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Why Vaccines do not work in Chagas Disease

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Prophylaxis of Chagas disease is currently carried out by vector control. Recent reports show that the domestic triatomine bug *Triatoma infestans*, the most important vector of *Trypanosoma cruzi* in Latin America, has been virtually eradicated from the whole State of São Paulo, Brazil, by the use of insecticides[1]. Active drugs for mass treatment of the disease are not available, and control of the vector by housing improvement is a long-term costly enterprise that depends more on political than technical decisions. What then is the possible role of a vaccine in this context?

There are several reasons why immunization against *T. cruzi* is recurrently mentioned as an alternative for Chagas disease control. In particular, most endemic countries seem reluctant to take the political decision to launch expensive control programmes based on insecticide spraying. Perhaps their view is also coloured by the spectre of malaria, where control based on specific therapy and insecticide spraying has met many difficulties, such that hopes for control in many endemic areas now rest on the widely sought development of new vaccines.

The requirements for a vaccine in Chagas disease and the array of antigens used for immunization have been reviewed elsewhere[2]. So far, all attempts to vaccinate animals with the various *T. cruzi* antigens (live attenuated or non-proliferative organisms; killed intact parasites; purified surface membrane glycoproteins; cell homogenates or subcellular fractions) resulted in a lack of protection or in only partial protection to a challenge infection. In human Chagas disease, heart and digestive tract lesions often appear late in the course of infection (10–20 years after infection), and no evidence has been provided that these lesions are correlated with the severity of the early stage of the disease. For these reasons, only vaccines affording total protection in animals would be acceptable for further investigation. Why are the vaccination attempts against *T. cruzi* at best only partially protective?

Two Types of Antibody

Recent work is throwing light on the host immune response to immunizing antigens in Chagas disease and the reasons for their inefficacy[3,4]. Two types of antibody with different functional activities have been described in this disease. Protective or 'lytic antibodies' (LA) are associated with host resistance in active infections; such antibodies are detected by a complement-mediated lysis (CoML) or an immunofluorescence test performed with viable *T. cruzi* blood forms (BTry) or trypomastigotes derived from tissue culture. In contrast, 'conventional serology antibodies' (CSA), which are involved in the serological diagnostic tests for Chagas disease, are not associated with host resistance and are not detected by CoML. Chronically infected patients or animals display both LA and CSA, whereas in immunized animals only CSA are found. Interestingly, in a certain percentage of chagasic patients submitted to specific treatment, with nitrofuran or 2-nitro-imidazole derivatives, the CSA persist as 'dissociated' sera but the LA disappear — a result which strongly suggests that the patients are parasitologically cured[4].

Thus it appears that active infections induce protective antibodies that recognize epitopes from living BTry (LA), and also produce non-protective antibodies (CSA) that only recognize epitopes present on fixed parasites. In contrast, immunization raises only CSA (Fig. 1). The different binding capability of both antibodies has been directly demonstrated by incubating living BTry from X-irradiated infected mice (which are devoid of membrane-bound antibodies) with sera from either infected or immunized mice and then incubating them with a second ligand, an anti-mouse fluoresceinated gamma-globulin. Only BTry incubated with sera from chronically infected animals were fluorescent[3].

The implications of these findings in regard to immune protection in Chagas disease are very important. Since CSA induced by the different antigens cannot bind to BTry, they are liable to be poor mediators of immune effector mechanisms which depend on the presence of antibodies on the target cell surface. Sera from chronically infected patients or animals are the only ones that mediate destruction of BTry by antibody-dependent cellular cytotoxicity (ADCC), whereas sera from immunized animals or 'dissociated' sera from treated patients do not induce parasite lysis[5]. Phagocytosis of BTry by mouse peritoneal macrophages is significantly enhanced by sera from *T. cruzi* infected humans, mice or rabbits, as compared with sera from vaccinated animals or 'dissociated' sera[6]. As mentioned above, CSA is also unable to mediate complement-lysis, another reputed control mechanism of *T. cruzi* infection[3].

The reason why only LA but not CSA bind to viable trypomastigotes is not yet clear but may be related to their different

affinity to the target cells[5]. In relation to the lytic activity, immune IgG fragments Fab and Fab' are as effective as the intact IgG in transforming BTry into activators of the alternative complement pathway (ACP)[7]. This phenomenon suggests that specific IgG binds to BTry surface membrane components which normally inhibit complement activation, thus inducing *T. cruzi* lysis by the ACP. A study of *T. cruzi* infected mouse sera fractions obtained by affinity chromatography has shown that the lytic activity as demonstrated by CoML was related to peaks of IgG_2 isotypes, whereas the indirect immunofluorescence test with fixed parasites was positive with all sera fractions[8].

In summary, experimental evidence points to the presence in *T. cruzi* BTry of surface antigens responsible for the host resistance in active infections. Although this resistance is strong enough to prevent a new outbreak of acute parasitaemia after a challenge infection, it still allows the development of a residual subpatent parasitaemia by the superimposed infective stages[9]. However, this acquired resistance is not matched by the partial protection afforded by the antigens used for vaccination in Chagas disease which usually results in a variable patent parasitaemia. Whether these surface antigens would induce total protection in naive hosts is a matter of speculation.

More recent work has shown that CoML positive sera from chronic chagasic patients or infected mice recognize by immunoprecipitation a 160 kDa surface polypeptide from *T. cruzi* trypomastigotes, whereas sera from immunized mice and treated patients lacking LA could not recognize this polypeptide[10]. Isolation of this 160 kDa antigen, seemingly involved in the resistance, would be the next logical step in this interesting sequence.

Intriguingly, protection against challenge was also provided by immunization of mice with metacyclic trypomastigotes grown in acellular medium and killed by heating at 50°C or by merthiolate[11]. The sera from the immunized mice presented positive CoML and recognized by immuno-precipitation surface proteins of 77, 82 and 88 kDa. This finding, if confirmed, could facilitate the yield of protective antigens from culture-derived parasites which present a much more simple surface antigenic make-up than BTry[10,11].

Prospects for Vaccination

What are the prospects for vaccination against *T. cruzi* using isolated relevant antigens? In the first place it should be possible to test directly the hypothesis that only antigens inducing LA are likely to build up a steady resistance against *T. cruzi*. There is no guarantee that this would occur, because several evasion mechanisms have apparently evolved in *T. cruzi* which may circumvent the host immune response[12,13]. In addition, we cannot exclude the possibility that LA are merely markers of underlying complex events which take place during immunization by *T. cruzi* antigens. If eventually those purified antigens would indeed induce a strong protective effect, the practical implications of this finding are difficult to predict. For the sake of vaccination this purified antigen would have to afford long-lasting total protection and not bring about autoimmune aggression[9].

Finally, the use in humans of this presumptive vaccine (or others) will generate a series of crucial questions such as the problems of trials in volunteers and the ethical dilemma of 'keeping vaccinated and controls exposed to the hazards of infection in an area of active transmission'[2]. Nevertheless, despite all these difficulties the search for a vaccine for Chagas disease will probably be pursued by a number of researchers. After all, curiosity and challenge are what really move them. In addition, it is important (as we can learn from the malaria experience) to have people engaged on anticipating problems that may emerge in the future.

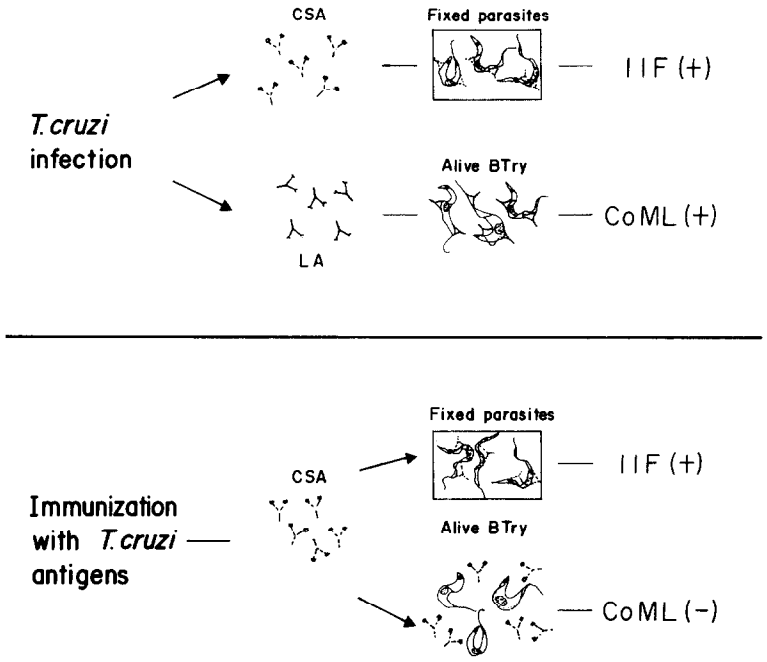

Fig. 1. Scheme of the humoral response induced by ongoing active infections and immunizations with T. cruzi *antigens, respectively. In infected animals LA (which bind to living BTry) and CSA (which bind only to fixed parasites) are detected, resulting in positive IIF and CoML positive tests. In immunized animals only CSA is detected and only IIF is positive. BTry – living blood forms; LA – lytic (or protective) antibodies; CSA – conventional serology antibodies; CoML – complement-mediated lysis; IIF – indirect immunofluorescence test.*

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