SUMMARY

Antigenic Targets in Leishmania Donovani Parasite and Infected Macrophages

Indian visceral leishmaniasis (kala-azar), one of the major tropical diseases, is caused by *Leishmania donovani*. The parasite is a kinetoplastid protozoan, which resides and replicates obligatorily within the macrophages of vertebrates. In intra llular infections including leishmaniasis, malaria and tuberculosis, infection specific antigenic changes on the surface of infected cell surface have been a focus of attention for design of new diagnostic, therapeutic and prophylactic tools and for a better understanding of the complex host-parasite interactions.

Monoclonal antibodies generated earlier in the lab, against promastigote and infected macrophages at early post infection times were, used for this study. Considering the importance of the later stages of infection, which involve the transformation of promastigotes into amastigotes, the fate of these antigenic determinants with the progression of infection was examined taking above antibodies. The reactivity of the panel was also analyzed with the infected tissues and isolated amastigotes and the antibodies were used to compare the antigenic profiles of tissue derived, axenic and *in vitro* cell-derived amastigotes. The relevance of the MAbs in controlling the intracellular infection was also probed.

Several MAbs reacted with the *in vitro L. donovani* infected macrophages. MAbs 44G3, 33E8, and 38C10 reacted with the infected cell surface at 4 hr, 24 hr and 48 hr of post infection, while MAb 31D6 recognized the infected cell surface only at early stages. Though reactivity of 31D6 was completely lost by 24 hr post infection, it continued to recognize the intracellular parasite even at 48 hr time point. The reactivity of MAb 8G2 with the infected cell surface increased with the progression of infection.

MAb 44G3 recognized a 22 kDa antigen in the infected macrophage membrane. Though this antigen was secreted by the parasite in the culture supernatant, it did not associated passively with the infected cells and its appearance in the macrophage membrane was dependent on the viability of the parasite. MAb 33E8 reacted with 48 kDa and 65 kDa components in the infected macrophage membrane. Of these the 65 kDa band could be detected non specifically in the unspent M199 medium and in the membrane preparation of uninfected macrophages. The epitopes recognized by MAbs 44G3 and 33E8 are not related to the previously described immunodominant molecule LPG since the MAbs recognized their respective antigens in the lysates of LPG deficient

R2D2 promastigotes and in the membrane of macrophages infected with this mutant. Additionally, this also suggested that the appearance of these epitopes on the infected cell membrane did not depend on the presence of LPG.

MAbs 31D6, 33E8, 38C10, 38E7, and 8G2 showed strong reactivity with both spleen and lesion sections, while MAb 44G3 specifically reacted with the lesion sections only. When analyzed with the infected tissue isolated amastigotes, a similar reactivity profile was observed, with 44G3 exhibiting specific recognition of lesion isolated amastigotes. In the immunoblots, this MAb specifically reacted with a 22 kDa antigen in the lesion and not with the spleen isolated amastigotes. However, the 22 kDa antigen was recognized with the promastigotes transformed from both lesion and spleen amastigotes. Additionally, 44G3 reacted with a faint 70 kDa band in the amastigotes isolated from infected spleen and lesion. The level of 22 kDa antigen increased as the spleen amastigotes transformed into promastigotes, while the 70 kDa band disappeared. MAb 33E8 reacted with polydisperse bands in both the amastigotes and promastigotes derived from infected spleen and lesion. MAb 31D6 identified a 44 kDa antigen in the promastigotes and 55kDa antigen in the spleen amastigotes.

The axenic and *in vitro* cell transformed amastigotes derived from both spleen and lesion derived parasites, did not showed any difference with respect to the 22 kDa antigen. In terms of their reactivity with MAbs 44G3, 33E8, 31D6, 8G2 and 38C10, these amastigotes were more closer to their respective promastigotes than the tissue isolated amastigotes. However, with respect to the reactivity of CA7AE, these amastigotes resembled more with the tissue-derived amastigotes.

Pretreatment of promastigotes with the membrane reactive MAbs resulted in the inhibition of intracellular survival of promastigotes within the macrophages. For MAbs 33E8, 31D6 and 8G2 this inhibition could be attributed to the inhibitory effect of antibody pretreatment on the invasion of macrophages by the promastigotes. However, the level of inhibition of intracellular growth of the parasite due to MAb 44G3 pretreatment could not be correlated with the level on invasion inhibition by this antibody, thus suggesting that the epitope recognized by MAb 44G3 is important for the survival of parasite in the macrophages.

Most of the antigens recognized by the MAbs were found to be cross reactive amongst various amastigotes of different strains of *L. donovani*. Also the reactivity profile of the MAbs with *L. major* promastigotes was similar to that observed with *L.*

donovani promastigotes. The observed cross reactivity of MAbs 44G3 and 33E8 extended to the infected cell membrane.

The highlights of the present study are:

i) MAbs identified some *Leishmania* stage-specific antigens, as well as those common to parasite and infected cells at later stages of infection. These might provide useful targets for therapeutic interventions of leishmaniasis.

ii) The 22 kDa antigen is differentially expressed on lesion and spleen amastigotes. This observation might provide a base for further understanding of yet unexplored mechanisms that underlie tissue tropism in PKDL associated with Indian kala-azar.