Skin-Sensitizing Antibodies in *Schistosoma mansoni* Infected Baboons: Evidence for the Presence of Two Types[1] (36761)

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The stimulation of reaginic (homocytotropic or skin-sensitizing) antibodies by parasitic helminth infection in a variety of host–parasite systems has been well documented (1–13). The antibody involved, in those cases in which it has been characterized, has many features in common with the type of antibody in humans responsible for reactions of immediate hypersensitivity. Some of the most important of these include: (a) the ability to fix to specific target cells in the skin and other tissues of the same or closely related species and remain fixed for extended periods, generally with a half-life of fixation of about 10 to 14 days; (b) the ability to react with a specific antigen (allergen) while fixed to the cells, with the resultant release of mediator substances, chiefly vasoactive amines such as histamine and serotonin; (c) heat lability, 56° for 1 to 4 hr being generally sufficient for inactivation; and (d) susceptibility to inactivation by reduction with sulfhydryl reducing agents, such as 2-mercaptoethanol.

It is now generally recognized that the majority of reaginic activity in human serum resides in a distinct immunoglobulin class, IgE (14, 15); antibodies homologous to human IgE have been demonstrated in several other species, including the rat (16), rabbit (17), guinea pig (18), dog (19) and rhesus monkey (20). In certain rodents the existence of two types of homocytotropic antibodies has been documented (21–23). One of these appears to be the homolog of human IgE, while the other has been identified as IgG. Regarding the situation in primates, there is also considerable evidence that skin-sensitizing activity in man is not confined to the IgE class, but that IgG might also be involved (24–31). Reid, Minden and Farr (24, 27) and Reid (29) fractionated human reaginic sera and was able to show skin-sensitizing activity in fractions apparently devoid of IgE. By the use of the technique of short-term passive cutaneous anaphylaxis, Parish (28) demonstrated the presence in certain human sera and serum fractions of skin-sensitizing antibodies differing from classical reagins in that they did not require extended periods for fixation to target cells, and remained fixed for only short periods. He suggested that they probably belonged to the IgG class. Additionally, it has been found that anti-IgG releases histamine from leukocyte preparations from some atopic patients, indicating the presence of cell-bound IgG (32). Further study localized IgG predominantly on neutrophil granulocytes and monocytes (33), with trace amounts on basophil granulocytes, the cell type to which IgE is bound (31) and which contains the majority of the leukocyte histamine (34). Evidence is presented below that two types of skin-sensitizing antibodies are produced in baboons subsequent to infection with the human parasite, *Schistosoma mansoni*.

Methods. Animals. Laboratory born and reared baboons (*Papio cynocephalus*), or animals from East Africa shown to be free of schistosomiasis by stool examination and serologic tests, were infected with 100 to

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3600 cercariae of an East African strain of *S. mansoni*. Maintenance of the parasite in the laboratory and methods of exposure have been described (35). The animals were bled for serum at weekly intervals during the early stages of the infection, and at biweekly or monthly intervals thereafter. Serum was stored frozen at —20°.

Passive cutaneous anaphylaxis (PCA). Aliquots (0.05 ml) of the serum or fraction to be assayed were injected intradermally in the abdominal skin of rhesus (*Macaca mulatta*) or African green (*Cercopithecus aethiops*) monkeys. Generally, the same animal was used for the determination of both short-term and long-term reactivity. After the desired latent period (2 hr after the serum injections for short-term, 48 hr for long-term), 2% Evans blue dye (0.5 ml/kg) was injected intravenously. This was followed immediately by the intravenous injection of soluble schistosome antigen. The latter was prepared by homogenizing 200 to 300 adult *S. mansoni* of both sexes, obtained from experimentally infected mice, in 2 ml of phosphate buffered saline (PBS) (pH 7.2) in an ice bath, extracting the mixture overnight at 4°, and centrifuging at 10,000*g* for 30 min. A rabbit anti-human IgE serum, prepared as described previously (36), diluted 1:100 and 1:1,000, and known positive and negative sera were included in each assay to assess the reactivity of individual monkeys. Anti-human IgE, by virtue of its cross-reactivity with cell-fixed monkey IgE, produces an allergic-type skin response (37), and thus simulates the response obtained with a PCA positive serum. At the site of injection of a reactive serum the combination of antigen with cell-fixed antibody results in the release of vasoactive substances, followed by vasodilation and extravasation of the dye. The size and intensity of the resulting blue spot was subjectively scored on an arbitrary scale from 1 to 4. No evidence of Arthus reactivity was noted at any of the injection sites.

Serum fractionation. Ion exchange chromatography. The globulin portion was obtained by precipitation at 40% saturation with neutralized ammonium sulfate. The precipitation was repeated three times, the final precipitate dissolved in 0.005 *M* sodium phosphate buffer (pH 7.8) and exhaustively dialyzed against several changes of the same buffer. After clarification by centrifugation (10,000*g* for 10 min), 110 mg was applied to a 2.5 × 45 cm column of DEAE-cellulose, previously equilibrated with the 0.005 *M* buffer. Elution of the first peak with the 0.005 *M* buffer was followed by either stepwise elution of the remaining proteins with 0.025, 0.035 and 0.2 *M* phosphate buffer at a constant pH of 7.8, or by a linear gradient from 0.005 to 0.3 *M*. Fractions were concentrated by negative pressure dialysis against PBS and stored frozen.

Gel filtration. Five milliliters whole serum were applied to a 2.5 × 100 cm column of Sephadex G-200 and eluted with PBS. Fractions were collected and treated as above.

Determination of heat lability. Serum or serum fractions were heated in a water bath for 4 hr at 56 and 60°, followed by centrifugation at 10,000*g* for 30 min. Samples were stored frozen until assayed.

Determination of sensitivity to reduction. Sera were dialyzed at full strength and diluted 1:4 against 0.1 and 0.2 *M* 2-mercaptoethanol for 48 hr, followed by alkylation by dialysis for 48 hr against 0.01 *M* iodacetamide. Control sera were dialyzed against PBS for a comparable period. All samples were then dialyzed against several changes of PBS during 72 hr. Samples were centrifuged and stored as above.

Results. All of 6 animals exposed to 100 cercariae were positive in long-term PCA, becoming positive between weeks 5 and 7 after exposure and remaining positive for 2 to 4 wk. One of these was positive only in short-term PCA, when tested 3 wk after exposure. An additional 3 animals, exposed to larger numbers of cercariae (1000–3600), were reactive only in short-term PCA at varying times, from 4 wk to 11 mo after exposure. Two other heavily infected animals displayed both types of reactivity, at 18 and 32 mo after exposure, respectively. The serum of one of these, initially exposed to 1200 cercariae, plus two of the other short-term reactive sera, were selected for further study.

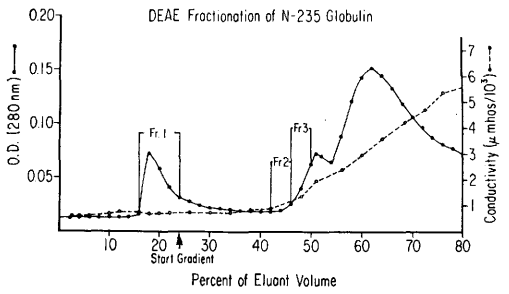

FIG. 1. Elution profile of the globulin portion of a dually reactive serum fractionated on DEAE-cellulose.

Ion exchange chromatography. A typical elution pattern, in which a linear gradient was used, is shown in Fig. 1. Only short-term reactivity was seen in the fractions eluted with 0.005 *M* buffer (Fr. 1 in Fig. 1), while both types of reactivity was present in the second peak (Fr. 3) obtained with serum from the dually reactive animal. The short-term reactivity in Fr. 3 could have been due to material tailing from the first peak, or to a high level of long-term antibodies which became rapidly fixed to the tissues (38). No reactivity was seen in higher molarity fractions of the other two sera, obtained 3 wk after exposure to 100 cercariae and 6 wk after exposure to 1000 cercariae, respectively.

Gel filtration. The serum from the animal displaying dual reactivity was fractionated on Sephadex G-200. As shown in Fig. 2, there was considerable overlap in the localization of the two types of reactivity. However, the earlier elution of long-term reactivity suggests a slight size difference in the molecules responsible for the two types of reactivity.

Heat sensitivity. Heating of the same serum used in the gel filtration fractionation at 56° for 4 hr had no discernible effect upon either the short- or long-term reactivity. Heating at 60° for 4 hr essentially eliminated both types. However, there were indications that aggregation or other forms of denaturation was occurring at the higher temperature, *i.e.*, DEAE fractions of some sera which were previously unreactive, became weakly reactive after heating. It was therefore not possible to accurately assess the effects of heating at this temperature upon the low molarity DEAE fractions showing only short-term reactivity.

Reduction. Very weak long-term reactivity remained after reduction of the above dually reactive serum with 0.1 *M* 2-mercaptoethanol; this was removed by the more complete reduction achieved with 0.2 *M* 2-mercaptoethanol. Short-term reactivity was essentially abolished by both regimens; very weak reactivity was noted only in one diluted aliquot reduced with 0.2 *M* 2-mercaptoethanol.

Discussion. The classes of antibody responsible for the two types of skin-sensitizing activity described here have not as yet been identified. Most of the material in the 0.005 *M* elutate from DEAE is IgG. However, it is recognized that trace amounts of IgE can contaminate such preparations (39), and in order to firmly establish the identity of the antibody classes involved, it will be necessary to perform absorption studies with class-specific anti-globulin antisera.

That the antibodies involved in long-term PCA might have a possible protective function in schistosomiasis has been demonstrated by the transfer of reagin-positive infected rat serum into the skin of normal rats, followed by exposure to cercariae at the same site (2). However, no protection could be shown in rhesus monkeys by two groups of investigators using a similar homologous transfer of reaginic monkey serum into normal monkey skin followed by percutaneous

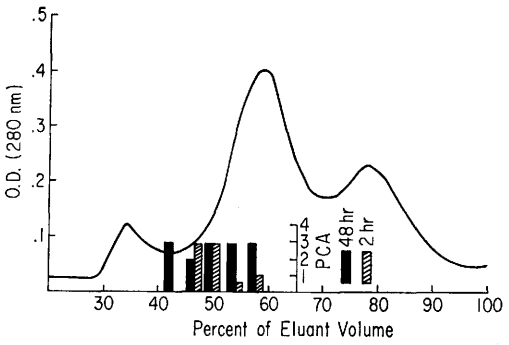

FIG. 2. Gel filtration (Sephadex G-200) elution profile of a serum with dual skin-sensitizing capacity, showing localization of the short-term and long-term reactivity.

challenge at the same site (3,5). The experiments in both instances were designed to assay only long-term reactive antibodies; thus, the finding of short-term skin-sensitizing acitvity in *S. mansoni* infected baboons, as reported here, reopens the question of a possible protective function for this type of antibody in primate schistosomiasis. The possibility of a homocytotropic antibody-mediated barrier to a skin and tissue penetrating parasite remains as an attractive working hypothesis.

Summary. Both short-term and long-term skin-sensitizing antibodies are present in the serum of baboons infected with the human parasite, *Schistosoma mansoni*. The two types were separated by ion exchange chromatography; a molecular size difference was indicated by gel filtration. Both types are heat stable at 56°, although not at 60°, for 4 hr, and both are inactivated by reduction and alkylation.

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