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Circulating antigens and immune complexes in *Schistosoma mansoni*-infected rats

CHARACTERIZATION BY RADIOIMMUNOPRECIPITATION-PEG ASSAY (RIPEGA)

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SUMMARY

Circulating schistosome antigens (CSA) and circulating immune complexes (CIC) were investigated in rats infected with *Schistosoma mansoni*. The radioimmunoprecipitation–polyethylene glycol (PEG) assay (RIPEGA), with ^{125}I-labelled anti-*S. mansoni* anti-serum, detected CSA during two distinct periods of the infection; the first between the 11th and the 14th week of infection and the second between the 11th and 14th week after infection. The CH_{50} deviation test revealed the presence of CIC in sera from infected rats, approximately at the two periods when CSA were detected. At 6 weeks of infection, the levels of CIC in infected rats were not different from those in control rats. However, a more sensitive method characterized IgG2a in C1q-binding CIC from infected rats. At weeks 5 and 6, IgE immune complexes were also detected in the serum from infected rats. In fact, the use of RIPEGA on the material eluted from infected rat serum after passage through an anti-IgE immunosorbent showed the presence of schistosome antigen at week 4, and at higher levels at week 6. Levels of 50% haemolytic complement in infected rat serum were lowered between the 2nd and the 4th week, the 5th and the 8th week and after the 12th week of infection. The possible role played by CIC in the protective mechanisms to a *S. mansoni* challenge infection in rats is discussed.

INTRODUCTION

Schistosoma mansoni infection in the rat has been extensively studied, and offers several biological and immunological features which render this host an ideal model for the study of immunity to schistosomes. A decline in the worm burden of infected animals occurs between the 4th and the 8th week after exposure to cercariae (Smithers & Terry, 1965b). This worm expulsion, called 'self-cure phenomenon', appears to be an immunological process mediated by a population of thymus-derived lymphocytes (Phillips *et al.*, 1975; Cioli & Dennert, 1976). This initial phase of immunity is followed between the 4th and the 11th week by the acquisition of resistance to a challenge infection as measured by the liver perfusion technique (Sadun & Bruce, 1964; Smithers & Terry, 1965b; Maddison *et al.*, 1970; Knopf, Nutman & Reasoner, 1977; Philipps, Reid & Sadun, 1977) or by the lung recovery method (Perez, Clegg & Smithers, 1974). The exact mechanism of this phase of acquired immunity is still unclear, but recent experiments *in vitro* suggest the involvement of antibodies and non-sensitized cells (Dean, Wistar & Murrel, 1974; Perez & Smithers, 1977; Capron *et al.*, 1975).

In the present study we have used different techniques to investigate circulating antigens and antigen-antibody complexes during the course of *S. mansoni* infection of inbred rats. Since recent *in vitro*

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studies have indicated that IgE immune complexes and IgG2a antibodies might play a role in the rat's effector mechanisms, particular emphasis was given to their detection.

MATERIALS AND METHODS

Parasite and animals. A Puerto Rican strain of *S. mansoni* was maintained as previously described (Capron *et al.*, 1974). Inbred Fischer/Ico male rats (Iffa Credo, L'Arbresle, France), weighing 180–200 g at the beginning of the experiments, were used in the present work. Rats were exposed to 800 cercariae, according to the ring method of percutaneous infection (Smithers & Terry 1965a). Animals were individually bled through the retro-orbital sinus at different intervals post-exposure, depending on the experiment. Blood was allowed to clot for 2 hr at room temperature and for 2 hr at 4°C. The serum was centrifuged at 800 *g* for 5 min. For the complement studies, individual sera were used immediately. For the investigation of circulating schistosome antigen (CSA) and circulating immune complexes (CIC), sera were pooled and stored at −20°C. Control rats were maintained and bled under the same conditions as infected ones.

Reagents. A goat IgG, specific for the epsilon chain of rat IgE, and a rabbit anti-rat IgG2a antiserum were kindly supplied by Dr H. Bazin (Bazin, Beckers & Querinjean, 1974). A hyperimmune rabbit anti-*S. mansoni* antiserum was prepared against a whole extract of adult schistosomes (Capron *et al.*, 1968). The Ig fraction from the antiserum was prepared by affinity chromatography with the whole soluble extract of *S. mansoni* antigen and radioiodinated according to the method previously described (Morrison, Bayse & Webster, 1971). C1q complement component was isolated from fresh normal human serum by the method of Volanakis & Stroud (1972).

CH_{50} deviation assay. The 50% haemolytic complement (CH_{50}) deviation assay recently described for the detection of CIC in human sera (Santoro, Wattre & Capron, 1977b) was used with minor modifications. For the test, 0·1 ml samples of the rat serum were mixed with 0·1 ml of 0·2 M EDTA and incubated for 30 min at 37°C. 3·0 ml of complement (serum from normal rats stored at −70°C) diluted 1 : 25 in veronal buffer were added and the tubes were incubated at 37°C for 2 hr. This was solution (A). Serial dilutions of solution (A) were made in veronal-buffered saline containing magnesium and calcium (Levine, Osler & Mayer, 1953). 1·5 ml of optimally sensitized, thrice-washed 2% sheep red blood cells were added, and the tubes were incubated for 45 min at 37°C. After centrifugation at 1700 *g* for 10 min at 4°C, the optical density of the released haemoglobin in the supernatant was measured at 540 nm. Standards of osmotically 50% lysed cells and complement alone were always used.

The complement remaining after the first incubation (solution A) was calculated:

$$CH_{50}\text{ units} = \frac{\text{amount of solution (A) to 50\% haemolysis}}{\text{25 (inverse of complement dilution)}}.$$

The normal rat serum used as complement was treated in the same way. Its level of CH_{50} was used as a base for the final results, which were expressed in CH_{50} units fixed (CH_{50} uf) to CIC present in rat serum.

$$CH_{50}\text{ uf} = (CH_{50}\text{ u of normal rats}) - (CH_{50}\text{ u of tested samples}).$$

Anti-IgE immunosorbent. Goat IgG anti-rat IgE, specific for epsilon chain, was coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) using a previously described method (Axen, Porath & Ernback, 1967). To 1·0 g of swollen Sepharose (3·5 ml) were added 5·0 ml of the anti-rat IgE solution (4·0 mg/ml), and the coupling reaction was allowed to take place at room temperature for 2 hr with slow stirring. 90% of the IgG was bound to the Sepharose. Unbound material was washed away with the coupling buffer (0·1 M $NaHCO_3$ buffer solution containing 0·5 M NaCl) and any remaining active groups were inactivated with 0·5 M 4-aminobutyric acid dissolved in 0·05 M Na_2CO_3 buffer at room temperature for 2 hr. The gel was washed three times with 100 ml 0·1 M acetate buffer containing 1·0 M NaCl, pH 4·0, followed by a wash with 100 ml 0·1 M borate buffer containing 1·0 M NaCl, pH 8·0. After a final wash with 0·5 M NaCl solution, the gel was packed in a K9-15 column (Pharmacia). 2·0 ml of infected or uninfected rat serum was allowed to react with an anti-rat IgE–Sepharose gel for 1 hr at room temperature. After binding, the column was washed with 0·5 M NaCl until the optical density was zero. Bound, free or complexed IgE was eluted with 15 ml of 0·2 M glycine–HCl buffer, pH 2·8, for 15 min, and then dialysed overnight against PBS, pH 7·2. After dialysis against distilled water for 24 hr, the material was lyophilized, redissolved to the initial volume in normal rat serum and tested for schistosome antigens.

C1q *immunosorbent.* C1q-binding CIC present in infected rat serum were isolated by affinity chromatography (Svehag & Burger, 1976), using the procedure described above for the goat anti-rat IgE, with minor modifications. Bound immune complexes were eluted with 0·2 M EDTA.

PEG precipitation. Precipitation with different concentrations of polyethylene glycol (PEG, mol. wt. 6000) (Creighton, Lambert & Miescher, 1973) was used to study the solubility of IgE present in normal and infected rat sera. After centrifugation, the pellet was dissolved in distilled water to restore to the intial serum volume.

Ultracentrifugation. Serum from normal and infected rats was centrifuged at 120,000 *g* for 3 hr. The upper two-thirds of the supernatant fluid were collected, the lower third discarded, and the pellet was resuspended to the initial volume in normal rabbit serum, which was the medium used in the radioimmunoassay for rat IgE (Carson, Metzger & Bazin, 1975).

Radioimmunoassay for IgE. Rat IgE levels after PEG precipitation or ultracentrifugation were determined by the method

previously described (Carson *et al.*, 1975; Rousseaux-Prevost, Bazin & Capron, 1977). For the PEG precipitation, the results were expressed as the percentage of PEG-precipitated IgE as compared with the corresponding total serum IgE.

Immunoelectrophoresis for IgG2a. Rat IgG2a was investigated in C1q-binding CIC by immunoelectrophoretic analysis (Scheidegger, 1955; Bazin *et al.*, 1974).

Radioimmunoprecipitation–PEG assay (RIPEGA). The RIPEGA, recently described for the detection of specific CIC and circulating antigen in carriers of hepatitis B antigen (Santoro *et al.*, 1977c), was used to investigate schistosome antigens in the serum from infected rats, in elution patterns from anti-IgE immunosorbent and in the supernatant and redissolved pellet after ultracentrifugation of the serum from normal and infected rats. For the test, 0·2 ml of samples, diluted 1/5 in borate buffer (0·1 M, pH 8·4), were mixed with 0·2 ml of radioiodinated anti-*S. mansoni* (anti-Sm) rabbit IgG (10,000 ct/min), giving ^{125}I-labelled anti-Sm Ab. After 4 hr at room temperature, 3·0 ml of 7% PEG in borate buffer were added and the samples were left overnight at 25°C. After centrifugation (1500 *g*, 20 min, 4°C), the precipitate was washed in 7% PEG and the radioactivity was measured in a well-type scintillation counter. All tests were done in triplicate. Results were expressed as percentage of precipitated ^{125}I-labelled anti-Sm Ab as compared with the radioactivity precipitated in a 'trichloracetic acid control' tube, in which 3·0 ml 20% trichloracetic acid were added to 0·2 ml normal rat serum (diluted 1/5 in borate buffer), mixed with 0·2 ml of ^{125}I-labelled anti-Sm Ab (10,000 ct/min).

Complement studies. 50% haemolytic complement (CH_{50}) of rat serum was measured according to the technique previously described (Lagrue *et al.*, 1967). The C3 level was determined by the automated nephelometric test (Autoanalyzer, Technicon).

RESULTS

Circulating schistosome antigens

Detection of circulating schistosome antigens (CSA) was performed on pooled serum from three groups of ten normal and twenty *S. mansoni*-infected rats, during the course of the infection, by the use of RIPEGA with ^{125}I-labelled anti-Sm Ab (Fig. 1). For all groups of infected rats, two peaks were noticed; the first between the 4th and the 6th week after infection and a second peak between the 11th and the 14th week. Between the 6th and the 11th week a significant decrease was observed.

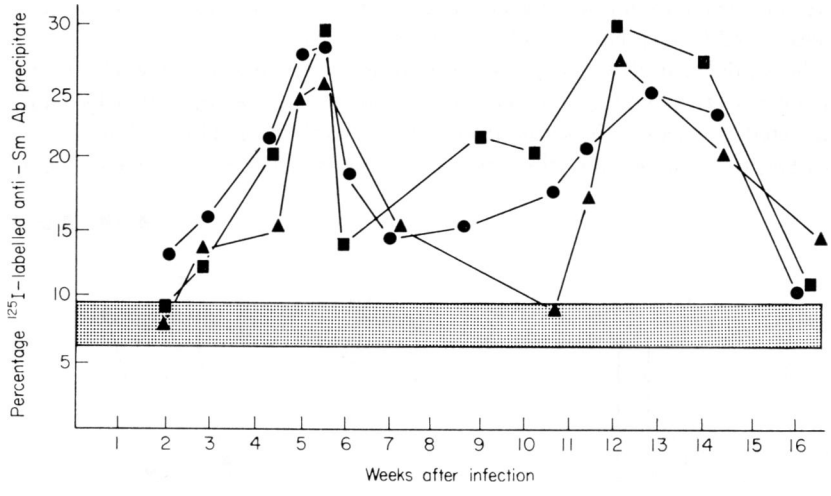

FIG. 1. Circulating schistosome antigens (CSA) during the course of *S. mansoni* infection in three different groups of twenty rats measured by the radioimmunoprecipitation–PEG assay (RIPEGA) with ^{125}I anti-*S. mansoni* antibodies. The results for the control rats were always lower than 10% during the same period (shown by shaded area).

Circulating immune complexes

Circulating immune complexes (CIC) were assayed by the CH_{50} deviation test in pooled serum from both normal and *S. mansoni*-infected rats studied above for CSA (Fig. 2). A close correlation was observed between levels of CSA and CIC. Infected rats presented two peaks of complement-binding CIC. The first appeared between 3 and 5·5 weeks after infection and the second between 8 and 15 weeks.

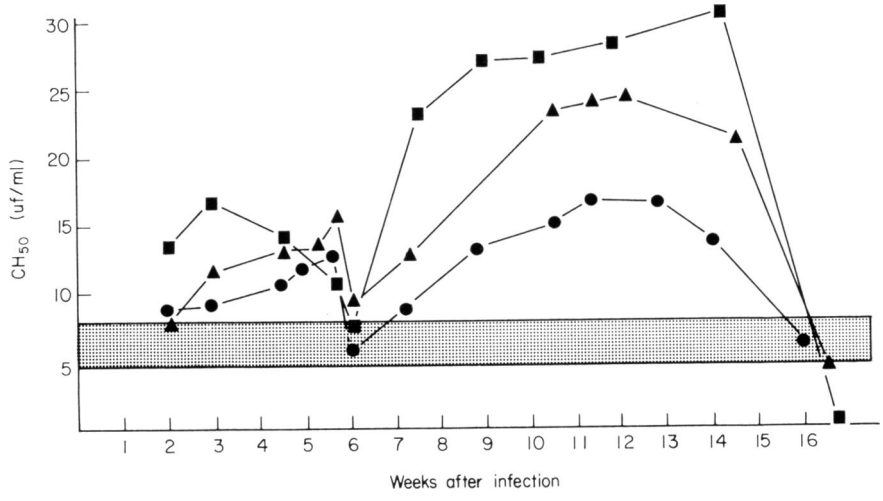

FIG. 2. Circulating immune complexes (CIC) during the course of *S. mansoni* infection in three different groups of twenty rats quantified by the CH_{50} deviation test. The results for the thirty control rats were lower than 8 during this period (shown by shaded area).

At 6 weeks, the levels of complement-binding CIC in the serum from infected rats were not different from the levels in control rats.

IgE immune complexes

Sera from normal and infected rats were passed through an anti-IgE immunoabsorbent column and the IgE eluate was examined for schistosome antigens by RIPEGA. Fig. 3 shows that antigens were detected at weeks 4 and 6, with the highest level at week 6.

A study of the solubility in PEG, of IgE in serum from normal and from 6 week-infected rats showed that, at PEG concentrations between 10 and 16%, the percentage of precipated IgE was higher in the serum from infected rats than in the serum from normal rats (Fig. 4). This preliminary result suggested that 6 weeks after an *S. mansoni* infection, rat serum contained IgE in aggregated or complexed form.

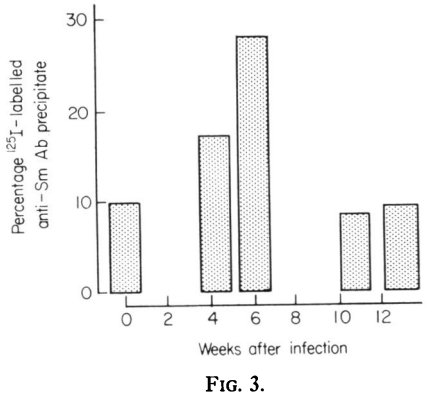

FIG. 3.

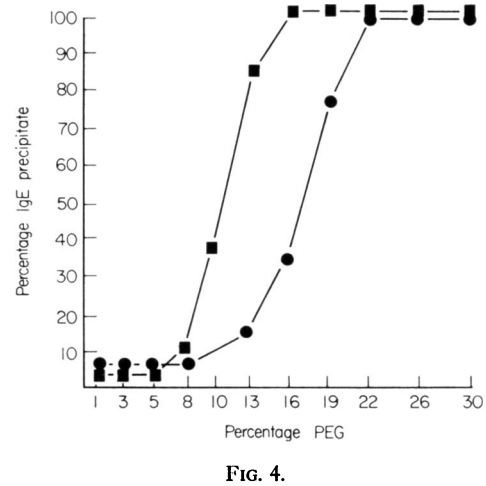

FIG. 4.

FIG. 3. Circulating IgE immune complexes at various times of infection detected by the use of the RIPEGA in the desorbed material from the anti-rat IgE immunosorbent. Week 0 = control rat.

FIG. 4. Solubility of 6 week infected rat IgE (■) and normal rat IgE (●) in various concentrations of polyethylene glycol (PEG).

Moreover, 6 week *S. mansoni*-infected rat serum, when centrifuged at 120,000 *g* for 3hr, presented in a redissolved pellet significant amounts of IgE and *S. mansoni* antigens in comparison with normal rats (Table 1). In fact, 13% of the total serum IgE (quantified by radioimmunoassay) and 68·6% of the total CSA (detected by RIPEGA) were found in the centrifuged pellet of 6 week infected rats.

IgG2a immune complexes

Sera from rats infected for 5 and 6 weeks were passed through a C1q immunoabsorbent column. The CIC eluate was examined for the presence of IgG2a by immunoelectrophoresis using a monospecific anti-rat IgG2a antiserum. Fig. 5 shows that IgG2a was present at both weeks. Twice the amount of eluted CIC was obtained at week 5 than at week 6.

TABLE 1. Characterization of total IgE and schistosome antigens in ultracentrifuged serum of rats infected for 6 weeks with *S. mansoni*

	Pellet*		Supernatant*	
Rats	IgE† (ug/ml)	CSA‡ (%)	IgE	CSA
Infected	0·75	13·4	5·68	10·6
Uninfected	0·03	7·3	0·16	7·8

* After ultracentrifugation at 120,000 *g* for 3 hr.
† Total IgE.
‡ Schistosome antigens (percentage ^{125}I-labelled anti-Sm Ab precipitate).

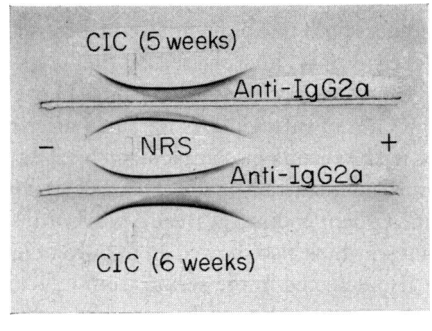

FIG. 5. Characterization of IgG2a in C1q-binding CIC from rats infected, after 5 and 6 weeks, with *S. mansoni*. (NRS) Normal rat serum.

Complement levels

Complement investigations were performed on fresh individual serum from three other groups of ten normal and ten *S. mansoni*-infected rats. Levels of 50% haemolytic complement during the course of the infection are shown in Fig. 6. Infected rats showed three decreased zones of haemolytic complement. A first zone between the 2nd and the 4th week, a second between the 5th and the 8th week and a third following the 12th week of infection. Concerning C3, in our experimental conditions, no difference was observed during the course of infection between values of normal rat serum and levels of infected rats.

DISCUSSION

In the present study CSA and CIC were detected in the sera from rats infected with *S. mansoni*. RIPEGA with specific ^{125}I-labelled anti-Sm Ab demonstrated CSA in two distinct periods of infection. This

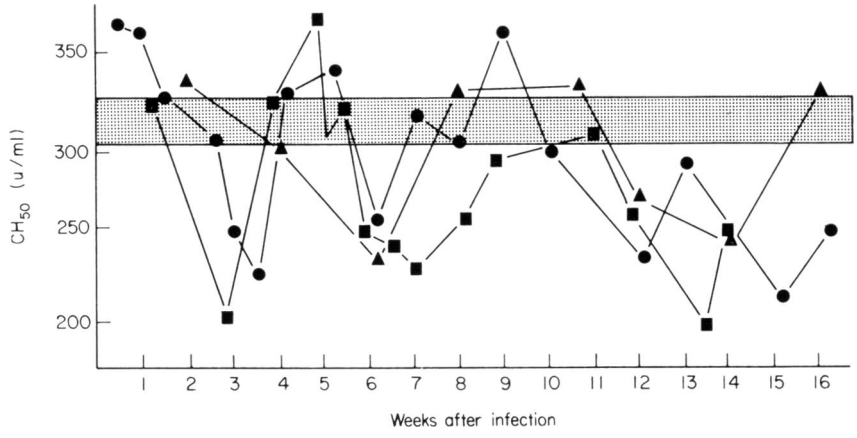

FIG. 6. 50% haemolytic complement during the course of *S. mansoni* infection in three different groups of ten infected rats and in thirty control rats (shown by shaded area).

sensitive and reproducible method, proposed for the detection of specific CIC in other infections (Santoro *et al.*, 1977c; Fruit *et al.*, 1977) gave satisfactory results when applied to the investigation of circulating antigens in rat schistosomiasis.

The CH_{50} deviation test revealed the presence of CIC in serum from infected rats approximately at the period when CSA were detected. The peaks of CSA and CIC were situated between the 3rd and the 6th week and between the 8th and the 14th week of infection respectively. Although the CH_{50} deviation test can detect CIC of any antigen origin, the relationship observed with the CSA argues for the *S. mansoni* specificity of the detected complement-binding CIC. Moreover, with the RIPEGA, a primary antibody-binding assay, one can detect both free or complexed CSA.

At 6 weeks of infection, the levels of CIC investigated by the CH_{50} deviation test in the serum from infected rats were not different from the levels in control rats. However, during that period we were able to characterize IgG2a in the CIC of infected rats obtained after passage through a C1q immunosorbent column. This difference in the results of complement-binding CIC is probably due to a higher sensitivity of the C1q immunosorbent than that of the CH_{50} deviation test. Moreover, the amount of CIC eluted from the C1q immunosorbent column at week 5 was twice that at week 6. This supports well the results of the CH_{50} deviation test, which also showed an important level of CIC at 5 weeks of infection.

IgE immune complexes were also detected in the serum from infected rats. RIPEGA on the material eluted from the anti-IgE immunosorbent column characterized schistosome antigens at week 4 and 6, with a higher level at week 6. Both ultracentrifugation and PEG precipitation methods confirmed the presence of aggregated or complexed IgE in the serum from 6 week infected rats. The maximal amount of *S. mansoni*-specific IgE antibody in the serum from infected Fischer rats, observed by Rousseaux-Prévost *et al.* (1978), represents 18% of the total IgE level, whereas 13% of the total IgE is present in the ultracentrifugation pellet of 6 week infected rat serum. These results suggest that most of the specific IgE antibody is likely to be in a complexed form at this period. The presence of non-specific aggregated IgE cannot be ruled out at this stage of our experiments and further work is in progress to clear this point.

In the first peak of CSA (between the 4th and the 6th week of infection), two types of CIC were demonstrated in rats infected with *S. mansoni*: (a) complement-binding CIC, constituted in part by IgG2a complexes and (b) non-complement-binding CIC, mainly represented in this work by IgE complexes. The presence of CSA and CIC at this period is probably the consequence of the initial phase of the 'self-cure phenomenon', which takes place, in the Fischer rat, just before their appearance, when the major part of the worm population disappears from the liver. In the second peak of CSA (between the 11th and the 14th week of infection), only complement-binding CIC were observed. Complexed IgE was not detected at this period. The reason why complement-binding CIC were present in infected rat serum beyond week 11 is unclear. The parasitic origin of these complexes can be, in part, supported

by the presence of CSA, which is probably released by residual worms. However, it is possible also that CIC of non-parasitic origin could be formed at this period in the serum from infected rats and detected by the CH_{50} deviation test. The involvement of IgE and IgG2a in the antigen-antibody complexes present in the serum from infected rats does not imply that other immunoglobulin classes or subclasses could not participate in other CIC.

Three periods of decreased haemolytic complement were observed in the serum from infected rats, between the 2nd and the 4th week, the 5th and the 8th and after the 12th week of infection. These are probably due to the consumption of complement by immune complexes in the circulation, but are not related to the third complement component, since levels of C3, in our experiments, did not change during the course of infection. In human schistosomiasis as well, C3 levels were negatively correlated to CIC (Santoro *et al.*, 1977a).

Two periods of immunity against *S. mansoni* reinfection in rats have been observed, the first at week 7, with 70% of protection, the second at week 15 (67% protection), with a significant decrease of immunity around week 13 (Capron *et al.*, 1977b). It is worth noting that the two CIC peaks appeared just before the maximal peaks of immunity to a challenge infection. This relationship suggests a possible role for CIC in immunity, in association with effector cells (Capron *et al.*, 1975). At least one of the two types of CIC demonstrated in the serum from infected rats, IgE and IgG2a complexes, may be associated with unsensitized cells. Indeed, complexed IgE has been shown to interact with normal macrophages in *in vitro* cytotoxicity to schistosomula (Capron *et al.*, 1975, 1976, 1977a,b). The presence of significant amounts of CSA and IgE in the redissolved ultracentrifugation pellet from 6 week infected rat serum may account for the cytotoxicity against schistosomula induced by macrophages activated by this material (Capron *et al.*, 1977a). Concerning the other type of CIC detected in infected rat serum, IgG2a complexes, it is noteworthy that this immunoglobulin was recently characterized as an opsonic antibody involved in *in vitro* adherence and cytotoxicity of rat eosinophils to schistosomula (Capron *et al.*, 1978).

Although immune complexes in human and murine schistosomiasis have been suggested to be responsible for the observed renal injury (Andrade, Andrade & Sadigursky, 1971; Hoshino-Shimizu *et al.*, 1976; Natali & Cioli, 1976), in rat schistosomiasis CIC may play an important role in the effector mechanisms of the immune response to schistosomes.

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