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Blocking antibodies and vaccine strategy in schistosomiasis

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Immune effector mechanisms against schistosomes are very diverse. Depending on the experimental model and the method of immunization, some components of the immune response can appear more important than others.

The rat seems particularly suitable to studies on antibody-mediated immunity [1]. Rats immunized either by an active infection or with live attenuated material develop high levels of protective antibodies. The real question remains the exact basis of protective immunity in man. At this stage it would be unwise to draw conclusions, but in terms of antibody-mediated immunity, several striking similarities appear between rats and men, in their response to schistosomes. All the effector mechanisms against schistosomula targets in vitro have been demonstrated in both species: complement-dependent «lethal» antibody, IgG- or IgE-dependent eosinophils, IgE-macrophages or more recently IgE antibodies in cooperation with platelets. For a long period the real biological significance of multiple in vitro killing mechanisms was legitimately debated. The high levels of protection induced either by passive transfer of antibodies or cells exhibiting defined in vitro effector function, or by direct immunization with the corresponding target molecules or anti-idiotypes now shows that, at least in rats, in vitro ADCC mechanisms correlate well with in vivo immunity.

In the present report, we will analyze how regulatory mechanisms identified in rat experimental schistosomiasis both during experimental infection or by using monoclonal antibody probes, are also found during human schistosomiasis. The existence of blocking antibodies of defined isotypes and their association with the susceptibility to reinfection might represent an important constraint on potential vaccines. For this reason, an alternative approach using anti-idiotype vaccine will be presented.

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I. Blocking antibodies in rat experimental schistosomiasis

Immune complexes were known for almost ten years, as able to modulate in vitro the cytotoxic activity of eosinophils, both in human infection, as shown by Butterworth [2] or during experimental rat schistosomiasis [3]. More recently, the production of *S. mansoni*-specific monoclonal antibodies has allowed some explanation for such immunologic mechanism [4, 5]. Rat IgG_{2c} monoclonal antibodies (IPLSm3) which did not exhibit any killing activity for schistosomula, specifically inhibited the eosinophil-dependent cytotoxicity mediated by monoclonal IgG_{2a} antibodies (IPLSml), both in vitro and in vivo [5]. The blocking effect of IgG_{2c} was dual, both at the surface target antigen (38,000 M.W.) and at the effector cell level by competition for the Fcγ receptor [5, 6].

This blocking effect of rat IgG_{2c} mAb was mainly effective on IgG-mediated mechanisms, not only on eosinophil-dependent cytotoxicity but also on complement-dependent «lethal» antibody-mediated killing. However, no inhibitory role could be detected on IgE-dependent killing assays neither with macrophages nor with eosinophils. This would indicate either that the target antigens of IgG_{2a}-eosinophil and lethal antibody cross-reacted with the 38,000 M.W. antigen and were different from the target antigens of IgE-mediated mechanisms (which was indeed demonstrated thereafter). Alternatively, we could show a direct inhibitory role of IgG_{2c} on the FcγR itself and not on Fcε receptor as shown by a specific inhibition of peroxydase released by IgG_{2a} and not by IgE [6].

This inhibitory role of the IgG_{2c} isotype was not restricted to monoclonal antibodies. Indeed it was shown that IgG_{2c} depletion of immune rat serum by protein A absorption led to an increase in eosinophil mediated cytotoxicity in vitro. The in vivo relevance was confirmed by the kinetic study of the presence of cytophilic IgG antibodies bound to eosinophils from *S. mansoni* infected rats, according to their status of immunity to reinfection: the presence of IgG_{2a} corresponding to the period of immunity, whereas the detection of IgG_{2c} was linked to the decrease in immunity to reinfection [6].

The demonstration of such mechanisms in rats raises the question of the possible existence in human schistosomiasis of blocking antibodies modulating the efficiency of immune effector mechanisms [7]. Indeed, in extensive studies performed in an endemic area of schistosomiasis in Kenya, Butterworth et al. reported that the comparison of various parameters of the immune response, among which antibodies mediating eosinophil-dependent killing of schistosomula, anti-schistosomula IgE antibodies and blood eosinophil counts, showed no significant differences, between the two groups of children defined as resistant or susceptible to posttherapeutic reinfection [8]. The demonstration in experimental models that defined antibody isotypes might block the expression of immunity, prompted us to investigate the presence of such blocking antibodies, and their possible role in preventing immunity to reinfection.

II. Blocking IgM antibodies in human schistosomiasis

These studies were performed on human sera obtained from *S. mansoni* infected patients before and after treatment with oxamniquine. Among these subjects, two subgroups could be considered as resistant or susceptible to reinfection, according to the previously defined criteria [8].

The techniques used to demonstrate the existence of blocking antibodies of given isotypes were mainly: the protein A (SpA) absorption of human sera which led to SpA effluents (containing IgM, IgA and IgG_3) and SpA eluates (containing IgG_1, IgG_2 and IgG_4). More precise serum fractionation of IgG and IgM were performed with a fast protein liquid chromatography (FPLC) system. The effector function of these various fractions was evaluated on *S. mansoni* schistosomula in the presence of human eosinophils.

Evidence from fluorescence studies on schistosomula sections indicated the presence in sera from infected patients, of IgG and IgM antibodies specifically directed against schistosomula surface. To determine the antibody isotype required in the cytotoxicity assay, human sera were fractionated either by absorption on protein A-sepharose or by Ig separation on chromatography column (FPLC system). Detectable levels of cytotoxicity were observed, when *S. mansoni* schistosomula were incubated with purified eosinophils in the presence of heat-inactivated total immune sera. When the serum was fractionated, the cytotoxic activity was detected in the IgG containing fractions (SpA eluate fraction or purified IgG fraction). Moreover, the IgG fractions produced significantly higher levels of cytotoxicity than the total sera ($p < 0.01$). These results suggested that the depletion of some inhibitory factor not retained on protein A could lead to an increase in IgG-mediated killing. No cytotoxic effect was observed neither in the presence of SpA effluent fractions nor with IgM fraction purified by FPLC.

Since IgM antibodies were able to bind to target surface, it was interesting to study their activity in eosinophil-dependent cytotoxicity mediated by IgG antibodies. *S. mansoni* schistosomula were preincubated with various dilutions of SpA effluents for 2 h at 37° C. After 2 washings, total immune sera or SpA eluates and eosinophils were added. The percentage of cytotoxicity was compared to the killing activity induced by total immune serum or by SpA eluate fraction, after preincubation of schistosomula with medium. As shown in Fig. 1, the preincubation of schistosomula with SpA effluents of immune sera markedly inhibited the IgG-dependent cytotoxicity mediated by total immune serum or SpA eluates in a dose-dependent manner ($p < 0.01$).

The target specificity of IgG and IgM antibodies was determined. Immunoprecipitation of ^{125}I-labelled schistosomula antigens with protein A binding antibodies (IgG antibodies) from infected human serum demonstrated the precipitation of 30–40,000 M.W. antigens in 100% of the sera tested. However, additional bands with 20–25,000 M.W. range were revealed (66%) of sera

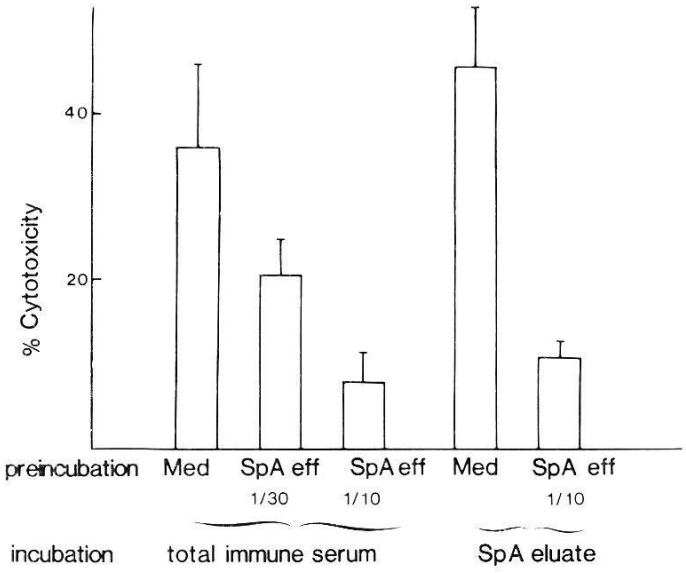

Fig. 1. Inhibitory role of IgM containing SpA effluents on IgG dependent cytotoxicity by human eosinophils. Schistosomula were preincubated for 2 h at 37°C and washed twice with medium and then incubated with homologous serum or fractions. SpA effluent and SpA eluate represent the unbound and bound fractions (IgM and IgG antibodies respectively). The percentage of cytotoxicity was measured after 48 h contact with human eosinophils (mean of 9 experiments ± SD).

tested). Surface antigens precipitated by IgM antibodies from infected human serum ranged between 30–40,000 M.W. (100% of sera tested) and also between 20–25,000 M.W. (20% of sera tested). However, the predominant IgM antibody response was against the 32,000 M.W. antigen. Adsorption experiments, in which the detergent extract was previously adsorbed by protein A binding-IgG antibodies demonstrated a cross-inhibition between IgG and IgM antibodies.

In order to investigate the respective epitopes recognized by IgG and IgM antibodies, we used a radioimmunoassay method in which the capacity of IgG and IgM fractions to inhibit antigen binding of two rat monoclonal antibodies (IgG_{2a} and IgG_{2c} directed against the 38,000 Ag) was tested. The preincubation of wells precoated with schistosomula antigen in the presence of IgG and IgM fractions of infected serum inhibited both the binding of ^{125}I-labelled IgG_{2a} and IgG_{2c}.

The specificity of epitope recognized by IgG and IgM antibodies in infected serum was tested by cross-inhibition experiments against the 38,000 antigen. The preincubation of antigen-coated plates in the presence of IgG fraction inhibited the binding of homologous ^{125}I-labeled IgM antibodies. In the same conditions, higher levels of inhibition were obtained when cold IgM antibodies were preincubated before labeled IgG, suggesting higher affinity of IgM for the targets.

These results suggest that IgG and IgM antibodies in human infected sera were able to immunoprecipitate antigens of 3 h-schistosomula surface ranging between 30 and 40,000 M.W. A major band of 32,000 M.W. was recognized by

IgM antibodies. The latter result suggests that the 32,000 antigen was more extensively labelled or that the presence of this antigen on the older parasite induced high levels of antibodies directed against the 32,000 antigen [9]. However, the findings indicated a blocking effect at the level of membrane target antigen. In addition, there is a competition between IgG and IgM containing fractions of the same antigen as shown by the radioimmunoassay. A second and more likely possibility is that the blocking effect of IgM antibodies could be effective at another target antigen level: at 38,000 M.W. (discriminated in the 30–40,000 M.W. region [10] defined by protective rat monoclonal antibodies and recognized by 95% of infected human sera [11]. Such hypotheses are confirmed by the inhibition by immune serum, IgG and IgM fraction of binding to antigen of two rat monocolonal antibodies (IgG_{2a}, IgG_{2c}) recognizing the 38,000 M.W. schistosomulum surface antigen.

These observations suggest that the target epitope presented on the 38,000 M.W. antigen could elicit the production of IgG and IgM antibodies which have antagonistic activities. Very stricking were the similarities between human infection and rat experimental schistosomiasis, since the same molecule (38,000 M.W.) may elicit both the production of effector (IgG_{2a}) and blocking antibodies (IgG_{2c}) [5].

The biological relevance of such IgM blocking antibodies was assessed by the evaluation of anti-38,000 IgM antibodies in the sera from individuals classified as resistant or susceptible to reinfection using previously defined criteria [8]. Such antibodies were measured in an IgM capture assay in order to avoid the competition with the IgG antibodies. Results presented in Fig. 2 showed that the mean levels of IgM antibodies in susceptible individuals were significantly higher than those present in resistant subjects, in the pretreatment and 12 month serum samples but did not differ significantly at 5 weeks after treatment. However, for the susceptible population (defined as those individuals having more than 100 eggs per gram of faeces) there was a positive association between the level of IgM antibodies and the number of eggs at 12 months after treatment.

Although our evidence is derived from in vitro studies, these findings indicate that the susceptibility to reinfection to *S. mansoni* in human infection might be explained at least in part by the presence of specific IgM blocking antibodies. In addition, they suggest indirectly the important role of the effector IgG antibodies directed against the 38,000 M.W. antigen. The fact that these antibodies are a necessary but not a limiting factor in immunity and the presence of blocking antibodies supports the hypotheses that the acquisition of immunity might reflect the loss of a blocking response, rather than the acquisition of an effector response. The availability of this type of information would be of considerable importance in defining the nature of vaccine susceptible to induce the production of both effector and blocking antibodies. However, another approach to explain the prevention of immunity may involve the idiotypic regulation whereby the protective antibodies were blocked by antibodies

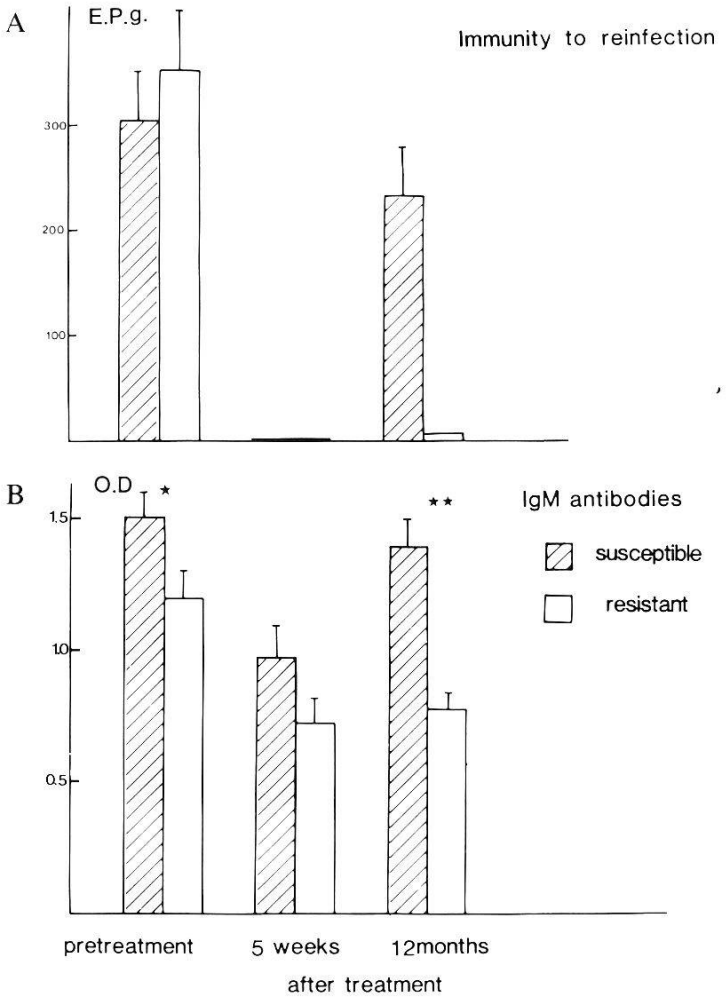

Fig. 2. Relationships between the presence of IgM antibodies against the 38,000 schistosomulum surface antigen and the status of immunity to reinfection. Sera were selected from susceptible (n = 21) and resistant (n = 32) individuals using previously defined criteria. Fig. 2 A represents the number of eggs per gram (Epq) calculated by examination of duplicate Kato preparations. Fig. 2 B represents the level of anti-38,000 IgM antibodies measured by a capture assay. Results are presented as mean ± SEM (* $p<0.01$, ** $p<0.001$).

directed against the antigen-specific combining sites of effector antibodies (i.e. anti-idiotype antibodies).

III. Anti-idiotype strategy

To evade the problem of the existence of blocking antibodies, we considered, as an alternative approach the possibility of immunization with anti-idiotype antibodies [12] instead of using specific antigens.

Monoclonal antibodies were produced against IPLSml, the protective monoclonal antibody AB1, directed against the gp38. Antiidiotype antibodies AB2, were selected by their capacity to inhibit the binding of radioiodinated, AB1 to its 38,000 target antigen, representing therefore antiparatope antibodies which according to the Jerne's theory can be considered as the internal image of the original epitope. The majority of positive hybridoma supernatants con-

tained antibodies of the IgM class. Sera from LOU rats immunized with a purified AB2 preparation contained specific antischistosome antibodies (AB3), detected on schistosomula sections and which bound to gp38. These AB3 antibodies were strongly cytotoxic for schistosomula in the presence of rat eosinophils. Further inhibition or depletion experiments revealed that AB3 were of the IgG_{2a} subclass, similarly to AB1. The in vivo relevance of this mechanism was demonstrated by showing that AB3 conferred significant levels of protection by passive transfer, whereas rats actively immunized by AB2 demonstrated a marked protection (50 to 76% to challenge infection).

These results clearly demonstrate that immunization with an antiidiotype mAb can reproduce several parameters of acquired immunity observed in experimental rat schistosomiasis. Several lines of evidence suggests that the AB2 produced might represent an internal image of the original epitope: AB3 exhibited the same specificity for the gp38, the same isotype and the same effector function (in vitro and in vivo), as the original AB1. Thus immunization with anti-idiotype antibodies represents an alternative approach to immunization against pathogens.

To envisage the possibility of using such immunization procedures in humans, we have investigated the existence of cross-reacting idiotypes in human schistosome infection, by using an inhibition assay: human sera were tested for their capacity to inhibit the binding of labelled AB1 to Ab2 coated on microplates. Very high levels of inhibition could be obtained for some human sera (up to 84%) indicating that human antibodies possess common idiotopes with the rat protective mAb directed against the gp38.

At this stage of our research, several points remain to be clarified, in particular the possible relation between blocking antibodies and their anti-idiotypic activities or the link which could exist between paratopic specificity of anti-GP38 antibodies and expression of immunity.

Whatever the importance of the research which is still to be pursued, it seems however that our description of the existence both in experimental and human schistosomiasis of antibody isotypes blocking the effector response against defined surface targets might open a new lead in our understanding of the mechanisms regulating immunity to reinfection against schistosomes and possibly other parasites.

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