4. DISCUSSION
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High level of antileishmanial antibodies was shown to be present in the sera of all the active cases of Indian KA (Table 3.1.2). However, considerable variations were noted in the serum antileishmanial antibody titres which could be a reflection of duration and severity of the illness (Ghose et al, 1980; 1994). The PKADL patients, on the other hand, displayed a more restricted serological response which is consistent with the localized nature of the disease profile. Persistence of antileishmanial antibodies was demonstrated in the sera of treated KA cases 1-2 years after apparent clinical recovery. These results agree well with the earlier observations where significant amounts of antileishmanial antibodies were demonstrable in the sera of KA patients even after 1 year of successful chemotherapy (Haldar, 1982). Similar results were also obtained by Hailu (1990) where antileishmanial antibodies were shown to persist in the sera of 10% of the Ethiopian VL patients even after 50 months of clinical recovery following treatment. Persistence of detectable levels of antileishmanial antibodies in some of the recovered patients could actually be due to the presence of residual parasites. In fact, persistence of leishmanial parasites was documented in recovered leishmaniosis patients (Aebischer, 1994) as well as in experimental animal models (Muller, 1992; Aebischer et al 1993). Recent studies demonstrated that lymph node dendritic cells may carry leishmanial parasites and, serve as a depot for sustained
release of antigen to stimulate both B and T cells (Moll et al., 1995; reviewed by Ahmed and Gray, 1996). However, these considerations do not rule out the possibility of reinfection in the recovered patients as they continue to live in the leishmania endemic areas.

Earlier studies showed that antileishmanial antibodies of IgG class should be of more importance than IgM class for the detection of VL or PKADL cases (Ghose et al., 1980; Haldar et al., 1981). However, persistence of serum IgG antibodies raises an important issue regarding their applicability to detect an active infection. The question was addressed here by the determination of IgG subclass-specific antileishmanial antibody levels in the sera of active as well as treated kala-azar cases. Results demonstrate that antileishmanial antibodies in active KA sera belong to the IgG1, IgG2 and IgG3 subclasses, with very little IgG4 (Fig. 3.1.4). Comparable increase of different IgG subclass antibody levels, although lesser in magnitude than those obtained in KA sera, was also noted in PKADL sera. These results are in partial agreement with the information available so far from studies with Sudanese (El Amin et al., 1986) and Brazilian (Zwingenberger et al., 1990) KA patients, in which elevation of serum IgG1 and IgG3, but not IgG2, levels was noted. However, in a recent study (Deplazes et al., 1995) antileishmanial antibodies of both IgG1 and IgG2 subclasses were shown to be stimulated in canine visceral leishmaniasis. In certain chronic parasitic diseases, the IgG4 isotype
response was found to predominate and was also accompanied by the elevation of serum IgE and cytokine (IL-4) levels (Iskander et al, 1981; Hussain and Ottesen, 1986). Although, elevation of IgE in KA sera was documented in this (Table 3.1.4) as well as in earlier studies (Zwingenberger et al, 1990), data presented here show minimal involvement of IgG4 during the active phase of disease in Indian kala-azar patients.

The IgG1 and IgG3 antibodies are known to possess high levels of complement-fixing and opsonizing activities (Burton et al, 1986). Thus, the elevation of total (Zwingenberger et al, 1990) as well as leishmania specific IgG1 and IgG3 antibodies may be responsible for the observed lowering of C3 levels (Ghose et al, 1980), tissue damage and other inflammatory reactions (Veress et al, 1977) noted in active KA. IgG2 antibodies, on the other hand, are poorly complement-fixing and opsonizing (Unkeless et al, 1988) and therefore, are less likely to contribute to such abnormal pathology. Earlier studies (El Amin et al, 1986; Zwingenberger et al, 1990) failed to document any significant rise of IgG2 level in the sera of Brazilian and Sudanese VL patients, although antileishmanial antibodies of IgG2 subclass could be demonstrated in majority of Indian KA sera (Fig. 3.1.4). It would be of interest to determine whether the synthesis of IgG2 antibodies is also reflected in the rise of total IgG2 levels in the sera of these patients.
Results presented here demonstrate that both IgG1 and IgG2 antileishmanial antibodies decreased following antimonial therapy, the decrease being more marked for IgG1 than IgG2 subclass. The IgG3 antibody level, however, showed some degree of persistence in the sera of treated KA cases. These results are only in partial agreement with the findings of Elassad et al (1994) where L. donovani specific IgG1 and IgG3, but not IgG2, antibody levels were shown to decrease significantly in the sera of KA patients following chemotherapy. In another study, antileishmanial IgG2 level was shown to remain elevated in the sera of L. infantum infected dogs, while the IgG1 antibody level disappeared almost completely following chemotherapy and subsequent recovery (Deplazes et al, 1995). Therefore, the nature of the parasite antigen as well as the host-parasite interaction do seem to play a key role in the regulation of different IgG isotype response in the infected hosts (Heiner, 1984). All these considerations suggest that determination of subclass-specific antileishmanial antibody levels may help in following the course of leishmanial infection as well as in the differentiation between an active infection and past exposure to leishmanial antigens.

The ELISA data generated in this study was based on the use of crude soluble antigen (CSA) derived from L. donovani promastigotes as the coating antigen. Initial experimentation (Table 3.1.1) had shown that the CSA was marginally more sensitive than the whole cell promastigotes (used in the
native form or after formalinization) as the coating antigen. It is conceivable that the use of intact whole cell promastigotes in the ELISA primarily allowed the recognition of parasite surface antigens by the immune sera whereas solubilized fractions of both cell surface and intracellular antigens present in the CSA preparation were involved in ELISA reaction between the CSA and immune sera. The fact that the whole cell promastigotes as well as the CSA were equally effective as the coating antigen in generating comparable ELISA results suggest that antibody responses in KA patients were probably stimulated against both cell surface as well as intracellular antigens of the parasite.

The SDS-PAGE analysis of the whole cell promastigotes and the CSA preparation showed differences in their banding profiles (Fig. 3.1.3). The whole cell promastigotes (strain ASI) showed an intense banding pattern around 54 kD region which was missing in the CSA preparation. In fact, presence of an intensely stained band around 54 kD region was a common feature of all the three different L. donovani strains which showed considerable similarities in their SDS-PAGE profiles (Fig. 3.1.1 & Fig. 3.1.2). Although, similarity in molecular mass of subunit proteins cannot be considered as a definitive proof of structural identity between different leishmanial isolates, the homology observed between the SDS-PAGE patterns of different strains of L. donovani suggests structural relatedness amongst these strains. It may be mentioned here that two of these strains (BI2302 and Mohan) were maintained
through animal passages while the strain ASI was maintained through in vitro cultures only. Incidentally, the in vitro passaged strain ASI failed to induce any significant degree of visceral infection when inoculated into hamsters or BALB/c mice. Therefore, the SDS-PAGE analysis, clearly, appears inadequate to distinguish between the virulent and avirulent strains of *Leishmania donovani*.

The overall similarity between the three *L. donovani* strains in respect to their SDS-PAGE banding profiles is also reflected in their immunoblot reactivity patterns which were strikingly similar (Fig. 3.1.9). The three different *L. donovani* strains used in this experiment were isolated from three different individuals showing clinical manifestation of KA (LD1 & LD2) and PKADL (LD3). The fact that serum from a KA patient recognized *L. donovani* strains isolated from KA or PKADL patients equally well and vice versa suggest that the leishmanial organisms involved in KA or PKADL cases may not necessarily be different. On the other hand, variability in the immunoblot profiles generated with different KA and PKADL sera against a given *L. donovani* strain (Fig. 3.1.7) may reflect certain diversity in the host's antibody response towards different leishmanial antigens. Despite these variabilities, sera from KA patients recognized certain common leishmanial antigen bands, particularly in the region of 60-63 kD and 28-29 kD (Fig. 3.1.7). Leishmanial parasites are known to express a 63 kD surface glycoprotein (gp63) (Bouvier et al, 1985) which is a major antigen recognized by
sera from patients with different forms of leishmaniasis, (Dos Santos et al, 1987; Heath et al, 1987; Reed et al, 1987; Bogdan et al, 1990). The 60-63 kD band(s) in our immunoblot results (Fig. 3.1.7) is likely to arise as a result of recognition of this major surface glycoprotein in different stages of deglycosylation (Fong and Chang, 1982). The 28-29 kD band needs special mention as it is recognized by sera from a large number of KA cases including the stibanate unresponsive ones (Fig. 3.1.7, lane e and Fig. 3.1.8). This band appears to represent a soluble antigen(s) as bands in identical position are also discernible in immunoblot patterns generated with different KA sera against the CSA (Fig. 3.1.10). A 28 kD cross-reactive antigen was identified in the parasite L. donovani chagasi which was recognized by sera from Brazilian patients infected with L. donovani chagasi, Trypanosoma cruzi as well as Mycobacteria, but not by control sera (Reed et al, 1987). Although the 28 kD antigen reported here was not recognized by control sera, its cross-reactivity pattern with sera from patients with unrelated parasitic and mycobacterial diseases remains to be established.

Qualitative analyses of immunoblot data suggest that certain leishmanial antigens are preferentially recognized by a particular subclass of antibodies. To our knowledge, this is the first study in which attempts were made to analyze leishmanial antigens with respect to their reactivity pattern to antibody molecules of different heavy-chain isotypes.
Thus, the 28 kD band appears to be preferentially recognized by the IgG2 isotype, while 20 to 22 kD and 60 to 63 kD bands are better recognized by IgG1 and IgG2 isotypes. Although there is suggestive evidence (Hammarstrom and Smith, 1986) to show that the IgG2 response is produced predominantly against carbohydrate antigens while IgG1 and IgG3 are generally stimulated against protein antigens of certain bacteria, such a generalization may not necessarily be true for all microbial antigens. In fact, leishmanial antigens recognized by IgG1 antibodies appear to be different from those recognized by IgG3, the antigen recognition pattern of the latter isotype being remarkably similar among different KA and PKADL patients studied. Thus, it appears that the isotype switch of Ig-heavy chain may depend on the nature of the antigen(s) or its epitopes and is regulated by cytokines released as a result of a complex interaction between the antigen epitopes, their receptors and major histocompatibility complex of various antigen-processing cells (Vercelli et al, 1989; Splawski and Lipsky, 1991).

Antileishmanial antibodies were earlier shown to persist in KA patients even after several months of chemotherapy and clinical recovery (Haldar et al, 1983; Hailu, 1990). Immunoblot data (Fig. 3.1.12) show that the antileishmanial activity demonstrable in the serum of a cured KA patient is, more or less, restricted to IgG3 antibodies which recognize antigens around the 14 to 34 kD region.
KA patients are known to show T cell unresponsiveness to leishmanial antigens during the active stage of the disease (Haldar et al., 1983; Ho et al., 1983; Sacks et al., 1987; Carvalho et al., 1989). This is also evident from the data presented in this study which show impairment of the in vitro proliferative response of lymphocytes from active KA patients towards both leishmanial antigens (data not shown) and, at least, partially towards T cell mitogens (PHA-P and/or Con A) (Table 3.1.6). Further, a significant reduction in the number of CD2\(^+\), CD4\(^+\) cells as well as in the CD4\(^+\)/CD8\(^+\) cell ratio was observed in the peripheral blood of active KA cases (Table 3.1.5). These results are compatible with those obtained with Kenyan (Koech, 1987) and Brazilian (Carvalho et al., 1992) visceral leishmaniasis cases where a similar reduction in the number of CD4\(^+\) cells as well as in the CD4\(^+\)/CD8\(^+\) cell ratio was noted. In contrast, CD4\(^+\)/CD8\(^+\) cell ratio in the peripheral blood of Mediterranean VL cases was found to remain within the normal limits (Cillari et al., 1991). It is difficult to explain such variable findings which may arise as a result of ethnic differences amongst the VL cases belonging to different geographic region. Alternatively, involvement of different variants of the parasite *Leishmania donovani* in different parts of the world may give rise to such differences in the host response. It is also evident from this and earlier studies (Carvalho et al., 1992) that alteration in the CD4\(^+\) and CD8\(^+\) cell population is a manifestation of the disease process, as these values
tend to return to normalcy several months after successful chemotherapy. These results are also consistent with the earlier reports demonstrating restoration of T cell responsiveness in Indian KA cases several months after clinical recovery (Haldar et al., 1983; Sacks et al., 1987; Neogy et al., 1988). It should be mentioned here that no significant alteration in CD4⁺/CD8⁺ cell ratio was noted in the peripheral blood of patients with post kala-azar dermal leishmaniasis (Ghosh et al., 1995), who usually show normal T cell responsiveness to mitogen as well as leishmanial antigen (Haldar et al., 1983; Neogy et al., 1988).

The impairment of T cell responsiveness in active KA cases cannot be explained solely on the basis of reduction of their CD2⁺, CD4⁺ cell numbers in the periphery. Conflicting reports are available regarding the mechanism of this immunosuppression. In Kenyan visceral leishmaniasis, it has been suggested that the immunosuppression is due largely to increased levels of T suppressor cells and reduced levels of T helper cells during the active stage of the disease (Koech, 1987). Removal of adherent cells from the peripheral blood mononuclear cells of Kenyan VL patients restored lymphoproliferative response in vitro to both mitogens and antigens (Koech et al., 1987). On the other hand, Carvalho et al. (1989) failed to restore antigen-specific lymphoproliferative response in active VL cases following depletion of adherent cell population.
Results presented with VL patients showed considerable variation in their immunological responsiveness towards leishmanial antigens as well as mitogens. Variations in the leishmanial antigen recognition pattern was also evident in the sera of KA patients. As mentioned earlier, such variability may arise as a result of the variation in the genetic background, nutritional status and other factors of the human host. Further, differences in the parasitic burden and state of illness may also give rise to such variability in the host’s immunological responsiveness. Admittedly, there are limitations in the design of experiments involving KA patients to address such questions. Therefore, studies were carried out by first establishment of an experimental animal model of visceral leishmaniasis in mice and studying alterations in their pathophysiological and immunobiological status with the progression of the disease. BALB/c mice was chosen for the establishment of experimental infection for the following reasons: (a) availability of genetically defined strain of the animal host, (b) initial susceptibility of BALB/c mice to leishmanial infection (Lsh<sup>S</sup> background), and (c) convenience in handling and cost effectiveness of the model.

Results presented in this study demonstrate that intracardial inoculation of BALB/c mice with <i>L. donovani</i> amastigotes led to a progressive infection with massive hepatosplenomegaly and histopathological changes in liver
that are comparable to those seen in man. Influx of mononuclear cells with occasional granulomatous response was noted in the liver of mice during the early stage of infection. Granuloma formation was, however, accompanied with obliteration of hepatic cellular architecture in animals during the late stage of infection (Fig. 3.2.10A). Hepatic granuloma formation is shown to be dependent upon chemotactic factors that induce the migration of granulocytes, lymphocytes and monocytes from the microvasculature (McElrath et al, 1988). In fact, formation of liver granuloma may be associated with resolution of liver parasite burden (Fig. 3.2.10) which is also supported by earlier studies in murine leishmaniasis model (Squires et al, 1990). Similar type of histopathological changes were noted in the liver of Indian kala-azar patients (Sengupta et al, 1956).

Although the liver parasite load started declining around 60 day following infection, parasite load in the spleen of infected animals continued to rise throughout the study period. These results, however, differ from those reported earlier (Murray et al, 1982) with BALB/c mice where parasite burden in both liver and spleen showed a decline during the late stage of infection following an early increase (upto about 4 week period after infection). These differences between the two studies may arise as a result of subline variations amongst the BALB/c strains used in these experiments. Alternatively, intracardial route of inoculation with the amastigotes followed in this study as against the
intravenous route of inoculation followed in earlier studies (Murray et al, 1982; Reiner, 1982; Squires et al, 1990), may give rise to such variations in the progression of the infection pattern.

As in the case of VL in man (Ghose et al, 1980), *L. donovani* infection in BALB/c mice led to the production of plenty of serum antileishmanial antibodies of IgG class. Such antibodies could be detected in the sera of animals as early as on day 10 following infection. With the progression of the disease, the antibody level continued to increase which could be detected equally well by using the whole cell promastigotes of the inoculating strain (BI2302) or the laboratory passaged strain (ASI) or the CSA preparation derived from the strain ASI (Fig. 3.2.11).

Despite the presence of high levels of circulating antibodies, *L. donovani* infected mice showed a gradual impairment of their lymphoproliferative response to *in vitro* stimulation with mitogen (Con A) as well as leishmanial antigen. The degree and specificity of such impairment were, however, dependent on the stage of illness and inversely related to the increase in the serum antileishmanial antibody titres or splenic parasitic burden. These results, if extrapolated to human VL cases, may explain the apparently conflicting results available in the literature on the variability of the lymphoproliferative response in these patients to T cell mitogen stimulation (Haldar et al, 1983; Ho et al, 1983; Sacks et al, 1987). Thus, it is likely that
patients during the early stage of the disease with moderate levels of antibodies (equivalent to the stage of 20 to 50 day infection period in BALB/c mice) would show variable degree of lymphoproliferative response to mitogen stimulation, although such response towards leishmanial antigens would be generally absent. Patients during the late stage of illness (equivalent to 60-120 days following infection in mice) with high levels of circulating antibodies are not likely to show lymphoproliferative response towards both leishmanial antigens and mitogens. On the other hand, individuals at a very early stage of infection (equivalent to day 10 following infection in mice) are expected to produce lymphoproliferative responses to both leishmanial antigen and mitogens. Presumably, such individuals at this early stage of infection are not likely to show clear cut clinical symptoms and their antileishmanial antibodies will not be detectable serologically by the conventional tests.

Results presented in this study somewhat differ from those reported by other workers (Murray et al, 1982; Nickol and Bonventre, 1985) where gradual restoration of lymphoproliferative response to both mitogen and leishmanial antigen was noted in L. donovani infected mice. Such restoration of proliferative response was apparently related to the gradual decrease of the parasite burden in the liver and spleen of mice 6 to 8 weeks following L. donovani infection (Murray et al, 1982; Nickol and Bonventre, 1985). All these considerations suggest that, unlike the intravenous
route of inoculation used by earlier workers, intracardial route used in this study led to a progressive infection in BALB/c mice with features similar to those encountered in man (Wilcocks and Manson-Bahr, 1972) as well as in hamsters (Stauber, 1966; Dasgupta, 1997).

As noted in the case with visceral leishmaniasis in man and hamsters, the observed impairment of lymphoproliferative response to leishmanial antigen/mitogens during the acute phase of infection in BALB/c mice could, at least, be partially reversed by the removal of adherent cell populations (Fig. 3.2.17 and Fig. 3.2.18). In an earlier study, Nickol and Bonventre (1985) demonstrated that the adherent spleen cell population in L. donovani infected mice was responsible for unresponsiveness of their splenic lymphocytes to mitogen as well as leishmanial antigen. These workers were able to identify the adherent cell population as macrophage-like cells with suppressor activities. It is quite possible that these "adherent suppressor" cells generated in the leishmania infected animals released some soluble factors to mediate the observed inhibition of lymphoproliferative response. Recently, immunosuppression associated with certain parasitic diseases (like malaria, trypanosomiasis etc.) was shown to be mediated through the production of nitric oxide by "suppressor macrophages", which down regulate the T cell proliferative response to various parasite antigens and mitogens (Sternberg and Mcguigan, 1992; Rockett et al, 1994). Interestingly enough, activated T helper cell clones from L.
major infected mice did not produce any nitric oxide (Thuring et al, 1995). Other possible mechanisms suggested to explain the observed immunosuppression in *L. donovani* infected mice include: (a) defective processing and presentation by leishmania infected macrophages (Fruth et al, 1993; Prina et al, 1993), (b) the elaboration of suppressive mediators like prostaglandins etc by the leishmania infected animals (Saha et al, 1995).

Sera obtained from mice during the course of leishmanial infection showed variable pattern of reactivity towards leishmanial antigens (Fig. 3.2.12 and Fig. 3.2.13). Out of multiple antigens recognized by infected sera, bands around 60-63 kD, 50-54 kD, 28-29 kD and 14-18 kD regions need special mention. It may be noted that the leishmanial promastigotes contained a major polypeptide around 54 kD region (Fig. 3.1.1 and Fig. 3.1.2). This major polypeptide may represent the structural protein tubulin of leishmania which is composed of 55 kD subunit protein(s) (Dwyer, 1981). On the other hand, Lezama-Davila and Gallagher (1995) recently demonstrated that the major glycoprotein (gp63) of the parasite *L. mexicana mexicana* migrated in the region of 50 kD in SDS-PAGE under reducing condition. Whatever may be the case, the fact that this major leishmanial protein(s) was recognized by sera from *L. donovani* infected mice as well as KA and PKADL patients makes it an attractive antigen complex for further analysis. Analysis of leishmanial antigens by SDS-PAGE also gave rise to band(s) around 16 kD region which
appears to be recognized by *L. donovani* infected sera (Fig. 3.1.9, Fig. 3.2.12 and Fig. 3.2.13). Recently, Suffia et al (1995) identified two nuclear proteins of 14 and 18 kD from *L. infantum* that were recognized by sera from all the VL patients tested. Further studies showed that these antigens also contributed towards the induction of cell-mediated immune response in these patients by stimulating proliferative response of their lymphocytes *in vitro*. Our results are consistent with these data since leishmanial antigen fractions F8 and F9 (belonging to the 14 to 20 kD region) induced proliferative response of splenic lymphocytes from immune mice in T cell immunoblotting experiments (Fig. 3.2.21). Apart from these antigens, several other leishmanial fractions (containing antigens in the 26 to 32 kD and 46 to 60 kD regions) also induced lymphoproliferative response *in vitro*. These results were extended by another T cell immunoblotting experiment (Fig. 3.2.22) carried out with fractionated protein antigens of subunit molecular weights 28 kD, 30 kD, 42 kD and 63 kD. Out of these, the 28 kD, 30 kD and 42 kD antigens showed the capacity to induce lymphoproliferative response *in vitro* (Fig. 3.2.22). Interestingly enough, the 63 kD antigen failed to show any significant lymphoproliferative activity in these experiments. In fact, conflicting informations are available in the literature regarding the ability of leishmanial gp63 antigen to stimulate proliferative response *in vitro* of immune T cells (Nascimento et al, 1990; Mendonca et al, 1991;

Our results appear to be consistent with those reported by earlier workers who showed the involvement of various leishmanial antigens in stimulating T lymphocyte response (Melby and Sacks, 1989; Melby et al., 1989; Reed et al., 1990; Laskay et al., 1991; Bahrenscheer et al., 1995; Suffia et al., 1995). Reed et al. (1990) identified two glycoproteins of 30 and 42 kD from *L. chagasi* which elicited strong proliferative response *in vitro* to PBMC from cured VL cases with the production of IL-2. Marked *in vitro* lymphoproliferative response of PBMC from CL patients to stimulation with *L. aethiopica* antigen fractions of molecular masses 36-46 kD, 27-33 kD and ≤ 20 kD was reported by Laskay et al. (1991). In another study, lymphocytes obtained from cured VL patients were found to be stimulated in response to *L. chagasi* semipurified antigens of high molecular weight as well as low molecular weight (≤ 20.5 kD) antigens. Recently, Bahrenscheer et al. (1995) showed that T cells from individuals, who had earlier been cured of VL, recognized and responded to a wide range of antigens which included the ones in the molecular weight range of 13-16 kD, 16-22 kD, 26-34 kD and 42-56 kD.

Results obtained in this as well as earlier studies (discussed as above) suggest that the induction of protective immunity against *Leishmania* is probably mediated by multiple antigens of the parasite. Therefore, studies were initiated
to determine the vaccination efficacy of different whole cell antigen preparations (Fig. 3.3.1). Since there appears to be no general rule governing the relative efficacies of different routes of immunization in the induction of protective immunity against infectious diseases (Liew et al., 1985), the subcutaneous route of immunization was preferred due to its easy applicability and acceptibility as compared to those of the other routes of immunization. All the three antigen preparations showed moderate level of protection (39 to 52%) against leishmanial challenge. This may be due to the fact that only two of these antigen preparations (HK and FK) were able to stimulate moderate level of antibody production whereas none of these were able to produce marked cell-mediated immune response. Similar results were obtained by earlier workers who used killed leishmanial antigen preparations to induce only moderate level of protection in immunized animals (Holbrook and Cook, 1983; Jarecki-Black et al., 1986; Greenblatt, 1988; Modabber, 1990). Admittedly, killed organisms are poor antigens for the stimulation of cell-mediated immune response which usually require live, replicating antigen preparations (Rivier et al., 1993). In fact, use of radioattenuated parasites as vaccines yielded improved level of protection against leishmanial challenge in the mouse model (Alexander, 1982; Howard et al., 1982; Rivier et al., 1993). Further, such protection was shown to be correlated well with the stimulation of lymphocytes that were able to activate intracellular killing of Leishmania (Rivier
An alternate approach to improve the vaccination efficacy of killed antigens is based on the use of appropriate adjuvants or immunomodulators. Thus, addition of agents like *C. parvum* (Mitchell and Handman, 1983), BCG (Fortier *et al.*, 1987), glucan (Holbrook *et al.*, 1981; Holbrook and Cook, 1983) were shown to increase the protective efficacy of killed promastigote antigens. Further, administration of some of these immunomodulating agents alone were shown to exert antileishmanial effect by reducing parasitic burden in the *Leishmania* infected animals (Smrkovski and Larson, 1977; Fortier *et al.*, 1987). The immunomodulator of microbial origin (protein A) used in this study also induced moderate level of reduction (34%) of the liver parasitic burden in *L. donovani* infected animals (Fig. 3.3.4). However, a combination of protein A with the antileishmanial drug stibanate led to marked reduction (80%) in the parasitic load as compared to the 63% reduction noted with the drug alone. Comparable results were obtained by Dasgupta (1997) where treatment of *L. donovani* infected hamsters with protein A led to significant reduction in their organ parasite load. All these results, therefore, demonstrate the beneficial effect of the use of the immunomodulator protein A with the antileishmanial drug as a combination therapy. Earlier studies already established protein A as a potent immunostimulant and anticancer agent having little side effect, even on multiple administration.
(Ray et al, 1984). The immunomodulatory effect of protein A is likely to be mediated through the production of IFN-gamma (Catalona et al, 1981; Decatenzeiler and Menezes, 1988) and related cytokines which activate macrophages, thereby leading to destruction of intracellular parasites. In fact, various cytokines like IL-2, IL-12, IFN-gamma, GMCSF were used directly as immunotherapeutic agents to treat leishmania infected hosts with variable degree of success (Murray, 1990; Sypek et al, 1993; Murray and Hariprashad, 1995; Murray et al, 1995). Furthermore, use of these cytokines in combination with the antileishmanial drugs considerably improved their therapeutic efficacies (Badaro et al, 1990; 1994a; 1994b; Murray, 1990; Murray et al, 1991; Nabors et al, 1995).

Current information available from the murine leishmaniasis model suggest that the Th1 subset of CD4+ cells (secreting IL-2 and IFN-gamma) are host protective, whereas Th2 subset (producing IL-4 and IL-5) are disease exacerbating (Scott et al, 1988; Heinzel et al, 1989; Liew et al, 1989; Holaday et al, 1991; Locksley and Louis, 1992; reviewed by Nabors, 1997). Differential recognition of Th1 and Th2 cell types by different leishmanial antigens was documented recently (Melby et al, 1989; Reed et al, 1990; Kemp et al, 1993; 1994a; 1994b; Bahrenscheer et al, 1995; Suffia et al, 1995; Nabors, 1997). It is possible that out of various leishmanial antigens, only the selected ones are responsible for the induction of protective immunity through stimulation of appropriate immune cell types in the host.