MODULATION OF CELLULAR IMMUNE RESPONSE BY CYTOKINES IN BANCROFTIAN FILARIASIS

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ABSTRACT

Human lymphatic filariasis is a chronic helminth infection caused by *Wuchereria bancrofti* and *Brugia malayi*. Wide spectrum of clinical manifestations are seen in different clinical groups of filariasis patients which comprises asymptomatic patients with circulating microfilaria (MF), individuals with chronic lymphatic obstruction (CP), Endemic normals (EN) who are asymptomatic and amicrofilaraemic or the relatively rare tropical pulmonary eosinophilia (TPE). The cellular immune response to this infection varies in different clinical groups of filarial patients, ranging from normal lymphocyte proliferative response in EN individuals to lymphocyte hyporesponsiveness in MF to total parasite antigen [*Brugia malayi* antigen (BMA)]. But in response to recombinant filarial antigen (pRJ51) the lymphocyte proliferation is restored in MF patients. Interestingly the lymphocytes from MFs responded normally to parasite antigen when EN serum was added in the culture whereas sera from MFs failed to revert the lymphocyte hyporesponsiveness. In order to study the molecular mechanisms responsible for parasite antigen specific lymphocyte hyporesponsiveness, we analysed both the Th1 and Th2 type cytokine gene expression profile in different clinical groups of filariasis patients. MF individuals expressed elevated Th2 type cytokines like IL-4, IL-5 and IL-10 and decreased levels of IL-2 and IFN- γ in response to parasite antigen. Chronic patients have elevated levels of both Th1 and Th2 type cytokines in response to parasite antigen. The EN individuals had a purely Th1 type pattern with absence of IL-4 and IL-5 expression. These studies clearly demonstrate the role of Th2 cytokine like IL-10 in antigen specific hyporesponsiveness seen in MF patients. Any methods to arrest the progression of this disease should concentrate on the means to revert the Th2 type into Th1 type response in the MF patients either by Th1 type cytokine therapy or by using recombinant filarial antigen which stimulates the Th1 response. Further the recombinant filarial antigen which induces Th1 type cytokine response could be used for immunoprophylactic studies.

KEYWORDS : Cytokine, Filariasis, *Wuchereria bancrofti*, RT-PCR, recombinant antigen

INTRODUCTION

The lymphatic filariae, consisting of *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* infect some 120 million persons world wide. Filariasis causes the high level of clinical morbidity and is the second leading cause of permanent and long-term disability among other diseases. It is an impediment to socio-economic

development in 73 endemic countries of the tropics and sub-tropics. (1). Infection with filarial parasites may cause acute or chronic obstruction of the lymphatics that ultimately leads to elephantiasis. To understand the molecular basis of parasite specific anergy in human lymphatic filariasis caused by the nematode *Wuchereria bancrofti*, cellular proliferation and cytokine levels were investigated using *Brugia malayi* antigen (BMA) and recombinant filarial antigen (pRJ51). The study population was selected from individuals residing in Chennai, India, an area endemic

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for bancroftian filariasis. Ten patients with microfilaraemia (MF), ten patients with chronic lymphatic obstruction (CP), and ten uninfected individuals residing in Chennai (endemic normals [EN]) were included in this study.

Cellular immune response to *Brugia malayi* antigen (BMA) and recombinant filarial antigen (pRJ51).

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by ficoll diatrizoate gradient centrifugation. The PBMC proliferation was performed by [³H] thymidine incorporation assay. In order to evaluate the state of cellular responsiveness of the various study populations, base line data was generated on the stimulation index (S.I.) values of the PBMCs derived from the different study groups for both mitogen phytohemagglutinin (PHA), purified protein derivative (PPD), parasite antigens (BMA) and a 58kDa recombinant *W. bancrofti* parasite protein antigen (pRJ51) (2,3). BMA driven proliferation of PBMCs from MF individuals was significantly lower than that seen with CP individuals and EN individuals (Fig 1). In contrast, there was no significant difference in PPD and PHA induced proliferation of PBMCs among all patient groups. The 58kDa recombinant filarial protein induced a lymphocyte proliferative response in all the clinical group of filariasis patients (Fig. 1). Further, the proliferative response towards the recombinant filarial protein (pRJ51) was significantly higher than that of BMA in MF patients. The possible reason being that the recombinant antigen is free from several down regulatory peptides associated with the unfractionated antigen (BMA) and is free of post translational modifications that add phosphoryl choline or carbohydrates and might interfere with induction of immune response. This result demonstrates that recombinant antigens can be used as candidate antigens for future immune response and vaccine studies in filariasis.

Effect of cytokines or serum on the lymphocyte proliferative response

Interleukin-1 (IL-1) is one of the soluble costimulatory molecules necessary for the activation of antigen presenting cells (4). Antigen presentation in the absence of costimulatory molecules could lead to hyporesponsiveness. In order to find

out the functional role of IL-1 and a Th1 type cytokine i.e., IFN- γ in lymphocyte hyporesponsiveness in filariasis, we studied the PBHC proliferative response in MF to BMA in the presence of IL-1 or IFN- γ and also serum from EN, MF or CP patients. The addition of recombinant IFN- γ along with BMA did not reverse the hyporesponsiveness in MF individuals (Fig. 2). When the serum from EN or CP were added to MF PBMC cultures, the S.I. values improved. But the addition of serum from MF patients did not improve the proliferation index. Among the filariasis infected patients, the CP serum had lesser suppressive effect than the MF serum. This study indicates that the MF patients serum lack costimulatory molecule(s) other than IL-1 (eg: CD80, CD86, CD28) which might be downregulated by some parasite antigen resulting in lymphocyte hyporesponsiveness.

Cytokine profile in different clinical groups of filariasis patients

In human lymphatic filariasis, previous studies on MF patients have revealed that parasite antigens evoked a Th2 response in the absence of a Th1-type response (5-8). T cell lines derived from Indonesian subjects generated to *Brugia malayi* antigen produced high levels of IL-4 relative to IFN- γ (8). It has been demonstrated that individuals with active *B. malayi* infection had lower levels of filarial antigen driven IFN- γ secretion when compared to ENs and CP patients (9). IL-10 is one of the cytokines belonging to Th2 subset that inhibits the cytokine produced by Th1 cells. The role of IL-10 in the downregulation of antigen responsiveness in parasitic infections has been reported in filariasis by us and in other parasitic infections (5,7,10,11).

To understand the role of cytokines in antigen specific hyporesponsiveness in MF patients, we studied the levels of expression of cytokine genes IL-2, IFN- γ , IL-4, IL-5 and IL-10 by RT-PCR in different clinical groups of filarial patients. The PCR products were detected by southern hybridization using fluorescein labelled cytokine probes. The OD values were determined using densitometer and were normalized with HPRT OD values of the respective samples.

Parasite antigen simulated expression of IL-4 and IL-5 were similar in MF and CP patients (Fig 3). In response to parasite antigen, EN individuals did not express IL-4 and IL-5 genes. The MF individuals were unable to respond to parasite

Fig.1 : Lymphocyte proliferative response in different clinical groups of filarial patients

PHA Phytohaemagglutinin

PPD Purified protein derivative

BMA *Brugia malayi* antigen

pRJ51 Recombinant filarial antigen

Fig.2 : Effect of IL-1 α , IL-1 β , IFN- γ and filarial patient serum in lymphocyte proliferation of MF patients

antigen and exhibited poor expression of IL-2 or IFN- γ , suggesting that, in the MF individuals, there is a defect in the production of Th-1 type cytokines (Fig 3). In EN individuals, there was parasite antigen induced expression of IL-2 and IFN- γ , suggesting a clear polarized Th1 subset differentiation pathway. When the IL-10 production in response to parasite antigen was examined among the different groups, it was seen that only

the MF individuals had an IL-10 response (Fig 3), lending credence to the hypothesis that IL-10 is the cytokine responsible for the downregulation of the Th1 responses in these MF individuals. Also there was a significant negative correlation ($p < 0.001$ by Spearman rank) between IL-10 mRNA expression and PBMC proliferation in the MF individuals.

Fig.3 : Th 1 and Th2 type cytokine gene expression in filarial patients in response to BMA

Th shift and disease development

It is clear that in human helminth infection there is an alteration in the balance of Th cells towards Th2 cells. In significant proportion of resistant individuals living in endemic areas, the PBMC proliferative response to parasitic antigen was higher and the cytokine production are of Th1 type. (Figs 1 and 3). In established murine Schistosomiasis, infection correlates with Th2 response while protective immunity induced by vaccination with irradiated cercaria correlates with elevated Th1 cytokine production and is partially ablated by treatment of the vaccinated mice with monoclonal antibodies against IL-2 receptor and IFN- γ (10, 12). Thus in parasitic diseases Th2 type cytokine production generally leads to progressive and persistent infection and Th1 type of cytokine production leads to resistance to parasitic infection.

Th1 to Th2 shift in MFs. Individuals living in the endemic areas (EN) show a strong proliferative and Th1 response to parasitic antigen (Figs 1 and 3). In filariasis, the disease establishes in susceptible individuals wherein, the adult worms reside in the lymphatics and release the microfilaria in the blood stream. During this time parasite antigen specific T cell proliferation diminishes with enhanced IL-10 production i.e., Th2 type response. This hyporesponsive state is unstable and in many individuals it eventually breaks down, leading to expression of both Th1 and Th2 types of cytokines. This non-tolerant condition is associated with normal T-cell proliferative response and lymphatic pathology seen in chronic patients.

CONCLUSION

Thus the modulation of lymphocyte response in patients with MF are likely to be multifactorial with a predominant Th2 response to filarial antigen contributing the major component of

The mechanism involving the shift in response towards Th2 predominance in helminth infection is unclear but such a shift could result from structural elements in helminth derived antigens or from the chronic antigenic load that occur in helminth infection (10,12).

There is a clear cut evidence that there is a

hyporesponsiveness. The parasite antigen specific hyporesponsiveness is associated with enhanced IL-10 production. With the advent of recombinant DNA technology, the recombinant filarial antigens can be screened for Th1 type cytokine inducing capability and eventually could be used for immunoprophylactic studies.

REFERENCES

1. WHO/CTD/TDR Lymphatic Filariasis infection and disease control strategies, Report of meeting held at Malaysia, August 1994.
2. Theodor, J.G., Kaliraj, P., Jayachandran, S. and Jayaraman, K. (1993) Cloning, overexpression and evaluation of a recombinant fusion protein of *Wuchereria bancrofti* towards its application as a diagnostic agent for bancroftian filariasis J. Helminth. 106, 413-420.
3. Regunathan, J., Jayaraman, K. and Kaliraj, P. (1997) Cellular immune response studies in bancroftian filariasis. J. Helminth. (In Press).
4. Weaver, C.T. and Enanue, E.R. (1990) The costimulatory function of antigen presenting cells. Immunol. Today. 11, 49-59.
5. King, C.L., Mahanty, S., Kumaraswami, V., Abrams, J.S., Ragunathan, J., Jayaraman, K., Ottesen, E.A. and Nutman, T.B. (1993) Cytokine control of parasite specific anergy in human lymphatic Filariasis Preferential induction of regulatory T helper type 2 lymphocyte subsets, J.Clin. Invest. 92, 1667-1673.
6. Mahanty, S., King, C.L., Kumaraswami, V., Ragunathan, J., Maya, A., Jayaraman, K., Abrams, J.S., Ottesen, E.A and Nutman, T.B. (1993). IL-4 and IL-5 secreting lymphocyte populations are preferentially stimulated by parasite-derived antigens in human tissue invasive nematode infections, J Immunol. 151, 3704-3711.
7. Mahanty, S., Mollis, S.N., Ravichandran, M., Abrams, J.S., Kumaraswami, V., Jayaraman, K., Ottesen, E.A. and Nutman, T.B. (1996) High levels of spontaneous and parasite antigen-driven Interleukin-10 production are associated with antigen-specific hyporesponsiveness in human lymphatic filariasis. The Journal of Infectious Diseases, 173, 769-773.
8. Yazdanbakhsh, M., Sartono, E., Kruize, Y.C., Kurniawan, A., van der Pouw-Kraan, T., van der Meide, P.H., Selkirk, M.E., Partono, F., Hintzen, R.Q., van Lier, R.A. et. al. (1993) Elevated levels of T cell activation antigen CD27 and increased interleukin-4 production in human lymphatic filariasis. Eur. J. Immunol 23, 3312-3317.
9. Maizels, R.M., Sartono, E., Kurniawan, A., Partono, F., Selkirk, M.E. and Yazdanbakhsh, M. (1995), T cell activation and the balance of antibody isotypes in human lymphatic filariasis, Parasitol. Today. 11, 150-56.
10. Urban, J.F.J., Madden, K.B., Svetic, A., Cheever, A., Trotta, P.P., Gause, W.C., Katona, I.M. and Finkelman, F.D. (1992) The importance of Th2 cytokines in protective immunity to nematodes. Immunol. Rev. 127, 205-210.
11. Ravichandran, M., Mahanty, S., Kumaraswami, V., Nutman, T.B. and Jayaraman, K. (1997) Elevated IL-10 mRNA expression and down regulation of Th1 type cytokines in microfilaremic individuals with *Wuchereria bancrofti* infection. Parasite. Immunol. 19, 69-77.
12. Sher, A. and Coffman, R.L. (1992) Regulation of immunity to parasites by T cells and T cell-derived cytokines., Annu. Rev. Immunol. 10, 385-409.