Cloned T Cells Internalize Peptide from Bound Complexes of Peptide and Purified Class II Major Histocompatibility Complex Antigen*

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Antigen presentation to helper T cells involves the formation of a trimolecular complex consisting of a class II major histocompatibility complex (MHC) antigen combined with an antigenic peptide on the surface of an antigen-presenting cell and a T cell receptor (TCR) on the T cell. The fate of the MHC class II peptide, or TCR moieties of the ternary complex following antigen presentation is unknown. Using radio-labeled complexes of affinity-purified murine MHC class II molecules and peptides corresponding to T cell epitopes of myelin basic protein (MBP), this report presents evidence that the binding of preformed relevant MHC class II-peptide complexes to cloned T cells in vitro results in internalization of the peptide moiety. Neither the restricting MHC class II molecule nor the TCR moiety of the trimolecular complex was internalized by T cells. The specificity of peptide internalization was demonstrated using complexes of syngeneic MHC class II with an irrelevant MBP peptide analog and by cloned T cells restricted for a different epitope of the same MBP antigen. Furthermore, the peptide translocation mediated by MHC class II and TCR was demonstrated by antibody-blocking experiments using anti-class II and anti-TCR monoclonal antibodies. The peptide internalization by T cells was markedly reduced when binding was performed at 4 °C as compared with 37 °C. In addition, a significant inhibition of peptide translocation was observed in the presence of a metabolic inhibitor (sodium azide) but not in the presence of cytochalasin B. These results together demonstrate that the in vitro interaction of soluble MHC II-peptide complexes with cloned T cells is an active process associated with uptake of the antigenic peptide.

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MHC II peptide binding site, which because of sequence homology is inferred to be structurally and functionally similar to the peptide binding cleft of the MHC I molecule (5, 6). The MHC II-peptide complexes thus formed are then expressed on the surface of the antigen-presenting cell where they are recognized by TCRs on the surface of T cells (7–9). Although the trimolecular model of antigen presentation (MHC class II-antigen-TCR) is widely accepted, the fate of components of the ternary complex upon separation of apposed APC and T cell is still unknown. This report presents evidence that the in vitro binding of complexes, containing affinity-purified murine class II MHC and synthetic antigenic peptide, to cloned murine T cells was associated with internalization of only the peptide component of the ternary complex.

MATERIALS AND METHODS

Murine T cell clones (A/J.2 and 4R.3.9), restricted for IA*–MBP(1–14) complexes, were obtained from the laboratory of Patricia Jones, Stanford University (Stanford, CA). The HS-1 murine T cell clone, which recognizes IA*–MBP(89–101), was obtained from the laboratory of S. Sircar, University of Vermont (Burlington, VT). The hybridoma cell line producing 10–2.16 monoclonal antibody against murine IA* and IA+ was obtained from American Type Culture Collection (Rockville, MD). Affinity-purified α/β-specific anti-TCR (H57-597) hamster monoclonal antibody and isotype match antibodies were purchased from PharMingen (San Diego, CA). Radioactive sulfur labeling reagent and 125I were purchased from Amersham Corp.

Maintenance and Culture of Cloned Murine T Cells—A/J.12 and 4R.3.9 cloned T cells restricted by IA* and MBP(1–14)A+ peptide were stimulated with MBP(1–14)A+ peptide and freshly irradiated SJL/J mouse spleen cells every 10 days. Similarly, the HS-1 cloned T cells restricted by IA* and MBP(89–101) peptide were pulsed with MBP(89–101) peptide and freshly irradiated SJL/J mouse spleen cells every 10 days. Residual antigen-presenting cells were removed by subjecting cloned T cells to a 19% metrizamide density gradient centrifugation, followed by two washes in RPMI 1640 medium, 2–5 × 109 T cells were cultured in RPMI 1640 + 10% fetal bovine serum (HyClone Laboratories Inc.) in the presence of 0.75–8.0 × 106 freshly irradiated A/J or SJL/J spleenocytes, 1 unit/ml recombinant human interleukin-2 (Genzyme Corp.), 26.6 μM MBP(1–14)A+ or 5 μg/ml MBP(89–101), 10 μM HEFPS, and 50 μM 2-mercaptoethanol in a final volume of 15 ml at 37 °C for 10 days. Prior to various experiments, cells were subjected to metrizamide density gradient centrifugation twice, washed in RPMI 1640, and resuspended in medium containing 10% fetal bovine serum.

Affinity Purification of Murine IA+ and IA—IA+ and IA− were purified from Nonidet P-40 extracts of membranes, prepared from CH27 cells and SJL/J mouse spleen cells, respectively, using an affinity column prepared by coupling monoclonal antibody, 10–2.16 (specific for IA* and IA+), with Sepharose 4B beads by the standard cyanogen bromide-coupling method as described earlier (10). Briefly, a high speed (100,000 × g) membrane fraction was detergent-extracted in a buffer containing 10 mM Tris-HCl, pH 8.3, 0.5% Nonidet P-40, 0.1 M NaCl, 5 mM EDTA, 0.02% sodium azide, and 1 mM PMSF. The lysate was recycled over the pre-equilibrated antibody column at 4 °C for 16 h. The column was washed with 10 bed volumes of deoxycholate buffer containing 10 mM Tris-HCl, pH 8.3, 0.5% deoxycholate, 0.1 M NaCl, 5 mM EDTA, 0.02% sodium azide, and 1
mm PMSF, followed by 5 bed volumes of PBS containing 1% n-octyl-β-D-glucopyranoside (OG) buffer. Finally, the IA molecules were eluted with 20 mM phosphate buffer, pH 11, containing 0.1 M NaCl, 1% OG, 0.02% sodium azide, and 1 mM PMSF. Each fraction was neutralized with 1 M acetic acid to a final concentration of 12 mM, and the MHC II class II molecules were concentrated using an Amicon Centriprep-10 concentrator. Affinity-purified IA" and IA molecules were characterized by 12% SDS-polyacrylamide gel electrophoresis.

**Synthesis of Peptides**—The rat myelin basic protein peptide analogs, MBP(1-14)A4 with the sequence Ac-ASQARPSQRHGSKY, MBP(1-14)A4A6 with the sequence Ac-ASQARPSQRHGSKY, and MBP(1-14)A4A6A4 with the sequence YFKKNIVTPRTPPP were synthesized by the standard solid phase methodology using side chain-protected Fmoc (N-(9-fluorenylemethyl)carbonyl) amino acids and an Applied Biosystems 431A automated peptide synthesizer. The deprotected, crude carboxyl-terminal peptide amidies were purified by reverse-phase high pressure liquid chromatography, and the homogeneity and identity of the purified peptides were confirmed by mass spectroscopic analysis.

**Radiolabeling of Peptides and IA Molecules**—Radiolabeling of peptides was achieved by the standard chloramine-T labeling procedure (11). Typically, 2.5 mg of peptide in a 500-ml volume was incubated with 2 mCi of Na[125I] in 0.1 M sodium phosphate buffer, pH 7.7. The labeled peptides were separated from free [125I] by Sephadex G-10 gel filtration chromatography. Specific activities of various MBP peptides ranged from 0.5 to 2.7 × 10⁶ cpm/μg. Radiolabeling of class II molecules was performed either by [125I] or by sulfur labeling reagent. Labeling of IA" with [125I] was achieved using Pierce IODO-BEADS and the manufacturer's recommended procedure. Preloaded beads were washed by preparing with 50 mM phosphate buffer, pH 7.5 (1 ml of buffer for two beads), and dried on Whatman paper. Six beads were added to 50 μg of 0.1 mg/ml IA" in 100 mM phosphate buffer, pH 7.5, containing 1 mM of [125I]. The reaction mixture was incubated at room temperature for 10 min. Labeled IA" was separated from the beads, and residual free [125I] was removed by extensive dialysis against PBS at 4 °C. The specific activity of [125I]-labeled IA" was in a range of 0.4-0.5 × 10⁶ cpm/μg. [35S] Labeling of IA" with sulfur labeling reagent was performed according to the manufacturer's recommended procedure (Amersham). Briefly, 1 ml of [35S] sulfur labeling reagent (1 mCi) was evaporated to dryness under a gentle stream of argon. One mg of IA" at a concentration of 1 mg/ml in 0.1 M borate buffer, pH 8.5, containing 0.5% OG was added to the dry sulfur labeling reagent. The reaction mixture was mixed and incubated at room temperature for 30 min, and the labeling was stopped by adding 100 μl of 0.1 M borate buffer, pH 8.5, containing 0.2 M glycine. The excess reagent was removed by dialysis of the labeled IA" against PBS containing 0.5% OG. The specific activity of IA" with [35S] label was 1.98 × 10⁶ cpm/μg.

**Peptide Binding Assay**—Peptide binding to murine class II molecules was analyzed by silica gel thin layer chromatography (TLC) as described earlier (12, 13). IA" or IA" at a concentration of 200-500 pg/ml was incubated with a 50-fold molar excess of radiolabeled peptide at 37 °C for 48 h. The excess unbound peptide was removed by extensive dialysis against PBS containing 0.1% OG detergent at 4 °C for 36 h. One μl of complex was applied in triplicate onto a 5-cm plastic-supported silica gel TLC plate (from EM sciences) and run in a solvent system of 5% ammonium acetate in 50% methanol. The plate was dried, and the distribution of radioactivity was estimated at RT 0-0.2 for calculating the percent of IA" or IA" occupied with labeled peptide. The percent of class II occupied with labeled peptide ranged from 25 to 35.

**T Cell Binding Assay**—Purified complexes of either radiolabeled peptide and nonradiolabeled IA molecules or [125I]-MBP and IA molecules with tritiated bovine serum albumin solution at 37 °C in a CO2 incubator. For antibody blocking experiments, cells were co-incubated with radiolabeled complexes in the presence of varying amounts of affinity-purified monoclonal antibodies. Purified isotype-matched standard control monoclonal antibodies were used in experiments to verify the extent of aggregation in the final preparation was measured by high speed centrifugation and by Sephadex G-200 gel filtration chromatography of complexes of [125I]IA" and unlabeled MBP(1-14)A4 peptide in a separate experiment and was found to be approximately 60%. Complexes of IA" with [125I]MBP(1-14)A4 were prepared, and the effect of complex concentration on T cell binding was measured (Fig. 1). Incubation of 4R3.9 T cells with an increasing concentration of complexes showed a linear increase in the number of molecules associated per T cell. No significant decrease in T cell viability was observed up to a complex concentration of 5 μg/ml. However, incubation of T cells with a complex concentration greater than 5 μg/ml showed a decrease in T cell viability. At a complex concentration of 25 μg/ml the viability was reduced to 70%. Based on this observation, a complex concentration of 5 μg/ml or less was used in all subsequent experiments.

To demonstrate that the recognition of complexes by cloned T cells is peptide-specific, two acetylated N-terminal MBP peptide analogs, MBP(1-14)A4 and MBP(1-14)A4A6A4, were used as controls.

**RESULTS**

Specific association of MHC class II and antigenic peptide with MBP-specific cloned T cells was demonstrated using complexes of radiolabeled peptide and affinity-purified murine MHC class II molecules. Since T cells are very sensitive to detergent, all cell binding experiments used complexes that were extensively dialyzed against detergent-free PBS. The extent of aggregation in the final preparation was measured by high speed centrifugation and by Sephadex G-200 gel filtration chromatography of complexes of [125I]IA" and unlabeled MBP(1-14)A4 peptide in a separate experiment and was found to be approximately 60%. Complexes of IA" with [125I]MBP(1-14)A4 were prepared, and the effect of complex concentration on T cell binding was measured (Fig. 1). Incubation of 4R3.9 T cells with an increasing concentration of complexes showed a linear increase in the number of molecules associated per T cell. No significant decrease in T cell viability was observed up to a complex concentration of 5 μg/ml. However, incubation of T cells with a complex concentration greater than 5 μg/ml showed a decrease in T cell viability. At a complex concentration of 25 μg/ml the viability was reduced to 70%. Based on this observation, a complex concentration of 5 μg/ml or less was used in all subsequent experiments.

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synthesized and purified. Replacement of the native lysine residue at position 4 by alanine has been shown by others (14) and confirmed in our laboratory to confer increased binding to IAα. Whereas both peptide analogs bind equally to IAα, only MBP(1-14)A4 stimulated AJ1.2 T cells. Both peptides were radiolabeled with 125I at the position 14 tyrosine residue and complexed with IAα. The cell association of IAα-MBP(1-14)A4 and IAα-MBP(1-14)A3A4A6 complexes to AJ1.2 T cells restricted by IAα and MBP(1-14) is presented in Fig. 2A. A significant increase in the cell association of relevant IAα-MBP(1-14)A4 complexes to AJ1.2 T cells was observed compared with the cell association of irrelevant IAα-MBP(1-14)A3A4A6 complex. The binding was sustained for at least 24 h at 37 °C without any decrease in T cell viability. When the binding of complexes with T cells at 4 and 37 °C was examined using 4R3.9 T cells restricted by IAα and MBP(1-14) peptide, a significant decrease in the number of cell-associated molecules was observed at 4 °C (Fig. 2B). The specificity of cell association of complexes was further demonstrated by incubating IAα-MBP(1-14)A4 complexes with another MBP-specific murine T cell line, HS-1, restricted for IAα-MBP(89-101) as shown in Fig. 2C. Although the percent of occupancy of IAα with MBP(89-101) and of IAα with MBP(1-14)A4 was very similar, only IAα-MBP(89-101) complexes showed a sharp increase in the number of cell-associated molecules with time. In all these experiments, the amount of cell-associated radioactivity increased to a maximum at 5-6 h, during which time there was no decrease in cell viability. The number of molecules associated with single T cells was calculated in each case from the specific activity of labeled peptide and was found to be in the range of 500,000-600,000.

To demonstrate that the uptake of radiolabeled peptide by T cells was mediated directly via TCRs and class I molecules, antibody-blocking experiments using affinity-purified anti-(αβ)TCR monoclonal antibodies or in control experiments, equivalent amounts of isotype-matched antibodies co-incubated under identical conditions did not show any significant inhibition of radiolabeled peptide uptake by 4R3.9 T cells.

In all these experiments, the number of labeled peptide molecules associated with single T cells was substantially higher than the number of TCRs (20,000-50,000) reported on single T cells (15, 16). To demonstrate that the increased number of labeled peptide molecules/T cell was not due to dissociation of the prebound peptide or release of free 125I from the tyrosine residue of the labeled peptide moiety at 37 °C, the stability of the purified complexes was studied. IAα-MBP(1-14)A4 complexes containing radiolabeled peptide were incubated in the absence of T cells in RPMI-1640 medium containing 10% serum. At various time intervals, complexes were analyzed by silica gel TLC, and the dissociation of complexes was measured. As shown in Fig. 4B, complexes of IAα and radiolabeled MBP(1-14)A4 were fully stable for the 6-h incubation period without any significant dissociation. The rate constant measurements (kd) indicate that the complexes were 100% stable during the entire incubation period. In addition, complexes at time 0 and at the end of the 6-h incubation period were subjected to silica gel TLC to ensure there was no detectable release of bound peptide or free 125I in the medium (Fig. 4A).

Since the number of complex molecules associated with a single T cell (400,000-600,000) did not correspond to the reported number of TCRs (20,000-50,000) on the T cell surface, further experiments were designed to permit separate calculation of the average number of IAα heterodimer and peptide moieties associated with single T cells following the complex incubation. This was achieved using preformed complexes of known specific activity containing either 125I-labeled IAα and unlabeled MBP(1-14)A4 peptide or complexes containing unlabeled IAα and 125I-labeled MBP(1-14)A4 peptide.

**Fig. 2. Specificity of the association of MHC-peptide complex with cloned T cells.** Complexes of IAα and IAα with various radiolabeled MBP peptide analogs were prepared and purified as described under "Materials and Methods." In panel A, the antigen specificity was shown by incubating resting AJ1.2 T cells with IAα-MBP(1-14)A4 complexes (○) or with IAα-MBP(1-14)A3A4A6 complexes (□). The specific activity of MBP(1-14)A4 was 2.7 × 105 cpm/μg and that of MBP(1-14)A3A4A6 was 1.4 × 106 cpm/μg. Based on the concentration of IAα and the specific activity of IAα-bound peptide, MBP(1-14)A4 and MBP(1-14)A3A4A6 occupied 41 and 35% of the IAα molecules, respectively, as measured by silica gel TLC assay. Panel B represents the binding of IAα-MBP(1-14)A4 complexes to 4R3.9 T cells at 37 °C (□) and at 4 °C (○). Panel C represents the binding of IAα-MBP(89-101) complexes (△) and IAα-MBP(1-14)A4 complexes (△) to HS-1 T cells. The specific activity of the radiolabeled peptide MBP(1-14)A4 in panels B and C was 0.75 × 105 cpm/μg.
The results of such an experiment are shown in Fig. 5A. Approximately 20,000–30,000 molecules of the IA\(^b\) moiety of the IA\(^a\)-peptide complex were associated with single AJ1.2 T cells compared with 400,000–500,000 molecules of peptide moiety/T cell. This result was again confirmed in a separate experiment with 4R3.9 cloned T cells, specific for the same N-terminal MBP peptide fragment. In this study, T cells were incubated with a double labeled complex prepared from \(^{35}S\)-labeled IA\(^a\) and \(^{125}I\)-labeled MBP(1–14)A\(^4\) peptide. The calculated number of cell-associated IA\(^a\) or peptide moiety of the complex bound per T cell is shown in Fig. 5B. The results obtained from the experiment using double labeled complexes were similar to those obtained with single labeled complexes.

To examine the fate of TCRs on the T cell surface following class II-peptide complex treatment, cells were monitored for TCRs by FACS analysis using anti-TCR(\(c\)u\(B\)) monoclonal antibody followed by fluorescein isothiocyanate-conjugated secondary antibody. The flow cytometry results presented in Fig. 6 revealed no decrease in cell surface TCRs before and after treatment with relevant complex, suggesting no down-regulation of T cell receptors during binding of complex and internalization of peptide molecules.

Finally, to address the question whether the peptide uptake by T cells from the trimolecular complex is an energy-dependent phenomenon or is mediated by microfilaments, the effect of sodium azide and cytochalasin B on the cell association of the IA\(^a\) and peptide moieties of the doubly labeled complex was examined (Fig. 7). Sodium azide is known to inhibit the electron transport system and therefore can be used as an inhibitor of energy-dependent cellular uptake mechanisms. Cytochalasin B, however, is a fungal product widely used as an inhibitor of cell function via its binding to the actin moiety of actinomycin and inhibition of microfilament contraction (17). As shown in Fig. 7A, cloned 4R3.9 T cells incubated with double labeled complexes in medium containing 0.2% sodium azide showed a significant decrease in the uptake of peptide moiety, whereas 4R3.9 T cells incubated with up to 4 mM cytochalasin B did not show any significant decrease in peptide uptake (Fig. 7B). Similar results were obtained with cloned AJ1.2 T cells incubated with double labeled complex in the presence of each inhibitor. The viability of T cells was measured at various time periods, and cells were found to be fully viable in all these experiments.

**DISCUSSION**

Antigen presentation to T cells involves the interaction of MHC-peptide complexes on the surface of APC with TCRs on T cells (1, 2). In *in vivo*, such presentation leads to the activation of T cells (18). In *in vitro*, however, the recognition of purified MHC class II-peptide complexes by T cells in the absence of APCs and costimulatory signals results in induction of nonresponsiveness (19). In both cases the fate of the antigenic peptide, MHC class II, and TCR molecules following presentation is not defined. In this report, using radiolabeled complexes of MHC class II and antigenic peptides we observed internalization of the peptide molecules from the trimolecular complexes. A direct association of labeled complexes (containing radiolabeled peptide, radiolabeled class II, or both) with T cells was demonstrated using murine cloned T cells. AJ1.2 and 4R3.9 T cells have been shown to recognize and respond to peptide containing the first nine amino acids of MBP in context to murine IA\(^a\) (20). Both clones also recognize and respond to MBP(1–14) in association with IA\(^a\). Similarly, the HS-1 T cell clone restricted for IA\(^a\) and MBP(90–101) has
been characterized (21). Complexes were prepared and purified, and the complete removal of unbound peptide was confirmed by analyzing complexes by silica gel TLC and autoradiography. Incubation of T cells with increasing concentrations of purified complexes resulted in an increase in cell association of these molecules as shown in Fig. 1. Incubation of cloned T cells with higher concentrations of complexes was associated with decreased cell viability. The possibility that mature T cells stimulated with very high levels of TCR occupancy in the absence of costimulatory signals first become functionally anergic and then die, whereas cells stimulated with lower levels of TCR occupancy in the absence of costimulation manifest only the first phenotype, has been speculated upon earlier (22). Recent results from our laboratory indicate that cells incubated with 50 μg/ml or higher concentration of complexes for 15 h lead to complete cell death.2

Based on the specific activity of the peptide, the number of molecules associated with a single T cell was calculated and found to be approximately 600,000. The number of TCR molecules on a single T cell was reported to be 20,000–30,000 (15, 16). Thus, the observed number of molecules associated per T cell in our experiments was significantly higher than the reported number of TCRs on T cells. Such an increased number of cell-associated molecules may be the result of one or more of the following possibilities: (i) internalization of the peptide moiety; (ii) rapid dissociation of labeled peptide from the MHC-peptide complexes; or (iii) release of free 125I from the tyrosine residue. The dissociation kinetics of IAa,125I-MBP(1-14)A4 complex was performed at 37 °C and analyzed by TLC at times of 0–6 h. Since no detectable labeled peptide or free 125I was observed for up to 6 h of incubation of the complex as shown in Fig. 4A, the increased number of T cell-associated radiolabeled molecules is not due to either dissociation of peptide from the MHC-peptide complexes or release of free 125I in medium containing 10% serum.

The specificity of peptide uptake is demonstrated by three different approaches. (i) The binding of complex and the internalization of the peptide moiety are peptide-specific, as shown by using an analog of MBP(1-14)A4 peptide where

\[ \text{MBP(1-14)A} \]

\[ \text{IAa} \]

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The effect of sodium azide and cytochalasin B was examined to determine if the uptake of peptide by T cells is due to an active process and not due to a microfilament-mediated phenomenon. The internalization of peptide molecules from the ternary complexes is an active process as the uptake was significantly reduced at 4 °C. Similarly, the number of cell-associated radiolabeled peptides was markedly decreased when complexes were incubated with T cells in the presence of varied concentrations of sodium azide ranging from 0.01 to 0.2%. Sodium azide is known to inhibit the electron transport system and therefore can be used as an inhibitor of energy-dependent cellular uptake mechanisms. In contrast, incubation of T cells with relevant complex in the presence of cytochalasin B up to 4 mM did not show any significant inhibition of cell-associated peptide molecules/T cell. Cytochalasin B is a fungal product widely used as an inhibitor of cell function via its binding to the actin moiety of actinomyein and inhibition of microfilament contraction (17). Cytochalasin B at concentrations between 0.02 and 0.2 μg/ml has been shown to be slightly stimulatory to lymph node cells, whereas at higher concentrations of 5-10 μg/ml or above, it shows inhibition of lymphocyte responses (26). Incubation of 4R3.9 T cells with up to 4 mM cytochalasin B did not show any significant decrease in peptide uptake by these T cells.

Estimates of $k_d$ for dissociation of MHC-peptide complex from TCR have been reported both for recombinant soluble MHC-I peptide complexes (27) and recombinant soluble MHC-II-peptide complexes (28). In the latter study, the $k_d$ value ranged from $4 \times 10^{-3}$ to $6 \times 10^{-3}$ for soluble, monomeric 1εA-peptide complex bound to TCR on T cells or T hybridomas. These data would suggest that very little MHC-II-peptide complex will bind to T cells at the concentrations of complexes used in our study. However, the present study uses purified native MHC II with the transmembrane domain intact. Therefore, one would not expect an appreciable fraction of the complex to exist in a soluble, monomeric form in the absence of detergent. In fact, we have shown that the native MHC II used in our studies is 60-90% aggregated. Similar binding of purified MHC-II-peptide complexes to several T cell clones was observed in other studies from our laboratory (10, 23). We propose that the aggregated material is multivalent and binds with a higher apparent affinity to multiple TCR on the T cell surface, and therefore lower concentrations of relevant complex effectively bind to the cognate T cell. Another possibility is that the MHC-II-peptide complex binds to the TCR with high affinity, but a rapid translocation of the peptide moiety into the cell results in a rapid release of the empty MHC II molecule from the ternary complex, resulting in a lower apparent $k_d$.

The results presented in this study suggest that the in vitro interaction of purified MHC-II-peptide complex with T cell receptor on cloned T helper cells leads to internalization of only the peptide moiety, most likely by an energy-dependent translocation process. Although the function served by the proposed internalization of the peptide moiety of the cognate complex is not clear at present, one may speculate that it could facilitate disengagement of apposed antigen-presenting cell and T cell after interaction and/or that it may be involved in immunoregulation of T cell function. A model for T cell activation has been proposed in which the last step is a decline in the enhanced adhesiveness between APC and T cell prior to detachment (27). The proposed disengagement would be hindered by multiple attachments remaining between the apposed cells as a result of intercellular MHC-peptide-TCR ternary molecular bridges stabilized by CD4 or CD8 molecules that are known to cross-link MHC to the CD3-TCR complex.

![Figure 7](image-url)
Internalization of the peptide moiety may serve to destabilize these bridges and facilitate separation.

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