A Review

α - Galactosyl Epitope on *Trypanosoma Cruzi, Leishmania Mexicana* and *L. Braziliensis*. Ultrastructural Localization and Possible role of Antibodies Against this Epitope

José Luis Avila and Antonio Bretaña
Instituto de Biomedicina. Apartado 4043. Caracas 1010A. Venezuela

ABSTRACT

Using colloidal gold immunocytochemistry - monoclonal antibody gal-13 (specific for lipid-linked galactosyl(α1-3)galactose residues, anti-laminin and anti-nidogen antibodies - and lectin cytochemistry (***Bandeiraea simplicifolia*** IB_4), both techniques specific for demonstrating galactosyl(α1-3)galactose residues we have found terminal disaccharide residues on the ***Tripanosoma cruzi*** external surface of mouse blood - and Vero cell-derived trypomastigotes. Although intact epimastigotes moderately stained only with human antibodies, disrupted epimastigotes strongly immunoreacted with human as well as with rabbit antibodies. Staining was also detected in the lips of the flagellar pocket, and on the parasitic side exactly opposite to the flagellar pocket in amastigote and promastigote forms of American *Leishmania*. The presence of abundant galactosyl(α1-3) galactose residues on Trypanosomatid family members suggest a specific unknown role in parasite physiology for this terminal disaccharide.

KEYWORDS
α-galactosyl, ultrastructure, *T. cruzi*, *Leishmania mexicana*, *Leishmania braziliensis*, antibodies.

Biochemical demonstration of α-galactosyl epitopes.

The presence of Gal(α1-3)Gal residues in *T. cruzi* trypomastigotes have been clearly demonstrated, using biochemical methods, linked to glycolipids by Avila *et al* (1) and linked to glycoproteins by Travassos and Milani (2) and by Couto *et al* (3).

As regard the presence of α-galactosyl epitope on lipids, recent work has demonstrated the existence of glycoinositolphospholipids (GIPL) in *T. cruzi* epimastigotes and trypomastigotes as well as in *Leishmania mexicana* and *Leishmania braziliensis* promastigotes.

The GIPL of eukaryotic cells are functionally important components in the outer leaflet of the plasma membrane. These glycolipids have the general structure, glycan-glucosamine-phosphatidylinositol, and appear to act predominantly as membrane anchors for a diverse family of cell surface proteins (4). McConville *et al* (5) described the major glycolipids of *Leishmania major*. These glycolipids belong to a family of GIPLs, which contain 4-6 saccharide residues linked to alkylacylphosphatidylinositol or lysoalkylphosphatidylinositol. The general structure of the GIPL is: $R\text{-}3Gal_f(\beta 1\text{-}3)Man_p(\alpha 1\text{-}3)Man_p(\alpha 1\text{-}4)GlcN_p(\alpha 1\text{-}6)$ alkylacyl-PI or lysoalkyl-PI, where R= OH for GIPL-1; R= Gal(α1 - for GIPL-2; R= $Gal_p(\alpha 1\text{-}6)$ Gal $_p(\alpha 1\text{-})$ for GIPL-3 and R= $Gal_p(\alpha 1\text{-}3)Gal_f$ (1 - for GIPL-A. Using *L. major* GIPL as standards, Avila *et al* (6) have partially identified *T. cruzi*, *L. mexicana* and *L. braziliensis* GIPLs. These molecules were characterized by their comigration by high-performance thin-layer chromatography with purified *L. major* GIPLs, gas-liquid chromatography of the monosaccharides released after aqueous HF treatment, N-acetylation and methanolysis, sensitivity to exoglycosidases and antibody absorption on several specific natural haptens. The major GIPLs of these trypanosomatids include tetraglycosyl-, pentaglycosyl - and hexaglycosyl-phosphatidylinositol. Singh *et al* (7) have also demonstrated the presence of three different glycosyl-phosphatidylinositols in *T. cruzi* epimastigotes, with chemical structures close to that described

in our experiments, main component been inositol phosphate ceramide (TCP-2). The major long chain base appeared to be C_{18} sphinganine having a C16:0 fatty acid linked to the long chain base via amide linkage.

The presence in *T. cruzi* of protein-linked Gal (α1-3) Gal epitope have been demonstrated on at least two parasitic proteins, namely Gp-25 (2) and Gp-85 (3).

Morphological demonstration of α-galactosyl epitopes.

Two different approaches have been used for the demonstration of α-galactosyl epitopes on *Trypanosoma cruzi* and *L. mexicana* and *L. braziliensis*: a) Colloidal gold immunocytochemistry studies using: 1) Purified human anti-Gal (α1-3) Gal antibodies, 2) Rabbit anti-laminin antibodies, 3) Rabbit anti-nidogen antibodies, 4) mouse monoclonal antibody Gal-13, specific for terminal lipid-linked α-galactosyl residues and b) Lectin binding studies using biotinylated *Bandeiraea simplicifolia* isolectin B_4, all these macromolecules reacting specifically with Gal(α 1-3)Gal epitopes as previously demonstrated (8, 9).

Figure 1 shows the immunocytochemical localization of anti-laminin antibodies on specific sites, located close to and on the flagellar veil, in circulating *T. cruzi* trypomastigotes obtained from infected mouse. A totally different results are obtained when Vero-cell derived trypomastigotes are submitted to same treatment. In this latter case, anti-laminin immunoreactivity is present in a diffuse form all over parasitic surface (Fig. 2). A similar immunocytochemical localization is obtained when human anti-Gal(α1-3)Gal antibodies are used on Vero cell-derived trypomastigotes (Fig. 3), suggesting some identity between epitopes being recognized by both antibodies. Note that plasma membrane immunoreactivity is localized on an amorphous material close to the trypomastigote cell membrane (Fig. 4a), some immunoreactivity being detected on detached filopodia (Fig. 4b). To check the specificity for α-galactosyl epitopes we used the isolectin *B. simplicifolia* B_4. Fig. 5 demonstrates intense binding on several intra-cellular structures (outer surface of plasma membrane, lysosomes). As regard *T. cruzi* epimastigotes, Fig. 6 shows that human anti-Gal(α1-3)Gal immunoreactivity, although moderate, is located in a disperse form on parasitic plasma membrane. Figure 7 shows rabbit anti-nidogen antibody immunoreactivity of *T. cruzi* epimastigotes. As reported for human anti-Gal(α1-3)Gal antibodies, staining is sparse and distributed all along the parasitic surface. This low immunoreactivity of anti-nidogen antibodies contrast with that obtained for American *Leishmania* amastigotes, which have diffuse staining on parasitic plasma membrane (Fig. 8). Interestingly, staining for Gal(α1-3)Gal in American *Leishmania* seems to be tightly attached to the external surface of the plasma membrane, a fact that contrasts with that obtained for *T. cruzi*. Anti-nidogen pattern on amastigotes also contrast with that obtained when *Leishmania* promastigotes and amastigotes reacts with human anti-Gal(α1-3)Gal, rabbit anti-laminin antibodies or *B. simplicifolia* B_4, immunoreactivity being localized on the external lips or close to the to the flagellar pocket (Fig. 9 and 10) or on the side opposite to the flagellum (Fig. 11). Our results, also presented elsewhere (10, 11), ultracytochemically confirm the presence of α-galactosyl residues in specific sites of *T. cruzi* trypomastigote and epimastigote forms and in *Leishmania* promastigote and amastigote forms.

Similar results, using fluorescein-labelled lectins have been recently obtained by Gazzinelli *et al* (12), who additionally showed that Gal(α1-3-)Gal residues are present in all monoxenic and heteroxenic trypanosomatids studied, and in fact gal(α1-3)gal antibody levels are also elevated in *T. rangeli* - infected patients (13).

Possible role of galactosyl(α1-3)galactose antibodies.

Galactosyl (α1-3) galactose (antiGal) antibodies are similar to EVI antibodies.

AntiGal is a natural antibody which constitutes as much as 1% of circulating IgG in humans and displays a distinct specificity for the structure Gal(α1-3)Gal (14). This glycosyl structure has been found on various tissues of many non-primate as well as New World primate mammals. in contrast, the antiGal antibodies are present in discrete titres in sera from Old World monkeys, apes and humans but not in sera from non-primate or New World primate mammals. Such finding suggests that the mechanism which controls antibody self reactivity may control expression of natural antiGal antibodies in different mammals species (15). In sera from chagasic patients, the antiGal

antibody titres are approximately 4- to 5-fold higher than in sera from healthy or bacteria-infected individuals (16-18).

The natural antiGal antibodies were first identified in sera of chagasic patients by Muniz and Santos (19). These authors showed that antibodies with *T. cruzi* agglutinating activity bind to a polysaccharide fraction present on the surface of this hemoflagellate. Because such antibodies were absorbed from chagasic sera with sheep red blood cells, they were called heterophile antibodies.

Cossio *et al* (20) described the ocurrence of antibodies in sera from chagasic patients which react with determinants present on mouse heart endothelium cells, vascular structures and insterstitium (EVI antibodies) as well as reacting with *T cruzi* antigens.

It was latter shown by Szarfman *et al* (16) that laminin, was the major binding site for EVI antibodies on mouse tissue. Further work by Avila *et al* (21) reported elevated titres of anti-nidogen antibodies in human chagasic patients. As the majority of antibodies which bind to nidogen also cross-react with laminin (22), we suggested that the epitopes shared by nidogen and laminin are not genetically determined protein structures rather than post-translational modifications common to both proteins.

Latter on, Towbin *et al* (23) demonstrated that binding of chagasic antibodies to mouse purified laminin is specifically blocked by Gal(α1-3)Gal residues or adsorption with rabbit and sheep erythrocytes, which are rich in Gal(α1-3)Gal residues as well as by treating laminin with α-galactosidase and periodate but not by alkali, pronase, or the reduction of disulfide bonds.

The heterophile nature of EVI antibodies or anti-galactosyl(α1-3)galactose antibodies present in chagasic sera was demonstrated not only by their affinity to rabbit and sheep red blood cells but also because they fail to bind to the respective human structures (EVI) and molecules (laminin) (16, 23).

Noteworthy, when anti-gal(α1-3)gal antibody titers are compared between patients having chagasic and dilated cardiomyopathies none difference in antibody values or percentage of anti-gal positive patients is found, although they are significantly elevated compared with control subjects, suggesting the existence in tropical areas of another anti-gal antibody elevated cardiomyopathy.

Terminal α-galactose groups, and possibly Gal(α1-3)Gal epitopes, may exist in some normal or pathological human tissues. This was demonstrated by lectin binding to some extracellular structures of peripheral nerves (24), which is of particular interest in view of mAb reactions with neurones and *T. cruzi* (25) and ocurrence of nerve injury as late complication of Chagas' disease (26).

The possibility also exist that infected cells (macrophages, neurons, muscle cells) due to the intracellular residence of parasites, may change their surface structure by inserting parasite-derived epitopes or changing host components by new posttranslational modifications, and in fact we have demonstrated the presence of α-galactosyl residues on the surface of *T. cruzi*-infected cardiac cells (27) as well as intense *T. cruzi* invasion of cultured human neuroblastoma cells and further expresion of anti-α-galactosyl epitope on plasma membrane of infected cells (Avila *et al*, unpublished results).

The autoimmunity observed in Chagas disease may therefore not be to cross-reacting structures (28, 29) present per se, but to a modified structure arising in the infected individuals, this would be an example of acquired molecular mimicry.

Antibodies directed at parasite antigens are known to induce the lysis of cells infected with *T. cruzi* by the action of antibody and complement and/or antibody-dependent cellular cytotoxicity (30). Then, α-galactosyl antibodies could induce cellular damage or lysis by interacting with naturally or adquired α-galactosy epitope-bearing tissues. This possibility of course would increase in acute or chronic chagasic patients where elevated serum α-galactosyl antibody levels are present (18). Using *L. major* GIPL as standards, Avila *et al* (6) have demonstrated that elevated levels of antibodies against terminal disaccharide structure exist in chronic chagasic patients, Gal(α1-3)Gal being the more immunoreactive disaccharide residue. Furthermore, using immunochemical techniques, they have characterized these antibodies and demonstrated that they are reacting with Gal (β1-3)Gal and Gal(α1-6)Gal respectively for those *T. cruzi*

GIPLs migrating together with *L. major* GIPL-1, GIPL-2 and GIPL-3 respectively. As discussed before for Gal(α1-3)Gal antibododies, anti-Galf(β1-3)Man and -Gal(α1-6)Gal antibodies through cross-reactions may interact with exposed terminal α-galactosyl residues existing on normal or *T. cruzi*-infected cells.

Galactosyl(α1-2)galactose antibodies in chagasic patients.

Since Gal(α1-2)Gal residues have been recently shown to be a structural unit of a phosphatidylinositol anchor for inserting variable surface glycoproteins of *T. brucei* (31) and certain proteins of *T. cruzi* (32) into their cell surface, it was hypothesized that specific Gal(α1-2)Gal antibodies may have arisen in *T. cruzi*-infected human subjects from previously sensitized B lymphocyte clones, which could be stimulated to produce specific antibodies by the presence of terminal Gal(α1-2)Gal residues in infecting parasites, and indeed we have reported significantly elevated Gal(α1-2)Gal antibody levels in 66% of chronic chagasic cardiomyopathy patients and that other acute or chronic Kinetoplastidae infections such as *T. rangeli*-infection, localized (LCL), mucocutaneous (MCL), diffuse cutaneous (DCL) or visceral leishmaniasis did not induce significantly elevated Gal(α1-2)Gal antibody levels (33). To investigate whether results in chronic Chagas' disease patients were associated with degree of myocardial damage, patients were subdivided into several groups (34). Among chagasic groups there was no significant difference in anti-Gal(α1-2)Gal antibody levels or in percentage of patients having elevated antibody levels, although Gal(α1-2)Gal antibody levels were significantly higher when any of the chagasic groups were compared with healthy human subjects or with humans having other dilated cardiomyopathies.

Due to the close structural relationship existing between terminal Gal(α1-3)Gal, Gal(α1-2)Gal and Gal(α1-3)Man oligosaccharide residues the question is: are increased human chagasic antibodies totally specific for each these chemical structures or are they cross-reactive?

Analysis of results of our laboratory (17, 18, 21-23, 33-35) suggests that in human healthy individuals, as well as in *T. cruzi*-infected subjects, there are at least three types of terminal α-galactosyl-reactive antibodies (Table 1). One is detected by ELISA when rabbit neutral glycosphingolipids-enriched for ceramide pentasaccharide (18), murine laminin (16, 17) or murine nidogen (21) are used as antigen. These antibodies are mainly IgG and highly specific for the disaccharide Gal(α1-3)Gal epitope as they are almost totally absorbed by rat, rabbit and guinea pig RBC as well as for murine laminin or nidogen, all these structures being rich in exposed Gal(α1-3)Gal-bearing residues (22). The second antibodies are detected using an American *Leishmania* glycoinositolphospholipid preparation bearing terminal Gal(α1-3)Man or Gal(β1-3)Man disaccharide as antigen (35). These antibodies are mainly IgM, and also bound to Gal(α1-3)Gal-linked synthetic antigens, but did not to the same residues present in rabbit, rat and guinea-pig RBC or in murine laminin or nidogen, their immunoreactivity being equally blocked by low concentrations of Gal(α1-3)Man, Gal(β1-3)Man. They are strongly elevated in 89% and 84% of DCL and LCL patients (35). The third type is Gal(α1-2)Gal antibodies, whose immunoreactivity is not blocked by > 200 mM Gal(α1-3)Man or Gal(β1-4)Man and are elevated only in 38% and 28% of DCL and LCL patients (33). The fact that they are unabsorbed neither by rabbit, rat or guinea-pig RBC or murine laminin and nidogen or by pronase-treated human O RBC nor their binding affected by methyl-α-galactopyranoside, melibiose and stachyose, adds further evidence for fact that these latter antibodies are indeed different from Gal(α1-3)Gal antibodies. Interestingly, Gal(α1-2)Gal antibodies strongly absorb on an Man(α1-2)Man-affinity column suggesting the possibility that so-called anti-Gal(α1-2)Gal antibodies are strictly anti-Man(α1-2)Man antibodies, and in fact this structure has been described very recently in *T. cruzi* trypomastigotes 1G7-antigen (36). Given the polyclonal nature of anti-Gal(α1-3)Gal antibodies (22), our results suggest that among three different antibodies we have demonstrated at elevated levels in chronic chagasic cardiomyopathy sera, a considerable proportion of antibody clones might have a different affinity for the protein-(2, 3) or lipid-linked (1) α-galactosyl epitopes present on *T. cruzi* membrane. Furthermore, individual differences in the overall affinity of the α-galactosyl epitopes on *T. cruzi* may determine the capacity of this family of antibodies to prevent infection and destroy the invading parasite. Supporting this view, Almeida *et al* (37) have recently demonstrated binding of anti-Gal(α1-3)Gal to *T. cruzi* and the complement-mediated lysis of trypomastigotes by this specific antibody and

that chagasic anti-Gal(α1-3)Gal had a lower affinity for Gal(α1-6)Gluc bound to agarose than for Gal(α1-3)Gal(β1-4)GlcNac, and was several times more effective in immunolysis of trypomastigotes than corresponding anti-Gal(α1-3)Gal from normal human serum. This demonstrates a considerable diversity in the recognition of Gal(α1-3)Gal epitopes by natural antibodies.

Possible role of α-galactosyl antibodies in host defense mechanisms.

Gazzinelli *et al* (38) have recently proposed that anti-Gal(α1-3)Gal antibodies are responsible in acute Chagas disease patients for the direct lysis of *T. cruzi* blood forms independent of either the classic or alternative complement pathways.

Additionally, the binding of the anti-Gal(α1-3)Gal antibodies to trypomastigotes may reflect a general process facilitating the sequestration of parasites by the reticuloendothelial phagocytic cells, and in fact participation of antibodies in the resistance against *T. cruzi* has been demonstrated at the chronic phase of Chagas disease by protection after passive transfer of immune serum (39) or immunoglobulin fractions (40).

Due to cross-reactions betwen Gal(α1-2)Gal and Gal(α1-3)Gal antibodies the possiblility exist that they interact with natural or induced terminal α-galactosyl residues existing on normal or *T. cruzi*-infected cells respectively.

ACKNOWLEDGMENTS

Thanks are due to Marisol Contreras-Bretaña, María Argelia Polegre and Angela Avila afor technical help. This research was supported by CONICIT (Project S1-2113), Consejo de Desarrollo Científico y Humanístico (Project 10.35.2242/90) and Fundación Polar.

RESUMEN

Usando la técnica inmunocitoquímica del oro coloidal - unido a anticuerpos humanos y de conejo anti-laminina y anti-nidogen, y al monoclonal gal-13 (específico para el residuo galactosil(α1-3)galactosa unido a lípidos) - así como la lectina ***Bandeiraea simplicifolia*** IB_4 - ligada a peroxidasa de rábano, todas estas técnicas específicas en la demostración de residuos galactosil(α1-3)galactosa, hemos encontrado estos residuos disacarídicos sobre la superficie externa de tripomastigotes obtenidos de sangre de ratón o de cultivo de células Vero. Si bien epimastigotes intactos se tiñeron pobremente con anticuerpos humanos, epimastigotes rotos inmunoreaccionaron fuertemente con estos anticuerpos, así como con los de conejo. Se detectó también tinción en los labios de la bolsa flagelar y en el lado exactamente opuesto a la salida del flagelo en amastigotes y promastigotes de ***Leishmania*** americanas. La presencia de abundantes residuos galactosil(α1-3)galactosa en miembros de la familia Trypanosomatidae sugiere un papel específico desconocido de estos residuos disacarídicos en la fisiología parasitaria.

REFERENCES

1. Avila, J. L., Rojas, M. and Galili, U. (1989) Immunogenic gal (α1-3) carbohydrate epitopes are present on pathogenic American ***Trypanosoma*** and ***Leishmania.*** J Immunol **142**: 2828-2834.

2. Travassos, L. R. and Milani, S. R. (1988) Anti-α-galactosyl antibodies in chagasic patients. Possible biological significance. Brazilian J Med Biol Res **21**: 1275-1286.

3. Couto, A. S., Goncalves, M. F., Colli, W. Lederkremer, R. M. (1990) The N-linked carbohydrate chain of the 85-Kilodalton glycoprotein from ***Trypanosoma cruzi*** trypomastigotes contain sialyl, fucosyl, and galactosyl (α1-3) galactose units. Mol Biochem Parasitol **39**: 101-108.

4. Ferguson, M. A. J. and Williams, A. F. (1990) Cell-surface anchoring of proteins via glycosyl-phosphatidylinositol structures. Ann Rev Biochem **57**: 285-320.

5 McConville, M. J. and Bacic, A. (1989) A family of glycoinositol phospholipids from ***Leishmania major***. Isolation, characterization, and antigenicity. J. Biol Chem **264**: 4683-4688.

6. Avila, J. L., Rojas, M. and Acosta, A. (1991) Glycoinositol phospholipids from American ***Leishmania*** and ***Trypanosoma*** spp.: Partial characterization of the glycan cores and the human humoral immune response to them. J. Clin Microbiol **29**: 2305-2312.

7. Singh, B. N., Costello, C. E., Beach, D. H. and Holz, G. G. Jr. (1990) Characterization of glycosyl phosphatidylinositol lipids of parasitic protozoans: ***Leishmania mexicana mexicana*** promastigotes, ***Trypanosoma cruzi*** Peru epimastigotes and ***Tritrichomonas foetus***. Indian J Biochem Biophys **27**: 411-415.

8. Galili, U., Macher, B. A., Buehler, J, and Shohet, S. B. (1985) Human natural anti-α-galactosyl IgG.

II. The specific recognition of α (1-3) - linked galactose residues. J Exp Med 162: 573-582.
9. Goldstein, I. J., Blake, D. A., Ebisu, S., Williams, T. J., and Murphy, L. A. (1981) Carbohydrate binding studies on the *Bandeiraea simplicifolia* I isolectins. J Biol Chem 256: 3890-3893.
10. Bretaña, A., Avila, J. L., Arias-Flores, M., Contreras, M., and Tapia, F. J. (1986) *Trypanosoma cruzi* and American *Leishmania* spp: Immunocytochemical localization of a laminin-like protein in the plasma membrane. Exp Parasitol 61: 168-175.
11. Bretaña, A., Avila, J. L., Contreras-Bretaña, and M., Tapia, F. J. (1992) American *Leishmania* and *Trypanosoma cruzi*: Galactosyl (α1-3) galactose epitope localization by colloidal gold immunocytochemistry and lection cytochemistry. Exp Parasitol 74: 27-37.
12. Gazzinelli, R. T., Romanha, A. J., Fontes, G., Chiari, E., Gazzinelli, G. and Brener, Z. (1991) Distribution of carbohydrates recognized by the lectins *Euonymus europaeus* and Concanavalin A in monoxenic and heteroxenic Trypanosomatids. J. Protozool 38: 320-325.
13. Avila, J. L., Rojas, M., Velazquez-Avila, G. and Rieber, M. (1987) Antibodies to laminin in *Trypanosoma rangeli*-infected subjects. Parasitol Res 73: 178-179.
14. Galili, U., Buehler, J., Shohet, S. B. and Macher, B. A. (1987) The human natural anti-Gal IgG. III. The sublety of immune tolerance in man as demonstrated by crossreactivity between natural anti-Gal and anti-B antibodies. J Exp Med 165: 693-704.
15. Galili, U., Clark, M. R., Shohet, S. B., Buehler, J. and Macher, B. A. (1987) Evolutionary relationship between the natural anti-Gal antibody and the Galα1-3Gal epitope in primates. Proc Nat Acad Sci USA 84: 1369-1373.
16. Szarfman, A., Terranova, V. P., Renard, S. I., Foidart, J. M., Lima, M. F., Scheinman, J. I. and Martin, J. R. (1982) Antibodies to laminin in Chagas' disease. J Exp Med 155: 1161-1171.
17. Avila, J. L., Rojas, M. and Rieber, M. (1984) Antibodies to laminin in American cutaneous leishmaniasis. Infect Immun 43: 402-406.
18. Avila, J. L., Rojas, M. and Towbin, H. (1988) Serological activity against galactosyl (α1-3) galactose in sera from patients with several kinetoplastida infections. J Clin Microbiol 26: 126-132.
19. Muniz, J. and Santos, M. C. F. (1950) Heterophile antibodies in American trypanosomiasis. O Hospital 38: 601-610.
20. Cossio, P. M., Diez, C., Szarfman, A., Kreutzer, E., Candiolo, B. and Arana, R. M. (1974) Chagasic cardiopathy. Demonstration of a serum gamma globulin factor which reacts with endocardium and vascular structures. Circulation 49: 13-21.
21. Avila J. L., Rojas M, Velázquez-Avila G, von der Mark H, and Timpl r, (1986) Antibodies to basement membrane protein nidogen in Chagas' disease and American cutaneous leishmaniasis. J. Clin. Microbiol. 24: 775-778.
22. Avila, J. L., Rojas, M., Velázquez-Avila, G., and Rieber, M. (1987) Antibodies to basement membrane proteins nidogen and laminin in sera from streptococcal-related diseases and juvenile rheumatoid arthritis patients. Clin Exp Immunol 70: 555-561.
23. Towbin, H., Rosenfelder, G., Wieslander, J., Avila, J. L., Rojas, A., Szarfman, A., Esser, K., Nowack, H., and Timpl, R. (1987) Circulating antibodies to mouse laminin in Chagas' disease, American cutaneous leishmaniasis and normal individuals recognize terminal galactosyl (α1-3) galactose epitopes. J Exp Med 166: 419-432.
24. Gulati, A. K., Zalewski, a. A., Sharma, K. B., Ogrowsky, D. and Sohal, G. S. (1986) A comparison of lection binding in rat and human peripheral nerve. J Histochem Cytochem 34: 1487-1494.
25. Wood, J., Hudson, L., Jessel, T., and Yamamoto, M. (1982) A *T. cruzi*-human cross-reactive protein antigens. Monoclonal antibody defining antigenic determinants on subpopulations of mammalian neurones and *Trypanosoma cruzi* parasites. Nature 296: 34-38.
26. Köberle, F. (1968) Chagas' disease and Chagas' syndromes: the pathology of American trypanosomiasis. Adv Parasitol 6: 63-116.
27. Avila, J. L. and Salma, N. (1991) Interaction between various strains of *T. cruzi* and cardiac primary cultures. Acta Cient Venez 42: 185.
28. Kierszenbaum, F. (1986) Is there autoimmunity in Chagas' disease? Parasitol Today 1: 4-6.
29. Hudson, L. (1986) Autoimune phenomena in chronic chagasic cardiopathy. Parasitol Today 1: 6-9.
30. Laguens, R. P., Meckert, P. C., and Chambo, J. G. (1988) Antiheart antibody-dependent cytotoxicity in the sera of mice chronically infected with *Trypanosoma cruzi*. Infect Immun 56: 993-997.
31. Ferguson, M. A. J., Homans, S. W., Dwek, R. A. and Rademacher T. W. (1988) Glycosylphosphatidylinositol moiety that anchors *Trypanosoma brucei* variant surface glycoprotein to the membrane. Science 239: 753-759.
32. Lederkremer, R. M., Lima, C., Ramírez, M. I. and Casal, O. L. (1990) Structural features of the lipopeptidophosphoglycan from Trypanosoma cruzi common with glycophosphatidylinositol anchors. Eur J Biochem 192: 337-345.

33. Avila, J. L., Rojas, M., and Velázquez-Avila, G. (1992) Characterization of a natural human antibody with antigalactosyl (α1-3) galactose specificity that is present at high titers in chronic *Trypanosoma cruzi* infection. Am J Trop Med Hyg **47**: 413-421.
34. Avila, J. L., Rojas, M. and Carrasco, H. (1991) Increased galactosyl (α1-3) galactose antibody levels in patients with different clinical stages of tropical chronic dilatatory cardiomyopathies. Immunol (Life Sci Adv) **10**: 1-10.
35. Avila; J. L. and Rojas, M. (1990) A Galactosyl (α1-3) Mannose epitope on phospholipids of *Leishmania mexicana* and *L. braziliensis* is recognized by Trypanosomatid-infected human sera. J Clin Microbiol **28**: 1530-1537.
36. Güther, M. L. S., Cardoso de Almeida, M. L., Yoshida, N., Ferguson, M. A. J., (1992) Structural studies on the glycosylphosphatidylinositol membrane anchor of *Trypanosoma cruzi* 1G7-antigen. The structure of the glycan core. J Biol Chem. **267**, 6820-6828.
37. Almeida, I. C., Milani, S. R., Gorin, P. A. J. and Travassos, L. R. (1991) Complement-mediated lysis of *Trypanosoma cruzi* trypomastigotes by human anti-α-galactosyl antibodies. J Immunol **146**: 2394-2400.
38. Gazzinelli, R. T., Pereira, M. E. S., Romanha, A., Gazzinelli, G. and Brener, Z. (1991) Direct lysis of *Trypanosoma cruzi*: a novel effector mechanism of protection mediated by human anti-gal antibodies. Parasite Immunol **13**: 345-356.
39. Krettli, A. U. and Brener, Z. (1976) Protective effects of specific antibodies in *Trypanosoma cruzi* infections. J. Immunol **116**: 755-760.
40. Takehara, H. A., Perini, A., daSilva, M. H. and Mota I. (1981) *Trypanosoma cruzi*: role of different antibody classes in protection against infection in the mouse. Exp Parasitol **52**: 137-146.

TABLE 1

Comparative study of the immunochemical properties of human galactosyl(α1-3)galactose, galactosyl(α1-2)galactose and galactofuranosyl (β1-3) mannose antibodies by determining the sugar concentration inhibiting 50% antibody binding and percentage of soluble control activity after absorption on several natural and synthetic haptens.

Experimental conditions	Human antibodies against:		
	Gal(α1-3)Gal	Gal(α1-2)Gal	Gal(β1-3)Man
A) Sugar concentration (mM) inhibiting 50% antibody binding			
Gal(α1-3)Gal	0.4	8	4
Gal(α1-2)Gal	8	2	16
Gal(α1-6)Gal	60	50	30
Gal(α1-3)Man	60	>200	2
Gal(β1-3)Man	100	>200	2
Gal(β1-4)Man	200	>200	4
Man(α1-2)Man	10	2	50
Melibiose	10	>200	34
Methyl-α-galactopyranoside	50	>200	250
Methyl-β-galactopyranoside	100	>200	300
B) Percentage of residual activity after absorption			
Control (None)	100	100	100
Murine laminin (5 µg/ml)	0	100	90
Murine nidogen (5 µg/ml)	10	100	85
Rabbit RBC	12	100	89
Guinea-pig RBC	10	100	95
Rat RBC	42	96	84
Synsorb 14	2	33	8
Synsorb 90	10	54	34
Synsorb T	94	10	59

Data obtained from References 18, 33 and 35.

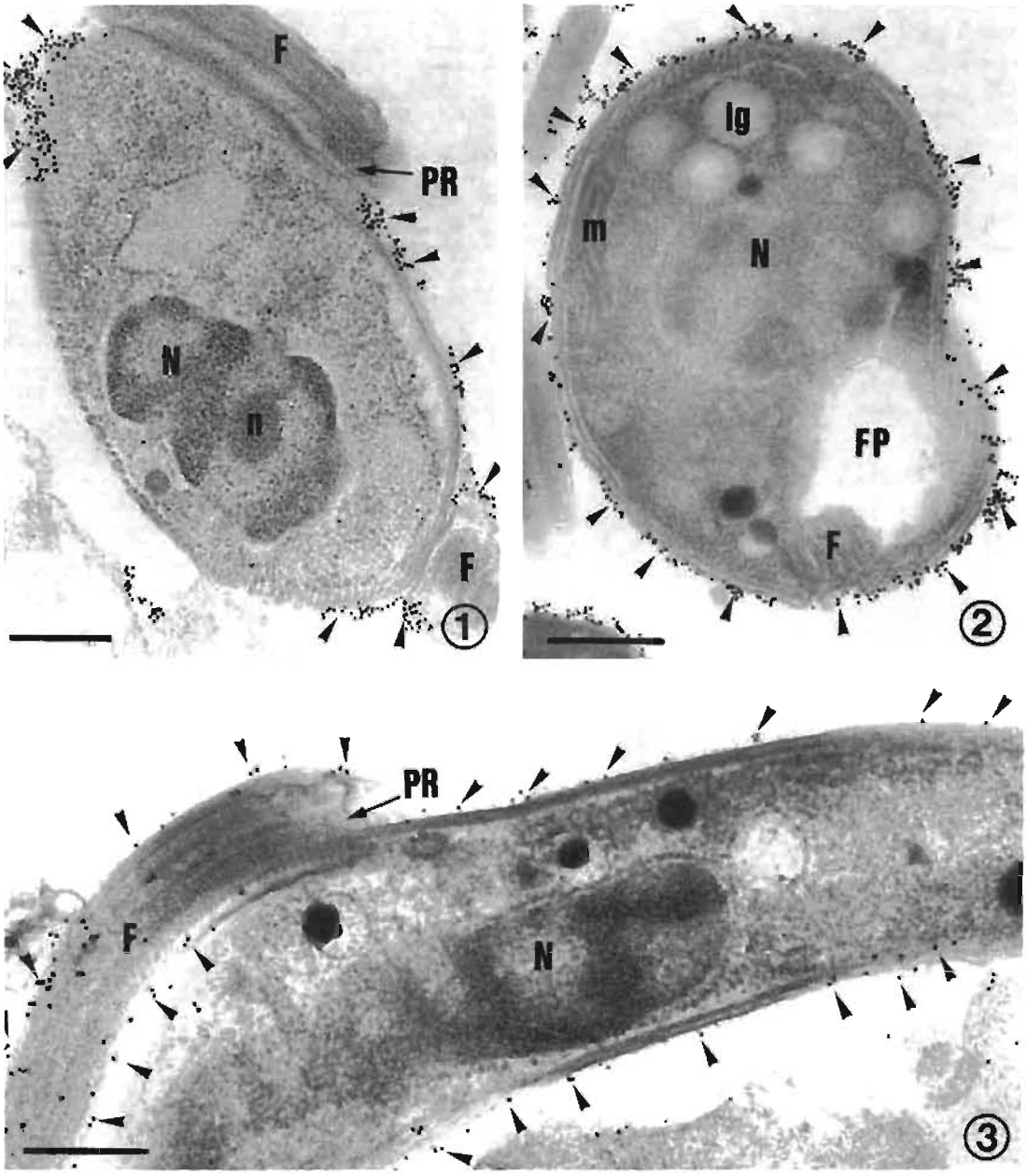

Fig. 1 Pre-embedding immunogold staining for laminin epitopes in *Trypanosoma cruzi* trypomastigote obtained from infected mouse. The colloidal gold particles (arrows) are distributed in specific areas on the external surface of the plasma membrane, the immunolabel is located close to and on the flagellar veil. (F) flagellum; (PR) paraflagellar rod; (N) nucleus; (n) nucleolus. Bar = 0.5 μm.

Fig. 2 Pre-embedding immunogold staining for laminin epitopes in *T. cruzi* trypomastigotes obtained from Vero-cell cultures. Tangential section of a trypomastigotes showing the immunolabel for laminin epitopes in a diffuse form (arrows), surrounding the whole external surface of the plasma membrane. (F) flagellum; (FP) flagellar pocket; (lg) lipid granules; (m) mitochondria; (N) nucleus. Bar = 0.5 μm.

Fig. 3 Immunogold staining for Gal(α1-3)Gal antibodies on *T. cruzi* trypomastigote obtained from Vero-cell cultures. The immunogold staining (arrows) is dispersed in all the surface of the plasma membrane and on the flagellum. (F) flagellum; (PR) paraflagellar rod; (N) nucleus. Bar = 0.5 μm.

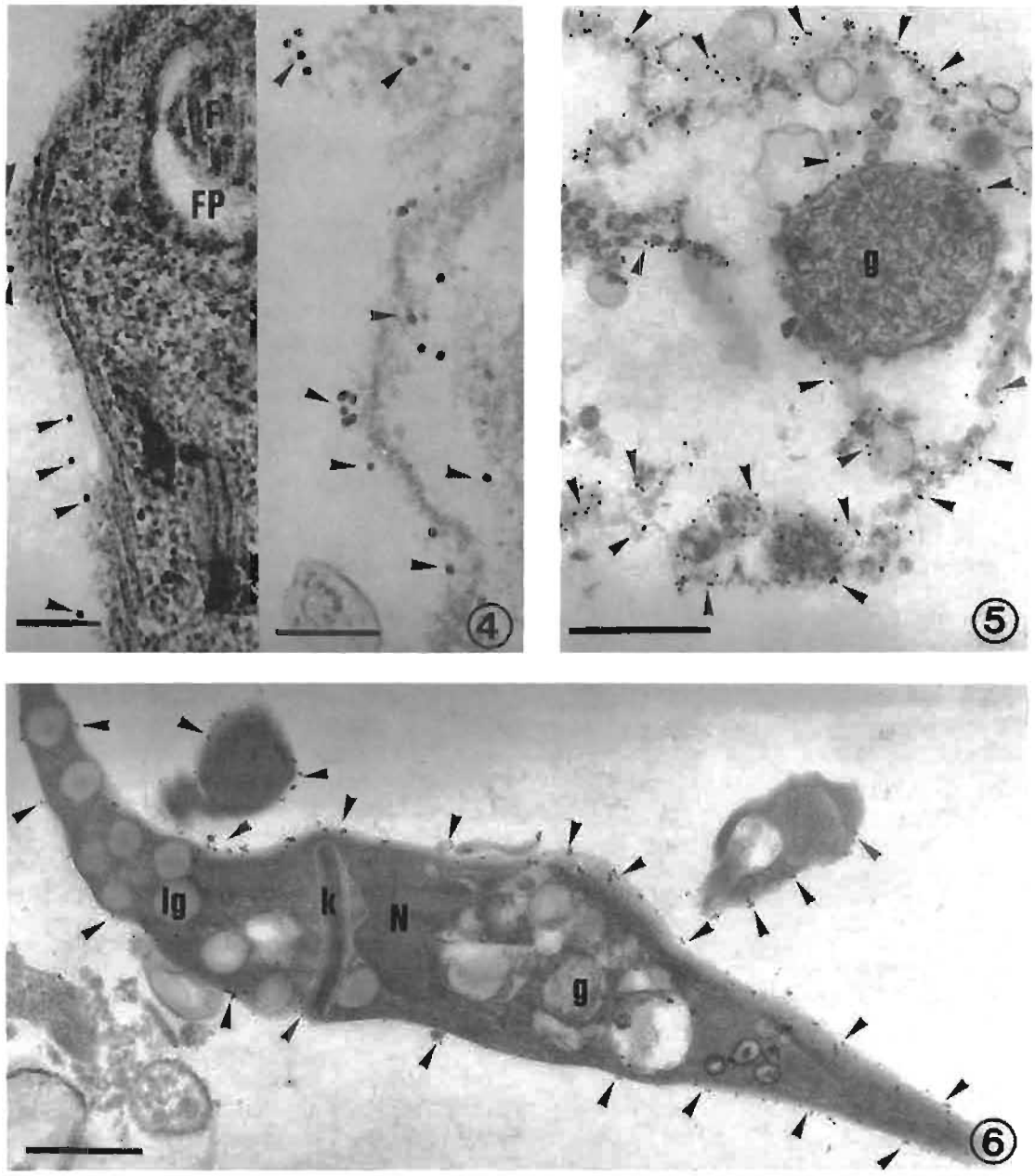

Fig. 4 *T. cruzi* trypomastigotes obtained from Vero-cell cultures. Fig. 4a shows a detail of parasitic plasma membrane immunostained for α-galactosyl residues using monoclonal antibody Gal-13. The immunolabel (arrows) is localized on an amorphous material attached to external face of cell membrane. Fig. 4b Immunogold particles (arrows) can be seen associated to filopodia detached from parasitic cell membrane. (F) flagellum; (FP) flagelallar pocket. Bars = 0.2 μm.

Fig. 5 Lectin-gold staining of Gal(α1-3)Gal residues usin isolectin *B. simplicifolia* B_4 in *T. cruzi* epimastigote in lysis process, obtained from axenic culture medium. Intense binding of colloidal gold particles (arrows) on several intra-cellular structures and on residues of the plasma membrane can be seen. (g) granule. Bar = 0.5 μm.

Fig. 6 Immunogold labeling of Gal(α1-3)Gal epitopes using human anti-Gal(α1-3)Gal antibodies in *T. cruzi* epimastigotes obtained from axenic culture medium. Moderate immunostaining (arrows) is located disperse on the whole surface of the plasma membrane. (N) nucleus; (g) granules; (lg) lipid granules; (k) kinetoplast. Bar = 1 μm.

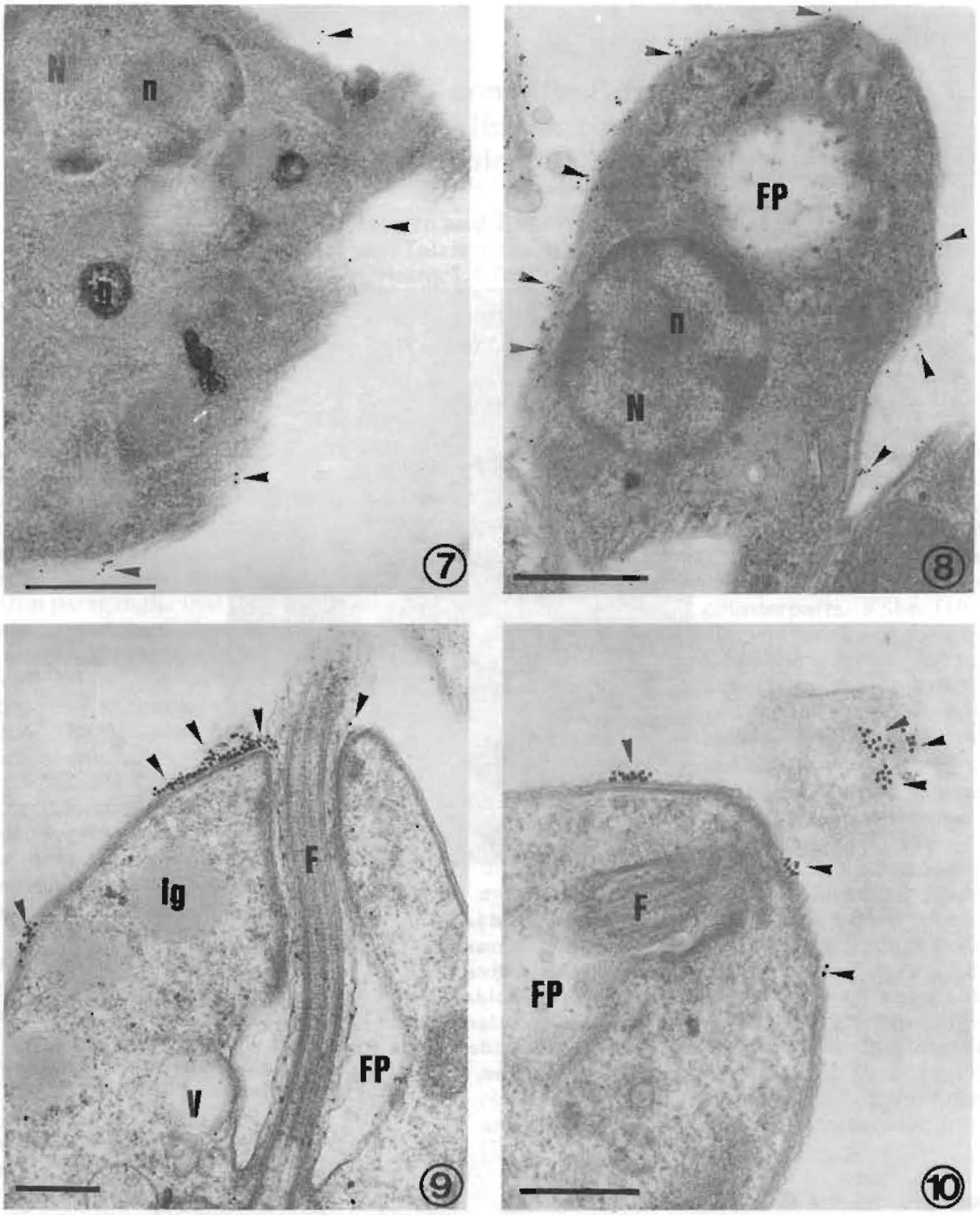

Fig. 7 Immunogold staining of rabbit anti-nidogen antibody-reacting sites in *T. cruzi* epimastigotes. Immunogold particles (arrows) are scarse and distributed all along the parasitic surface. (g) granules; (N) nucleus; (n) nucleolus. Bar = 0.5 μm.

Fig. 8 Immunoreactivity of rabbit anti-nidogen antibodies in *Leishmania mexicana* amastigotes obtained from infected hamsters. The colloidal gold particles (arrows) are distributed in patches on the surface of the parasite plasma membrane. (FP) flagellar pocket; (N) nucleus; (n) nucleolus. Bar = 0.5 μm.

Fig. 9 Pre-embedding immunogold staining for α-galactosyl epitopes using monoclonal Gal-13 antibodies in *L. mexicana* promastigotes obtained from axenic culture medium. Immunogold particles (arrows) attached to the external lips or close to the flagellar pocket can be seen. (F) flagellum; (FP) flagellar pocket; (lg) lipid granules; (V) vacuole. Bar = 0.5 μm.

Fig. 10 Pre-embedding lectin-gold labeling for Gal(α1-3)Gal residues using biotinylated isolectin *B. simplicifolia* B_4 in *L. mexicana* promastigotes, obtained from axenic culture medium. Colloidal gold particles (arrows) attached to external lips of the flagellar pocket can be seen. Also the gold particles are localized in low electron dense membranous bubbles associated with the lips of the flagellar pocket. (F) flagellum; (FP) flagellar pocket. Bar = 0.5 μm.

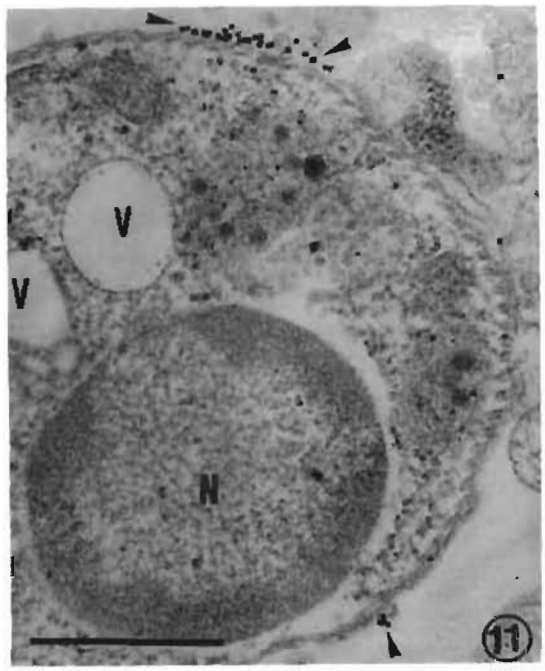

Fig. 11 Immunogold labeling for Gal(α1-3)Gal epitopes using human anti-Gal antibodies in *L. mexicana* amastigotes obtained from infected hamsters. The colloidal gold label (arrows) on the surface of the plasma membrane is located in the opposed side to the flagellum. (N) nucleus; (V) vacuoles. Bar = 0.5 μm.