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Production of 19S IgM Antibodies with Restricted Heterogeneity from Sharks

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ABSTRACT **Two of three nurse sharks, *Ginglymostoma cirratum*, immunized with streptococcal A-variant vaccine, produced over 7 mg/ml serum of 19S IgM antibody to the group-specific carbohydrate. The light chains of this specific antibody from one shark were separated into multiple bands by disc electrophoresis. The light chains from the other antibody-producing animal were much less heterogeneous and gave only one predominant band.**

Most antibodies consist of heterogeneous populations of molecules not amenable to structural studies of the combining site. However, in some rabbits, streptococcal (1) and pneumococcal (2) polysaccharides induce the formation of large amounts of IgG antibodies with restricted heterogeneity upon which studies of the combining site may be more successful. Sharks, on the other hand, appear to produce only one class of immunoglobulin, which resembles IgM (3), and thus may represent the best source of antibodies of the IgM class. This report describes the immunogenicity of streptococcal A-variant carbohydrate in three sharks and the heterogeneity of the antibodies produced.

MATERIALS AND METHODS

Immunization of sharks

2.5–5.0 kg nurse sharks (juvenile specimens of *Ginglymostoma cirratum*) were housed in free-flowing sea water (about 27°C) in Bimini Bay, Bahamas. The animals were immunized intravenously with streptococcal group A-variant (strain A 486) or group C (strain C 74) vaccines (4) as indicated in Table 1. On the days specified, approximately 100 ml of blood was drawn from the caudal vein into glass tubes and allowed to stand at room temperature (28°C) for 2–4 hr. Serum (usually about 70% of the blood volume) was removed by pipette and stored at −20°C until used.

Quantitative precipitin analysis

Streptococcal A-variant and C carbohydrates for use as antigens were isolated from cell walls by hot formamide digestion (5). In the precipitin reactions 0.1 ml of serum was mixed with 0.9 ml of various concentrations of antigen dissolved in 0.15 M NaCl–0.01 M Tris·HCl (pH 7.4)–0.01 M EDTA at 37°C for 1 hr and the mixture was kept for 24 hr at 2.5°C. The precipitates were washed twice (with centrifugation for 20 min at 1200 × *g*) with cold buffer and dissolved in 0.1 N NaOH. The absorbance at 280 nm was used to calculate shark antibody concentrations, assuming an extinction coefficient of 13.75 at 280 nm in a 1-cm light path for a 1% solution (6).

Immunochemical procedures

Previously described methods (6, 7) were used for analytical ultracentrifugation, extensive reduction and alkylation followed by Sephadex G-200 gel filtration in the presence of guanidine·HCl, and disc electrophoresis in urea-containing polyacrylamide gels.

RESULTS

Shark antibody levels

Sera obtained from each shark on day 1 (prior to the initial antigen injection) failed to give detectable precipitates with either of the isolated streptococcal carbohydrates. Some of the sera obtained after immunization yielded detectable precipitates, each of which gave a precipitin curve (Fig. 1) resembling those obtained with macroglobulin antibodies of other species (8–10). As Table 1 shows, two of the three sharks

TABLE 1. *Immunization schedules and serum antibody levels in nurse sharks immunized with streptococcal vaccines*

Shark number	Days of immunization*	Vaccine	Day of bleeding	Antibody concentration (mg/ml)
			1	<0.25
225	1, 2, 3, 5	A variant	38	2.3
	39		72	10.0
	76		116	9.1
			1	<0.25
226	1, 2, 3, 5	A variant	38	<0.25
	39		72	5.7
	76		116	7.4
			1	<0.25
227	1, 2, 3, 5	A variant	38	<0.25
	38, 39		72	<0.25
	76		116	<0.25
			1	<0.25
228	1, 2, 3, 5	C	38	<0.25
and	39		72	<0.25
230	76		116	<0.25

* Each injection consisted of 1 ml of vaccine given intravenously.

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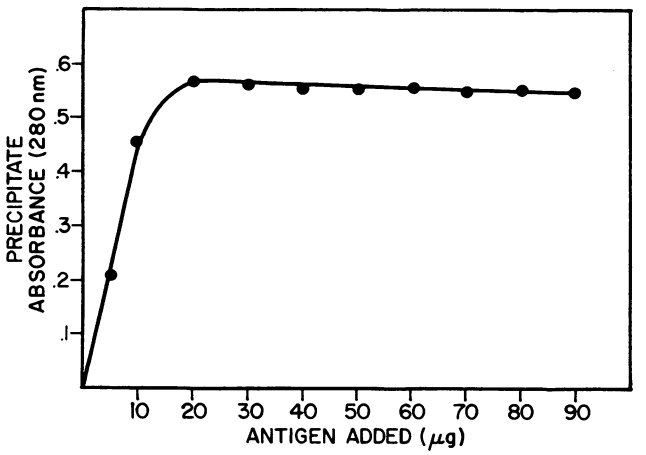

FIG. 1. Quantitative precipitin curve obtained using streptococcal A-variant carbohydrate and nurse shark antiserum (shark 225, day 72).

immunized with A-variant vaccine produced more than 7 mg of antibody per ml serum between 10 and 13 weeks after initial immunization, with the highest concentration being 10 mg/ml. One of the animals immunized with A-variant vaccine and both with C vaccine failed to yield detectable precipitating antibody. The extent of cross-reactivity of the shark antibodies was assessed by allowing the 72-day serum from sharks 225 and 226 to react with the C carbohydrate. Serum from shark 226 gave no detectable precipitation with this antigen, whereas the former contained almost 1.5 mg/ml precipitating antibody (or 15% as much as for the homologous antigen).

Properties of shark antibodies

To determine the relationship of the shark antibodies produced in response to the A-variant vaccine to the immunoglobulins of this species, we dissolved washed immune precipitates, formed at equivalence with A-variant carbohydrate, in 7 M guanidine·HCl and then subjected the solution to analytic ultracentrifugation in 3.5 M guanidine·HCl. The Schlieren pattern of a dissolved precipitate from shark 225 (Fig. 2) shows a single major sedimenting boundary with an

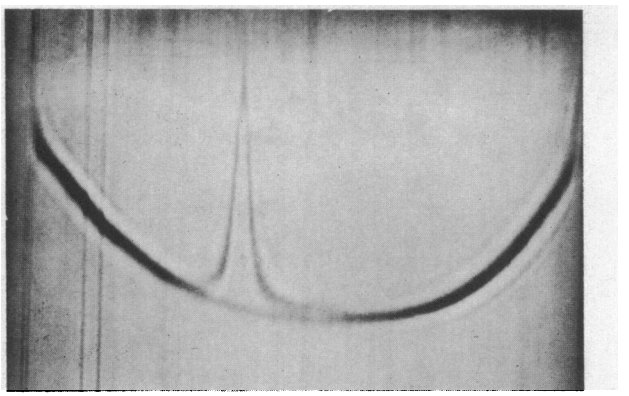

FIG. 2. Schlieren pattern of dissolved immune precipitate (shark 225, day 72) with streptococcal A-variant carbohydrate subjected to analytical ultracentrifugation in 3.5 M guanidine·HCl. Protein concentration, about 8 mg/ml. Antigen concentration, about 0.3 mg/ml.

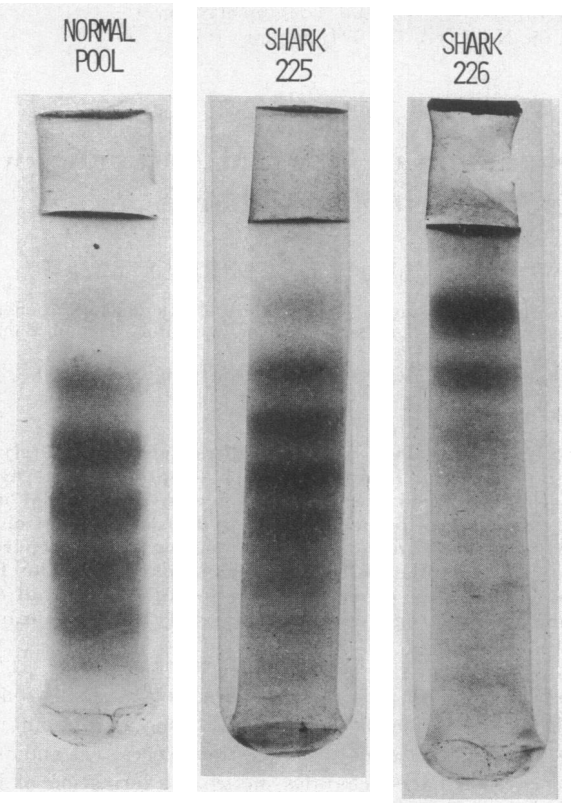

FIG. 3. Disc electrophoretic patterns of L chains from nurse-shark antibodies to streptococcal A-variant carbohydrate and from pooled normal nurse shark 19S immunoglobulin.

$s_{20,w}$ of 13.1 S (8 mg/ml). Each of the precipitates from sharks 225 and 226 gave similar results. Although the shark immune precipitates dissolved readily in 7 M guanidine·HCl, attempts to purify active antibody by Sephadex G-200 gel filtration in the presence of 5 M guanidine·HCl have been unsuccessful because the antibody is apparently labile in the guanidine·HCl (the isolated antibody no longer precipitated with the antigen). However, immunodiffusion in agar gels, employing rabbit antisera to nurse shark serum and to nurse shark immunoglobulin (7), has indicated that the precipitated nurse shark protein is a single antigenic species identical with nurse shark immunoglobulin. Thus, the isolated shark protein was considered to represent shark 19S antibody; the somewhat low sedimentation coefficient was presumably due to protein concentration effects and/or configurational changes in the guanidine·HCl solution. Similar sedimentation coefficients were obtained with similar concentrations of nurse shark 19S immunoglobulin in this solvent.

Electrophoretic heterogeneity of antibody light (L) chains

The dissolved immune precipitates discussed in the preceding paragraph were extensively reduced and alkylated (8) prior to gel filtration on Sephadex G-200 columns equilibrated with 5 M guanidine·HCl. The elution profiles from these columns were undistinguishable from those previously obtained (7) with nurse shark immunoglobulin. The separated L chains were concentrated, the guanidine was removed by dialysis against 10 M urea, and the L chains were subjected to disc electrophoresis in polyacrylamide gels (Fig. 3). The L chains from a pool of normal nurse shark immunoglobulin gave

several bands, as did the L chains from antibody isolated from shark 225; however, the L chains from shark 226 showed but a single predominant band, one minor band, and only traces of other bands.

DISCUSSION

These results extend the phylogenetic spectrum of animals capable of responding to streptococcal carbohydrates and illustrate two important factors relative to the immunogenic "uniqueness" previously described for these antigens in rabbits (1). First, two of three sharks immunized with A-variant vaccine produced rather high levels (>7 mg/ml) of antibody. Ultracentrifuge studies of dissociated antigen·antibody complexes showed the shark antibody to have a sedimentation rate similar to that of shark 19S IgM. The isolated shark antibody was antigenically similar to shark 19S IgM. These data indicate the shark antibodies to be of the 19S IgM class, in contrast to the almost exclusively 7S IgG antibodies elicited in rabbits. Secondly, one of the responder sharks produced antibody with L chains of considerably restricted electrophoretic heterogeneity suggestive of primary structural uniformity. It may therefore be possible (if larger sharks are used) to obtain from a single bleeding of a single shark as much as 5 g of specific 19S antibody of fair molecular uniformity; such studies are currently not possible with higher vertebrates.

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