Schistosoma mansoni: antigenic community between schistosomula surface and adult worm incubation products as a support for concomitant immunity

Colette Dissous and André Capron

Centre d'Immunologie et de Biologie Parasitaire, INSERM U 167 – CNRS ERA 422, Institut Pasteur, 15, rue Camille Guérin, 59019 Lille Cedex, France

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The use of protective monoclonal antibodies has enabled us to demonstrate antigenic community between a 38-kDa schistosomula surface molecule and a 115-kDa component derived from adult worms. Injection of adult worms in rats also led to the production of antibodies specific for the 38-kDa antigen, suggesting that the 115-kDa adult worm molecule could act as an inducer of the protective immune response raised against young invading parasites.

Schistosoma mansoni *Antigenic community* *Concomitant immunity*

1. INTRODUCTION

Schistosomes are digenetic trematodes which affect more than 200 million people in the world. Cercariae escape from infected snails, penetrate through the host skin, transform into schistosomula and mature in adult schistosomes. Infected mammalian hosts develop a variable immunity against young invading schistosomula of a challenge infection and it was given evidence that adult schistosomes provided the major stimulus for immunity to schistosomiasis [1], possibly by the release of antigenic components. However, the host resistance does not affect the established population of adult worms and such a situation has been termed 'concomitant immunity'.

Using the lactoperoxidase-radioiodination technique, we have previously characterized surface antigens of *Schistosoma mansoni* schistosomula and shown that several components ranging from 30–40 kDa were recognized by antibodies of infected rat, mouse, monkey and human sera [2]. It was also demonstrated that incubation products of adult worms can compete with the 30–40-kDa schistosomula surface antigens for specific antibodies [3]. This result suggested the presence of shared epitopes in the two parasite stages and corroborated previous data indicating that adult worms can be responsible for the induction of antibodies that bind to the schistosomula surface [4]. A rat protective monoclonal IgG2a antibody was recently available and shown to be specific for one of the characterized schistosomula surface antigens (38-kDa) [5]. This monoclonal antibody was used here to identify an adult worm component bearing the same antigenic determinant(s). The ability of adult worms to induce an antibody response against the 38-kDa surface antigen was also confirmed by immunization of rats with adult worms.

2. MATERIALS AND METHODS

2.1. *Parasites*

A Puerto Rican strain of *S. mansoni* was used. Schistosomula were obtained by penetration of cercariae through mouse skin [6]. Adult schistosomes were collected by portal perfusion of 6 weeks *S. mansoni*-infected golden hamsters (*Mesocricetus auratus*).

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2.2. *Antibodies*

Anti-*S. mansoni* monoclonal IgG2a was secreted by an IPLSm1 cell line, produced by hybridization of IR983F myeloma cells with spleen lymphocytes of Lou/C rats infected by cercariae for 35 days [7]. IPLSm1 antibodies were purified from ascitic fluids by anion-exchange chromatography on DEAE–Trisacryl (IBF). Infected rat sera were collected from animals 14 weeks after exposure to 1000 cercariae. Infected human sera were obtained from patients with parasitologically confirmed schistosomiasis.

Immunized rat sera were obtained from animals which have received weekly adult worms of *S. mansoni*. Six successive injections of 100 living adult worms were made intraperitoneally in each rat.

2.3. *Identification of antigens*

Fifty adult worms were incubated in 0.3 ml phosphate-buffered saline (PBS), pH 7.4, for 3 h at 37°C. Released products were separated from parasite bodies by a 10 min centrifugation at 5000 × *g* and subsequently labelled with 0.2 mCi ^{125}I using the lactoperoxidase-technique in [8]. Immunoprecipitation of antigens was performed with protein A–Sepharose, exactly as described for the isolation of schistosomula surface antigens [3,5].

3. RESULTS

Products released during in vitro incubation of adult schistosomes were radioiodinated by the lactoperoxidase technique, then immunoprecipitated by IPLSm1 antibodies. Results, presented in fig.1, show that the anti-*S. mansoni* monoclonal IgG2a recognized specifically a 115-kDa antigen derived from adult worms. When immunoprecipitations were performed using infected rat and human sera, a more complex electrophoretic pattern was obtained, showing particularly the presence of the 115-kDa schistosome molecule. These results suggest that mammalian hosts develop during infection an antibody response against an antigenic determinant common to adult schistosomes and schistosomula surface.

In other experiments, rats were injected weekly with adult schistosomes and the production of antibodies directed against the schistosomula surface was followed-up. Sera from immunized rats were

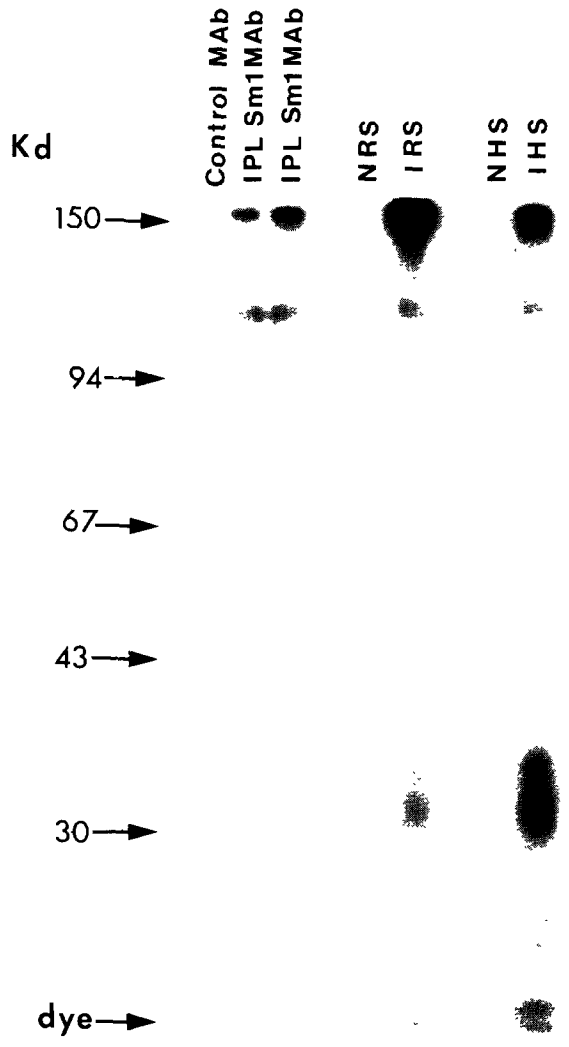

Fig.1. SDS–PAGE analysis of adult worm antigens: 40-μl aliquots of radioiodinated incubation products (equivalent to 5 adult worms) were immunoprecipitated by 20 μg of purified monoclonal IgG2a without anti-*S. mansoni* specificity (control MAb), 20 μg anti-*S. mansoni* monoclonal IgG2a (IPLSm1 MAb, two different preparations were used) or 10 μl infected rat and human sera (IRS and IHS). Normal sera were used as controls (NRS and NHS). Immune complexes were analyzed by SDS–PAGE; Kd, kilodalton.

allowed to react with a detergent extract from surface-radioiodinated schistosomula and surface antigens were detected as soon as 2 weeks after the initial injection of schistosomes. SDS–PAGE analysis of immune complexes (fig.2) revealed that immunized rat sera recognized the previously

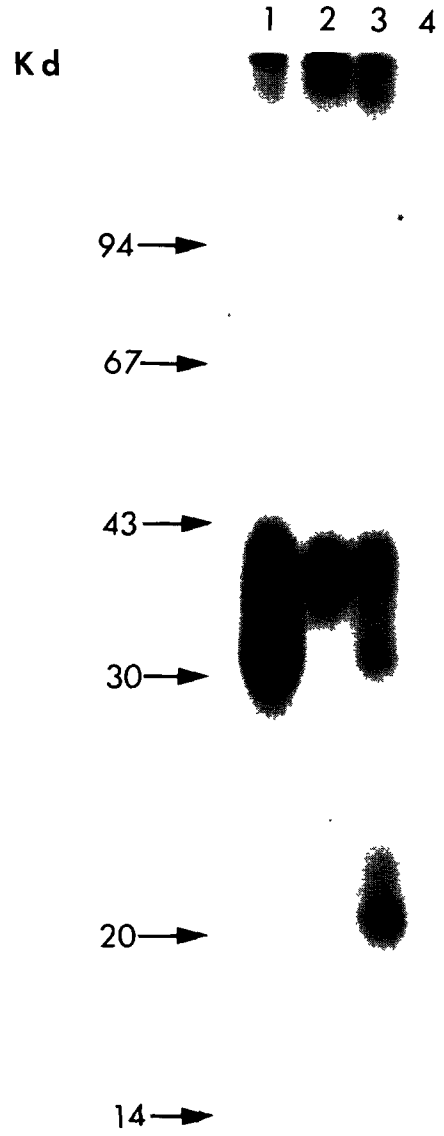

Fig.2. SDS–PAGE analysis of schistosomula surface antigens. Detergent extract of surface-radioiodinated schistosomula was prepared as in [3], then immunoprecipitated by 10 μl infected rat sera (1), 20 μg of IPLSm1 antibodies (2) or 10 μl immunized rat sera collected 7 weeks after the initial injection of adult worms (3,4). In (4), immune complexes were dissociated by boiling 2 min in SDS 2% and incubated with 20 μg IPLSm1 antibodies. Immune complexes were analyzed by SDS–PAGE.

described schistosomula surface antigens of 30–40 kDa (cf. lanes 1,3). The presence of antibodies binding the surface molecule defined by the monoclonal antibody, was confirmed by reisolating the 38-kDa surface molecule with IPLSm1 antibodies from dissociated immune complexes (cf. lanes 2,4). Moreover, immunoprecipitation of schistosome-released products by immunized rat sera also demonstrated an antibody response against the 115-kDa adult worm component, showing an electrophoretic pattern of dissociated immune complexes similar to that obtained with infected rat sera.

4. DISCUSSION

The concept of concomitant immunity was first introduced [9] in a situation in which hosts develop resistance against a challenge infection but cannot rid themselves of the established adult worm population. The slow development of immunity in infected hosts suggested that adult worms could provide the major immunogenic stimulus and direct evidence of this was given by the same authors who showed that monkeys could be protected from infection by cercariae following transfer of adult worms [1].

Mechanisms of acquired immunity have been studied and different cytotoxicity systems were described in which specific antibodies could mediate in cooperation with cells or complement the killing of schistosomula [10]. The demonstration of these mechanisms mainly stated the reason for studying schistosomula targets and we previously identified 30–40-kDa schistosomula surface antigens by immunoprecipitation with rat, mouse, monkey and human sera. These antigens were also shown to be accessible at the surface of living parasites [2]. Among these antigens of very close M_r, only the 38-kDa component was precipitated by the rat monoclonal IgG2a [5], an antibody which can mediate in vitro killing of schistosomula and protect rats from infection by *S. mansoni* [7]. The 38-kDa antigen was identified at the surface of cercariae but not at the surface of lung-stage schistosomula [5]. These data suggested that the 38-kDa molecule could correspond to an important target antigen for the first larval stages of *S. mansoni*.

The presence of shared antigens between extracts of adult worm and schistosomula membranes has already been demonstrated [11,12] and the ability of adult worm membrane extract to in-

duce antibodies against the schistosomula surface has been reported [4]. As we also observed that incubation products of adult worms compete with the 30–40-kDa antigens for infected host antibodies [3], we used the monoclonal antibody to identify the adult worm component susceptible to induce an antibody response against the 38-kDa target antigen. Results presented here show that a 115-kDa adult worm antigen was precipitated by the monoclonal IgG2a, as well as by antibodies produced during rat and human schistosomiasis. In control experiments, we also demonstrated that a schistosomula detergent extract was able to inhibit the binding of the monoclonal antibody to the adult worm antigen.

The finding that the epitope defined by the IgG2a antibody was expressed in adult worms and schistosomula on different molecular structures was not so surprising. Indeed, using monoclonal antibodies, antigens of different M_r were characterized in the various parasite stages [12,13]. From these data, it might be supposed that monoclonal antibodies were specific for definite sugar moieties linked to different protein structures. Here, such a hypothesis has been strengthened by preliminary results which demonstrated that the 38-kDa schistosomula antigen, as well as the 115-kDa adult worm antigen could no more be precipitated by IgG2a antibodies following treatment by periodate [14].

Results obtained from injection of adult worms in rats also gave evidence that the adult schistosome could act as an inducer of the antibody response against the 38-kDa schistosomula surface antigen, a finding which was in agreement with the important delay observed in the appearance of antibodies following infection. In rat, antibodies specific for 30–40-kDa antigens were detected just after the animal rejected most of its worm population (i.e., 4 weeks after infection), and in mouse the antibody response was shown to appear 7 weeks after the initial infection [2]. Recent studies also indicated that human hosts, in which adult worms are known to survive for many years, present a high antibody response against the 38-kDa antigen, even after long periods following infection (unpublished).

Since the schistosome surface membrane has a rapid rate of turnover [15], we attempted to know whether the 115-kDa released antigen originated from the schistosome surface. Results of external labelling with ^{125}I (using lactoperoxidase or Bolton–Hunter reagent) did not indicate a surface location of the 115-kDa adult worm antigen but did not exclude it as a membrane component which can be unexposed or masked at the parasite surface. These data can also corroborate the observation that adult worms are unaffected by the host immune response.

In conclusion, the use of the monoclonal antibody has enabled us to demonstrate an antigenic community between a 115-kDa adult worm molecule and a 38-kDa schistosomula surface molecule. These antigens, found in two different molecular structures bearing the same epitope defined by protective antibodies, might represent, respectively, inducer and target antigens efficient in acquired immunity, thus providing a new molecular support for the concept of concomitant immunity.

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