CHAPTER II

Differential sensitivities of primary and secondary T cell responses to antigen structure
Chapter II

INTRODUCTION

A viable T-dependent humoral response hinges on the ability of an antigen to appropriately prime specific B and T lymphocytes. Subsequent progression is then driven by the efficiency with which such antigen-activated B cells can recruit help from the antigen-specific T helper cell pool (1). Antigen-activated B and T lymphocytes engage in a cognate interaction, leading to reciprocal proliferation and differentiation into effector and memory populations. Thus, a productive T-dependent immune response requires an optimal activation of both antigen specific B and T lymphocytes. For B cell activation, the primary requirement is, recognition of an antigen (mostly in its native form) through Ab receptor (BCR). In contrast for the activation of T cells, which do not recognize the antigen in its soluble form, requires help of an antigen presenting cell (APC). The APC takes up the antigen, processes it in the intracellular compartment and presents the antigen in the form of peptide fragments complexed with MHC molecule for T cell recognition. Thus, this suggests, recognition of an antigenic determinant by a T cell should be independent of the structural milieu of its location.

While activated T cells play a pivotal role in driving humoral responses, it has recently been shown in our laboratory that, the primed T cells are also critical in defining the spectrum of B cell fine-specificities of the positively selected population from the multitude of clonotypes initially activated (3). The available pool-size of primary antigen-activated T cells serves as a rate-limiting determinant that enforces a competitive process to select for high affinity clones (3). It is the successful subset, which dominates the population of B cells contributing to the later stages of a primary humoral response (3). Thus, effective priming of CD4 T cells by an antigen is critical in elaborating not only the quantitative aspects of a humoral response, but also the antibody specificities that constitute it.
This present work is in continuation of the earlier investigations to understand the poor immunogenicity of the peptide CysCT3, where the immunodominant epitope (DPAF segment) exists in a relatively rigid cyclic loop (Fig-1, chapter-1), compared to the parent peptide, PS1CT3 (3-6). This work demonstrates that the poor immunogenicity of CysCT3 is an outcome of its poor priming of the T cells and that the efficacy of T cell priming in such constructs can be significantly affected by the imposition of a conformational constraint on the flanking B cell epitope. Interestingly however, although T cell priming was significantly diminished, secondary T cell responses were relatively unaltered. In addition to confirming differences in requirements for primary and secondary T cell activation, these results also suggest that the efficiency of priming by individual T cell epitopes on a multi-determinant antigen may be sensitive to the structural milieu in which they occur.
RESULTS

Immunogenicity of peptides PS1CT3 and CysCT3

The salient features of these two peptides has already been discussed in chapter 1 (page-1). To compare the immunogenicity of the above two peptides, groups of BALB/c mice were immunized with a single dose of either peptide PS1CT3 or peptide CysCT3 and specific serum antibody levels monitored as a function of time. As shown in Fig. 1 the anti-CysCT3 titers were significantly lower than that obtained against peptide PS1CT3. This was true over the entire range of time points evaluated. It would thus appear that peptide CysCT3 is markedly less immunogenic than peptide PS1CT3.

Peptide CysCT3 primes T cells poorly

To examine whether the differences in immunogenicity could have resulted from differences at the level of T cell priming, proliferative responses of lymph node cells (LNCs) from mice immunized with either peptides were measured. While LNCs from peptide PS1CT3-immunized mice yielded significant proliferative responses, those from CysCT3-primed mice were markedly diminished (Fig. 2). This was true regardless of whether the challenge antigen employed was the homologous peptide (Fig. 2A) or a peptide representing the CT3 segment of these immunogen molecules (Fig. 2B).

The poor T cell priming ability of peptide CysCT3 could be either due to altered secondary structure of the neighboring B cell epitope segment, or, a consequence of the nature of substitutions performed. To discriminate between these two possibilities a linearized analog of peptide CysCT3 was synthesized, where the cysteine-sulfhydryl groups were blocked as acetamidomethyl-derivatives (peptide Cys(Acm)CT3).
Immunization of BALB/c mice with peptide Cys(Acm)CT3 followed by an examination of LNC proliferative responses revealed that this analog was far more potent at priming T cells than peptide CysCT3 (Fig. 2). Thus, it would appear that the attenuated ability of peptide CysCT3 to prime specific T cells is a consequence of the altered conformation of the flanking B cell epitope.

**Poor B cell immunogenicity of peptide results from inefficient T cell priming**

It was of interest to ascertain whether the poor antibody response to peptide CysCT3 in Fig. 1 was due to insufficient T cell priming. For this, mice were first preprimed with peptide CT3 seven days prior to immunization with peptide CysCT3. Prepriming with peptide CT3 has been shown to function by increasing the frequency of antigen-activated T cells available at the time of antigen exposure to naive B cells (5). An analysis of anti-CysCT3 IgG levels in sera collected subsequently revealed that CT3-prepriming resulted in a marked improvement in anti-CysCT3 titers over that from mock-preprimed mice (Fig. 3). This clearly suggests that the poor B cell immunogenicity of peptide CysCT3 was likely to be due to the presence of a limiting amount of T cell help. Such a possibility could further be validated by comparing primary anti-peptide IgG titers obtained in mice immunized with either peptide CysCT3 or Cys(Acm)CT3. In such experiments we observed that peptide Cys(Acm)CT3 was at least five-fold more immunogenic, in terms of IgG antibody levels, than peptide CysCT3 (Fig. 4).

**Peptides PS1CT3 and CysCT3 are equally competent at eliciting secondary T cell responses**

Although the analogs under study here differed markedly in their abilities to prime for a T cell response, a comparison of their potencies at recalling a preprimed...
population of antigen-specific T cells was analysed. For this, LNCs from mice primed with either peptide CT3 or PS1CT3 were challenged in vitro with varying concentrations of either peptide PS1CT3 or peptide CysCT3 and the resulting proliferation measured. As shown in Fig. 5, recall proliferative responses elicited by peptide CysCT3 were comparable to that by peptide PS1CT3 both in PS1CT3- (panel A) and CT3- (panel B) primed LNCs. Thus, although peptides PS1CT3 and CysCT3 differ in their abilities to prime T cells they are equally proficient at recalling a preprimed antigen-specific T cell population.

Pretreatment of CT3-primed LNCs with chloroquine abolished recall responses to both peptides PS1CT3 and CysCT3 (stimulation index values <2.0), confirming our earlier observations (6) that PS1CT3 and its analog peptides require processing prior to appropriate presentation in association with MHC Class II molecules.
DISCUSSION

A productive immune response requires optimal activation of both antigen-specific B and T lymphocytes. Recognition - a prerequisite for activation - by B lymphocytes involves a direct encounter between the B cell antigen receptor (BCR) and target antigen, the latter in its native form (11). Consequently, both qualitative and quantitative aspects of a humoral response can be expected to be influenced by the structural context in which a given antigenic determinant is present. In contrast, T cell recognition is restricted to peptides in an extended conformation - generated by processing of the parent antigen within APCs, and presented in association with MHC Class II molecules (12,13). As a result, recognition of an antigenic determinant by T cells is expected to be independent of the structural milieu of its origin. Although true in principle, activation of an antigen-specific T cell is - nevertheless - variably affected by factors that influence the efficacy with which an APC can present the appropriate ligand to it. Thus, for example, extent of antigen uptake by APCs, rate and specificity of antigen processing, and affinity of the resulting peptides for the MHC Class II molecule have all been shown to serve as regulatory influences on T cell activation (14-16).

The present study describes a surprising effect of conformation of a flanking domain on in vivo priming by a T cell epitope. Peptide CysCT3, where the neighboring B cell epitope sequence was held in a disulfide-bonded loop, was distinctly less immunogenic than its parent peptide PS1CT3 where the PS1 segment existed in a random distribution of conformations. The poor anti-CysCT3 IgG antibody response was shown to result from its inability to prime T cells as effectively as peptide PS1CT3. Furthermore, it could also be demonstrated that the attenuated T cell priming efficacy of peptide CysCT3 was purely a consequence of altered structure of the PS1 segment rather than due to the nature of substitutions performed. Interestingly however, while primary T
cell responses were sensitive to the structural environment of the CT3 segment, both peptides PS1CT3 and CysCT3 proved equally competent at recalling secondary T cell responses in vitro. This latter result confirms a difference in requirements for activation of primary and secondary T cell responses (17).

The mechanism by which an altered conformation of the PS1 segment inhibits T cell priming by peptide CysCT3 is presently unclear. However, it is likely that the difference is experienced at the level of antigen presentation, presumably by dendritic cells and/or macrophages - the principal cell types implicated in priming of CD4+ T cells (19). Such a variance could arise either as a consequence of altered antigen uptake, or alterations at the level of antigen processing. Although, both peptides PS1CT3 and CysCT3 elicit near identical recall responses may appear to rule out dramatic differences in processing of the two peptides, but the possibility of minor differences that exert their influence at the level of primary but not secondary T cell responses. Furthermore, the findings of comparable recall efficiencies of peptides PS1CT3 and CysCT3 regardless of whether the LNCs were primed with peptide CysCT3, PS1CT3 or CT3 strongly point towards quantitative rather than qualitative differences in antigen presentation from the two peptides.

Thus, above results demonstrate that, the structural environment in the vicinity of a T cell epitope can affect its ability to prime T cells there by raising some interesting implications of it. This is particularly true with respect to the more complex multiepitope antigens such as proteins. It is generally believed that immune responses are initiated by presentation of epitopes for CD4+ T cells by antigen non-specific APCs (19). The poor efficiency of non-specific uptake mechanisms, leading to low concentrations of peptide fragments generated intracellularly, has been proposed to account for the selective immunodominance of peptide epitopes in primary CD4+ T cell responses (19).
Results presented here suggest that, local environmental influences, by modifying T cell priming efficacies of individual T cells epitopes, can add yet another variable in defining the repertoire of T cell specificities generated in a primary response to protein antigens. Indeed, in this connection, a recent study has identified the existence of a predictive correlation between immunodominant T cell epitopes, and nearby structurally unstable segments in protein antigens (20).
Figure 1: Relative immunogenicity of peptides PS1CT3 (○) and CysCT3 (●). Figure shows a representative of five independent experiments.
Figure 2: Primary T cell proliferative responses induced by peptide PS1CT3 and its analogs. LNCs from mice primed with either peptide PS1CT3 (○), peptide CysCT3 (●) or peptide Cys(Acm)CT3 (△); recall with the homologous peptide (panel A), or with peptide CT3 (panel B). Values are presented as stimulation index (mean ± S.E. of quadruplicate sets). Figure shows a representative of five independent experiments.
Figure 3: Immunogenicity of peptide CysCT3 in CT3-preprimed mice. Group 1: mock primed with adjuvant alone; Group 2: preprimed with peptide CT3. Seven days latter both groups were immunized with peptide CysCT3. Sera were collected at weekly intervals and anti-CysCT3 IgG titers measured by ELISA. Data shown here are for sera obtained from individual mice day 14 post CT3-immunization. The figure is a representative of three independent experiments.
Figure 4: Relative Immunogenicity of peptide Cys(Acm)CT3 and CysCT3
Figure 5: T cell proliferative recall responses to peptides PS1CT3 and CysCT3. LNCs from mice primed with either peptide PS1CT3 (panel A) or peptide CT3 (panel B), recall with either peptide PS1CT3 (○) or peptide CysCT3 (●). Values are presented as stimulation index (mean ± S.E. of quadruplicate sets).
REFERENCES


