SUMMARY & CONCLUSIONS
Summary and Conclusions

The present study was carried out to study immunobiology of pyelonephritis in ascending acute and chronic mouse model. The salient features of present investigation are summarized below:

1. Different laboratory strains and uroisolates of *E. coli* were scanned for production and expression of heat shock proteins. All the isolates were found to express two heat shock proteins of 45 and 65 kDa respectively.

2. Bacteria were given heat shock treatments by exposing to different time and temperatures, keeping one parameter constant at a time. Exposure to 42°C for 1 hr resulted in expression of hsp 65 without any loss of viability.

3. The maximum expression of hsp 65 was observed in strains belonging to serotypes 04 and 06. Expression of other proteins was found to be depressed in strain 06 on exposure to 42°C. This strain because of this unique characteristic was used for purification of hsp 65.

4. Hsp 65 from *E. coli* 06 was first run on preparative gel and transferred on to nitrocellulose paper. The material was eluted and injected in rabbits with freund's adjuvant. The preparation was found to be immunogenic.

5. Hsp-65 was purified by affinity chromatography using antiserum raised in rabbits and purity of preparation was checked by SDS-PAGE on 10 percent gel.
6. Acute and chronic infection was set up in female BALB/c mice by introducing infection with *Escherichia coli* strain O6 : K13: H1 by ascending route.

7. Peritoneal, blood and kidney macrophages and neutrophils from infected and control mice were collected using standard methods.

8. Ability of different cell populations for phagocytosis was measured and a decrease in phagocytic ability was observed on progression of disease in all cell populations. This decrease was more marked in renal cell populations as compared with blood and peritoneal cells.

9. The above mentioned cells were stimulated *in vitro* by exposing to hsp-65. Luminol dependent chemiluminescence response was measured and the results showed.
   a) LDCL response of infected mice was significantly high when compared with uninfected mice in all cell populations.
   b) LDCL response increased on progression of disease and was maximal in all cell population in chronic stage i.e. after 150 days of infection.
   c) LDCL response was highest in case of renal macrophages and neutrophils as compared to blood monocytes, blood neutrophils and peritoneal macrophages at all time points measured during infection.
   d) A decrease in time required to attain peak LDCL value was observed in all the cell populations on progression of disease.
e) Decrease in time period was more marked in case of renal neutrophils and macrophages than in peritoneal macrophages, blood monocytes and neutrophils.

10. Cell populations when stimulated in vitro with hsp-65, showed significant increase in peak LDCL response in comparison to uninfected cells however this response was less marked than that observed with whole bacteria stimulated cells.

11. Macrophages from peritoneum and lymphocytes from spleens of infected as well as normal control mice were separated and checked for production of different interleukins like IL-1, IL-2, IL-4 and IFN-γ following stimulation with different antigens like hsp-65, OMPs and porins from *E. coli* as well as non specific antigens like Con A and PHA. Results showed are:

a) With all antigens IL-1 secretion was observed however it was maximum following hsp-65 stimulation in vitro.

b) For splenic lymphocytes of infected animals, hsp-65 was observed to be the best stimulator for IL-2 production out of all the antigens studied.

c) IL-4 secretion was not observed with any of the antigen used to stimulate splenic lymphocytes indicating that TH2 type of response is not operative with hsp-65 antigen.

d) IFN-γ secretion was observed from splenic lymphocytes when stimulated with hsp-65, porins, OMPs as well as non specific antigen PHA. However, IFN-γ secretion was maximum with hsp-65.
e) Secretion of interleukins studied that is IL-2 and IFN-γ decreased respectively at 150th post infection day (chronic stage of the disease) in comparison to 7th post infection day (acute stage of disease) whereas secretion of IL-1 increased with progression of disease.