A BIOLOGICAL ROLE FOR HAEMAGGLUTINATION ACTIVITY OF *LEISHMANIA* PROMASTIGOTES AND AMASTIGOTES

SVOBODOVÁ M.*, CAPO C.* & MEGE J.M.*

Summary :
Leishmania promastigotes and axenic amastigotes possess a haemagglutination activity (HA). *Leishmania* attachment to human macrophages was studied after a 30 min incubation in the presence of 10 mM carbohydrates at 37 °C. Galactosamine, sialic acid, heparin, mannose, and NAc-mannosamine impaired the attachment of promastigotes and amastigotes to monocyte-derived macrophages and the myelomonocytic cell line THP 1 whereas other carbohydrates had no effect. Preincubation experiments showed that mannose inhibits the macrophage receptor, whereas galactosamine acts on promastigotes. Moreover, the HA is considerably decreased after incubation with macrophages. Our results suggest that promastigotes of different Leishmania species and axenic amastigotes possess a lectin-like receptor with similar specificity, which is in some way involved in the attachment to vertebrate host cells.

KEY WORDS : *Leishmania*, amastigotes, phagocytes, attachment, lectin activity, *Leptomonas*.

Résumé : LE RÔLE BIOLOGIQUE DE L'ACTIVITÉ HÉMAGGLUTINANTE DES LEISHMANIES
L'adhérence des Leishmanies aux macrophages humains a été étudiée après une incubation de 30 mn à 37 °C en présence de carbohydrates à 10 mM. La galactosamine, l'acide sialique, l'héparine, le mannose, la N-acétylmannosamine inhibent partiellement l'adhérence de promastigotes ou d'amastigotes aux macrophages issus de monocytes et aux cellules de la lignée myélomonocytaire THP 1 alors que d'autres carbohydrates sont sans effet. Des expériences de préincubation montrent que le mannose inhibe les récepteurs macrophagiques et que la galactosamine affecte spécifiquement les promastigotes. De plus, l'activité hémagglutinante des Leishmanies est fortement diminuée après incubation avec les macrophages, ce qui suggère que leur activité « lectin-like » est probablement impliquée dans leur adhérence aux cellules-hôtes.

MOTS CLÉS : Leishmania, *amastigotes, phagocytes, attachment, activité « lectin-like »*, Leptomonas.

INTRODUCTION

Leishmania are trypanosomatid parasites of mammals including man. They are transmitted by the bite of sandflies in the form of flagellated, extracellular promastigotes, which gain entry into mononuclear phagocytes where they transform into aflagellated amastigotes, multiply and eventually infect new host cells. For succesful infection and spread of the pathogen, both promastigotes and amastigotes have to attach to phagocytes before they are taken in. The promastigote-host cell interaction has been extensively investigated; the amastigote-host cell interaction apparently involves distinct mechanisms (for review see Mosser & Rosenthal, 1994). Promastigotes bind to host cells directly or after opsonisation and a wide range of molecules is involved in this interaction; e.g. the major surface antigen lipophosphoglycan (LPG) (Kelleher *et al.*, 1992; Mosser & Handman, 1992) and the metalloproteinase gp63 of the parasite (Russel & Wilhelm, 1986); fibronectin, mannose, CR3, CR1, and advanced glycosylation endproducts receptors of macrophages (Wyler *et al.*, 1985; Blackwell, 1985; Dasilva *et al.*, 1989; Wilson & Pearson, 1986; Mosser *et al.*, 1987). Considerably less is known about the amastigote-phagocyte interaction. LPG, CR3, fibronectin and heparin-binding activity have been proposed as mediating amastigote adhesion (Kelleher *et al.*, 1995; Guy & Belosevic, 1993; Wyler et al., 1985; Love *et al.*, 1993). Among the multiple, redundant mechanisms of *Leishmania* adhesion to macrophages, carbohydrates and lectin-like receptors are involved (Chang, 1981; Bray, 1983; Hernándes *et al.*, 1986). We have previously described a haemagglutination activity (HA), found on promastigotes of different *Leishmania* species and also on axenic amastigotes of *L. mexicana*, which is specific for aminosugars and some glycoconjugates (Svobodová *et al.*, in press). High activity found on amastigotes suggested a role for it in the vertebrate stage of the parasite life-cycle. Therefore, we decided to study the inhibitory ability of different carbohydrates for *Leishmania* binding to human phagocytes. A relatively wide range of carbohydrates was chosen for the study, including inhibitors of the hae-

* Unité des Rickettsies, Faculté de Médecine, Marseille, France.
Correspondence: M. Svobodová Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague, Czech Republic.
Fax: 420 2 29 97 13.
e–mail milena@mail.natur.cuni.cz.

magglutination activity (galactosamine being the most potent one). Related sugars without inhibitory effects served as controls. We suggest that promastigotes and amastigotes possess a lectin-like receptor involved in their attachment to vertebrate host cells.

MATERIALS AND METHODS

Parasites

Four *Leishmania* strains were used: *L. infantum* (MHOM/TU/81/ITMAP 263), *L. major* (MHOM/IL/68/LV 561, LRC-L 137 JERICHO II; MRHO/SU/58/NEAL-P), *L. mexicana* (MNYC/BZ/62/M 379); and *Leptomonas pyrrhocoris* (IPYR/CS/79/LP 9). Promastigotes were grown in RPMI 1640 (Gibco-BRL) supplemented with 20 % foetal calf serum (FCS); seven days old subcultures were used. Axenic amastigotes of *L. mexicana* were obtained as described (Bates, 1994); briefly, freshly isolated promastigotes were transformed to metacyclic promastigotes in Schneider's *Drosophila* medium (Gibco-BRL), pH 5,5, subcultured and transformed to axenic amastigotes in the same medium at 32 °C.

Host cells

Macrophages were obtained by differentiation of human peripheral blood mononuclear cells (PBMC) as described (Molloy *et al.*, 1994). Briefly, PBMC from volunteers were obtained by Ficoll gradient centrifugation and cultured for 8 h at 37 °C (in 5 % CO_2) in Lab-Tek chamber slides (Nunc) at cell density 5×10^5 cells per chamber. Nonadherent cells were removed and the remaining monocytes were allowed to differentiate into macrophages in RPMI 1640 supplemented with 20 % FCS and L-glutamine at 37° (in 5 % CO_2). The macrophages were used for the binding studies after seven days of culture. The leukemic human cell line THP 1 was cultured in RPMI 1640 with 2 mM L-glutamine and 25 mM Hepes supplemented with 10 % FCS, penicillin (100 IU/ml) and streptomycin (50 µg/ml).

Inhibition experiments

500 mM stock solutions of carbohydrates (glucose, galactose, mannose, glucosamine, galactosamine, mannosamine, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, N-acetyl-D-mannosamine, sialic acid; purchased from Sigma) were prepared in 20 mM Tris, 150 mM NaCl, pH 7.6, adjusted to neutral where appropriate, and sterilized by filtration (0,2 µm disposable filter). Heparin was prepared to contain 10,000 IU (approx. 1.2 mg/ml), mannan 1,250 mg/ml. Because RPMI 1640 contains approx. 10 mM glucose, the effect of this carbohydrate was first tested in Tris. Before use, parasites and host cells were washed three times in RPMI 1640. THP 1 cells were incubated for 30 min with promastigotes or amastigotes (10/1 parasite/host cell ratio) in the presence of 10 mM carbohydrate while gently shaking in a 37 °C waterbath, cytocentrifuged, fixed with methanol and stained with Giemsa. In the case of macrophages, 10^6 promastigotes and carbohydrate at a final concentration of 10 mM were added to the macrophages. After 30 min of incubation (37 °C, 5 % CO_2), nonattached promastigotes were removed by washing and slides were treated as above. The number of attached promastigotes on 100 host cells was scored microscopically. The inhibitory effect of each carbohydrate was expressed as the percentage of bound promastigotes compared to that in the control (100 %) not exposed to carbohydrate.

Preincubation experiments

In some experiments, promastigotes of *L. infantum* were preincubated with 10 mM carbohydrates in RPMI 1640 for 30 min at 25 °C, washed three times, added to THP 1 cells and treated as described above. In other experiments, macrophages were preincubated with 10 mM carbohydrates for 30 min (37 °C, 5 % CO_2 in air), and washed three times before addition of promastigotes.

Effect of inhibitor concentration

L. infantum promastigotes were allowed to bind to THP 1 cells at different concentrations (0.1-100 mM) of NAc-mannosamine to test if the inhibition was saturable. After 30 min incubation the cells were treated as described above.

Adsorption of HA

Promastigotes from seven day old subcultures were washed three times in saline. They were lysed by freezing and thawing three times in liquid nitrogen (10^8 cells/ml Tris). The lysates were centrifuged at 2,000 g for 3 min and supernatants used in haemagglutination tests. Fifty µl doubling dilutions of lysates were prepared in Tris in U-well microtitre plates to give final dilution ranges of 1:2 to 1:2,048. Fifty µl of native rabbit red blood cells (2 % in saline) were added and agglutination was scored after 1 h incubation at room temperature. The end point titre was defined as the last dilution causing visible agglutination of RBC. This dilution was deemed to contain about one haemagglutination unit (HAU). Lysates in Tris containing about three HAU were double diluted in microtitre plates and added to RPMI-washed macrophages in Lab-Tek (100 µl of lysate per chamber) as well as to previously

washed Lab-tek chambers without macrophages (control of nonspecific binding). After 2 h at 4 °C, the lysates were transfered to microtitre plates and adsorption of the HA activity was measured by comparing the level of HA in chambers with and without macrophages. The experiments were repeated three times for *Leishmania* strains LV 561, Neal-P and ITMAP 263.

Statistical analysis

Percentage values derived from each experiment were used in multifactorial analysis of variance (ANOVA). For comparison of carbohydrate influence on binding, LSD multiple range tests were used.

RESULTS

Inhibition of binding of *Leishmania* promastigotes to THP 1 and macrophages

The initial attachment of promastigotes to host cells in controls was 163 ± 10 per THP 1 cells, 101 ± 8 per macrophage (stat. sign. difference between host cells, P = 0.007). The results (Table I) were processed by ANOVA to reveal the influence of carbohydrate, parasite strain and host cell type on the binding of *Leishmania* promastigotes to host cells. Both the strain (*L. infantum* ITMAP 263, *L. major* LV 561 and Neal-P) and the host cell type have no effect on the binding (P = 0.06, F-ratio 2.785 and P = 0.36, F-ratio 0.859, resp.), while the carbohydrate is the factor influencing the binding to the host cell (P = 0.0001, F-ratio 6.574). Six out of twelve carbohydrates tested show significant inhibition (about 25 %) of promastigote binding to host cells: galactosamine, heparin, sialic acid, mannose, NAc-mannosamine and, to a lesser degree, NAc-glucosamine. None of the carbohydrates enhanced the binding (Table I). Concentration experiments show that increasing the carbohydrate concentration above 10 mM does not increase the inhibition (Fig. 1).

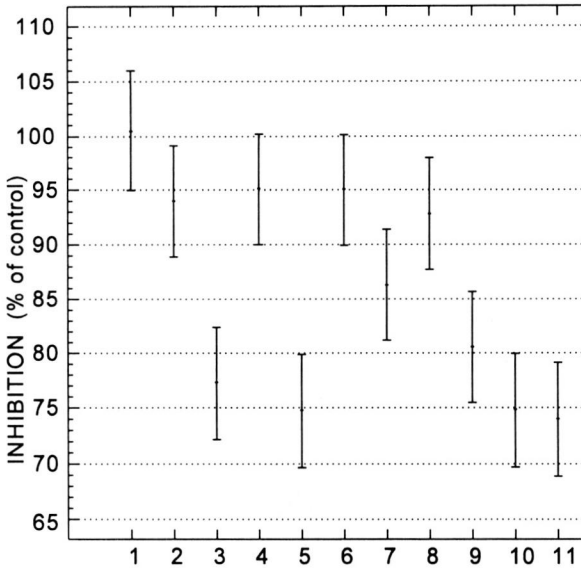

Fig. 2. — Carbohydrate inhibition of *Leishmania* promastigote attachment to host phagocytes. Data summarized for macrophages and THP 1 cells, four strains of *Leishmania* were used. 95 % LSD intervals for means, n = 21.
1. glucose, 2. galactose, 3. mannose, 4. glucosamine, 5. galactosamine, 6. mannosamine, 7. NAc-glucosamine, 8. NAc-galactosamine, 9. NAc-mannosamine, 10. sialic acid, 11. heparin.

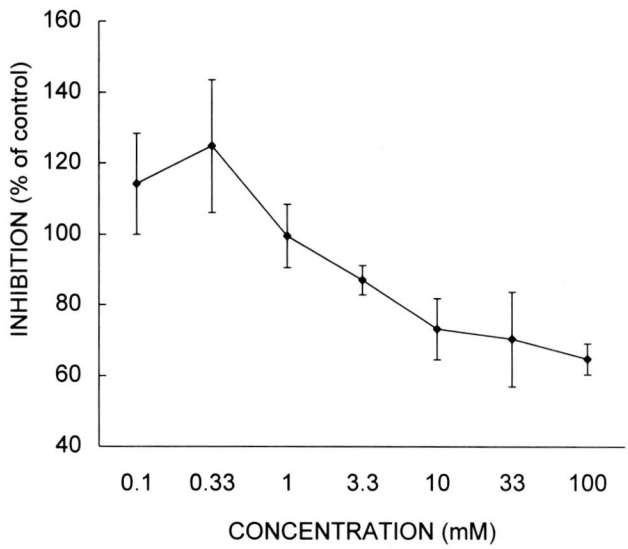

Fig. 1. — Effect of NAc-mannosamine concentration on the binding of *L. infantum* to THP 1 cells. Promastigotes were incubated 30 min with different concentrations (0.1-100 mM) of NAc-mannosamine. The inhibition of attachment is expressed in mean (n = 3 ± SE).

Binding of *L. mexicana* axenic amastigotes and promastigotes to THP 1 cells

The pattern of carbohydrate inhibition of *L. mexicana* binding to THP 1 cells was similar to three strains used previously (Table I, P = 0.18); the data could thus be summarised (Fig. 2). Inhibition by selected carbohydrates was then compared between promastigotes (four strains) and axenic amastigotes of *L. mexicana* (Table II).
The initial binding of amastigotes to THP 1 cells was 122 ± 13 and does not differ significantly from the binding of promastigotes (P = 0.38). Heparin had the same inhibitory effect on both promastigotes and amastigotes, and galactosamine had an even higher inhibitory effect on amastigote than on promastigote binding. The inhibitory effect of other carbohydrates on amastigote binding was not significant.

Specificity of inhibition for *Leishmania* promastigotes

Inhibitors of *Leishmania* promastigote binding were used for a strain of the insect pathogen *Leptomonas*

	Leishmania strain						
	ITMAP 263		LV 561		Neal-P		M379
Carbohydrate	THP1	mø	THP1	mø	THP1	mø	THP1
Glucose(#mannan)	98 ± 13	87 ± 8	107 ± 4	110 ± 19	104 ± 3	96 ± 9	78 ± 1#
Galactose	103 ± 13	102 ± 4	102 ± 5	85 ± 9	82 ± 9	93 ± 8	92 ± 6
Mannose	70 ± 4	89 ± 9	82 ± 9	85 ± 6	76 ± 11	64 ± 10	76 ± 11
Glucosamine	93 ± 16	86 ± 3	117 ± 14	106 ± 5	81 ± 15	93 ± 15	91 ± 2
Galactosamine	72 ± 7	69 ± 14	75 ± 6	71 ± 9	84 ± 20	76 ± 10	76 ± 2
Mannosamine	79 ± 3	92 ± 6	109 ± 8	93 ± 6	97 ± 19	97 ± 15	97 ± 9
NAc-glucosamine	74 ± 14	80 ± 4	84 ± 6	90 ± 5	84 ± 9	105 ± 18	87 ± 10
NAc-galactosamine	83 ± 19	100 ± 12	118 ± 4	92 ± 7	78 ± 17	97 ± 6	82 ± 2
NAc-mannosamine	74 ± 12	98 ± 10	80 ± 4	76 ± 10	62 ± 11	91 ± 8	83 ± 4
Sialic acid	75 ± 1	81 ± 6	73 ± 2	74 ± 4	55 ± 5	78 ± 5	88 ± 11
Heparin	76 ± 8	76 ± 4	84 ± 1	64 ± 5	65 ± 5	77 ± 15	77 ± 2

Table I. – Inhibition of *Leishmania* promastigotes binding to host phagocytes. THP 1 cells and macrophages (mø) were exposed to parasites in the presence of 10 mM carbohydrates (100 IU/ml heparin, 0.25 mg/ml mannan). In % of control ± SE, $n = 3$ for each experiment.

Carbohydrate	Promastigotes	Amastigotes	*Leptomonas*
Mannose	76 ± 4	88 ± 2 @	62 ± 5* @
Glucosamine	95 ± 7	93 ± 6	n.d.
Galactosamine	77 ± 5	68 ± 3* @	93 ± 2 @
NAc-glucosamine	82 ± 5	78 ± 4	80 ± 3*
NAc-mannosamine	75 ± 4 #	83 ± 5	95 ± 4 #
Sialic acid	73 ± 4	85 ± 7	79 ± 2*
Heparin	75 ± 3 #	75 ± 5* @	107 ± 14 @ #

* Statistically significant difference within group.
@, # Statistically significant difference between groups (# promastigotes-*Leptomonas*, @ amastigotes-*Leptomonas*).

Table II. — Comparison of carbohydrate inhibition of binding of *Leishmania* promastigotes, amastigotes and *Leptomonas* to THP 1 cells. Promastigotes of for strains ($n = 12$) of *Leishmania*, axenic amastigotes ($n = 5$) and *Leptomonas* promastigotes ($n = 3$) are compared using LSD multiple range tests. % of control ± SE.

pyrrhocoris to test the specificity of inhibition. Its initial binding to THP 1 cells was 223 ± 16 and does not differ from the binding of *Leishmania* (P = 0.08). Multiple range tests showed that mannose, sialic acid and NAc-glucosamine have statistically significant inhibitory effect on *Leptomonas* binding to THP 1 cells, while heparin and NAc-mannosamine do not inhibit the binding when compared to promastigotes (Table II). When the influence of inhibitors was compared for *Leptomonas* and amastigotes, even more pronounced differences were found. Three carbohydrates differed significantly in their ability to inhibit binding to THP 1 cells: mannose (*Leptomonas* binding inhibitor), galactosamine and heparin (amastigote binding inhibitor) (Table II).

Carbohydrate preincubation of parasites and/or host cells

To test if the potential inhibitor binds to carbohydrate-binding receptors on parasite or host cell surfaces, preincubation experiments with *L. infantum* promastigotes and macrophages were performed. The difference in the inhibitory effect of carbohydrates between preincubated promastigotes and preincubated macrophages is significant (ANOVA, P = 0.01), being most pronounced for mannose which binds to macrophages only. Galactosamine and NAc-mannosamine bind to promastigotes, sialic acid acts rather on macrophages and heparin has a similar effect on both types of cells (Table II). Fig. 3 clearly shows the opposite effect that mannose and galactosamine have on promastigotes and macrophages.

Adsorption of haemagglutination activity on the macrophage surface

Promastigote lysates of three strains (*L. infantum* ITMAP 263, *L. major* LV 561 and Neal-P) were used to test if the haemagglutination activity is adsorbed to macrophage surface. The incubation of the lysates diminished the HA for about three HAU, showing that the HA binds to macrophage surface.

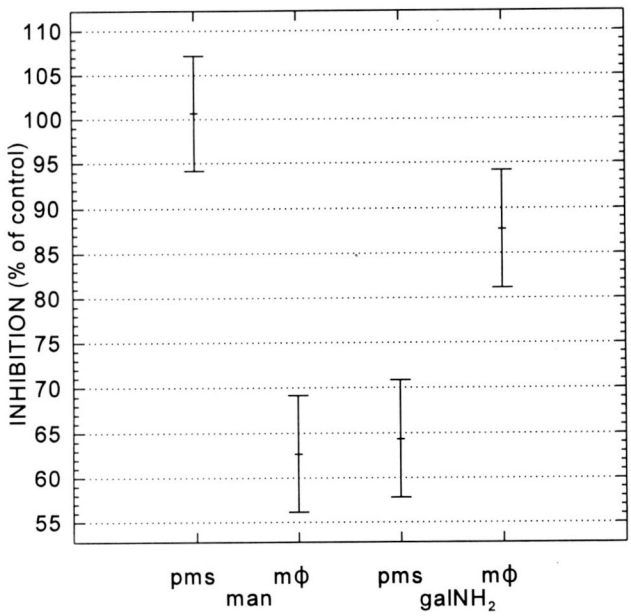

Fig. 3. — The opposite effect of galactosamine (galNH2) or mannose (man) preincubation of *Leishmania infantum* promastigotes (pms) or macrophages (mø). 95 % LSD intervals for means, n = 3.

Carbohydrate	Promastigotes preincubated	Macrophages preincubated
Mannose	101 ± 7 #	63 ± 3* #
Galactosamine	64 ± 2* #	88 ± 1 #
NAc-mannosamine	70 ± 1*	83 ± 7
Sialic acid	88 ± 10	73 ± 13
Heparin	76 ± 1*	77 ± 3

*, Statistically significant difference within group
#, Statistically significant difference between groups

Table III. — Preincubation with carbohydrates. Promastigotes or macrophages were preincubated with 10 mM carbohydrates, washed and allowed to interact. n = 3, % of control ± SE.

DISCUSSION

We have previously demonstrated the presence of haemagglutination activity on a wide range of *Leishmania* strains including axenic amastigotes (Svobodová *et al.*, in press). The higher level of HA and its presence on amastigotes suggested the haemagglutinin could play a role in the vertebrate part of parasite's life cycle. In this study, six out of eleven carbohydrates tested (galactosamine, sialic acid, heparin, mannose, NAc-mannosamine, NAc-glucosamine) significantly diminished the binding of promastigotes to host phagocytes, whereas other did not affect the binding. In the case of amastigotes, out of seven carbohydrates tested galactosamine and heparin had significant effects on the binding to THP 1 cells. The pattern of inhibition was similar for mature macrophages and monocyte-like cells. Moreover, the haemagglutinin function is shared by several species of *Leishmania* subgenus, as it was detected in all tested species of *Leishmania*: *L. donovani, L. infantum, L. tropica, L. major, L. aethiopica, L. mexicana* and *L. amazonensis* (Svobodová *et al.*, in press). The HA is not identical to LPG, because purified LPG from *L. major* did not inhibit haemagglutination (Rangarajan *et al.*, upublished).

Several authors have previously studied the effects of carbohydrates on *Leishmania* binding to host cells. Unfortunately, the use of different methods, carbohydrates, host cells and *Leishmania* strains precludes interpretation as to which of these factors is responsible for different results. We as well as Bray (1983) have used a relatively low concentration of 10 mM; others used concentrations five to twenty times higher (Hernándes *et al.*, 1986; Chang, 1981; Channon *et al.*, 1984), introducing the risk of nonspecific effects on the cells. In our case, at a concentration of 33 mM NAc-mannosamine, some THP 1 cells had disrupted membranes after cytocentrifugation. Moreover, in some studies the presence of the carbohydrates in the test system does not allow distinction between the effect on parasite and/or host cell. This led to different conclusions. Chang (1981) who first demonstrated the influence of carbohydrates on *Leishmania* binding to macrophages, suggested the presence of lectin-like molecules on macrophages, whereas Hernández *et al.* (1986) on promastigotes. Bray (1983) found NAc-glucosamine/glucosamine-specific lectin-like molecules on promastigotes rather than on macrophages. In our case, glucosamine did not significantly affect the binding of *L. mexicana* M 379 promastigotes to host cells and the effect of NAc-glucosamine was the weakest among sugars with significant inhibitory activity. Chakraborty & Das (1988) failed to demonstrate any inhibitory effect of NAc-glucosamine pretreatment of promastigotes on blood clearance of *L. donovani*.

The specificity of the interaction for *Leishmania* was confirmed using a related kinetoplastid, the insect pathogen *Leptomonas pyrrhocoris* without HA. Mannose diminished the binding of *Leptomonas* promastigotes to THP 1 cells, whereas galactosamine did not, confirming the presence of galactosamine-specific lectin-like molecules on the *Leishmania* surface. This result is in accordance with preincubation experiments: preincubation of macrophages with galactosamine had no effect on promastigote binding, whereas mannose was significantly inhibitory. When promastigotes were preincubated, the effect was opposite: only galactosamine inhibited the attachment. Thus, it can be concluded that promastigotes and axenic amastigotes of *Leishmania* possess a lectin-like activity on their sur-

face which is specific for galactosamine. For promastigotes, NAc-mannosamine is a second specific inhibitor, since it has no inhibitory activity for *Leptomonas* binding nor does it affect macrophages in preincubation studies.

The effect of other inhibitors is more complex. Sialic acid exerts its activity rather on macrophages than on promastigotes, although this difference was not statistically significant; it has no effect on amastigote binding. In some studies, sialic acid was suggested to be toxic to cells at a concentration of 5 mM. Its nonspecific influence may also be due to its negative charge.

The level of inhibition of *Leptomonas* binding to THP 1 cells by NAc-glucosamine, which is similar to that of *Leishmania*, proves that this carbohydrate binds to host cells. The potential receptor is the mannose/fucose receptor, which is also specific for NAc-glucosamine (Shepherd *et al.*, 1981).

Heparin was the most potent inhibitor of *Leishmania* promastigote and amastigote binding to host cells. Preincubation experiments showed a similar effect on both parasite and host cells. Human monocytes are known to possess a heparin-binding protein (Leung *et al.*, 1989); heparin bound to host cells during preincubation could prevent the later binding of *Leishmania* heparin-binding activity. This could also explain the lack of any effects of heparin on *Leptomonas* binding. Similarly to us, Love *et al.* (1993) found that heparin diminishes the binding of *Leishmania* to host cells; in our case, not only amastigote but also promastigote binding is affected. The presence of heparin-binding activity was demonstrated on promastigotes (Mukhopadhyay *et al.*, 1989), but heparin enhanced the binding of *Leishmania* to macrophages (Butcher *et al.*, 1992). It was suggested that two distinct heparin-binding activities, differing in affinity, exist in *Leishmania* (Love *et al.*, 1993); however, we and others (Peters *et al.*, 1995) were not able to demonstrate high affinity heparin-binding receptors on amastigotes.

Mannose was the only inhibitor of binding which influenced host cells, and our results suggest the presence of a mannose receptor on THP 1 cells, although blood-isolated monocytes lack this receptor (Rouleux-Bonnin *et al.*, 1995). Mannose inhibits the binding of promastigotes only, and the mannose receptor is not involved in amastigote binding, as demonstrated previously (Peters *et al.*, 1995).

The HA is adsorbed on macrophage surface; similar carbohydrates inhibit the HA and attachment to host phagocytes. Therefore, it can be concluded that the lectin activity found in Leishmania promastigotes and axenic amastigotes can account for about 25 % of the attachment of the both parasite life stages to vertebrate host phagocytes. Its specificity for galactosamine is interesting, because in vertebrates this carbohydrate is known only in N-acetylated form. However, the lack of inhibitory activity of NAc-galactosamine, glucosamine and mannosamine confirms the specificity of the inhibition. Further studies are required to show if the lectin activity is identical to previously described heparin-binding activity of *Leishmania*.

AKNOWLEDGEMENTS

We thank Dr. Anthony Molloy for numerous helpful discussions and practical help, Dr. Daniel Frynta and Dr. Martin Černý for advice in statistical analysis. M. S. was supported by TEMPUS mobility and by Grant Agency of the Czech Republic (205/95/0641).

REFERENCES

BATES P.A. Complete developmental cycle of *Leishmania mexicana* in axenic culture. *Parasitology*, 1994, *108*, 1-9.

BLACKWELL J.M. Role of macrophage complement and lectin-like receptors in binding *Leishmania* parasites to host macrophages. *Immunology Letters*, 1985, *11*, 227-232.

BRAY R.S. *Leishmania mexicana mexicana* : attachment and uptake of promastigotes to and by macrophages *in vitro*. *Journal of Protozoology*, 1983, *30*, 314-322.

BUTCHER B., SKLAR L.A., SEAMER L.C. & GLEW R.H. Heparin enhances the interaction of infective *Leishmania donovani* promastigotes with mouse peritoneal macrophages. *Journal of Immunology*, 1992, *148*, 2879-2886.

CHAKRABORTY P. & DAS P.K. Role of mannose/N-acetylglusosamine receptors in blood clearance and cellular attachment of *Leishmania donovani*. *Molecular and Biochemical Parasitology*, 1988, *28*, 55-62.

CHANG K. *Leishmania donovani*-macrophage binding mediated by surface glycoproteins/antigens: characterization *in vitro* by a radioisotopic assay. *Molecular and Biochemical Parasitology*, 1981, *4*, 67-76.

CHANNON J.Y., ROBERTS M.B. & BLACKWELL J.M. A study of the differential respiratory burst activity elicited by promastigotes and amastigotes of *Leishmania donovani* in murine resident peritoneal macrophages. *Immunology*, 1984, *53*, 345-355.

CRANE M.ST.J. & DVORAK J.A. Influence of monosaccharides on the infection of vertebrate cells by *Trypanosoma cruzi* and *Toxoplasma gondii*. *Molecular and Biochemical Parasitology*, 1982, *5*, 333-341.

DASILVA R.P., HALL B.F., JOINER K.A. & SACKS D.L. CR1, the C3b receptor, mediates binding of infective *Leishmania major* metacyclic promastigotes to human macrophages. *Journal of Immunology*, 1989, *143*, 617–622.

GUY R.A. & BELOSEVIC M. Comparison of receptors required for entry of *Leishmania major* amastigotes into macrophages. *Infection and Immunity*, 1993, *61*, 1553-1558.

HERNÁNDEZ A.G., RODRIGUEZ N., STOJANOVIC D. & CANDELLE D. The localization of a lectin-like component on the *Leish-*

mania cell surface. *Molecular Biology Reports*, 1986, *11*, 149-153.

Kelleher M., Bacic A. & Handman E. Identification of a macrophage-binding determinant on lipophosphoglycan from *Leishmania major* promastigotes. *Proceedings of the National Academy of Sciences USA*, 1992, *89*, 6-10.

Kelleher M., Moody S., Mirabile P., Osborn A.H., Bacic A. & Handman E. Lipophosphoglycan blocks attachment of *Leishmania major* amastigotes to macrophages. *Infection and Immunity*, 1995, *63*, 43–50.

Leung L., Saigo K. & Grant D. Heparin binds to human monocytes and modulates their procoagulant activities and secretory phenotypes. Effect of histidine-rich glycoprotein. *Blood*, 1989, *73*, 177-184.

Love D., Esko J.D. & Mosser D.M. A heparin-binding activity on *Leishmania* amastigotes which mediates adhesion to cellular proteoglycans. *Journal of Cell Biology*, 1994, *123*, 759-766.

Molloy A., Laochumroovorapong P. & Kaplan G. Apoptosis, but not necrosis, of infected monocytes is coupled with killing of intracellular *Bacillus* Calmette-Guerin. *Journal of Experimental Medicine*, 1994, *180*, 1499-1509.

Mosser D.M., Vlassara H., Edelson P.J. & Cerami A. *Leishmania* promastigotes are recognized by the macrophage receptor for advanced glycosylation endproducts. *Journal of Experimental Medicine*, 1987, *165*, 140-145.

Mosser D. M. & Handman E. Treatment of murine macrophages with interferon-γ inhibits their ability to bind *Leishmania* promastigotes. *Journal of Leukocyte Biology*, 1992, *52*, 396-376.

Mosser D.M. & Rosenthal L.A. Divergent strategies used by the promastigote and amastigote forms of *Leishmania* to invade mammalian cells, *In* : Strategies for intracellular survival of microbes. Bailliere's clinical infectious diseases Vol 1 Nr 2, Russel D. G. (ed), Bailliere Tindall London, 1994, 191-212.

Mukhopadhyay N.K., Shome K., Saha A.K., Hassell J.R. & Glew R. H. Heparin binds to *Leishmania donovani* promastigotes and inhibits protein phosphorylation. *Biochemical Journal*, 1989, *264*, 517-525.

Peters C., Aebischer T., Stierhof Y.D., Fuchs M. & Overath P. The role of macrophage receptors in adhesion and uptake of *Leishmania mexicana* amastigotes. *Journal of Cell Science*, 1995, *108*, 3715-3724.

Rouleux-Bonnin F., Monsigny M. & Legrand A. Transcriptional expression of mannose receptor gene during differentiation of human macrophages. *Biochemical and Biophysical Research Communications*, 1995, *217*, 106-112.

Russel D.G. & Wilhelm H. The involvement of the major surface glycoprotein (gp63) of Leishmania promastigotes in attachment to macrophages. *Journal of Immunology*, 1986, *136*, 2613-2620.

Shepherd V.L., Lee Y.C., Schlesinger P.H. & Stahl P.D. L-Fucose-terminated glycoconjugates are recognized by pinocytosis receptors on macrophages. *Proceeding of the National Academy of Sciences USA*, 1981, *78*, 1019-1022.

Wilson M. & Pearson R.D. Evidence that *Leishmania donovani* utilizes a mannose receptor on human mononuclear phagocytes to establish intracellular parasitism. *Journal of Immunology*, 1986, *136*, 4681-4688.

Wyler D.J., Sypek J.P., McDonald J.A. *In vitro* parasite-monocyte interactions in human leishmaniasis: possible role of fibronectin in parasite attachment. *Infection and Immunity*, 1985, *49*, 305-311.

Reçu le 28 février 1997
Accepté le 25 avril 1997