T LYMPHOCYTE RECOGNITION OF PEPTIDE ANTIGENS: EVIDENCE FAVORING THE FORMATION OF NEOANTIGENIC DETERMINANTS*

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Although T lymphocyte responses to exogenous antigens are exquisitely specific, there is little information on the nature of the antigenic determinant interacting with T cell receptors. Several studies have suggested that antigenic conformation is not important for T cell recognition, and that the primary sequence alone determines the antigenic epitopes (1–3). This proposal is based on observations that immune T cells react identically to undenatured and denatured protein antigens, whereas antibodies can discriminate between these two forms of the same antigen. One explanation for these observations is that T cells, unlike B cells, require that antigens be "processed" by macrophage-like stimulator cells before they can be recognized. It is assumed that one feature of antigen processing by macrophages is that larger proteins are degraded to small peptide fragments that represent the moiety recognized by T cells, and that the same fragments are produced from denatured or undenatured proteins. In addition, we previously (4, 5) found that the specific responses to small peptide antigens were determined in large part by the amino acid side chains. Conformation did not seem to be critical for the major epitopes, as several analogues containing single residue substitutions had no effect on antibody binding but dramatically altered T cell responses (4). These observations are consistent with the proposal that the epitopes recognized by T cells are defined mainly by the amino acid sequence of a peptide antigen and that conformation has a relatively lesser role, if any, in formation of the antigenic determinant. If this is correct, it might be predicted that inverting the residue sequence of a small peptide antigen would have little effect on its antigenicity, as the same amino acids are present in the same spatial order and the side chains are identical. To test this prediction, we examined T cell responses to the small fibrino-peptide fragment Bβ7-14 and a peptide with the inverted sequence of Bβ7-14.

Materials and Methods

Animals. Inbred strain 2 and strain 13 guinea pigs were obtained from Biological Systems, Toms River, NJ.

Antigens. The peptide antigens Bβ7-14, inverted Bβ7-14, Bβ7-15, and inverted Bβ7-15,

Bβ7-14	NH$_2$-Glu^7-Glu^8-Gly^9-Phe^{10}-Phe^{11}-Ser^{12}-Ala^{13}-Arg^{14}-OH
Inverted Bβ7-14	NH$_2$-Arg-Ala-Ser-Phe-Phe-Gly-Glu-Glu-OH
Bβ7-15	NH$_2$-Glu^7-Glu^8-Gly^9-Phe^{10}-Phe^{11}-Ser^{12}-Ala^{13}-Arg^{14}-Gly^{15}-OH
Inverted Bβ7-15	NH$_2$-Gly-Arg-Ala-Ser-Phe-Phe-Gly-Glu-Glu-OH

FIG. 1.   Primary amino acid sequence of peptide antigens Bβ7-14, inverted Bβ7-14, Bβ7-15, and inverted Bβ7-15.

whose sequences are shown in Fig. 1, were synthesized and purified as described previously (4). Purified protein derivative of tuberculin (PPD) was purchased from Connaught Medical Research Laboratory, Willowdale, Ontario, Canada.

Preparation of Cells.   2–6 wk after immunization with 400 μg of each peptide antigen in complete Freund's adjuvant (Difco Laboratories, Detroit, MI) guinea pigs was injected intra-peritoneally with 25 ml of sterile mineral oil (Marcol 52; Exxon Corp., Linden, NJ), and the resulting peritoneal exudate was harvested 3–4 d later. A T lymphocyte-enriched peritoneal exudate lymphocyte (PEL) population was prepared by passing cells over a rayon wool adherence column (6).

In Vitro Assay of DNA Synthesis.   Immune PEL (3–4 × 10^5 cells/well) were cultured in round-bottomed microtiter plates (Cooke Laboratory Products Div., Dynatech Laboratories, Inc., Dynatech Corp., Arlington, VA) with soluble peptide antigen (5–40 μg/well) or PPD (10 μg/well), in a total volume of 0.2 ml, or RPMI 1640 medium (Grand Island Biological Co., Grand Island, NY) containing L-glutamine (300 μg/ml), penicillin (100 U/ml), 2-mercaptoethanol (5 × 10^{-5} M), and 5% heat-inactivated normal guinea pig serum. After incubation for 2 d at 37°C in 5% CO$_2$ in air, 1 μCi of tritiated thymidine ([^{3}H]TdR) (sp act 6.7 Ci/mmol; Research Products, International Corp., Prospect, IL) was added to each well. The amount of radioactivity incorporated into cellular DNA was determined after an additional 18-h incubation with the aid of a semiautomated microharvesting device (Titer-Tek; Flow Laboratories, Inc., Rockville, MD). Each [^{3}H]TdR counts per minute value represents the mean of triplicate cultures, and the standard error was always within 10% of the mean. The representative experiments shown were each performed two to six times with similar results, and 40 μg of the peptide antigens was the dose producing the highest proliferative responses.

Results and Discussion

We previously found that Bβ7-14 was immunogenic and antigenic for strain 2 guinea pig T cell responses, but that strain 13 animals were completely unresponsive to this antigen (7). Accordingly, strain 2 animals were immunized with Bβ7-14 or the inverted sequence peptide, and in vitro T cell responses were assessed with Bβ7-14 and inverted Bβ7-14 (Table I). Strain 2 Bβ7-14 immune T cells were stimulated with Bβ7-14 but showed no response to the inverted Bβ7-14. Moreover, T cells from strain 2 animals immunized with inverted Bβ7-14 failed to respond to either Bβ7-14 or inverted Bβ7-14. It is clear that inverting the amino acid sequence of Bβ7-14 results in a peptide that is neither antigenic nor immunogenic for strain 2 T cell proliferative responses. Therefore, not only are the residue side chains critical for T cell responses, but the order conferred by the $\propto$-carbon backbone is critical as well. This is particularly interesting as both peptides are symmetrical with respect to the Phe residues in positions 10 and 11 in Bβ7-14. We previously (4) found that Phe^{10}, as well as Arg^{14}, contributed to the clonal specificity of T cell responses to Bβ7-14, whereas substitutions for Phe^{11} eliminated antigenicity and immunogenicity and substitutions for Gly^7 and Glu^8 altered immunogenicity but not the specificity of the response. Thus, the major differences between these two peptides as far as T cell responses are concerned are the changes of Ala^{13} and Arg^{14} in Bβ7-14 to Glu.

One explanation for these observations may be that T cells recognize the confor-

TABLE I

Antigenicity and Immunogenicity of Bβ7-14 and Inverted Bβ7-14

Strain 2 guinea pig immunized*	None	Antigen in Culture		Inverted Bβ7-14
		PPD	Bβ7-14	
Bβ7-14	937	*142,287*	*19,413*	426
Inverted Bβ7-14	2,204	*264,783*	1,386	1,725

* Strain 2 guinea pigs were immunized with Bβ7-14 or inverted Bβ7-14 and the immune T cell-enriched PEL cultured in vitro with PPD (10 μg/well), as a positive control, and Bβ7-14 and inverted Bβ7-14 (40 μg/well) as described in Materials and Methods. The [³H]TdR counts per minute was determined on the 3rd d of culture, and each value represents the mean counts per minute from triplicate cultures. Italicized values indicate those cultures in which positive stimulation occurred.

mation of the peptide as well as side chains. Because the properties of these peptides with respect to size and charge are identical, however, it is less likely that a dramatically altered conformation is responsible for these antigenic differences, particularly as Bβ1-14 shows only random conformation in solution (8). One problem with this argument, though, is that the shape of the peptide as it is actually bound by the receptor is unknown. However, if the peptide is sufficiently malleable to be "shaped" by the receptor, then it should not matter if the sequence is inverted. Although it is difficult to conclusively rule out a conformational role for the antigen specificity of T cell recognition, we feel that this is less likely for the reasons given above and those mentioned in the introduction to this paper. We therefore favor the interpretation that T cells exhibit polarity in the "reading" of peptide antigens such that the ordering of the residues is critical. The simplest way to achieve polarity is that one end of the peptide is "fixed" with reference to self and that T cells read from the self-associated end of the peptide. This would be consistent with formation of a neoantigenic determinant created by the association of exogenous antigens with self moieties in some fashion. This association may be a covalent or noncovalent attachment of peptide antigens to a self carrier, which may or may not be macrophage Ia antigen (9, 10).

If the interpretation that exogenous peptide antigens become associated with self is correct, it is likely that for Bβ7-14 this association occurs via the carboxyl terminus. The reasoning for this is that (*a*) altering or deleting residues from the amino terminus of Bβ7-14 has no effect on the antigenicity or fine specificity of T cell responses to Bβ7-14; and (*b*) altering or deleting the carboxyl terminal Arg[14] has profound effects on the clonal specificity of T cell responses and on immune response (Ir) gene control. If this is the case, then further changes at the carboxyl terminus may dramatically influence T cell recognition. Accordingly, a Gly residue was added to the carboxyl end of Bβ7-14 to form the peptide Bβ7-15. Because Gly[15] does not contribute a major side chain, it should serve primarily as a spacer such that any purported attachment of Bβ7-14 to self would be at a greater distance than with native Bβ7-14. We found that Bβ7-15 was immunogenic and antigenic for strain 13 T cell responses (Table II), despite the fact that strain 13 guinea pigs fail to respond to Bβ7-14. In strain 2 animals, Bβ7-15 elicits the same responses as Bβ7-14. As before, the inverted Bβ7-15 peptide was neither antigenic nor immunogenic for guinea pig T cell responses in either strain. It would therefore appear that for strain 13 T cell recognition, the addition of a single Gly residue to the carboxyl end of Bβ7-14 converts the peptide to

TABLE II

Antigenicity and Immunogenicity of Bβ7-15 and Inverted Bβ7-15

Guinea pig	Immunized	None	Antigen in culture		
			PPD	Bβ7-15	Inverted Bβ7-15
Strain 13	Bβ7-15	1,145	*119,773*	*8,456*	1,653
	Inverted Bβ7-15	486	*114,593*	521	419
Strain 2	Bβ7-15	1,128	*56,054*	*30,789*	1,168
	Inverted Bβ7-15	446	*99,103*	555	727

See legend, Table I.

an immunogenic form and changes Ir gene control. According to the preceding hypothesis, this could indicate that the spacing of Bβ7-14 relative to "self" is essential to create antigenic determinants capable of being recognized by T cells.

Our interpretation of these observations is that Ir gene control of T cell responses may function at several levels. First, it is possible that for some peptide antigens there may be no association with self macrophage components, and consequently no immunogenic moiety is created for T cell recognition. Second, even though an appropriate immunogenic moiety is formed between exogenous peptides and macrophages, there may be few or no T cell clones with the complementary recognition structures for those particular antigenic determinants. We have recently obtained evidence for the latter proposal and found that in some cases Ir gene control does not reside with macrophages, but represents a functional absence of responder T cells (11). Thus, the use of peptide antigens has provided several new insights into the nature of T cell recognition. Further studies using this system should provide greater definition of the immunogenic complex and of those cellular structures that specifically interact with exogenous antigens.

Summary

To more precisely define the nature of exogenous antigenic determinants recognized by T cells, the response to fibrinopeptide fragment Bβ7-14 and a peptide of the inverted amino acid sequence of Bβ7-14 was examined. Strain 2 guinea pig T cells immunized with Bβ7-14 showed in vitro proliferative responses with Bβ7-14, but failed to respond to the inverted Bβ7-14 peptide. Moreover, the inverted Bβ7-14 peptide was nonimmunogenic and failed to prime strain 2 T cells for responses to native or inverted Bβ7-14. These results suggest that T cell recognition of peptide antigens involves more than simple interactions with amino acid side chains and that the ordering of the amino acids within the peptide is critical. One interpretation of these results is that T cells exhibit polarity in reading of antigenic determinants and peptides become associated with self in some fashion to form a neoantigenic determinant. To test this possibility, a Gly residue was added to the carboxyl end of Bβ7-14 (Bβ7-15), which is the likely site of attachment to self. It was found that strain 13 guinea pigs, which are totally unresponsive to Bβ7-14, produced T cell responses to Bβ7-15. This observation is consistent with the interpretation that Gly spaces the Bβ7-14 away from self to form an antigenic determinant complementary to strain 13 T cell antigen recognition structures. These results are discussed with respect to several mechanisms for immune response gene control of T cell responses.

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