Brief Definitive Report

Prevention of Autoimmune Diabetes in Non-Obese Diabetic Mice by Treatment with a Class II Major Histocompatibility Complex-blocking Peptide

By Ursula Hurtenbach, Eduard Lier, Luciano Adorini, and Zoltan A. Nagy*

*From the Preclinical Research, Sandoz Pharma Ltd., 4002 Basel, Switzerland; and the *Department of Inflammation/Autoimmune Diseases, Hoffmann-La Roche Inc., Nutley, New Jersey 07110*

Summary

The role of antigen presentation as a possible mechanism underlying major histocompatibility complex (MHC) association of autoimmune disease has been studied in non-obese diabetic (NOD) mice. By screening for inhibition of antigen presentation to NOD T cell hybridoma, we have selected a synthetic peptide, yIYYTVHAAHAYTYt (small letters denote D amino acids), that efficiently blocks antigen presentation by the NOD class II MHC molecule $A\alpha^{g7}A\beta^{g7}$ (A^{g7}) in vitro. The inhibition is MHC selective, in that it does not affect antigen presentation by the E^d and E^k molecules, and has only a marginal effect on presentation by the A^d molecule. This peptide also inhibits the priming for A^{g7}-restricted T cell responses in vivo, and prevents the spontaneous development of diabetes in female NOD mice, when administered chronically from 3 wk of age on. Chronic treatment with a control peptide, KMKMVHAAHAKMKM, that fails to bind to A^{g7} has no effect on the disease. These data indicate that antigen presentation by the A^{g7} molecule plays a pivotal role in the induction of autoimmune diabetes. Furthermore, the results demonstrate that interference with antigen presentation by a class II molecule can prevent the onset of spontaneous autoimmune disease associated with the same molecule.

Susceptibility to autoimmune diseases is associated with certain class I or II alleles of the MHC (1). Similarly, animal models of autoimmune diseases often exhibit MHC linkage (2–6). Recently, two sets of findings have shed light on the, thus far enigmatic, mechanisms underlying MHC–disease association. First, MHC molecules have been shown to possess a binding site capable of interacting with antigenic peptides in an allele-dependent, selective manner (7–10). Second, the intramolecular mapping of MHC–disease linkages has revealed that in certain instances susceptibility or resistance depends on particular amino acid residues localized within the peptide-binding site (11–14). Thus, the most likely explanation for MHC–disease association would be that the disease-inducing autoantigen is selectively presented by the particular allelic form of MHC molecule that confers disease susceptibility (15). Since, however, the antigens involved in most autoimmune diseases are unknown, this hypothesis is not directly testable. Here, we have taken the approach of MHC blockade (16–18) to test the above hypothesis experimentally. Using the non-obese diabetic (NOD) mouse as an autoimmune disease model (19), we demonstrate that a peptide capable of blocking antigen presentation by the NOD class II MHC molecule A^{g7}, in vitro and in vivo, can also prevent the spontaneous development of type I diabetes linked to this class II molecule (6).

Materials and Methods

Mice. NOD mice, originally derived from Dr. E. Leiter's colony (The Jackson Laboratory, Bar Harbor, ME), were bred under specific pathogen-free conditions in the Sandoz animal facilities. Diabetes occurs in 65–72% of female and 15–24% of male mice in this colony.

Treatment of NOD Mice with Peptides. To inhibit in vivo priming for T cell responses, 500 nmol of peptide in HBSS was injected subcutaneously into the flanks of mice daily for 4 d. The immunogen was injected into the hind footpads in CFA on day 2 of peptide treatment. To prevent diabetes, mice were injected with 500 nmol of peptide daily, intraperitoneally and subcutaneously, at multiple sites. Mice were treated from 3 to 23 wk of age with decreasing frequency of injections as described in Results. Groups of mice injected with HBSS (solvent) were included to control for stress effect. To prevent peptide-induced hypersensitivity symptoms, chronically treated mice received 3 mg/kg of 4-(1-methyl-4-piperidylidene)-4H-benzo(4,5)cyclohepta(1,2-b)thiophen-10(9H)-1H fumarate (ketotifen; Zaditen; Sandoz, Basel, Switzerland; reference 20), injected together with the peptide for the last 2 wk of treatment.

T Cell Hybridoma. Hybridomas 2D12.1, 2G7.1, and 1H11.3 were generated by fusing hen egg white lysozyme (HEL)-immune

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/93/05/1499/06 $2.00
Volume 177 May 1993 1499–1504

lymph node cells with the TCR α/β^- variant of the BW5147 thymoma as described previously (21). Fine specificity of the hybridoma was determined by response to a panel of overlapping HEL peptides in the presence of APC (21; and L. Adorini, unpublished results). Peptide competition for antigen presentation was tested by incubating 2.5×10^5 NOD splenocytes or 2.5×10^4 LK cells per well as APC, with antigenic and competitor peptides (as shown in Fig. 1), in 96-well flat-bottomed microtiter plates for 24 h. Culture medium was RPMI 1640 supplemented with 10% FCS. The cells were then irradiated (2,000 rad) and washed before adding 5×10^4 hybridoma cells per well (in 0.2 ml). After 24 h of culture, 50-μl aliquots of supernatant were transferred to microculture wells containing 10^4 CTLL cells. IL-2 production was assessed by thymidine incorporation into CTLL during the last 4 h of the 24-h culture.

Peptides. Peptides were prepared by the solid phase method and purified as described previously (17).

ELISA Methods. Peptide-specific antibodies were detected by the ELISA method. Microtiter wells were coated with 250 ng peptide in 50 μl PBS at 4°C overnight. The plates were then washed four times with PBS/0.2% Tween 20, and blocked with 2% BSA in PBS at 37°C for 30 min. After washing twice in PBS/Tween 20 and four times in PBS, the plates were incubated with 50 μl/well of the test sera, diluted in PBS + 1% BSA + 0.2% Tween 20, at 37°C for 1 h. Subsequently, the plates were washed with PBS/Tween 20, and incubated with either phosphatase-coupled goat anti-mouse Ig, anti-IgG, or anti-IgM (Southern Biotechnology Associates, Inc., Birmingham, AL), each at 1:500 dilution, at 37°C for 2 h. After washing, 100 μl/well of *p*-nitrophenyl-phosphate disodium (1 mg/ml) was added and incubation followed for 45 min. Color reaction was measured in an ELISA counter (Titertek, Dynatech Labs, Inc., Chantilly, VA) at OD 405 nm. Background (test sera in uncoated wells) was subtracted from the mean of duplicate OD values. Serum IgE was measured as described (22). IgE determinations were performed by Dr. C. Heusser (Ciba-Geigy Inc., Basel, Switzerland).

Results

To identify peptides that block antigen presentation by the A^{g7} molecule, we screened a panel of peptides for inhibition of A^{g7}-restricted antigen presentation to T cell hybridoma. Since NOD mice were found to give a strong A^{g7}-restricted T cell response to HEL peptide 8–29 (L. Adorini, unpublished observation) and to sheep insuline (23), hybridoma of these two specificities were produced for peptide screening. Because of the similarity between the A^d and A^{g7} class II molecules (24), peptides known to bind to the A^d molecule (25) were included in the panel. Based on the screening results, we selected for further studies one peptide that binds (P1, ŷTYTVHAAHAYTYt, in one-letter code; small letters denote D amino acids; 25) and another that fails to bind (P2, KMKMVHAAHAKMKM; 25) to A^{g7}. The effect of these two peptides on antigen presentation by four different class II molecules is shown in Fig. 1. P1 inhibited the presentation of HEL 8–29 by A^{g7} strongly (IC_{50}, ~1 μM), that of OVA 323–339 by A^d weakly (IC_{50}, ~20 μM), and had no effect on the presentation of either HEL 1–18 by E^k or HEL 105–120 by E^d. P2 inhibited antigen presentation by A^d and E^k, but not that by A^{g7} and E^d. Thus, both peptides have satisfied the specificity criteria for a selective MHC blocker.

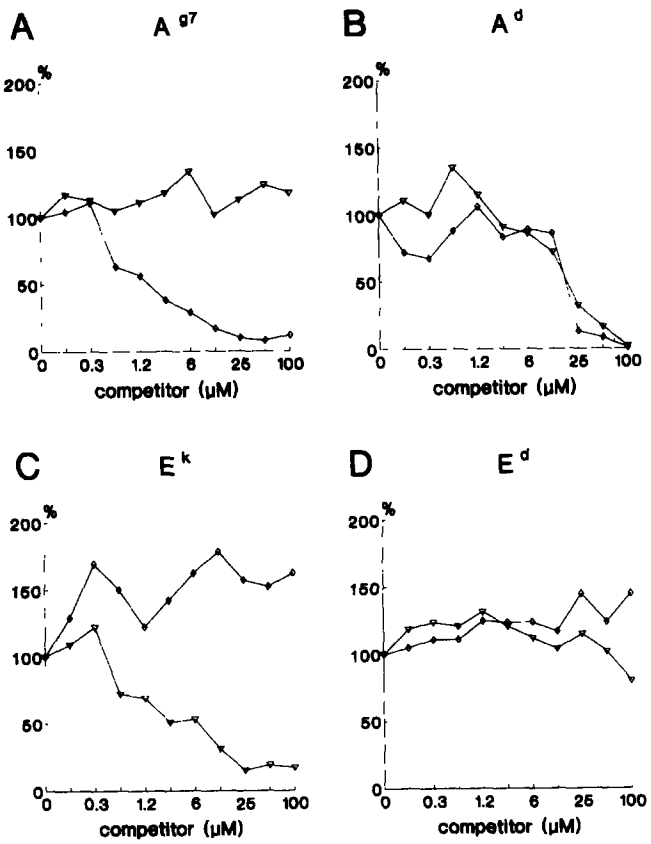

Figure 1. Selective inhibition of antigen presentation by P1 (*diamonds*) and P2 (*triangles*). Results represent antigen-induced IL-2 production by T cell hybridoma (100% is the response in the absence of P1 or P2). (*A*) APC, NOD splenocytes; antigen, 80 nM of HEL 8–29; hybridoma, 2D12.1 specific for HEL 8–29 presented by A^{g7}; 100% is 191,682 cpm; background (IL-2 production in the absence of antigen) is 266 cpm. (*B*) APC, LK lymphoma cells; antigen, 1 μM of OVA 323–339; hybridoma, DO11.10 specific for OVA 323–339 on A^d; 100% is 40,287 cpm; background is 1,895 cpm. (*C*) APC, LK cells; antigen, 2 μM of HEL 1–18; hybridoma, 2G7.1 specific for HEL 1–18 on E^k; 100% is 117,150 cpm; background is 296 cpm. (*D*) APC, LK cells; antigen, 500 nM of HEL 105–120; hybridoma, 1H11.3 specific for HEL 105–120 on E^d; 100% is 281,444 cpm; background is 775 cpm.

P2 was shown by Lamont et al. (25, 26) to be relatively resistant to proteases, in vitro and in vivo, and P1 was rendered similarly resistant by including D amino acids at the NH_2 and COOH termini.

Since we planned to use P1 for prevention of diabetes, it was important to ascertain that this peptide was suitable for chronic treatment, i.e., it exhibited immunosuppressive effect when applied in soluble form (18). We therefore tested whether P1 administered in isotonic aqueous solution could interfere, in vivo, with the induction of A^{g7}-restricted T cell responses. Mice were injected daily with 500 nmol of P1 for 4 d, and immunized on day 2 of treatment with 10 nmol of OVA 323–335 or HEL 8–29 in CFA. 9 d after immunization, the draining lymph nodes were removed, and antigen-specific T cell proliferation was determined (17). The representative experiment in Fig. 2 demonstrates a substantial inhibition of T cell response to OVA 323–335 in P1-treated mice, in com-

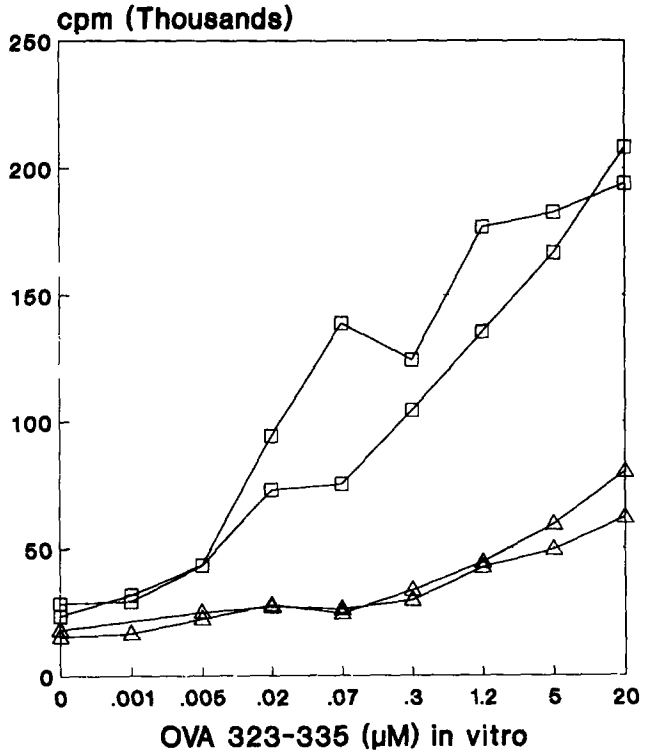

Figure 2. In vivo inhibition of T cell priming to OVA 323–335 by P1. The data represent antigen-specific proliferation of T cells from draining lymph nodes explanted 9 d after immunization. OVA 323–335-specific proliferation of lymph node cells from NOD mice treated with P1 (*triangles*) or solvent only (*squares*), as described in Materials and Methods, is shown. Curves represent the response of individual mice.

parison with the controls treated with the solvent only. A similar treatment with P2 had no immunosuppressive effect (data not shown). P1 also inhibited T cell priming to HEL 8–29 (data not shown).

It was then investigated whether a chronic treatment of prediabetic NOD mice with P1 would interfere with the development of diabetes. The treatment schedule was based on the observation that young NOD mice develop lymphocytic infiltration in the islets of Langerhans from the age of 3–4 wk (27). Thus, T cell recognition of a putative self-antigen presented by the A^{g7} molecule could occur from this age on. We therefore treated 3-wk-old NOD mice with 500 nmol of the A^{g7} blocker P1 five times per week for 3 wk, followed by 500 nmol five times per week for 3 wk, and 500 nmol twice a week until the age of 22–23 wk. Control groups of animals were treated either with the nonblocking peptide P2, or with solvent only. In the first experiment (Fig. 3 *A*), the P1-treated group of mice did not develop diabetes until 16 wk of age, and thereafter, the frequency of diabetic animals did not exceed 16% for the whole period of treatment. In the control groups, diabetes onset was between 13 and 16 wk of age, and the plateau level of 60% diabetic animals was reached by 22 wk of age. By Fisher's exact test, the diabetes incidence at 22 wk of age was significantly different between the P1- and P2-treated group ($p = 0.036$), and between the P1- and solvent-treated group ($p = 0.025$). The variation of diabetes onset observed in the control groups reflects most likely family differences that occur within the same NOD colony (U. Hurtenbach, unpublished observation). After the cessation of P1 treatment at week 22, the frequency of diabetic animals increased in parallel with the control groups. In this experiment, mortality occurred in both peptide-treated groups (6/18 P1-treated and 1/12 P2-treated mice died) after 10–18 wk of treatment, preceded by symptoms of peptide-induced immediate-type hypersensitivity (28), such as lack of movement upon prompting, shallow breathing, and prostrate posture. In the second experiment (Fig. 3 *B*), the mice received P1 mixed with an antianaphylactic compound (ketotifen) after showing the first signs of postinjection fatigue (after 20 wk of treatment). This group of mice did not develop diabetes until the end of treatment, furthermore, the mice remained symptomless, and no mortality occurred. Treatment with ketotifen alone had no influence on the disease. Thus, treatment with the A^{g7} blocker P1 resulted in a delayed onset and a significantly decreased frequency or complete absence of diabetes.

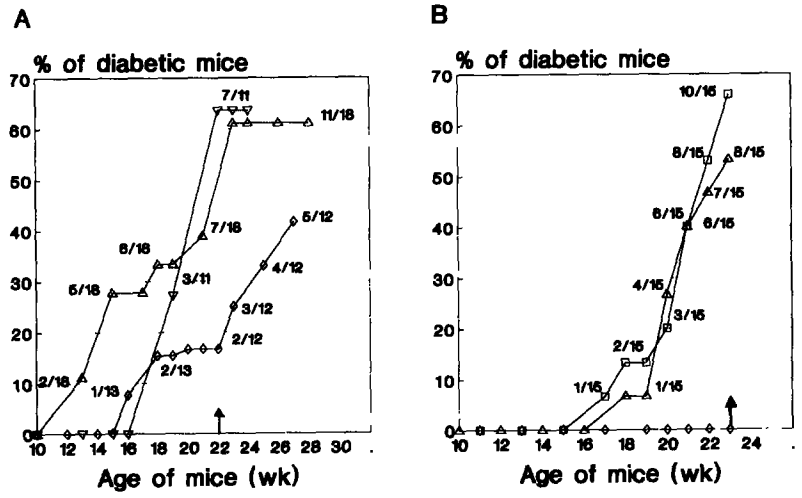

Figure 3. Prevention of spontaneous autoimmune diabetes in NOD mice by P1. (*A*) Groups of female mice were treated from 3 to 22 wk of age with P1 (18 mice; *diamonds*), P2 (12 mice; *inverted triangles*), or solvent only (18 mice; *triangles*). (*B*) Mice were treated with P1 for 20 wk followed by P1 supplemented with 3 mg/kg of ketotifen until 23 wk of age (13 mice; *diamonds*), ketotifen only (15 mice; *squares*), or solvent only (15 mice; *triangles*). Arrows indicate the cessation of treatment. Diabetes incidence was monitored from 10 wk of age. Mice with at least 200 mg/dl of glucose in blood were considered diabetic. The number of diabetic per total mice is shown for each group (except where 0) at each time point of determination.

Since T lymphocytes are activated by antigenic peptides bound to MHC molecules, class II MHC–blocking peptides are expected to induce immunity against themselves unless they are under self-tolerance (17) or rendered nonimmunogenic. Immune responses against MHC blockers could lead to accelerated elimination and thereby decreased efficacy of these substances, as well as to diverse side effects, such as the immediate hypersensitivity observed in this study. Previous results have shown that a single dose of class II–blocking peptides, when applied in soluble form, fails to induce a proliferative T cell response (18). However, the immunogenicity of chronically applied, soluble MHC-binding peptides has not yet been assessed. We therefore tested whether long-term treatment of NOD mice with P1 and P2 (from Fig. 3 *A*) would lead to immunization against these peptides. The data in Fig. 4 demonstrate the presence of antibodies in the sera of both groups of peptide-treated mice. Antibodies were detectable up to a serum dilution of 1:200, i.e., their titer was low. Mice treated with the class II–binding P1 produced IgG but no IgM antibodies. The number of antibody-positive mice increased with the duration of treatment. However, approximately half of this group remained antibody negative after 13 wk of treatment. Interestingly, mice treated with the nonbinding P2 peptide also mounted an antibody response, which was exclusively of IgM isotype. Approximately half of the mice were also nonresponders in this group. The isotype difference of P1- and P2-specific antibodies probably reflects the presence and absence, respectively, of class II–restricted T cell help in these responses. In the experiment in Fig. 3 *B*, only 3 of 13 P1-treated animals produced antibodies against the peptide (data not shown). Thus, chronic application of soluble peptides can induce a low titer IgM or IgG antibody response, although the majority of treated animals may remain nonresponder. In view of the observed hypersensitivity symptoms, we also determined serum IgE levels and found a substantial elevation in mice treated with P1 (8.29 ± 2.7 μg/ml) or P2 (6.9 ± 3.76 μg/ml), over the solvent-treated (1.78 ± 1.67 μg/ml) or untreated (0.68 ± 0.85 μg/ml) animals. These data suggest that IgE antibodies against the peptides may be responsible for the observed immediate hypersensitivity reactions.

Discussion

The data presented herein demonstrate a correlation between the capacity of a peptide to block antigen presentation by the A^{g7} molecule in vitro, to inhibit the priming for A^{g7}-restricted T cell responses in vivo, and to interfere with the development of spontaneous autoimmune diabetes in NOD mice. Since the development of diabetes is genetically linked to the A^{g7} class II allele (6), the data provide support for the hypothesis that the mechanism underlying disease linkage to a particular class II allele is the presentation of a disease-inducing antigen by the molecule encoded by this allele.

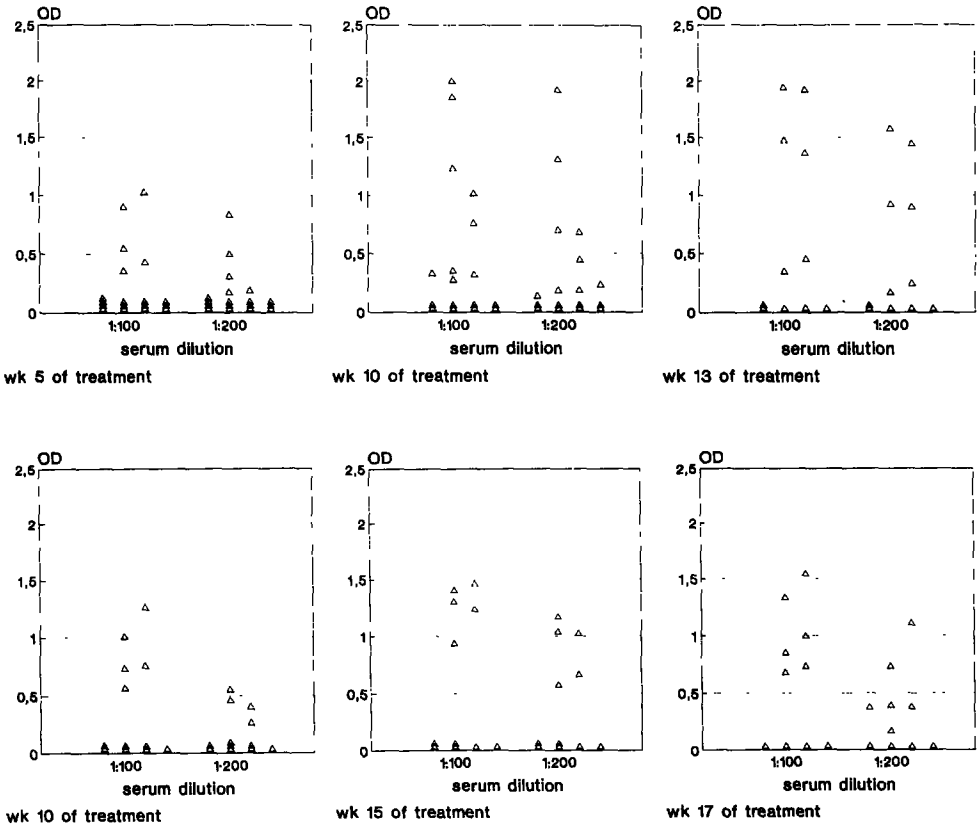

Figure 4. Antibody response of NOD mice after chronic treatment with P1 (*top*) and P2 (*bottom*). IgG antibodies to P1 and IgM to P2 are shown (P1 induced no IgM and P2 no IgG antibodies; data not shown). Each triangle represents antibodies of an individual mouse at the time point and serum dilution indicated.

Previous studies have demonstrated that class II MHC-binding peptides can selectively inhibit the induction of T cell responses to other peptides binding to the same MHC molecule (17, 18). These findings have been extended to an autoimmune disease model, namely experimental autoimmune encephalomyelitis (EAE). Thus, EAE induced by peptides of myelin basic protein can be prevented by peptides competing with the pathogenic ones for the same class II MHC-binding sites (26, 29, 30). Since the antigens involved in most autoimmune diseases are unknown, the MHC molecule that presents putative disease-inducing antigens is, at present, the only target available for selective immunosuppressive intervention. Our data demonstrate the feasibility of an MHC-binding site-directed immunosuppressive approach, for the first time, in a spontaneously occurring, MHC-linked autoimmune disease model. It should be pointed out that the pathogenesis of NOD diabetes, similarly to human type I diabetes, commences with lymphocytic infiltration of the islets of Langerhans at young age, followed by selective depletion of the insulin-producing β cells (19). Furthermore, the first domain of the A^{g7} β chain exhibits sequence homology with the DQ β chains conferring susceptibility to type I diabetes in humans (11, 24). Thus, the close resemblance of the NOD disease model to a human autoimmune disease raises the possibility that the immunosuppressive principle applicable in this model may also be effective in human autoimmune conditions. Our data also suggest that MHC blocking may not permanently suppress disease, that is, the symptoms can return after the cessation of treatment. Thus, a continuous treatment may be necessary, or alternatively, this immunosuppressive strategy may preferentially be applied in diseases occurring as episodes interrupted by symptom-free periods, e.g., rheumatoid arthritis and multiple sclerosis. An important observation in this study is that peptides, although not immunogenic when applied short term in soluble form (18), can induce antibody production and severe immunological side effects in a fraction of animals upon long-term treatment. Thus, although 14 residue–long peptides proved to be suitable reagents for testing the validity of MHC blockade as an immunosuppressive strategy, they will probably not become suitable drugs. Therefore, the pharmacological application of this strategy should solve the problem of immunogenicity, perhaps by the development of smaller, possibly nonpeptidic MHC-blocking molecules.

We thank Drs. F. Gaeta and A. Sette for making peptides available for our screening, Dr. H. Fliri for peptide synthesis, Dr. C. Heusser for IgE determinations, and Ms. C. Maurer and Ms. S. Trembleau for technical assistance.

Address correspondence to Ursula Hurtenbach, Department of Inflammation, Preclinical Research, Building 386, Room 732, Sandoz Pharma Ltd., 4002 Basel, Switzerland.

Received for publication 12 January 1993.

References

1. Svejgaard, A., P. Platz, and L.P. Ryder. 1983. HLA and disease 1982. A survey. *Immunol. Rev.* 70:193.
2. Vladutin, A.O., and N.R. Rose. 1971. Autoimmune murine thyroiditis relation to histocompatibility (H-2) type. *Science (Wash. DC).* 174:1137.
3. Günther, E., H. Odenthal, and W. Wechsler. 1978. Association between susceptibility to experimental allergic encephalomyelitis and the major histocompatibility system in congeneic rat strains. *Clin. Exp. Immunol.* 32:429.
4. Wooley, P.H., H.S. Luthra, J.M. Stuart, and C.S. David. 1981. Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates. *J. Exp. Med.* 154:688.
5. Griffiths, M.M., R.J. Eichwald, J.H. Martin, C.B. Smith, and C.W. DeWitt. 1981. Immunogenetic control of experimental type II collagen induced arthritis. I. Susceptibility and resistance among inbred strains of rats. *Arthritis Rheum.* 24:781.
6. Hattori, M., J.B. Buse, R.A. Jackson, L. Glimcher, M.E. Dorf, M. Minami, S. Makino, K. Moriwaki, H. Kuzuya, H. Imura, W.M. Strauss, J.G. Seidman, and G.S. Eisenbarth. 1986. The NOD mouse: recessive diabetogenic gene in the major histocompatibility complex. *Science (Wash. DC).* 231:733.
7. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature (Lond.).* 329:506.
8. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. The foreign antigen binding site and the T cell recognition regions of the class I histocompatibility antigens. *Nature (Lond.).* 329:512.
9. Brown, J.H., T. Jardetzky, M.A. Saper, B. Samraoui, P.J. Bjorkman, and D.C. Wiley. 1988. A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. *Nature (Lond.).* 332:845.
10. Garrett, T.P.J., M.A. Saper, P.J. Bjorkman, J.L. Strominger, and D.C. Wiley. 1989. Specificity pockets for the side chains of peptide antigens in HLA-Aw68. *Nature (Lond.).* 342:692.
11. Todd, J.A., J.I. Bell, and H.O. McDevitt. 1987. HLA-DQβ gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature (Lond.).* 329:599.
12. Baisch, J.M., T. Weeks, R. Giles, M. Hoover, P. Stastny, and J.D. Capra. 1990. Analysis of HLA-DQ genotypes and susceptibility in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 322:1836.

13. Nepom, G.T., P. Byers, C. Seyfried, L.A. Healey, K.R. Wilske, D. Stage, and B.S. Nepom. 1989. HLA genes associated with rheumatoid arthritis. Identification of susceptibility alleles using specific oligonucleotide probes. *Arthritis Rheum.* 32:15.
14. Hiraiwa, A., K. Yamanaka, W.W. Kwok, E.M. Mickelson, S. Masewicz, J.A. Hansen, S.F. Radka, and G.T. Nepom. 1990. Structural requirements for recognition of the HLA-Dw14 class II epitope: a key HLA determinant associated with rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA.* 87:8051.
15. 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.
16. 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.
17. 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.
18. 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.
19. Castano, L., and G.S. Eisenbarth. 1990. Type-I diabetes: a chronic autoimmune disease of human, mouse and rat. *Annu. Rev. Immunol.* 8:647.
20. Martin, U., and D. Roemer. 1978. The pharmacological properties of a new orally active antianaphylactic compound: ketotifen, a benzocycloheptathiophene. *Drug Res.* 28:770.
21. Adorini, L., A. Sette, S. Buus, H.M. Grey, M. Darsley, P.V. Lehmann, G. Doria, Z.A. Nagy, and E. Appella. 1988. Interaction of an immunodominant epitope with Ia molecules in T-cell activation. *Proc. Natl. Acad. Sci. USA.* 85:5181.
22. Ledermann, F., C. Schlienger, K. Wagner, and C. Heusser. 1991. A sensitive and efficient induction system for murine IgE. Single cell analysis at the clonal level. *J. Immunol. Methods.* 141:263.
23. Hurtenbach, U., and C.J. Maurer. 1989. Type I diabetes in NOD mice is not associated with insulin-specific, autoreactive T cells. *Autoimmunity.* 2:151.
24. Acha-Orbea, H., and H.O. McDevitt. The first external domain of the nonobese diabetic mouse class II I-A β chain is unique. *Proc. Natl. Acad. Sci. USA.* 84:2435.
25. 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.
26. Lamont, A.G., A. Sette, R. Fujinami, S.M. Colon, C. Miles, and H.M. Grey. 1990. Inhibition of experimental allergic encephalomyelitis induction in SJL/J mice by using a peptide with high affinity for IA^s molecules. *J. Immunol.* 145:1687.
27. Makino, S., K. Kunimoto, Y. Muraoka, and K. Katagiri. 1981. Effect of castration on the appearance of diabetes in the NOD mouse. *Exp. Anim. (Tokyo).* 30:137.
28. Soloway, P., S. Fish, H. Passmore, M. Gefter, R. Coffee, and T. Manser. 1991. Regulation of the immune response to peptide antigens: differential induction of immediate-type hypersensitivity and T cell proliferation due to changes in either peptide structure or major histocompatibility complex haplotype. *J. Exp. Med.* 174:847.
29. Wraith, D.C., D.E. Smilek, D.J. Mitchell, L. Steinmann, and H.O. McDevitt. 1989. Antigen recognition in autoimmune encephalomyelitis and the potential for peptide mediated immunotherapy. *Cell.* 59:247.
30. 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 major histocompatibility complex proteins. *Proc. Natl. Acad. Sci. USA.* 86:9470.