5. SUMMARY

1. Increased production of antileishmanial antibodies of IgG class was demonstrable in the sera of KA patients during the active phase of the disease. The IgG response consisted predominantly of IgG1 isotype followed by IgG2 and IgG3 with negligible amounts of IgG4 isotype. Presence of antileishmanial antibodies of IgE class was also noted in the sera of active KA patients. Antileishmanial antibody level of IgG3 isotype persisted in the sera even after 1-2 yr of apparent recovery. On the other hand, a sharp reduction in the antileishmanial antibody level of IgG1 subclass was noted in recovered cases of KA.

2. Antileishmanial antibodies stimulated in the sera of different KA patients showed variable pattern of reactivity to leishmanial antigens in immunoblot experiments. Despite this variability, majority of the sera recognized certain common bands, particularly around 60-63 kD and 28-29 kD regions. Sera obtained from patients during the late stage of infection (as well as in cases of stibanate-unresponsive KA patients), strongly recognized bands around 20 to 22 kD region in addition to other bands. Parallel immunoblot experiments, using sera from a KA and a PKADL patient, against different *L. donovani* isolates (one isolated from a PKADL patient and two from two different KA cases), demonstrated that the antigen-recognition pattern of the KA or PKADL sera against all the three different isolates were
remarkably similar. Predominant bands recognized by the KA/PKADL sera were located around 14-16 kD, 28 kD, 50-54 kD and 63 kD regions. Results also showed that all the KA sera recognized the 28-29 kD band(s) as the predominant antigen in the CSA preparation.

3. Further analysis of promastigote antigens recognizable by antileishmanial antibodies belonging to different subclasses of IgG demonstrated that the recognition of bands around the 60-63 kD and 20-22 kD regions were specific and mainly associated with the IgG1 subclass of antibodies. The IgG2 antibodies, on the other hand, strongly recognized the 28 kD band. It was also evident that antileishmanial antibodies of IgG2 isotype recognized the band(s) around 50-54 kD region. The IgG3 antibodies showed a broad reactivity pattern to leishmanial antigens, predominantly in the region between 14 to 34 kD which persisted even in recovering patients after successful chemotherapy. The subclass-specific immunoblot reactivity of serum from PKADL patients was found to be somewhat similar to that observed with the serum obtained from the cured KA patients, with broad reactivity being restricted primarily to the IgG3 subclass. Results of immunoblot experiments suggest that leishmanial antigens, particularly, those around 60 to 66 kD and 28 kD regions, may have diagnostic importance while bands around 20 to 22 kD region can be of potential prognostic value.
4. Suppression of the lymphoproliferative response of peripheral blood mononuclear cells obtained from active KA patients to \textit{in vitro} stimulation with leishmanial antigen was noted in this study. A reduced responsiveness of their peripheral blood lymphocyte to \textit{in vitro} stimulation with mitogens was also documented. Moreover, peripheral blood CD4\(^+\)/CD8\(^+\) cell ratio was shown to diminish during the active phase of the disease due to depletion of CD4\(^+\) cell population, though the number of CD8\(^+\) cells remained unchanged. However, the number of CD4\(^+\) cells as well as CD4\(^+\)/CD8\(^+\) cell ratio returned to normalcy following successful chemotherapy.

5. Experimentally induced visceral leishmaniasis in BALB/c mice showed an increase in the visceral organ (liver and spleen) weights with the progression of the infection. The magnitude of the increase in liver weight was about 2 fold of the initial value. In contrast, change in spleen weight was more prominent as about 21 fold increase in the mean spleen weight was demonstrable in animals at the late stage of infection as compared to that of control value.

6. The degree of parasitemia in the liver of \textit{L. donovani} infected mice showed a gradual increase from day 10 onwards and reached a maximum value around day 60 following which a decline in the liver parasite load was noted. The spleen parasite load, on the other hand, showed a gradual increase initially upto post infection day 20 or so and reached
high values during the late stage of illness.

7. Histopathologic examination of liver tissue sections from *L. donovani* infected BALB/c mice revealed that the radial distribution of hepatocytes around the central vein (as seen in normal mice) was altered as a result of infiltration of mononuclear and other cells around the central vein. Influx of mononuclear cells around the central vein was more abundant during the late stage of infection (around 120 day post infection period) with obliteration of cellular architecture of the liver which, in turn, was associated with tissue granulomatous response.

8. Longitudinal studies using *L. donovani* infected mouse model demonstrated that antileishmanial antibody levels in the sera of BALB/c mice increased during the course of leishmanial infection. Antileishmanial antibodies could be detected by ELISA as early as on day 10 following infection. Use of a laboratory passaged strain (ASI) or the inoculating strain (BI 2302) as coating antigen gave comparable results although, the inoculating strain (BI 2302) was more sensitive in the assay than that of the strain ASI.

9. Sera collected from mice on day 45 following infection recognised leishmanial antigens primarily around 50 to 63 kD region. The intensity of reactivity pattern as well as multiplicity of bands increased with the progression of infection as more antigens around 14-18 kD, 28-29 kD, 50-54
KD and 63 KD were recognised. These results also suggested that the inoculating strain (BI 2302) was more sensitive when used as antigen in immunoblot assay as compared to that of the strain ASI. Certain leishmanial antigens were identified which may be of considerable diagnostic (50-54 KD and 63 KD) as well as prognostic value (14-18 KD).

10. Longitudinal studies using L. donovani infected BALB/c mice demonstrated a gradual suppression of the lymphoproliferative response of the infected splenic lymphocytes to in vitro stimulation with mitogen as well as leishmanial antigen. However, removal (though partial) of adherent cells led to partial restoration of in vitro lymphoproliferative response to both mitogen and leishmanial antigen in BALB/c mice at late stage of L. donovani infection (around day 60 post infection period). Antileishmanial chemotherapy of infected mice also restored their lymphoproliferative response in vitro to both mitogen as well as leishmanial antigen.

11. Splenic lymphocytes derived from drug treated (leishmania immune) mice were shown to be stimulated in vitro with leishmanial antigen fractions F3 (46-60 KD), F6 (26-32 KD), F8 (17-20 KD) and F9 (14-16 KD). The other antigen fractions [F1 (>80 KD), F2 (61-80 KD), F4 (37-45 KD), F5 (33-36 KD), F7 (21-25 KD)], however, did not show any such ability. In a similar experiment; 42 KD (M42), 30 KD (M30) and 28 KD (M28) exhibited the ability to stimulate splenic
lymphocytes derived from immune mice while the 63 kD (M63) antigen failed to induce any such response.

12. Protective efficacies of different antigen preparations (heat-killed, formalin-killed and sonicated-killed) of *L. donovani* promastigotes were evaluated against challenge with leishmanial amastigotes in BALB/c mouse model. All the three antigen preparations conferred, more or less, comparable levels (39-52% reduction in the liver parasite burden) of protection against leishmanial challenge. Further, the HK and FK antigen preparations induce only weak to moderate levels of cell-mediated and humoral immune responses in the immunized hosts. However, the SK antigen preparation was unable to induce any significant level of immune response under comparable experimental conditions.

13. Treatment of *L. donovani* infected mice with the immunomodulator (protein A) led to moderate level of reduction (34%) of the parasite burden in the liver of these animals. However, a combination treatment of protein A with the antileishmanial drug stibanate led to marked reduction (80%) of the liver parasite load as compared to the 63% reduction noted in the liver of infected animals treated with the drug (stibanate) alone.