7. CONCLUSION......
While there has been numerous reports about the effect of systemic administration of IL-1β through intravenous or other routes on various body functions of animals and humans, there is very little information, if any, available on the ability of exogenously added IL-1β to modulate humoral immune response in vivo to different antigens. By using a weak adjuvant like incomplete Freund's adjuvant to emulsify antigens we have analysed the effect of co-injection of IL-1β on the humoral response of BALB/c (H-2\(^d\)) and C57Bl/6 (H-2\(^b\)) mice to complex mixture of *Shigella dysenteriae* antigens as well as defined antigens like Gelonin and Thyroglobulin. We find that IL-1β modulates antigen specific IgG1 type of antibody response to a significantly higher level against all the antigens tested. The response of the mice to all the antigens tested in the presence of IFA and IL-1β is as good as in the presence of CFA.

Since IL-1β is a pleiotropic molecule capable of inducing various biological effects simultaneously including inflammatory response, search was conducted to identify peptide fragments of IL-1β which could only modulate the IL-1β like IgG1 type response in vivo. For this, a computational method was used which took into consideration factors like local hydrophilicity, inverted hydrophobicity, acrophilicity, accessibility, flexibility and antigenicity besides the occurrence of β-turns, to predict domains of IL-1β molecule which are exposed on the surface and therefore are the most likely candidates to be involved in biological activity of the whole molecule. We got excellent results as two out of three domains predicted to be exposed (47-55 and 85-97) indeed had immunomodulating property. It was also found that these two domains are closely spaced in the whole molecule. But the domain 85-97 had the ability to induce inflammatory response such as Prostaglandin E2 synthesis. Peptides bearing the two domains independently and composite peptides bearing both the domains when used in vivo showed IL-1β like ability to modulate antigen specific IgG1 type of response. Another domain of IL-1β representing the N-terminal sequence 4-16 was also reported to have important role in the biological activity of the molecule. Therefore we also synthesised composite peptides bearing the three domains namely 4-16, 47-55 and 85-97 and found that it possessed IL-1β like immunomodulatory activity. Thus this composite peptide may have potential for use as a immunoadjuvant. This needs to be tested. This peptide assumes importance in the absence of any immunostimulating adjuvant for use in human, the immunostimulating property of this composite peptide coupled with lack of any inflammation associated with it, this peptide has a potential for use as an immunoadjuvant.
In order to understand how the 47-55 domain peptide modulates IL-1β like activity the binding of this peptide to IL-1 receptors on different cell types was tested in a competitive binding assay. This involved finding out the optimal binding of $^{[125]I}$-IL-1β to IL-1 receptor on Raji and EL4 cell surface. Since iodination of 47-55 peptide may affect its ability to bind to the receptor, the ability of a large excess of this peptide to displace $^{[125]I}$-IL-1β bound to its receptor on Raji, EL4 and Jurkat cells was tested. It was found that large excess of the eprptide was not able to displace $^{[125]I}$-IL-1β bound to its receptor to any detectable extent. The two domain peptide when present at 250μg/ml concentration could displace $^{[125]I}$-IL-1β bound to its receptor to the extent of 20%. Since IL-1β, IL-1α and IL-1 receptor antagonist bind to the same receptors and IL-1β alone exhibits immunomodulatory activity in vivo, the analysis of the domains of each of these three molecules would reveal some information. It was found that IL-1α and IL-1 receptor antagonist lack the sequence present in the 47-55 peptide of IL-1β. Further analysis of this domain present in the IL-1β molecules of all the species sequenced so far also showed that it is a highly conserved domain and five out of the nine amino acids present in this domain are conserved. Thus the nonapeptide representing the domain 47-55 was subjected to our scrutiny. All the amino acids except Valine present at position 47 of this sequence are hydrophilic. We therefore synthesised different mutants where Val$^{47}$ was replaced by amino acids which are hydrophilic but carry either positive or negative charges. The amino acids at other positions were also replaced to study the effect of mutation on the biological activity of the peptides. It was found that replacement of Valine at position 47 with Aspartic acid or Lysine at the same position considerably enhanced the IL-1β like modulatory activity of the peptides. Gly being the only amino acid with no side chain of its own, contribute nothing to the hydrophilicity of the peptide, we effected a mutation at this position with a hydrophilic Asp. The peptide has no immunomodulating property associated with it but is inflammatory to a small extent. While the change involving Glu$^{50}$ to Ile had lower immunomodulation, Asp$^{54}$→Ile resulted in no loss of immunomodulation. Circular dichroism study of these peptides in aqueous solutions even in the presence of 90% Trifluoro ethanol, an agent which induces α-helical formation, did not induce any structure in the peptides except Mut-1. Due to the presence of two opposite charges at two ends of the Mut-1 peptide possibly resulting in a mini-dipole, it showed a slight propensity to form an helix in trifluoroethanol. This stabilisation might be the reason for the high immunomodulation associated with it. The fact that the peptides did not compete with IL-1β to bind to its receptor and were not capable of forming any α-helical structure indicated
that the immunomodulatory effect of the peptide is not mediated by through binding to IL-1 receptor. But these peptides may be binding to some other molecules on the cell surface by induce fit mechanism or after passive transport to the cytoplasm of the target cell they may be binding to other molecules which is responsible for the observed effect.

Cyclosporin A, an immunosuppressive agent is known to exert its effect through two distinct separate effects on the release of endogenous IL-1β and IL-2 from their respective producer cells whose effect can be overcome by adding exogenous IL-1β and IL-2. Since T_h2 cells need no IL-2 but have IL-1 receptors on their surface we thought that IL-1β would alleviate the CsA suppression by inducing T_h2 to proliferate. This will be a good model system to monitor the efficacy of exogenously added IL-1β activity. We found that IL-1β can alleviate suppression of antigen specific or mitogen induced T cell response by Cyclosporin A. The composite peptide bearing the two domains 47-55 and 85-97 also alleviated Cyclosporin A induced suppression.

In conclusion, we by looking at the ability of human IL-1β to modulate humoral immune response against a wide range of antigens varying from complex S. dysenteriae to well defined gelonim, have devised an in vivo assay which takes into consideration the switch to IgG1 isotype antibodies. We have also devised a novel in vitro assay system for IL-1β which when added exogenously, has the ability to alleviate the suppression caused by Cyclosporin A. Different regions of IL-1β were predicted by computational methods to be exposed and tested for their IL-1β like bioactivity both in vivo and in vitro. Our observations regarding the immunomodulatory and non-inflammatory 47-55 peptide have corroborated others observations. We by effecting point mutations in this 47-55 domain have come up with a mutant Val^K7→Asp, which has high immunomodulatory property. The structural studies involving CD and preliminary NMR work have shown that structural differences brought about by this mutation are responsible for this enhancement. Later by devising novel composite peptides consisting of 4-16, 47-55 and 88-101 domains separated by diglycine bridges we could enhance the immunomodulatory properties keeping the inflammatory responses under check. The ability of these immunomodulating peptides to act independent of IL-1β receptors coupled with their non-inflammatory nature opens up a potential for these to be used as immunoadjuvants.