune responses manifested by delayed type hypersensitivity (DTH) to *Leishmania* antigens (Ag) and T cells producing interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukin 2 (IL-2) cytokines that activate macrophages and kill intracellular parasites [19]. The parasite actively secretes proteases and other molecules that affect host immune response (cells and cytokines) facilitating the infection process. In addition, the parasite possesses intracellular nonsecreted antigens viz. *LmS3arp* and *LimTXNPx*, members of conserved protein families, which are believed to contribute to the chronic immunopathology, observed in leishmaniasis [20]. Treatment relies on chemotherapy which is expensive and becoming compromised by emerging drug resistance. Hence the identification and evaluation of several *Leishmania* antigens which preferentially stimulate a Th1 response rather than a Th2 response against *Leishmania* may be a vital strategy for effective control of the disease.

Some basic trials have been made to rejuvenate the suppressed immune response in active leishmaniasis using components of the parasite itself. One of such studies highlights the efficacy of two critical antigens, 63kDa and 17kDa, in the reprogramming of $CD4^+$ T cells to produce IFN-γ in VL patients [21,22]. The study shows an increase in the frequency of IFN-γ producing $CD4^+$ T-cells after stimulation with 63kDa or 17kDa in presence or absence of recombinant IL-4. Another recent study reported 9-O-acetylated sialoglycoproteins to be important immunomodulator in Indian visceral leishmaniasis [23]. It shows that the sensitization of peripheral blood mononuclear cells (PBMCs) VL patients with 9-O-AcSGPs, a lectin, results in a mixed Th1/Th2 cellular response with the predominance of the Th1 response, indicating the ability of 9-O-AcSGPs to modulate the immune response in favor of the host. Another evolutionarily conserved antigen of *Leishmania* that has shown remarkable immunological properties is *L. infantum* is HSP70 [24]. Immunization of BALB/c mice with the MBP-HSP70 fusion protein preferentially induced a Th1 immune response. A dominant immune-response against recombinant *Leishmania* HSP70 detected in sera from Indian kala-azar patients indicated this antigen to be an immunodominant antigen [25]. The search for *Leishmania* antigens capable of eliciting a protective T cell response led to the identification of LACK (*Leishmania* homolog of receptors for Activated C Kinase). A portion of this antigen, LACK-D1 (a truncated form of 24 kDa), when administered as a vaccine with IL-12 before infection, protected susceptible mice [26]. It is interesting to consider the peculiar immunological properties of the LeIF protein, the *Leishmania*l homologue of the eukaryotic initiation factor 4A [27]. It stimulated proliferative responses and preferential Th1 cytokine production in PBMCs of patients. Proteins of *Leishmania* parasite that stimulate the production of IL-12 could be of significant interest either as immunotherapeutic components for leishmaniasis or as adjuvants. *Leishmania* eukaryotic initiation factor (LeIF) and its recombinant polypeptides induce the production of IL-12, IL-10 and the expression inducible-nitric oxide synthase (iNOS) by mouse myeloid dendritic cells [28].

With the main aim of identifying a defined antigen of *Leishmania* that could elicit a specific immune response in the host, Arora *et al*., [29] screened an expression cDNA library of *L. donovani* which was constructed from the poly(A)$^+$ mRNA of the parasite [25] with immune sera and *Leishmania* specific cell line (LSCL) established from the peripheral blood mononuclear lymphocytes of a naïve healthy individual. Three novel antigens were identified and characterized which could stimulate the LSCL *in vitro* and induce the release of a very high amount of IFN-γ. One of the recombinant antigens, rF14 when used along with an immunomodulator monophosphoryl lipid A (MPL-A), which is known to boost the cell mediated immune response in the host, showed a protective efficacy of 67% against a virulent challenge in the hamster model of visceral leishmaniasis [30]. Reactive nitrogen intermediates (RNI) and reactive oxygen intermediates (ROI) were found to be upregulated in the peritoneal exudates cells of hamsters immunized with this antigen- adjuvant combination. Also, there was a significant elevation of DTH response in immunized hamsters which demonstrated the activation of the cell-mediated responses that might be important for providing resistance against infection. The immunogenic properties of *L. donovani* LdP1 ribosomal protein homolog were analyzed in an animal model [31]. Although the prophylactic immunization with recombinant protein vaccine, rLdP1, was capable of inducing cellular response in immunized hamsters as seen by proliferation and upregulated expression of IL-12 transcripts by splenocytes as well as the IFN-γ release by LSCL *in vitro*, the animals were not protected against the virulent challenge of *L. donovani* promastigotes. At the same time, the animals immunized with dual dose of pVAXP1 DNA vaccine showed higher production of IFN-γ and IL-12 along with a significant suppression of IL-10. The immunized animals resisted the increase in spleen weight and parasite burden to a significant level suggesting the immunomodulatory potential of this formulation.

Unraveling the immunogenic properties of these antigens will provide clues to understand the immunopathology of disease and, perhaps more importantly, might act as immunotherapeutics in the treatment of the disease.

Helminthiasis

The development of mathematical models based on data from epidemiological surveys has led to a deeper understanding of how helminth infections persist in human communities [32-35]. The intensity of infection is the key variable in understanding the population biology of helminths and the morbidity they induce. Also, knowledge of intensity is crucial for the optimum use of anti-helminthic chemotherapy in the community. The nature and mode of action of various nematode-derived molecules with immunomodulatory properties are being studied, and their therapeutic efficacy in several helminth infections are also being undertaken.

Schistosomiasis

Schistosomiasis is an acute and chronic parasitic disease caused by blood flukes (trematode worms). People become infected when larval forms of the parasite, released by freshwater snails, penetrate the skin during contact with infested water. Estimates show that at least 206.5 million people required preventive treatment for schistosomiasis in 2016 (WHO, 2016) (http://www.who.int/mediacentre/factsheets/fs115/en/). The schistosomes have complex life cycles using two separate hosts to complete their development. Severe consequences of infection include bladder cancer or renal failure (*Schistosoma haematobium*) and liver fibrosis and portal hypertension (*Schistosoma mansoni*). Again, control is largely dependent on mass chemotherapy but the development of resistance to drugs is a major concern [10].

In recent years, considerable effort has been made to develop a protective vaccine against schistosome infection and several potential

candidate molecules have been identified. One such antigen is the integral membrane protein Sm23 that was originally identified as the target of a protective monoclonal antibody [36]. Sm23 is expressed in all schistosome life stages examined and in several tissues, including the adult tegument [37]. In infected mice and humans, the protein has been found to be highly immunogenic [38,39]. Since protection mechanisms against schistosomes depend on both humoral and cellular immune responses, the use of a defined vaccine candidate in combination with different immunomodulators and the characterization of both the humoral and cellular immune responses elicited by those combinations seem to be essential [40]. Siles-Lucas M *et al*., [40] have investigated the protection rates achieved against *S. mansoni* in mice after vaccination with the Sb14ζ recombinant antigen combined with three different immunomodulatory molecules: the commercial CpG containing polynucleotide (ODN 1826 [41]; an extract of *Polypodium leucotomos* (PAL; [42]), and the synthetically obtained amino alcohol AA2829 [43]. Sb14ζ-CpG-vaccinated mice showed high IFN-γ levels and the absence of IL-4 production. Sb14 ζ -CpG-vaccinated animals also exhibited the production of TNF-α and a balanced IgG1/IgG2a humoral response [40]. PAL, consisting of an extract from the plant *Polypodium leucotomos*, has been shown to down-regulate the Th2-like response induced by parasitic infections, such as *Trichinella spiralis* [44] and *Trichomonas vaginalis* [45]. Similarly, its use in an anti-schistosome vaccine is justified because Th2-type dominant responses have been related to a lack of protection against these parasites [46]. Both the diamine AA0029 and the amino alcohol AA2829, described as synthetic derivates from the immunomodulatory compound myriocin, have been tested previously for their immunomodulatory properties [43], and they have been shown to increase T helper $CD4^+$ and $CD8^+$ cell numbers and nitric oxide (NO) production from mouse immunocompetent cells after *in vitro* stimulation [43].

Ascariasis

It was reported in 2002 that an *Ascaris suum* extract could inhibit lung inflammation and hyper responsiveness. More recently, it was discovered that *A. suum* contains a protein, APAS3, which, upon immunization and challenge is able to induce Th2-dependent, eosinophilic airway hyper reactivity in BALB/c mice [47]. Interestingly, another *A. suum* product, PAS1, inhibits this response (Itami *et al*., 2005. Inhibition is associated with reduced Th2 cytokines (IL-4, IL-5) and chemokines [eotaxin, RANTES (regulated upon activation, normal T-cell expressed and secreted)], and considerably elevated IL-10 levels in bronchoalveolar lavage fluid. PAS1 is most active when administered prophylactically at both immunization and challenge stages; administration at immunization only reveals a partial effect, but PAS1 is completely ineffective when given only as the challenge.

Malaria

Malaria is the world's most important tropical parasitic disease. In 2016, there were an estimated 216 million cases of malaria, an increase of about 5 million cases over 2015 and deaths reached 445,000 (WHO, 2017) (http://www.who.int/malaria/publications/world-malaria-report-2017/en/). Unfortunately, the development of multiple drug resistant isolates of *Plasmodium* spp. and the increased resistance of its vector, the *Anopheles* mosquito, to DDT (dichloro-diphenyl-trichloroethane) underscores the importance of developing new chemotherapeutic means to control the spread of malaria. In this regard, it has always remained imperative to enhance the anti-parasitic efficacy of already existing anti-malarial agents.

The effective immune response to various stages of *Plasmodium* parasite demands simultaneous activation of both humoral as well as cell mediated arms of the immune system of the host. To further enhance the efficacy of most potent anti-malarial drug, chloroquine, against less susceptible isolates of *Plasmodium*, concomitant usage of chloroquine in combination with some potent immunomodulators capable of activating host immune system has been envisaged. Picroliv, a standardized fraction isolated from the ethanol extract of the root and the rhizome of *Picrorhiza kurroa* was found to be effective to correct liver damage induced by *Plasmodium berghei* infection in *Mastomys natalensis* (common African rat) [48]. It was also reported to possess strong immunostimulant activity and showed significant protection against challenge with *L. donovani* promastigotes in experimental golden hamsters [49]. One of the studies evaluated immunomodulatory effect of picroliv in animal models to establish its practical suitability in the treatment of infectious diseases [50]. The potential of combination therapy involving picroliv and chloroquine to combat multiple drug resistant isolate of *Plasmodium yoelii* in animal models was also evaluated. It upregulates the expression of co-stimulatory molecules CD80/86 on the surface of macrophages. Besides, it also activates macrophages for production of ROI and RNI in the immunized animals. The immunomodulator was also found to induce higher T cell proliferation as well as antibody production in the immunized animals [50]. This indirectly suggests that the immunomodulator helps in skewing of immune response in favor of Th1 type of T helper cells.

The immunogenicity of *Plasmodium berghei* recombinant protein rPbMSP1 formulated in alum was found to be immunogenic which induced high levels of specific anti- rPbMSP1 antibody [51]. MSP1 is a merozoite surface protein synthesized during schizogony and its products are localized on the surface of extracellular merozoites. There was elevation in serum IgG2 level and suppression of parasitemia which may be due to the enhancement of the effector mechanisms of protective immunity after rPbSMP1 administration. Few individual chimeric antigens incorporating B cell and T cell epitopes without a fusion protein have shown strong efficiency in eliciting a B cell response, which is the most important immune protection against blood-stage malaria parasites [52]. It has been demonstrated that a multivalent vaccine containing antigens from different stages of the parasite can prevent parasitic infection. The advantage of using multivalent peptides within a chimeric antigen arises from putting together the most immunopotentiating units of vaccine candidates and leaving out the non-antigenic sequences. Immunization of rabbits and mice with the purified 35 kDa antigen, named Malaria RCAg-1 in the presence of Freund's adjuvant strongly generated long-lasting antibody responses that recognized the corresponding individual epitope peptide in this vaccine as well as blood stage parasites. $CD4^+$ T cell responses were also elicited as shown by the enhancement of T cell proliferation, IFN-γ and IL-4 level [53]. The same study also showed that a polyepitope-chimeric antigen malaria vaccine M.RCAg-1 is favorable in eliciting both specific B and T cell responses. In addition, the significant correlation between the stimulation index (SI) and antibody levels, SI and IFN-γ, as well as SI and IL-4 from the immunized animals were observed. Targeting the host, using immunomodulatory compounds, might be a useful strategy to complement the direct anti-parasitic activity of standard anti-malarial drugs and present a valuable tool in the management of resistant parasites.

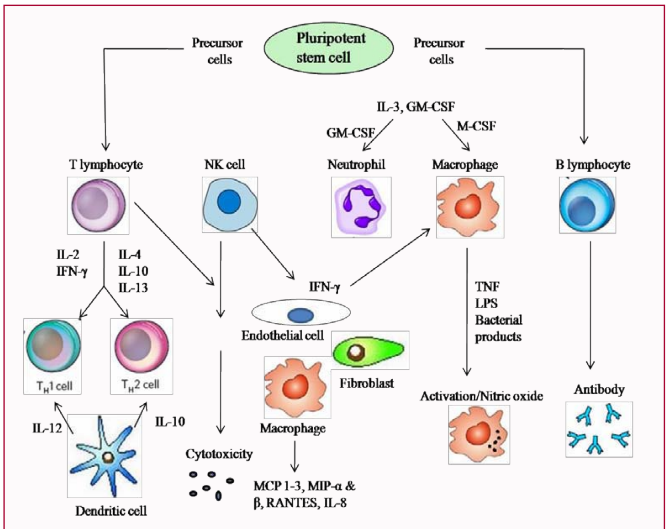

Figure 2: Interaction of cytokines and immune system cells [56].

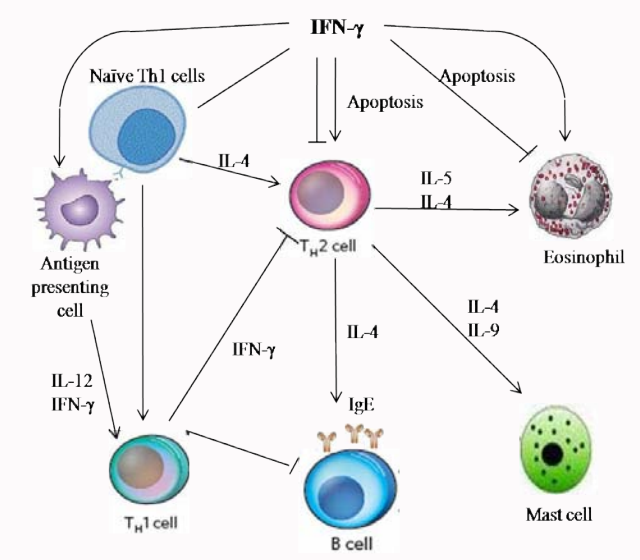

Figure 3: Effects of IFN-γ on T helper immune and allergic responses [57].

Cytokines as immunomodulatory agents

Cytokines are intimately involved in host antimicrobial immune responses. Certain cytokines stimulate the production of other cytokines and interact in complex synergistic or antagonistic networks [7]. Recent advances in identification and cloning using monoclonal antibody and recombinant DNA technologies have led to the availability of ample quantities of cytokines with immunomodulatory activities. Ineffective immunity in parasitic infections is often associated with depressed Th1 cytokine response, and reduced production of IFN-γ. Interleukin-10 (IL-10) is thought to be a central mediator of the depressed IFN-γ response in diseases from intracellular pathogens [54]. T-helper lymphoid cells are crucial in orchestrating the appropriate cytokine responses and are of critical importance for the outcome of infectious diseases. Th1 cells produce IFN-γ, TNF-α and IL-2 and are required for the effective development of cell-mediated immune responses to intracellular microbes. Th2 cells produce IL-4 and IL-5 that enhance humoral immunity to T-dependent antigens and are necessary for immunity to helminth infection [55].

The evidence for a pathophysiological involvement of cytokines in several parasitic disease conditions has been steadily accumulating during the last decade. Several studies have been reported concerning the effects of newer cloned cytokines in infectious disease processes within past few years. The interaction of cytokines and immune system cells is represented in a simplified form in Figure 2 [56].

Interferons

Interferon gamma (IFN-γ) is an important mediator of host resistance during the acute and chronic phases of infection and is pivotal in protection against a variety of intracellular pathogens. IFN-γ is the principal Th1 effector cytokine which exerts direct inhibitory effects on Th2 cytokines (Figure 3) [57]. It induces IL-12 production by dendritic cells and macrophages [58,59]. It is a potent macrophage- activating factor produced by both $CD4^+$ and $CD8^+$ T cells besides cells of innate immune response like natural killer (NK) cells and can induce class I and class II MHC products. Numerous experimental studies have demonstrated an important role for IFN-γ in protection against several infections. Substantive experimental data and emerging clinical results suggested that interferon-gamma (IFN-γ), a T-cell-derived lymphokine with broad macrophage-activating effects, had considerable potential in the treatment of non-viral infections as a host defense enhancing antimicrobial agent. IFN-γ remains a promising host defense enhancing cytokine with still unexplored clinical potential.

Recombinant IFN-γ appears effective as adjunctive therapy in combination with conventional pentavalent antimonial drugs in visceral leishmaniasis and may also benefit certain other forms of cutaneous leishmaniasis [60]. One study reported that rIFN-γ conferred remarkable resistance against acute infection with *Toxoplasma gondii* in T cell-deficient (athymic nude) mice [61]. Peritoneal macrophages obtained from mice injected with rIFN-γ were activated and effectively killed tachyzoites of *T. gondii in vitro*. This suggests that rIFN-γ may be effective for therapy of toxoplasmosis in immunosuppressed patients who have impaired activity of T cell function.

Although immune-based therapy, mainly by administration of exogenous IFN-γ, is a promising path to treating these infectious diseases, it is still plagued by serious problems and dilemmas, such as occasionally severe side effects, and a very high cost, making it nearly impracticable for large-scale use in developing countries.

Tumor necrosis factor

TNF-α (Cachectin) is a protein produced mainly by macrophages, with a wide range of biological activities and may be important in inflammatory processes. In parasitic infection, administration of recombinant human TNF released from intraperitoneal osmotic pumps could effectively suppress the *Plasmodium chabaudi adami* infection in CBA mice [62]. In contrast, a single injection of rabbit antibody to TNF on Day 4 or 7 fully protects CBA mice infected with *P. berghei* from cerebral malaria without modifying the parasitemia [63]. In experimental *Trypanosoma cruzi* infections, treatment of macrophages with recombinant TNF plus lipopolysaccharide (LPS) resulted in a significant reduction in the number of intracellular organisms compared with mock-treated macrophages [64]. It is documented that TNF-α is host protective in experimental cutaneous leishmaniasis [65]. Injection of rTNF into the lesion of CBA mice infected with *L. major* significantly reduced the lesion development and activates macrophages to kill *Leishmania in vitro*. The involvement

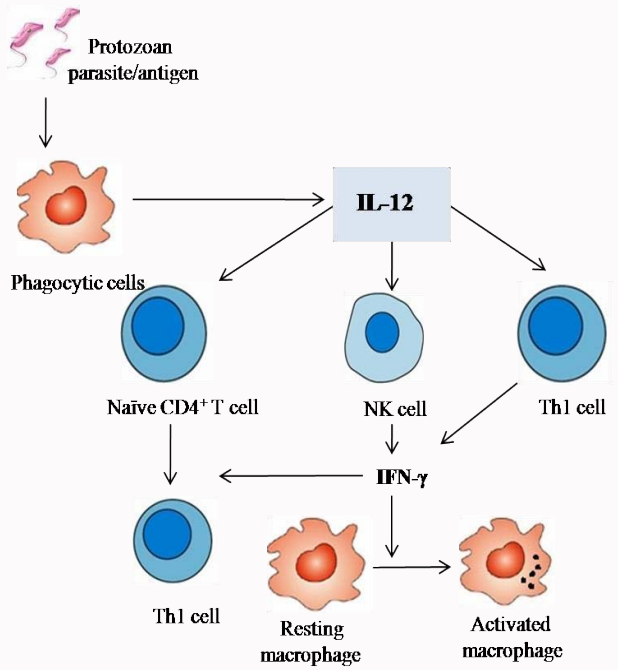

Figure 4: Effects of IL-12 in parasitic infections (http://www.ppdictionary.com/parasites_1.html).

of tumor necrosis factor (TNF) in resistance to *Toxoplasma gondii* infection was examined by means of experiments in which mice were treated with anti-TNF antibodies prior to infection with ME49, a low-virulence *Toxoplasma* strain. The study provides evidence that TNF produced endogenously during *T. gondii* infection plays a role in restraining trophozoite numbers and, in B6 mice infected orally, may prevent death [66]. TNF was able to activate IFN-γ-primed macrophages to exhibit anti-*T. gondii* activity *in vitro*.

Interleukins

Interleukin-12 (IL-12) directly stimulates T-cells and NK cells to produce interferon-γ (IFN-γ).

IFN-γ leads to disease resolution because it can activate macrophages (Figure 4) (http://www.ppdictionary.com/parasites_1.htm). Recombinant IL-12 was useful in the treatment of blood-stage malaria infection. Treatment during the first 5-6 days of infection delayed the onset of parasitemia, significantly reduced the peak of parasitemia, and prevented lethal infection in the *P. chabaudi*-susceptible mice [67]. IL-12 was also effective in correcting malarial anemia [68,69]. One of the recent studies shows that in an NK cell/macrophage co-culture system, IL-12 and IL- 18 drive the production of sufficiently high levels of IFN-γ and TNF for the subsequent induction of macrophage anti-leishmanial activities [70].

Administration of recombinant IL-12 protected BALB/c mice against challenge with *P. yoelli* sporozoites [71] and protected rhesus monkeys against challenge with *P. cynomolgi* through IFN-γ and NO-dependent elimination of the parasite [72]. Another study reported that IL-12 enhances protective immunity in mice immunized with engendered by immunization with recombinant 14 kDa *S. mansoni* fatty acid-binding protein through an IFN-γ and TNF-α dependent pathway [73]. The co-administration of exogenous IL-12 enhanced protective immunity engendered by rSm14 and higher levels of IFN-γ, TNF-α and IgG2a were observed in mice immunized with rIL- 12/rSm14 with no detection of IL-10.

Interleukin-10 (IL-10) is a pleiotropic immunomodulatory cytokine, produced by a wide variety of cells, including activated Th2 cells, monocytes and macrophages, B cells, thymocytes, and keratinocytes [74-76]. IL-10 plays a pivotal role in the establishment and maintenance of a class of immune response by suppressing Th1-dependent cell-mediated immunity and augmenting Th2-dependent immune responses [77]. Through the prevention of macrophage activation, as well as *via* direct interaction, IL-10 has been shown to prevent antigen-specific T cell stimulation, proliferation, and cytokine production indirectly by reducing the Ag presenting ability of monocytes [78]. *L. donovani* induces endogenous secretion of murine IL-10, which in turn facilitates the intracellular survival of the protozoan and selectively impairs PKC-mediated signal transduction [79]. Preincubation of the infected macrophages with neutralizing anti-IL-10 MAb significantly blocked the inhibition of nitric oxide and murine TNF-α release by the infected macrophages.

Clinical implications of immunomodulators

Objectives of immunomodulation as observed by modern researchers are more diverse than were thought. Both immunostimulation and immunosuppression are equally important in clinical medicine. During chemotherapy, immunopotentiation is the strategy followed when the host defense mechanisms are to be activated under conditions of impaired immune response. Reducing the dose and duration of chemotherapy in certain infections like Leishmaniasis or malaria with the adjunct use of immunomodulators will go a long way in not only reducing the toxicity of the drug but also will reduce the chances of development of drug-resistance as well as further transmission by early clearance of pathogen from circulation. Major clinical implications are not restricted to alleviating the human suffering but also handling the epidemics on a larger scale from public health point-of-view.

Concluding Remarks

The appearance of drug-resistant strains of several pathogens manifests the need for the development of innovative therapeutic modalities, which elicits proper host immune response. This becomes essential in infections caused by intracellular pathogens since chemotherapies and conventional vaccines are often partially or totally ineffective against such pathogens. In this review, we have focused on the implications of various immunomodulatory agents during parasitic infections. Selective stimulation by suitable immunomodulators of immune cells and cytokines important in protective effector mechanisms against a given infection will play an exceedingly vital role. The development of molecules with immunomodulatory and anti-parasitic action may pave the way for their use as an adjunctive therapy for the management of parasitic diseases in the coming times.

References

1. Masihi KN, Lange W. Immunomodulators and Nonspecific Host Defence Mechanisms Against Microbial Infections. Oxford, New York: Pergamon Press, 1988: 1–462.
2. Masihi KN, Lange W. Immunotherapeutic Prospects of Infectious Diseases. Berlin: Springer-Verlag, 1990: 1–401.
3. Masihi KN. Immunotherapy of Infections. New York: Marcel Dekker, 1994: 1–508.
4. Masihi KN. Immunostimulants as anti-infectives. In: Nijkamp FP,

Parnham MJ, editors. Textbook of Immunopharmacology. Basel: Birkha¨user Verlag, 1999: 309–325.

5. Agarwal SS and VK Singh. Immunomodulators: A review of studies on Indian medicinal plants and synthetic peptides Part 1: Medicinal plants. PINSA, B65. 1999; 179-204.

6. Nadkarni KM. Indian Materia Medica. Reprinted Vol. 1, Bombay Popular Prakashan, India. 2000; pp: 300-302.

7. Masihi KN. Immunomodulators in infectious diseases: panoply of possibilities. International Journal of Immunopharmacology. 2000; 22: 1083–1091.

8. Jatawa AS, Paul R and Tiwari A. Indian medicinal plants: A rich source of natural immuno-modulator. Int. J. Pharmacol. 2011; 7: 198-205.

9. Maizels RM. Parasite immunomodulation and polymorphisms of the immune system. Journal of Biology. 2009, 8: 62.

10. DP Knox. Parasite Vaccines: Recent Progress in, and Problems Associated with their Development. The Open Infectious Diseases Journal. 2010; 4: 63-73.

11. Luster AD, Alon R and von Andrian UH. Immune cell migration in inflammation: present and future therapeutic targets. Nat Immunol. 2005; 6: 1182-1190.

12. Scapini P, *et al*. The neutrophil as a cellular source of chemokines. Immunol Rev. 2000; 177: 195-203.

13. Liles WC. Immunomodulatory approaches to augment phagocyte-mediated host defense for treatment of infectious diseases. Semin Respir Infect. 2001; 16: 11-17.

14. Knox DP. Development of vaccines against gastrointestinal nematodes. Parasitology. 2000; 120: S43-S61.

15. S Gupta, SC Sharma and VML Srivastav. Efficacy of Picroliv in combination with miltefosine, an orally effective anti*Leishmania*l drug against experimental visceral Leishmaniasis. Acta Tropica. 2005; 94: 41–47.

16. Arif K, Aijaz AK, Dwivedi V, Ahmad MG, Hakeem S and Owais M. Coadministration of tuftsin augments antitumor efficacy of liposomised etoposide against fibrosarcoma in Swiss albino mice. Mol. Med. 2007; 13: 5–6.

17. Khan MA, and Owais M. Immunomodulator tuftsin increases the susceptibility of Cryptococcus neoformans to liposomal amphotericin B in immunocompetent BALB/c mice. J Drug Target. 2005; 13: 423–429.

18. Khan MA, Khan A and Owais M. Prophylactic use of liposomized tuftsin enhances the susceptibility of Candida albicans to fluconazole in leukopenic mice. FEMS Immunol. Med. Microbiol. 2006; 46: 63–69.

19. Herwaldt BL. Leishmaniasis. Lancet. 1999; 354: 1191–1199.

20. Santarem N, Silvestre R, Tavares J, Silva M, Cabral S, Maciel J, *et al*. Immune Response Regulation by *Leishmania* Secreted and Nonsecreted Antigens. Journal of Biomedicine and Biotechnology, J Biomed Biotechnol. 2007; 2007: 85154-85164.

21. Pranati, Bimal S, Pandey K, Sinha PK, Gupta AK, Gingh SK, *et al*. *Leishmania* donovani: immunomodulatory role of 63kDa antigen in the promotion of IFN- gamma response (VL vs HIV-VL co-infection). Am J Immunol. 2006; 2: 52-57.

22. Singh SK, Bimal S, Dinesh DS, Gupta AK, Sinha PK, Bimal R, *et al*. Towards Identifying Immunogenic Targets in Visceral Leishmaniasis: Role of 17kDa and 63kDa Phosphoproteins. American Journal of Immunology. 2005; 1: 96-100.

23. Angana Ghoshal, Sumi Mukhopadhyay, Bibhuti Saha, Chitra Mandal. 9-O-Acetylated Sialoglycoproteins Are Important Immunomodulators in Indian Visceral Leishmaniasis. Clin Vaccine Immunol. 2009; p. 889–898.

24. Rico AI, *et al*. Characterization of the immunostimulatory properties of *Leishmania* infantum HSP70 by fusion to the Escherichia coli maltose-binding protein in normal and nu/nu BALB/c mice. Infect. Immun. 1998; 66: 347–352.

25. Arora SK, Melby PC, Sehgal S. Lack of serological specificity of recombinant heat shock protein of *Leishmania* donovani. Immunol Cell Biology. 1995; 73: 446-451.

26. Mougneau E, *et al*. Expression cloning of a protective *Leishmania* antigen. Science. 1995; 268: 563–566.

27. Skeiky YAW, *et al*. A recombinant *Leishmania* antigen that stimulates human peripheral blood mononuclear cells to express a Th1-type cytokine profile and to produce interleukin 12. J. Exp. Med. 1995; 181: 1527–1537.

28. Koutsoni O, Barhoumi M, Guizani I, Dotsika E. LiEIF and its recombinant polypeptides enhance the maturation of mouse dendritic cells and the production of the protective IL-12 cytokine. BMC Proceedings. 2011; 5: p41.

29. Arora SK, Pal NS, Mujtaba S. *Leishmania* donovani: identification of novel vaccine candidates using human reactive sera and cell lines. Experimental Parasitology. 2005; 109: 163–170.

30. Bhardwaj S, Vasishta RK, Arora SK. Vaccination with a novel recombinant *Leishmania* antigen plus MPL provides partial protection against *L. donovani* challenge in experimental model of visceral Leishmaniasis, Exp Parasitol. 2009; 121: 29–37.

31. Arora SK, Masih S, Vasishta RK. Efficacy of *Leishmania* donovani ribosomal P1 gene as DNA vaccine in experimental visceral Leishmaniasis. Exp Parasitol. 2011; 129: 55-64.

32. Bundy DAP. This wormy world— then and now. Parasitol. Today. 1997; 13: 407–408.

33. Crompton DWT. Gastrointestinal nematodes. In Topley & Wilson's 'Microbiology and Microbial Infections'. Ed. Cox FEG, Kreier JP, Wakelin D. 1998; 5. Arnold: London,1998: pp. 561–84.

34. Weidong P, Xianmin Z, Crompton DWT. Ascariasis in China. Adv. Parasitol. 1998; 41: 110–148.

35. Hlaing T. Epidemiological basis of survey design, methodology and data analysis for ascariasis. In Ascariasis and its prevention and control. Ed. Crompton DWT, Nesheim MC and Paulowski ZM. Taylor and Francis: London, 1989: pp. 351–368.

36. Harn DA, Mitsuyama M, Huguenel ED, David JR. *Schistosoma mansoni*: detection by monoclonal antibody of a 22,000-Da surface membrane antigen which may be blocked by host molecules on lung stage parasites. J Immunol. 1985; 135: 2115–2120.

37. Oligino LD, Percy AJ, Harn DA. Purification and immunochemical characterization of a 22 kilodalton surface antigen from *Schistosoma mansoni*. Mol Biochem Parasitol. 1988; 28: 95–103.

38. Reynolds SR, Shoemaker CB, Harn DA. T and B cell epitope mapping of Sm23, an integral membrane protein of *Schistosoma mansoni*. J Immunol. 1992; 149: 3995–4001.

39. Koster B, Hall MR, Strand M. *Schistosoma mansoni*: immunoreactivity of human sera with the surface antigen Sm23. Exp Parasitol. 1993; 77: 282–294.

40. Siles-Lucas M, Uribe N, L´opez-Ab´an J, Vicente B, Orfao A, Nogal-Ruiz JJ, et al. The Schistosoma bovis Sb14-3-3ζ recombinant protein cross-protects against *Schistosoma mansoni* in BALB/c mice. Vaccine. 2007; 25: 7217–7223.

41. Kumar S, Jones TR, Oakley MS, Zheng H, Kuppusamy SP, Taye A, *et al*. CpG oligodeoxynucleotide and Montanide ISA 51 adjuvant combination enhanced the protective efficacy of a subunit malaria vaccine. Infect Immun. 2004; 72: 949–957.

42. Martinez-Fernandez AR, Nogal-Ruiz JJ, Lopez-Aban J, Ramajo V, Oleaga

A, Manga- Gonzalez Y, *et al*. Vaccination of mice and sheep with Fh12 FABP from Fasciola hepatica using the new adjuvant/immunomodulator system ADAD. Vet Parasitol. 2004; 126: 287–298.

43. Olmo ED, Plaza A, Muro A, Martinez-Fernandez AR, Nogal-Ruiz JJ, Lopez-Perez JL, *et al*. Synthesis and evaluation of some lipidic aminoalcohols and diamines as immunomodulators. Bioorg Med Chem Lett. 2006; 16: 6091–6095.

44. Dea-Ayuela M, Rodero M, Rodriguez-Bueno R, Bolás-Fernández F and Martínez-Fernández AR. Modulation by Anapsos (*Polypodium leucotomos* extract) of the antibody responses against the nematode parasite *Trichinella spiralis*. Phytotherapy Research. 1999; 13: 566–570.

45. Nogal-Ruiz JJ, Gomez-Barrio A, Escario JA and Martinez-Fernandez AR. Effect of Anapsos in a murine model of experimental trichomoniasis. Parasite. 2003; 10: 303–308.

46. Hewitson JP, Hamblin PA and Mountford AP. Immunity induced by the radiation-attenuated schistosome vaccine. Parasite Immunology. 2005; 27: 271–280.

47. Itami DM, *et al*. Modulation of murine experimental asthma by *Ascaris suum* components. Clin Exp Allergy. 2005; 38: 873-879.

48. Chander R, Dwivedi Y, Rastogi R, Sharma SK, Garg NK, Kapoor NK, and Dhawan BN. Evaluation of hepatoprotective activity of picroliv (from Picrorhiza kurroa) in *Mastomys natalensis* infected with *Plasmodium berghei*. Indian J. Med. Res. 1990; 92: 34–37.

49. Pui RP Saxena, Sumati PY Guru, DK Kulshreshtha, KC Saxena and BN Dhawan. Immunostimulant activity of Picroliv, the iridoid glycoside fraction of Picrorhiza kurroa, and its protective action against *Leishmania* donovani infection in hamsters. Planta Medica. 1992; 58: 528–532.

50. Dwivedi V, Khan A, Vasco A, Fatima N, Soni VK, Dangi A, et al. Immunomodulator Effect of Picroliv and its Potential in Treatment Against Resistant *Plasmodium yoelii* (MDR) Infection in Mice. Pharmaceutical Research. 2008; 25: 2312-2319.

51. Omar AW, Roslaini AM, Ngah ZU, Azahari AA, Zahedi M and Baharudin O. A recombinant 19 kDa *Plasmodium berghei* merozoite surface protein 1 formulated with alum induces protective immune response in mice. Tropical Biomedicine. 2007; 24: 119–126.

52. Good MF. Towards a blood-stage vaccine for malaria: are we following All the leads? Nat Rev Immunol. 2001; 1: 117–125.

53. Cai Q, Peng G, Bu L, Lin Y, Zhang L, Lustigmen S, *et al*. Immunogenicity and *in vitro* protective efficacy of a polyepitope *Plasmodium* falciparum candidate vaccine constructed by epitope shuffling. Vaccine. 2007; 25: 5155– 5165.

54. Gong J-H, Zhang M, Modlin RL, Lindsey PS, Iyer D, Lin Y, Barnesi PF. "Interleukin-10 downregulates Mycobacterium tuberculosis-induced Th1 responses and CTLA-4 expression". Infection and Immunity. 1996; 64: 3; 913-918.

55. Hubbell JA, Thomas SN, Swartz MA. Swartz. Materials engineering for Immunomodulation. Nature. 2009; 462: 449-460.

56. Masihi KN. Fighting infection using immunomodulatory agents. Expert Opin Biol Ther. 2001; 1: 641-653.

57. Leonardo K Teixeira, Bruna PF Fonseca, Bianca A Barboza, João PB Viola. The role of interferon-γ on immune and allergic responses. Mem Inst Oswaldo Cruz, Rio de Janeiro. 2005; 100: 137-144.

58. Snijders A, Kalinski P, Hilkens CM, Kapsenberg ML. High-level IL-12 production by human dendritic cells requires two signals. Int Immunol. 1998; 10: 1593-1598.

59. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell. 2000; 100: 655-669.

60. Murray HW. Interferon-gamma and host antimicrobial defense: Current and future clinical applications. The American Journal of Medicine.1994; 97: 459-467.

61. Y Suzuki, K Joh and A Kobayashi. Tumor necrosis factor-independent protective effect of recombinant IFN- gamma against acute toxoplasmosis in T cell-deficient mice. The Journal of Immunology. 1991; 147: 2728-2733.

62. Clark IA, Hunt NH, Butcher GA & Cowden WB. Inhibition of murine malaria (*Plasmodium* chabaudi) *in vivo* by recombinant IFN-gamma or tumor necrosis factor and its enhancement by butylated hydroxyanisole. J. Immunol. 1987; 139: 3493.

63. Grau GE, Fajardo LF, Piquet PF, Allet B, Lambert PH & Vassalli P. Tumor necrosis factor (cachectin) as an essential mediator in murine cerebral malaria. Science. 1987; 237: 1210.

64. Wirth JJ & Kierszenbaum F. Recombinant tumor necrosis factor enhances macrophage destruction of *Trypanosoma cruzi* in the presence of bacterial endotoxin. J. Immunol. 1988; 141: 286.

65. Liew FY, Parkinson C, Millott S, Severn A and Carrier M. Tumour necrosis factor (TNFa) in Leishmaniasis I. TNFa MEDIATES HOST PROTECTION AGAINST CUTANEOUS Leishmaniasis. Immunology. 1990; 69: 570-573.

66. Johnson LL. A Protective Role for Endogenous Tumor Necrosis Factor in *Toxoplasma gondii* Infection. INFECTION AND IMMUNITY. 1992; P. 1979-1983.

67. Muniz-Junqueira MI. Immunomodulatory therapy associated to anti-parasite drugs as a way to prevent severe forms of Malaria. Current Clinical Pharmacology. 2007; 2: 59-73.

68. Stevenson MM, Tam MF, Wolf SF, Sher A. IL-12 induced protection against blood-stage *Plasmodium* chabaudi AS requires IFN-γ and TNF-α and occurs via a nitric oxide dependent mechanism. J Immunol. 1995; 155: 2545-2556.

69. Stevenson MM, Su Z, Sam H, Mohan K. Modulation of host responses to blood-stage malaria by interleukin-12: from therapy to adjuvant activity. Microbes Infect. 2001; 3: 49-59.

70. Prajeeth CK, Haeberlein S, Sebald H, Schleicher U, Bogdan C. *Leishmania-Infected Macrophages* Are Targets of NK Cell-Derived Cytokines but Not of NK Cell Cytotoxicity. Infection and immunity. 2011; p. 2699–2708.

71. Sedegah M, Finkelman F, Hoffman SL. Interleukin 12 induction of interferon γ- dependent protection against malaria. Proc Natl Acad Sci USA. 1994; 91: 10700-10702.

72. Hoffman SL, Crutcher JM, Puri SK, *et al*. Sterile protection of monkeys against malaria after administration of interleukin-12. Nat Med. 1997; 3: 80-83.

73. Fonseca CT, Brito CF, Alves JB, Oliveira SC. IL-12 enhances protective immunity in mice engendered by immunization with recombinant 14 kDa *Schistosoma mansoni* fatty acid-binding protein through an IFNγ and TNFα dependent pathway. Vaccine. 2004; 22: 503–510.

74. De Waal MR, Abrams J, Bennett B, Figdor C, and De Vries J. IL-10 inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J. Exp. Med. 1991; 174: 1209–1220.

75. Go NF, BE Castle, R Barrett, R Kastelein, W Dang, TR Mosmann, *et al*. Interleukin 10, a novel B cell stimulatory factor: unresponsiveness of X chromosome linked immunodeficiency B cells. J. Exp. Med. 1990; 172: 1625–1631.

76. Mizuno T, Sawada M, Marunoouchi T, Suzumura A. Production of interleukin-10 by mouse glial cells in culture. Biochem. Biophys. Res. Commun. 1994; 205: 1907–1915.

77. Mosmann TR and Moore KW. The role of IL-10 in cross regulation of TH1 and TH2 responses. Immunol. Today. 1991; 12: A49–A53.

78. Fiorentino DF, A Zlotnik, P Vieire, TR Mosmann, M Howard, KW Moore and A O'Garra. IL-10 acts on the antigen presenting cells to inhibit cytokine production by TH1 cells. J. Immunol. 1991; 164: 3444–3450.

79. Bhattacharyya S, Ghosh S, Jhonson PL, Bhattacharyya SK, Majumdar S. Immunomodulatory Role of Interleukin-10 in Visceral Leishmaniasis: Defective Activation of Protein Kinase C Mediated Signal Transduction Events. Infection and Immunity. 2001; p. 1499–1507.