RESEARCH NOTE

Use of Synthetic Peptides Derived from Adult Worm Proteins of *Schistosoma mansoni*, in the Diagnosis of Schistosomiasis

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It has become increasingly evident that in intestinal schistosomiasis, parasitological techniques frequently fail to reveal low-intensity infections. This happens in countries with a low level of transmission as Venezuela, where approximately 80% of individuals eliminate less than 100 eggs/g of feces (B Alarcón de Noya et al. 1992 *Mem Inst Oswaldo Cruz 87*: 227-231). In this and other countries as China (Yu Sen Mai et al. 1992, p. 29-38. In NR Bergquist, *Immunodiagnostic Approaches in Schistosomiasis*, John Wiley & Sons), control programs have incorporated serology as a way of improving diagnosis and treatment of the affected populations. Several serologic tests such as the circumoval precipitin test, indirect immunofluorescent assay, and immunoenzymatic assays with crude antigens, have been preferred among the antibody detection methods. However, none of them fulfill all of the following requirements: low cost, simplicity, high specificity and sensitivity, and correlation with active infection and worm burden (B Alarcón de Noya et al. 1996 *Epidemiol Infect 116*: 323-329). As alternative, the detection of circulating antigens such as the circulating cathodic antigen and the circulating anodic antigen by monoclonal antibodies (A Deelder et al. 1989 *Am J Trop Med Hyg 40*: 268-272, N De Jonge et al. 1990 *Trans R Soc Trop Med Hyg 84*: 815-818, 1991 *85*: 756-759) have shown promising results only in areas of moderate and high transmission of schistosomiasis (H El-Morshedy et al. 1996 *Am J Trop Med Hyg 54*: 149-153). Therefore, alternative methods need to be developed and synthetic peptides, either for the detection of antibodies or antigens, could be one of the approaches.

Previously, other authors (A Ruppel et al. 1985 *Clin Exp Immunol 62*: 499-506, 1990 *Trop Med Parasitol 41*: 127-130, M Klinkert et al. 1991 *Trop Med Parasitol 42*: 319-324) and ourselves (O Noya et al. 1995 *Parasite Immunol 17*: 319-328), have identified two prominent molecules of the adult worm of *Schistosoma mansoni*, Sm 31-cathepsin B and Sm 32-asparaginil endopeptidase, which besides being highly antigenic in infected individuals, are exoantigens actively eliminated to the blood stream (Y Li et al. 1996 *Parasitol Res 82*: 14-18). Both molecules were cloned by M Klinkert et al. (1989 *Mol Biochem Parasitol 33:* 113-122), and based on their sequence, we chemically synthesized peptides of the complete molecules.

Our aims in the present study were (i) the identification of the most antigenic peptides using human sera, in order to develop an antibody-detection method; (ii) to evaluate their immunogenicity in rabbits, in order to get polyclonal and monoespecific antibodies, able to recognize the native Sm 31 and Sm 32 molecules, in an antigen based capture assay currently in process.

Serum samples were collected from 51 infected individuals from the Carabobo and Aragua states, Venezuela, and 25 negative controls from the same area.

Adult worm antigen (AWA) was obtained from adult worms (strain JL, kindly provided by Dr Italo Cesari, IVIC, Caracas, Venezuela) collected from hamsters and processed as previously described (Noya et al. *loc. cit.*). AWA was separated by SDS-PAGE, electrotransferred onto nitrocellulose paper and exposed to immune rabbit for the immunoblot as previously described (Noya et al. *loc. cit.*).

Based on the protein sequences of the Sm 31 and Sm 32 described by Klinkert (*loc. cit.*), we chemically synthesized 17 polymerized molecules of the Sm 31 and 22 of the Sm 32. They were manu-

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ally synthesized using a solid phase (B Merrifield 1963 *J A Chem Soc 85:* 2149-2154, R Houghten 1985 *Proc Natl Acad Sci 82*: 5131-5135) under the t-boc strategy, at the Instituto de Medicina Tropical, Universidad Central de Venezuela.

Each group of two or three rabbits were immunized with a pool of 5 to 6 different peptides following a protocol of 0, 15 and 30 days. Each dose corresponded to 250 mg of each peptide, diluted in 0.5 ml of complete Freud adjuvant for the first and incomplete adjuvant, the second and third doses. Bleeding was carried out before the first immunization and ten days after the third dose.

Antigenicity and immunogenicity of peptides were evaluated using a dot-blot assay developed in our laboratory (multiple antigen blot assay, MABA) (O Noya & B Alarcón de Noya 1998 *J Immunol Lett*, submitted). Briefly, peptides are immobilized onto a nitrocellulose membrane, using an acrylic template. Two-mm width nitrocellulose strips, containing small squares of the total number of peptides of each of the molecules investigated, are exposed to immune human or rabbit sera. Thereafter, a second antibody conjugated to horseradish peroxidase was added and developed by a chemiluminiscent substrate (Luminol^{R}). Positive reactions to the different antigens are recorded as small black squares in a film.

Antigenicity of synthetic peptides with human sera was evaluated by MABA. Table, summarizes the sensitivity and specificity of the more reactive peptides. It is evident that peptides derived from the Sm32 were poorly antigenic, suggesting that we were not able to imitate the conformation of the important epitopes of the original molecule. Indeed the strongest reactive peptide was the IMT-70 from the Sm32, which only reached a 29% sensitivity. Conversely, peptides IMT-164, 178 and 180 derived from the Sm31, showed 49, 49 and 86% sensitivity, respectively, with a 100% specificity. Furthermore, when we combined the results of the IMT-164 and 180, the sensitivity increased to 96%.

These results indicate the potential of an antibody-based detection technique, using combination of peptides IMT-164 and 180 from the Sm31 molecule, based on their high sensitivity and specificity. As far as we know, there are not previous results using synthetic peptides in the diagnosis of schistosomiasis. There is a previous report (M Klinkert et al. 1992, p. 59-70. In N Bergquist, *Immunodiagnostic Approaches in Schistosomiasis*, John Wiley & Sons) using recombinant peptides from Sm31 in which the maximum sensitivity obtained was 68.3%. We currently evaluate these synthetic peptides, by the classical immunoenzymatic assay and by a latex agglutination test.

In order to develop an antigen-detecting technique based on the capture of circulating Sm31 and Sm32 molecules excreted by adult worms, we obtained immune sera from rabbits immunized with peptides. Among those, the IMT-12, 14 and 64 of the Sm32, were the responsible of the specific recognition of the parasite molecule demonstrated by western blot.

In the case of Sm31 molecule, peptides IMT-172 and 180 were the responsible of the recognition of the native molecule. This was demonstrated not only by western blot, but also by immunocytochemistry of cross-sections of adult worms.

These highly reactive and specific antibodies are being evaluated in an antigen-capture assay. We expect that with the mixture of antibodies against different epitopes and molecules to increase the sensitivity reached by previously developed antigen capture assays (Li *loc. cit.*). Finally, we can conclude that synthetic peptides are a promising approach on the search of sensitive and specific diagnostic assays, based either on the detection of antigens or antibodies. This is of particular relevance in areas of low and moderate intensity of infection, together with the evaluation of efficacy of chemotherapy.

TABLE

Frequency of recognition by multiple antigen blot assay of Sm31 and Sm32 derived synthetic peptides from patients with schistosomiasis

Peptide code	Molecule	*Schistosoma mansoni* infected individuals Positive/Evaluated	(%)	Negative controls Positive/Evaluated
IMT-6	Sm32	3/21	14	0/5
IMT-12	Sm32	5/21	24	0/5
IMT-70	Sm32	6/21	29	0/5
IMT-26	Sm32	3/21	14	0/5
IMT-164	Sm31	25/51	49	0/20
IMT-172	Sm31	18/51	35	0/20
IMT-176	Sm31	13/51	25	0/20
IMT-178	Sm31	25/51	49	0/20
IMT-180	Sm31	44/51	86	0/20