

CH-87-5 CHEMICAL CHARACTERIZATION OF EPITOPES COMMON TO TRYPANOSOMA CRUZI
AND MAMMALIAN NERVOUS CELLS

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Abstract

Infection with T. cruzi results in chronic lesions of many mammalian tissues, including muscle and nerve. These lesions are thought to be of autoimmune origin. Although there is general agreement about the autoimmune nature of the lesions, their origin is not understood. It is possible that there is immune sensitization to self-antigens in the infected host, either due to their presentation in an unusual context, or because of dysfunction of the immune system in the infected animal. On the other hand, common epitopes on the parasite and host tissues could be responsible.

In an effort to understand these phenomena, we have isolated monoclonal antibodies (MABs) that recognize determinants both on T. cruzi and host brain. To do this, mice were injected with either T. vespertilionis or T. dionisii (subgenus Schizotrypanum, isolated in Great Britain) neither of which are natural murine parasites. The rationale in this approach was that natural infection with T. cruzi results in such severe immunodepression that relevant hybridomas are difficult to isolate. It was reasoned that the xenoparasites might set up a mild self-curing infection with less immunodepression. Using this approach, ten hybridomas secreting cross-reactive MABs were isolated.

Five of these MABs also cross-react with immunocytochemically identified cells of the mammalian nervous system in mouse cerebellar cell cultures and thin sections. All the MABs are of the IgG isotype. Surprisingly, all five of the cross-reactive MABs recognize complex lipid or glycolipid antigens, or antigens which are associated with lipids. We have begun preliminary studies to characterize antigens recognized by two of the MABs, VESP 6.2 and VESP 8.2. A very small population of living neurons is recognized by VESP 6.2, while VESP 8.2 reacts with glial cells (astrocytes). Both antibodies cross-react with different lipid fractions isolated from human, bovine and mouse brain and isolated from Trypanosoma cruzi.

VESP 6.2: Chemical reactions indicated that the sulfate group of the lipids is an important part of the epitope recognized by the monoclonal antibody. Lipid extracts of mouse brain contained all the antigenic species present in the parasite. One of the antigens isolated from brain extracts was identified as sulfo-galactosyl-ceramides (Sulf-Gal-Cer). The specificity of VESP 6.2 for these isolated lipid antigens was demonstrated by three different methods: i) HPTLC immunostaining, ii) solid phase radioimmunoassay, iii) lysis of artificial liposomes.

The T. cruzi sulfated lipid antigens were shown to be of parasite origin rather than scavenged from the culture medium. They could be radiolabelled with ³⁵S sulfate. Furthermore, lipid extracts from two T. cruzi strains (C1 and Brazil D1) grown in different media contained the same antigens while the media contained either no antigens or different species.

VESP 8.2: Galactosyl-ceramides (Gal-Cer) isolated from both T. cruzi and different mammalian nervous tissue, and Sulf-Gal-Cer isolated from the brain were identified as antigens. The analysis of fatty acids of the antigenic glycolipids indicated that 2-hydroxy fatty acids are an important part of the epitope recognized by the cross-reacting antibody. The specificity of VESP 8.2 for these isolated glycolipids was demonstrated by HPTLC immunostaining and solid phase radioimmunoassay.

The Gal-Cer isolated from T. cruzi were not found in the media components used for the parasite culture indicating that the antigens were of parasite origin rather than scavenged from the culture medium

We do not know what, if any role the cross-reactive antigens described here might play in the pathology of Chagas' disease. Tests performed with sera from chronically infected mice were positive with the lipid antigens isolated from both T. cruzi and mammalian brain. Controls with normal mouse sera showed no reaction.

Since most of the sulfated antigens common to the parasite and the nervous tissue have not yet been defined chemically we do not yet know their distribution. However the Sulf-Gal-Cer and Gal-Cer antigens have a broad distribution and could be target antigens in the generation of the pathology. Sulf-Gal-Cer are particularly present in myelin and in the mucosa of the digestive tract, known targets in the chronic disease.

Although Gal-Cer and Sulf-Gal-Cer are small molecules, they are strongly antigenic and could be important target antigens in demyelinating diseases of mammals. Strong antigenic stimulation, such as chronic infection with T. cruzi acting as a "carrier" for hapten glycolipid epitopes, may lead to immune responses against these autologous antigens. Thus, some pathology of Chagas' disease may be due to autoimmunity of the infected mammalian host against glycolipid antigens.

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