CHAPTER 5

DISCUSSION
5.1 Introduction

Rigorous structural analysis of the three antibodies has provided a description of the conformational features associated with polyclonal immune response against PS1. The antibodies exhibited similar CDR conformation while recognizing the common immunodominant epitope on the antigen. However, in the absence of the antigen, these antibodies display dissimilar paratope structures. The epitope exhibits a β-turn conformation for the otherwise flexible peptide antigen when bound to any of the antibodies. This chapter discusses the implications of the observations made in this study, in the light of present knowledge regarding immune responses.

5.2 Common conformation of immunodominant epitope

The Fab-peptide crystal structures provide a description of epitope-paratope interactions and conformational features of PS1 when bound to the three independent antibodies. In all the complexes, the major energetic contribution to antibody binding of PS1 comes from interactions formed by the four residues DPAF. The murine immune response against PS1 progresses in such a way that, although the primary response is directed against diverse determinants, the antibodies in the secondary response are unanimous in their specificity for the DPAF stretch (Agarwal et al., 1996). This immunodominant nature of DPAF is in accordance with the observation that same residues exhibit maximum interactions with the paratope in all the three cases.
Historically, immunodominance of an epitope has been attributed to properties such as static accessibility, hydrophilicity and mobility that relate to the exposed regions on the molecular surface in the case of protein antigens (Hopp, 1993). For peptide antigens, the inherent flexibility could facilitate adaptability while binding to diverse paratopes from the pre-immune repertoire and, thus, be instrumental in eliciting an immune response (Getzoff et al., 1988; Berzofsky, 1985). It was shown that the peptide PS1CT3, against which mAbs were generated, does not have a single definite conformation in aqueous solution as observed from the proton-decoupled $^{13}$C Nuclear Magnetic Resonance and Circular Dichroism studies (Agarwal et al., 1996; Nayak et al., 1998). PS1 did not indicate propensity for any regular secondary structure, when analyzed experimentally and computationally. Therefore, a priori, it was anticipated that the ability of PS1 to take up a multitude of conformations would be relevant in defining the immunodominant nature of the DPAF epitope. However, contrary to this, DPAF exists in a similar conformation in all the three complexes.

The DPAF segment adopts a $\beta$-turn conformation when bound to each of the three antibodies. Diverse peptide epitopes, when bound to corresponding antibodies, exist in a variety of different conformations including $\alpha$-helical, extended as well as $\beta$-turn. However, the $\beta$-turn conformation is observed statistically more often (Wilson and Stanfield, 1994). In this conformation, all the side chains are directed outward on the same side of the main chain and the side chains of non-consecutive amino acids come in close proximity. This enables a large number of side chain interactions with the antibody, ensuring higher specificity. It may, therefore, be surmised that the structural properties that make this particular sequence immunodominant are optimally presented by adopting the $\beta$-turn
conformation. A correlation between chemical composition and immunogenicity has also been established for this immunodominant epitope (Nakra et al., 2000). Ability of the DPAF epitope to adopt common β-turn conformation could constitute another key reason for the immune system to promote this epitope over others as the focus of the secondary response.

A similar conformation was adopted by an immunodominant epitope of a peptide antigen from influenza virus haemagglutinin while binding to two independent monoclonal antibodies (Churchill et al., 1994). In the present study, we have shown that the epitope adopts a common conformation even while binding to three independent antibodies indicating broader implications in the context of flexible immunodominant epitope. It is apparent that the immune system evolves high affinity receptors only to a single conformation of the epitope even though linear peptides adopt multiple conformations in solution. To generate an immune response against every epitope on a flexible antigen, all of which may not be optimal, will be extremely demanding on cellular and nutrient resources. Focussing on a single epitope would be a more effective and ingenious way of neutralizing the antigen than addressing the conformational repertoire of the antigen in a one-to-one fashion.

5.3 Diverse antibodies exhibit conformational convergence

Differences exist in the sequences and quaternary arrangements of the superdomains in the three antibodies. However, the backbone conformations of the hypervariable loops are remarkably similar in the bound state. In other words, a similar set of CDR conformations is utilized to stabilize a common conformation of the epitope. Churchill et al. (1994) had observed similar main chain conformations
of antibody CDRs in two antibodies recognizing the same immunodominant peptide epitope. On the other hand, for overlapping but non-identical epitopes, different conformations have been observed when bound to two distinct antibodies (Stanfield et al., 1999). It appears that the selection pressures during antibody maturation filter out a common conformational solution to the problem of binding the same epitope. This kind of structural convergence under the pressures to conserve function - so to say - has been often observed in molecular associations concerning other physiological processes (Denessiouk et al., 1998; Delano et al., 2000; Charbonnier et al., 1997).

The antibodies from the preimmune B-cell pool are known to possess paratopes that exhibit broader specificity and have the ability to bind disparate antigens with low affinity (Manivel et al., 2000). During maturation, the immune system employs somatic mutations to change the paratope and selects those mutated antibodies, which show an improvement in the complementarity with inducing antigen. It is, therefore, expected that the antibody response in an individual against a flexible epitope would involve a set of genetically heterogeneous antibodies, which bind to different parts and conformations of the epitope through diverse CDR conformations and interactions. Indeed, the affinity-based selection of non-homologous peptides generated from a combinatorial library using anti-p24 (HIV-1) antibody revealed that the peptide epitopes adopt different conformations forming independent critical contacts with the antibody while binding to the same site (Keitel et al., 1997). However, contrary to expectations, the regulatory and selection processes of the immune system choose a similar set of conformations in both antigen and antibody, as optimal for achieving the twin goals of specificity and affinity.
The studies show that the antibodies in a secondary response bind to an immunodominant epitope adopting a common conformation while the antigen by itself exists as a flexible molecule (Agarwal et al., 1996; Nayak et al., 1998). If the conformational polymorphism of the epitope in solution were to be responsible for the polyclonality of the immune response, antibodies would have matured to recognize the epitope in different conformations. Instead, antibodies adopt a common scaffold to recognize a single conformation of the antigen in the bound form, even though they have different paratope conformations in the unliganded form. This relates to the instructional theory, as against the clonal selection theory, and is consistent with the observations made by Wedemayer et al. (1997) while comparing the antigen recognition by germline antibody with that by its affinity matured descendent. The instructional theory regarding antigen-antibody recognition emphasized that the antibody is moulded using antigen as a template (Breinl and Hurowitz, 1930; Pauling, 1940).

The comparison of the variable region sequences has led to the possibility that PC283 could have evolved from a progenitor distinct from that of the other two antibodies. Hence, it is likely that two independent naive mature B cells have evolved -through somatic mutation - into a set of three combining sites, which show a similar conformation of CDRs in the bound state. If a flexible peptide binds to separate naive B-lymphocytes, one would naturally expect them to lead to independent paratopes. Therefore, the observed conformational convergence in the antigen bound state indicates that the selection and maturation of the paratopes must be under tight regulation.

From a large available repertoire, similar sets of CDR conformations are chosen to bind DPAF in a common conformation. If the immune system had
permitted sampling of the entire gamut of paratopes available in an unrestricted manner and then subjected all of them to somatic hypermutation to improve the complementarity, there would be significant chance that some of the selected paratopes would have the ability to bind to self antigens. Probably, only a restricted set of competent paratope structures are selected for binding to a given antigen in order to prevent such a breakdown in the discrimination between self and non-self. This could also imply that the immunodominant nature of this epitope facilitates survival of the antibodies directed towards this epitope by successfully competing out those directed against other determinants. In either case, the observed convergence in CDR conformations might serve to amplify the resolution between self and non-self. Thus, the development of an appropriate set of antibody paratopes to recognize a particular antigen appears to be dictated by the fundamental tenet of self non-self discrimination rather than the anticipated stereochemical features alone.

5.4 Plasticity of interactions facilitates conformational convergence

The complementarity achieved for the minimal epitope is similar in the three antibodies. The affinities between the antibodies and the epitope as measured through dissociation constants have been found to be in the order of $10^{-7}$ M (Manivel et al., 2000). Thus, the immune system has successfully selected and matured a set of antibodies that can bind the DPAF epitope with high specificity and affinity. Structures of the complexes exhibit similar main chain conformation for the epitope as well as the antibody CDRs. There are a number of common interactions in the three complexes. The flexible nature of the peptide allows compensation for changes in the paratope by changing its conformation in such a way that the
interactions with the CDR residues are optimized. Also, dissimilar amino acids are present in the three paratopes at equivalent positions interacting with the epitope. A solvent molecule plays a critical role in improving antigen-antibody complementarity in one case but not in the other two cases. Thus, there is a degree of plasticity evident in the epitope-paratope association modulated by the nature and conformations of certain side chains and the solvent interactions.

It has been earlier observed that the antibody paratope, especially the germline paratope, does show a degree of plasticity (Keitel et al., 1997; Manivel et al., 2000). The ability of different peptides to bind to the same antibody either by changing structure, or through presence of cementing water molecules, is documented (Keitel et al., 1997; Ochoa et al., 2000). Plasticity has been shown to be a physiologically relevant key feature in the binding of T cell receptor to MHC-peptide complexes (Garcia et al., 1998), and the molecular associations outside the immune system as well (Chen and Sigler, 1999). The degree of plasticity seen in the three Fab-peptide complexes described here is qualitatively similar to that observed in other physiological interactions.

The comparison of the antigen-antibody interactions in the three cases suggests that the sequence variations in the CDRs do not lead to significant differences in the main chain conformation due to the observed plasticity in interactions. Thus, the conformational convergence of the epitope and the paratopes may have a direct correlation with the plasticity of antigen-antibody interactions.

5.5 Different modes of activation for antigen recognition

It is possible that PC282 and PC287 may have matured from the same
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progenitor. In which case, it is intriguing that they exhibit independent structures in the absence of the antigen and yet converge over to a common structure when bound to the antigen. In the present case, CDR H3 exhibits variability in the unbound state. However, in the antigen bound form, the conformation of CDR H3 is the same in both the antibodies. Although PC283 could not be crystallized in the antigen-free state, there are indications to suggest that the paratope in this case may exhibit flexibility.

The entire ensemble of structures shows that the center of the peptide binding groove in the three antibodies is highly hydrophobic. In the absence of the antigen it might, therefore, require shielding from the solvent. This may have been achieved by movement of CDR H3 over the center of the paratope in case of PC282. Similar conformational change is not possible in case of PC287; this shielding is, therefore, achieved, to an extent, by the flipping of the aromatic ring of Tyr94L. Such a shielding is perfectly achieved on PS1 binding. Thus, the hydrophobic interactions between peptide and antibody could constitute a major driving force for recognition, as expected. It can, therefore, be surmised that the hydrophobic nature of the peptide provides enormous strength in selectivity and may define an additional property relevant for immunodominance in this case. The importance of this hydrophobic character also provides a rationale for the relationship between chemical composition and immunodominant character of the epitope.

The three antibodies exhibit different levels of structural alterations to ultimately arrive at a similar set of CDR conformations to bind the immunodominant epitope in a common conformation with comparable affinities in the physiological range. One involves more of a lock and key mechanism (PC287), the other involves
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an induced fit mechanism (PC282) and the third, probably, involves a disorder to order transition (PC283). Even if the two antibodies for which the crystal structure has been determined- with bound antigen and without- were to be considered, interesting implications emerge. It is obvious that the paratope is already pre-organized for receiving the antigen in one case (PC287), while a significant change in the conformation is necessary in the other (PC282). It has been earlier shown that pre-organization of the antigen-combining site can lead to drastic improvement in the affinity during antibody maturation (Wedemayer et al., 1997). However, in the present case, the affinities exhibited by the two antibodies towards the peptide are comparable (Manivel et al., 2000). The water structure in the vicinity of the paratope is different in the antigen bound and unbound forms of the two antibodies. Thus, it is possible that the reorganization of solvent may have been exploited to compensate for conformational changes in order to achieve similar binding affinities.

To summarize, in the thesis, the binding interactions between a conformationally flexible epitope and three distinct antibodies have been examined. The study revealed that there exists conformational convergence of the epitope as well as that of the paratope. In addition to this, the analysis disclosed that the similarity in conformational and thermodynamic features -in spite of divergent paratope sequences - is due to plasticity and varying degrees of conformational changes and solvent reorganization associated with the antigen-antibody interactions. While the conformational convergence of the epitope could be correlated with its immonodominance, the observation that the antibody also moulds itself in accordance with the optimal conformation of the peptide, which is otherwise flexible in solution, describes a unique feature of antibody maturation.
responses. In addition to this, directed maturation against an energetically favored conformation of an epitope may be an ingenious way of limiting the structural repertoire of the paratope while effectively neutralizing the antigen. It is therefore tempting to suggest that restricted conformational repertoire on antigen binding may be helpful in minimizing the probability of the generation of self-reactive antibodies and thus enhancing self non-self resolution.

Overall it appears that maturation of T-dependent humoral responses represent a uni-directional optimization process where, structurally diverse epitope-specific germline antibodies are guided along a pathway where they all adapt towards binding epitope in a unique conformation. The driving force behind such a mode of maturation would be improved specificity and self vs. non-self discrimination. Thus, the patterns observed in the ensemble of structures described here, contribute significantly in understanding the relationship between structural and regulatory features in an immune response.