View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by NIRT Institutional Repository

Ann. Natl. Acad. Med. Sci. (India), Vol. 21 No. 3, July-Sept., 1985

Detection of Circulating Filarial Antigen

P.R. Narayanan and R.S. Paranjape

Tuberculosis Research Centre, Madras-600 031,

Abstract

Affinity binding to specific solid phase antibody, immune complex binding to Raji cells or PEG precipitation of immune complexes was used in conjunction with Western blotting to detect circulating filarial antigen in filarial sera. A high molecular weight antigen was present in free as well as complex form. PEG precipitation method revealed specific filarial antigens in the low molecular weight region also.

Introduction

The diagnosis of filarial infections can be made with certainty by the detection of filarial parasite in the body. The demonstration of filarial parasite especially in bancroftian filariasis is often difficult due to behavioural peculiarities of the parasite. The adult parasite remains in relatively inaccessible sites in the body and the larvae appear in the blood only during the night. An alternative approach to the diagnosis, namely, demonstration of filaria specific antibodies has been employed, though with limited success. The cross reactivity of non-filarial helminth antigens with filarial antigens imposes severe restriction on the specificity of antibody assays, especially due to the non-availability of species or stage specific filarial antigens. (Kagan, 1980 and Paranjape, *et al,* 1985). In the light of this, a recent approach to antigen detection has yielded considerable success in specific diagnosis of filariasis. Many antigen detection system have been reported for bancroftian filariasis. (Dasgupta *et al.,* 1984; Hamiltion *et al.,* Prasad *et al.,* 1983; Senarath *et al,* 1984). Attempts to recognize and characterize the antigens in circulation have been successful (Paranjape, *et al.,* 1984). In this paper we arc reporting the application of three different methods for detection of circulating antigens and antigen-antibody complexes.

Material and Methods

Sera of patients with microfilaremia were stored at –70°C till use. Control serum was obtained from normal North American individuals.

Affinity binding methods

Fifty or 200 ul plasm was rotated overnight at room temperature with 450 ul of a 2.5% suspension of rabbit anti BmA sorbent. Subsequently the sorbent was washed with PBS pH 7.4. The last wash was carried out in PBS containing 0.5 M NaCl. Washings were done by centrifugation at 2000 rpm at 4°C. The sorbent was aspirated dry after the last washing. The pellet was vortexed and boiled in 50 ul of SDS sample buffer for five minutes. The sorbent was then microfuged and 25 ul of supernatent was applied to the gel, The extracted antigens were separated on SDS-PAGE slab gel (7.5% to 12.5%) in a discountinuous tris-HCl buffer system., (Laemmeli, 1970). The gel was run in 0.025M tris-glycine (pH 8.4) for 4 hr at 25 mA constant current. The antigens separated in the SDS-PAGE were blotted onto Nitrocellolose paper. Electro-blotting was performed in a Bio-Rad transblot ceil at a constant current of 0.1 to 0.15 mA per gel at 4-10°C for 14 to 18 hr. (Tourbine *et al.,* 1979)

The NCP with the antigens bound to it was incubated with 5 uCi of ^{125}I-labeled rabbit anti-BmA in 20 ml Tris-HCl buffer containing 5% heat inactivated fetal calf serum for 2 hr at room temperature with gentle rocking. This incubation was followed by three washes in wash buffer containing 10 mM EDTA and one wash in buffer. containing 0.5 M KI. The NCP was dried and exposed to X-ray films, (DEF-2), in Kodak X-Omatic cassettes with intensifying screen. The films were exposed for one day or three days at –70°C, developed and fixed.

Antigens detection in immune complexes using Raji cell assay

The patients' sera were diluted 1 : 1 in RPMI-1640 and incubated with 1 X 10^7 viable Raji cells at 37°C for one hour. The supernatant after initial centrifugation was saved and applied in antigen detection assay to detect non-complexed antigens. After incubation the cells were washed thrice with RPMI-1640 and subsequently incubated with Citrate buffer (pH 3.0) for 10 minutes. The suspension was microfuged and the supernatant medium was applied to SDS-PAGE and tested for filarial antigen by immunoblotting.

Polyethyelene glycol precipitation method

The immune complexes present in the sera (0.2 ml) were precipitated with polyethylene glycol (2.4% final concentration) (PEG-6000) and washed the precipitate was washed twice in cold. Precipitate was finally aspirated dry and

dissolved in 50 LJ of SDS-sample buffer, of which 25 ul was applied on the SDS-PAGE and processed further in the same way as described for other two methods.

Results

Affinity binding methods

Using Ra BmA on a solid phase as a trap, it was possible to extract the antigen and to elute it, subsequently to be detected by immunoblotting. The figure la shows the antigen specific band (indicated by arrow) in autoradiogram of the sera of macrofilaremic patients. This band was absent in control samples. The bands in control samples are of non-specific nature since these bands were obtained even when buffer alone (Blank Control) was used.

Raji cell assay

When two positive (microfilaremic) and one control sample were used for detection of immune complexed and free antigen, it was possible to detect parasite specific antigen in both free and complexed form in one serum and only in the free form in other serum (Fig. 1 b).

PEG-Precipitation method

Immune complexes in the serum were precipitated by 2.4% PEG-6000. The precipitate was autoradiographically analysed and it yielded a number of bands specific for filarial parasite (Fig. 1c). The bands were present in both the high and low molecular weight regions. The antigen detected in affinity binding method as well as Raji cell binding was also detectable by the PEG precipitation method.

Discussion

There are a number of antigen detection methods available for the diagnosis of filariasis. (Dasgupta, *et al,* 1984; Hamilton, *et al;* Prasad *et al,* 1983; Sevarath, *et al.,* 1984) However, there has not been sufficient effort made to identify the antigens in circulation. We have described in our earlier publication the application of affinity binding method for detection and characterization of circulating filarial antigen, (Paranjape *et al.,* 1984). However, affinity binding method could detect only one predominant circulating antigen in microfilaremic sera. Since a number exo-secretory antigens of filarial parasite have been

characterised (Kaushal *et al.,* 1982), it is evident that other or modified methods need be applied for the detection of other antigens.

When we used binding of immune complexes to Raji cells for eluting the antigens, we were again able to find the same predominant circulating antigen in the serum. The antigen was found in both free and complexed form. This shows that in some patients probably this antigen does not elicit antibody response and hence remains free in circulation. It will be of interest to analyse sera from various clinical categories of filarial disease and to study the antibody levels against this antigen.

The high molecular weight antigen was not only detected in affinity binding method, but also by Raji cells as well as polyethylene glycol precipitation. Polyethylene glycol precipitation yielded the predominant high molecular weight circulating antigen, but of more interest were many antigens in the low molecular weight region that were not seen in other methods. There antigens may either be more sensitive and get destroyed in harsh eluting conditions or they are not bound in sufficient quantity for being able to detect them in final immunoblot analysis. The other possibility is that these antigens may be present in complexes involving IgE and IgA class of antibodies and hence are not detectable in both first and second methods.

Thus, by applying three different methods we have been able to detect circulating antigens in microfilaremic sera. It is significant to note that each method has its limitations and hence, as many diverse methods as possible should be employed for detection and characterisation of the whole complement of circulating antigens. Knowing the identity of them is necessary to identify the right antigens for application in the immunodiagnosis of filariasis.

Acknowledgement

We acknowledge Dr. Eric A Ottesen, Dr. Rabia Hussain and other staff members of Laboratory of Parasitic Diseases, where major portion of the above work *was* carried out and thank Mr. P. Karthigayan for secretarial assistance.

References

Dasgupta, A., Bala, Shukal and Datta, S. N. (1984). Lymphatic filariasis in man: demonstration of circulating antigens in Wuchereria bancrofti infection. *Parasite Immunol.* **6** : 341-34:.

Hamilton, R. G.. Hussain, R. and Ottesen, E. A. Immunoradiometric assay for detection of filarial antigens in human serum. *J. Immunol;* (in press).

Kagan, I.G. (1980). Serodiagnosis of parasitic diseases. In Manual of Clinical Microbiology, 3rd Edition, Eds. Lennette, E.H., Balros, A., Mausler, Ju, W.L.J. and Tsuant, J. P., Am. Soc. for Microbiology, Washington DC, p. 724.

Kaushal, N. A., Hussain, R., Nash, T. E.. and Ottesen, E. A. (1982). Identification and characterization of excretory secretory products of Brugia malayi adult filarial parasite. *J. Immunol* **129** : 338-343.

Laemmeli, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature.* **227** : 680.

Paranjape, R. S., Hussain. R., Nutman, T. and Ottesen, E. (1984). Detection of High molecular weight circulating parasite antigen in Bancroftian filariasis. *Fed. Proceedings,* **43(6)** : Abstract No. 578.

Paranjape, R. S., Kumaraswami, V., Prabhakar, R. and Narayanan, P. R. (1985). Increased serum levels of Antifilarial (IgA) antibodies in patients with Topical Pulmonary Eosinophilia. *Lung India,* **3** : 27-29.

Prasad, G. B. K. S., Reddy, M.V.R. and Harinath, B. C. (1983). Detection of filarial antigen in immunocomplexes in bancroftian filariasis by ELISA. *India J. Med. Res.* **78** : 780.

Senarath Dissanayaka, Forsyth, K. P., Ismail, M. M. and Mitchell, G. F. (1984). Detection of circulating antigen in Bancroftian filariasis by using a monoclonal antibody. *Am. J. Trap. Med. Hyg.,* **33(6)** : 1130-1140.

Towbin, H. T., Stachlin, T. and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocelluloso sheets; procedures and some application. *Proc. Nat. Acad. Sci.,* **76** : 4350.

Fig. a. b and c represent autoradiogramms obtained using affinity binding, Raji cell binding and PEG precipitation. The bands shown by arrows represents parasite specific components. (B-Blank, C-North American Control Serum and mf (+) – microfilaremic serum).