

THE 90kDa SURFACE ANTIGEN OF METACYCLIC FORMS OF
TRYPANOSOMA CRUZI

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Studies on the immunity induced by metacyclic trypomastigote stages of Trypanosoma cruzi carried out in our laboratory have shown that: a) mice immunized with metacyclics (G strain) killed by heating or merthiolate treatment resist acute infection upon challenge with trypomastigotes derived from the insect vector or axenic cultures, b) antibodies present in the serum of immunized mice are capable of lysing metacyclics in a complement-dependent fashion, c) surface antigens specific of infective metacyclic forms appear to be involved in the protective immunity.

In order to identify and characterize the parasite antigens that may be contributing to the immune response against metacyclics we raised monoclonal antibodies directed to the surface components of these developmental stages. In a series of experiments using G strain, one of the monoclonals (1G7) was found to be stage-specific and to have complement-mediated trypanolytic activity. This monoclonal antibody reacted with a metacyclic surface polypeptide with an apparent molecular mass of 90kDa (pI4.6). This protein, which is also one of the major antigens precipitated by the serum of mice immunized with killed metacyclics and protected against acute T. cruzi infection, appears to be one of the main products of protein synthesis by metacyclic trypomastigotes, as suggested by metabolic labeling studies.

To test the functional activity of 1G7 we performed "in vitro" parasite neutralization assays and also "in vivo" experiments of passive transfer of antibody. For these studies we used metacyclics of Tulahuen strain, which are antigenically very similar to the G strain but unlike this latter produce high parasitemias in mice. Mice inoculated intravenously with Tulahuen metacyclics previously treated with 1G7 developed parasitemia levels which were much lower than those of control animals that received the parasites preincubated with normal mouse serum. Fab fragments of 1G7 were as effective as the intact immunoglobulin. The metacyclic neutralizing effect of the monoclonal antibody 1G7 was confirmed by passive transfer experiment. When mice were injected with 0.5 mg of 1G7 30 minutes before challenge with metacyclic trypomastigotes, a high percentage of them (80%) showed markedly reduced levels of parasitemia.

The mechanism of this antibody-mediated reduction of parasitemia levels is not known. Although the metacyclics are lysed "in vitro" in the presence of antibody and complement, it remains to be determined to what extent, if any, this lytic effect contributes for the parasite destruction "in vivo". Another possibility could be a blocking effect of 1G7 on the parasite entry into the host cells. We have observed that the treatment of metacyclics of G or Tulahuen strain with 1G7 (250 ug/ml) before incubation with cultured Vero cells results in 40-45% inhibition of parasite invasion. The unrelated monoclonal antibody 3D11 reacting with a sporozoite surface antigen of Plasmodium berghei has no such effect.

How prevalent is 1G7-reactive antigen among T. cruzi strains? To answer that question we examined the reactivity of the monoclonal antibody 1G7 with metacyclics of 11 isolates of different sources from distinct geographical regions. By Western blot technique the 90kDa antigen was detected in all strains except Y and CL. However, both Y and CL metacyclics do have a 90kDa antigen immunologically related to the 90kDa polypeptide present in other strains. This antigen, though lacking the 1G7-specific epitope, is recognized by another monoclonal antibody (5E7) which is also metacyclic-specific. Furthermore, a weak band of 90kDa was revealed in Y and CL strains by precipitating the extracts of surface iodinated metacyclics with mouse polyclonal monospecific antiserum to the 90kDa polypeptide. We believe that the variant 90kDa protein of Y and CL strains is present on the parasite cell surface in small amounts. When the surface iodinated proteins of metacyclics of different T. cruzi strains were analysed by SDS-PAGE, the 90kDa band could be seen in all strains as one of the major iodinated components, except for Y and CL strains.

The 90kDa metacyclic antigen which is ubiquitous among different T. cruzi isolates appear to be absent in epimastigotes, tissue culture-derived trypomastigotes and amastigotes since the antiserum to the 90kDa polypeptide failed to recognize any of these developmental stages.

The 90kDa antigen is of interest from the point of view of immunoprophylaxis. Immunization of mice with purified 90kDa polypeptide induced the production of antibodies that recognized the native meta-

cyclic surface antigen. In addition, the purified antigen was found to stimulate the "in vitro" proliferation of 90kDa protein-primed mouse lymph node cells (L3T4+ helper T cells) in a dose-dependent manner while it had no effect on non-primed T cells. On the other hand, these 90kDa antigen-primed cells failed to proliferate in the presence of the unrelated Leishmania antigen GP10/20.

Preliminary studies have shown that the 90kDa antigen is also recognized by sera of Chagasic patients while no reaction is observed with normal human sera.

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