

# Disease Inhibition by Major Histocompatibility Complex Binding Peptide Analogues of Disease-associated Epitopes: More than Blocking Alone

By Marca H. M. Wauben, Claire J. P. Boog, Ruurd van der Zee, Irma Joosten, Angelique Schlieff, and Willem van Eden

---

*From the Institute of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, University of Utrecht, 3508 TD Utrecht, The Netherlands*

## Summary

Peptide analogues of disease-associated epitopes were studied for inhibition of experimental allergic encephalomyelitis (EAE) and adjuvant arthritis (AA) in Lewis rats. EAE- and AA-associated analogues were selected as competitors because of their *in vitro* inhibitory activity on proliferation of encephalitogenic and arthritogenic T cells. Although the EAE-associated competitor had a superior major histocompatibility complex (MHC) binding affinity, the AA-associated competitor was a better inhibitor of the *in vitro* proliferation of arthritogenic T cells. Furthermore, although *in vivo* EAE was inhibited by both competitors, AA was only inhibited by the AA-associated competitor. Remarkably, in contrast to what was expected of a regular MHC competitor peptide, the AA-associated peptide analogue also prevented AA upon immunization before disease induction and appeared to induce T cell responses that crossreacted with the original disease-associated epitope. Therefore, it is concluded that antigen-specific regulatory mechanisms were involved in synergy with MHC competition. The integration of both qualities into a single "competitor-modulator" analogue peptide may lead to the development of novel, more effective, disease-specific immunomodulatory peptides.

CD4<sup>+</sup> helper T cells are activated upon recognition of antigen, provided the antigen is processed and its peptides are presented by cells expressing the proper MHC class II products (1–3). Competition between various peptides for binding to the same MHC molecule already has been demonstrated to occur not only *in vitro* but also *in vivo* (4–9). These findings, added to the fact that predisposition to most autoimmune diseases occurs in association with particular MHC molecules (10), have prompted several groups to investigate the possibility of inhibiting the formation of the trimolecular complex formed by MHC, peptide, and TCR in order to develop a new strategy of immunological intervention in autoimmunity. One of the best studied experimental animal models is the experimental allergic encephalomyelitis (EAE)<sup>1</sup> model. EAE is a CD4<sup>+</sup> T cell-mediated disease (11–13) that can be induced by immunization with myelin basic protein (MBP) or peptides derived from MBP. Both in mice and rats,

CD4<sup>+</sup> MBP-specific helper T cell clones have been isolated that transfer the disease into naive recipient animals (11, 13). Coadministration of anti-MHC class II antibodies has prevented the induction of EAE in mice by blocking MHC recognition of potentially encephalitogenic T helper cells (14, 15). Based on these findings, several groups have searched for peptides that could inhibit, by competition for MHC class II binding, the stimulus that is turning on the dangerous anti-self T cell responses. First reports dealing with this peptide immunotherapy approach have indicated it to be successful in the EAE model in mice. Coimmunization of nonencephalitogenic peptide analogues, designed on the basis of known encephalitogenic MBP T cell epitopes, together with MBP or MBP peptides reduced the clinical signs of EAE (16, 17). However, as also suggested by Janeway (18), because of the close structural relationships between inhibitors and encephalitogenic peptides, one could question whether MHC blockade was the sole mechanism of the successful immunological intervention in the disease process, or that immune responses elicited or suppressed by the analogue peptides themselves were interfering with the anti-self immune responses.

In the present study, we asked ourselves whether the approach of blocking peptides would work in more complex

---

<sup>1</sup> Abbreviations used in this paper: AA, adjuvant arthritis; EAE, experimental allergic encephalomyelitis; MBP, myelin basic protein; SMPS, simultaneous multiple peptide synthesis.

experimental diseases, in which the disease is not induced by an unnatural exposure to a well-defined self-peptide. Furthermore, when peptide immunotherapy is only based on competition for MHC binding, it should be possible to use the same competitor peptide in different experimental models, provided that in these models the same MHC restriction elements are involved. To study these questions we selected two experimental models in rats, the adjuvant arthritis (AA) model and the peptide-induced EAE model. The AA model, an experimental model for rheumatoid arthritis, is relatively complex compared with the peptide-induced EAE model, because the disease is not induced by an immunization with a defined self-antigen but by immunization with whole *Mycobacterium tuberculosis* (Mt), comprising many distinct antigens (19). However, among T cell lines obtained from Mt-immunized Lewis rats, so far exclusively lines or clones with specificity for the 180–188 amino acid sequence of the mycobacterial 65-kD heat-shock protein (M-65 180–188) have been shown to be arthritogenic in vivo (20–23). In the EAE model in Lewis rats, encephalitogenic T cells recognizing the 72–89 amino acid sequence of guinea pig MBP (MBP 72–89) dominate the immune response (24). Analysis of the MHC restriction element of an arthritogenic T cell clone (A2b), responding to the mycobacterial 180–188 epitope, and an encephalitogenic T cell line (Z1a), responding to the MBP 72–85 epitope, revealed that both were MHC class II RT1 B<sup>L</sup> (I-A) restricted (25). Based on these observations, our aim was to design competitor peptides that could inhibit in vivo, by competition at the level of MHC binding, triggering of autoaggressive T cells in both models. For the design of competitor peptides we used both the 180–188 amino acid sequence of the mycobacterial 65-kD protein, and the 72–85 amino acid sequence of MBP. First, we selected in vitro single alanine-substituted analogues of peptide 180–188 and MBP 72–85 (peptide 1020) that were not stimulatory for, respectively, clone A2b and Z1a, and that showed in vitro an efficient inhibition of the antigen-induced proliferative responses of both T cell clones. Coimmunization of both the AA- and EAE-associated competitor peptides together with the encephalitogenic MBP 72–85 peptide (peptide 1020) resulted in a strong inhibition of EAE. However, in the AA model, only coimmunization of the AA-associated competitor peptide together with Mt/IFA resulted in strong disease inhibition, while, although the EAE-associated competitor peptide had a higher binding affinity for MHC class II molecules, no significant disease inhibitory activity of the EAE-associated competitor peptide was seen. In animals immunized with the AA-associated competitor peptide, draining lymph node lymphocytes showed proliferative responses against the competitor peptide itself, which coincided with raised responses to the original peptide 180–188. Preimmunization with the AA-associated competitor peptide before disease induction inhibited development of AA, while the induction of EAE was not affected. This study shows that besides MHC blockade, as shown in the peptide-induced EAE model, analogues of a disease-related epitope may elicit an additional antigen-specific inhibitory mechanism.

## Materials and Methods

**Rats.** Male inbred Lewis rats, 160–190 g (6–8 wk of age), were obtained from the University of Limburg (Maastricht, The Netherlands).

**T Cell Lines.** The isolation, maintenance, and properties of the A2b helper T cell clone have been described previously (21). Briefly, an arthritogenic T cell clone, A2, reactive to Mt, was first isolated from draining lymph nodes of a Lewis rat immunized with Mt in IFA (20). Subcloning of the A2 line revealed the presence of an arthritogenic T cell clone, A2b, which recognized the 180–188 amino acid sequence of the mycobacterial hsp65 (23). The isolation, maintenance, and properties of the encephalitogenic Z1a helper T cell line have been described previously (26). Briefly, T cell line Z1a reactive to the 72–85 amino acid sequence of guinea pig MBP was isolated from the draining lymph nodes of a Lewis rat 9 d after immunization into each footpad with guinea pig MBP in CFA. The CD4<sup>+</sup> T cell line ATL was isolated from the popliteal lymph node cells of a Lewis rat 10 d after footpad immunization with 100 µg peptide A183 in CFA. Subcloning of the ATL line revealed the presence of the ATL11 T cell clone specific for both peptide 180–188 and A183. T cell lines were cyclically restimulated in vitro for 3 or 4 d with irradiated (3,000 rad) thymocytes as APCs and 10 µg/ml heat-killed Mt for clone A2b, 10 µg/ml MBP for T cell line Z1a, or 10 µg/ml peptide A183 for clone ATL11, and propagated for 6 or 7 d in IMDM (Gibco Laboratories, Grand Island, NY), supplemented with 10% FCS, 10% EL-4 supernatant (IL-2 source), 2 mM glutamine, 2-ME, antibiotics, and 1% nonessential amino acids.

**Antigens.** The single alanine-substituted peptide analogues of peptide 180–188, the 180–188 amino acid sequence of the mycobacterial 65-kD protein (M-65 180–188), and of peptide 1020, the 72–85 amino acid sequence of guinea pig MBP (MBP 72–85), were prepared by automated simultaneous multiple peptide synthesis (SMPS). The SMPS set-up was developed using a standard autosampler (221; Gilson Co., Inc., Worthington, OH) as described elsewhere (27). Shortly, for the concurrent synthesis of 30 peptides, standard Fmoc chemistry with Pfp-activated amino acids (Dhbt for serine and threonine) in a sixfold molar excess and Hobt as catalyst were employed. Peptides were obtained as COOH-terminal amides from 7.5 mg resin/peptide (0.21 meq/g, PAL TM resin; Milligen, Etten-Leur, the Netherlands). Large quantities of synthetic peptide 180–188 (M-65 180–188), TFGLQLELT, peptide A183 (M-65 180–188, Leu183→Ala), TFQAQLELT, peptide A184 (M-65 180–188, Gln184→Ala), TFGLALELT, peptide 1020 (MBP 72–85), QKSQRSQDENPV, peptide 1028 (MBP 72–85, Asp81→Ala), QKSQRSQAENPV, and peptide 1029 (MBP 72–85 Glu82→Ala), QKSQRSQDANPV were synthesized by standard solid phase Fmoc chemistry (23, 28). The activities of in vitro defined noninhibitory and inhibitory peptides synthesized by the SMPS method were confirmed with the alanine-substituted peptides (A184, 1029 and A183, 1028) prepared by conventional solid phase synthesis. Heat-killed Mt H37Ra was derived from Difco Laboratories (Detroit, MI). The 65-kD *M. bovis* BCG recombinant protein (M-65) was cloned and purified as described previously (23, 29).

**Lymphocyte Proliferation Assay.** Proliferative responses of the T cell lines were measured in flat-bottomed microtiter plates in triplicate cultures (21). Each well contained 2 × 10<sup>4</sup> T cells, irradiated (3,000 rad) syngeneic thymocytes (10<sup>6</sup> cells/well) as APCs, and various amounts of antigens in 0.2 ml IMDM (Gibco Laboratories, Grand Island, NY) supplemented with 2% rat serum, glutamine, 2-ME, and antibiotics. Polyclonal responses of freshly isolated lymphocytes from draining lymph nodes or spleens were

measured with  $2 \times 10^5$  lymphocytes/well in culture medium supplemented with 10% FCS (Seralab, Sussex, England) instead of 2% rat serum. The cells were cultured for 3 d and pulsed for 18 h with [ $^3\text{H}$ ]thymidine. Cells were harvested on fiberglass filters, and [ $^3\text{H}$ ]thymidine incorporation was measured.

**Competitive Proliferation Assay.** To assess the capacity of non-stimulatory substituted peptides to inhibit antigen-induced proliferation of clone A2b or Z1a, varying concentrations of competitor peptides were added to the culture containing T cells and irradiated thymocytes 2 h before the addition of a suboptimal dose of the stimulatory antigens. The T lymphocyte proliferation assay was performed as mentioned above.

**MHC Binding Assay.** The MHC-peptide binding studies were performed using a direct binding assay (30). Briefly, Rat RT1 B<sup>L</sup> molecules were affinity purified from cell lysates of the Con A-activated MHC class II-positive Z1A T cell line using the mAb Ox6 coupled to Sepharose-4B beads. Peptide 1020 (MBP 72–85) was biotinylated with a twofold molar excess of *N*-hydroxy-succinimide (NHS)-LC-Biotin (Pierce Chemical Co., Rockford, IL). For competition studies purified MHC class II molecules (2  $\mu\text{M}$ ) were incubated with 100 nM of biotinylated peptide 1020 and a dose range of 0–100  $\mu\text{M}$  nonlabeled competitor peptides for 48 h at room temperature in the presence of a protease inhibitor mix. The MHC-peptide mixtures were analyzed by SDS-PAGE under nonreducing conditions. The proteins were then blotted onto nitrocellulose. After blocking the blot was incubated with biotinylated streptavidin-HRP complexes (Amersham Corp., Arlington Heights, IL). Detection of the presence of labeled peptide was by enhanced chemiluminescence using the Western blot ECL kit (Amersham Corp.). Exposure was overnight on Hyperfilm-ECL (Amersham Corp.).

**Induction and Clinical Evaluation of AA.** AA was induced by inoculation of 0.1 ml of a 1:1 emulsion of heat-killed Mt (5 mg/ml)/IFA and PBS intracutaneously at the base of the tail. All rats were examined daily, in a blind set-up, for clinical signs of arthritis. The severity of arthritis was scored by grading each paw from 0 to 4 based on erythema, swelling, and deformity of the joints. The highest achievable score was 16 (31). Statistical analysis was performed with the Student's *t* test. The in vivo effect of peptides A183, A184, 1028, and 1029 was investigated by coimmunization at the base of the tail of 0.1 ml of a 1:1 emulsion of Mt (5 mg/ml)/IFA together with 250  $\mu\text{g}$  peptide/PBS.

**Induction and Clinical Evaluation of EAE.** EAE was induced by injecting 0.05 ml of a 1:1 emulsion of the encephalitogenic peptide 1020 (72–85 amino acid sequence of guinea pig MBP)/PBS (1 mg/ml) and CFA (4 mg/ml Mt) in each hind footpad. Clinical signs of EAE were monitored daily on a scale of 0 to 4: 0, no signs; 0.5, weight loss; 1, limp tail; 2, hind leg weakness; 3, paraplegia; 4, paraplegia with forelimb weakness, moribund condition. The in vivo effect of peptides 1028, 1029, and A183 was investigated by adding these peptides (500  $\mu\text{g}$ /animal) to the 1020/CFA emulsion.

**Immunization with Peptide A183 before Disease Induction.** Rats were immunized, 7 d before disease induction, at the base of the tail in case of the EAE model and in the hind footpads in case of the AA model, with 100  $\mu\text{l}$  of a 1:1 emulsion of peptide A183/PBS (1 mg/ml) or with dimethyl-dioctadecylammonium bromide (DDA)/PBS (10 mg/ml). In the AA model, the disease was induced by intracutaneous injection at the base of the tail of 100  $\mu\text{l}$  Mt/IFA (5 mg/ml). In the EAE model, the disease was induced by subcutaneous injection in both hind footpads of 50  $\mu\text{l}$  of a 1:1 emulsion of peptide 1020/PBS (1 mg/ml) and CFA (Mt, 4 mg/ml). To circumvent the use of a mycobacteria-containing adjuvant, DDA

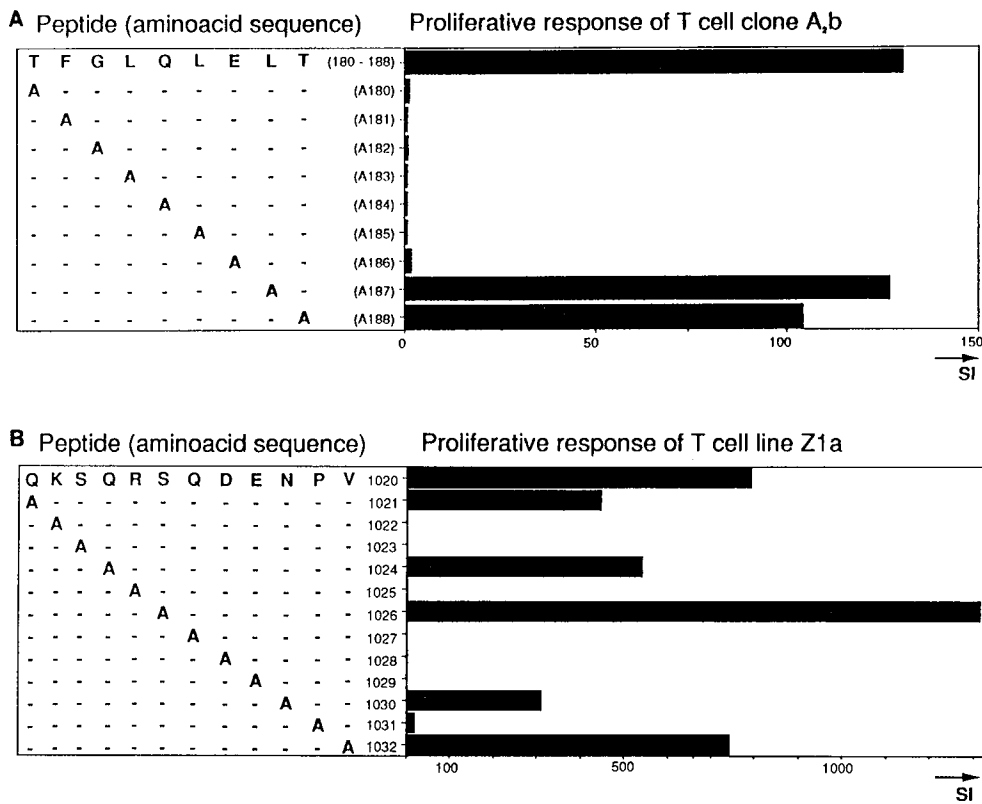
was used as an alternative adjuvant, as described by Snippe and Kraaieveld (32). DDA was obtained from Eastman Kodak Co. (Rochester, NY).

## Results

**Identification of Nonstimulatory Alanine-substituted Peptide Analogues.** For the design of competitor peptides based on the two disease-associated T cell epitopes, the 180–188 amino acid sequence of the mycobacterial 65-kD protein (M-65 180–188) and the 72–85 amino acid sequence of guinea pig MBP (MBP 72–85), we first analyzed which residues within these epitopes were essential for stimulation of, respectively, T cell clone A2b and T cell line Z1a. Single amino acid-substituted analogues of both T cell epitopes were generated in which every residue was replaced by an alanine residue. Proliferative responses of T cell clone A2b and T cell line Z1a in the presence of the substituted peptide analogues were determined. As can be seen in Fig. 1 A, T cell clone A2b responded in addition to the original peptide 180–188, only to the alanine-substituted peptide analogues A187 and A188. Fig. 1 B shows that T cell line Z1a responded not only to the original MBP peptide 1020 (MBP 72–85), but also to the alanine-substituted peptide analogues 1021, 1024, 1026, 1030, and 1032, whereas a slight response against peptide 1031 was seen.

**Competitive Inhibition of the Proliferative Response of Clone A2b by Nonstimulatory Substituted Peptides.** The analysis of the substituted peptide analogues provided us with non-stimulatory peptides with unknown MHC binding characteristics. An indirect method for studying MHC binding is to investigate whether a nonstimulatory peptide can inhibit antigen-induced proliferative responses in vitro. Because both T cell clone A2b and T cell line Z1a were MHC class II RT1 B<sup>L</sup> (I-A) restricted (25), clone A2b was used to test the inhibitory capacity of nonstimulatory 180–188 (M-65)- and 1020 (MBP 72–85)-derived analogue peptides. As can be seen from Fig. 2 A, addition of peptide analogue A181 (M-65 180–188, Phe181→Ala) or A183 (M-65 180–188, Leu183→Ala) induced a concentration-dependent inhibition of the proliferative response against peptide 180–188 (0.5  $\mu\text{g}/\text{ml}$ ). Peptide A182 (M-65 180–188, Gly182→Ala) showed only a slight concentration-dependent inhibition, while the alanine substitutions at positions 180, 184, 185, and 186 within the 180–188 peptide failed to inhibit the proliferative response induced by peptide 180–188. Of the 1020 (MBP 72–85) analogues, only addition of peptide 1028 (MBP72–85, Asp81→Ala), induced a strong concentration-dependent inhibition of the peptide 180–188-induced proliferation of clone A2b (Fig. 2 B).

**Comparison of the Inhibitory Activity of Peptide A183 and 1028 on T Cell Proliferation.** We selected the analogue of peptide 180–188 (M-65 180–188), peptide A183 (Leu183→Ala), and the analogue of peptide 1020 (MBP 72–85), peptide 1028 (Asp81→Ala), to compare their efficacy in inhibiting proliferation of both clones A2b and Z1a. Fig. 3 A shows that peptide A183 inhibited the proliferation of clone A2b more efficiently than the nonrelated peptide 1028. However, Fig. 3 B shows that the peptide 1020-induced proliferation of Z1a



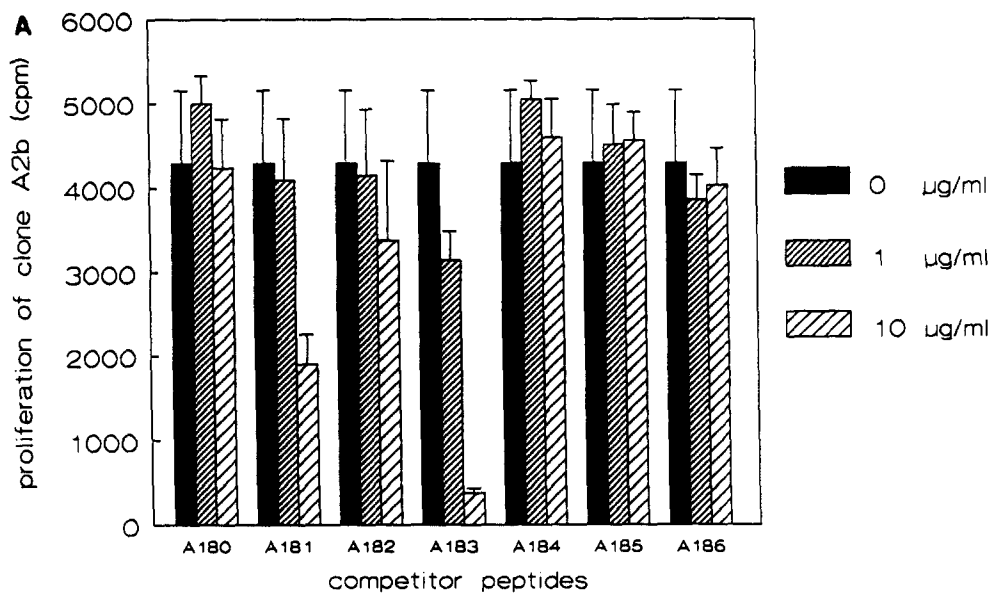
**Figure 1.** Proliferative responses ( $[^3\text{H}]$  thymidine incorporation) of clone A<sub>2</sub>b and T cell line Z1a to, respectively, peptide 180-188 and peptide 1020 single alanine-substituted analogue peptides. The responses of T cell clone A<sub>2</sub>b are shown in the presence of single alanine-substituted peptide analogues of peptide 180-188 (2-3  $\mu\text{g}/\text{ml}$ ) (A). The responses of T cell line Z1a are shown in the presence of single alanine-substituted peptide analogues of peptide 1020 (30-50  $\mu\text{g}/\text{ml}$ ) (B). Data are expressed as stimulation indices. SI, mean cpm of triplicate samples in the presence of antigen/mean cpm of triplicate samples in the absence of antigen.

was inhibited more efficiently by the analogue peptide 1028 compared with the nonrelated peptide A183.

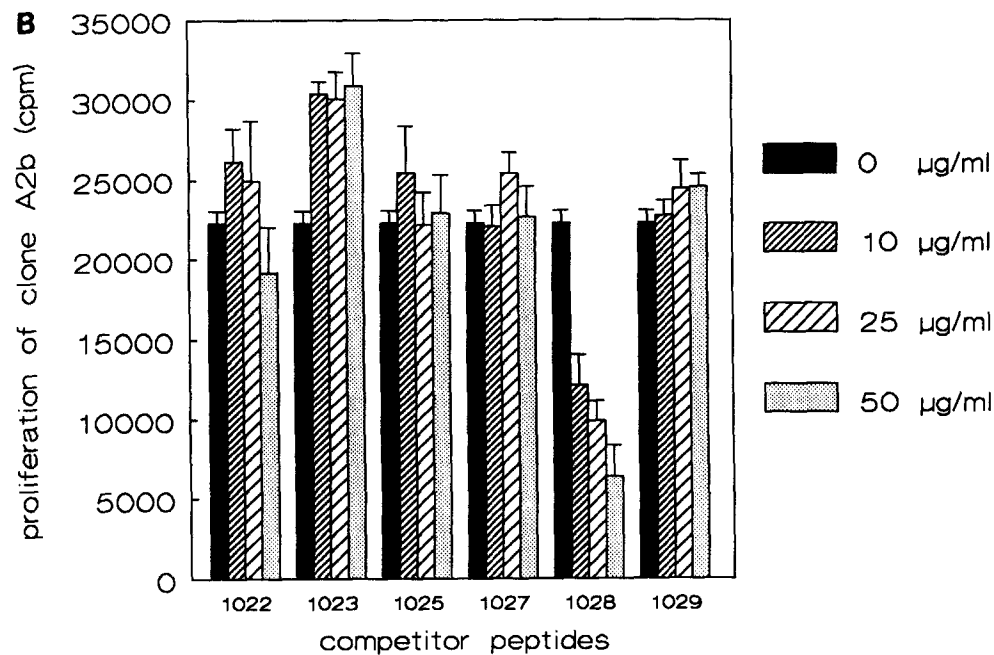
**MHC Binding Affinity of the Competitor Peptides.** Because the proliferative responses of both clones A<sub>2</sub>b and Z1a, were inhibited most efficiently by their homologous epitope-related peptide analogues, it is possible that this inhibition was not merely based on competition at the level of peptide-MHC binding, but that also antigen-specific mechanisms at the level of the TCR were involved. To determine the MHC binding affinities of both competitor peptides and to discriminate MHC binding from TCR-specific effects, we performed direct peptide-MHC binding experiments on isolated MHC class II molecules. Purified MHC class II molecules (RT1 B<sup>L</sup>) were incubated with the biotinylated marker peptide 1020 together with various concentrations of the nonlabeled competitor peptides A183 or 1028 for 48 h. Binding of the labeled marker peptide was analyzed by nonreducing SDS-PAGE followed by blotting. Biotinylated peptide-MHC binding was detected by chemoluminescence. This method enables competition studies in which the relative affinity of a given peptide as compared with the marker peptide can be assessed in a semi-quantitative way (30). Fig. 4 A shows the competitive inhibition of MHC binding of biotinylated peptide 1020 by increasing concentrations of peptide 1028. At 40-fold molar excess of competitor 1028, the binding had decreased by ~90%. The competitive inhibition by peptide A183 (Fig.

4 B) appeared to be less efficient, at 40-fold molar excess the binding has decreased by ~50%. Therefore, the MHC binding affinity of the EAE-associated analogue peptide 1028 is higher than the binding affinity of peptide A183. The fact that, despite the lower MHC binding affinity of peptide A183, the proliferation of clone A<sub>2</sub>b was inhibited more efficiently by peptide A183 compared with peptide 1028 (Fig. 3 A) is likely due to inhibitory mechanisms other than MHC competition.

**EAE Blocking Activity of Peptides A183 and 1028.** Because both peptides A183 and 1028 inhibited the antigen-induced proliferation of T cell clone A<sub>2</sub>b and T cell line Z1a, and because both cells were critical for disease development of AA or EAE, respectively, we subsequently studied the ability to use peptides A183 and 1028 as competitor peptides during disease induction in both models. Peptides A184 and 1029 were selected as noncompetitive control peptides (See Fig. 2, A and B). EAE was induced with the encephalitogenic peptide 1020 (MBP 72-85) (50  $\mu\text{g}/\text{rat}$ ) emulsified in CFA. Coimmunization with peptide 1028 (500  $\mu\text{g}/\text{rat}$ ) resulted in a complete inhibition of EAE, while coimmunization of peptide A183 (500  $\mu\text{g}/\text{rat}$ ) resulted in almost complete inhibition of EAE (Table 1). Only two of eight animals coimmunized with A183 showed clinical signs of EAE, and the maximal score of these two animals was only 0.5 (weight loss without any signs of paralysis). In contrast, all animals coimmunized



**Figure 2.** Competition for antigen presentation between nonstimulatory single alanine-substituted peptide analogues and peptide 180–188. Nonstimulatory alanine-substituted peptide analogues of peptide 180–188 (A) and peptide 1020 (B) were preincubated with APCs and A2b T cells 2 h before the addition of the stimulatory peptide 180–188. Competition was evaluated in a proliferation assay by determining the reduction of proliferation in the presence of a varying concentration of competitor peptides and a suboptimal concentration of peptide 180–188 (0.5 µg/ml). The background value in the test was (A) 211 cpm and (B) 164 cpm. Data are expressed as the mean cpm of triplicate cultures.



**Table 1.** Coimmunization of 1028 or A183 Inhibits Peptide-induced EAE

Immunization*	Incidence <sup>†</sup>	Day of onset <sup>‡</sup>	Duration <sup>§</sup>	Maximal severity <sup>¶</sup>
1020 (50 µg) + PBS	8/8	12.9 ± 1.4	5.8 ± 1.7	2.0 ± 1.1
1020 (50 µg) + 1028 (500 µg)	0/8	–	–	–
1020 (50 µg) + A183 (500 µg)	2/8	13.0 ± 0	1 ± 0	0.1 ± 0.2
1020 (50 µg) + 1029 (500 µg)	8/8	11.8 ± 1.0	5.5 ± 0.9	2.6 ± 0.8

\* EAE was induced by subcutaneous injection of 1020 (50 µg) + CFA (Mt 400 µg) emulsified with PBS, 1028 (500 µg), A183 (500 µg), or 1029 (500 µg). Rats were observed daily and graded on a four-point scale.

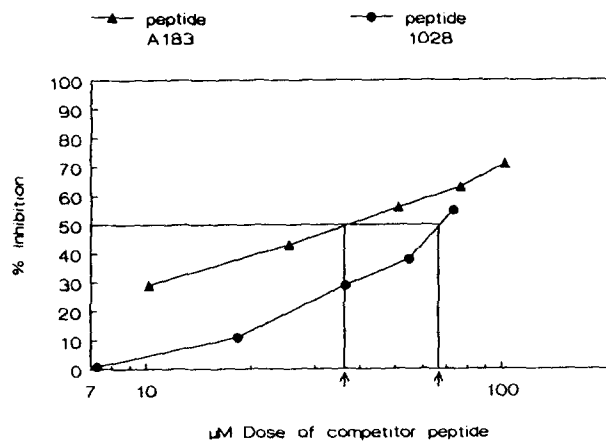
<sup>†</sup> Number with disease/number tested.

<sup>‡</sup> Average day of disease onset of those animals that developed disease (±SD).

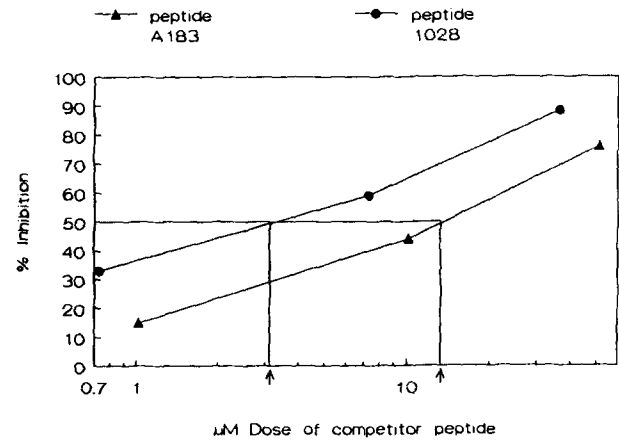
<sup>§</sup> Average duration of the disease in days of those animals that developed disease.

<sup>¶</sup> Value represents the mean of the maximum EAE score for each experimental group.

A



B

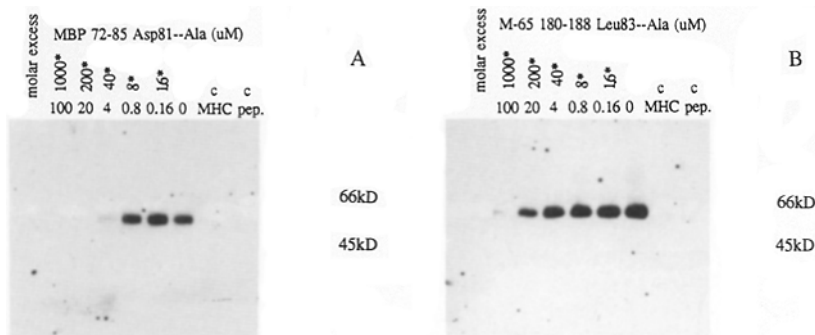


**Figure 3.** Inhibitory activity of peptides A183 and 1028 on T cell proliferation. Competition assays were performed as described for Fig. 2. The inhibitory activity of peptides A183 and 1028 are expressed as percent inhibition of the peptide 180–188 (0.49  $\mu$ M)-induced proliferation of clone A2b (A) or the peptide 1020 (3.55  $\mu$ M)-induced proliferation of Z1a (B).

with control peptide 1029 developed severe EAE, comparable to the control group immunized with peptide 1020/PBS in CFA (Table 1). At day 35 after EAE induction, popliteal lymphnode cells of rats immunized with the encephalitogenic peptide 1020/CFA together with either PBS, peptide A183, peptide 1028, or peptide 1029 were isolated and assessed for their antigen specificity in a proliferation assay. Table 2 shows in a representative experiment polyclonal T cell responses of animals coimmunized with the competitor peptides 1028 or A183, in which, as compared with the control groups immunized with PBS or peptide 1029, decreased responses against the encephalitogenic peptide 1020 were seen. These results were compatible with *in vivo* competitive inhibition of the priming of a T cell response against the encephalitogenic peptide 1020 and subsequent prevention of EAE development.

**Inhibitory Activity of Peptides A183 and 1028 in the AA Model.** However, the results of the coimmunization experiments in the AA model were not compatible with such a competitive inhibition mechanism. Arthritis induction with Mt/IFA in the presence of the strongest MHC binding competitor peptide, peptide 1028 (MBP 72–85, Asp81→Ala),

showed no significant disease inhibition as compared to the control groups (Table 3). In contrast, the weaker MHC binding but AA-related competitor analogue of peptide 180–188 (M-65 180–188), peptide A183 (Leu183→Ala), reduced upon coimmunization with Mt/IFA the arthritis severity and incidence very efficiently (Fig. 5). No significant effect on disease severity was seen after coimmunization with control peptide A184 (Fig. 5). At day 43 after AA induction, when the clinical signs of arthritis in the affected groups had subsided, inguinal lymph node cells of animals immunized with Mt/IFA together with either PBS, peptide A183, or peptide A184 were isolated and assessed for their antigen specificity in a proliferation assay. Table 4 shows the proliferative responses of the inguinal lymph node cells of an animal of each experimental group. In contrast to the decreased polyclonal responses against the encephalitogenic peptide in animals coimmunized with the competitor peptides A183 or 1028 in the peptide-induced EAE model (Table 2), in the more complex AA model no inhibition of the responses against Mt or the mycobacterial 65-kD protein was observed in rats coimmunized with peptide A183 or 1028 (Table 4). How-



**Figure 4.** Competitive inhibition of MHC binding of biotinylated peptide 1020 by peptides 1028 and A183. Competition studies for binding to 2  $\mu$ M RT1 B<sup>d</sup> MHC molecules were performed with different concentrations (0–100  $\mu$ M) of nonlabeled peptide 1028 (MBP 72–85, Asp81→Ala) (A) or peptide A183 (M-65 180–188, Leu183→Ala) (B), and 100 nM of biotinylated peptide 1020. The assays were performed as described in Materials and Methods.

**Table 2.** Proliferative Responses of Popliteal Lymph Node Cells at Day 35 after EAE Induction

Antigens	Dose $\mu\text{g/ml}$	Rats immunized at day 0 with:			
		1020/PBS	1020/A183	1020/1028	1020/1029
MBP	10	6.1	2.9	3.1	7.8
	25	11.0	3.3	5.3	8.2
1020	10	5.1	2.0	1.4	8.5
	25	11.4	2.0	1.5	12.1
	50	15.0	1.9	1.1	22.1
1028	50	1.8	1.2	10.0	6.0
1029	50	1.3	0.9	0.7	18.5
A183	50	1.5	34.0	1.3	0.9
Maximum EAE		3	0	0	3

Data are expressed as SI. For disease induction, rats were immunized at day 0 with peptide 1020 (50  $\mu\text{g}$ ) + CFA (Mt 400  $\mu\text{g}$ ) emulsified with PBS, A183 (500  $\mu\text{g}$ ), 1028 (500  $\mu\text{g}$ ), or 1029 (500  $\mu\text{g}$ ).

ever, coimmunization of the immunogenic AA-related peptide A183 resulted not only in proliferative responses against peptide A183 itself, but, interestingly, also against peptide 180–188, while in none of the other groups were responses against peptide 180–188 observed. From these polyclonal proliferative responses it was not clear whether the same T cells that recognized peptide A183 also responded to peptide 180–188. For this reason we generated a T cell clone, ATL11, against peptide A183. In agreement with our findings at the polyclonal level, this T cell clone recognized, besides peptide A183, also the original peptide 180–188 (M-65 180–188) (Table 5).

**Table 3.** Coimmunization of 1028 or 1029 during Arthritis Induction with Mt

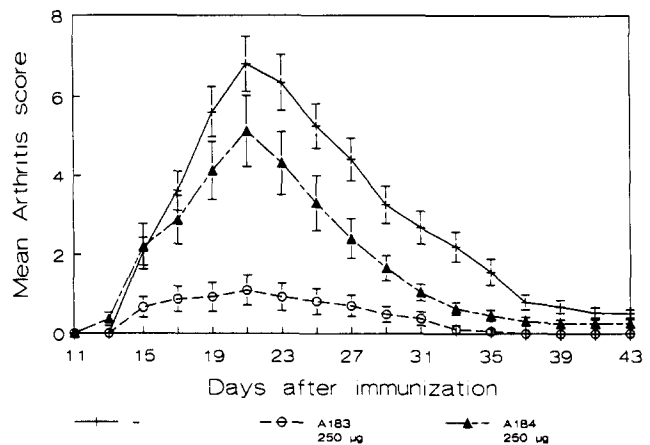
Immunization*	Incidence <sup>†</sup>	Duration <sup>§</sup>	Maximal severity <sup>  </sup>
Mt + PBS	6/6	7.8 $\pm$ 1.3	5.8 $\pm$ 1.9
Mt + 1028 (250 $\mu\text{g}$ )	4/5	6.8 $\pm$ 1.6	4 $\pm$ 1.6
Mt + 1029 (250 $\mu\text{g}$ )	4/5	8.7 $\pm$ 1.2	5.2 $\pm$ 2.0

\* AA was induced by intracutaneous injection of CFA (Mt 5 mg/ml) emulsified with PBS, 1028 (250  $\mu\text{g}$ ), or 1029 (250  $\mu\text{g}$ ).

<sup>†</sup> Number with disease / number tested.

<sup>§</sup> Average duration of the disease in days of those animals that developed disease.

<sup>||</sup> Value represents the mean of the maximum AA score / rat for each experimental group.



**Figure 5.** Coimmunization of competitor peptide A183 during arthritis induction. Shown is the reduction of arthritis severity after disease induction with Mt in the presence of peptide A183. In the AA model, rats were coimmunized with Mt/IFA together with PBS (control group  $n = 37$ ); peptide A183, 250  $\mu\text{g}/\text{rat}$  (A183 group,  $n = 18$ ); or peptide A184, 250  $\mu\text{g}/\text{rat}$  (A184 group,  $n = 19$ ). Rats were followed for disease development from day 11 after immunization until day 43. The results shown are the mean arthritis scores of each group. SE is indicated by a bar.

*Preimmunization with Peptide A183 before Disease Induction Inhibited AA but Not EAE.* In the AA model coimmunization of the AA-related peptide A183 together with Mt/IFA inhibited the development of AA more efficiently than the stronger MHC binding, but non-AA-related, peptide 1028 (Table 3, Fig. 5). Furthermore, in the AA model no evidence for the inhibition of priming for critical disease-associated T cell responses in vivo was seen after addition of the competitor peptides A183 or 1028 during disease induction with Mt/IFA (Table 4). However, animals coimmunized with pep-

**Table 4.** Proliferative Responses of Inguinal Lymph Node Cells at Day 43 after Arthritis Induction

Antigens	Dose $\mu\text{g/ml}$	Rats immunized at day 0 with:				
		Mt/-	Mt/1028	Mt/1029	Mt/A183	Mt/A184
Mt	10	36.9	36.0	30.6	36.5	23.5
65 kD	25	3.0	5.4	6.1	4.4	5.1
180–188	10	1.6	1.9	1.3	3.7	1.2
	25	1.3	1.7	1.2	3.9	1.4
A183	10	1.5	1.8	1.4	3.8	1.2
	25	0.9	1.3	0.9	4.0	1.3
A184	25	0.9	0.8	1.3	1.0	1.5
1028	25	0.9	5.2	2.9	1.3	1.2
1029	25	1.4	1.6	6.2	1.2	1.4

Data are expressed as SI. For disease induction, rats were immunized at day 0 with Mt/IFA emulsified with PBS or 250  $\mu\text{g}$  A183, A184, 1028, or 1029 in PBS.

**Table 5.** Antigen Specificity of T Cell Clone ATL11

Antigens	Dose	Proliferative response
-	-	390 ± 10
Con A	2.5	87,995 ± 1,726
180-188	1	82,720 ± 3,520
	10	123,194 ± 1,974
A183	1	86,256 ± 1,552
	10	139,568 ± 2,318
A184	1	508 ± 102
	10	362 ± 37

tide A183 together with Mt/IFA showed, besides a response against peptide A183 itself, also an increased response against the original peptide 180-188, which plays a crucial role in AA development (Table 4). All these results suggested that in vivo peptide A183 probably interfered with the induction of AA by an antigen-specific mechanism rather than MHC blockade. However, to exclude MHC blockade and to study the antigen-specific protective mechanism of peptide A183, we tested whether preimmunization with peptide A183 in DDA, a nonmycobacteria-related adjuvant (32), at a relatively low dose (50 µg/rat compared with 250 µg/rat in the coimmunization experiments), 7 d before disease induction with Mt/IFA or peptide 1020 (MBP 72-85)/CFA, could influence the subsequent development of AA or EAE. First, before any macroscopical signs of AA were observed in Mt/IFA-immunized animals, polyclonal T cell responses of animals preimmunized with PBS/DDA or peptide A183/DDA were assessed. Table 6 shows the results of one representative rat of each group. No differences in the popliteal lymph node responses against Mt or the mycobacterial 65-kD protein were observed between the different animals, although it is clear that in the A183-preimmunized group responses against the original peptide 180-188 already were detected, while in the control group the response against peptide 180-188 was not yet detectable. Furthermore, as can be seen in Fig. 6, rats preimmunized with peptide A183 showed a significantly reduced disease severity compared with the control group, which had received PBS/DDA at day -7 ( $p < 0.05$ ). In contrast, preimmunization at day -7 with peptide A183 did not interfere with the development of peptide-induced EAE (maximal EAE score of  $2.2 \pm 1.2$ ; data not shown).

## Discussion

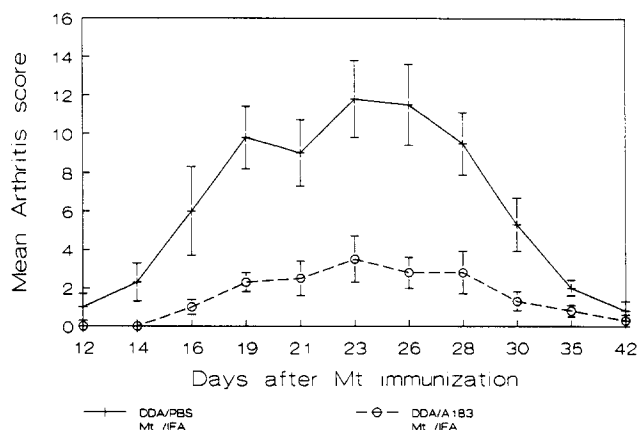
Of inbred rats, Lewis rats especially were found to be susceptible to a number of experimental autoimmune diseases, such as EAE (12), AA (19), experimental autoimmune uveoretinitis (EAU) (33), etc. Therefore, the development of competitor peptides, specific for Lewis MHC class II molecules, would enable the study of such peptides in different autoim-

**Table 6.** Proliferative Responses of Popliteal Lymph Node Cells at Day 10 after Arthritis Induction

Antigens	Dose	Rats immunized with:	
		Day-7 DDA/PBS Day 0 Mt/IFA	Day-7 DDA/A183 Day 0 Mt/IFA
	µg/ml		
Mt	10	15.1	18.6
65 kD	10	5.1	5.1
	50	7.9	7.0
180-188	10	1.4	3.8
	50	1.6	3.9
A183	10	1.1	3.5
	50	0.8	4.3
A184	10	0.7	1.2
	50	0.9	1.4

Data are expressed as SI. Rats were immunized in the hind footpads at day -7 with PBS/DDA or 50 µg A183/DDA. Arthritis was induced by tail immunization at day 0 with Mt/IFA. Polyclonal lymph node responses were measured at day 10.

mune models. In the present report we have described the design of such competitor peptides based on two disease-associated Lewis rat T cell epitopes: the 180-188 T cell epitope of the mycobacterial 65-kD heat-shock protein, which appeared to be critical in AA and which was recognized by the arthritogenic T cell clone A2b, and the 72-85 T cell epitope of guinea pig MBP recognized by the encephalitogenic T cell line Z1a. Both Z1a and A2b were restricted by the MHC class II RT1 B<sup>L</sup> locus products (25). First, single



**Figure 6.** Inhibition of adjuvant arthritis by preimmunization with peptide A183. Rats were immunized at day -7 with PBS/DDA (control group  $n = 4$ ) or with 50 µg peptide A183 DDA (A183 group,  $n = 4$ ) and at day 0 with Mt/IFA. Rats were followed for disease development from day 11 after immunization until day 43. The results shown are from a representative experiment and expressed as the mean arthritis score of each group. SE is indicated by a bar.



alanine-substituted analogues of both epitopes were tested to define nonstimulatory analogues (Fig. 1, *A* and *B*). These nonstimulatory analogue peptides were tested in competition assays *in vitro* to study their capacity to inhibit antigen-induced proliferation of clone A2b and Z1a. In contrast to most previous studies (4, 5, 8), these competition assays were performed with nonfixed viable APCs to approach the *in vivo* situation as well as possible. The AA-associated peptide analogue of 180–188, peptide A183, showed, compared with the EAE-associated analogue 1028, a stronger inhibition of the antigen-induced proliferation of T cell clone A2b (Fig. 3 *A*). However, the response of T cell line Z1a was inhibited most efficiently by the EAE-associated analogue peptide 1028 (Fig. 3 *B*). Because this functional inhibition assay is a rather indirect method to measure competition for MHC binding, we also performed direct MHC-peptide binding assays on isolated MHC molecules to define the MHC binding affinity of both competitor peptides. It appeared that the EAE-associated competitor peptide 1028 had a higher MHC binding affinity as compared with the AA-associated competitor peptide A183 (Fig. 4, *A* and *B*). Therefore, it could well be possible that the stronger inhibition of the antigen-induced proliferation of clone A2b by the AA-associated competitor A183 resulted not only from a successful competition with the stimulatory antigen at the level of MHC binding, but that alternative antigen-specific mechanisms were involved. These mechanisms may include the induction of anergy (34), in which the inhibitor peptide induces T cell tolerance, or the very recently described antigen-specific mechanism of TCR antagonism (35), in which the TCR becomes occupied by the analogue peptide-MHC complex without any signal transduction.

In the *in vivo* experiments addition of the AA-associated competitor peptide A183 to the EAE-inducing preparation of peptide 1020 (MBP 72–85)/CFA resulted in a strong inhibition of EAE, while addition of the EAE-associated competitor 1028 completely inhibited EAE development (Table 1). In the animals coimmunized with the competitor peptides during EAE induction, polyclonal lymph node responses against the encephalitogenic peptide 1020 were found to be inhibited (Table 2). These data demonstrated that not only the disease-associated competitor peptide 1028 but also the AA-associated peptide A183 was capable of inhibiting EAE induction by *in vivo* competitive inhibition of the priming of a T cell response against the encephalitogenic peptide 1020. This was in accordance with the data of Lamont et al. (36), which also showed in the mouse that a fully unrelated peptide was inhibitory to peptide-induced EAE.

In contrast to the findings in the EAE model, in the more complex AA model only coimmunization of the AA-associated competitor peptide A183 resulted in a significant disease reduction ( $p < 0.001$ ), while the stronger MHC binding EAE-associated peptide 1028 did not. Because of the failure of peptide 1028 to interfere in the AA model, it is not likely that the disease induction by addition of peptide A183 was only inhibited by competition for MHC binding. A possible clue for the mechanism that could be relevant for the dramatic

*in vivo* AA-suppressing activity of the AA-associated peptide A183 was obtained by the observation of unexpected T cell responsiveness after administration of peptide A183 during AA induction. In rats protected from AA by coimmunization with peptide A183, in addition to responses to peptide A183, increased responses against the native peptide 180–188 were seen, while responses against the mycobacterial 65-kD protein or Mt were not affected (Table 4). Although the 180–188 epitope of the mycobacterial 65-kD protein has been described as an important AA-associated epitope (23), at the polyclonal level, responses against this epitope were often hard to detect and normally not present at day 43 after Mt immunization, while responses against Mt or the 65-kD protein were very obvious (37). Therefore, in all other experimental groups, coimmunized with Mt together with PBS, the EAE-associated competitor 1028, or with the control peptides, no responses against peptide 180–188 were detected at day 43 after immunization (Table 4). However, be that as it may, coimmunization of peptide A183 together with Mt resulted in both disease reduction and triggering of T cell responses against peptide A183 and the original disease-associated peptide 180–188. From the polyclonal lymph node responses, however, it could not be concluded that the same T cells recognized both peptides A183 and 180–188, or that different T cell populations responded to the different peptides. For that reason we generated a T cell clone against peptide A183 and revealed that these T cells indeed recognized not only peptide A183, but also the original peptide 180–188 (Table 5).

In support of our hypothesis, that peptide A183 did interfere in the AA model not only by MHC blockade but in addition by antigen-specific immunomodulatory mechanisms, were also our findings that even rats preimmunized with peptide A183 7 d before AA induction featured reduced disease (Fig. 6) whereas, in the EAE model, in which the disease was induced with the encephalitogenic peptide 1020/CFA, no effect of such pretreatment with peptide A183 was observed. Arthritis inhibition was, in this situation, probably not due to MHC blocking by residual peptide A183, because a relative low dose (50  $\mu\text{g}$ ) was used compared with the coimmunization experiments (250–500  $\mu\text{g}$ ), and because in the peptide-induced EAE no inhibitory effect was seen. The exact mechanisms of the antigen-specific immunomodulatory qualities of peptide A183 in the AA model, however, remain to be elucidated. The strong *in vitro* inhibition by peptide A183 on the peptide 180–188-induced proliferation of the arthritogenic T cell clone A2b suggests that the TCR antagonist mechanism (35) could play an important role. However, such a T cell antagonist mechanism fails to explain the preimmunization experiments *in vivo* in which AA was inhibited by a relatively low dose of peptide A183 injected 1 wk before disease induction. Furthermore, in the *in vitro* experiments we have obtained additional experimental support for an active immunoregulatory mechanism of peptide A183 interference in the AA model. In the AA model it has already been demonstrated that immunization with attenuated arthritogenic A2b cells induced protection by triggering disease-specific regulatory T cells (38). Furthermore, Offner et al.

(39) recently demonstrated that T cells recognizing different MBP epitopes can induce a crossreactive immunity against a TCR idiotope that is shared by these cells. Therefore, it is possible that the T cells recognizing both the competitor peptide A183 and the original peptide 180-188, and which we have shown to become activated in our in vivo immunization experiments with peptide A183, play a crucial role. Because of the close relationship of such cells with the disease-inducing 180-188-specific T cells not only at the level of antigen specificity but possibly also at the TCR level, they may induce regulatory antiidiotypic responses directed against

shared TCR idiotopes, thereby preventing the development of AA. However, alternative explanations such as that peptide A183 induces anergy in the arthritogenic 180-188-specific T cell population or the specific triggering of other regulatory suppressive mechanisms are not to be excluded.

Irrespective of the actual mechanism, the disease-suppressive properties of such novel competitor-modulator peptides would make them attractive devices for the treatment of human autoimmune diseases, where we may have to deal with antigens of a relatively complex nature. In that situation we may need peptides harboring activities exceeding MHC blocking alone.

---

We thank Dr. E. J. Hensen (Utrecht), Dr. J. D. A. van Embden (Bilthoven), and Prof. I. R. Cohen (Rehovot) for valuable discussions, and Dr. S. Buus (Copenhagen) for his collaboration in the development of the direct peptide-MHC binding assay.

Part of the work was supported by a grant from Pasteur-Merieux (Lyon, France). The research of Dr. I. Joosten has been made possible by a fellowship of the Royal Netherlands Academy of Arts and Sciences.

Address correspondence to W. van Eden, Institute of Infectious Diseases, P.O. Box 80165, 3508 TD Utrecht, The Netherlands.

Received for publication 26 May 1992.

## References

1. Unanue, E.R. 1984. Antigen presenting function of macrophages. *Annu. Rev. Immunol.* 2:395.
2. Schwartz, R.H. 1985. T lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. *Annu. Rev. Immunol.* 3:237.
3. Buus, S., A. Sette, and H.M. Grey. 1987. The interaction between protein derived immunogenic peptides and Ia. *Immunol. Rev.* 98:115.
4. Babbitt, B.P., G. Matsueda, E. Haber, E.R. Unanue, and P.M. Allen. 1986. Antigenic competition at the level of peptide-Ia binding. *Proc. Natl. Acad. Sci. USA.* 83:4509.
5. Buus, S., and O. Werdelin. 1986. Oligopeptide antigens of the angiotensin lineage compete for presentation by paraformaldehyde treated accessory cells to T cells. *J. Immunol.* 136:459.
6. Lehmann, P.V., F. Cardinaux, E. Appella, S. Muller, F. Falcioni, L. Adorini, and Z.A. Nagy. 1989. Inhibition of T cell response with peptides is influenced by both peptide-binding specificity of major histocompatibility complex molecules and susceptibility of T cells to blocking. *Eur. J. Immunol.* 19:1071.
7. Adorini, L., S. Muller, F. Cardinaux, P.V. Lehmann, F. Falcioni, and Z.A. Nagy. 1988. In vivo competition between self peptides and foreign antigens in T cell activation. *Nature (Lond.)* 334:623.
8. Lamont, A.G., M.F. Powell, S.M. Colon, C. Miles, H.M. Grey, and A. Sette. 1990. The use of peptide analogs with improved stability and MHC binding capacity to inhibit antigen presentation in vitro and in vivo. *J. Immunol.* 144:2493.
9. Muller, S., L. Adorini, A. Juretic, and Z.A. Nagy. 1990. Selective in vivo inhibition of T cell activation by class II MHC binding peptides administered in soluble form. *J. Immunol.* 145:4006.
10. Todd, J.A., H. Acha-Orbea, J.I. Bell, N. Chao, Z. Fronek, C.O. Jacob, M. McDermott, A.A. Sinha, L. Timmerman, L. Steinman, and H.O. McDevitt. 1988. A molecular basis for MHC class II associated autoimmunity. *Science (Wash. DC)* 240:1003.
11. Zamvil, S., P. Nelson, J. Trotter, D. Mitchell, R. Knobler, R. Fritz, and L. Steinman. 1985. T cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination. *Nature (Lond.)* 317:355.
12. Ben-Nun, A., and I.R. Cohen. 1982. Experimental autoimmune encephalomyelitis (EAE) mediated by T cell lines: process of selection of lines and characterization of the cells. *J. Immunol.* 129:303.
13. Holoshitz, J., A. Frenkel, A. Ben-Nun, and I.R. Cohen. 1983. Autoimmune encephalomyelitis (EAE) mediated and prevented by T lymphocyte lines directed against diverse antigenic determinants of myelin basic protein. Vaccination is determinant specific. *J. Immunol.* 131:2810.
14. Steinman, L., J.T. Rosenbaum, S. Sriram, and H.O. McDevitt. 1981. In vivo effects of antibodies to immune response gene products: prevention of experimental allergic encephalomyelitis. *Proc. Natl. Acad. Sci. USA.* 78:7111.
15. Sriram, S., D.J. Topham, and L. Carroll. 1987. Haplotype specific suppression of experimental allergic encephalomyelitis with anti-Ia antibodies. *J. Immunol.* 139:485.
16. Sakai, K., S.S. Zamvil, D.J. Mitchell, S. Hodgkinson, J.B. Rothbard, and L. Steinman. 1989. Prevention of experimental encephalomyelitis with peptides that block interaction of T cells with MHC proteins. *Proc. Natl. Acad. Sci. USA.* 86:9470.
17. Wraith, D.C., D.E. Smilek, D.J. Mitchell, L. Steinman, and H.O. McDevitt. 1989. Antigen recognition in autoimmune

- encephalomyelitis and the potential for peptide mediated immunotherapy. *Cell*. 59:247.
18. Janeway, C.A. 1989. Immunotherapy by peptides? *Nature (Lond.)*. 341:482.
  19. Pearson, C.M. 1956. Development of arthritis, peri-arthritis and periostitis in rats given adjuvant. *Proc. Soc. Exp. Biol. Med.* 91:95.
  20. Holoshitz, J., Y. Naparstek, A. Ben-Nun, and I.R. Cohen. 1983. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. *Science (Wash. DC)*. 219:56.
  21. Holoshitz, J., A. Matitiau, and I.R. Cohen. 1984. Arthritis induced in rats by clones of T lymphocytes responsive to mycobacteria but not to collagen type II. *J. Clin. Invest.* 73:211.
  22. Van Eden, W., J. Holoshitz, Z. Nevo, A. Frenkel, A. Klajman, and I.R. Cohen. 1985. Arthritis induced by a T-lymphocyte clone that responds to *Mycobacterium tuberculosis* and to cartilage proteoglycans. *Proc. Natl. Acad. Sci. USA.* 82:5117.
  23. Van Eden, W., J.E.R., Thole, R. Van der Zee, A. Noordzij, J.D.A. Van Embden, E.J. Hensen, and I.R. Cohen. 1988. Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. *Nature (Lond.)*. 331:171.
  24. Chou, Y.K., A.A. Vandembark, R. Jones, G. Hashim, and H. Offner. 1989. Selection of encephalitogenic rat T lymphocyte clones recognizing an immunodominant epitope on myelin basic protein. *J. Neurosci. Res.* 22:181.
  25. Boots, A.M.H., M.J. van Lierop, M.H.M. Wauben, P.J.S. van Kooten, E.J. Hensen, and W. van Eden. 1991. CD4 rat × rat and mouse × rat T cell hybridomas produced by fusion of established T cell lines and clones to W/Fu (C58NT)D. *J. Immunol. Methods.* 144:1.
  26. Ben-Nun, A., H. Wekerle, and I.R. Cohen. 1981. The rapid isolation of clonable antigen specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Eur. J. Immunol.* 11:195.
  27. Van der Zee, R., M.H.M. Wauben, T.H.A. Lots, and W. van Eden. 1992. Simultaneous multiple peptide synthesis (SMPS) for the analysis of T cell epitopes. *J. Cell. Biochem.* 16D:83.
  28. Steward, J.M., and J.D. Young. 1984. Solid Phase Peptide Synthesis. Pierce Chemical Co., Rockford, IL.
  29. Thole, J.E.R., W.J. Keulen, J. de Bruyn, A.H.J. Kolk, D.G. Groothuis, L.G. Berwald, R.H. Tiesjema, and J.D.A. van Embden. 1987. Characterization, sequence determination, and immunogenicity of a 64-kilodalton protein of *Mycobacterium bovis* expressed in *Escherichia coli* K-12. *Infect. Immun.* 55:1466.
  30. Joosten, I., M.H.M. Wauben, E.J. Hensen, and S. Buus. 1992. A novel method for the analysis of binding between peptides and purified MHC class II molecules. *J. Cell. Biochem. Suppl.* 16D:33.
  31. Trentham, D.E., A.S. Townes, and A.H. Kang. 1977. Autoimmunity to collagen: an experimental model of arthritis. *J. Exp. Med.* 146:857.
  32. Snippe, H., and C.H. Kraaieveld. 1989. The immunoadjuvant dimethyldioctadecyl-ammoniumbromide. In *Immunological Adjuvants and Vaccines*. G. Gregoriades, A.C. Allison, and G. Poste, editors. Plenum Press NI, London. 47-59.
  33. Gery, L., M. Mochizuki, and R.B. Nussenblatt. 1986. Retinal specific antigens and immunopathogenic processes they provoke. *Prog. Retinal. Res.* 5:75.
  34. Schwartz, R.H. 1990. A cell culture model for T lymphocyte clonal anergy. *Science (Wash. DC)*. 248:1349.
  35. DeMagistris, M.T., J. Alexander, M. Coggeshall, A. Altman, F.C.A. Gaeta, H.M. Grey, and A. Sette. 1992. Antigen analog-Major histocompatibility complexes act as antagonists at the T cell receptor. *Cell.* 68:625.
  36. Lamont, A.G., A. Sette, R. Fujinami, S.M. Colon, C. Miles, and H.M. Grey. 1990. Inhibition of experimental encephalomyelitis induction in SJL/J mice by using a peptide with high affinity for IA<sup>b</sup> molecules. *J. Immunol.* 145:1687.
  37. Hogervorst, E.J.M., C.J.P. Boog, J.P.A. Wagenaar, M.H.M. Wauben, R. van der Zee, and W. van Eden. 1991. T cell reactivity to an epitope of the mycobacterial 65-kDa heat-shock protein (hsp65) corresponds with arthritis susceptibility in rats and is regulated by hsp 65-specific cellular responses. *Eur. J. Immunol.* 21:1289.
  38. Lider, O., N. Karin, M. Shinitzki, and I.R. Cohen. 1987. Therapeutic vaccination against adjuvant arthritis using autoimmune T cells related with hydrostatic pressure. *Proc. Natl. Acad. Sci. USA.* 84:4577.
  39. Offner, H., M. Vainiene, D.P. Gold, W.J. Morrison, R.Y. Wang, G.A. Hashim, and A.A. Vandembark. 1991. Protection against experimental encephalomyelitis, idiopathic autoregulation induced by a nonencephalitogenic T cell clone expressing a cross-reactive T cell receptor V gene. *J. Immunol.* 146:4165.