Summations

Man will occasionally stumble over the truth, but most of the time he will pick himself up and continue on.

-- Sir Winston Churchill

Summations

The dream of a disease free earth is illusory, however, few infectious diseases which harry humanity may be consigned to obvillion like small pox. The 21st century shall hopefully see many of these diseases being eradicated. Investigating the immune system under a virus infection involves many theories and techniques that should go hand in hand.

The present work has attempted to throw some light in certain fields. Initially, it was shown that it is possible to predict a potential antigenic determinant from the linear sequence of JEV Egp. The antigenic determinants (B cell epitopes) were predicted by methods based on physico-chemical properties and even neural networks for β-turn prediction. These epitopic regions were analyzed for their homology amongst different strains of JEV and flaviviruses for insights about their cross reactive or specific nature. Peptides were synthesized that represented the epitopes and their antigenicity was checked along with their ability to elicit JEV reactive polyclonal immune response. One region was selected for indepth study for structure and antigenicity studies by synthesis of overlapping peptides. Various ELISA conditions that would affect the structure of the overlapping peptides helped in determining the structural conditions for optimal antigenicity. Molecular modeling studies and these practical experiments were done in tandem to arrive at the a stable conformation required for antigenicity. The antigenicity was shown by binding of these peptides with anti-JEV MAbs. Significantly, peptides linked covalently to the substratum (plastic surface) were more reactive than the normal coated peptides. Stabilized structures of the otherwise random orientation of the peptide could be one of the explanations for higher reactivity. The studies show that it is indeed possible to delineate an epitope capable of reacting with the JEV reactive monoclonal antibodies, hence antigenicity.

In the above studies there was an observation of a cross reactive anti-JEV MAb binding to this epitope which was surprising since it belonged to specific region by homology patterns. This observation was studied in more detail by raising MAbs against this epitope. In addition, a MAb is an excellent reagent in immunology. The MAb generated against this epitope was seen to be binding to the virus. It was further shown that it could neutralize the virus. The fact that this peptide was capable of eliciting neutralizing antibody proved beyond doubt that this epitope was virus neutralizing and specific determinant. In addition, studies with a different JEV strain (Sri Lankan isolate) showed that this MAb was specific for virus

neutralization (Indian strain) and not for binding. Hence, it also proved that antigenicity was different from virus neutralization. It could be interpreted that the chosen peptide/epitope was able to attain a structure that was near native as a consequence of which strain specific virus neutralization antibodies are seen.

Once it was empirically shown that the B cell epitope was capable of eliciting neutralizing antibody response, this peptide was used for studies which would make the epitope closer towards a candidate for peptide based vaccine. This was tested by linking the peptide to T helper epitope that was derived from Egp of the same virus based on our earlier published studies. The purpose of incorporating T cell epitope from Egp served dual purpose, one because it is known that the immune system maintains a pool of T memory cells and the endemicity of JEV is well known. Hence, this peptide which can prime the immune system can capitalize on the continuous stimulation in the JEV endemic areas. The T helper activity was demonstrated by measuring the IgM and total Ig response at various time intervals. Since the dependence of T helper activity on the haplotype in the population is known, it is mandatory to check the response in different haplotypes. Hence, studies were done using different mouse strains. It was shown that this peptide had a bias towards one particular haplotype which is not at all surprising. A final chimeric peptide was synthesized that had a more efficient T cell epitope.

The final chimeric peptide had a more efficient T helper epitope and was assayed as a candidate peptide vaccine. The experiments with the final chimeric peptide were done along with the commercially available JEV vaccine. Haplotype dependence was also checked with this chimeric peptide. A vaccine should be able to elicit efficient responses in general population, hence outbred strains of mice was also used for study. Antibody response was also compared with mice immunized with B cell peptide only. As expected from earlier experiments response was better in C3H ($H\text{-}2^k$) mice that were immunized with chimeric peptide. However, the antibody response `lagged' in terms of time and antibody titre as compared to vaccine. Finally all immunized mice were challenged with live virus. The vaccine immunized mice were 100% protected irrespective of the strain of mice and only chimeric peptide immunized C3H mice showed 50% mortality as reflected by the efficient antibody response. Hence, the chimeric peptide containing single copy of T and B cell was able to protect mice (C3H) against lethal virus challenge. Immune response to peptides may be affected by the individual's haplotype, which may lead to unresponsiveness in a population to

a vaccine based on a single peptide. Hence, it would be imperative to incorporate many epitopes so that all the immunodominant protective clones of both B and T cells are stimulated. Peptide vaccines offer advantages like ease of manipulation and addition of new epitopes.

Though the immune response was not comparable with vaccine it is significant that only a single copy of both T and B cell epitope is capable of eliciting protective immune response. In protection the role of challenge virus is not ruled out. As a matter of fact, this is essentially prime and boost strategy where priming was done by peptide and boosting effect was due to challenge virus dose. This has been shown to be a mechanism by other workers also. In other words, the peptides need not elicit an efficiently protective immune response. The approach that has been followed here can help develop peptide based vaccines against JEV. The peptides may be inoculated to prime the individuals and since JEV is endemic in many areas of the globe, the natural infection may give rise to the protective immunity in the individual. It would be essential to have multiple T cell epitopes to be included in the future peptide based vaccine in order to overcome genetic restriction of the T cell recognition of peptides. Similarly even multiple B cell epitopes would helpful for the overall effectivity of the vaccine. Incorporation of CTL epitopes might be beneficial in a peptide based vaccine since all the arms of the immune system would be stimulated.

Presently, there is no peptide based commercial vaccine. This doesn't mean that peptides have been neglected for their poor responses. Successes have been slow in the field of peptide vaccines which might be due to factors like, our limited understanding about the immune system. The real success story of peptides as vaccines has been against FMDV. A single dose of peptide was capable of protecting swine from lethal infection. Peptide based vaccines that are in developmental and clinical trial stages include malaria and HIV vaccine. At the same time since there are numerous new generation vaccines being developed, the safety and efficacy of these vaccines should be addressed (**Capron *et al*, 1994**).

The work has undoubtedly identified a neutralizing epitope that can be incorporated in a peptide based vaccine. In spite of this there are other studies that need to be done. The predictive programs should be modified and developed so that the outputs are accepted with more confidence. This is possible only with our increasing understanding of immunology and biophysics and also better processing speeds of computers like parallel proessing and neural networks. It would be worthwhile to study immune response against

more than one T and B cell epitopes so that the best combinations can be decided and incorporated. Also multiple copies of these epitopes could be synthesized on a single molecule in order to make it more immunogenic as in multiple antigen peptide. One could also introduce certain CTL epitopes so that the immune response triggered is a complete one. Sometimes it has been shown that attaching a lipid moiety can drastically increase the immune response against peptides. One needs to study the immune response against such novel vaccine candidate in more detail. It would also be interesting to check if the immune response is preferentially of T_h1 or T_h2 types. This would help us in the trying to mimic the immune response in case of natural infection. Cytokine profiles would have to be assayed for this purpose. It is now possible to modify the type of immune responses that are particularly suited for the specific purpose. Finally since these studies were aimed at developing vaccines, these must be tested in higher mammals especially in this case, swines as it is a amplifying host of JEV in case of its natural life cycle.

The time has come to make use of the new knowledge not only in the field of immunology but also in diverse fields to design a new and more effective vaccine. The 21st century shall see the development of *designer vaccines*. The pace may be slow but the developments in this field promises to be lively ones. To conclude, the lines of Confucius are most appropriate '*It does not matter how slowly you go so long as you do not stop*'.

REFERENCES

Capron A, Locht C, Fracchia GW (1994) Safety and efficacy of new generation vaccines. ***Vaccine***; 12: 667