CHAPTER FIVE

SUMMARY
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The salient findings of the present study are summarized below:

1. Five local strains of *E. histolytica* were axenized in laboratory. Crude soluble antigen (CSA) was prepared from these strains as well as from reference strain NIH:200 by sonication and centrifugation of the sonicated material at 30,000xg for 30 minutes.

2. CSA when subjected to Sephacryl-S-300 gel filtration chromatography showed four fractions. Protein was found mainly in FI and FII fraction whereas carbohydrate was the major constituent in fraction III.

3. Physico-chemical analysis of CSA and its fractions suggests that CSA and FI antigens were predominantly glycoprotein in nature.

4. 10% SDS-PAGE analysis of CSA and its fractions showed several discrete polypeptide bands ranging from >200 kDa to 20 kDa. Similar banding patterns with lesser number of polypeptide subunits were observed in FI proteins. However, discrete polypeptides in the molecular weight of 180 kDa to 30 kDa and 140 kDa to 22 kDa were revealed with FII and FIII proteins respectively. Only few minor bands were seen with FIV protein.

5. Comparison of antigenic activity amongst CSA and its fractions, revealed that the maximum antigenic activity was present in the highest molecular weight fraction i.e. in FI.

6. Major immunoreactive polypeptides of CSA and FI antigens against the whole trophozoite antibody were observed in the 10 to 170 kDa region. However, differences in immunoreactivity of the two antigens were noticed at 116 and 14 kDa for FI antigen and at 84, 30 and 20 kDa for CSA.

7. The efficacy of active immunization with CSA and its four fractions showed that antigenicity and immunogenicity were closely associated with high molecular weight proteins, as 91.6% protection was observed in the FI antigen, whereas the FII and
Fill antigens and CSA provided only 41%, 33% and 41% protection, respectively.

8. Studies on immunofluorescence and Immuno blot assay suggest that the FI antigenic molecules are expressed on the surface of the parasite.

9. The two subfractions of FI antigen which resolved after ion-exchange chromatography, and SDS-PAGE analysis revealed multiple banding patterns.

10. Six stable hybrid cell lines producing anti-FI monoclonal antibodies (viz., MAbs NICED 11 to 16) were generated. Isotype characterization of the MAbs revealed that NICED 13, 14, 15 and 16 belonged to IgM class whereas NICED 11 and 12 were of IgG1 and IgG2a subclasses, respectively.

11. In end point titration, NICED 11 MAb showed the maximum antibody and as well as agglutination titer.

12. With the exception of NICED 11 MAb, multiple banding patterns were observed in immunoreactivity analysis against FI antigen. NICED 11 MAb showed a highly immunoreactive band at 29 kDa region. So, for further studies, NICED 11 MAb was selected.

13. In ELISA and immunoblot assay, MAb NICED 11 reacted intensely with the isolates of *E. histolytica* but did not show any reactivity with other non pathogenic amoebas as well as enteric parasites.

14. The results of immunofluorescence assay suggest that NICED 11 MAb specific antigenic epitopes were present mainly on the surface of the trophozoites.

15. Using NICED 11 MAb as a detecting antibody, a PAb-MAb based multilayer ELISA was developed. The system was found highly sensitive and specific as it could detect 8 ng of amoebic antigen. The system showed complete correlation with microscopic examination in the groups comprised of known cases of amoebiasis and controls. The test was found even 20% better than CIEP. This test can be useful for the routine serodiagnosis of present cases of amoebiasis.

16. To avoid delay, a much simpler and quicker Dot-immunobinding assay has been
developed for the detection of amoebic antigen in stool samples. The test will be good for large field surveys.

17. The direct demonstration of 29 kDa antigen against NICED 11 MAb in all the amoebic stool samples suggests the relation of this antigen with the disease and of single antigen in diagnosis of the disease.

18. The results of immunoblot assay of NICED 11 MAb against various amoebic strains definitely suggests that NICED 11 MAb has propensity to differentiate the pathogenic and nonpathogenic strains of *E. histolytica*. However, this requires further analysis with more number of strains.

19. The homogeneity of purified 29 kDa polypeptide antigen was confirmed by SDS-PAGE. The role 29 kDa antigen in human amoebiasis was confirmed by ELISA.

20. Increase in circulating antibody in experimental animals by immunizing with the 29 kDa antigen reveals the humoral antibody response elicited by the 29 kDa antigen in amoebiasis.

21. Cell mediated immune response was studied in control and the 29 kDa primed experimental animals using macrophage migration inhibition (MMI) test. The average migration index in 29 kDa antigen primed animals was found 0.67 as compared to 1.0 in control animals.

22. The protective effect of 29 kDa antigen was studied in both *in vitro* amoeba agglutination inhibition test and *in vivo* challenge experiments. *In vivo* challenge experiments revealed only 7.1% of immunized animals developed amoebic liver abscess indicating a 92.9% protective efficacy as compared to the control animals.