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

B.P. Nayak*

Immunology Group, International Center for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110 067, India

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Abstract Here we examined T cell responses to two analogs of a chimeric peptide encoding a known B and a known T cell epitope. In one of the analogs, the B cell epitope existed in a random conformation whereas it was restricted within a disulfide-bonded cyclic loop in the other. Immunization of these peptides in mice revealed that the latter peptide was significantly poorer at priming T cells and our preliminary results suggest this could be the result of differential processing of the two analogs. While primary T cell responses were sensitive to the influence of conformation, secondary responses were not discriminatory for the two antigens confirming the differences in activation requirements for primary and secondary T cell responses. Further, our studies also suggest that the priming efficacy of a T cell epitope is influenced by its structural environment.

Key words: Synthetic peptide; T cell priming; Secondary structure

1. 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 [2].

While activated T cells play a pivotal role in driving humoral responses, we have recently shown that primed T cells are also critical in defining the spectrum of B cell fine specificities that are positively selected from the multitude of clonotypes that are 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 that 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.

The present report continues our earlier investigations on modulation of B cell responses using analogs of a chimeric peptide encoding a known B and a known T cell epitope [3–7]. We show here 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, our results also suggest that the efficiency or priming by individual T cell epitopes on a multi-determinant antigen may be sensitive to the structural milieu in which they occur.

*Fax: (91) (11) 616 2316.
E-mail: bishnu@icgebnd.ernet.in

Abbreviations: MHC, major histocompatibility complex; LNC, lymph node cells; APC, antigen presenting cell(s); i.p., intraperitoneal

2. Materials and methods

2.1. Materials

Horseradish peroxidase-labeled anti-mouse IgG (heavy chain-specific) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). F-moc amino acid derivatives were purchased from Novabiochem (Laufelfingen, Switzerland).

2.2. Peptide synthesis

Peptides were synthesized on a Milligen 9050 automated peptide synthesizer using F-moc chemistry [8]. Crude peptide was purified to at least 95% purity by reverse phase HPLC. For the synthesis of peptide CysCT3, the side chain protecting group used for cysteine was the acetamidomethyl (Acm) group. Subsequent to synthesis and cleavage from solid support, simultaneous deprotection of the cysteine side chains and oxidation to form intramolecular disulfide bonds was achieved with iodine in acetic acid [9].

The correct identity of all peptides synthesized was ascertained both by amino acid analysis and by mass spectrometry.

2.3. Animals and immunizations

Female BALB/c mice (6–8 weeks old) were obtained from the small animal facility at the National Institute of Nutrition (Hyderabad, India). Immunizations with either peptide PS1CT3 or CysCT3 were i.p. at a dose of 50 μg/mouse as an emulsion in Freund's complete adjuvant (CFA). In experiments with CT3 prepriming, peptide CT3 at a dose of 50 μg/mouse (CFA, base of tail) was administered 7 days prior to immunization with peptide CysCT3.

ELISA and LNC proliferation assays were essentially as described earlier [6].

3. Results

3.1. Immunogenicity of peptides PS1CT3 and CysCT3

Peptide PS1CT3 is a chimeric peptide with the sequence HQLDPAFGANSTNPDGGDIEKKIAKMEKASSVFNVVNS. The first 15 residues of this sequence (segment PS1) represent a well characterized B cell epitope derived from the envelope protein of hepatitis B virus [10]. Residues 18–38 (segment CT3) correspond to a promiscuous T cell epitope present on the circumsporozoite protein of the malaria parasite *Plasmodium falciparum* [11]. Separating the B and T cell epitopes is a spacer of two glycine residues at positions 16 and 17. Prior analysis of peptide PS1CT3 by circular dichroism spectroscopy had revealed that this peptide exists in a random distribution of conformations in aqueous solutions [3]. Immu-

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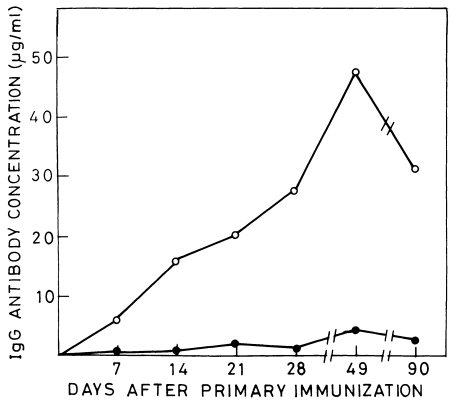

Fig. 1. Relative immunogenicity of peptides PS1CT3 and CysCT3. Groups of five mice each were immunized either with peptide PS1CT3 (○) or with peptide CysCT3 (●) as described in Section 2. Blood was collected at indicated time points and sera within a group pooled. Peptide-specific IgG antibody levels were quantitated by ELISA. The figure shows a representative of five independent experiments.

nization of BALB/c mice with peptide PS1CT3 has been shown to result in a T-dependent primary and secondary IgG response that is exclusively directed against a tetrapeptide epitope between positions 4 and 7 (sequence: DPAF) [3]. In the present study we also employed an analog of peptide PS1CT3 where positions 1 and 10 were substituted with cysteine residues. Subsequent air oxidation resulted in the generation of an intramolecular disulfide bond, to yield a peptide where the first 10 residues were locked within a cyclic loop (peptide CysCT3).

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 were monitored as a function of time. As shown in Fig. 1 the anti-CysCT3 titers were significantly lower than those 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.

3.2. 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 peptide 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). Furthermore, challenge of CysCT3-primed LNCs with peptide PS1CT3 resulted in stimulation index values identical to those obtained with peptide CysCT3 as the challenge antigen (data not shown).

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 the substitutions performed. To discriminate between these two possibilities we synthesized a linearized analog of peptide CysCT3 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. 3). 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.

3.3. 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 7 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 those from mock-preprimed mice. 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. This possibility could be further validated by comparing primary anti-peptide IgG titers obtained in mice immunized with either peptide CysCT3 or Cys(Acm)CT3. In these experiments we observed that peptide Cys(Acm)CT3 was at least five-fold more immunogenic, in terms of IgG antibody levels, than peptide CysCT3 (data not shown).

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

Although the analogs studied here differed markedly in their abilities to prime for a T cell response, we also compared their potencies at recalling a preprimed population of antigen-specific T cells. For this, LNCs from mice primed with either peptide CT3 or PS1CT3 were challenged in vitro with varying

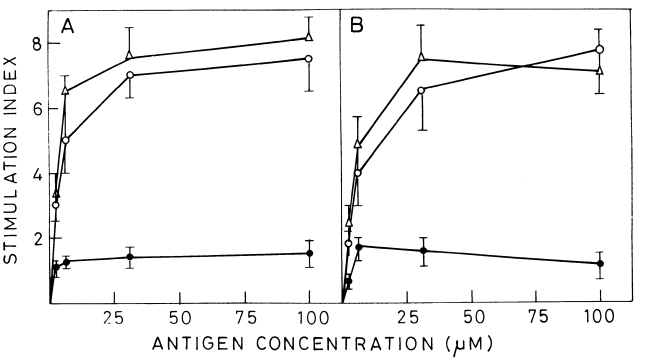

Fig. 2. Primary T cell responses induced by peptide PS1CT3 and its analogs. LNCs from mice primed with either peptide PS1CT3 (○), peptide CysCT3 (●) or peptide Cys(Acm)CT3 (△) were cultured in vitro with the indicated concentrations of either the homologous peptide (A) or peptide CT3 (panel B). Lymphocyte proliferation was measured as counts of [^{3}H]thymidine incorporated. Mean background counts varied between 1500 and 2000 cpm. Values are presented as the stimulation index (mean ± S.E.M. of quadruplicate sets), which represents the ratio of counts obtained at a given concentration of antigen over that obtained in the absence of any challenge antigen. The figure shows a representative of five independent experiments.

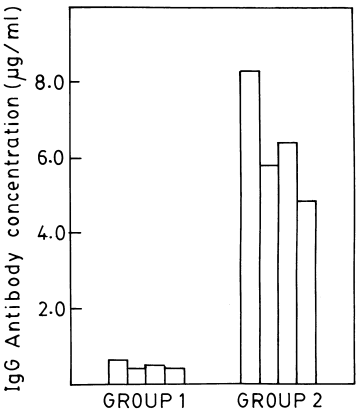

Fig. 3. Enhanced immunogenicity of peptide CysCT3 in CT3-preprimed mice. Groups of four mice each were either preprimed with peptide CT3 (group 2) or mock-primed with adjuvant alone (group 1). Seven days later, both groups were immunized with peptide CysCT3. Sera were collected at weekly intervals and anti-CysCT3 IgG titers measured by ELISA. Data shown here are for sera obtained from individual mice at 14 days after CT3 immunization.

concentrations of either peptide PS1CT3 or peptide CysCT3 and the resulting proliferation was measured. As shown in Fig. 4, recall proliferative responses elicited by peptide CysCT3 were comparable to those 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.

4. 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 [12]. 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 antigen-presenting cells (APCs), and presented in association with MHC class II molecules [13,14]. 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 [15–17].

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 the altered structure of the PS1 segment rather than due to the nature of the 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 [18].

The mechanism by which an altered conformation of the PS1 segment inhibits T cell priming by peptide CysCT3 is currently 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 variance could arise as a consequence of either altered antigen uptake or alterations at the level of antigen processing. Although our observations that both peptides PS1CT3 and CysCT3 elicit nearly identical recall responses may appear to rule out dramatic differences in processing of the two peptides, we cannot ignore the possibility of minor differences that exert their influence at the level of primary but not secondary T cell responses. Furthermore, the finding of comparable recall efficiencies of peptides PS1CT3 and CysCT3, regardless of whether the LNCs were primed with peptide CysCT3, PS1CT3 or CT3, strongly points towards quantitative rather than qualitative differences in antigen presentation from the two peptides.

Our demonstration here that the structural environment in the vicinity of a T cell epitope can affect its ability to prime T cells raises some interesting implications. This is particularly true with respect to the more complex multi-determinant antigens such as proteins. It is generally believed that immune responses are initiated by presentation of epitopes for $CD4^+$

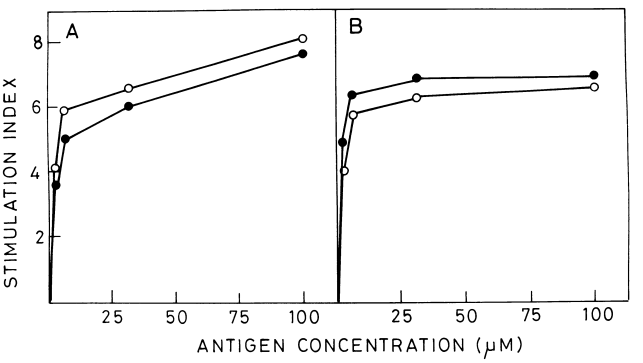

Fig. 4. T cell recall responses to peptides PS1CT3 and CysCT3. LNCs from mice primed with either peptide PS1CT3 (A) or peptide CT3 (B) were challenged with indicated concentrations of either peptide PS1CT3 (○) or peptide CysCT3 (●) and proliferation measured as described for Fig. 2. Data presentation is as described for Fig. 2. The figure is a representative of three separate experiments.

T cells by antigen non-specific APCs [20]. 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 $CD4^+$ cell responses [20]. The results presented here suggest that local environmental influences, by modifying T cell priming efficacies of individual T cell 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 [21].

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