CR1 MEDIATES BINDING OF L. MAJOR METACYCLIC PROMASTIGOTES TO HUMAN MACROPHAGES.

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Parasites of the genus Leishmania are intracellular pathogens surviving in the phagolysosomal system of macrophages (1). During development either in the sandfly or in axenic cultures, promastigotes differentiate from a non infective form undergoing logarithmic growth (LP) into a infective metacyclic stage (MP) (2) that can be distinguished by its distinctive morphology, relative resistance to complement mediated lysis, minimal triggering of oxidative burst and ability to survive within macrophages.

Internalization of Leishmania by macrophages requires specific ligand-receptor interactions, and the use of different receptors on the cell membrane might offer means of evading cell activation and subsequent parasite killing. We compared phagocytosis of MP and LP by human macrophages, based on the hypothesis that differences in their mechanism of binding to macrophages might be involved in determining the MP ability of intracellular survival.

We have shown that while LP attach relatively well to macrophages in absence of exogenous complement, fresh serum opsonization is an absolute requirement for efficient MP attachment (fig.1) (3). As we mentioned above, MP are the promastigotes stage resistant to complement mediated lysis, certainly meaning that they have contact with molecules of the complement system during natural infection (4). This, together with their dependency on C3 opsonization to enter macrophages, clearly establishes a role for the complement system during *in vivo* infections.

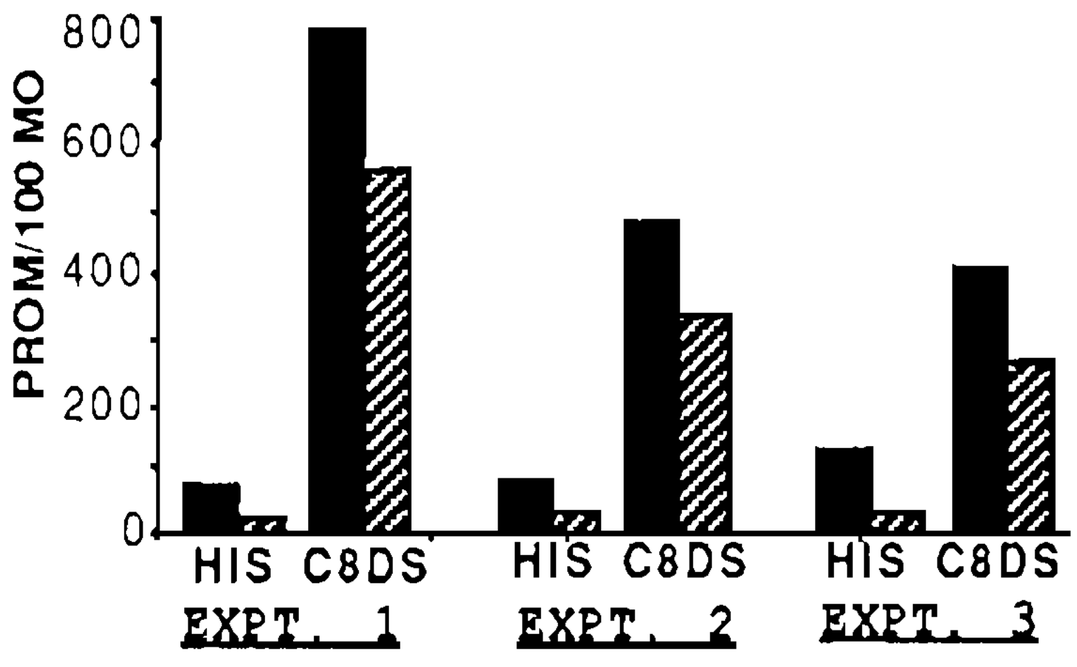

Fig. 1- 3 experiments showing binding assays of L. major promastigotes to human MO. LP(■) and MP(▨) were pre-treated for 1h with heat inactivated serum (HIS) or C8 deficient fresh serum (C8DS) before being added to the cells.

Several studies have already implicated the receptor for the iC3b fragment of C3 (CR3) and the mannose-fucose receptor (MFR) in binding and ingestion of Leishmania promastigotes (5,6). Use of complement receptors is generally thought to be advantageous for the parasite, because binding of C3-bearing particles to macrophages complement receptors fails to trigger a respiratory burst (7). Indeed, increased deposition of C3 on the promastigote surface has been associated with enhanced survival inside macrophages (8,9).

Other receptors involved in L. major uptake include the receptor for advanced glycosylation end products (10) and the fibronectin receptor (FR) (11). However, in all these studies, binding assays were performed using non-defined populations of promastigotes under serum free conditions. Our complement receptor inhibition assays showed that when comparing binding of defined populations of promastigotes, CR3 is the major complement receptor mediating binding of the non infective LP, in fresh serum independent assays (fig. 2A) (3).

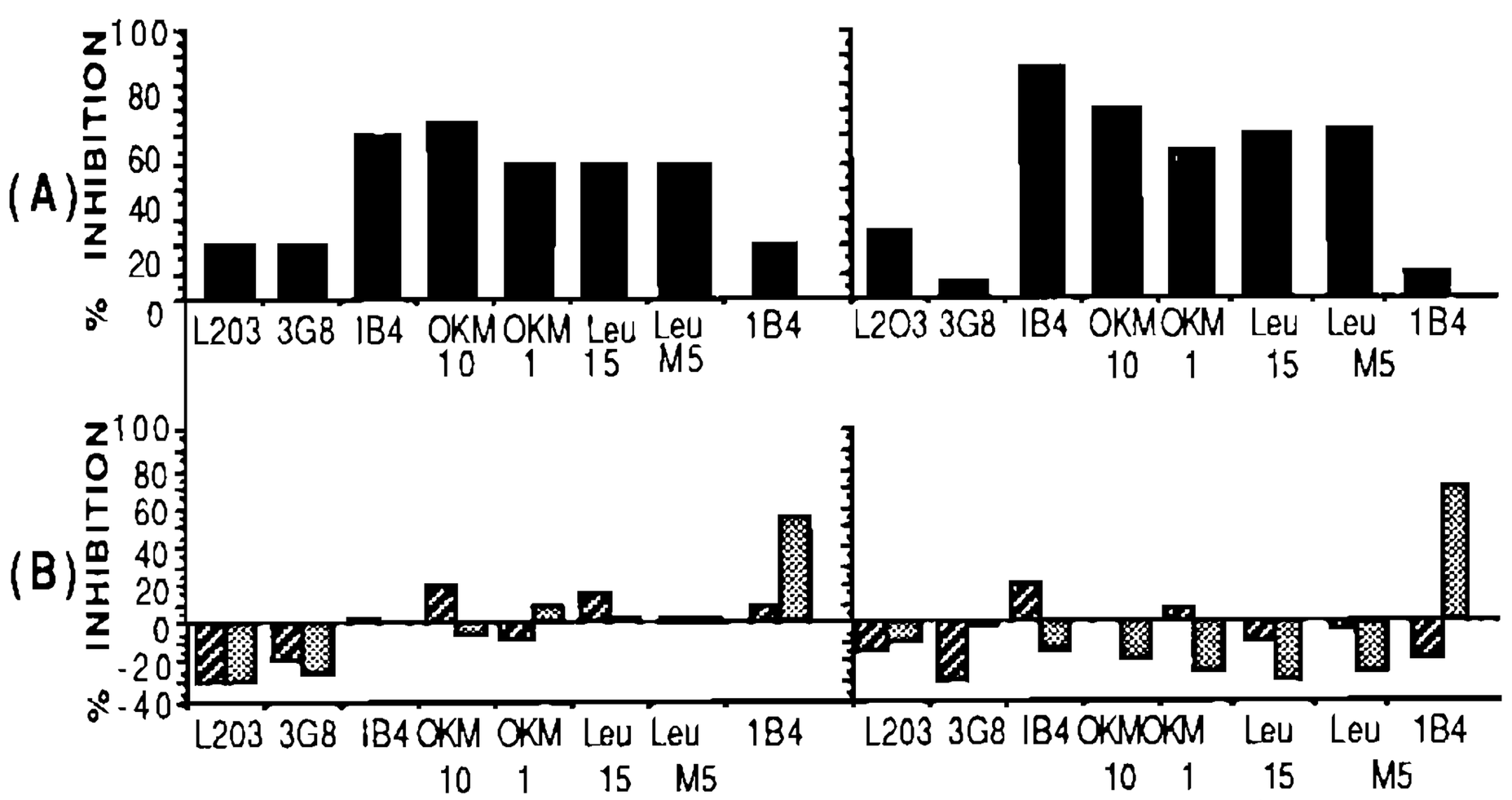

Fig.2- 2 experiments showing the effect of C receptors down-modulation on L.major binding to human MO. Cells were plated on plastic surfaces covered with anti- CR3 mAb (IB4, OKM10, OKM1 and Leu15), anti-p150.95 mAb (LeuM5), anti-CR1 mAb (1B4) or irrelevant mAb (L203, an anti-human HLA and 3G8, an anti-human Fc receptor. Parasites were pre incubated in (A)- heat inactivated serum (LP-▮) or (B)- C8 deficient fresh serum (LP- ◪ and MP-▩) and then added to the cellsfor 30 min. Slides were washed, stained and counted for determination of % inhibition of attachment/ingestion relative to controls.

Also, experiments where anti-CR3 monoclonal antibodies (mAb) were added in the fluid phase, suggest that LP bind to CR3 independent of iC3b, to a site close to or coincident with the lectin-binding site of the receptor(3). Attachment of LP to macrophages can be inhibited by mAb OKM1, which binds to the lectin-binding site of CR3, and not by mAb OKM10, that is directed against the iC3b binding site (12) (fig.3).

This is consistent with recent work showing that the Arg-Gly-Asp containing Leishmania glycoprotein GP63 binds directly to macrophage CR3 (13). Another aspect of LP attachment to the host cell, is the fact that down-modulation of the macrophage iC3b binding protein p150.95 by mAb LeuM5 inhibits their uptake (fig. 2A)(3). The presence of residual iC3b on these parasites (14) offers a ligand for p150.95 and is one possible explanation for this result.

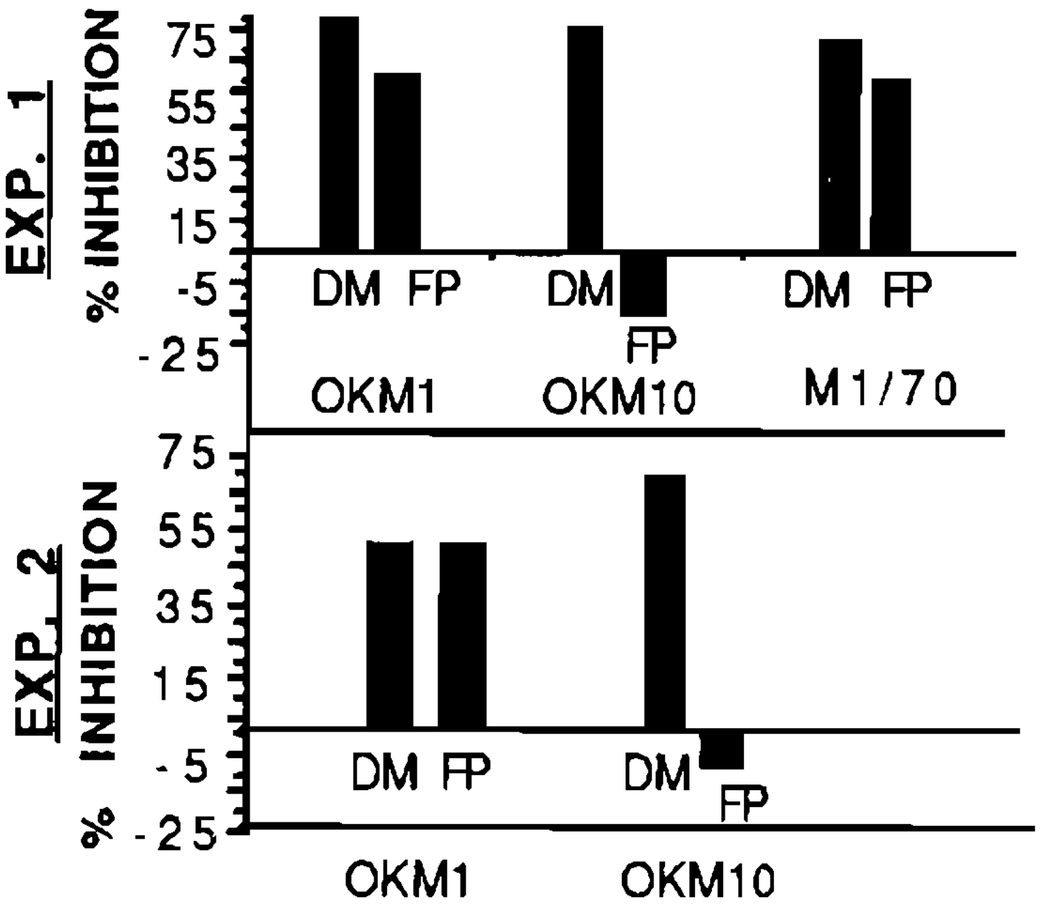

Fig. 3- LP, pre-treated with heat inactivated serum, were added to to human MO that had been pre-incubated for 15 min with anti-CR3 mAb (OKM1, OKM10 and M1/70) added in the fluid phase (FP) or MO plated on the same anti-CR3 mAb for down-modulation of the receptor (DM). No inhibition was observed when either LP or MP were pre-treated with C8 deficient fresh serum. (not shown in the figure)

The infective MP though, use primarily the CR1 receptor(3) (fig. 2B), which is consistent with data showing that the predominant form of C3 on L. major is C3b. (14). In the same paper, the authors reported the acceptor molecule for C3 on the parasite surface as being the lypophosphoglycan (LPG). LPG is also known to undergo developmental changes that have been associated with the acquisition of virulence by MP. This molecule on the MP surface has increased molecular weight and is immunologically distinct (15). Recent work in our laboratory suggests that during promastigotes differentiation, more polysaccharide repeats are added to the lipid and glycan core, considerably increasing the size of the molecule (Sacks, D., et al., manuscript in preparation). The developmental modification of LPG on MP, might then be involved in turning other potential ligands for complement receptors unavailable for the host cell membrane and the C3b deposited on the LPG would be the functional ligand to interact with CR1. This hypothesis is reinforced by the finding that on LP_ that still have the shorter version of LPG, both CR1 and CR3 are used for uptake (fig. 4)(3).

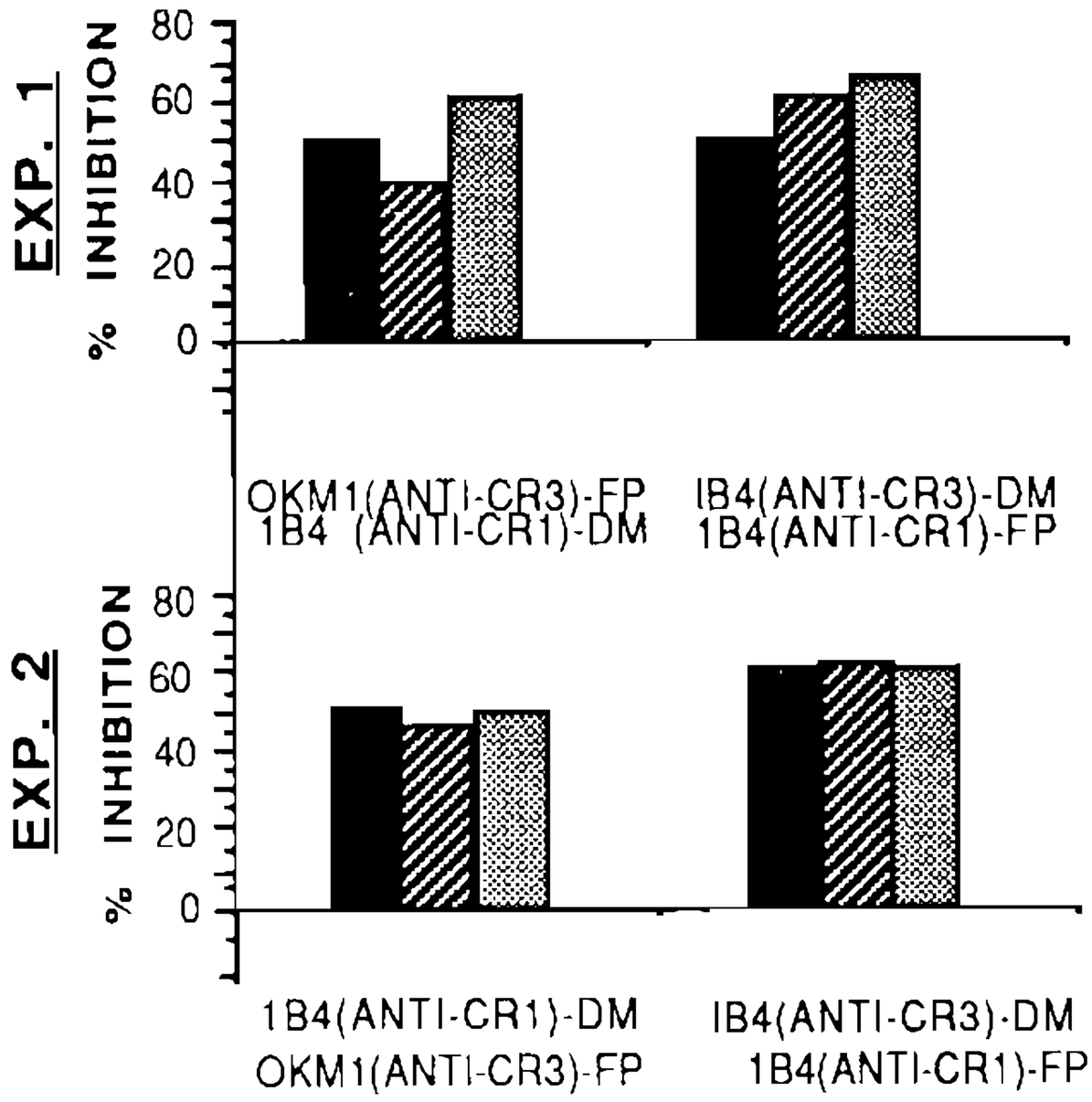

Fig.4- Effect of simultaneous inhibition of CR3 and CR1 on L.major binding to human MO. Cells were plated on slides covered either with anti-CR3 mAb(IB4) or anti-CR1 mAb(1B4). Residual C receptor activity was then blocked by adding 30 ug/ml of anti-CR3 mAb OKM1 to the cells that had been plated on anti-CR1 (OKM1-FP + 1B4-DM) or alternatively, by adding 30ug/ml of 1B4 to the cells plated on anti-CR3 (IB4-DM + 1B4-FP). LP pre-treated with heat inactivated serum (■) or C8 fresh deficient serum (▩) and MP pre-treated with fresh C8DS (▩) were added for 30 min. Slides were washed and processed as described in Fig. 2 legend.

Since CR1 binding does not trigger internalization of the ligand (16), we are currently investigating the possibility of collaboration from other macrophage receptors in uptake of MP. Experiments using mannan to block the MFR show that this receptor does not seem to interfere with binding of either stage (3). The FR is a interesting possibility, since binding of the FR can efficiently activate CR1 to mediate internalization of the ligand(17) . Thus, there might be a role for the FR in uptake of the complement opsonization dependent MP.

The differences in receptors usage by MP and LP seem to reflect on their ability to trigger the release of oxygen radicals, highly toxic for both stages. H2O2 production by macrophages stimulated by MP is negligible, whereas LP elicit a strong respiratory burst on these cells. MP fail to trigger the burst even after fresh serum treatment, when attachment indexes have at least a 10-fold increase. In contrast, fresh serum opsonized LP elicit production of higher levels of H2O2, roughly proportional to their increase in binding (R. da Silva, manuscript in preparation). Ligation of the lectin-binding site of CR3 triggers the oxidative burst(18), possibly indicating that macrophage triggering by LP is mediated via CR3. By the other hand, the CR1 mediated phagocytosis of MP would enable them to enter the host cell while avoiding cellular activation and thereby contributing to their enhanced infectivity.

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