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ONCHOCERCIASIS IN THE UNITED REPUBLIC OF CAMEROON: THE DEVELOPMENT OF ELISA FOR IMMUNODIAGNOSIS

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In Progress Report No. 2, December 1983, I had described the preparation of proteins of O. volvulus adult worms by brief treatment with collagenase followed by extraction with triton X-100/ Tris buffer. I had also screened 132 onchocerciasis sera by ELISA for high titer antibodies. These sera and the worm extracts were to be used for the construction of a human anti-O. volvulus antibody affinity column for the isolation of O. volvulus antigens. I report here that 2.0 mg of O. volvulus antigens have been purified by such an affinity column. These results pave the way for the generation of monoclonal antibodies specific for O. volvulus proteins which are antigenic in the human host.

Summary of Experiments

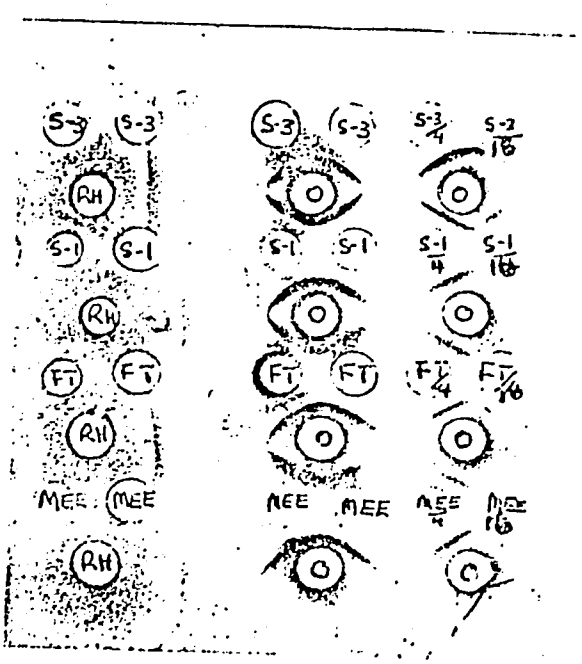
Experiment 1. Evaluation of O. volvulus extracts for antigenic activity.

Detergent extracts of O. volvulus proteins ISS-1, ISS-3, FT-1 and MEE-1 (refer to last progress report) were analyzed for antigenic activity by a) Cuchterlony agarose gel diffusion (Fig. 1)

Fig. 1.

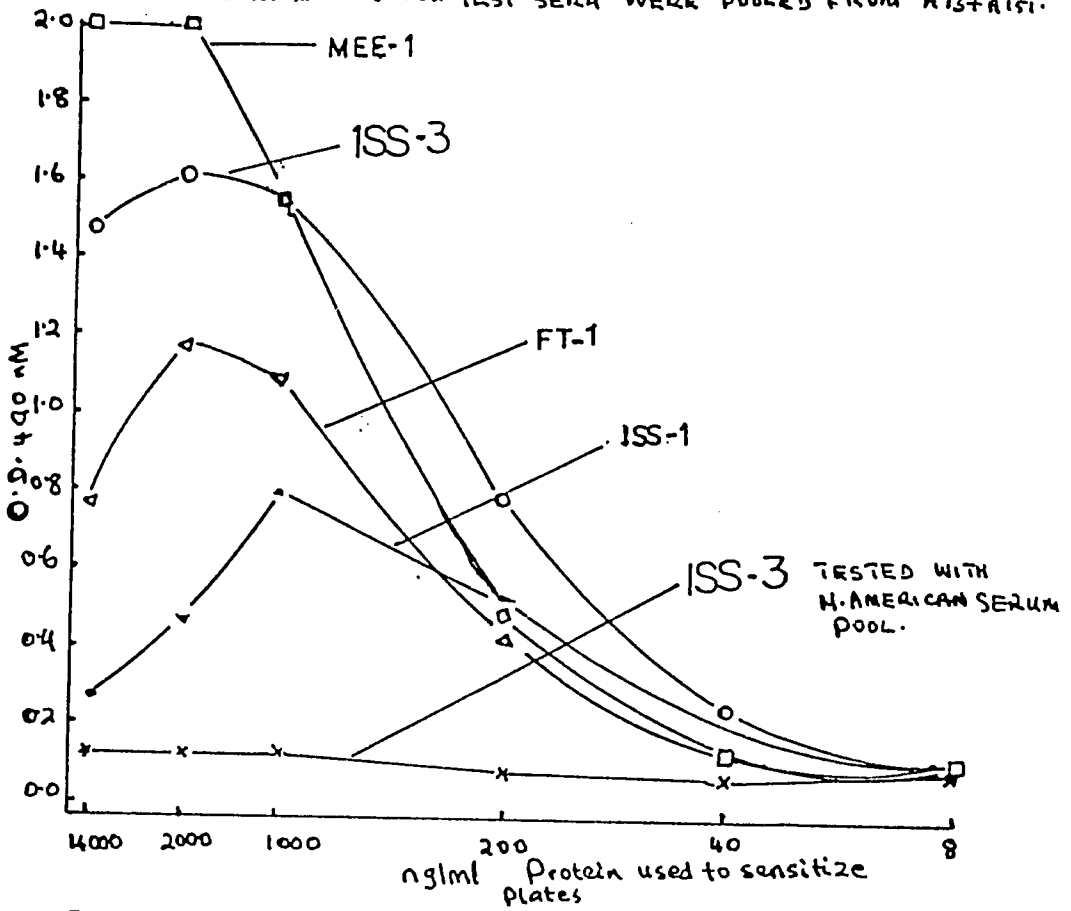
Legend:

S-3 = ISS-3 O. volvulus antigen
S-1 = ISS-1 " " "
FT = FT-1 " " "
MEE = MEE-1 " " "
O = Human anti-O. volvulus serum pool of A73 + A151.
RH = Rabbit anti-whole Human serum.



and b) by ELISA (Fig. 2).

Figure 2: Antigenic titers of O. volvulus preparations as determined by ELISA. ANTI-O. VOL. TEST SERA WERE POOLED FROM A73+A151.

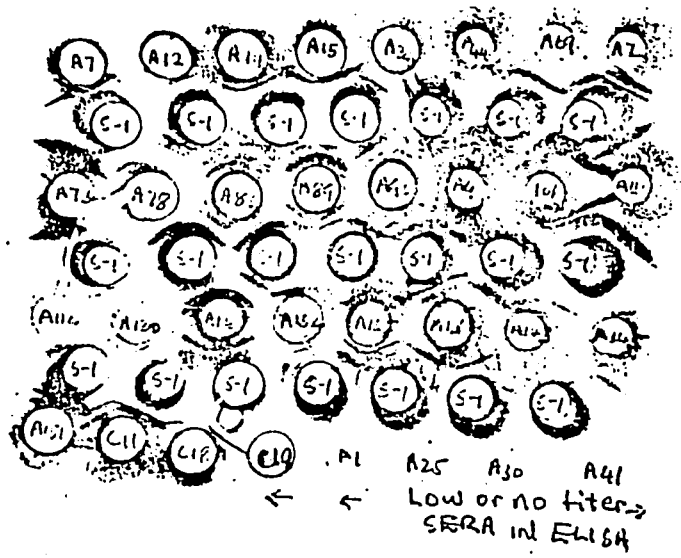


In the previous report, I had met difficulties extracting sufficient quantities of proteins from relatively large amounts of O. volvulus adult worm fragments. I later found that homogenization of the worms for a considerably longer time after collagenase treatment yielded 7 times more proteins. The ISS-3 preparation obtained in this way was chosen for the isolation of O. volvulus antigens because of its protein content (7 mg/ml for 5.0 ml) and ELISA binding activity.

Experiment 2. Selection of high titer anti-O. volvulus human sera for antibody affinity column.

Of the 132 serum samples which I screened in ELISA, 26 titered at

1:4,000 - 16,000 and detected at least 1 of 4 precipitin bands in Ouchterlony double diffusion tests against ISS-1 (Fig. 3).
Fig. 3.



Sera (such as C19) which reacted poorly in ELISA also reacted poorly in these gels. Parasitological profile indicated that A7, A72 and A89 were co-infected with either ascaris or schistosomiasis and they were eliminated along with C19. This left 22 sera to be used for the subsequent studies.

Experiment 3. Construction of human anti-O. volvulus antibody affinity column and rabbit anti-whole human serum antibody affinity column.

Equal volumes of 14 of the 22 human sera were pooled to 3.0 ml. This pool of serum and 10.0 ml of high titered rabbit anti-whole human serum were separately precipitated in 33% saturated ammonium sulfate. The pellet obtained following centrifugation in a Sorvall Centrifuge (20,000 RPM/15 mins at 4°C) was dissolved in PBS, dialyzed extensively in the same and then in 0.1 M $NaHCO_3$ buffer, pH 9.3. Human anti-O. volvulus Ig at 26 mg/ml (2 ml) and rabbit anti-whole human serum at 13 mg/ml (4 ml) were coupled to 2.0 and 4.0 ml, respectively, of periodate-activated sephacryl S-300. The results of coupling efficiency are shown (Table 1).

able 1.
Results of coupling efficiency.

Test sample	mg/ml protein in bead supernatant before coupling	after coupling	mg protein coupled/ml beads.	Total bead volume (ml)
anti-O. volvulus	26 mg/ml	8 mg/ml	18	2
anti-L. loa	12 mg/ml	1 mg/ml	11	2
anti-Hu. serum	13 mg/ml	1 mg/ml	12	4

Experiment 4. Isolation of O. volvulus antigens by a) positive selection on a human anti-O. volvulus antibody column and then by b) negative selection on a rabbit anti-whole human antibody column.

5.0 ml of ISS-3 at 7.0 mg/ml were shaken with 2.0 ml of human anti-O. volvulus Ig-sephacryl (18 mg protein/ml beads), packed into a syringe, washed extensively and then eluted with 0.1M sodium acetate, pH 3.5. The eluate was neutralized immediately to pH 7.2 using drops of 4 M Tris-HCl, pH 7.2, and then dialysed extensively in 0.1M Tris-HCl, pH 7.2, containing 0.15M NaCl and finally in 0.01M Tris-HCl/.015 M NaCl, pH 7.2. The dialysate was lyophilized and reconstituted in 0.1M Tris-HCl/.15M NaCl, pH 7.2. Four ml of this solution of about 0.5 mg/ml protein was shaken with 4 ml of rabbit anti-whole human Ig-sephacryl (12 mg/ml) and then packed into a syringe. The void volume plus 2 ml x 3 washes with the

packing buffer were pooled, dialysed in).01M Tris-HCl/.015M NaCl and then lyophilized. O. volvulus antigens isolated in this way were designated AP-1 (for affinity isolated antigen number one). The yield was 2.0 mg protein. This was reconstituted in 2.0 ml of 10 mM Tris/ .015M NaCl, pH 7.2.

Experiment 5: Characterization of AP-1 by gel diffusion and SDS-PAGE.

A. By Ouchterlony double diffusion gels (Fig. 4).

Fig. 4.

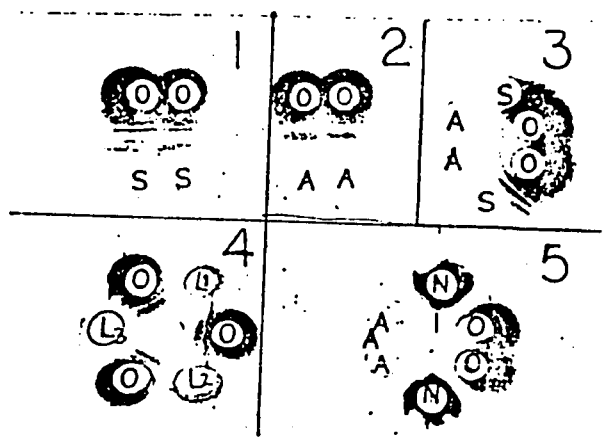


Fig.4 Panel 1: double immunodiffusion of the total O. volvulus worm extract (S) against human anti-O. volvulus serum pool (O);

Panel 2: O. volvulus antigens (A) isolated by human antibody affinity column and diffused against immune human serum pool (O);

Panel 3 indicates considerable measure of identity between S and A antigen preparations;

Panel 4: Affinity purified antigen (A) isolated reacts strongly with human anti-O. volvulus serum pool (O), but apparently not with L. loa sera L_1, L_2 or L_3;

Panel 5: crossreactivity test between A and antigenic extracts of Ascaris Suum (N).

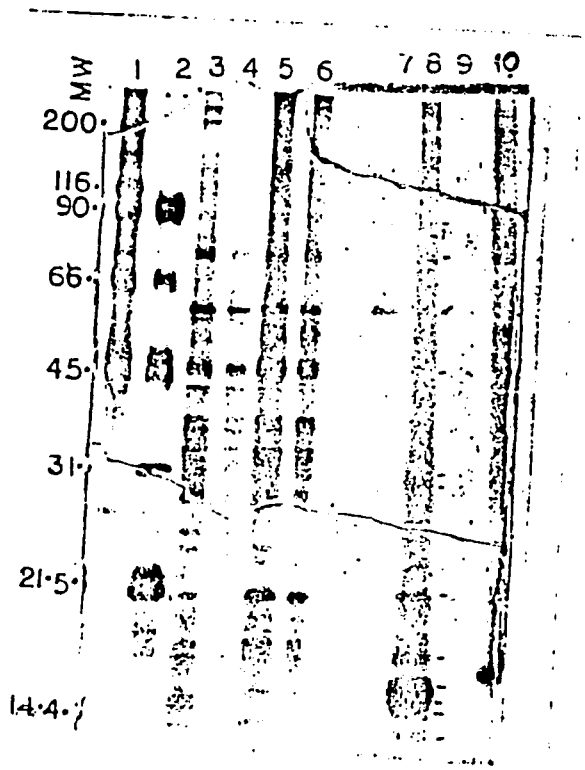
This experiment gave inconclusive results because the bands running between the N-N wells are formed between A and O sera, but not intensely enough for final judgement. In cross-inhibition assays in ELISA, however, up to 50 ug/ml of N does not inhibit the binding of antibody O to 1 ug/ml

of affinity isolated O. volvulus antigens (A). In the same test, only 10 ug/ml of either S or microfilarial extract inhibits antibody binding to A by > 90% (data available but not enclosed).

B. By SDS-PAGE under non-reducing conditions (Fig. 5).

Results:

Fig. 5



Legends:
Lanes 1 and 2 = High and low mol. wt. standards (BIO-RAD); Lanes 3 - 6 = total detergent extracts of O. volvulus worms obtained by freeze-thaw (Lanes 1 and 2) and by homogenization after brief treatment with collagenase (Lanes 3 and 4); Affinity isolated antigens (Lane 8); resolution is lost by an apparent proteolysis and by protein overloading.

Legends (contd.)

About 7-8 light and intense bands corresponding to Ouchterlony gel analysis (Fig. 4, Panel 2) can be identified. Worm extract which has passed through a first anti-O. volvulus column loses most of its antigens: it hardly binds to a second such column (Lanes 7 and 9). Lane 11 = material eluted from a rabbit anti-whole human serum antibody affinity column shoould contain contaminating human proteins initially present in the total O. volvulus worm extract.

Comments:

The considerable smear evident in the gel lane 8 (affinity isolated antigen) is likely to originate from two sources: A) the brief treatment of the worms with collagenase before extraction; B) prolonged handling at room temperature during the various fractionation steps. In addition to this sampl.. latter preparations have been obtained from worms extracted without collagenase digestion, and to which the enzyme inhibitors phenylmethyl sulfonyl fluoride (PMSF) and trysil have been added at 5-fold larger concentrations. These samples have not been analyzed by SDS-PAGE. I think it unlikely that small amounts of proteolysis would significantly affect the immunogenicity of the constituent proteins with respect to raising monoclonal antibodies. But it is still important · keep these antigens as native as possible, and of course it is easier to analyze an uncracked SDS-PAGE gel.

Respectfully submitted,

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