Bioscience Reports, Vol. 18, No. 3, 1998

Macrophage Mannosyl Fucosyl Receptor: Its Role in Invasion of Virulent and Avirulent *L. Donovani* Promastigotes

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Received March 3, 1998; accepted June 5, 1998

The interaction of leishmania parasites with macrophages is known to be receptor mediated. Previous study from this laboratory (*J. Parasitol.* **82**:632, 1996) showed the significant involvement of LPG and gp63 receptors in the recognition of virulent strains onto the macrophages. The role of carbohydrate receptors—the other major receptors besides LPG and gp63 receptors, in the recognition of both virulent (strains AG83 and GE1) and avirulent (strain UR6) leishmania onto the host macrophages has been the major focus of the present investigation. Various neoglycoproteins were used as efficient ligands to preblock the carbohydrate receptors on the macrophage surface. Similarly, various sugar specific lectins were used to preblock the corresponding carbohydrate ligands on the parasite surface. When these preblocked macrophages or parasites were used to study their mode of recognition, it was obvious from the findings that avirulent leishmania promastigotes possibly use the mannosyl fucosyl receptors (MFR) more avidly for their initial attachment and subsequent internalization into the macrophages whereas the virulent leishmania exhibits limited use of this receptor. When a macrophage-like cell line (J774), lacking in MFR, was purposely selected to test the previous findings, as expected, the attachment of avirulent promastigotes (UR6) onto the cell line was found to be negligible when compared to the peritoneal macrophages. Thus, it appears that avirulent leishmania promastigotes probably utilize MFR significantly for their initial recognition and subsequent internalization by macrophages.

KEY WORDS: Fucose, Leishmania, macrophage, mannose, receptor, virulence

INTRODUCTION

Leishmania species are all obligate intracellular parasites existing within the phagolysosomes of their host's macrophages. Although the macrophage is equipped with a battery of antimicrobial responses, Leishmania must have evolved strategies to establish and sustain intramacrophage infection [20]. Successful infection of the macrophage is achieved through a series of distinct phase: adherence and entry, establishment of intracellular infection and long-term sustenance of infection. Considerable interest has been directed towards the identification of the parasite ligand and macrophase receptor pairings operating in attachment and entry of Leishmania into macrophages.

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0144-8463/98/0600-0129$15.00/0 © 1998 Plenum Publishing Corporation

Macrophages possess a variety of receptors e.g. complement receptors (CR3 and CR1), carbohydrate receptors (mannose-fucose receptor or MFR), fibronectin receptors and a receptor for nonenzymatic glycosylation end products that are implicated in the binding and internalization of Leishmania [2, 6, 17, 21, 22, 28, 30, 31, 32]. Although the role of MFR on the macrophages was not examined before for the invasion of virulent and avirulent strains of Leishmania donovani promastigotee a recent report [23] shows the utilization of MFR for invasion of virulent and attenuated strains of Mycobacterium tuberculosis.

In our previous study, we have demonstrated that the entry of virulent and avirulent *L. donovani* promastigotes via CR3 receptors of macrophages differs significantly [5]. In this study we sought to characterize the involvement of carbohydrate receptors of macrophage and macrophage-like cell line (J774) in the recognition of virulent and avirulent strain of *L. donovani* promastigotes. Experiments with different neoglycoproteins and lectins were performed to block the macrophage carbohydrate receptors and parasite ligands. A clear understanding of this particular receptor-ligand interaction may be essential to understand the ultimate intracellular fate of both the virulent and avirulent Leishmania parasites within the macrophages.

MATERIALS AND METHODS

Media and Chemicals

RPMI 1640 and heat inactivated fetal bovine serum (FBS) were obtained from Difco, U.S.A., Penicillin, streptomycin, gentamycin, MEM and M199 from Gibco laboratories, Grand Island, N.Y., Hepes (N-2-hydroxyethyl piperazine-N-2-ethanesulphonic acid) and L-glutamine from Sisco, India, Lactoperoxidase, glucose oxidase (Type V), p-aminophenyl-α-D-mannopyranoside, p-aminophenyl-α-D-fucopyranoside, p-aminophenyl-α-D-glucopyranoside, p-aminophenyl-α-D-galactopyranoside, Bovine serum albumin, EDC_1 [1-ethyl-3(3-dimethyl aminopropyl) carbodimide hydrochloride], Concanavalin A, Ulex eurapeus, Wheat germ agglutinin and Ricinus communis agglutinin were obtained from Sigma Chemicals, St. Louis, U.S.A.

Animals

Male Balb/c mice, 20-22 gms body weight were obtained from the animal facility of our Institute.

Parasites

L. donovani strain UR6 (MHOM/IN/1978/UR6) is a nonpathogenic strain. The cells were grown at 22°C on blood agar slants, 100 ml of which contained 1 g glucose, 1.5 g agar, 3.7 g of brain heart infusion powder and 2–3 ml of fresh rabbit blood. The cells were maintained at 72 hr intervals at 22°C [18].

L. donovani strain AG83 (MHOH/IN/1983/AG83) and GE-1 (MHOH/IN/89/GE-1) are pathogenic strains maintained in susceptible Balb/c mice. Just before use heavily infected spleen from 3 month old mice were minced and added into culture medium 199 supplemented with 20% heat inactivated serum (FBS), 0.15 M Hepes, 100 μg penicillin/ml and 100 μg streptomycin/ml. The culture was kept for 5 days at 22°C when transformed promastigotes of AG83 or GE-1 emerged. The preparation was purified by subsequent culture for 2 weeks.

Raising of Mouse Model

Our colony of Balb/c mice (20–22 gm) was used to maintain *L. donovani* strain AG83 and GE-1 by intravenous injection of promastigotes every three months. The culture of promastigotes was centrifuged at 2300 × g in SS34 rotor (Sorvall RCIIB refrigerated centrifuge) for 10 mins. The pellet was washed twice and resuspended in 2–4 ml sterile PBS. Each animal was injected intravenously in tail vein with approximately 2×10^7 promastigotes. The spleen of mice infected for three months were used as source of parasites.

Preparation of Macrophage Cultures

Peritoneal macrophages were harvested from 3 month old male Swiss albino mice (20–22 g) as previously described [19]. 4% Thioglycollate medium (1 ml) was injected into each mouse intraperitoneally. After 4 days macrophages were collected from peritoneal fluid with 5 ml of PBS. The cells were harvested by centrifugation at 2300g in a SS34 rotor (Sorvall RCIIB), refrigerated centrifuge at 4°C for 10 min and washed twice with PBS. The macrophages were separated from other cells by adherence on coverslips at 37°C in RPMI medium for 4 hrs. Using this technique, 85% of the macrophages were viable, as determined by trypan blue exclusion test. About 10^6 macrophages/coverslip were cultured in 500 μl RPMI 1640 medium supplemented with 20% FBS, L-glutamine (4 mM), Hepes (25 mM), streptomycin (100 μg/ml) and penicilllin (100 μg/ml). After 4 hrs adherence at 37°C, non-adherent cells were washed off with PBS.

The macrophage-like cell line J774 was maintained and subcultured in MEM medium plus 10% FBS. Before the experiment, cells were scrapped and adhered for 2 hrs on coverslips at 37°C. After incubation, cells were washed to remove unattached cells.

Infection of Macrophage Cultures

Peritoneal macrophages and macrophage-like cell line were challenged with promastigotes (both pathogenic or nonpathogenic) in RPMI 1640 medium at macrophage to parasite ratio 1:20 and kept at 37°C for 1 hr. After incubation the excess parasites were washed off thoroughly from each of the slides and the slides were air-dried, stained with Giemsa and examined under microscope for 2 parameters, viz., (i) number of parasites adhering to the outer surface of each macrophage and (ii)

number of parasites inside each macrophage. Experiments were repeated thrice and at least 100 macrophages were counted in each experiment.

Preparation of Neoglycoproteins

Synthetic glycoproteins like mannose-bovine serum albumin (BSA), fucose-BSA, galactose-BSA and glucose-BSA were prepared by coupling p-aminophenyl-α-D-mannopyranoside, p-aminophenyl-α-D-glucopyranoside with BSA through water soluble 1-ethyl-3(3-dimethylaminopropyl)-carbodimide hydrochloride [7]. Briefly, BSA was added to sugar solution (1:100) at pH 4.75. Carbodimide solution was then added drop-wise to the sugar-BSA mixture at pH 4.75. This solution was kept at room temperature for 4 hrs and then the reaction was quenched with 1(M) Na-acetate buffer, pH 5.5. Finally, it was dialysed against distilled water. The mannose, fucose, galactose and glucose-BSA conjugates were found to contain 60, 50, 55 and 40 mole of sugar residues per mole of BSA respectively. Sugar was assayed by phenol-sulphuric acid method [10] while protein was assayed by Lowry's method [15].

Binding Assay of Mannose-BSA

Iodination of mannose-BSA was done by standard chloramine T method to a specific radioactivity of $(3\text{–}5) \times 10^6$ cpm/μg. Binding of mannose-BSA to peritoneal macrophage were performed in RPMI medium plus 20% FCS. Macrophages (10^6 cells) were cultured for different time periods e.g. 1 hr, 3 hr and 12 hr, washed with PBS, cooled to 4°C and incubated for 2 hrs with ^{125}I-mannose-BSA (10^6 cpm) in a total volume of 200 μl in the absence or presence of cold mannose-BSA (2 mg/ml). After incubation, the cells were washed thoroughly, solubilized in 1 ml of 0.5(N) NaOH and then taken for counting [25, 26]. Protein was determined using Lowry method [15].

Surface Labelling of *L. donovani* Promastigotes

Parasites were surface labelled by using the lactoperoxidase/glucose oxidase enzymatic coupling of ^{125}I to exposed tyrosine residues [13]. Specific activity of ^{125}I labelled parasites was about 3.0×10^5 cpm/10^8 parasites.

Binding of Surface Labelled Promastigotes to Peritoneal Macrophages

Thioglycollate elicited peritoneal macrophages (10^7 cells) after 2 hr adherence to coverslips were washed with PBS and then incubated with surface labelled parasites (5×10^4 cpm) for 1 hr with or without 200 μg of different neoglycoproteins e.g. mannose-BSA, fucose-BSA, galactose-BSA or glucose-BSA. The cells were then throughly washed with PBS and counts taken after solubilizing with 1 ml of 1(N) NaOH.

Attachment and Internalization of *L. donovani* Promastigotes to Macrophages in Presence of Neoglycoproteins

The thioglycollate elicited peritoneal macrophages after 2 hr adherence to coverslips were washed with PBS and then incubated with virulent or avirulent *L. donovani* promastigotes for 1 hr in presence or absence of the various neoglycoproteins. After incubation the excess promastigotes were removed and the cells were washed with PBS. The cells were then air dried, fixed, stained with Giemsa and counted for the number of attached and internalized parasites.

Assay of Binding and Internalization of Lectin-treated *L. donovani* Promastigotes to Macrophages

Binding and internalization of lectin treated *L. donovani* promastigotes to macrophages were carried out using a non-agglutinating concentration of different lectins incubated with different strains of Leishmania. The non-agglutinating concentration was first established microscopically. This concentration was then used to block the parasites surface ligands. The treated promastigotes were incubated with peritoneal macrophages for 1 hr and washed thoroughly to remove excess parasites. The coverslips containing the cells were then air-dried, fixed, stained and counted for number of promastigotes bound and internalized into the macrophages. Macrophages incubated with untreated promastigotes were used as control in each case.

RESULTS

Kinetics of Expression of the Mannosyl-fucosyl Receptor (MFR) on Perotoneal Macrophages

The presence of MFR on thioglycollate elicited peritoneal macrophages was examined using ^{125}I-mannose-BSA. Macrophages cultured for different time points (1 hr, 4 hr and 12 hr), were incubated at 4°C with ^{125}I-mannose-BSA in presence or absence of cold mannose-BSA for 2 hr. From Fig. 1, it appears that appreciable expression of MFR was reached in macrophages cultured for 1 hr and 4 hr and a two-fold increase in MFR expression was noticed in case of macrophages cultured for 12 hr. It may be mentioned that when galactose-BSA was used as control, negligible binding was seen. A similar sugar specificity of MNF receptor is already established by Stahl *et al.* [24].

Binding of Surface Labeled *L. donovani* Promastigotes to Macrophages

To determine the contribution of carbohydrate receptors in binding of virulent and avirulent promastigotes to macrophages, different neoglycoproteins (man-BSA, fuc-BSA, gal-BSA) were used as inhibitors of parasite-uptake through these receptors. In general, from Table I, it can be seen that the inhibition was high with man-BSA and fuc-BSA for all the strains but for avirulent UR6 strain better inhibition

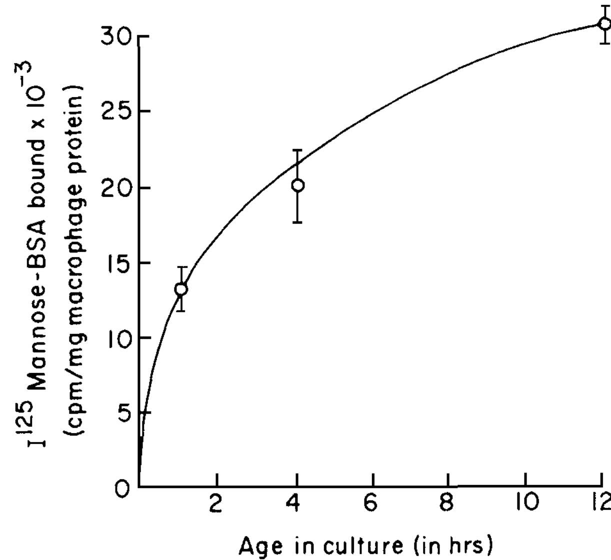

Fig. 1. Expression of the mannosyl-fucosyl receptor on peritoneal macrophages as a function of the age of macrophage culture. Specific binding of ^{125}I-mannose-BSA was determined by comparing binding in presence and absence of cold mannose BSA. When galactose-BSA was used as control, binding was found to be negligible. Results are shown as mean of three values (N = 3). (○) mannose-BSA.

Table 1. Inhibition in the binding of surface labelled *L. donovani* promastigotes to murine thioglycollate elicited peritoneal macrophages in presence of various neoglycoproteins

	% inhibition		
Inhibitors	UR6	AG83	GEI
Mannose-BSA	62 ± 6	30 ± 5	40 ± 4
Fucose-BSA	49 ± 5	29 ± 7	25 ± 3
Galactose-BSA	14 ± 2	11 ± 4	10 ± 3

Inhibition studies were performed by using various neoglycoproteins to block the respective carbohydrate receptors on the macrophage surface and % inhibition was calculated by comparing with respective controls (i.e. in absence of competitors). Peritoneal macrophages (10^6 cells) after 2 hrs adherence to coverslips were incubated with surface labelled promastigotes (5×10^4 cpm) for 1 hr in presence or absence of neoglycoproteins at an optimum concentration (200 μg). After incubation the cells were washed, solubilized with 1(N) NaOH and radioactive counts taken. Results are shown as Mean ± S.D. (N = 3). The cpm bound for UR6, AG83 and GE-1 are 5.1×10^3, 4.96×10^3 and 5.98×10^3 respectively in the absence of competitor.

was noticed in comparison to those of virulent strains (AG83 and GE-1). Gal-BSA showed a lower inhibitory effect for all the three strains.

Light Microscopic Studies of Macrophages Infected with *L. donovani* Promastigotes

To examine separately the inhibitory effects of neoglycoproteins in the attachment and internalization of *L. donovani* promastigotes onto the macrophage surface, giemsa stained parasites were counted. Fig. 2 shows a higher inhibition in the attachment and internalization of avirulent promastigotes to the macrophages when compared with their virulent counterparts. A similar phenomenon was observed with binding studies using radio-labelled promastigotes. For both avirulent and virulent strains inhibitory effect with man-BSA was found to be highest followed by fuc-BSA, gal-BSA showed insignificant inhibition.

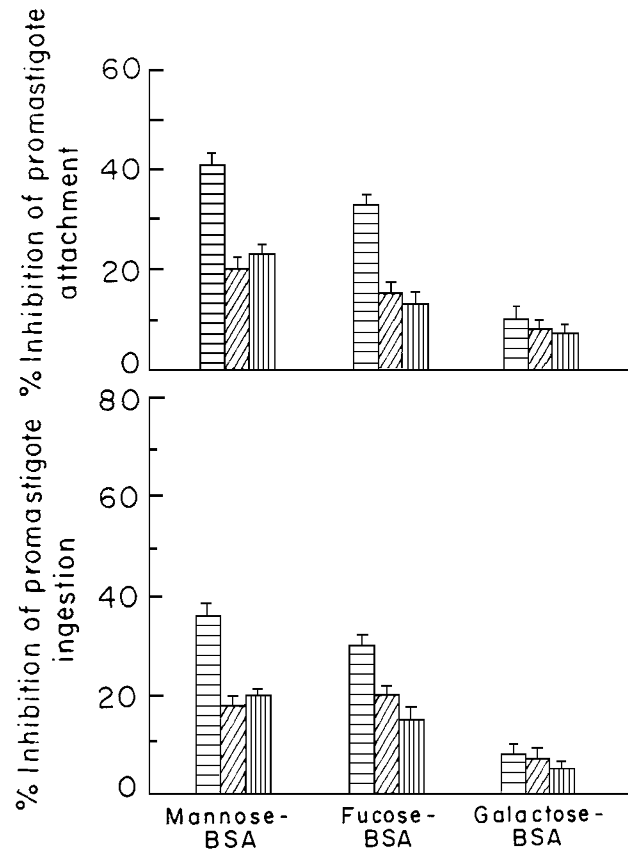

Fig. 2. Light microscopic studies on the inhibitory effect of neoglycoproteins in the attachment and internalization of different strains of *L. donovani* promastigotes to macrophages. Results are shown as Mean ± S.E. (N = 3). (▤) UR6 strain; (▨) Ag8a3 strain; (▥) GE-1 strain.

Table 2. Agglutination of virulent strains of *L. donovani* promastigotes with different plant lectins

Lectins	Sugar specificity	Agglutination as % of control (± S.D.)		
		UR6	GE-1	AG83
None		100	100	100
Concavalin A	mannose-glucose	576 ± 50*	450 ± 52*	290 ± 40
Ulex europaeus	L-fucose	454 ± 58	375 ± 63	200 ± 20
Wheat germ agglautination	N-acetyl glucosamine	342 ± 80	140 ± 20	210 ± 30
R. communis agglutinin	galactose,	600 ± 56*	700 ± 70*	750 ± 53

The assay mixture contained 1.4 ml of PBS, 20 μl of 1 mg/ml lectin and 20 μl of promastigotes (1.5×10^8 cells/ml). Agglutination was measured at 340 nm and expressed as % agglutination compared to that of control without lectin. Specificity of lectins were tested by using specific inhibitors. Agglutination studies were performed by using both virulent and avirulent strains of *Leishmania donovani* promastigotes and different sugar specific lectins.

*Statistical analysis (paired, one tail, student's t test) of the differences in agglutination as % of control, between the strains UR6, GE-1 and Ag83 were tested for all the four lectins. Especially, between UR6 and GE-1, the higher agglautination with ConA was seen for UR6 ($p < 0.001$, $n = 4$) but the lower agglutination was seen for the same strain with R. Communis agglutinin with a borderline significance ($p < 0.05$, $n = 4$).

Interaction of Virulent and Avirulent Leishmania Strains with Different Lectins

From the inhibitory studies with neoglycoproteins it appears that the virulent promastigotes do not utilize the carbohydrate receptors significantly for its entry into macrophages (Fig. 2) This difference might well be due to a difference in the availability or exposure of sugar residues on the parasite surface. The availability of sugar residues exposed on the surface of parasites was monitored by agglutination of parasites with different lectins specific for various sugars. This study showed a higher level of mannose/glucose and fucose residues on the surface of avirulent promastigotes while higher concentration of galactose was found on the virulent strains (Table 2).

Inhibition of Attachment and Internalization of Lectin-treated *L. donovani* Promastigotes to Macrophages

Table 3 demonstrates the effect of the lectin-treated promastigotes in its entry into macrophages. Non-agglutinating concentration of the lectins were used to block the carbohydrate residues on the surface of the parasites. Non-agglutinating concentration was chosen by agglutination studies with parasites and different concentration of lectins. The avirulent strain treated with ConA and Ulex eurapaeus showed maximum inhibitory effect, compared to that of virulent strains, indicating

Table 3. Lectin-mediated inhibition in the attachment and internalization of *L. donovani* promastigotes to macrophages

	In the attachment			In the Internalization of		
Inhibitors	UR6	AG83	GE-1	UR6	AG83	GE-1
Concanavalin A	38 ± 4	18 ± 3.2	22 ± 2.5	37 ± 2.2	17 ± 3.6	27 ± 1
Ulex europaeus	29 ± 2.6	14 ± 3.2	15 ± 1.2	28 ± 4	16 ± 2.2	15 ± 3
R. Communis agglutinin	7 ± 2	6 ± 1.4	5 ± 1.7	1 ± 1	4 ± 1.2	5 ± 1

In these studies the surface ligands of virulent and avirulent strains of *L. donovani* were blocked with non-agglutination concentration of different lectins. These treated promastigotes were then incubated with peritoneal macrophages for 1 hr (37°C) at a ratio of 20:1. AFter incubation the cells were washed, air-dried, fixed, stained with Giemsa and counted for the number of promastigotes attached and internalized into the macrophages. % inhibition of attached and internalized parasites were calculated using macrophages incubated with untreated parasties as control. Results are shown as Mean ± S.D. ($N = 3$).

mannose or fucose terminating ligands on the avirulent parasite surface play significant role in their recognition by macrophages.

Comparison of Phagocytosis of *L. donovani* by Cell-line Derived Macrophages and Peritoneal Macrophages: To verify our observations that MFR plays a major role in the interaction of avirulent *L. donovani* promastigotes with macrophages, we used a macrophage-like cell line (J774) which is known to be devoid of MFR. Fig. 3 shows that although overall attachment and internalization of both avirulent and virulent leishmania promastigotes seem to be less in J774.1 cell line compared to the peritoneal macrophages, the effect appeared to be more pronounced with avirulent strains than that with the virulent ones. This clearly demonstrates the importance of MFR on macrophages in the endocytosis of avirulent promastigotes.

DISCUSSION

The nature of interaction between leishmania and its host cell the macrophage, is critical to establish a new infection [1]. The interaction between macrophage and the parasite involves initial attachment to macrophage surface and its subsequent internalization. Many groups have identified putative receptor-ligand interactions that mediate the attachment of promastigotes to their host cells. These interactions include both the direct binding of the parasite-derived ligands to host cell receptors and the binding of the parasite-associated host-derived opsonins (complement and fibronectin) to their respective receptors [3]. In this study we examined the contribution of carbohydrate receptors in the recognition of both virulent and avirulent parasites by the macrophages. In parallel study, we also examined the contribution of the corresponding ligands on the surface of these parasites, which are being used for their attachment onto the macrophage surface and their subsequent internalization.

Receptors for glycoprotein with terminal mannose (MFR) has been recognized in rat alveolar macrophages [25], mouse peritoneal macrophages [27], cultured bone-marrow derived macrophages [24] and human monocyte derived macrophages [30].

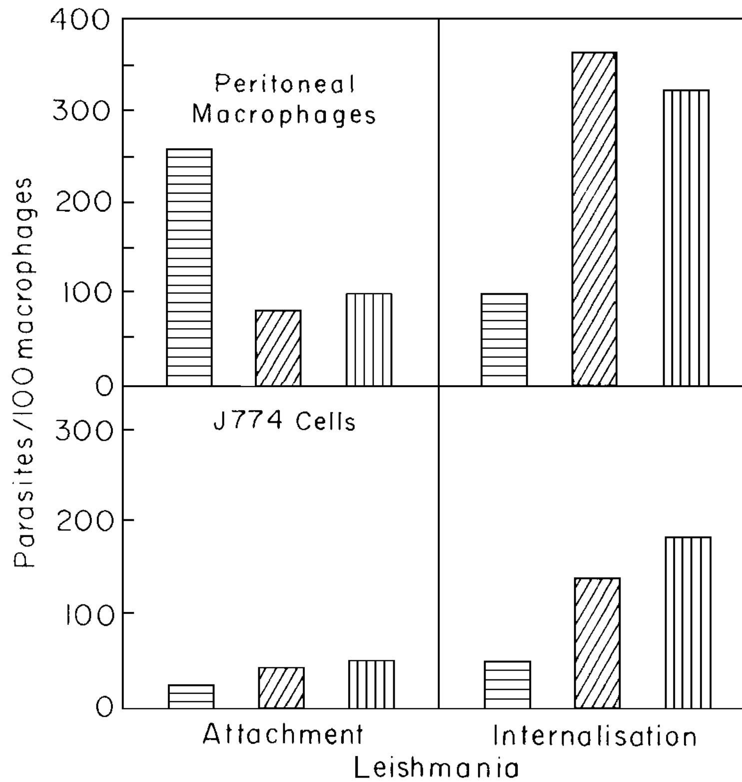

Fig. 3. Comparison of phagocytosis of *L. donovani* promastigotes after 1 hr by peritoneal macrophages and cell-line (J774) macrophages. Results are shown as mean of three values (N = 3). (▤) UR6 strain; (▥) GE-1 strain; (▨) AG83 strain.

In the present report, we examined the kinetics of expression of MFR in murine peritoneal macrophages using ^{125}I-mannose BSA. Although appreciable expression of MFR was noticed on the macrophages cultured for 1 hr and 3 hr, a two-fold increase in their expression was found in case of macrophages cultured for overnight (Fig. 1). Stahl *et al.* [24] had also noticed appreciable expression of MFR in peritoneal macrophages cultured for 90 mins. Also by using galactose-BSA as negative control, they demonstrated that MFR is specific for mannose and fucose terminating glycoproteins and not galactose terminating ones.

Next, we prepared various neoglycoproteins which could be used as efficient ligand in an attempt to preblock the respective carbohydrate receptors on the macrophage surface. The effect of blocking the carbohydrate receptors with these neoglycoproteins on the binding of virulent and avirulent promastigotes onto the macrophage surface was then examined [Fig. 2, Table 1]. Since the binding of avirulent promastigotes was inhibited considerably with mannose-BSA and fucose-BSA, it was concluded that this avirulent strain probably use MFR significantly in its entry into the macrophages. The virulent *L. donovani* promastigotes on the other hand, showed a lesser inhibitory effect and hence probably do not use the MFR as

significantly as the avirulent ones, although the role of other receptors in the phagocytosis cannot be ruled out. Many investigators have shown that involvement of MFR to some extent in the entry of Leishmania into the macrophages [7, 30, 31] whereas Wilson *et al.* [28] have provided evidence that MFR plays an important role in the attachment and ingestion of *L. donovani* promastigotes to human macrophages. Blackwell *et al.* [2] have demonstrated that in the absence of serum a combined effect of CR3 and MFR is required for the entry of Leishmania into macrophages. On the other hand in presence of serum, CR3 and MFR acts independently.

Since a difference in the utilization of MFR by different *L. donovani* strains was seen, it appears that expression of the surface carbohydrates in these strains are different. With a view to probe the expression of surface carbohydrates on the parasites various carbohydrate-specific lectins were selected. Lectin-binding studies have already demonstrated the presence of mannose on the surface of Leishmania promastigotes [11, 14, 29]. From the studies with lectin agglutination of avirulent *L. donovani* it appears that high levels of mannose/or glucose, fucose, N-acetylglucosamine are present on its surface compared to that of the virulent strains (Table 2). Even between the virulent strains a striking difference in the expression of these carbohydrate receptors were noticed. Although galactose residues were found to be high in both virulent and avirulent strains, entry of both virulent and avirulent parasite was found to be negligible when gal-BSA was used as an inhibitor (Table I). This high expression of galactose could very well be due to the presence of galactose containing glycoproteins or glycolipids in the form of LPG and gp63, the presence of which on leishmania surface have already been documented [2]. It is possible that these Leishmania parasites utilize their respective receptors other than the carbohydrate receptors for their entry into macrophages [31].

To support our previous observation in relation to the preblocking of macrophage receptors with specific neoglycoproteins which showed the contribution of MFR to be maximum in the entry of avirulent strain of Leishmania, the parasite surface carbohydrate ligands were blocked with the non-agglutinating concentration of lectins and these treated parasites were then used for attachment and internalization studies with macrophages. Results obtained (Table 3) showed similar trends as observed previously (Fig. 2). From the observation it is obvious that avirulent Leishmania possibly uses the MFR significantly in its initial attachment and subsequent internalization into murine peritoneal macrophages, but the virulent Leishmania exhibits limited use of this receptor. Thus from our studies we can summarize that the virulent and avirulent strains probably use different receptors avidly on the same host for its internalization although molecular mechanism remains unclear.

It is reported that all species of Leishmania synthesize a complex family of low molecular weight glycoinositol-phospholipids (GIPLs) containing branched or linear glycan chains [12, 16]. In the *L. donovani* promastigotes the predominant GIPL′ belong to the hybrid series, which consists of a branched glycan of α1-6 linked and α1-3 linked mannose residues attached to Manα1-4GlcN 1-PI, whereas *L. donovani* amastigotes contain another series of GIPLs, known as type 1, which contain 1-6 linked mannan residue only [16]. But, whether the different strains of parasites express a different profile of Leishmania glycoinositol phospholipids remains an

open question. Such possibility was examined by De-Majumder [8], who focussed attention onto the glycophosphosphingolipids (GSPL), a class of GIPLs in which the lipid is anchored to the cell membrane via ceramides. When such a ceramide-anchored GSPL antigen was isolated from the lipid extract of *L. donovani* promastigotes (avirulent UR6 strain) and purified by affinity chromatography this glycolipid antigen was found to contain galactose, mannose, myoinositol, phosphate, ceramide and hexosamine but no sialic acid. But, similar glycolipid was also isolated from the *L. donovani* promastigotes (virulent, AG83 strain and WHO reference strain DD8) indicating that there may be no gross difference in the profile of Leishmania GSPL between virulent and avirulent strains [8]. However, the involvement of cell surface carbohydrate between non-infective and infective development stages of *L. donovani* promastigotes is already known [49]. As judged from specific lectin-induced agglutination, D-galactose and dGalNAc were found to decrease in going from infective stationary phase to non-infective decline phase of a particular strain. In this report, it is obvious from Table 2 that the presence of D-galactose as judged from RCA agglutination, is lower in the avirulent strain (UR6) than the virulent ones (AG83 and GE1), when compared to the same stationary phase of growth. The presence of mannose and fucose both, on the other hand is found to be higher in the avirulent strain compared to the virulent ones. It is possible that the total molar ratio of Man:Gal vary from strain to strains.

In an attempt to test our hypothesis that avirulent promastigotes utilize MFR most significantly for their entry into macrophages, we purposely selected a cultured macrophages-like cell line (J774) lacking in MFR [24] to study the entry pattern of both virulent and avirulent promastigotes. It was observed that the recognition of avirulent *L. donovani* (UR6) by this cell was insignificant (approx. ten fold less) compared to that of peritoneal macrophages (Fig. 3). But, phagocytosis of both virulent and avirulent Leishmania by J774 cell-line was much lower when compared to that of peritoneal macrophages. These results are in close agreement with previous report [4] in which interaction involving Leishmania mexicana and J774 cell line was studied. When the attachment and internalization pattern of both virulent and avirulent leishmania was compared only for peritoneal macrophages it was observed that avirulent parasites attach more avidly on the macrophage surface whereas the virulent parasites are internalized or phagocytosed rapidly within the macrophages. It is possible that the recycling time of the receptors which are being used avidly by the virulent strains is much faster than that of the receptors which are significantly used by the avirulent strains. As a result, the virulent leishmania are so rapidly phagocytosed that only a fraction can be detected on the surface at a given time point whereas the avirulent Lieshmania gets stuck on surface because of poor internalization [5].

The present study provides evidence for a major contribution of MFR in the binding and subsequent internalization of avirulent *L. donovani* by macrophages. In case of virulent *L. donovani* this role of MFR is somewhat subdued. Thus it appears that virulent and avirulent parasites probably utilize different receptors for their entry into the macrophages. This structure function relationship of Leishmania with the macrophages may be the basis of their survival or destruction within the host.

ACKNOWLEDGMENT

Financial assistance by Department of Science and Technology and University Grants Commission in the form of a fellowship to one of used (R.C.) is gratefully acknowledged. Thanks are due to Dr. Santu Bandyopadhyay for generous supply of celline macrophages (J774).

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