IMMUNE EVASION STRATEGIES BY FILARIAL NEMATODES

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Abstract

Approximately 120 million people are infected with the major forms of filarial parasites causing the diseases - lymphatic filariasis. Though infections by these parasites are non-fatal, but they are associated with high rates of morbidity and disability. Helminths are experts in regulating or modifying host immune responses, developing complex mechanisms to diminish host protective responses and promote their long-term infections. Filarial nematodes have evolved a range of strategies to escape and down-regulate the host's immune system for infecting their hosts chronically. These evasion strategies are based on mutual survival of both the parasite and the host. This paper discusses about the immunomodulatory mechanism adopted by the filarial parasites, the range of cells that are targeted during filarial infection and can be utilized to determine new anti-inflammatory therapeutics.

Keywords: Lymphatic filariasis, cell mediated immunity, immunomodulation,

Introduction

Lymphatic filariasis (LF) is a major cause of morbidity causing social stigma and significant obstruction to economic development to emerging countries and has been identified as the second leading cause of permanent and long-term disability world-wide.Lymphatic filariasis is a long-lasting, chronic infection and has been reported to cause profound immunosuppression in actively infected humans and animal models. Filarial worms are tissue-invasive, vector-borne parasitic nematodes that cause tremendous morbidity worldwide. The causative agents of lymphatic filariasis are *Wuchereriabancrofti*, *Brugiamalayi*, and *Brugiatimori*. Out of these three species, *Wuchereriabancrofti*accounts for nearly 90 percent of cases and is prevalent throughout the tropics and some sub-tropical areas world-wide. An estimated 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis; of which, almost 25 million men have genital disease (most commonly hydrocele) and almost 15 million, mostly women, have lymphoedema or elephantiasis of the leg (GPELF, 2011). Several research studies have been carried out for their effective control and eradication. In order to target in on molecular level, it is necessary to explore the response of the host on the cellular front. Therefore, it is mandatory to develop an understanding of the immune reactions shown by the host after the infection. The immune

response against filarial parasites includes a notable range of innate as well as adaptive immune pathways for the induction of extremely influential effector mechanisms. These potentially pathogenic responses by the host immune system should be in equilibrium with immunoregulatory mechanisms.

Immune response in filariasis

In general terms, immunological profile differs between people with microfilaraemia, chronic filarial pathology and tropical eosinophilia syndrome (Freedman, 2002). Asymptomatic microfilaraemic people (carriers) have microfilaria (mf) in their bloodstream generally but show no outward signs of filarial disease. Carriers (mf +ve) are likely to harbour fecund adults in the lymphatics and they appear to be immunologically tolerant to the parasite antigens. Microfilaraemics have reduced cellular immunity to filarial antigens and their lymphocytes stimulation index in lymphocyte proliferation tests is usually very low.These individuals have shown low serum antifilarial antibody levels and a poor production of cytokines in in vitro studies against filarial antigens(Arndts et al., 2012). The mean level of specific IgG4 is significantly higher in microfilaraemic groups than in amicrofilaraemic groups, whereas the mean level of specific IgGl is significantly higher in amicrofilaraemic, symptomatic cases than in microfilaraemic, symptomatic cases (Jaoko et al., 2006). In contrast, a second group of individuals display chronic pathology (CP), such as elephantiasis, that are generally amicrofilaraemic. It is thought that most of the patients of this category are immunologically resistant to incoming L3 and some may also be able to kill adult nematodes. However, there may be an immunopathological consequence of this immunity (Neilsen et al., 2002). People with elephantiasis usually have normal or increased cellular immunity to filarial antigens with stimulation indexes between 5 and 25. Das Bidyut et al., (1996) found that circulating tumour necrosis factor (TNF) is increased during acute filarial episodes suggesting that TNF may play a role in inducing clinical systems. A third, small proportion of the populations termed ‘endemic normal’s (EN) although continually exposed to infective mosquito bites, remain both symptom and mf free. These individuals may generate immune response that prevent the establishment of infection and progression of disease and have the most marked T-cell response to filarial antigens (Freedman, 1998). In individuals with tropical pulmonary eosinophilia (TPE), there is a severe hypersensitivity response with marked eosinophilia, extreme levels of serum IgE and high titres of antifilarialIgG and IgE. Paranjape et al., (1985) found that although the mean level of anti-filarial IgG antibody is very high, a small number of patients who had clinical TPE had lower than expected antibody

titres. During the natural course of infection, humans are simultaneously exposed to different life-cycle stages, making it difficult to distinguish between an immune response mounted against one particular stage of the parasite and a cross-reactive immune response that may be life-cycle stage independent.

Antibody response in filariasis

The longevity of the nematode parasites reflects their ability to induce defects in the host immune system by incorporating modulation of parasite-specific and even generalized B and T cell responses (Gillan et al., 2005). Many studies have addressed the role of antibody as a prerequisite for immunity to filarial nematodes both *in vivo* and *in vitro*. Antibodies have been shown to be involved in microfilarial killing and are also likely to play a role in clearance of L3 (Babu et al., 1999). The levels of various classes of immunoglobulins have been reported to be increased in the host sera infected with various filariid species (Lawrence, 2001). Qualitative analysis has indicated significant antibody responses during the course of filarial infection and loss of motility or killing of larvae *in vitro* in presence of antisurfaceIgG and IgM (Rajan et al., 2005). Increased levels of specific IgA in filarial infections have been reported. Themicrofilaraemic patients were found to be deficient in total as well as filaria-specific IgA. Similarly, a positive correlation between protective immunity and increased levels of filaria-specific IgA was observed in human bancroftianfilariasis (Sahu et al., 2008). The consequences of antifilarialIgM response in filarial infection are becoming clear; previously it was thought that IgM antibodies are related to mf clearance. Significantly greater levels of IgM antibodies are observed in amicrofilaraemicsymptomiatic cases in response to adult parasite antigens than the patients with microfilaraemia. Lower concentrations of IgM antibodies in other forms of clinical disease, endemic normal and non-endemic normals (Ata et al., 1993).Activated macrophages from primed mice carried high levels of IgM on their surfaces, and IgM have been shown to be essential for cell adherence to *Brugia* L3 (Rajan et al., 2005).

Isotype and subclass dominance or restriction is a prominent characteristic of the immune response to a variety of different antigens. The antibody isotype ratios to both pathology and immunity in filarial infections plays a very impotant role. Since IgG4 and IgE are often directed at the same antigenic determinants, the ratio of these two isotypes will be a major factor in determining whether antigen triggers an IgE mediated hypersensitivity response, or whether excess IgG4 can act as an antiallergic blocking antibody (Hussain et al., 1992). Putatively immune (endemic normals, EN) individuals have lower antibody response to worm

extract than individuals with active infection. The EN group exhibit lower parasite-specific isotype responses (IgG1, IgG2, IgG3, IgG4 and IgE). Active microfilaraemic infection is also associated with lower levels of IgG1, IgG2 and IgG3 than in other patient categories. Both quantitative and qualitative analysis of different subclasses of IgG directed against filarial antigens showed that the chronic symptomatic patients have minimal or no IgG4 responses to parasite antigens but very prominent IgG2 and IgG3 responses (Kurniawan et al., 1993) in contrast to a markedly high level of IgG4 in asymptomatic microfilaraemic 'carriers'. The route of infection may also have a strong impact on the type of immune response elicited, as mf inoculated intravenously induce high levels of IgE in addition to IgG1, IgG2a, IgG2b, IgG3 (Lawrence et al., 2001).

Cell mediated immune response in filariasis

First evidence for the involvement of cellular immune response in filariasis came from the studies of Bagai and Subrahmanyam (1970) who demonstrated cellular adhesion to dead and disintegrating microfilariae in pleural cavity of albino rats which developed latency after a period of patent microfilaraemia. Later, the presence of serum dependent cell-mediated mechanisms that damage the infective larvae of filarial nematodes were reported *in vitro*. These findings led to the assumption that such a mechanism should be operative *in vivo* also. Rogers et al. (1975) followed the histological changes in affected lymph nodes of *B. pahangi* infected cats and showed that a strong cell mediated response was elicited by nematode antigens early after infection. Delayed type hypersensitivity reaction was observed in human filariasis using antigen derived from homologous or heterologous filarial species. The use of experimental models aided the attempts to understand the relationship between filarial infection and regulation of immune responses. There is a correlation between altered non-specific/specific immunological reactivity and the presence of circulating microfilariae. T-cell mediated immune responses are involved in the rapid clearance of primary L3 infections and different life stages of parasite stimulates contrasting Th-cell subsets (Babu et al., 2007).L3 and adult worm exclusively stimulate Th-2 cell response with high IL-4, IgG1 and IgE production. Microfilariae injected intraperitoneally initially induce a strong Th1 cell response with the induced secretion of interferon (IFN)-γ which continues during infection, although both IL-4 and IL-5 responses develop later (Pearlman et al., 1993).

Macrophages play crucial roles in the immune response, as they can initiate, modulate and also be final effector cells during immune responses to helminth infections. Studies have suggested an important role of macrophages and their products, viz. nitric oxide (NO) during

helminth infections (Reyes andTerrazas, 2007). Immunocompetent mice which are refractory to infection with infective larvae showed enhanced recovery of adult worms when treated with aminoguanidine, an inhibitor of nitric oxide. In filariasis, *in vitro* studies have demonstrated that both larval and adult *B. malayi* are susceptible to reactive nitrogen intermediates (RNI), generated either via activation of macrophages or chemical donors of NO (Taylor et al., 2005).

Role of *Wolbachia* in host parasite interaction and pathology

Starting from the mid-1970s, electron microscopy studies showed the presence of intracellular bacteria in the body of various species of filarial nematodes infecting both humans and animals. *Wolbachia* are speculated to play an important role in nutrition and metabolism or in subverting the host immune responses. *Wolbachia* not only represent a useful target for the control of filariasis, they alsoseem to play an important role in the immunology and pathogenesis of filarial diseases (Brattig et al., 2004). The mammalian host is exposed to *Wolbachia* and their products as a consequence of degeneration of infective larvae, adult worms or microfilariae and/or as a result of their destruction by the host's immune mechanisms. The exposure of molecules from endosymbionts to the mammalian host was confirmed by the presence of IgG antibodies against the *Wolbachia* surface protein (WSP) (Suba et al., 2007). The recombinant *Wolbachia* hsp60 can stimulate potent inflammatory responses such as production of TNF-α, IL-6 from macrophages. Wolbachial lipopolysaccharide (LPS)-like molecule is the major mediator of inflammatory responses induced directly by the parasite. In addition to inflammatory mediators, wolbachial components also appear to play a very important role in induction of anti-inflammatory mediators as a feedback loop to limit the inflammatory burst (Haarbrink et al., 2000). *Wolbachia*-derived, as well as parasite-derived molecules contribute to the host's cellular hyporesponsiveness. The persistent low-level release of bacterial molecules may induce down-regulation of inflammatory mediators, thus contributing to the anergic state or hyporesponsiveness in chronic infections (Debrah et al., 2006).

Immunoregulation in filariasis

The filarial parasite releases a number of proteins into its ambience, some of which are biologically active and considered to play an important role in the maintenance of infection and its survival through modulation of immune components (King, 2002).The host immune responses strongly polarize towards a Th2/Treg phenotype with the consequential suppression

of T cell proliferative responses (Maizels&Yazdanbakhsh, 2003). Peripheral blood mononuclear cells (PBMC) of microfilaraemic individuals fail to proliferate in response to specific filarial antigen, but treatment of individuals with diethylcarbamazine causes a reassertion of T cell responsiveness. However, PBMC of both EN and patients with chronic pathology proliferate to varying degrees. Hypo-responsiveness in filariasis have been attributed to variety of factors such as decreased production of Th1 cytokines such as interferon (IFN)-γ, enhanced release of anti-inflammatory cytokines such as IL-10, intense down regulation of filaria specific T-cell proliferation, diminished activity of antigen presenting cells (APC), modulation of signal-transduction pathways, parasite induced apoptosis, up-regulation of CTLA-4 and induction of T-regulatory cells in animals and humans harbouring filarial parasite(Babu&Nutman, 2014). The immune down regulation is not confined to a single arm of immunity. Production of both Th1 (IFN-γ) and Th2 (IL-5) inflammatory cytokines are suppressed where IL-4 remains intact and IL-10 levels are increased. The antigen-specific Th1 type hyporesponsiveness observed in microfilaraemic individuals is associated with high levels of spontaneous and parasite antigen-driven IL-10 secretion (Mahanty&Nutman, 1995). T regulatory cells (Tr1 or Th3) secrete high levels of the cytokines, IL-10 and TGF-β, which strongly down-regulate proliferation and IFN-γ response in microfilaraemic (mf+) patients (King et al., 1993). *In vitro* blockage of neutralizing antibodies against IL-10 and/or transforming growth factor β (TGF-β) from lymphocytes was partially able to restore the ability of these cells to mount filaria-specific proliferative response suggesting that parasite-specific Th2 type responses help regulate Th1 subpopulations (Cooper et al., 2001).

Although the resident adult parasites in the lymphatics and mf in the bloodstream are the prime candidates for eliciting immunosuppression, the L3, the infective form of filarial parasites is the critical stage for infection and presumably the target of protective immunity (Devaney &Osborne, 2000). However, it is difficult to assign the role of individual life cycle stages in skewing the immune response in human infection, as the individuals living in an endemic area are exposed to different life cycle stages simultaneously. Since dendritic cells (DC) are one of the first cells to encounter invading L3 in subcutaneous region initiation of down-regulation gets started with them, followed by other regulatory cells (Hoerauf et al., 2005). It has been demonstrated that following the immediate entry of L3, there is a dominance of pro-inflammatory cytokine and up-regulation of activated T cells surface markers CD69 and CD71, with significant increase in the frequency of T cells expressing pro-

inflammatory Th1 cytokines like IFN-γ, TNF-α, GM-CSF, IL-1β, and IL-8 (Babu and Nutman, 2003). T cell response occurs in both the CD4+ and CD8+ T cell compartment and was restricted to the effector/ memory pool (CD45RO+). The host factors influencing immunoregulation, induce Treg populations that suppress effector T cell responses, inhibit protective immunity and thus promote parasite survival (Taylor et al., 2009).

Conclusion

This article focuses onthe immune responseagainst the filarial infection and how these filarial nematodes modulate the host's immune system to favour their survival.It has explored about the immune regulation, general immunological concepts about regulatory T cells, activated macrophages, TLR stimulation by nematodes.Filarial parasites produce a number of immunomodulatory molecules to subside the anti-parasite and immunopathological responses at various levels. These responses vary from early initiating events in innate immunity to the later effector mechanisms in established adaptive responses. Further studies are required in order to explore the immunomodulatory effects of nematode-derived products against filariasis and sincere efforts should be made to develop new therapeutic approaches by understanding the mechanisms and pathways by which such molecules execute the immune evasion strategies.

Acknowledgement

Author of this manuscript would like to acknowledge her colleagues for providing suggestions and useful insight during the preparation of this manuscript.

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