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Immune Damage to Liposomes Containing Lipids from *Schistosoma mansoni* Worms (38125)

CARL R. ALVING, KENNETH C. JOSEPH, HERBERT B. LINDSLEY, AND MAURICE J. SCHOENBECHLER

Departments of Immunology and Medical Zoology, Walter Reed Army Institute of Research, Washington, D. C. 20012

Rhesus monkeys infected with *Schistosoma mansoni* have previously been used as a model to study the immune response in schistosomiasis (1, 2). IgG and homocytotropic "reagin-like" antibodies have both been detected (1). The antigens involved in those reactions had a complex array of specificities and because of this they have not yet been clearly characterized (2).

The purpose of the present study was to determine whether antibody activity against lipid antigens appeared during the course of schistosomal infection. Lipids are ubiquitous constituents of cell membranes, and account for about one-third of the dry weight of adult *S. mansoni* (3). Compared to proteins, lipids are less complex and more easily characterized (4). Antibody interactions with a wide variety of glycolipid and phospholipid antigens from various sources have been demonstrated (5).

Investigation of lipid antigens has been hampered to some extent in the past because of the relative insolubility of these substances. Since 1968, lipid spherules containing trapped glucose (liposomes) have been employed in the study of immunological reactions against lipids (6). Complement-dependent damage to the liposomes in the presence of specific antibody was determined by measuring the release of trapped glucose (6). In the present paper, membranes from adult worms were subjected to an extraction procedure designed to separate, at least partially, different types of lipids. Lipids derived from the worms were tested for the ability to serve as antigens by incorporating them into liposomes. Immunological activity in monkey serum was then assayed by measuring complement-dependent damage resulting in liposomal glucose release.

Methods. The life cycle of *S. mansoni* was maintained at Walter Reed Army Institute of Research, and adult worms were obtained as previously described (7, 8).

Five monkeys were exposed percutaneously to 500 cercariae each. A second exposure, at the times indicated below, involved either 10,000 cercariae (animal nos. 721, 784, and 51) or 1000 cercariae (animal nos. 58 and 59). The latter animals were also exposed a third time to 10,000 cercariae. Sera were collected at various times (see Fig. 1) and were routinely heated at 56° for 60 min. Fresh guinea pig serum was used as a complement source. In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care,' as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animals Resources, National Academy of Sciences.

Lipid extracts were prepared from about 2 g (dry weight) of mixed male and female adult worms in the following way. They were first homogenized in water and the membranes extracted by the Bligh–Dyer method (9–11). The chloroform phase, containing about 215 μmoles of phosphate, was applied to a silicic acid column. The column was developed first with 175 ml of chloroform (fraction 1) and then with 700 ml of acetone/methanol (9/1), (fraction 2, or F2). Analysis by thin layer chromatography (4), appropriate sprays (4) and phosphate measurement (12) showed that the F2 contained most of the glycolipid, less than 5% of the original phospholipid, and a relatively high concentration of an unidentified brown pigmented lipid. Fraction 1 (which

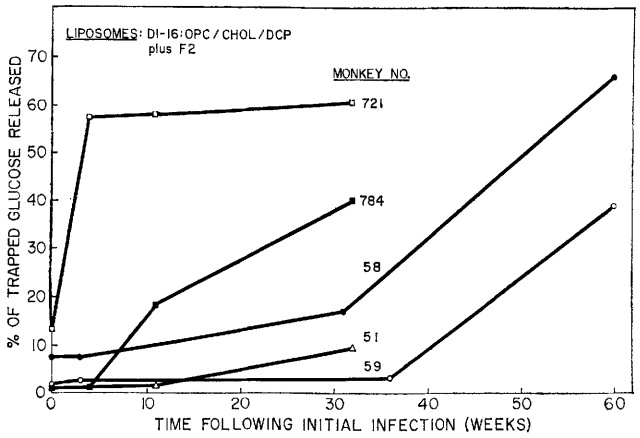

FIG. 1. Time course of serological activity of 5 infected monkeys against schistosomal lipids. Each point corresponds to a separate reaction cuvette which contained 4.7 μl of liposomes, 50 μl of appropriate monkey serum, 120 μl of fresh guinea pig serum as a complement source, 325 μl of 0.15 *M* NaCl and 500 μl of glucose assay reagent (10, 12). Glucose release was measured at room temperature (ca. 22°) 30 min after starting the reaction. See the text for further details.

was discarded) contained all of the sterols, sterol esters, free fatty acids, and triglycerides.

The methods for preparation of liposomes and assay of complement-dependent glucose release have been published (11, 13). The liposomes were made from dipalmitoyl lecithin (di-16:0 PC; Calbiochem, Los Angeles, CA), cholesterol (CHOL) and dicetyl phosphate (DCP; K&K Laboratories, Inc., Plainview, NY) in molar ratios of 2/1.5/0.22. F2 was added in chloroform along with the other lipids (before swelling the liposomes) in an arbitrary concentration corresponding to the amount removed from a volume of Bligh–Dyer extract which originally contained 4 μmoles of phosphate.

Results. Sera, which were collected from the five monkeys at various stages following infection, were assayed for activity against F2-containing liposomes. These results are given in Fig. 1. All of the animals were reexposed either once or twice. Nos. 721, 784 and 51 were reexposed once, at 24 weeks; nos. 58 and 59 were both reexposed twice, at 36 and 52 weeks. Sera from four out of 5 monkeys eventually reacted strongly with the liposomes. There was a wide variation in the time at which this occurred. Two monkeys (nos. 721 and 784) showed a response, before reexposure, at 11 weeks. The other 2 monkeys which responded (nos. 58 and 59) did so only after subsequent exposures. Only one monkey (no. 721) had maximum activity before the first reexposure. This serum reached its highest levels within 3 weeks and remained elevated for at least 28 weeks.

More detailed immunological analysis of one of these sera (no. 58, 60 weeks) is shown in Fig. 2. Different concentrations of serum were reacted with liposomes which contained F2 (+F2). A sigmoidal curve was obtained which resembled those previously observed with glycolipid antigens (6, 11). Significant glucose release did not occur in control experiments in which the F2 was omitted (−F2) or when heat-inactivated complement was used (+F2, heated C'). In another control, serum which was adsorbed with a homogenized suspension of adult worms (+F2, "adsorbed") also lacked activity. Except for a difference in the plateau of maximum glucose release, identical results were also observed with serum from monkey no. 59 at 60 weeks (not shown).

Fractions containing IgG or IgM were obtained by column chromatography of serum no. 58 (60 weeks) on Sephadex G200. An antibody titration curve of the IgM fraction is shown in Fig. 3 (+F2). This resulted in a sigmoidal curve of glucose release which accounted for all the activity in the original serum. The control preparation of liposomes which lacked F2 (−F2) did not have activity. The IgG fraction is not shown, but it com-

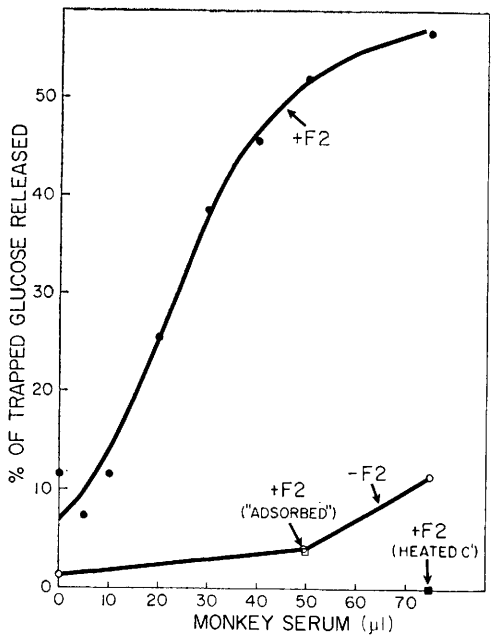

FIG. 2. The interactions of infected-monkey serum with liposomes. The assay procedure was the same as described in the legend of Fig. 1, except that the quantity of monkey serum was varied as shown on the abscissa. The reaction volume was kept constant (1.0 ml) by adjusting the amount of 0.15 *M* NaCl. Two liposome preparations were used which either contained F2 (●—●) or, as a control, lacked it (O—O). "Heated C" refers to guinea pig serum which had previously been heated at 56° for 60 min (■). In one case, monkey serum was used which had been "adsorbed" twice at 20° with 4 mg of a homogenized suspension of adult worms (□). The worms were removed following each adsorption by centrifuging at 27,000 *g* for 10 min.

pletely lacked activity even when 8 mg. of protein was present.

Discussion. The data presented here demonstrate that a previously unsuspected immune response against a lipid antigen (or antigens) may occur as a result of infection of monkeys with *S. mansoni*. Although the specific antigenic material present in the F2 extracted from the worm has not yet been identified, the possible substances are limited. The F2 (a) was protein-free, (b) was enriched with glycolipids, (c) contained less than five percent of the initial phospholipids, and (d) had a brown pigment.

In some studies in the past (see, for example, Refs. 1 and 2) a crude protein extract which had been partially delipidized by the method of Chaffee, *et al.* (14) was employed as a schistosomal antigen source. For slide flocculation studies this crude extract was adsorbed onto "cholesterol-lecithin crystals" (7, 2). It is interesting to note, however, that the glycolipids, such as those present in the F2 in this study, are relatively insoluble in ether and might not be removed by the Chaffee procedure. Furthermore, the liposomes in this report were prepared from lecithin and cholesterol. It is possible, therefore, that the lipids in the F2 might account for some of the antigenic activity which was previously attributed entirely to proteins (2). Due to the different techniques employed, however, the lipids in the F2 also might represent new specificities which before now have not been assayed.

Previous studies of the composition of cercariae, adult worms and eggs have established that most of the total lipid consists of a variety of phospholipids, sterol (mainly cholesterol), sterol esters, triglycerides, and free fatty acids (3, 15, 16). In the present study,

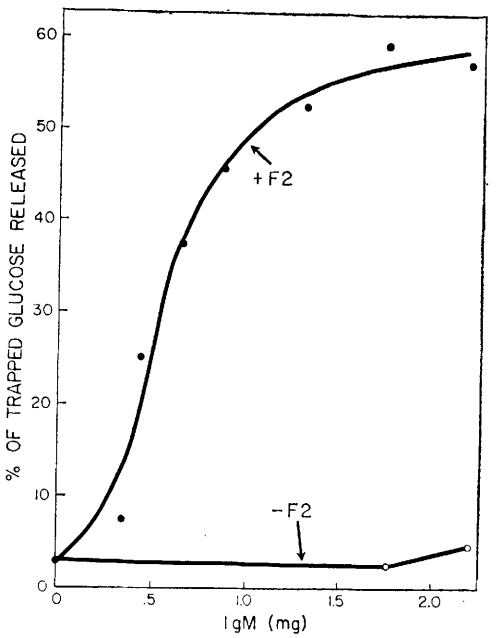

FIG. 3. The effect of an IgM-containing fraction from infected-monkey serum on liposomes. The assay procedure is described in the legends of Fig. 1 and 2. The amount of protein added (mg) was based on the measurement of the total protein present in an "IgM fraction" from Sephadex G-200.

except for a small amount of contaminating phospholipid, all of the above substances appeared either in fraction 1, which was discarded, or remained on the silicic acid column. Detailed analysis and isolation of the constituents of the fraction 2 (F2) are now in progress. Preliminary findings (unpublished) have established the presence, besides the pigment, of at least three glycolipids and two phospholipids. Any or all of these might conceivably serve as haptens in liposomes. The present study demonstrates the feasibility of utilizing liposomal model membranes to investigate further the haptenic activity of these substances.

Summary. A protein-free lipid extract ("F2") consisting mainly of glycolipids, a small amount of phospholipids and an unidentified brown pigment, was obtained from *Schistosoma mansoni* adult worms. The F2 was tested for the presence of haptenic molecules by incorporation into liposomal model membranes containing trapped glucose. Sera from five infected monkeys were assayed for serological activity against the F2 in liposomes at different times following infection. Complement-dependent damage leading to liposomal glucose release was observed with sera from four out of five monkeys. Glucose release did not occur when the F2 was omitted from the liposomes nor when heat-inactivated complement was used. All of the activity was removed by adsorption of monkey serum with adult schistosomes. The IgM-containing fraction of serum accounted for all of the antibody activity. It was concluded that a complement-dependent immune response against lipids may be observed during the course of schistosomiasis in monkeys. The antibody activity can be detected by utilizing liposomal model membranes which contain schistosomal lipids.

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