Brief Definitive Report

Exogenous Peptides Compete for the Presentation of Endogenous Antigens to Major Histocompatibility Complex Class II-restricted T Cells

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Summary

Antigen-presenting cells (APC) transfected with a construct encoding the hen egg-white lysozyme (HEL) amino acid sequence 1–80 constitutively present HEL peptides complexed to major histocompatibility complex (MHC) class II molecules to specific T cell hybridomas, indicating that endogenous cellular antigens can be efficiently presented to class II–restricted T cells. Here we show that exogenous peptide competitors added to HEL-transfected APC can inhibit the presentation of endogenous HEL peptides to class II–restricted T cells. The inhibition is specific for the class II molecule binding the competitor peptide, and it affects to the same extent presentation of exogenous or endogenous HEL peptides. These results, demonstrating that an exogenous competitor can inhibit class II–restricted T cell activation induced by endogenous as well as exogenous antigen, suggest lack of strict compartmentalization between endogenous and exogenous pathways of antigen presentation. Since autoreactive T cells may recognize endogenous, as well as exogenous antigens, the results have implications for the treatment of autoimmune diseases by MHC blockade.

T cells recognize antigen as peptides bound to MHC-encoded molecules on the surface of APC (1). Two classes of MHC molecules are involved in antigen presentation to T cells: class I molecules, expressed on the surface of the majority of nucleated cells (2), and class II molecules, expressed mainly on B cells, macrophages, and dendritic cells (3). Although class I and class II MHC molecules appear to have similar antigen-binding sites (4), they are loaded with peptides at different intracellular locations (5–8) and interact with different T cell populations, $CD8^+$ or $CD4^+$, respectively (9). Two separate pathways of antigen processing and presentation have been proposed, leading to selective association of peptides from endogenous cellular antigens to class I molecules and of peptides from exogenous protein antigens to class II MHC molecules (10). However, peptides derived from endogenous cellular antigens can bind to class II molecules and be presented to T cells (11–14).

Here we demonstrate inhibition by an exogenous MHC-binding competitor of class II–restricted T cell activation induced by endogenously derived antigenic peptides, suggesting lack of strict compartmentalization between the two pathways. These results imply that administration of exogenous class II blockers may also inhibit presentation of endogenous antigens, including those potentially able to activate class II–restricted autoreactive T cells leading to MHC-linked autoimmune diseases.

Materials and Methods

Antigens. The synthesis, purification, and analysis of HEL peptides have been previously described (15).

Cell Cultures. The establishment of class II–restricted, HEL-specific T cell hybridomas has been described (15). Cultures containing 5×10^4 T hybridoma cells and the indicated number of APC were set up in microtiter plates with or without antigen in 0.2 ml of RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 2 mM L-glutamine, 50 μM 2-ME, 50 μg/ml gentamicin, and 10% FCS (Seromed). After 24 h of culture, 50-μl aliquots of supernatant were assayed for the presence of T cell growth factors by [^{3}H]thymidine incorporation in 10^4 CTLL cells.

HEL-transfected APC. The preparation of plasmids, constructs, and transfected cells are described in detail elsewhere (Moreno, J., D. A. A. Vignali, F. Nadimi, S. Fuchs, L. Adorini, and G. J. Hämmerling, manuscript submitted for publication). Briefly, exons 1 and 2 of HEL were ligated to exons 5, 6, 7, and 8 of H-$2K^k$, and the entire hybrid sequence was ligated into the plasmid pHβAPr-1-neo, containing the human β-actin promoter, to obtain the construct pJAM2β-neo. LK-35.2 and A20 cells were transfected with linearized plasmid DNA and selected in medium containing G418. A20 cells transfected with genomic I-Aα^k and I-Aβ^b clones

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plus either pJAM2β-neo (A20.KB-HEL) or pUC19-neo (A20.KB) were selected for I-Aα^{k}β^b expression and cloned by FACS® (Becton Dickinson & Co., Mountain View, CA) at one cell/well. LK-35.2 cells transfected with pJAM2β-neo were cloned by limiting dilution. Clones were screened for their ability to stimulate appropriate class II–restricted, HEL-specific T cell hybridomas in the absence of exogenous HEL.

Results and Discussion

We tested the presentation of an endogenous cellular antigen to class II–restricted T cells by transfecting APC with a construct containing the first two exons of HEL, coding for HEL residues 1–80, linked to the transmembrane and cytoplasmic exons of the K^k gene, all under the β-actin promoter (Moreno et al., manuscript submitted for publication). This construct, (HEL[1-80]-K^k), was transfected in LK-35.2 cells (a B cell hybridoma expressing I-A^{k,d} and I-E^{k,d} molecules) or cotransfected together with genes coding for I-Aα^k β^b molecules in A20 cells (a B cell lymphoma expressing I-A^d and I-E^d molecules). LK-35.2 cells transfected with HEL[1-80]-K^k (LK-HEL) induce, in the absence of added antigen, lymphokine production by HEL-specific, class II–restricted T cell hybridomas, such as 2B6.3 recognizing the HEL peptide 25–43 in association with I-A^k molecules, or 2G7.1 specific for the HEL peptide 1–18 complexed to I-E^k molecules (15), but they fail to activate A.744 cells, a T cell hybridoma recognizing the HEL sequence 46–61 together with I-Aα^{k}β^b hybrid class II molecules (16). Conversely, the same HEL[1–80]-K^k construct cotransfected together with genes coding for I-Aα^{k}β^b molecules in A20 APC (A20.KB-HEL cells) fails to activate T cell hybridomas 2B6.3 and 2G7.1, but it stimulates A.744 cells (Fig. 1). Lack of cross-stimulation of T cell hybridomas by HEL-transfected APC expressing different class II molecules indicates that presentation of endogenous HEL peptides to T cells is MHC class II restricted.

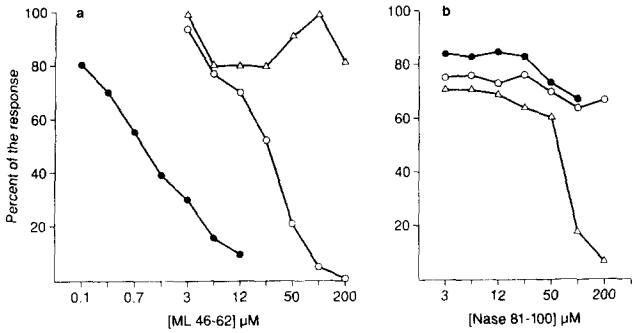

Figure 2. Ability of exogenous peptides to inhibit class II–restricted presentation of endogenous antigen to T cells. LK-HEL cells, 2 × 10^4/well (○, △), or A20.KB-HEL cells, 10^3/well (●), were incubated for 24 h with the indicated concentrations of ML 46-62 (*a*) or Nase 81–100 (*b*), then 5 × 10^4 cells/well of T hybridomas A.744 (●), 2B6.3 (○), or 2G7.1 (△) were added, and the culture was continued for a further 24 h before assessing interleukin production as described in Fig. 1. The control responses in the absence of competitor were, in panels *a* and *b*, respectively: 47,807/103,497 (A.744), 174,586/295,143 (2B6.3), and 162,294/234,866 (2G7.1) cpm. Background incorporation of [^{3}H]thymidine into CTLL cells was 251/690 cpm.

T cell activation depends on the transfected HEL sequence, since the T cell hybridoma 2C8.4, recognizing the HEL sequence 112–129 together with I-A^k molecules (15), is not activated by LK-HEL cells (data not shown).

We then tested the ability of exogenous peptide competitors to inhibit the presentation of endogenous antigen to class II–restricted T cells. The mouse lysozyme (ML) peptide 46–62 binds to I-A^k (17) and to I-Aα^{k}β^b (16), but not to I-E^k molecules (18). Incubation of HEL-transfected, living APC with ML 46–62 inhibits, dose-dependently, the activation of T cell hybridomas 2B6.3, recognizing the HEL peptide 25–43 with I-A^k molecules and A.744, recognizing the

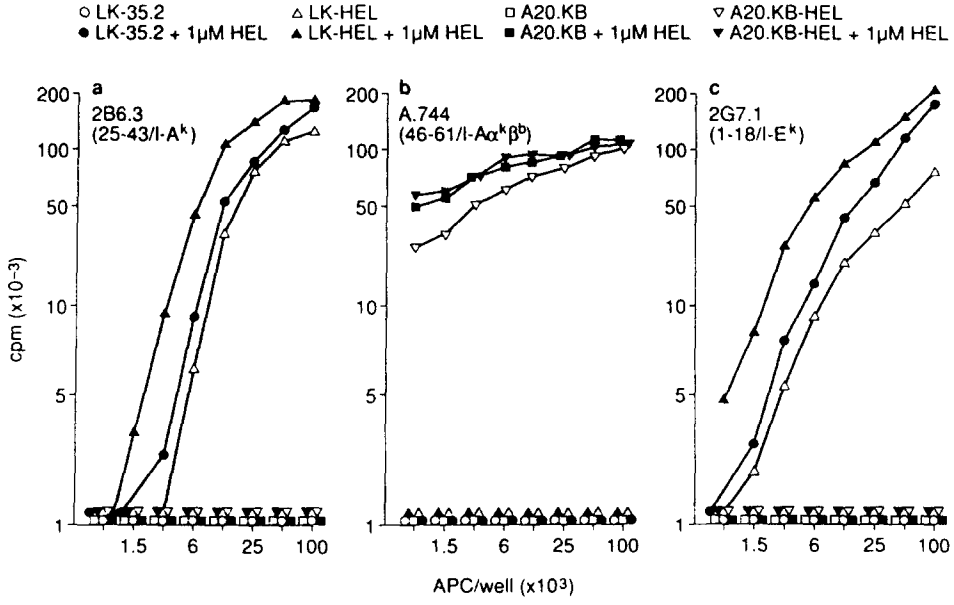

Figure 1. Activation of class II–restricted T cell hybridomas by endogenous antigen. Graded numbers of different APC, LK-35.2 (*circle*), LK-HEL (*triangle*), A20.KB (*square*), and A20.KB-HEL (*inverted triangle*) were cultured with 1 μM HEL (*closed symbols*) or without (*open symbols*), and with 5 × 10^4 cells/well of the indicated T cell hybridomas. After 24 h, antigen-specific T cell growth factor production was determined by ^{3}H-labeled thymidine incorporation into CTLL cells. Results are expressed as arithmetic mean of cpm from triplicate cultures. Background proliferation of CTLL cells was 250 cpm.

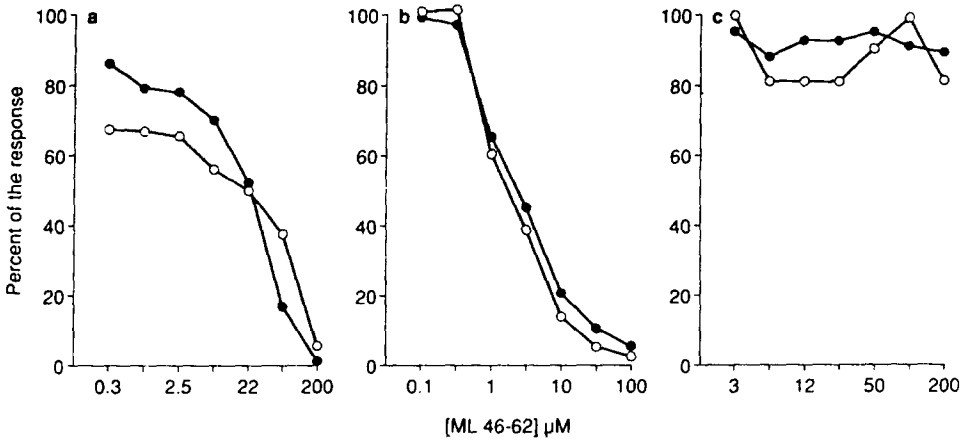

Figure 3. Capacity of the exogenous peptide ML 46–62 to inhibit presentation to I-A^k- and I-$A\alpha^k\beta^b$-restricted T cells of endogenous and exogenous antigenic peptides. The concentration of exogenous HEL peptide required to induce, in the presence of a given number of the appropriate APC, approximately the same degree of T cell activation as HEL-transfected APC was determined in pilot experiments. The indicated concentrations of ML 46–62 were incubated in *a* with 5×10^3 LK-HEL cells (○) or with 5×10^3 LK-35.2 cells and 10 μM HEL peptide 25–43 (●), in *b* with 0.5×10^3 A20.KB-HEL cells (○) or with 0.5×10^3 A20.KB cells and 1 μM peptide HEL 46–61 (●), and in *c* with 2×10^4 LK-HEL cells (○) or with 2×10^4 LK-35.2 cells and 2 μM HEL peptide 1–18 (●). After 24 h of culture, 5×10^4 cells/well of hybridomas 2B6.3 (*a*), A.744 (*b*), and 2G7.1 (*c*) were added, and after a further 24 h of culture, interleukin production was assessed as described in Fig. 1. Control responses in the absence of competitor were: *a*, 101,827 (○) and 177,326 (●); *b*, 24,520 (○) and 19,571 (●); *c*, 162,294 (○) and 301,171 (●) cpm. Background incorporation of [^{3}H]thymidine into CTLL cells was 840 cpm.

HEL peptide 46–61 together with I-$A\alpha^k\beta^b$ molecules. ML 46–62 does not affect recognition of the endogenously derived peptide corresponding to the HEL sequence 1–18 by the I-E^k-restricted T cell hybridoma 2G7.1 (Fig. 2 *a*). These results demonstrate that an exogenous peptide competitor selectively inhibits T cell recognition of endogenous antigen restricted by the class II molecules to which the competitor binds. ML 46–62 inhibits more efficiently the T cell response restricted by I-$A\alpha^k\beta^b$ than that restricted by I-A^k molecules, suggesting that it binds with higher affinity to the former class II molecule. Conversely, incubation of HEL-transfected APC with the I-E^k-binding peptide Nase 81–100 (19) selectively inhibits activation of the I-E^k-restricted T cell hybridoma (Fig. 2 *b*), further strengthening the interpretation that inhibition by exogenous competitors is in fact due to competition between peptides for binding to MHC class II molecules. The competition for the presentation of endogenous HEL peptides exerted by the exogenous competitor ML 46–62 is related to the number of HEL-transfected APC presenting the endogenous antigen. A 10-fold increase of A20.KB-HEL APC increases almost correspondingly interleukin production by the T cell hybridoma A.744. Accordingly, the inhibition induced by the competitor is ~10-fold decreased, consistent with the higher number of class II endogenous HEL peptide complexes present in culture (data not shown).

To assess the ability of an exogenous competitor to inhibit the presentation of endogenous vs. exogenous antigen, we compared the capacity of ML 46–62 to compete for the presentation of endogenous and exogenous HEL peptides. Results in Fig. 3 show that the exogenous competitor ML 46–62 inhibits equally well the activation induced by endogenous or exogenous antigenic peptides of T cell hybridomas 2B6.3 and A.744, whereas it has no effect on the I-E^k-restricted response of hybridoma 2G7.1.

These results indicate that an exogenous competitor can inhibit presentation to T cells by MHC class II molecules of peptide antigens not only from exogenous but also from endogenous origin, suggesting lack of strict compartmentalization between endogenous and exogenous pathways of antigen presentation. It is possible that in the case of endogenous HEL presentation, the competitor either prevents intracellular loading of class II with peptide or that unloading of antigenic peptide and loading of competitor occur. In the case of exogenous antigen, unloading and reloading could take place during endocytosis and recycling of class II molecules (20), or at the cell surface.

The capacity of an exogenous competitor to inhibit in vitro presentation to T cells of endogenous, as well as exogenous, antigens suggests that in vivo MHC blockade based on the administration of exogenous peptide competitors (21) could inhibit presentation to class II–restricted T cells of endogenous cellular antigens, likely the most relevant in the induction of autoreactive T cells leading to HLA-associated autoimmune diseases.

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