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IMMUNOPROTECTION IN CHAGAS' DISEASE

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Chagas' disease is transmited either by blood transfusion or by an hematophagous vector, in addition to accidental or congenital transmission, which, however, do not have the same epidemiological importance. Like any other infectious disease, the epidemiological characteristics of Chagas' disease should determine the strategy for its control.

Thus, Chagas' disease acquired through blood transfusion while being an iatrogenic disease should be dealt as such. The mere implementation of the available diagnostic and prophylatic measures (Dias, 1984, this Symposium) should reduce transfusional Chagas' disease to a medical rarity if not to medical negligence.

Transmission by the hematophagous vector, the most important means of transmission, is a domestic event restricted to rural areas. Precarious human housing favors the proliferation of the vector, which transmits the infection from man to man. Transmission from sylvatic animals to man seems to be of negligible importance at the moment. Therefore, at the present moment, controling Chagas' disease means controling the domiciliated vector, a task which is being undertaken in Brazil with apparent success (Silveira, 1984, this Symposium).

Whithin this epidemiological framework one may ask whether a vaccine against Chagas' disease is indeed necessary.

I tend to answer this question affirmatively.

Although not a substitute for vector control, a vaccine may be desirable for several other reasons.

The most cogent reasons relate to our incomplete knowledge of the biology of the sylvatic vector. At the moment we cannot predict what will happen when extensive florestal areas are colonized and a new dynamics of the sylvatic-reservoir association develops. Can Chagas' disease become a florestal disease similar to cutaneous leishmaniasis in its epidemiological features? Can the sylvatic vector adapt itself well to the human dwelling in florestal areas following the disruption of its natural habitat? Can some sylvatic vectors feed ubiquitously on animals and man? Can the triatomid vector develop resistance to inseticides under the prevailing conditions of their utilization?

Available evidence seems to permit disregarding these possibilities as excessively pessimistic. However, the catastrophic consequences of the malaria-control triumphal attitude of the fourties recommend extreme caution whenever dealing with epidemiological and vector uncertainties.

Therefore, it seems to me that we should have at hand a reliable vaccine in case unpredictable circunstances lead to partial or total failure in vector control.

In addition to that, a vaccine against Chagas' disease would be pretty much welcome by such risk groups as blood bank users or scientific personnel working with *Trypanosoma cruzi*. Also the development of a vaccine against Chagas' disease would have a scientific merit of its own by furthering our knowledge of the disease and bringing about a subsidiary technology of unexpected applications.

If a vaccine is thus necessary, which should its characteristics be?

Brener & Camargo (1982) postulated that an ideal vaccine against Chagas' disease should have the following characteristics: *a)* it should not produce infection; *b)* it should confer total protection; *c)* it should not induce autoimmune agression; *d)* it should not induce immunosupression. The authors justified itens *a* and *b* by stating that since "a mild acute phase is not necessarily followed by a mild chronic disease" one could not be satisfied neither with a vaccine causing an infection by itself nor with a vaccine producing a mere attenuation of the acute phase. Requirements *c* and *d* derived from the admited properties of *T. cruzi* extracts and fractions of inducing autoimmunity and immunosupression.

Following these parameters, Brener & Camargo surveyed up to 1980 the Chagas' vaccine literature pointing out that most vaccination attempts failed to meet requirements *a* and *b* and largely overlooked requirements *c* and *d*.

Past failure is probably discouraging further attempts of vaccination with crude preparations of *T. cruzi*. In recent years not much has been added to this subject except that the search for purified antigens is beginning to substitute crude preparations as vaccinating agents. Even the few reports available on the utilization of killed or attenuated trypomastigotes in immunization protocols were done with the ultimate aim of identifying specific cellular antigens.

Authors are following different strategies in the search for antigens capable of inducing immunoprotection but are generally looking for surface antigens (Araujo & Remington, 1981; Araujo & Tighe, 1984; Nogueira, Unkelless & Cohn, 1982; Snary, 1980; Yoshida, 1983).

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As suggested by Brener & Camargo (1982), surface antigens would be good candidates for eleciting protective antibodies, which, on their turn, "may act either by favoring the immunologically-mediated destruction of the parasite or by preventing the parasite from entering the host's tissue cells".

The search of antigens and their corresponding antibodies, which may act either as inhibitors of the penetration or as mediators of parasite destruction, has been handicapped by the complexity of the tests used for the evaluation of their efficacy. However, recent developments seem to have simplified this task: Andrews & Colli (1982) have successfully standardized the conditions for evaluation of parasite penetration into cultured cells while others (see for review Krettli, Cançado & Brener, 1984, this Symposium), have definitely stablished that the complement-mediated immuno lysis (CML) is a reliable aid in the search for lytic anti-living trypomastigote antibodies and their corresponding antigens.

Colli and colaborators, which are following the "inhibition of penetration" strategy, initially reported that sera from Chagas' disease patients and from rabbits inocculated with trypomastigotes but not epimastigotes of *T. cruzi* were capable of considerably reducing the penetration of trypomastigotes into cultured cells (Zingales et al., 1982). They reasoned that trypomastigote antigens, differently from epimastigote ones, were inducers of antibodies capable of inhibiting cell penetration and proceeded to analyse surface proteins of trypomastigotes originated in cell cultures. They have found (Andrews, Katzin & Colli, 1984) that a few proteins are restricted to trypomastigotes and that, among those, some are removable by trypsin treatment which considerably reduces the flagellates' ability for cell penetration. Colli et al. (1983) have also shown that N-acetyl-glucosamine added to culture media inhibited to about 80% trypomastigotes' penetration, a fact also reported by Crane & Dvorak (1982). The authors correlated these findings with the observation that a protein, Tc85, occurring in trypomastigotes and removable by trypsin (Andrews, Katzin & Colli, 1984) was known to possess N-acetyl-glucosamine (Katzin & Colli, 1983). Therefore, protein Tc85 became a serious candidate for the antigenic role of eliciting antibodies inhibitors of cell penetration. This putative role seems to be reinforced by more recent experiments in which it has been shown that among several monoclonals directed against surface components of trypomastigotes only two were capable of inhibiting cell penetration and that, coincidently, these two monoclonals specifically recognized (by immunoprecipitation) the Tc85 protein (Alves, Abuin & Colli, personal communication). Unfortunatelly, these monoclonals have not been tested for passive immunization nor the Tc85 protein has been tested for active immunization (Colli, personal communication).

These results are very encouraging but there may still be some obstacles in the path of a vaccine inhibitory of cell penetration. Doyle et al. (1984) have reported that clones from the same human isolate may differ considerably as to their penetration capabilities and also as to their susceptibility to the inhibitory effect of N-acetyl-glucosamine on cell penetration. This indicates that different cell and trypomastigote receptors may be involved in the process. This may preclude the utilization of a single antigen as a universal vaccinating agent.

Besides the group of Colli and colaborators there are reports from other groups working on cell surface components/cell penetration (Villalta & Kierszenbaum, 1983; 1984), but most groups are actually devoted to finding antibodies capable of promoting the immuno-destruction of the parasite. Most of these groups use the CML-test to monitor the lytic activity of poli or monoclonal antibodies obtained in different experimental conditions. This test has already proved its reliability and furthering it Martins et al. (in Krettli, 1984, this Symposium) have recently disclosed that a 160 kDa surface protein of trypomastigotes is apparently the target for the lytic antibodies present in infected mice.

Segura et al. (1977) who had reported increased survival rates in mice vaccinated with a flagellar fraction of epimastigotes have recently used this fraction as an immunizing agent to produce monoclonal antibodies (Subias et al., 1984). Among the monoclonals obtained at least two were found to possess lytic activity against blood trypomastigotes. Coincidently, in passive immunization experiments, these two monoclonals afforded considerable protection against a trypomastigotes' lethal challenge (50-70% of survivals). The authors have not yet reported on the antigenic target of these monoclonals but are considering their utilization for antigen purification.

Andrews et al. (1985) have vaccinated mice with live tissue culture trypomastigotes rendered non-proliferative by treatment with 8-MPO (8-methyl-psoralen) a DNA cross-linking agent. All vaccinated (10) mice survived a lethal challenge and presented no parasitemia throughout the duration of the experiment. However, two mice yielded positive hemocultures before and after challenge whereas one mice was found positive only after the challenge. Therefore, this vaccination attempt also failed to meet requirements *a* and *b* of an ideal vaccine. These results do not differ qualitatively from previous ones using Actinomycin D or X-rays to abolish the proliferative capacity of the immunizing form (see Brener & Camargo, 1982). However, Andrews et al. advanced some new information by reporting that sera of vaccinated mice exhibited lytic activity in the CML-test and also that these sera were capable of inhibiting cell penetration in vitro. Moreover, the authors reported on the several surface proteins recognized by immune sera.

Yoshida et al. (1984), instead of using blood or tissue culture trypomastigotes preferred to use metacyclic trypomastigotes from axenic cultures as immunizing agents. Metacyclics killed either by heating at 50°C or by merthiolate were inocculated in mice at weekly intervals. After six weeks the animals received a non-lethal challenge of metacyclic trypomastigotes collected from the urine of infected triatomids. Contrary to controls, parasitemia in vaccinated mice was always negative although hemocultures were

positive. The absence of an acute phase in vaccinated mice was underlined by the presence of lytic antibodies in the sera of all of them. This vaccination protocol obviously does not meet requirement *b* of an ideal vaccine but has been used by Yoshida (1985) for a different purpose, namely to identify trypomastigote proteins recognizable by immune sera but not by normal sera or by sera from animals immunized with epimastigotes. Yoshida indeed found that three trypomastigote proteins are immunoprecipitated exclusively by lytic sera: 77 kDa, 82 kDa and 88 kDa proteins. Monoclonals directed to this surface proteins were also found to lysis metacyclic trypomastigotes (Yoshida, personal communication).

In addition to these studies on different models for the characterization of antigens of eventual protective value there have been some straightforward attempts of vaccination with purified antigens.

Scharfstein et al. (1983) isolated a highly purified surface 25 kDa glycoprotein present in extracts of all evolutive stages of different strains of *T. cruzi*. This protein was recognized by immunoprecipitation by 96% of the sera from patients of Chagas' disease. Due to the universal occurrence of antibodies against these antigens the authors decided to verify its protective value. Brener (personal communication) tested on rabbits the effect of various immunization schedules using the 25 kDa protein as antigen. The results, however, were discouraging. No protection has been achieved and although a certain delay was found in the onset of the parasitemia this has not prevented the full course of the chronic evolution.

Snary et al. (1981) and Snary (1983) have also reported on the vaccination of mice with purified antigens of *T. cruzi* following up the initial observations of Scott & Snary (1979) on the same subject.

Snary (1983) utilized two surface glycoproteins as antigens: a 90 kDa protein present in all evolutive stages of *T. cruzi* and a 72 kDa protein restricted to epimastigotes and metacyclic trypomastigotes.

Mice vaccinated with the 72 kDa antigen developed lower parasitemias than controls and survived to a lethal challenge with metacyclic but not blood trypomastigotes. Mice vaccinated with the 90 kDa glycoprotein survived to both challenges and always presented lower parasite blood counts than controls.

Krettli & Brener (1982) utilized Snary's 90 kDa preparation in a study on comparative immunoprotection reporting that vaccinated mice presented lower parasite blood levels but nevertheless always developed an acute phase and a death rate not different from controls.

Thus, vaccination attempts with the purified antigens available to date still do not afford sterile immunity but clearly open a new trend in vaccination studies in Chagas' disease.

If we compare the 70 years of literature on immunoprotection before 1980 with that of the last four years we immediatelly notice a strategy change. Presently, the search for specific and well defined antigens of eventual protective value has taken the place of the numerous and sometimes unwarranted attempts of vaccination with all sorts of preparations of *T. cruzi*.

The current trend may or may not lead to a vaccine but it is on the right path anyway.

However, our problems will not end simultaneously with the successful production of an experimental vaccine. Many problems have to be solved before considering its utilization in humans. A vaccine against Chagas' disease may require an epidemiological evaluation different from that of other infectious diseases. The absence of acute phase symptomatology in vaccinated individuals may not be a sufficient criterion for a Chagas' disease vaccine whilst chronic phase symptomatology may take decades to set in. Therefore, it is time to begin thinking about reliable criteria to evaluate the efficacy of a vaccine against Chagas' disease.

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