Selective Immunosuppression by Administration of Major Histocompatibility Complex Class II-binding Peptides. II. Preventive Inhibition of Primary and Secondary In Vivo Antibody Responses

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Summary

The self-mouse lysozyme peptide corresponding to residues 46-62 (ML46-62) binds to the major histocompatibility complex (MHC) class II molecules I-A^k and it selectively inhibits, when coinjected with antigen, priming of I-A^k–restricted, antigen-specific T cells. We demonstrate that administration of ML46-62 also inhibits in vivo antibody responses induced by I-A^k–restricted T helper cells. ML46-62 is able to prevent the primary anti–hen egg white lysozyme (HEL) antibody response induced by the entire HEL molecule in B10.A(4R) mice, expressing only I-A^k molecules, but not in mice of H-2^d haplotype. ML46-62 also strongly decreases, in B10.A(4R) mice, the antibody response to ribonuclease A, a protein antigen unrelated to the MHC blocker, indicating that MHC blockade is the mechanism leading to inhibition of antibody response. This is further supported by the concomitant decrease, in vivo, of complex formation between immunodominant HEL peptides and I-A^k molecules, preventing I-A^k–restricted T cell induction. Administration of ML46-62 after antigen priming does not affect ongoing antibody responses, as expected from MHC blockade. A single injection of ML46-62 at the time of protein antigen priming precludes not only the primary, but also the secondary antibody response to a subsequent challenge with soluble protein, even when the challenge is performed several months after priming. Coinjection of antigen and MHC antagonist inhibits production of all antibody isotypes equally well, suggesting that MHC class II blockade affects both Th1- and Th2-type T helper cells. Therefore, these results indicate that administration of MHC class II–binding peptides can efficiently and selectively prevent the induction of T cell–dependent primary and secondary in vivo antibody responses by blocking antigen presentation to class II–restricted T helper cells.

Peptides bound to class II MHC molecules on the surface of APCs are the ligands for antigen-specific receptors of $CD4^+$ T cells (1). Each MHC class II molecule can bind different peptides, indicating that the interaction between peptides and class II molecules exhibits a low degree of selectivity (2). One consequence of the broad-range specificity of peptide–MHC interactions is that peptides of different sequences may compete for presentation by the same MHC molecule to T lymphocytes.

Peptide competition for antigen presentation has been demonstrated in vitro, by showing that antigens binding to the same MHC molecules can compete with each other for presentation to T cells (3–6). A direct correlation has been demonstrated between the capacity of a peptide to bind to purified MHC class II molecules and its ability to compete for presentation of antigenic peptides binding to the same class II molecules (7, 8). Peptide competition for the MHC class II binding site also occurs in vivo. This has been demonstrated first by competition between self- and non-self peptides for T cell activation, showing that T cell responses to antigenic peptides can be inhibited by coinjection of antigen and an unrelated MHC class II–binding competitor peptide (9). Subsequently, several groups have used a similar strategy to prevent induction of T cell–mediated autoimmune diseases like experimental allergic encephalomyelitis in mice (10, 11) and rats (12), or autoimmune carditis in mice (13).

These results suggest that in vivo competition for antigen presentation may result in MHC blockade preventing T cell activation. Recently, using an ex vivo system, we have demonstrated that lymph node cells (LNC)[1] from mice immunized

[1] *Abbreviations used in this paper:* HEL, hen egg-white lysozyme; LNC, lymph node cells; RNase, bovine ribonuclease.

with hen egg white lysozyme (HEL) display at their surface antigenic peptide–class II complexes able to stimulate, in the absence of any further antigen addition, HEL peptide-specific, class II–restricted T cell hybridomas. Coadministration of antigen and MHC class II–binding peptide competitors selectively inhibits the in vivo formation of antigenic complexes and prevents antigen presentation by LNC to class II–restricted T cells, indicating competitive blockade of class II molecules in vivo (14). MHC class II blockade can be induced by administering MHC antagonists either in a depot (9, 14, 15) or in soluble form (16), and this could represent a promising approach to selectively induce immunosuppression of undesired immune responses (17). However, several points still need to be addressed to evaluate the practical feasibility of this form of immunointervention.

In the present paper we have examined the effect of MHC class II blockade on the in vivo antibody responses to T cell–dependent antigens. Injection of a nonimmunogenic MHC class II–binding self-peptide at the time of antigen priming can inhibit both primary and secondary antibody responses to protein antigens. This inhibition is associated with decreased formation of antigenic peptide–MHC class II complexes in vivo, resulting in inhibition of class II–restricted T cell proliferation. All antibody isotypes are equally inhibited, suggesting that MHC blockade inhibits activation of both Th1- and Th2-type T helper cells. As for T cell activation, the inhibition of antibody responses is selective for the MHC class II molecules binding the competitor peptide, and its extent depends on the molar ratio between antigen and competitor.

Materials and Methods

Mice. 2- to 3-mo-old DBA/2, BALB/c, C3H, B10.D2 (Iffa Credo, L'Arbesle, France) and B10.A(4R) (Olac, Bicester, UK) mice of either sex were used.

Antigens. HEL, recrystallized three times, and bovine RNase were obtained from Sigma Chemical Co. (St. Louis, MO). Peptides were synthesized by the solid-phase method on phenylacetamidomethyl-polystyrene support using side chain protection, coupling procedures, and an automated apparatus (model 430A; Applied Biosystems, Inc., Foster City, CA) as described (9). Crude peptides were purified by preparative HPLC on a C18 reverse-phase column. The peptides showed correct amino acid ratios upon hydrolysis in 6 N HCl and the expected molecular ions in fast atom bombardment mass spectrometry. Sequences were confirmed by gas-phase microsequencing.

Immunizations. Mice were immunized subcutaneously at the tail base and into the hind footpads, or by intraperitoneal injection with the indicated amount of antigen emulsified in IFA or CFA containing H37Ra mycobacteria (Difco Laboratories, Inc., Detroit, MI). For the induction of secondary antibody responses, mice were challenged by injecting intraperitoneally 100 μg/mouse of soluble antigen in PBS.

ELISA. Polyvinyl microtiter plates (Falcon model 3012; Becton Dickinson & Co., Oxnard, CA) were incubated overnight at 4°C with 50 μl/well of HEL or RNase at 10 μg/ml in 60 mM sodium carbonate–bicarbonate buffer, pH 9.6. After washing with PBS, the plates were blocked by incubation with PBS containing 1% BSA for 1 h at 37°C. Plates were then washed three times with PBS containing 0.1% Tween 20 (PBS-Tw). Appropriately diluted sera (50 μl/well) were titrated in PBS-Tw containing 1% BSA (PBSA-Tw), and incubated for 90 min at 37°C. Plates were then washed and incubated for 1 h at 37°C with a mixture (100 ng/ml each) of the following biotin-conjugated goat anti–mouse isotype-specific antibodies (Southern Biotechnology Associates Inc., Birmingham, AL): anti-IgM, -IgG1, -IgG2a, -IgG2b, and -IgG3. After washing, the bound antiisotypic antibodies were revealed by an additional 1-h incubation with alkaline phosphatase–conjugated streptavidin (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) diluted 1:20,000. The plates were washed again and incubated with the developing substrate *p*-Nitrophenylphosphate disodium (Sigma Chemical Co.) in diethanolamine buffer, pH 9.6 (100 μl/well). The reaction was stopped by adding 50 μl/well NaOH 3 N, and absorbance was read at 405 nm using an automated microplate ELISA reader (Titertek Multiskan® MCC/340; Flow Laboratories, Helsinki, Finland). Standard curves were generated using pooled sera from mice immunized with the corresponding antigen. The amount of HEL-specific antibodies was calculated using affinity-purified anti-HEL antibodies and results expressed as serum antibody concentration in mg/ml. The serum anti-RNase antibody concentration was expressed as arbitrary U/ml. Anti-HEL and -RNase IgM, IgG1, IgG2a, IgG2b isotypes were determined as described above using biotinylated isotype-specific antibodies. Standard curves were obtained using calibrated serum pools revealed with antiisotypic developing reagents, and results expressed as relative U/ml.

T Cell Proliferation Assay. LNs draining the injection sites were removed, and 4×10^5 cells/well were cultured in 96-well culture plates (Costar Corp., Cambridge, MA) in synthetic HL-1 medium (Ventrex Laboratories, Portland, ME) supplemented with 2 mM L-glutamine and 50 μg/ml gentamicin (Sigma Chemical Co.) with the indicated antigen concentrations. Cultures were incubated for 3 d in a humidified atmosphere of 5% CO_2 in air and were pulsed 10 h before harvesting with 1 μCi [^{3}H]TdR (40 Ci/nmol; the Radiochemical Center, Amersham, UK). Incorporation of [^{3}H]TdR was measured by liquid scintillation spectrometry.

Assay for Antigen Presenting Activity of LNC from HEL-primed Mice. The antigen presenting activity of LNC from HEL-primed mice was assessed as previously described (14) using the following T cell hybridomas: 1C5.1 (I-A^k, HEL46-61) (14); 3B11.1 (I-A^k, HEL34-45) (Adorini, L., unpublished results); and 1H11.3 (I-E^d, HEL108-116) (8). Briefly, mice were immunized into the hind footpads with the indicated amount of antigen emulsified in IFA or CFA. 5–6 d later, the draining popliteal LNC were removed, irradiated (2,400 rad), and cultured in triplicate at the indicated cell doses with appropriate HEL-specific T cell hybridomas (5×10^4 cells/well) in 96-well culture plates (Costar Corp.). Culture medium was RPMI 1640 (Gibco, Basel, Switzerland) supplemented with 2 mM L-glutamine, 50 mM 2-ME, 50 μg/ml gentamicin (Sigma Chemical Co.), and 10% FCS (Gibco). After 24 h of culture, 50-μl aliquots of supernatants were transferred to microculture wells containing 10^4 CTLL cells and, after an additional 24-h incubation, the presence of T cell growth factors, mainly IL-2, was assessed by [^{3}H]TdR incorporation during the last 4 h of culture. For the anti-Thy-1 cytotoxic treatment, LNC (6×10^6/ml) were incubated with 1.5 μg/ml of anti-Thy-1.2 mAb (Becton Dickinson & Co., Mountain View, CA) for 30 min on ice. After washing, cells were resuspended in rabbit complement (low-tox M®; Cedarlane Laboratories, London, Canada) at a final dilution of 1:12, incubated 45 min at 37°C, washed, and then irradiated. T cell depletion was assessed by cytofluorimetric analysis with biotinylated anti-TCR mAb (18).

Results

ML46-62 Inhibits the Anti-HEL Antibody Response in H-2^k but Not in H-2^d Mice. The mouse lysozyme (ML) peptide 46-62 binds strongly to I-A^k molecules but fails to bind to I-E^k, I-A^d, and I-E^d molecules (5, 9, 16). We have previously shown that administration of this peptide selectively blocks, in vivo, T cell activation induced by I-A^k-binding antigenic peptides (9, 14). To examine the effect of MHC class II blockade on T cell–dependent antibody responses, B10.A(4R) mice, expressing only the I-A^k class II molecule, were immunized intraperitoneally with HEL (35 pmoles/mouse) alone, or mixed with either 5 or 0.5 nmoles of ML46-62. The anti-HEL antibody response was quantitated by ELISA at different times after priming. As shown in Fig. 1 *A*, administration of ML46-62 (5 or 0.5 nmoles/mouse) inhibits almost completely the anti-HEL antibody response. The inhibitory effect was slightly reduced at 14-fold competitor excess over HEL, as compared with 140-fold excess. As expected from its binding specificity, ML46-62 administered at 5 nmoles/mouse to DBA/2 (H-2^d) mice does not affect the anti-HEL antibody response (Fig. 1 *B*), demonstrating that this peptide is devoid of any nonspecific immunosuppressive properties. Therefore, as for T cell priming in vivo, inhibition of the antibody response by administration of class II–binding competitor peptides is selective for the MHC class II molecules to which the competitor binds, and its extent depends on the molar ratio between antigen and competitor.

Inhibition of the Primary and Secondary Antibody Response to HEL and RNase, an Antigen Unrelated to the MHC Blocker. Having shown that ML46-62 is able to selectively block the primary anti-HEL antibody response in B10.A(4R) mice, we tested its ability to inhibit both primary and secondary antibody responses to HEL and to RNase, an antigen unrelated to ML46-62. As shown in Fig. 2, ML46-62 administration induces a 5–10-fold reduction of the primary antibody responses to both protein antigens. This inhibition is long-lasting and can be observed over a period of 20 wk. Upon challenge with the corresponding soluble protein antigens, a 20-fold increase of antigen-specific serum antibody is induced, but in mice injected with ML46-62 at the time of priming, the secondary antibody response was 10–20-fold lower than control responses. This effect is specific since administration of a non-I-A^k–binding peptide (HEL64-77) does not affect the anti-HEL (Fig. 2 *A*) or the anti-RNase (data not shown) antibody responses. In Fig. 2 *B*, the competitor peptide was administered in IFA 1 d before immunization with RNase, indicating that separate administration of antigen and competitor also inhibits primary and secondary antibody responses. The results indicate that administration of class II–binding competitor peptides unrelated to the antigen can efficiently inhibit both primary and secondary in vivo antibody responses induced by Th whose activation is restricted by the blocked class II molecule.

Effect of MHC Blockade on Anti-HEL and Anti-RNase Ig Isotypes. The inhibitory effect of ML46-62 was tested, so far, by measuring the whole humoral response. We next determined whether different antigen-specific Ig isotypes were all similarly affected. Results in Table 1 show that, in the primary and secondary anti-HEL antibody responses, IgG1 is the dominant isotype, and that all Ig isotypes measured are inhibited by administration of ML46-62. Conversely, injection of HEL64-77 does not significantly affect any of the anti-HEL Ig isotypes. This inhibition is more pronounced in the secondary response, and it is higher for the dominant isotype. Similar results are obtained measuring anti-RNase Ig isotypes. In this case, unlike HEL, the primary antibody response also includes IgM antibodies. In the primary response, IgG1 and IgM are predominantly reduced, whereas in the secondary response, all isotypes are inhibited. Collectively, these results indicate that inhibition of antibody response by MHC class II blockade affects all Ig isotypes. Since IgG1 are

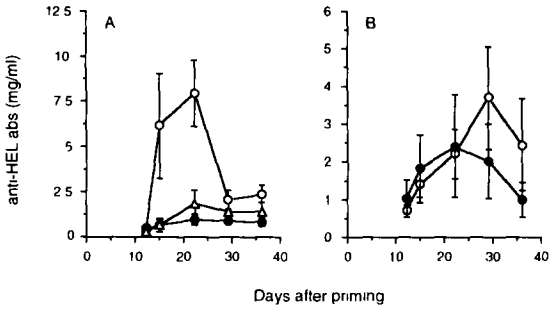

Figure 1. Inhibition of the primary anti-HEL antibody response by administration of ML46-62. B10.A(4R) (*A*) or DBA/2 (*B*) mice were immunized intraperitoneally with CFA only (▲) or with an emulsion containing 35 pmoles HEL alone (○), or mixed with 5 nmoles (●) or 0.5 nmoles (△) of ML46-62. The anti-HEL antibody response was measured by quantitative ELISA in individual mice at the indicated days after priming by ELISA. Data are expressed as mean ± SEM anti-HEL serum antibody concentration (mg/ml) from three to four mice per group.

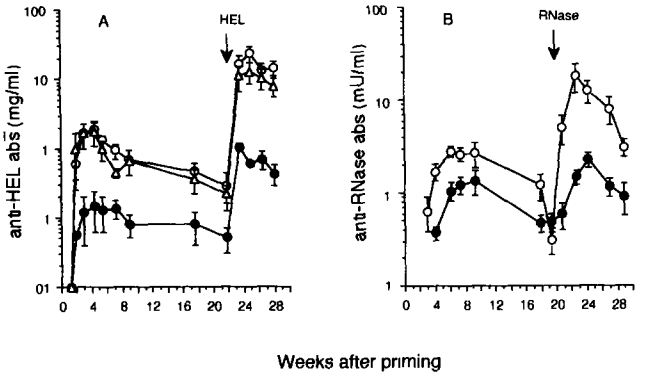

Figure 2. Inhibition of primary and secondary anti-HEL and anti-RNase antibody responses by administration of ML46-62. (*A*) B10.A(4R) mice were immunized intraperitoneally with 35 pmoles HEL alone (○), or mixed with either ML46-62 (●) or HEL64-77 (△), (5 nmoles/mouse). (*B*) Mice were injected 1 d before antigen priming with IFA alone (○) or with 30 nmoles ML46-62 (●) in IFA, and then immunized with RNase, 210 pmoles/mouse in CFA. Mice were then challenged with the corresponding soluble antigen (100 μg/mouse) about 20 wk after priming (*arrows*). Results are expressed as mean anti-HEL antibody concentration (mg/ml) from three to four mice per group, or as mean ± SEM anti-RNase antibody serum concentration expressed in arbitrary mU/ml from six mice per group.

Table 1. *ML46-62 Inhibits Different Antibody Isotypes Induced by HEL and RNase in B10.A(4R) Mice*

Antigen	Competitor	Challenge	Serum antibody concentration			
			Ig M	IgG1	IgG2a	IgG2b
HEL	-	-	<5	1,915 ± 318	34 ± 29	196 ± 101
HEL	ML46-62	-	<5	153 ± 91	5 ± 3.2	11.3 ± 6.6
HEL	HEL64-77	-	<5	1,762 ± 722	9.1 ± 4.5	119 ± 18
HEL	-	HEL	<5	14,840 ± 6,162	291 ± 199	874 ± 507
HEL	ML46-62	HEL	<5	724 ± 125	42 ± 24	92 ± 63
HEL	HEL64-77	HEL	<5	10,237 ± 4,004	171 ± 122	1,175 ± 597
RNase	-	-	13.5 ± 5.8	1,169 ± 231	117 ± 45	196 ± 71
RNase	ML46-62	-	5.3 ± 2.2	335 ± 110	83 ± 19	151 ± 43
RNase	-	RNase	<5	14,534 ± 6,212	1,443 ± 568	1,908 ± 629
RNase	ML46-62	RNase	<5	627 ± 224	316 ± 142	554 ± 329

Sera from the experiment described in Fig. 2 were tested for anti-HEL and anti-RNase Ig isotypes by ELISA, as described in Materials and Methods. Results shown refer to antibody isotypes detected in sera obtained at peak primary (day 31 for HEL, day 43 for RNase) and secondary (day 9 for HEL, day 21 for RNase) antibody responses. Results are expressed as serum antibody concentration (U/ml) ± SEM from three to five mice per group.

predominantly induced by Th2 and IgG2a/IgG2b by Th1 cells (19–21), MHC blockade appears to inhibit the helper function of both Th cell subsets.

Lack of Inhibition of Anti-HEL Antibody Response by Administration of ML46-62 in Primed Mice. We next analyzed the effect of administering ML46-62 1 d before immunization or 10 d after priming with HEL. As shown in Fig. 3 (*D* and *E*), administration to B.10A(4R) mice of the competitor peptide emulsified together with HEL, or in a separate depot 1 d before HEL priming, strongly inhibits the entire anti-HEL antibody response, as compared with controls (Fig. 3, *A* and *B*). Conversely, ML46-62 administered 10 d after HEL-CFA priming does not affect the ongoing anti-HEL antibody response (Fig. 3 *F*). Similar results were obtained when ML46-62 was administered 3 d after HEL priming (data not shown). These results confirm that the coadministration of antigen and competitor is not a prerequisite for inhibition of the antibody response, as previously shown with RNase (Fig. 2 *B*). It is interesting that once the immune response is initiated, the competitor peptide has no effect, a result consistent with the MHC blockade model.

Direct Evidence for MHC Blockade Induced In Vivo by ML46-62. To analyze the mechanisms involved in MHC blockade, we recently described an ex vivo system to detect complexes between antigenic peptides and MHC class II molecules generated in vivo (14). Using this assay we tested the ability of ML46-62 to selectively inhibit in vivo formation of antigenic complexes between I-A^k class II molecules and the two dominant HEL peptides 46-61 and 34-45 derived from HEL processing (22). H-2^k (C3H and B10.A[4R]) and H-2^d (BALB/c and B10.D2) mice were immunized with HEL or with an emulsion containing HEL and ML46-62 either in IFA or in CFA. 5 d later, irradiated LNC were tested for their ability to activate the I-A^k–restricted T cell hybridomas 1C5.1 (HEL46-61/A^k) and 3B11.1 (HEL34-45/A^k) or the I-E^d–restricted T cell hybridoma 1H11.3 (HEL108-116/E^d). As shown in Fig. 4, coinjection of HEL and the I-A^k–binding peptide ML46-62 markedly inhibits the antigen presenting activity of LN APC to the I-A^k–restricted T cell hybridomas, specific for HEL peptides 46-61 and 34-45, in both

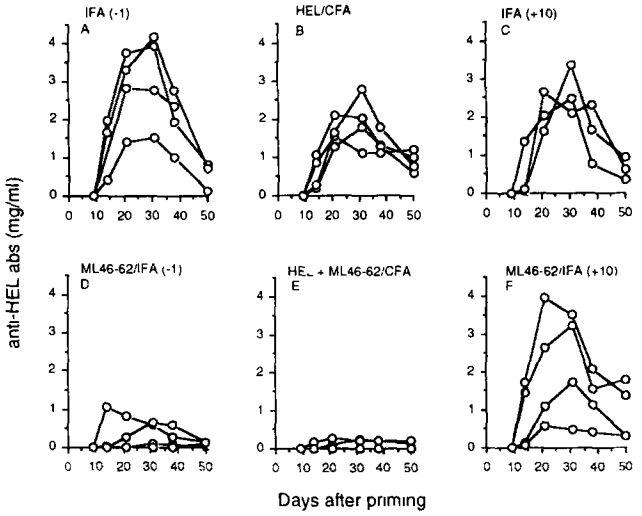

Figure 3. Lack of inhibition of the anti-HEL antibody response by administration of ML46-62 to primed mice. B10.A(4R) mice were injected with IFA alone or with ML46-62 (5 nmoles/mouse) in IFA, 1 d before (*A* and *D*) or 10 d after (*C* and *F*) immunization with 35 pmoles/mouse HEL/CFA. (*B* and *E*) Mice were immunized in CFA with HEL alone (*B*) or mixed with 5 nmoles/mouse ML46-62 (*E*). Results are expressed as anti-HEL serum antibody concentration (mg/ml) from three to four individual mice per group.

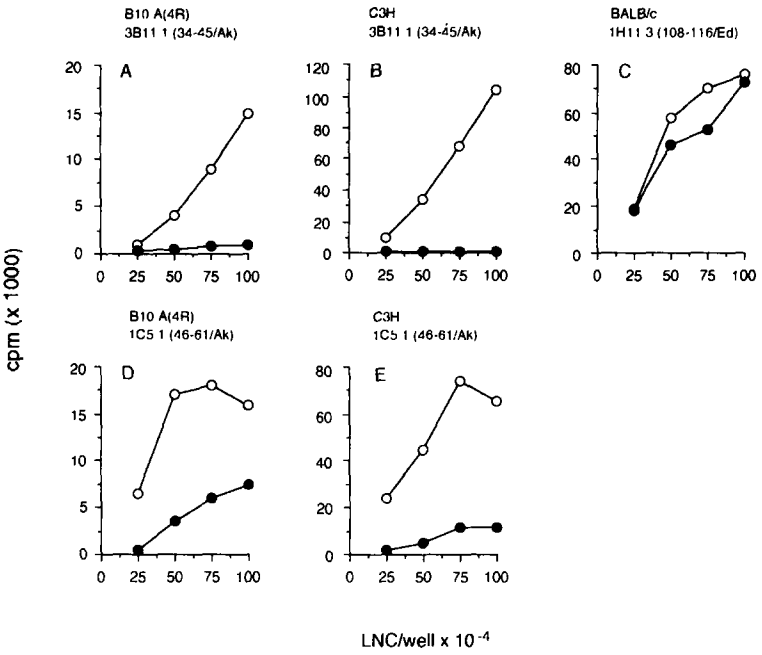

Figure 4. Selective inhibition of I-A^k–restricted antigen presentation by ML46-62. B10.A(4R) (*A* and *D*), C3H (*B* and *E*), and BALB/c (*C*) mice were immunized in IFA with 3 nmoles HEL alone (○) or mixed with 100 nmoles ML46-62 (●). 5 d later, LNC from three mice were pooled, irradiated and cultured with the T cell hybridomas 3B11.1 (*A* and *B*), 1C5.1 (*D* and *E*), or 1H11.3 (*C*). After 24 h, IL-2 production was measured by adding 50 μl aliquots of culture supernatant to 10^4 CTLL for an additional 24 h. [^{3}H]Thymidine (1 μCi/well) was added during the last 5 h of culture. IL-2 production induced in T cell hybridomas by addition of 3 μM HEL to 2.5 × 10^5 LNC/well from mice injected with HEL alone or mixed with the competitor peptide were respectively (cpm): 117,031, 129,413 (*A*); 128,927, 95,432 (*B*); 211,492, 218,803 (*C*); 105,126, 87,499 (*D*); and 100,203, 118,098 (*E*). Data are presented as mean cpm of thymidine incorporation from triplicate cultures. Background proliferation of CTLL cells was 258 cpm.

B10.A(4R) and C3H mice. Conversely, coinjection of ML46-62, nonbinding to I-E^d molecules, does not affect the antigen presenting activity of LNC from BALB/c mice to the I-E^d–restricted, HEL108-116–specific, T cell hybridoma 1H11.3. In addition, the ability of LN APC from competitor peptide–injected mice to process and present exogenous HEL added in vitro is not modified (see legend to Fig. 4).

To eliminate the possibility that inhibition of T cell hybridoma activation could be due to suppressor T cells induced by ML46-62 in the LNC population, T cell–depleted LNC from B10.A(4R) or B10.D2 mice immunized with HEL in CFA alone or mixed with ML46-62 were used as APC. Results in Fig. 5 show that the antigen presenting activity of T cell–depleted APC from HEL-primed B10.A(4R) mice to both I-A^k–restricted T cell hybridomas is strongly decreased

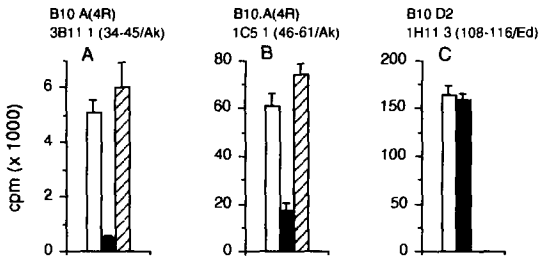

Figure 5. Inhibition of antigen presentation is not due to T cells. B10.A(4R) (*A*, *B*) and B10.D2 (*C*) mice were immunized with CFA containing 3 nmoles HEL alone (□) or mixed either with 100 nmoles ML46-62 (■) or 100 nmoles HEL64-77 (▨). 5 d later, LNC from two mice were pooled, treated with anti-Thy-1 mAb plus C', irradiated, and 10^6 cells/well cultured with the T cell hybridomas: 3B11.1 (*A*), 1C5.1 (*B*), and 1H11.3 (*D*). The experiment was then continued as in Fig. 4. Data are shown as mean (cpm) ± SEM from triplicate cultures. Background proliferation of CTLL was 545 cpm.

by coadministration of the I-A^k–binding competitor peptide, but not by the nonbinding peptide HEL64-77. The specificity of the inhibitory effect is indicated by lack of inhibition, after injection of the same emulsion in B10.D2 (H-2^d) mice, of the I-E^d–restricted presentation to the HEL108-116–specific T cell hybridoma 1H11.3. Therefore, these results demonstrate that inhibition of T cell hybridoma activation is selective for the MHC molecule to which the competitor peptide binds, and it does not depend on the presence of LN T cells, ruling out a possible role for suppressor T cells.

To correlate the inhibition of antigen presentation with the inhibition of T cell proliferation, C3H mice were immunized with HEL alone in CFA, or mixed with either ML46-62 or HEL64-77. 6 d later, the same LNC population was simultaneously tested for antigen presentation to both I-A^k–restricted T cell hybridomas 1C5.1 and 3B11.1, and for T cell proliferation to I-E^k– or I-A^k–restricted HEL peptides. As shown in Fig. 6, *A* and *B*, coadministration of a 16-fold excess of ML46-62 strongly decreases the antigen presenting activity of LN APC to both I-A^k–restricted T cell hybridomas, whereas, as shown previously, no inhibition is induced by the non-I-A^k–binding peptide HEL64-77. The same LNC populations were tested for T cell proliferation to four immunodominant HEL peptides. Results demonstrate that the proliferative response of T cells from mice coimmunized with HEL and ML46-62 to the I-A^k–binding peptides 46-61, 51-61, and 112-129 is inhibited as compared with control groups (Fig. 6, *D–F*), whereas no effect is observed on the T cell proliferation recalled by the I-E^k–binding peptide HEL1-18 (Fig. 6 *C*). Taken together, these results demonstrate that administration of the class II–binding peptide ML46-62 inhibits I-A^k–restricted antigen presentation to T cell hybridomas and antigen-specific proliferation of bulk T cells, indicating I-A^k blockade by the competitor peptide.

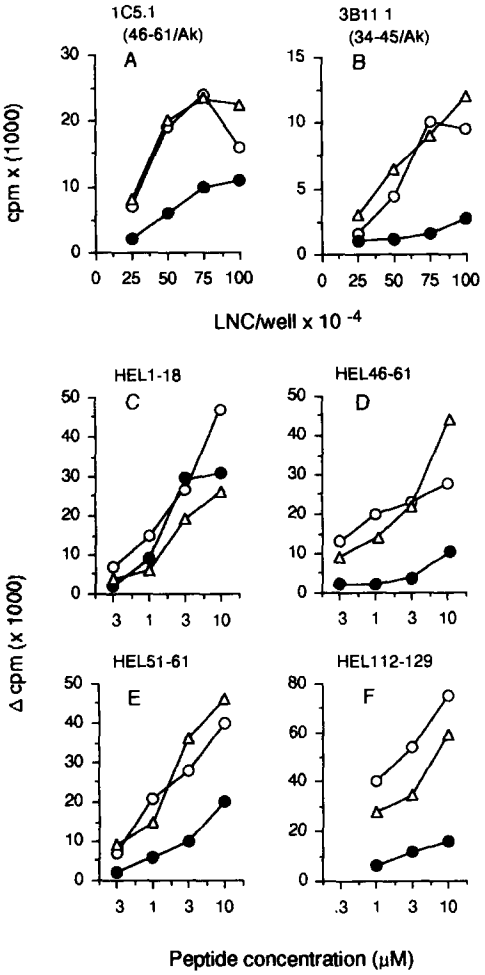

Figure 6. Inhibition of HEL peptides-I-A^k complex formation correlates with inhibition of I-A^k-restricted T cell proliferation. C3H mice were immunized in CFA with 3 nmoles HEL alone (O) or mixed either with 50 nmoles HEL64-77 (Δ) or 50 nmoles ML46-62 (●). 6 d later, LNC from two mice were pooled, and either irradiated and cultured with the T cell hybridomas 1C5.1 (*A*) or 3B11.1 (*B*), or restimulated in vitro with the indicated concentration of HEL1-18 (*C*), HEL46-61 (*D*), HEL51-61 (*E*), and HEL112-129 (*F*). (*A* and *B*) Results are expressed as in Fig. 4. (*C–F*), after 3 d of culture, 1 μCi [^{3}H]thymidine/well was added, and cells harvested 10 h later. Data are presented as mean Δ cpm from duplicate cultures. Backgrounds (no antigen) cpm were 12,379 (O), 8,970 (Δ), and 10,696 (●).

Discussion

Induction of immunosuppression by MHC class II antagonists has been demonstrated in a variety of experimental systems, by examining their effect on T cell activation, in vivo, either in response to conventional antigens (9) or in autoimmune disease models (10–13). However, MHC blockade has been evaluated mostly by its effect on T cell proliferation, and this may not reflect the entire immune response (23). Therefore, we have examined the effect of administering a class II–binding peptide on induction of T cell–dependent antibody responses to protein antigens.

The mouse self-lysozyme peptide ML46-62 binds to I-A^k molecules (5, 9, 16) and it selectively inhibits, when coinjected with antigen, priming of I-A^k–restricted, antigen-specific T cells (9). We have coinjected ML46-62 together with protein antigens, HEL or RNase, in B10.A(4R) mice, expressing only I-A^k as MHC class II molecules. Therefore, in this mouse strain, the T cell–dependent antibody response to protein antigens such as HEL or RNase can only be mediated by I-A^k–restricted T helper cells. In the case of HEL, different immunodominant T cell epitopes generated by HEL processing are presented by I-A^k molecules. Three major immunodominant HEL determinants recognized by I-A^k–restricted T cells are included in the HEL sequences 46-61 (22), 34-45 (22, 24), and 112-129 (25), and a minor one is located in the HEL region 25-43 (25). In the case of RNase, an immunodominant epitope able to induce I-A^k–restricted T cells is located in the region 43-56 (26). Since all these epitopes are potentially able to induce T helper cells, blocking the antigen-presenting function of the I-A^k molecule should result in inhibition of the antiprotein antibody response only if T cell responses to all these determinants are blocked. Results in the present paper demonstrate that this is the case: coinjection of ML46-62 and HEL in B10.A(4R) mice inhibits the entire anti-HEL antibody response. Similarly, coinjection of ML46-62 and RNase, an antigen unrelated to this class II–binding peptide, results in inhibition of the entire anti-RNase antibody response.

Administration of class II–binding peptides could inhibit in vivo T cell activation by antigen (23, 27-32) or MHC-related mechanisms (10, 11, 13, 14). Several lines of evidence indicate that the mechanism leading to inhibition of T cell–dependent antibody responses by administration of ML46-62 is in vivo blockade of MHC class II molecules and a consequent decrease in antigen-specific T cell responses. First, this self-peptide does not activate mouse T cells (9), therefore a direct blockade of T cell receptors by ML46-62 is unlikely. Second, since ML46-62 does not induce a T cell response, the inhibition of antibody production cannot be attributed to clonal dominance induced by the competitor peptide. Third, ML46-62 inhibits efficiently the antibody response induced by RNase, an unrelated protein antigen, indicating that inhibition of the antibody response does not result from induction of T cell tolerance or antigen-specific suppressor T cells. Fourth, injection of ML46-62 selectively inhibits antibody responses induced by T helper cells restricted by I-A^k, as expected from its binding specificity (5, 9, 16), thus excluding a possible induction of nonspecific suppressive mechanisms. Finally, administration of ML46-62 after antigen priming does not inhibit the ongoing antibody response, demonstrating its capacity to prevent T cell activation when coinjected with antigen, but not when injected subsequently, as predicted from the MHC blockade model.

MHC blockade as the mechanism leading to inhibition of antibody responses in vivo is also indicated by the concomitant in vivo inhibition of complexes formed between peptides derived from antigen processing and class II molecules. In addition to the 46-61 epitope (14), the most abundant naturally processed HEL epitope bound to I-A^k molecules (33), HEL34-45–I-A^k complexes formed in vivo are

readily detectable on LN APC. By monitoring complexes of both epitopes, we have demonstrated that administration of ML46-62 selectively inhibits the capacity of I-A^k molecules to present antigen to T cells. Inhibition of antigen presentation is associated with inhibition of I-A^k–restricted T cell proliferation to all immunodominant HEL epitopes tested, demonstrating the functional role of MHC blockade. Thus, inhibition of T cell–dependent antibody responses by administration of ML46-62 correlates with inhibition of antigenic complex formation in vivo and with inhibition of T cell priming, indicating MHC blockade as the mechanism leading to inhibition of antibody responses in vivo. This was previously postulated in a transgenic model where autoantibody production against the neo-self hepatitis B e antigen (HBeAg) was induced by a nontolerogenic T cell epitope of HBeAg and inhibited upon coadministration of an unrelated exogenous peptide (34).

Two types of T helper cells, Th1 and Th2, have been described in the mouse, based on their different lymphokine production pattern (35). Th1 cells, which produce IL-2 and IFN-γ, preferentially induce synthesis of antibodies expressing the IgG2a isotype. Conversely, Th2 cells, producing IL-4, -5, and -10, mainly elicit antibody responses of IgG1 and IgE isotype and cause B cell proliferation and differentiation (19–21, 36). Based on the fact that IgG1 and IgG2a/IgG2b isotypes were inhibited, as well as T cell proliferative responses, it is likely that MHC blockade inhibits activation of Th1- and Th2-type helper cells equally well. Therefore, the inhibition of clonal expansion of Th cells by blocking the initial antigen–TCR interaction seems to affect both types of Th cells, preventing B cell proliferation and differentiation as well as generation of antigen-specific memory cells. This is exemplified by the inhibition of the secondary immune response where the unresponsiveness in B cells to soluble antigen is likely to arise both from lack of antigen-specific T cell help, mainly of Th2-type, and lack of antigen-specific memory B cells able to endocytose soluble antigen and to present efficiently antigenic peptide–MHC complexes to primed T cells (37).

Tolerance induction by antigens administered in nonimmunogenic form can also inhibit T cell activation in vivo. It has been recently shown that antigen-specific unresponsiveness induced by pretreatment with aqueous antigen selectively tolerizes Th1-like but not Th2-like T cells (38, 39). Since human $CD4^+$ T cell clones specific for allergenic and helmintic antigens, exhibit Th2-like lymphokine production profile (40), in these cases tolerance induction may not affect pathogenic T cells. Conversely, as discussed above, MHC blockade can effectively inhibit the helper function of both Th1- and Th2-type cells.

In conclusion, we have demonstrated that administration of MHC class II antagonists can inhibit the induction of T cell–dependent primary and secondary antibody responses. Inhibition of in vivo antibody responses is associated to prevention of complex formation between antigenic peptides and class II molecules indicating MHC blockade as the mechanism hindering T helper cell activation. Therefore, MHC class II antagonists may induce selective immunosuppression in HLA-associated autoimmune diseases (17), including diseases, like myasthenia gravis, where autoantibodies have a direct pathogenic role (41).

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