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Immunology

Stimulation of T cells by antigenic peptide complexed with isolated chains of major histocompatibility complex class II molecules

(class II chains/peptide binding/T-cell stimulation)

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ABSTRACT **Major histocompatibility complex (MHC) class II molecules are heterodimeric glycoproteins with one α and one β polypeptide chain of similar molecular size. In this report, we describe the binding of an acetylated N-terminal peptide of myelin basic protein, [Ala^4]MBP-(1–14), to purified individual α and β chains of murine I-A^k molecules. Purified complexes of isolated single chains and antigenic peptide bind to cloned T cells restricted by I-A^k and [Ala^4]MBP-(1–14) tetradecapeptide. The binding is blocked by α/β anti-T-cell receptor (TCR) monoclonal antibody. Cell triggering as measured by an increase in extracellular acidification rate is observed when cloned T cells are exposed to purified complexes of isolated chains and antigenic peptide. This increase in the extracellular acidification rate is antigen specific and MHC-restricted, as chains alone or irrelevant chain–peptide complexes do not trigger an increase in the metabolic acidification rate. These results together demonstrate that *in vitro* cloned T cells are triggered by complexes of specific antigenic peptides and isolated individual chains of their cognate MHC proteins.**

Activation of CD4$^+$ T cells normally involves interaction of complexes of antigenic peptides and class II major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells and T-cell receptors (TCRs) on T cells. *In vitro* studies have demonstrated that peptides can bind to affinity-purified MHC class II molecules, and these complexes stimulate specific T-cell responses (1–3). It has been shown that fluorescence-labeled antigenic peptides bind to separate α and β chains of MHC class II molecules (4). This earlier work has raised two questions: (*i*) do MHC class II single-chain peptide complexes bind to TCRs on CD4$^+$ T cells, and (*ii*) can this putative binding trigger cloned T cells? The possibility of T-cell activation by MHC chain–peptide complexes was suggested by the reported ability of alloreactive I-A^k-specific cytotoxic T lymphocytes (CTL) to specifically lyse transfected L cells expressing either A^{k}b1/D^{d}c2 (5) or A^{k}a1/D^{d}c2 (6) MHC class II/class I hybrid molecules. Here we report the binding of N-terminal acetylated myelin basic protein (MBP) fragment [Ala^4]MBP-(1–14) [sometimes called "MBP(1-14)A^{4}"] to isolated and purified α and β chains of I-A^k. In addition, results presented here demonstrate that complexes consisting of isolated monomeric chains and antigenic peptide can recognize TCR and trigger MHC-restricted cloned T cells to a degree comparable to that obtained with the α/β dimer–peptide complexes, as measured by an increase in the metabolic acidification rate (7).

MATERIALS AND METHODS

Purification of Murine I-A^k and I-A^s. I-A^k and I-A^s were purified from Nonidet P-40 extracts of membrane prepared from cultured CH27 cells and SJL/J mouse spleen cells, respectively, by using an affinity support prepared with monoclonal antibody 10-2.16 (specific for I-A^k and I-A^s) coupled to Sepharose 4B beads by the standard cyanogen bromide coupling method. Briefly, a membrane fraction from high-speed centrifugation (100,000 × *g*) 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 phenylmethylsulfonyl fluoride (PMSF), and the lysate was recycled over the preequilibrated antibody column at 4°C for 16 hr. 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 phosphate-buffered saline (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 class II molecules were concentrated by using an Amicon Centriprep-10 concentrator. Affinity-purified I-A^k and I-A^s molecules were characterized by 12% one-dimensional SDS/polyacrylamide gel electrophoresis.

Isolation of α and β Chains of MHC Class II Molecules. Purified I-A^k was concentrated to 1 mg/ml with Amicon concentrators and applied onto a 12% preparative (16 cm × 18 cm) slab gel unit. The electrophoresis was conducted under nonreducing conditions for 16 hr at room temperature at constant volts (100 V). One lane was excised from the center of the gel and developed by silver staining. The stained gel lane was used as a guide to excise bands of α and β chains, and the proteins were electroeluted at 400 mA for 4 hr in an Amicon Electroeluter unit in the presence of 0.1% SDS. Eluted chains were concentrated, dialyzed against PBS containing 1% OG, and characterized on native and reduced SDS/polyacrylamide gels.

Synthesis of Peptides. The rat MBP peptide analog [Ala^4]MBP-(1–14) tetradecapeptide with the sequence Ac-Ala-Ser-Gln-Ala-Arg-Pro-Ser-Gln-Arg-His-Gly-Ser-Lys-Tyr, [Tyr^{89}]MBP-(89–101) tridecapeptide with the sequence Tyr-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro-Pro-Pro, and the ovalbumin (OVA) octadecapeptide [Tyr^{340}]OVA-(323–340) with the sequence Ile-Ser-Gln-Ala-Val-His-Ala-Ala-His-Ala-Glu-Ile-Asn-Gla-Ala-Gly-Arg-Tyr were synthesized by the standard solid-phase method using side chain-protected 9-fluorenylmethoxycarbonyl-conjugated amino acids and an Applied Biosystems 431A automated peptide

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Abbreviations: MHC, major histocompatibility complex; CTL, cytotoxic T lymphocyte; TCR, T-cell receptor; OG, *n*-octyl β-D-glucopyranoside; MBP, myelin basic protein; OVA, ovalbumin; CAE, cellulose acetate electrophoresis.

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synthesizer. Deprotected, crude peptide amides were purified by reverse-phase HPLC, and the homogeneity and identity of the purified peptides were confirmed by mass spectroscopic analysis.

Peptide Binding Assay. Peptide binding to α/β heterodimers or isolated chains of I-A^k was analyzed by TLC and by cellulose acetate electrophoresis (CAE) as described (8, 9). Briefly, synthetic [Ala^4]MBP-(1–14) and [Tyr^{340}]OVA-(323–340) peptides were ^{125}I-radiolabeled by the chloramine-T method at neutral pH. Free unbound ^{125}I was removed by centrifugal gel filtration, and the specific activities of [Ala^4]MBP-(1–14) and [Tyr^{340}]OVA-(323–340) peptides were calculated to be 1.73×10^6 and 1.65×10^6 cpm/μg, respectively. Intact I-A^k at a concentration of 200 μg/ml or each chain at a concentration of 100 μg/ml was incubated in a total volume of 100 μl with a 50-fold molar excess of radiolabeled peptide at 37°C for 48 hr. The excess unbound peptide was removed by extensive dialysis against PBS containing 0.1% OG detergent at 4°C for 36 hr. One microliter of complex was applied in triplicate onto a 5-cm silica gel TLC plate and run in a solvent system of 50% methanol/5% ammonium acetate. The plate was dried and the distribution of radioactivity was estimated at R_f 0–0.2 for calculating the percent of I-A^k or chains occupied with labeled peptide. Peptide binding was also measured by CAE in which 1 μl of complex was applied at the center of cellulose polyacetate paper strips (2.5 cm × 15.2 cm), and electrophoresis was performed at constant 350 V for 10 min in the presence of high-resolution Tris/barbital buffer [32.1% (wt/wt) Tris/13.7% (wt/wt) barbital/54.2% (wt/wt) sodium barbital, pH 8.1]. The strips were dried, and the origin was assayed for radioactivity to calculate the percent of I-A^k or chain occupied with labeled peptide.

Preparation of Chain–Peptide Complexes. Two types of complexes were prepared and purified. For peptide binding and T-cell binding assays, complexes of unlabeled heterodimer or individual chain with radiolabeled peptides were prepared as described above. For T-cell extracellular acidification rate measurements, unlabeled class II or chains and unlabeled peptide were used under identical conditions, and the complexes were dialyzed against bicarbonate-free, low-buffering RPMI 1640 medium (pH 7.4) containing 1 mM sodium phosphate, penicillin at 100 units/ml, and streptomycin at 100 μg/ml. The extent of aggregation in the final preparation was measured by high-speed centrifugation and by Sephadex gel-filtration chromatography of complexes of ^{125}I-labeled I-A^k and unlabeled [Ala^4]MBP-(1–14) peptide in a separate experiment and was found to be ≈60%.

T Cell-Binding Assay. Purified complexes of radiolabeled peptide and nonradiolabeled Ia dimer or monomers were incubated with 2.6×10^5 resting T cells in polypropylene tubes precoated with bovine serum albumin solution (1 mg/ml) at 37°C in a CO_2 incubator. For the antibody-blocking experiment, cells were first incubated with 500 μg of affinity-purified hamster anti-α/β TCR monoclonal antibody (H57-597 from PharMingen, San Diego) before the addition of labeled complexes. Purified hamster IgG isotype standard antibody, specific for 2,4,6-trinitrophenyl (PharMingen) was used as a negative control antibody. At the end of the incubation period, 15 ml of chilled PBS was added to each tube. The cells were resuspended gently and centrifuged at 2000 × g, and the supernatant was carefully removed. The washing procedure was repeated three times, and the radioactivity of the cell pellet was counted in a γ counter.

Measurement of T-Cell Metabolic Acidification Rate. Details of the loading of T cells into the microphysiometer for the acidification rate measurements have been described (7). Briefly, cells were rested from antigen pulsing for 10 days and cultured overnight in low-serum (2.5%) medium to lower their basal metabolic activity. The cells were harvested and resuspended in serum-free loading medium (low-buffering RPMI 1640 containing 10 mM Hepes, pH 7.3) at 1×10^7 cells per ml. T cells were loaded into disposable cell capsules (Molecular Devices, Menlo Park, CA) by centrifuging 0.1 ml of the cell suspension into the capsules using cell focusers (funnel-shaped inserts) to direct the cells into a 3.2-mm circle in the middle of the cell capsule membranes, which were precoated with particles of collagen to immobilize the cells. A top membrane is placed over the cells imbedded in collagen, and the cell capsule is loaded into the microphysiometer chamber, which is maintained at 37°C and perfused at 50 μl/min with low-buffering RPMI 1640 medium containing 1 mg of endotoxin-free human serum albumin per ml (Miles) but no added Hepes or bicarbonate. Extracellular acidification measurements were made in the microphysiometer as described by Parce et al. (10), Owicki et al. (11), and McConnell et al. (12) by collecting potentiometric measurements for 1 min every 2.5 min. Acidification rate data (μV/sec) were normalized to 100% prior to cell stimulation, which allows the comparison of data from cells in separate chambers. The microphysiometer was equipped with 200-μl sample loops for injection of soluble MHC–peptide complexes that were dialyzed against the same low-buffering medium.

RESULTS AND DISCUSSION

α and β chains of affinity-purified I-A^k were electroeluted from a nonreducing polyacrylamide gel and characterized by SDS/polyacrylamide gel electrophoresis (Fig. 1). Dissociation of α/β heterodimer into α and β chains can be achieved

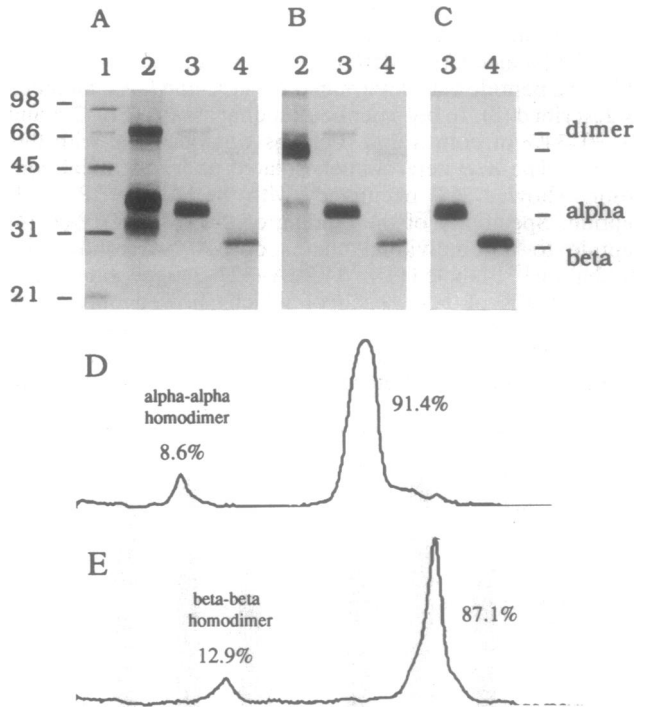

FIG. 1. Characterization of electroeluted α and β chains of I-A^k on SDS gel. Purified α and β chains of I-A^k were analyzed on a silver-stained 12% SDS gel. (*A*) Chains purified from a batch where dissociation of the heterodimer was obtained by incubation at 95°C for 5 min. Lanes: 1, molecular mass markers (reduced); 2, purified I-A^k (reduced); 3 and 4, α- and β-chain preparations, respectively (nonreduced). (*B* and *C*) Chains isolated after low-pH dissociation and analyzed under nonreducing and reducing conditions, respectively. Lanes: 2, I-A^k; 3, α chain; 4, β chain. Lanes 3 and 4 of the native gel from *B* were scanned on Microtek (model MRS-600 ZS) to calculate percent homodimers in the preparation, and are shown in *D* and *E*, respectively.

either by incubating purified I-A^k at 95°C for 5 min (Fig. 1*A*) or by exposing to pH 3.0 for 1 hr at 4°C (Fig. 1*B*). Both conditions resulted in significant dissociation of the dimer into monomers. When murine I-A^d and fluorescence-labeled OVA-(323–339) peptide was used (13), the peptide bound to individual α or β chains of I-A^d is stable at acidic pH as compared with the intact α/β–peptide complexes. Since incubation at high temperature is more likely to denature the MHC class II chains (14), low pH treatment was used as a method for monomer enrichment. Individual chains purified by the electroelution method were free of any cross-contamination. The chain preparations from different batches contained 5–15% homodimers. As expected, the homodimer bands migrated at a mobility close to the α/β heterodimer position. To make sure that the high molecular weight band at ≈60 kDa in the native gel is not due to any cross-contamination of chains, the chain preparations were analyzed by SDS/PAGE after reduction with 2-mercaptoethanol (Fig. 1*C*). Silver staining of the gel did not show any detectable cross-contaminant α or β band. The relative amount of homodimers present in the chain preparation was quantitated by scanning the native gel lanes for both α and β chains, which showed (Fig. 1 *D* and *E*) that the α and the β polypeptide preparations used in this study contain 8.6% and 12.9% homodimers, respectively.

Binding of peptide to purified α and β chains was carried out at pH 8.0, which has been reported to be optimal for the binding of [Ala^4]MBP-(1–14) peptide to I-A^k (9). The MBP peptide analog used in our study contains an alanine substitution at position 4 in place of a lysine residue. The replacement of Lys-4 with alanine in MBP-(1–14) has been shown earlier to increase peptide binding to I-A^k (15); this has been confirmed in our laboratory. Isolated chains were incubated with ^{125}I-labeled [Ala^4]MBP-(1–14) peptide, and quantitation of bound peptide was determined by TLC and CAE methods as described (8). In both peptide-binding assays (Fig. 2 *A* and *B*), 30–34% of both α and β chains was occupied with this peptide. The α/β heterodimer isolated under identical conditions showed 25% occupancy with the [Ala^4]MBP-(1–14) peptide. Specificity of the binding of the [Ala^4]MBP-(1–14) peptide to the individual chains of I-A^k was shown by incubating ^{125}I-labeled [Tyr^{340}]OVA-(323–340) peptide with α and β chains of I-A^k, each of which showed only 2–3% binding of peptide. Slightly increased binding of the [Ala^4]MBP-(1–14) peptide to individual chains of I-A^k compared with intact α/β heterodimeric molecule was observed consistently in three different experiments. This may be due to a more peptide-accessible conformation of chains vs. intact heterodimeric molecule or to the absence in isolated chains of prebound endogenous peptides that are known to be present in intact heterodimeric molecules (16, 17).

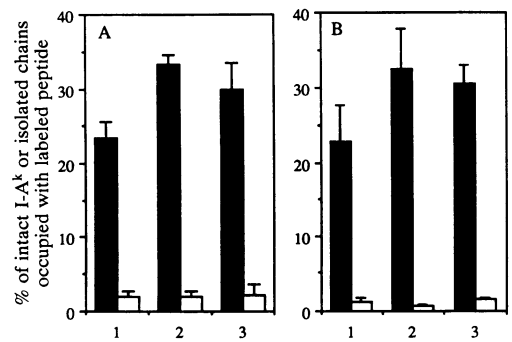

FIG. 2. Binding of ^{125}I-labeled [Ala^4]MBP-(1–14) peptide to isolated chains. I-A^k at 200 μg/ml and each chain at 100 μg/ml were incubated in a total volume of 100 μl with a 50-fold molar excess of radiolabeled peptide, and the complexes were purified as described in text. (*A*) TLC analysis. (*B*) CAE analysis. Bars: 1, intact I-A^k–[Ala^4]MBP-(1–14) complex; 2, α chain–[Ala^4]MBP-(1–14) complex; and 3, β chain–[Ala^4]MBP-(1–14) complex, ■, binding of ^{125}I-labeled [Ala^4]MBP-(1–14) peptide; open bars represent binding of ^{125}I-labeled [Tyr^{340}]OVA-(323–340) peptide. Each figure representation is an average of three data points.

The stability of single chain–peptide complexes at 37°C in PBS containing 1% OG is presented in Fig. 3. Incubation of β chain–[Ala^4]MBP-(1–14) complexes at 37°C for 1 hr led to 40% dissociation. In contrast, only 20% dissociation of α chain–[Ala^4]MBP-(1–14) complexes and 5% dissociation of I-A^k–[Ala^4]MBP-(1–14) complexes were observed over a period of 24 hr at 37°C. The dissociation observed for the α chain–[Ala^4]MBP-(1–14) and β chain–[Ala^4]MBP-(1–14) complexes is clearly biphasic. For the initial phase, β chain–[Ala^4]MBP-(1–14) complexes have a higher dissociation rate ($k_d = 4.31 \times 10^{-5}$ sec^{-1}) than α chain–[Ala^4]MBP-(1–14) complexes ($k_d = 6.2 \times 10^{-6}$ sec^{-1}). However, for the second phase of the dissociation, the slopes of the α/β chain–[Ala^4]MBP-(1–14) and α chain–[Ala^4]MBP-(1–14) complexes are almost identical with k_d values of 5×10^{-7} sec^{-1} and 7×10^{-7} sec^{-1}, respectively. The slope of the second phase of β chain–[Ala^4]MBP-(1–14) complexes had a k_d value of 6.2×10^{-6} sec^{-1}. The initial increased dissociation rate of I-A^k β chain–[Ala^4]MBP-(1–14) was different from dissociation rates reported for complexes of murine I-A^d subunits and fluorescence-labeled OVA-(323–339) peptide (13). There, the I-A^d β chain–OVA-(323–339) complexes showed a lower dissociation rate as compared with the rate for I-A^d α chain–OVA-(323–339) complexes. Biphasic peptide dissociation from α/β heterodimers has also been noted (18, 19).

Using complexes of radiolabeled peptide and unlabeled chains, direct binding of chain–peptide complexes to cloned T cells was measured, and the number of complex molecules associated with cells was calculated. The T-cell clone 4R3.9, which recognizes MBP-(1–14) in the context of I-A^k (20), was incubated with purified single-chain complexes containing ^{125}I-labeled [Ala^4]MBP-(1–14) peptide. Since T cells are very sensitive to detergent, for cell binding and stimulation assays

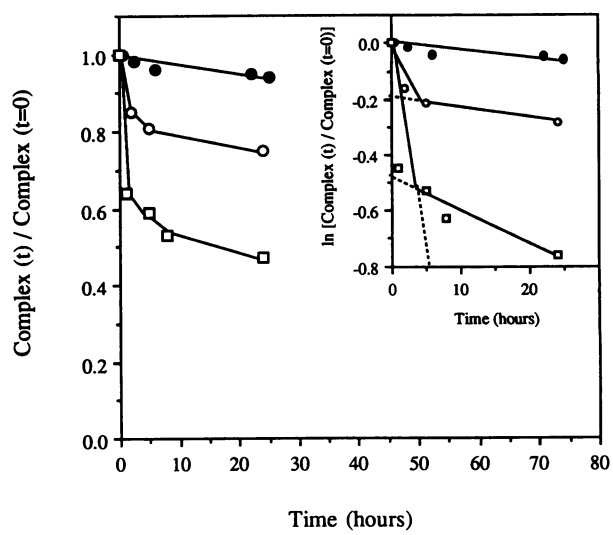

FIG. 3. Dissociation kinetics of chain–peptide complexes. Complexes of the α/β heterodimer and of isolated chains with ^{125}I-labeled [Ala^4]MBP-(1–14) peptide were prepared and purified as described in text. Purified complexes were incubated at 37°C; at various time intervals, 1 μl of complex was applied onto a silica gel TLC plate. The plate was run, and the percent dissociation was calculated. The value 1.0 represents the original complex at time zero. ●, I-A^k–[Ala^4]MBP-(1–14) complex; ○, α chain–[Ala^4]MBP-(1–14) complex; □, β chain–[Ala^4]MBP-(1–14) complex. The data represent an average of triplicate determinations. (*Inset*) Estimation of the dissociation rate constant, k_d.

we used complexes that were dialyzed against detergent-free low-buffering RPMI 1640 medium or PBS buffer. The results presented in Fig. 4*A* show that the binding of α chain–[Ala^4]MBP-(1–14) complexes to 4R3.9-cloned T cells is comparable to that of intact I-A^k–[Ala^4]MBP-(1–14) complexes. The β chain–[Ala^4]MBP-(1–14) complexes showed lower binding to 4R3.9 T cells. The association of MHC class II peptide complexes with the T cells was complete by 6 hr at 37°C; the number of complex molecules associated per T cell was calculated and found to be 2.55×10^6, 2.1×10^6, and 1.0×10^6 for I-A^k–[Ala^4]MBP-(1–14), α chain–[Ala^4]MBP-(1–14), and β chain–[Ala^4]MBP-(1–14) complexes, respectively. The number of complex molecules bound per T cell was higher than the reported number of TCRs on the T-cell surface, $2.5–5.0 \times 10^4$ (21, 22) to 1×10^5 (23), which could be due to the aggregation of the complexes in the absence of detergent. This aggregation may also account for the lower binding rate as compared with the binding of water-soluble I-E^k–moth cytochrome *c* peptide binding to a specific T-cell receptor (23). The specificity of the chain–peptide complex binding to the T cells was confirmed by incubating complexes of α and β chains of I-A^k and ^{125}I-labeled [Ala^4]MBP-(1–14) peptide with the HS-1 T-cell clone, restricted for I-A^s and MBP-(90–103) complexes (24). Preferential binding of all three complexes to 4R3.9-cloned T cells, restricted for I-A^k and MBP-(1–14) peptide, was observed as compared with the HS-1 T cells (Fig. 4*B*). In a positive control experiment, incubation of I-A^s–[Tyr^{89}]MBP-(89–101) complexes showed the expected strong binding to HS-1-cloned T cells. The binding of chain–peptide complexes to TCRs of 4R3.9 T cells was also confirmed by an antibody-blocking experiment in which cells pretreated with α/β anti-TCR monoclonal antibody failed to bind complexes (Fig. 4*C*). Cells preincubated with isotype-matched antibody under identical conditions showed no inhibition of binding. Similar experiments demonstrating the ability of anti-TCR Fab to compete with soluble MHC class II-peptide complexes have been reported earlier (23).

To determine whether isolated chain–peptide complexes trigger an *in vitro* T-cell response, we used a microphysiometer that measures extracellular acidification rate of ligand-mediated cell triggering. An increase in acidification rate on ligand binding to a wide variety of cellular receptors has been demonstrated with this microphysiometer (10–12). Recently, it has been confirmed that such extracellular acidification rate measurements can be used to detect the peptide-specific MHC-restricted response of a T-cell clone. The specificity of the T-cell response was demonstrated by the lack of response to irrelevant MHC–peptide complexes, MHC class II alone, and a 10-fold molar excess of peptide (7). In this report, we used preformed complexes of isolated chains of I-A^k with [Ala^4]MBP-(1–14) peptide to demonstrate the stimulation of T cells *in vitro*. A specific T-cell response, within minutes of the interaction of complexes with TCR, was observed when the resting 4R3.9 T cells were exposed to single chain–peptide complexes. A rapid, sustained increase in the acidification rate, characteristic of intact I-A^k–[Ala^4]MBP-(1–14) complexes and T-cell interaction, was observed when 4R3.9 T cells were exposed to α chain–[Ala^4]MBP-(1–14) complexes (Fig. 5*A*). The complexes of β chain–[Ala^4]MBP-(1–14) peptide also induced the increased acidification rate in 4R3.9 cells, but not to the same extent as the α-chain complex, which was slightly more potent. The intact I-A^k–[Ala^4]MBP-(1–14) complex gave the highest level of T-cell stimulation, which became more apparent about 2 hr after complex treatment. The lower increase in acidification rate induced by β chain–[Ala^4]MBP-(1–14) complexes was reproducible and could be correlated with the increased dissociation rate of this complex. The specificity of the I-A^k chain–peptide complex activation of T cells is demonstrated by the lack of increased acidification rates when an equimolar mixture of α and β is used with T cells as shown in Fig. 5 *A* and *B*. In several experiments, α and β chains without peptide gave no increase in acidification rate over medium alone. In the same set of experiments (Fig. 5*B*), several nonspecific MHC complexes, I-A^s–[Tyr^{89}]MBP-(89–101), I-A^s α chain–[Tyr^{89}]MBP-(89–101), and I-A^s β chain–[Tyr^{89}]MBP-(89–101) did not induce significant increases in the acidification rate. In a positive control experiment, the I-A^s–MBP-(89–101), I-A^s α chain–MBP-(89–101), and I-A^s β chain–MBP-(89–101) complexes did stimulate HS-1 cloned T cells, restricted for I-A^s and MBP-(89–101) (unpublished results).

Earlier reports indicate that MHC and peptide in planar membranes present antigen to T hybridoma cells, resulting in the antigen-specific release of interleukin 2 (IL-2) (25). In the case of T-cell clones, however, the antigen presentation by planar membranes does not induce detectable IL-2 production, but rather induces a long-lived state of nonresponsiveness to subsequent stimulation by antigen-presenting cells

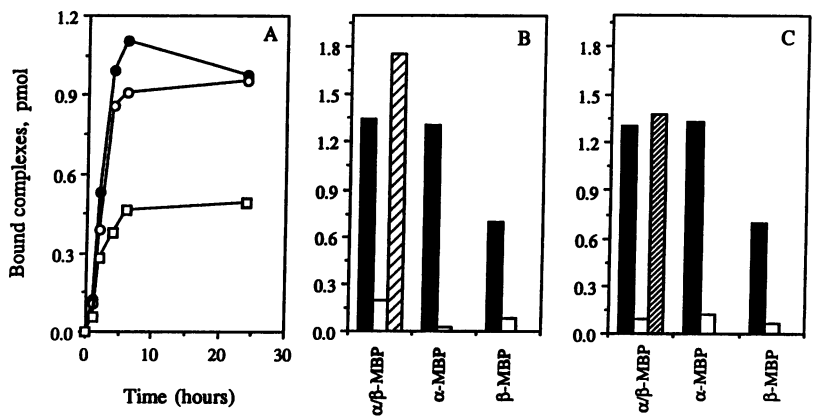

FIG. 4. Binding of individual chain–peptide complexes to cloned T cells. Complexes of radiolabeled [Ala^4]MBP-(1–14) peptide and α or β chains or intact I-A^k were prepared as described in the legend to Fig. 2. The percent occupancy of α/β heterodimer, α chain, and β chain, with labeled peptide was 26.5%, 35.2%, and 33.0%, respectively. Resting 4R 3.9 T cells (2.6×10^5; 10 days after antigen pulsing) were incubated at 37°C with 30 μg of I-A^k–[Ala^4]MBP-(1–14) peptide complexes or 15 μg of chain–[Ala^4]MBP-(1–14) peptide complexes in a total volume of 1 ml. At various times, cells were washed with RPMI medium and assayed for radiolabel, and the amount of bound complexes was calculated. (*A*) Binding of complexes to 4R3.9 T cells. ●, I-A^k–[Ala^4]MBP-(1–14) complex; ○, α chain–[Ala^4]MBP-(1–14) complex; □, β chain–[Ala^4]MBP-(1–14) complex. (*B*) Specificity of the binding after 6 hr of incubation. ■, Binding of various complexes to 4R3.9 T cells; □, binding of complexes to cloned HS-1 T cells; ▨, binding of I-A^s–[Tyr^{89}]MBP-(89–101) complexes to HS-1 clone as a positive control. (*C*) Effect of anti-α/β TCR monoclonal antibody (H57-597) (□) and isotyped matched antibody (▨) on the binding of complexes to T cells.

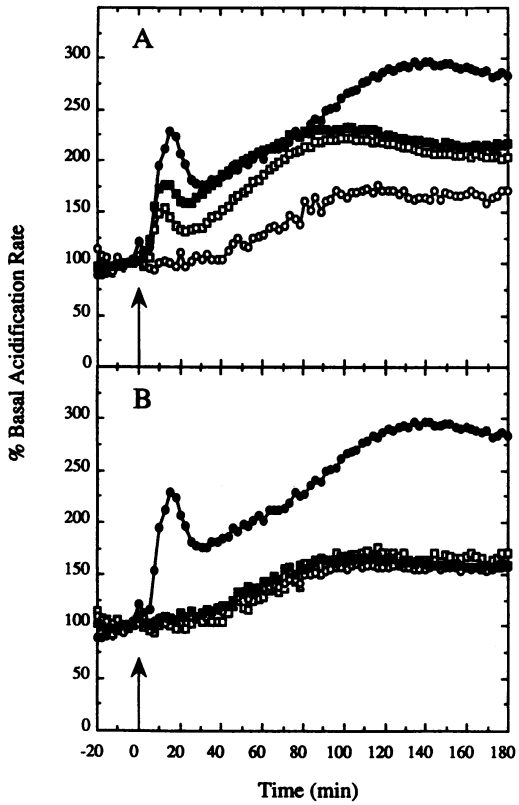

FIG. 5. Enhanced acidification rates of T cells induced by isolated chain–peptide complexes. Resting 4R3.9 T cells at day 10 after antigen pulsing were incubated overnight in low-serum medium (2.5% fetal calf serum) and washed in serum-free low-buffering RPMI 1640 medium containing 10 mM Hepes buffer (pH 7.4) just before use. T cells (1×10^6) were monitored for acidification rate over a 120-min period. Complexes were injected by using a 200-μl sample injection loop. At time zero, either 34 μg of I-A^k–[Ala^4]MBP-(1–14) peptide complex or 17 μg of chain–[Ala^4]MBP-(1–14) peptide complex was injected into separate chambers. The acidification rate for each channel was normalized to 100% 1 hr after cell loading. (*A*) Effect of chain–peptide complexes on 4R3.9 T-cell stimulation. ●, I-A^k–[Ala^4]MBP-(1–14) complex; ■, I-A^k α chain–[Ala^4]MBP-(1–14) complex; □, I-A^k β chain–[Ala^4]MBP-(1–14) complex; ○, equimolar mixture of I-A^k α chain and β chain without peptide. (*B*) Various controls. ●, I-A^k–[Ala^4]MBP-(1–14) complex; ■, I-A^s–[Tyr^{89}]MBP-(89–101) complex; □, I-A^s α chain–[Tyr^{89}]MBP-(89–101) complex; ○, I-A^s β chain–[Tyr^{89}]MBP-(89–101) complex.

and antigen, an anergic state (26). Also, it has been reported that IL-2 secretion and T-cell clonal anergy are induced by distinct biochemical pathways (27). Since no IL-2 was detected in the culture supernatant of 4R3.9 and other T-cell clones when exposed to appropriate MHC class II–peptide complexes (B.N., T.K., and S.D.S., unpublished results), the increase in the acidification rate may be correlated to the early events of T-cell anergy induction. The results presented here demonstrate that isolated chains of MHC class II molecules and antigenic peptide are recognized by MHC class II-restricted TCRs on T cells. In the case of the T-cell clone 4R3.9, this binding leads to a specific T-cell stimulation similar to that induced by intact heterodimer.

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