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Complex lipids as common antigens to *Trypanosoma cruzi*, *T. dionisii*, *T. vespertilionis* and nervous tissue (astrocytes, neurons)

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Summary

In a dot immunobinding test glycolipid and phospholipid antigens were recognized by anti-*T. dionisii* (DION) and anti-*T. vespertilionis* (VESP) monoclonal antibodies (mabs). Five of them crossreact with *T. cruzi* and cells of the central nervous system (CNS) (astrocytes, neurons). Indirect immunofluorescence (IIF) of neuraminidase treated *T. cruzi* cells indicated that two antigens were organized in a cryptic form.

Key words: *Trypanosoma cruzi;* glycolipids; astrocytes; neurons; monoclonal antibodies.

Introduction

Twenty million people in Latin-America are serologically positive for *T. cruzi,* the causative agent of Chagas' disease (WHO report, 1983). The disease is characterized by degeneration of cardiac muscle and of neurons.

Autoantibodies reacting with antigens common to parasite and host cells have been demonstrated in sera of chronically infected patients (reviewed by Hudson and Hindmarsh, 1985). The presence of crossreactive glycoprotein determinants on *T. cruzi,* neurons and glia (Wood et al., 1982; Snary et al., 1983) has been confirmed using mabs. Five different mabs reactive with *T. cruzi, T. dionisii,* and *T. vespertilionis* also crossreacted with astrocytes, neurons and a further population of cells from mouse cerebellum (Petry et al., in press).

Mabs might detect both glycoproteins and glycolipids as these often contain similar terminal carbohydrate sequences. Previous workers have charac-

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terized glycoconjugates in *T. cruzi* by using lectins (Schottelius and Uhlenbruck, 1983; Pereira et al., 1980). The high content of lipids (34%) and carbohydrates (16%) in the cell membrane (Da Silveira and Colli, 1981), and the presence of sialoglycolipids (Confalonieri et al., 1983) suggested that glycolipids might constitute an important class of parasite antigens. We found the expression of common epitopes in mouse cerebellar cells and *T. cruzi* by using anti-trypanosome mabs (Petry et al., in press). Based on these observations, we decided to examine standard glycolipids and phospholipids as antigens for the anti-trypanosome mabs.

Materials and Methods

Monoclonal antibodies

Mabs raised against *T. dionisii* and *T. vespertilionis*, and crossreacting with *T. cruzi* (Petry et al., 1986) and nervous cells (astrocytes, neurons) (Petry et al., in press) were used to characterize glycolipid or phospholipid antigens.

Dot immunobinding test

On the basis of the results from Da Silveira and Colli (1981) and from Confalonieri et al. (1983) we tested standard lipids such as sphingomyelin (Sm), phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), sulfatides (Sulf), cerebrosides (Cerebros) and a total ganglioside extract (Ganglios). All products were purchased from the Sigma Chemical Co. They were diluted in methylacetate-n-propanol-chloroform-methanol-0.25% aqueous potassium chloride (25:25:28:10:7; by vol.).

Dot immunobinding tests of standard lipids were performed as described by Hawkes et al. (1982). 10 μl samples of lipid standards at a concentration of 1 mg/ml were applied to acetate cellulose strips (Gelman Sciences, Michigan) and allowed to dry.

Neuraminidase treatment

Epimastigotes of *T. cruzi* strain Cl were treated with *Clostridium perfringens* neuraminidase (0.1 unit/ml; Sigma type VI) in 50 mM sodium acetate (pH = 6.0), 0.15 M NaCl, 9 mM CaCl, for 45 min at 37° C. After neuraminidase treatment the cells were washed with PBS and tested by IIF with the five mabs previously shown to be crossreactive with nervous cells. The IIF procedure for the characterization of mouse cerebellar cells is described elsewhere (Petry et al., in press).

Results

Out of 23 anti-trypanosome mabs tested against glycolipids and phospholipids, four mabs showed specific reactivity. DION 6.3 reacted weakly with cerebrosides, DION 10.1b with gangliosides, VESP 11.4 with sulfatides and VESP 14.2 with PC. VESP 5.6 and VESP 6.2 showed a strong preference for sulfatides as antigen, but they also reacted with other glycolipids. VESP 8.2 reacted with cerebrosides and weakly with PC. Two mabs, VESP 9.3 and DION 12.7, recognized the same lipid antigens: Sm, sulfatides and gangliosides. The reactions of the antitrypanosome mabs with standard lipids in the dot immunobinding test are shown in Fig. 1 and summarized in Table 1.

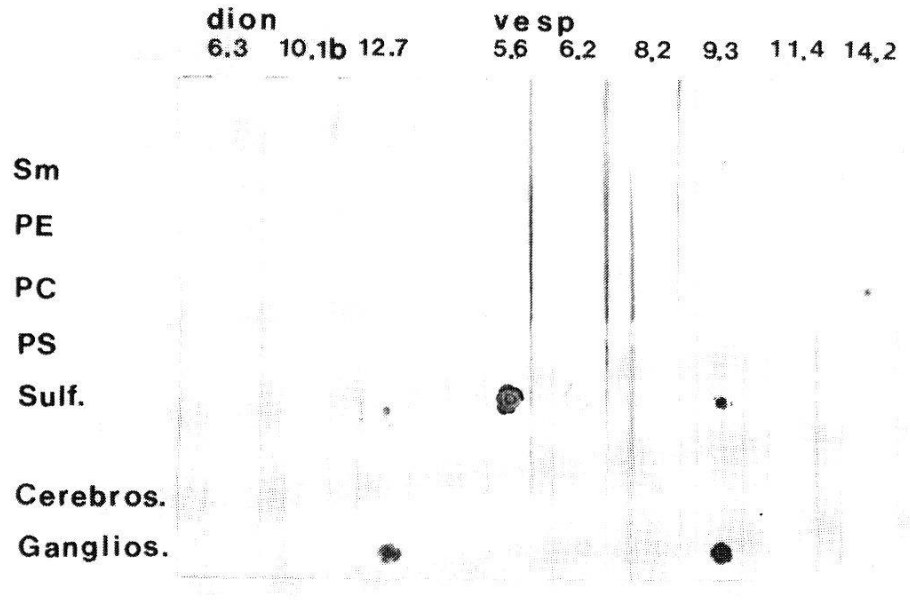

Fig. 1. Photograph of the dot immunobinding test with standard glycolipids and phospholipids illustrating specificities of monoclonal antibodies raised against *T. dionisii* and *T. vespertilionis*.

Table 1. Glycolipid and phospholipid antigen characterization of anti-trypanosome mabs crossreactive with trypanosomes and mouse cerebellar cells

	Monoclonal antibodies				
	DION		VESP		
	10.1b	12.7	6.2	8.2	9.3
Trypanosomes:					
T. cruzi	+	+	–	–	+
T. dionisii	+	+	–	–	+
T. vespertilionis	+	+	+	+	+
T. cruzi Cl[a]	+	+	+	+	+
Mouse cerebellar cells:					
astrocytes	–	+	–	+	+
neurons	+	–	*	–	–
Lipid standards:					
Sm	–	+	–	–	+
PE	–	–	–	–	–
PC	–	–	(+)	(+)	–
PS	–	–	–	–	–
sulfatides	–	+	+	–	+
cerebrosides	–	–	(+)	+	–
gangliosides	+	+	+	–	+

\+ positive reaction
– negative reaction
(+) weak reaction
* no clear assignment of cell type
[a] after neuraminidase treatment

The effect of neuraminidase treatment on the binding of mabs to *T. cruzi* Cl epimastigotes was measured by IIF. As a control non-neuraminidase treated parasites were also examined. Weak or no difference in IIF was observed in the reaction of mabs VESP 9.3, DION 10.1b and DION 12.7. Mabs VESP 6.2 and VESP 8.2 which did not react with *T. cruzi* Cl, however, did recognize an antigen on the parasite after neuraminidase treatment.

Discussion

Investigation of glycoconjugates on the surface of *T. cruzi* using lectins has suggested that they play an important role in both the cell cycle and the interaction of parasite with macrophages and other host cells (reviewed by Zingales and Colli, 1985).

A detailed analysis of *T. cruzi* lipids from whole cells (Oliveira et al., 1977) and from plasma membranes (Da Silveira and Colli, 1981) showed a high content of phospholipids. These phospholipids consisted of PE, PC, PI, and Sm. As shown in the present experiments, two of these phospholipids reacted as antigens by the dot immunobinding test with mabs. Mabs which reacted with two or more standard lipids always showed preference for one of these antigens. In most cases where crossreactivity was observed, sulfatides were recognized as one of the lipid antigens.

Although soybean or wheatgerm agglutinin (WGA) did not bind to surface glycoproteins extracted from epimastigote forms of *T. cruzi* Y strain (Katzin and Colli, 1983), receptors for such lectins were detected on the parasites in agglutination experiments (Schottelius and Uhlenbruck, 1983). This suggested that the lectin receptors might belong to a class of molecules other than glycoproteins, such as gangliosides (Katzin and Colli, 1983). The sensitivity of the epimastigote WGA receptor to neuraminidase treatment (Pereira et al., 1980) indicated indeed the presence of sialic acid on the surface membrane. Sialic acid (Schauer et al., 1983) bound to sialoglycolipids (Confalonieri et al., 1983) has been demonstrated in *T. cruzi* epimastigotes. These previous observations suggested, therefore, that gangliosides might be detected with anti-trypanosome mabs, and our results indicate that five out of nine mabs do react with total ganglioside extracts.

One mab (DION 10.1b) reacted specifically, but very weakly, with the total ganglioside extract. This mab crossreacted with *T. cruzi* and neurons from mouse cerebellum (Petry et al., in press). Mabs VESP 9.3 and DION 12.7 reacted with gangliosides, Sm and sulfatides. Antigens revealed by these mabs were found on *T. cruzi, T. dionisii, T. vespertilionis* and on astrocytes. A third mab (VESP 8.2) also positive for astrocytes (Petry et al., in press) recognized cerebrosides and PC. These observations may be correlated with the results obtained using mabs of the 0 series developed by Schachner and collaborators (Schachner, 1982) which are directed against antigens of the oligodendrocyte

cell surface. The 01 and 02 antibodies were shown to react with glycolipids comigrating with galactocerebrosides, while 03 and 04 react with glycolipids comigrating with sulfatides. Mab VESP 6.2 was specific for a cell type (which is not well assigned) of the mouse cerebellum, and showed a strong preference for sulfatides as antigens.

Mabs VESP 6.2 and VESP 8.2 do not react with *T. cruzi* epimastigotes (Petry et al., 1986). The antigens recognized by both mabs were exposed, however, when parasites were treated with neuraminidase, indicating that these antigens are organized in a cryptic form. We do not know if the crypticity of the antigens recognized on *T. cruzi* by VESP 6.2 and VESP 8.2 is effected by a second membrane component.

The mabs crossreacting with *T. cruzi* and with cells of the mouse CNS in IIF recognize gangliosides and other glycolipids as antigens. These mabs were raised against epimastigotes (DION 10.1b, VESP 6.2., VESP 8.2 and VESP 9.3) or metacyclic trypomastigotes (DION 12.7) of *T. dionisii* or *T. vespertilionis.* The immunopathogenesis of Chagas' disease, however, is induced by intracellular amastigotes and/or blood form trypomastigotes of *T. cruzi.* Antigen characterization of these developmental stages is currently underway.

Our preliminary observations about the antigenicity of glycolipids strongly suggest a role for the detected antigens in the immunopathology of Chagas' disease. A knowledge of the exact structure of crossreacting antigens will help us to understand the potential role of these molecules in the origin of *T. cruzi* associated autoimmunity.

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