Major histocompatibility (MHC) class II molecules are cell surface heterodimeric ($\alpha\beta$) glycoproteins that display processed antigens to T cell receptors (TCRs) of CD4-positive T cells. The present study describes that individual recombinant $\alpha$ and $\beta$ chains of human MHC class II molecules lacking the transmembrane region ($\alpha$-Tm and $\beta$-Tm) are capable of binding antigenic peptide and that these complexes of chain-peptide are recognized by TCRs to induce antigen-specific apoptosis in restricted T cells. The $\alpha$-Tm and the $\beta$-Tm of human HLA-DR2 (DRB5*0101) were cloned, expressed in Escherichia coli, and purified in large scale by conventional chromatographic methods. The in vitro binding of an immunodominant epitope from the myelin basic protein (MBP-(83–102)) to purified DR2$\alpha$-Tm and DR2$\beta$-Tm was demonstrated with biotinylated and fluorescein-labeled MBP-(83–102)Y83 peptide. The specificity of the MBP-(83–102)Y83 peptide binding to both DR2$\alpha$-Tm and DR2$\beta$-Tm was demonstrated in a competitive peptide binding assay. When exposed to a transformed T cell clone (SSBT) restricted to DR2(DRB5*0101) and MBP-(84–102) peptide, complexes of DR2$\alpha$-Tm and DR2$\beta$-Tm with MBP-(83–102)Y83 peptide were able to specifically recognize TCRs as measured by the increase in $\gamma$-interferon ($\gamma$-IFN) cytokine. Such recognition of TCRs by soluble $\alpha$-MBP-(83–102)Y83 and $\beta$-MBP-(83–102)Y83 complexes led to the induction of antigen-specific apoptosis in SSBT cells as measured by double fluorescence flow cytometry and electron microscopy. These results provide the first evidence that soluble complexes of antigenic peptide and individual chains of human MHC class II molecules lacking the transmembrane region can recognize TCRs and induce antigen-specific apoptosis in T cells. Since activated CD4-positive T cells are involved in pathogenesis of various autoimmune diseases, the apoptosis triggered by individual soluble chain-peptide complexes has significant potential for eliminating autoreactive T cells.

MHC$^1$ class II proteins are heterodimeric glycoproteins that bind peptides within the cell and present them at the cell surface for interaction with T cells (1, 2). Several in vitro studies have demonstrated that peptides can bind to affinity purified MHC class II molecules (3–7) and that these complexes stimulate specific T cell responses (8–10). In general, MHC class II molecules consist of a 34-kDa $\alpha$ polypeptide and a 28–30-kDa $\beta$ polypeptide chain noncovalently associated with each other. Furthermore, each polypeptide contains two distinct extracellular domains ($\alpha$I/II in the $\alpha$ chain and $\beta$I/II in the $\beta$ chain), a transmembrane region and a small cytoplasmic C terminus region. The crystal structure of MHC class II molecules reveals that the extracellular $\alpha$1 and $\beta$1 domains of both chains are involved in creating the peptide binding groove of MHC class II molecules (11–13).

The first observation for the binding of antigenic peptide to individual $\alpha$ and $\beta$ polypeptide of MHC class II molecules appeared in a study where fluorescence peptide was found to be associated with both chains when murine class II peptide complexes were subjected to SDS-gel electrophoresis under reduced conditions (14). Recent results from our laboratory have shown that electroeluted purified native $\alpha$ and $\beta$ polypeptide chains isolated from affinity-purified murine MHC class II proteins are capable of binding antigenic peptide (15) and that purified chain-peptide complexes can stimulate T cells in vitro as measured by an increase in extracellular acidification rate in a sensor-based assay (16). In earlier studies, the possibility of T cell activation by MHC chain-peptide complexes was also suggested by the ability of alloreactive IA$\alpha$-specific cytotoxic T lymphocytes to specifically lyse transfected L cells expressing either $\alpha$1b1D$^d$t2 (17) or Ak$^d$/D$^d$t2 (18) MHC class II/d class I hybrid molecules.

Although these studies show that native individual chains of murine MHC class II containing the transmembrane region are capable of binding peptide and can trigger T cells, no evidence of peptide binding to individual polypeptides of human MHC class II exists. In this report we describe that Escherichia coli expressed individual recombinant $\alpha$ and $\beta$ polypeptides of human HLA-DR2 (DRB5*0101) lacking the transmembrane region are capable of binding immunodominant epitopes from MBP. In addition, monomeric complexes of DR2$\alpha$-Tm and DR2$\beta$-Tm chains and MBP peptide can induce antigen-specific apoptosis in cloned T cells to a degree comparable with that of native $\alpha$$\beta$ dimer-peptide complexes.

**MATERIALS AND METHODS**

**Cell Lines, Antibodies, and Chemicals**—The hybridoma cell line L243, producing monoclonal antibodies against monomorphic human HLA DR molecules, was obtained from American Type Culture Collection, Bethesda, MD. Homozygous lymphoblastoid cell lines, GMO 3107 expressing HLA DR2 and GMO 8067 expressing HLA DR3, were obtained from the National Institute of General Medical Sciences (NIGMS) human genetic mutant cell repository (Coriell Institute of Medical Research, NJ). Immunopure biotinylated bovine serum albumin containing known amount of biotin molecules was purchased from Pierce Chemicals. Anti-human $\gamma$-IFN monoclonal antibody and rabbit anti-human $\gamma$-IFN polyclonal antibody were obtained from Endogen, Inc. Peroxidase-conjugated rabbit IgG was purchased from Jackson Immunoresearch Laboratories. Human $\gamma$-IFN was obtained from Boch-
ringer Mannheim. 3.3′,5′-Tetramethyl benzidine was obtained from Mois, Inc.

Cloning, Expression, and Purification of DR2αβ-Tm and DR2β-Tm—Cloning and expression of α-Tm and β-Tm of DR2 (DRB5*0101) in E. coli were described in our earlier report (19). Briefly, the plasmids p321929 and p33425 expressing DR2α-Tm and DR2β-Tm chains were transformed into the E. coli expression strain V8000. The complexes were expressed at 13°C and grown at 37°C in L-broth containing 0.4% glucose, 100 μg/ml ampicillin, and 15 μg/ml tetracycline. Cells were induced in mid-log growth by addition of isopropyl-β-D-thiogalactopyranoside to a final concentration of 0.4 mM and were harvested for inclusion body preparations.

Purification of DR2 α and β Chains Lacking the Transmembrane Region—At the end of the 72 h induction, the E. coli inclusion bodies were solubilized in 25 mM phosphate buffer, pH 7.4, containing 8 M urea and 20 mM dithiothreitol and purified by ion-exchange chromatography using High-Q-50 resin (Bio-Rad). The recombinant β chain was purified by one-step gel filtration chromatography using Sephacryl S-100 resin packed in an Pharmacia XK50. The α chain was expressed in E. coli lacking the transmembrane region from E. coli inclusion body preparations and was described recently (20). Briefly, the α chain E. coli inclusion bodies were solubilized in 25 mM phosphate buffer, pH 7.4, containing 8 M urea and 20 mM dithiothreitol and purified by ion-exchange chromatography using High-Q-50 resin (Bio-Rad). The recombinant α chain was expressed in E. coli lacking the transmembrane region from E. coli inclusion body preparations and was described recently (20). Each lane containing α and β chains were pooled and analyzed by SDS-gel electrophoresis using Laemli silver staining kit (Berk, CA). Individually pooled α and β chains were further purified by RP-HPLC and stained with silver at pH 10 with 2% recovery of 52 and 86%, respectively.

Purification of Human HLA DR2 and DR3 from Lymphoblastoid Cells—Purification of HLA DR2 from Epstein-Barr virus transformed lymphoblastoid cells was carried out as described earlier (21) with some minor modifications. Triton X-100 cell lysate was applied onto L243 coupled Sepharose 4B column, and the bound DR2 was eluted in phosphatase buffer containing 0.05% n-dodecyl β-maltoside (0.05% n-dodecyl β-maltoside) at pH 11.3. Fractions were immediately neutralized with 1 M acetic acid, and the DR2 pool was collected over a DEAE ion-exchange column in a phosphate buffer containing 0.5 M NaCl and 0.05% n-dodecyl β-maltoside, pH 6.0. Purified protein was then filtered through a 180-kDa cut-off membrane, dialyzed against PBS for 24 h at 4°C, and characterized by 13.5% SDS-polyacrylamide gel electrophoresis followed by silver staining. The purity of purified HLA DR2 was obtained by similar method in 0.01% Tween 80 detergent.

Synthesis and Modifications of MBP Peptide—The N-acetylated myelin basic protein peptide analogs MBP-(83–102)Y3 by the sequence Ac-YDENVPHVHFKNIVFTTPP, MBP-(124–143) was collected from each well to test for the increase in γ-IFN cytokine level. The detection of γ-IFN levels was performed by antibody enzyme-linked immunosorbent assay as described recently (19).

In Situ Terminal Deoxynucleotidyl Transferase Assay and Flow Cytometry—The quantitative detection of DNA strand break was performed by end labeling of 32P-labeled DNA using biotinylated dUTP followed by a fluorescent isothiocyanate-conjugated avidin detection system in a flow cytometer as described earlier with some modifications (23). Briefly, the transformed SSST donor T cells at a density of 1 × 106 cells/ml were incubated with equimolar amounts of DR2 dimer-peptide or chain-peptide complex preparations. Cells were fixed in 1% buffered formaldehyde and stored at 4°C. Cells were rehydrated in Hanks’ balanced salt solution and resuspended in 50 μl of terminal deoxynucleotidyl transferase reaction mixture containing 10 μl of terminal deoxynucleotidyl transferase buffer (1 mM potassium cacodylate, 125 mM Tris-HCl, pH 6.6, 1.25 mg/ml bovine serum albumin), 0.2 μl terminal deoxynucleotidyl transferase, 5 μl of CoCl2, and 0.5 μl of biotin-16-dUTP. The reaction mixture was incubated at 37°C for 30 min, and cells were rinsed in Hanks’ balanced salt solution and resuspended in 100 μl of staining solution containing 2.5 μg/ml fluorescein isothiocyanate-avidin in saline sodium citrate buffer. Cells were incubated for an additional 30 min at room temperature in the dark and resuspended in 1 ml of Hanks’ balanced salt solution and stored at 4°C. Double fluorescence measurements were carried out in a flow cytometer using LYSYS II software.

Transmission Electron Microscopy—SSST cells at a density of 2 × 106 cells/ml were incubated with 50 μg/ml of freshly prepared native DR2-MBP-(83–102)Y3 complexes or 25 μg/ml of DR2β-MBP-(83–102)Y3 complexes for the duration of 24 h. Complexes were immobilized in a 96-well microtiter plate. Bovine serum albumin-biotin with 8 biotin molecules per bovine serum albumin was used as a standard with a concentration range of 0.014–1.8 pmd (0.117–15 ng). The bound biotinylated peptide in complex preparations were detected colorimetrically using alkaline phosphatase-conjugated streptavidin and p-nitrophenol phosphate in 0.1 M diethanolamine as a substrate. Dissociation Kinetics of Chain-Peptide Complexes by SDS-Gel Electrophoresis—Stability of single chain-peptide complexes was measured by SDS-gel electrophoresis of various concentrations of radiolabeled MBP peptide. Complexes of HLA-DR2 and HLA-DR1 complexes were purified on 13.5% SDS-PAGE gels under nonreduced conditions. Gels were stained, dried, and auto-radiographed. Each lane containing the αβ dimer, α-Tm, or β-Tm polypeptide chain was cut and counted in a γ-counter.

Size-exclusion HPLC Analysis and Fluorescence Peptide Binding—Solutions of 5 μl α-Tm or β-Tm and 50-fold molar excess of CF-MBP-(83–102)Y3 peptide in PBS buffer, pH 9, were incubated at 37°C for 2 h. Complexes were purified from the excess of peptide by SE-HPLC using a Tosohaas TSK SW3000 column (0.75 × 60 mm) at the flow rate of 0.5 ml/min in PBS buffer at pH 9. The protein fraction has been collected from 20 to 40 ml, concentrated using a Centricon-10 microconcentrator (10-kDa cut-off membrane), and then analyzed by size-exclusion HPLC using the same column connected to UV diode array and fluorescence detector. Two signals were detected for each SE-HPLC chromatogram: fluorescence with excitation wavelength of 448 nm and emission wavelength of 525 nm and a UV signal at 278 nm. The fluorescence signal was used to measure the bound peptide, and the UV detection was used to calculate the protein concentration.

T Cell Receptor Occupancy Assay—The herpes simplex virus-transformed SSST human T cell line restricted to DR2 (DRB5*0101) and MBP-(83–102) was cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 10% fetal bovine serum (Hyclone) and 50 units/ml/human recombinant IL-2 (rIL-2) at 37°C. Every alternate day cells were transferred to fresh media. Various complex preparations were incubated in a microtitre plate at a density of 20,000 cells/μl/well in the absence of rIL-2. After 48 h of incubation at 37°C, the supernatants were collected from each well to test for the increase in γ-IFN cytokine level. The detection of γ-IFN levels was performed by antibody enzyme-linked immunosorbent assay as described recently (19).
MHC class II molecules consist of two individual polypeptide chains (an α and a β) of similar size that are noncovalently associated with each other. Recently reported crystal structure of MHC class II molecules shows that the extracellular α1 and β1 domains are involved in creating the peptide binding domain that can accommodate peptides of varied length (11–13). In the heterodimeric structure, it has been shown that the electrostatically resolved α and β chains independently bind the same peptides, although the two chains may or may not bind identical amino acid residues in the peptides (14, 24). In our previous reports, we demonstrated that electrophoretically purified α and β chains of murine MHC class II polypeptides can bind antigenic peptides like the native heterodimer, and equimolar amounts of single-chain-peptide complexes can trigger T cell response as measured by a sensor-based assay (15, 16, 25).

The limited availability of purified native chains by the tedious electroelution method led us to investigate recombinant MHC class II chains for further studies. In this report we describe our successful effort to demonstrate that (i) E. coli expressed individual α and β polypeptide chains of human MHC class II (HLA-DR2) lacking the transmembrane region are equally capable of binding an immunodominant epitope from myelin basic protein (MBP-(83–102)Y83) and (ii) complexes of DR2-α-MBP-(83–102)Y83 and DR2-β-MBP-(83–102)Y83 can induce antigen-specific apoptosis in restricted cloned T cells. The selection of human HLA-DR2 antigen is based on its predominant involvement in the autoimmune disorder multiple sclerosis (MS) (26). Similarly, the peptide of MBP (MBP-(84–102)) used in this study is considered a major immunodominant epitope for human MS (27). The peptide analog of MBP used in our study contains a tyrosine residue at position 83 and was found to have increased binding affinity to HLA-DR2 without loss of TCR recognition.2 The increased binding of N terminus tyrosine containing peptide was also observed in several other MHC class II-peptide complexes.3

The cloning and expression of human HLA-DR2 α-Tm and β-Tm was carried out as described recently (19). The expression of recombinant individual α and β chain of HLA-DR2 represent 30% of the total cell protein. In contrast to individual α and β polypeptide chain, the recombinant expression of heterodimeric MHC class II molecules in E. coli was totally unsuccessful. The insoluble denatured inclusion body preparations were solubilized in 8 M urea, purified on a scale of 50–100 mg by conventional chromatography methods as described earlier (20), and stored in PBS containing an 8 M urea solution. Prior to peptide loading, the α and β chains were dialyzed against PBS buffer. Binding of various biotinylated MBP peptides to purified α and β polypeptide chains were carried out by antibody captured plate assay using chain-specific rabbit polyclonal antibodies and enzyme-conjugated streptavidin as described recently (7).

The optimum pH for maximum peptide occupancy was measured by incubating a known amount of each chain with 50-fold molar excess of MBP peptides. Three MBP peptides were selected for the optimization of peptide binding to individual chains. Their affinities toward HLA-DR2 have been shown in the order of MBP-(84–102) > MBP-(124–143) > MBP-(1–14) (28). The epitope MBP-(1–14) had no affinity toward HLA-DR2 and was used as a negative control. As shown in Fig. 1, the binding of MBP-(83–102)Y83 peptide to both α and β chain was maximum at pH 9.0. In contrast, the binding of MBP-(83–102)Y83 peptide to native DR2 heterodimer was found to be maximum at acidic pH (7). In case of the α chain, the binding pH consistently appears to be critical below or above pH 9. The second high binding epitope of MBP, (MBP-(124–143)) bound strongly to the β chain at basic pH and weakly to the α chain. Further increase in peptide concentration beyond 50-fold molar excess as well as increase in incubation period did not provide additional binding (data not shown). In negative controls, the MBP-(1–14) peptide did not bind to either the α or β chain like the native αβ DR2 heterodimer. Although the maximum binding of the MBP peptide to each individual chain was at pH 9.0, a significant amount of peptide remained bound at around physiological pH between 7 and 8.

The specificity of the binding of MBP-(83–102)Y83 peptide to individual α and β chain was demonstrated in a competitive binding experiment. Purified α or β chain was incubated with 50-fold molar excess of biotinylated MBP-(83–102)Y83 peptide in the presence of increasing concentration of nonbiotinylated MBP-(83–102)Y83 or MBP-(124–143) peptide. As shown in Fig. 1, C and D, the binding of biotinylated MBP-(83–102)Y83 peptide was 75–80% inhibited at 2–4-fold excess concentration of nonbiotinylated MBP-(83–102)Y83 peptide. The binding of biotinylated MBP-(83–102)Y83 peptide to chains was partially inhibited by MBP-(124–143) peptide. In other words, the MBP-(83–102)Y83 peptide appears to be more inhibitory than MBP-(124–143) peptide, which correlates with their affinities to DR2

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2 P. Mukku, S. Arimilli, I. Astafieva, and B. Nag, unpublished results.
3 B. Nag, S. Arimilli, P. V. Mukku, and I. Astafieva, unpublished results.

**Fig. 1.** Binding of biotinylated MBP peptide to α-Tm and β-Tm chains. Binding of biotinylated MBP peptides to purified chains was carried out using chain-specific polyclonal antibody and enzyme conjugated streptavidin. A and B represent the pH-dependent binding of biotinylated MBP-(83–102)Y83 (closed circles), MBP-(124–143) (open circles), and MBP-(1–14) (closed squares) peptides to α-Tm and β-Tm, respectively. C and D represent the competitive inhibition of biotinylated MBP-(83–102)Y83 peptide binding in the presence either nonbiotinylated MBP-(83–102)Y83 (closed circles) or MBP-(124–143) peptide (open circles) to α-Tm and β-Tm polypeptides, respectively. Each data point represents an average of triplicate determinations and arrows in A and B indicate the optimum pH for maximum binding.
The stability of the bound peptide in complexes of α-MBP-(83–102)Y<sub>83</sub> and β-MBP-(83–102)Y<sub>83</sub> was examined using <sup>125</sup>I-labeled MBP-(83–102)Y<sub>83</sub> peptide in SDS-gel electrophoresis under nonreduced conditions. Complexes of native DR2 heterodimer, DR2α and DR2β, with <sup>125</sup>I-MBP-(83–102)Y<sub>83</sub> peptide were prepared under fully optimized binding conditions and purified from unbound labeled peptide by G-75 gel filtration chromatography. The stability of three complexes was then monitored at 4 and 37°C for 72 h. As shown in Fig. 2, the bound <sup>125</sup>I-MBP-(83–102)Y<sub>83</sub> peptide remained associated with both α and β chains like the native αβ DR2 heterodimer.

Further characterization of single chain-peptide complexes with respect to aggregation level was performed by size-exclusion HPLC analysis. Although the recombinant α and β polypeptide chains used in our study lack the hydrophobic transmembrane region, due to inclusion body preparation of these polypeptides in E. coli, purified proteins tend to aggregate in solution in the absence of denaturing agent. The HPLC result presented in Fig. 3D shows that, prior to the peptide loading, almost all of β polypeptide preparation appeared in the aggregated state (600 kDa). In contrast, most of the α polypeptide appears to be in a state of αα homodimers (60 kDa) as shown in Fig. 3A. The existence of αα and ββ homodimers in purified native polypeptide chains was also observed in our earlier studies (15, 16). Upon peptide binding, however, a significant amount of purified complexes of both α and β chain-peptide shifted to the monomeric state with a molecular size of ~30 kDa (Fig. 3, B and E). In these experiments, carboxyfluorescein labeled MBP-(83–102)Y<sub>83</sub> peptide was used to monitor the bound peptide associated with various molecular size protein fractions. Results presented in Fig. 3C, E, and F, show that almost no fluorescence intensity was associated with highly aggregated proteins or with homodimers. In fact, the fluorescence intensity was only found to be associated with the monomeric form of both αα and ββ-MBP-(83–102)Y<sub>83</sub> peptide complexes. These results clearly demonstrate that bound peptide prevents aggregation of purified chains. Such prevention of aggregation of MHC class II dimers on cell surface by peptide binding has been reported recently (29). Similarly, in a separate study we have observed that bound peptide significantly inhibits aggregation of purified MHC class II heterodimers in solution. Calculated percent aggregation and associated bound peptide by size-exclusion HPLC of individual chains and their complexes are also presented in Fig. 3. The molar percent of total bound peptide data observed with CF-MBP-(83–102)Y<sub>83</sub> peptide in HPLC experiment correlates well with the biotinylated peptide binding results obtained with the antibody captured plate assay.

The recognition of αα and ββ-MBP-(83–102)Y<sub>83</sub> peptide complexes by TCR was performed using herpes saimiri virus-transformed SS8T cloned T cells. The SS8T cell clone was generated from an MS patient and was fully characterized for its restricted to HLA-DR2 (DRB5*0101) and MBP-(84–102) peptide (30). The TCR engagement by soluble αα and ββ-MBP-(83–102)Y<sub>83</sub> peptide complexes was monitored by an increase in γ-IFN cytokine in a dose-dependent manner. Such increase in γ-IFN production by T cells was correlated with the occupancy of TCRs on the surface of T cells in an earlier study (30) and was adopted for SS8T cells in our laboratory (19). As shown in Fig. 4, specific increase in γ-IFN was observed when SS8T cells were exposed to complexes of native DR2, αα or ββ polypeptide chain with the MBP-(83–102)Y<sub>83</sub> peptide in various control experiments, cells incubated with α or β chain alone and complexes containing irrelevant peptide (α-MBP-(124–143) or β-MBP-(124–143)) did not show any significant increase in γ-IFN level. Human T cells are known to express a low levels of MHC class II molecules on their surfaces and can be stimulated in the presence of antigenic peptide (31, 32). In order to dem-
onstrate that the observed level of increased γ-IFN is not due to the release of bound peptide in the culture medium, the MBP-(83–102)Y83 peptide was complexed with irrelevant HLA-DR3 as a control and showed no increase in γ-IFN level (Fig. 4A). Similarly, in a mock experiment, equivalent amount of MBP-(83–102)Y83 peptide incubated and passed through Sephadex G-75 column under identical purification conditions in the absence of chains, did not show any increase in γ-IFN level (data not shown).

Prolonged incubation of SS8T cells with relevant complexes of individual α and β chains led to the induction of apoptosis. Typically apoptosis is characterized by chromatin condensation and is associated with endonuclease activity. The endonuclease activity in apoptotic cells can be demonstrated by cleavage of cellular DNA. The quantitative detection of DNA strand breaks in this study was demonstrated by labeling the 3'-OH end of the fragmented DNA with biotinylated dUTP followed by fluorescein isothiocyanate-conjugated avidin detection system in a flow cytometer. Induction of apoptosis in T cells by DR2-MBP-(83–102)Y83, α-MBP-(83–102)Y83, and β-MBP-(83–102)Y83 complexes as measured by fluorescence-activated cell sorter analysis is shown in Fig. 5. In this flow cytometry assay, the DNA degradation is directly related with the biotin-dUTP incorporation and can be utilized in quantitative measurement of apoptosis. Cells incubated with DR2 alone, α chain alone, β chains alone, DR2-MBP-(124–143), α-MBP-(124–143) and β-MBP-(124–143) complexes were used as controls. Minimal incorporation of biotinylated dUTP was observed in various control experiments, whereas T cells incubated with either recombinant α-MBP-(83–102)Y83 or β-MBP-(83–102)Y83 complexes showed approximately 30–35% of the cells labeled with biotinylated dUTP similar to the native DR2-MBP-(83–102)Y83 complexes. The calculated percent T cell apoptosis by relevant chain-peptide complexes with respect to various controls is presented in Fig. 6A. Apoptosis of T cells by chain-peptide complexes appeared to be time-dependent as shown in Fig. 6B.

Finally, the chromatin condensation and cell shrinkage characteristics of apoptotic cells were demonstrated by transmission electron microscopy (Fig. 7). As compared with untreated T cells (Fig. 7A), SS8T cells treated with native DR2-MBP-(83–102)Y83, α-MBP-(83–102)Y83, and β-MBP-(83–102)Y83 complexes showed typical apoptotic cells (Fig. 7, B–D).

In summary, results presented in this report describe that individual recombinant polypeptide chains of human MHC...
requires further investigation. In a parallel study, we observed binding or TCR recognition. The physiological significance of MHC class II molecules are not involved in either peptide study demonstrates that the transmembrane region of human class IImolecules are capable of binding antigenic peptide and induce antigen-specific apoptosis in T cells. Furthermore, this class IImolecules are capable of binding antigenic peptide and induce antigen-specific apoptosis in T cells. Additionally, this study demonstrates that the transmembrane region of human MHC class II molecules are not involved in either peptide binding or TCR recognition. The physiological significance of single-chain peptide complexes is unknown at present and requires further investigation. In a parallel study, we observed that recombinant murine IAα chain complexed with rat MBP-(90–101) peptide was highly effective in preventing experimental allergic encephalomyelitis in mice, an animal model for human MS. Prevention and treatment of several autoimmune diseases in animal models by soluble native MHC class II-peptide complexes were demonstrated in our laboratory (33, 34). The T cell apoptosis reported here by recombinant soluble chain-peptide complexes may have significant clinical relevance in developing therapeutics for the elimination of autoreactive T cells in various autoimmune diseases in an antigen-specific manner.

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