Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 3290–3294, April 1991
Immunology

Class II-restricted presentation of an endogenously derived immunodominant T-cell determinant of hen egg lysozyme

(antigen presentation/antigen processing/self-antigen/major histocompatibility complex)

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Communicated by J. F. A. P. Miller, December 17, 1990 (received for review February 15, 1990)

ABSTRACT **An *in vitro* model was used to investigate the potential for different structural forms of endogenous antigen to be processed and presented by major histocompatibility complex class II molecules. For this purpose the class II-restricted presentation of an immunodominant epitope of hen egg lysozyme [HEL-(46–61)] was studied in class II-positive B-lymphoma cells (M12.C3) transfected with genes encoding HEL molecules either (*i*) secreted in high (hi) or low (lo) amounts as soluble antigen [sHEL(hi/lo)], (*ii*) localized within the endoplasmic reticulum (ER)/salvage compartment (ERHEL), or (*iii*) anchored on the cell surface as an integral membrane protein (mHEL). The corresponding sHEL, ERHEL, and mHEL gene products were expressed as predicted except that HEL determinants accumulated in the culture supernatant as well as on the cell membrane of mHEL-transfected cells. Class II-positive cells endogenously expressing all three forms of HEL antigen constitutively presented the immunodominant HEL-(46–61) determinant with differential efficiency (mHEL, sHEL > ERHEL) to a class II-restricted T hybridoma. A second T hybridoma recognized endogenous HEL-(46–61) determinants constitutively presented on sHEL(hi) and mHEL transfectants but not on sHEL(lo) or ERHEL transfectants. The formation of HEL-(46–61)/I-A^k complexes in the ERHEL and sHEL(lo) transfectants was therefore limiting. Mixing experiments with different antigen-presenting cells indicated that the HEL-(46–61) determinant was derived from endogenous antigen rather than by reuptake of shed or secreted HEL determinants. We conclude that MHC class II molecules can present some antigenic determinants derived from endogenous proteins that are sequestered in the ER/salvage compartment as well as distally transported in the form of secretory or membrane antigens.**

Major histocompatibility complex (MHC) class II molecules are the ligands for presentation of processed foreign antigens to T cells (1). Normally, class II-restricted antigens are derived from an exogenous source (2, 3) and the major site of class II loading with processed exogenous antigens is thought to reside in post-Golgi acidified endosomes (4–6). In contrast, endogenously derived peptides appear to assemble in the endoplasmic reticulum (ER) with class I MHC molecules (7–9). Thus, separate pathways of processing and presentation of antigens restricted by class I and class II molecules have been suggested (10–12). Nevertheless, determinants derived from certain endogenous antigens can be presented in association with class II rather than class I molecules (13–24). In most instances this process involves cell surface antigens (13–17, 24), although some viral proteins can use a cytoplasmic pathway of presentation following endosomal processing (18, 20). With possible exceptions (23), these examples may reflect the internalization of cell surface membrane antigens, which allows them to become accessible to endosomal processing, thereby resulting in presentation by class II molecules. Consequently it remains unclear to what extent the loading of class II molecules with endogenously derived nonmembrane antigens is a general phenomenon and whether the form of expression and intracellular localization of endogenous antigens influences their availability for processing and presentation. To evaluate these issues, we examined the "endogenous" presentation of an immunodominant epitope of hen egg lysozyme [HEL-(46–61)] in cells synthesizing HEL either secreted as soluble antigen (sHEL), localized within the ER/salvage compartment (ERHEL) (25), or anchored on the cell surface (membrane HEL, mHEL).

MATERIALS AND METHODS

Gene Constructs. The plasmids encoding sHEL(lo) (SAY1) and ERHEL (SAYMK2) have been described (26). The plasmid encoding sHEL(hi) (pSVG-MT/HEL) (27) was used in some experiments. The carboxyl-terminal sequence encoded within the ERHEL plasmid includes the residues Lys-Asp-Glu-Leu (KDEL), which have been characterized as a retention sequence allowing the retrieval of soluble proteins, including HEL (26), from the salvage compartment into the ER of transfected cells (26, 28, 29). Construction of the mHEL gene is given in the legend to Fig. 1.

Transfection. For transfections the I-A^k α- and β-chain genes and a selectable marker gene (pSV2neo) were first introduced by electroporation into class II-negative, M12.C3 B-lymphoma cells (30). Supertransfection of I-A^k-positive transfectants was also carried out by electroporation using a second selectable marker gene (pSV2gpt) and the constructs encoding ERHEL, sHEL(hi/lo), or mHEL. Sublines and clones of each of the transfectants expressing equivalent levels of I-A^k were generated by cell sorting or limiting dilution.

ELISA and Metabolic Labeling. The HEL content of culture supernatants and cell lysates was assayed by ELISA (27). For metabolic labeling, 10^7 cells were incubated for 30 min in 1.5 ml of methionine-free medium before addition of 200 μCi of [^{35}S]methionine (Amersham; >1000 Ci/mmol; 1 Ci = 37 GBq) and incubation for a further 2 hr (31). Immunoprecipitations used rabbit anti-HEL or anti-β_2-microglobulin antiserum (31) and the radiolabeled proteins were analyzed by SDS/12.5% PAGE and fluorography.

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Abbreviations: APC, antigen-presenting cell; ER, endoplasmic reticulum; HEL, hen egg lysozyme; IL-2, interleukin 2; MHC, major histocompatibility complex.

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Antigen-Presentation Assays. The T hybridomas 3A9 (ref. 32; a gift of P. Allen) and A2.2B2 (ref. 33; a gift of L. Glimcher) are restricted to I-A^k and specific for HEL-(46–61). The creation of this determinant from native exogenous HEL requires a chloroquine-sensitive intracellular processing event (32).

Cocultures of 5×10^4 antigen-presenting cells (APCs) and 10^5 T-hybridoma cells were incubated in 200 μl of medium for 24 hr either without added HEL or in the presence of graded concentrations of HEL (Sigma). Triplicate supernatants were then tested for interleukin 2 (IL-2) activity over a range of dilutions by measuring the proliferation of the IL-2-dependent cell line CTLL. For proliferation assays 0.1 μCi of the thymidine analogue 5-[^{125}I]iodo-2′-deoxyuridine (^{125}IdUrd) was added to 5×10^3 CTLL cells at 18 hr and the cultures were harvested 6 hr later. In antigen-reuptake experiments (Fig. 4*A*), test sources of HEL (cells or supernatants) were distributed in culture plate inserts containing a microporous membrane (Millicell-HA; Millipore) designed to fit inside wells of 24-well culture plates (Costar) with ≈2-mm clearance from the well floor. Reporter APCs on the floors of the wells were then tested for HEL-(46–61) presentation.

RESULTS

Expression of Mutant and Secreted HEL Molecules in Transfected B-Lymphoma Cells. The HEL gene constructs (Fig. 1) encode molecules destined either to be secreted (sHEL), expressed on the cell surface (mHEL), or trapped within the ER/salvage compartment (ERHEL). These constructs were transfected into I-A^k-transfected M12.C3 B-lymphoma cells and the transfectants were analyzed by flow cytometry. Staining for HEL determinants HyHel-5 and HyHel-10 or with a polyclonal rabbit antiserum reactive with native HEL revealed surface expression on cells transfected with the mHEL construct but not on untransfected M12.C3 cells or cells transfected with the ERHEL or sHEL constructs (data not shown). Thus, by serological evaluation the protein folding of mHEL reproduced the native conformation of wild-type HEL.

Transfectants were selected for equivalent levels of I-A^k expression and analyzed for HEL expression by ELISA of both detergent cell lysates and culture supernatants (Fig. 2*A*). Transfected cells expressing all forms of HEL contained cell-associated HEL in nanogram quantities per 10^6 cells. After 24 hr of culture there was no detectable HEL in the culture medium of ERHEL transfectants, whereas mHEL transfectants did release significant amounts of HEL. The intracellular localization of HEL determinants in the ERHEL transfectants was similar to the ER distribution of this molecule described in transfected COS cells (26) when visualized by confocal laser microscopy (data not shown). Two transfected cell lines expressing sHEL/I-A^k were also studied. The first, sHEL(lo)/I-A^k, produced low levels of intracellular (<1 ng per 10^6 cells) and secreted (<10 ng per 10^6 cells in 24 hr) HEL. The second, sHEL(hi)/I-A^k, produced high levels of intracellular (5–10 ng per 10^6 cells) and secreted (50–100 ng per 10^6 cells in 24 hr) HEL.

The apparent molecular sizes of the transfected gene products were as predicted (Fig. 2*B*) except that the cell-associated mHEL molecules migrated electrophoretically as a doublet, perhaps reflecting a noncovalent association of some mHEL molecules with complex carbohydrate ("glycanation"), as described for secretory HEL (34). A smaller form of HEL was detected in the culture supernatant of cells expressing mHEL (Fig. 2*B*), suggesting proteolytic sensitivity of the membrane-bound form of HEL, most likely in the region connecting the HEL molecule to the membrane.

Constitutive Presentation of Endogenously Derived HEL-(46–61) by Transfected Cells. Transfected cells expressing equivalent levels of I-A^k were examined for their ability to stimulate IL-2 production by two sensitive, I-A^k-restricted T hybridomas with previously defined specificity for HEL-(46–61) (Fig. 3). Both T hybridomas (3A9 and A2.2B2) produced maximal amounts of IL-2 following stimulation with I-A^k-positive M12.C3 B-lymphoma cells expressing mHEL in the absence of exogenous HEL (Fig. 3 *A*, *B*, and *H*).

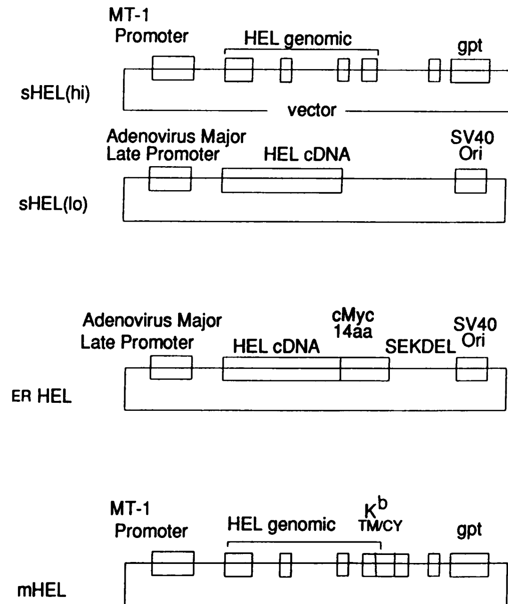

FIG. 1. HEL gene constructs. The sHEL(lo) plasmid encodes a wild-type HEL cDNA [129 amino acids (aa)] transcribed from the adenovirus major late promoter. SV40 Ori, simian virus 40 origin of replication. The ERHEL gene encodes 127 aa of the HEL cDNA followed by a 14-aa reporter sequence containing a linear epitope of c-Myc, and the 6 C-terminal residues SEKDEL. The sHEL(hi) plasmid (pSVG-MT/HEL) encodes the genomic HEL gene transcribed from the mouse metallothionein (MT-1) promoter, which is more active than the adenovirus major late promoter in B-cell transfectants. The mHEL gene comprises the complete HEL coding sequence joined in frame to a portion of the H-2K^b cDNA encoding a part of the connecting peptide as well as the transmembrane (TM) and cytoplasmic (CY) domains of this class I molecule. The hybrid mHEL gene is transcribed from a metallothionein promoter and is linked covalently to the selectable marker gene *gpt*. The predicted mHEL protein contains the complete 129-aa sequence of native HEL followed by the junctional residues ED and a further 86 aa derived from the connecting stalk (21 aa), transmembrane domain (24 aa), and cytoplasmic region (41 aa) of the murine class I molecule H-2K^b.

A similar degree of T-cell activation was observed when cells expressing sHEL(hi)/I-A^k were tested for constitutive expression of HEL-(46–61) (Fig. 3 *C* and *E*). The sHEL(lo)/I-A^k and ERHEL/I-A^k transfectants, however, stimulated only 3A9 and did not stimulate A2.2B2 unless provided with an exogenous source of HEL (Fig. 3 *D* and *G*). The constitutive presentation of the HEL-(46–61) determinant by the sHEL(lo)I-A^k transfectant was saturating for the 3A9 response (Fig. 3*D*), whereas there was no detectable response of A2.2B2 under the same conditions. The most likely explanation for the lack of detectable stimulation of A2.2B2 by sHEL(lo)/I-A^k transfectants was its less sensitive dose–response curve compared with that of 3A9 (Fig. 3*F*) rather than any unusual property of 3A9 *per se*.

Like sHEL(lo)/I-A^k cells, ERHEL/I-A^k transfectants did not stimulate the A2.2B2 T hybridoma unless HEL was added (Fig. 3 *B*, *D*, and *G*). Moreover, cells expressing ERHEL/I-A^k were not saturating in their stimulation of 3A9, as shown by the capacity of exogenous HEL to induce further IL-2 production by this T hybridoma (Fig. 3*G*). However, in preliminary experiments the ability of ERHEL/I-A^k transfectants to stimulate 3A9 constitutively required at least 1–5 ng of intracellular HEL per 10^6 cells (data not shown). Hence,

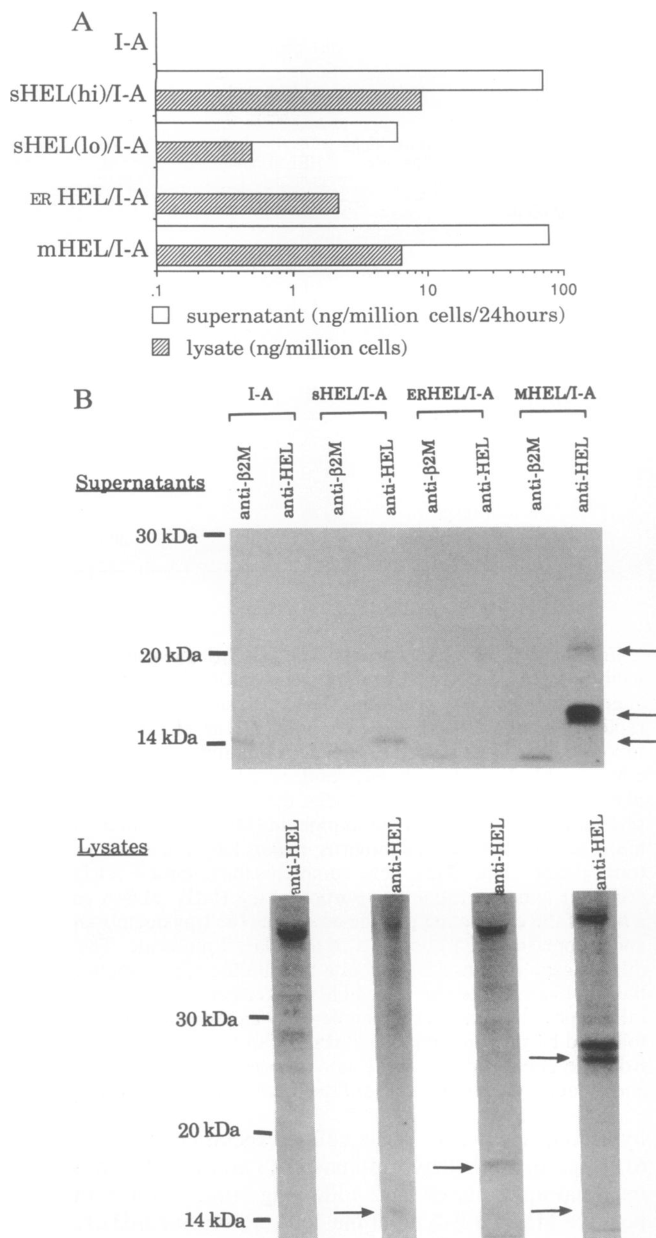

FIG. 2. Expression of mutant and secreted HEL molecules in transfected cells. Transfection of the M12.C3 B-lymphoma cells (30) with the constructs encoding the I-A^k α and β chains and either ERHEL, sHEL(hi/lo), or mHEL was carried out by electroporation (31). (*A*) ELISA of HEL determinants expressed in the detergent cell lysates (ng per 10^6 cells) or culture supernatant (ng per 10^6 cells in 24 hr) of homogeneous populations of cells expressing I-A^k (I-A) alone or in combination with sHEL(hi), sHEL(lo), ERHEL, or mHEL. Cells were cloned by limiting dilution or sorting to homogeneity by flow cytometry prior to analysis. (*B*) Fluorograph of [^{35}S]methionine-labeled proteins immunoprecipitated by anti-HEL or anti-β_2-microglobulin (β2M) from culture supernatants or detergent cell lysates of the indicated transfectants and separated by SDS/PAGE. Bands corresponding to HEL molecules are indicated by arrows. Predicted molecular masses of the HEL proteins are as follows: sHEL, 14.3 kDa; ERHEL, 16.4 kDa; mHEL, 23.5 kDa.

the submaximal response of 3A9 to ERHEL/I-A^k transfectants and the lack of response of A2.2B2 to ERHEL/I-A^k and sHEL(lo)/I-A^k transfectants was presumably due to the low expression of intracellular HEL in these APCs and to the differential sensitivity of the T hybridomas.

The quantitative expression of surface I-A^k (data not shown) and intracellular HEL determinants (0.5–2.5 ng per 10^6 cells, Fig. 2*A*) in the sHEL(lo)/I-A^k transfectants was comparable to that in the ERHEL/I-A^k transfectants, indicating a much greater efficiency of HEL-(46–61) presentation by APCs expressing sHEL(lo) compared with ERHEL/I-A^k. Culture supernatant containing a control form of secreted HEL identical in structure to ERHEL, but with the C-terminal residues KDAS, was processed and presented as HEL-(46–61) determinant to 3A9 (data not shown). Thus, the quantitative difference in presentation of HEL-(46–61) derived from sHEL and ERHEL molecules is unlikely to arise from marked intrinsic differences in processing susceptibility of these molecules.

We previously were unable to detect endogenous presentation of HEL-(46–61) in sHEL/I-A^k-transfected L cells (31) presumably because of the poor class II presentation function of L cells (mouse fibroblasts) and the subthreshold expression of secreted HEL molecules by these cells. In recent experiments we have observed that L cells expressing sufficient levels of ERHEL/I-A^k, sHEL/I-A^k, and mHEL/I-A^k do present endogenously derived HEL-(46–61) to 3A9 (data not shown). These findings confirm the dependence of the T-cell responses on the quantity endogenous antigen and indicate that the results obtained for the M12.C3 transfectants are not a peculiarity of B-cell tumor lines.

Collectively, these data suggest that endogenously derived HEL-(46–61) determinants are more readily available for I-A^k-restricted presentation in the mHEL/I-A^k and sHEL/I-A^k transfectants than in the ERHEL/I-A^k cells.

Presentation of HEL-(46–61) by mHEL/I-A^k and sHEL(lo)/I-A^k Transfectants Does Not Arise from Reuptake of Shed or Secreted HEL. The sHEL/I-A^k and mHEL/I-A^k transfectants accumulated immunoprecipitable HEL determinants in the culture supernatant. Therefore it was possible that the constitutive presentation of HEL-(46–61) was the result of reuptake and processing of shed/secreted HEL antigen. This conclusion was not consistent with the magnitude of the observed responses (Fig. 3 *D*, *E*, and *H*), given the minimum concentration of exogenous HEL required to trigger 3A9 (10–50 ng/ml) and A2.2B2 (0.1–1 μg/ml) using the I-A^k-transfected M12.C3 APCs (Fig. 3 *D*, *F*, and *G*). Moreover, coculture of I-A^k transfectants for 10 days in medium containing HEL at 0.1, 1, or 5 ng/ml did not result in detectable presentation of HEL-(46–61) determinants to 3A9 (data not shown). To address this question more formally, experiments involving mixed APCs were carried out. The sHEL(lo)/I-A^k, ERHEL/I-A^k, or mHEL/I-A^k transfectants were cultured in chambers containing a microporous membrane inserted in the wells of a 24-well cluster tray so that the membrane was just above the floor of the culture well. I-A^k-expressing reporter APCs (M12.C3 cells) were distributed on the floor of the same culture well so that HEL molecules were freely diffusible across the membrane separating the upper and lower chambers of the well. After 72 hr of coculture the insert containing the test source of HEL was removed and the reporter cells were assayed for presentation of HEL-(46–61) (Fig. 4*A*). No presentation of HEL-(46–61) was observed when the ERHEL/I-A^k, sHEL(lo)/I-A^k, or mHEL/I-A^k transfectants were the sole source of HEL in the upper chamber, whereas a control source of purified HEL was readily presented by reporter APCs to 3A9 under the same conditions (Fig. 4*A*).

To verify these findings under conditions of direct coculture of APCs, mixing experiments were performed in which M12.C3 cells expressing I-A^k but negative for mHEL were mixed with cells expressing mHEL but negative for I-A^k (Fig. 4*B*). Under conditions of several cell densities and various culture times (24 and 48 hr) there was no detectable stimulation of either A2.2B2 or 3A9 when 1:1 mixtures of these cell populations were used (Fig. 4*B* and data not shown). In contrast, cocultures of the T hybridomas with the mHEL/I-A^k transfectants were maximally stimulatory.

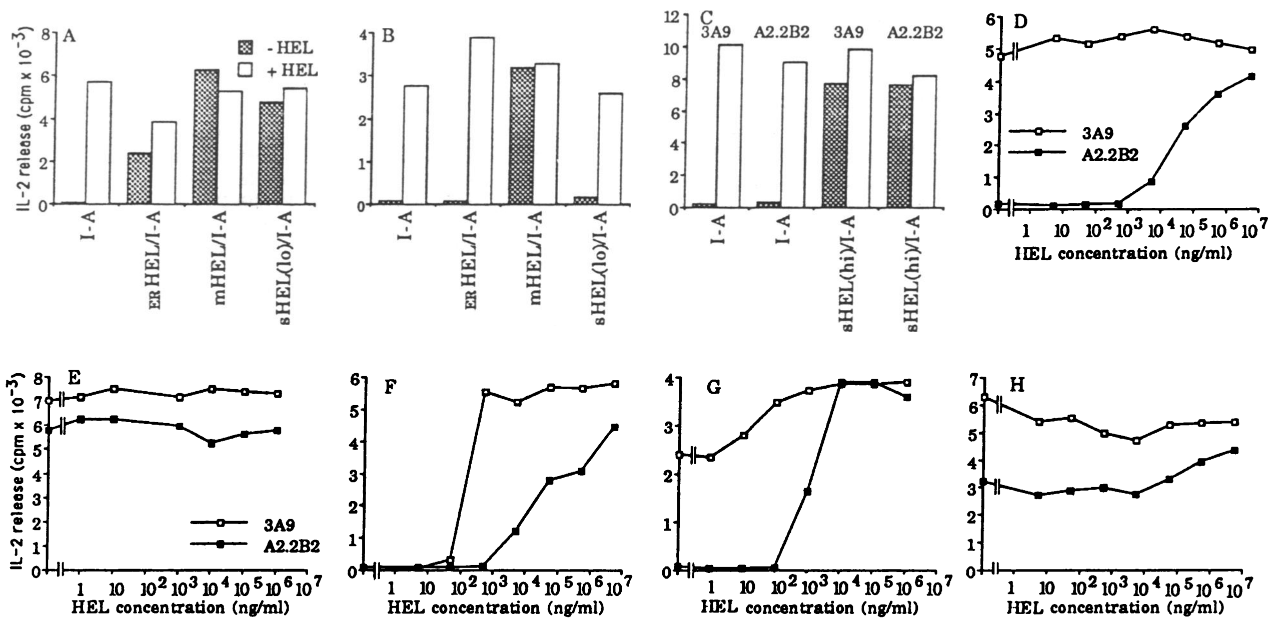

FIG. 3. Constitutive presentation of endogenously derived HEL-(46–61) by transfected cells. (*A* and *B*) M12.C3 B-lymphoma cells expressing I-A^k (I-A) alone or in combination with ERHEL, mHEL, or sHEL(lo) were tested for their ability to induce IL-2 production by the I-A^k-restricted, HEL-(46–61)-specific T hybridomas 3A9 (*A*) and A2.2B2 (*B*) in the presence or absence of added HEL (100 μg/ml). (*C*) IL-2 response of 3A9 and A2.2B2 stimulated by transfectants expressing I-A^k alone or sHEL(hi)/I-A^k in the absence or presence of added HEL. (*D*–*H*) Dose–response pattern of IL-2 production by the T hybridomas 3A9 and A2.2B2 following the addition of graded amounts of additional exogenous HEL to sHEL(lo)/I-A^k (*D*), sHEL(hi)/I-A^k (*E*), I-A^k M12.C3 (*F*), ERHEL/I-A^k-transfected cells (*G*), or mHEL/I-A^k (*H*) transfectants. Prior to these assays, cells were matched for I-A^k expression by flow cytometry. IL-2 activities shown (incorporation of ^{125}IdUrd by CTLL cells) were obtained from a 1 in 4 dilution of coculture supernatant and reflect the results obtained upon further dilution of the supernatant. The cpm obtained using 5-^{125}IdUrd are usually 2–5% of those obtained with [^{3}H]thymidine under the same conditions. Background counts rarely exceeded 100.

DISCUSSION

Recent evidence has suggested that determinants derived from some endogenous as well as exogenous antigens can be processed and presented in association with class II MHC molecules (13–24). The way in which membrane expression and intracellular localization of such endogenous antigens influence their availability for class II-restricted presentation, remains unclear, however. To clarify these issues, we studied the well-defined antigen HEL, since the activation of T cells by soluble HEL determinants such as HEL-(46–61) constitutes a paradigm of class II-restricted antigen presentation involving endosomal uptake and processing of exogenous antigen (2, 5, 32). Using transfectants expressing HEL molecules in secreted, ER-localized, or membrane-bound form, we have shown that T cells can recognize this epitope in a class II-restricted manner when it is presented either endogenously or via the classical endosomal pathway for exogenous antigen. Thus we support the view that the rigid class I/II dichotomy perceived for the MHC-restricted presentation of endogenous versus exogenous antigens is not absolute (18–23).

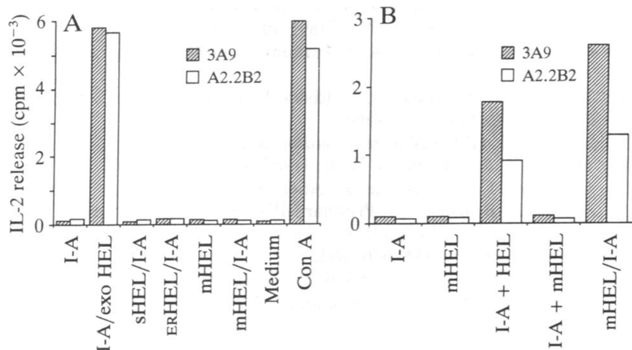

FIG. 4. Presentation of HEL-(46–61) by mHEL/I-A^k and sHEL(lo)/I-A^k transfectants is not due to reuptake of shed or secreted HEL. (*A*) The indicated sources of HEL [cells (10^5) or medium] were cultured in the chamber of a microporous membrane-containing insert separated from 10^5 I-A^k-expressing reporter APCs (M12.C3 cells) distributed on the floor of a 24-well culture plate. After 72 hr of coculture the insert was removed and the reporter cells were harvested in fresh medium and tested for their ability to induce IL-2 production by the T hybridoma 3A9. I-A, I-A^k transfectants without HEL; I-A/exo HEL, I-A^k transfectants with exogenous HEL (100 μg/ml); sHEL(lo)/I-A, sHEL(lo)/I-A^k transfectants; ERHEL/I-A, ERHEL/I-A^k transfectants; mHEL, mHEL transfectants; mHEL/I-A, mHEL/I-A^k transfectants; medium, medium alone; Con A, stimulation by direct culture with concanavalin A (10 μg/ml). (*B*) Combinations of the indicated APCs (5 × 10^4) were directly cocultured for 48 hr with the T hybridomas 3A9 and A2.2B2 and the culture supernatants were tested for IL-2 content as above. I-A, I-A^k transfectants alone; mHEL, mHEL transfectants alone; I-A + HEL, I-A^k transfectants pulsed with exogenous HEL (100 μg/ml); I-A + mHEL, I-A^k transfectants mixed with equal numbers of mHEL transfectants; mHEL/I-A, mHEL/I-A^k double transfectants.

Nevertheless there are some well-defined differences in the preferred pathway for processing and presentation of antigens for class I- and class II-restricted presentation. Class I molecules are believed to associate in the ER with processed antigens derived from the cytoplasm (7–9, 11). Class II molecules, on the other hand, are thought to associate in post-Golgi endosomes with processed antigens derived from the endosomal compartment (2–6, 11). The invariant chain (Ii) associates with newly synthesised class II molecules in the ER and may prevent the binding of antigen with class II molecules until the dissociation of class II/Ii complexes in a post-Golgi compartment (35, 36).

In the current experiments the ability of the ERHEL/I-A^k-transfected cells to present endogenously derived HEL-(46–61) clearly points to the existence of a mechanism for assembly of class II molecules with processed intracellular antigens derived from the ER/salvage compartment. Presentation of ERHEL determinants could arise from the generation and assembly of antigen determinants with class II

molecules in both the ER and the pre-Golgi sorting/salvage compartment, since molecules with the KDEL signal are known to shuttle between these compartments (25, 28, 29). Consistent with this possibility, protein degradation is known to occur in the ER (37) even though creation of endogenous HEL-(46–61) need not always involve proteolysis of HEL molecules (32). Moreover, there is evidence for (23) as well as against (12) the loading of class II molecules with peptide antigen in the ER. On the other hand, if HEL was derived from the cytoplasm or from leakage of small amounts of ERHEL antigen into the Golgi and post-Golgi vacuoles, then processing and presentation of HEL-(46–61) might occur later in cellular transport. In principle it should be possible to experimentally distinguish endosomal from nonendosomal processing of ERHEL by studying the chloroquine and brefeldin A sensitivity of ERHEL processing. However, when such experiments were performed in our system, the results were difficult to interpret because of the multiple treatments of APCs necessary to eliminate constitutive class II/HEL-(46–61) complexes from the cell surface (by antibody or peptide blocking) prior to drug manipulation of APCs. Hence we could not resolve definitively whether the constitutive HEL-(46–61) presentation by the ERHEL transfectants involved loading of class II molecules in the ER or in a distal vacuolar compartment. The relatively inefficient presentation of HEL-(46–61) derived from ERHEL molecules could therefore arise from (*i*) failure to reveal the HEL-(46–61) determinant in the ER, (*ii*) competition for class II assembly between HEL-(46–61) and Ii in the ER (35, 36), or (*iii*) the limited availability of ERHEL antigen leaking from the ER into the endosomal compartment. Nevertheless, irrespective of the site of class II antigen loading, the data do demonstrate that endogenous self-antigens localized within the pre-Golgi compartment are potentially accessible for class II-restricted presentation.

The implication from the finding with ERHEL transfectants is that the mechanisms of constitutive presentation of HEL-(46–61) in the sHEL/I-A^k and mHEL/I-A^k transfectants may also involve class II assembly with HEL determinants created during pre-Golgi vesicular transport as well as during transport distal to the ER/salvage compartment. The greater efficiency of presentation by the mHEL transfectants probably results from the greater level of expression of HEL determinants by these transfectants and the potential for mHEL molecules to undergo endocytosis from the cell surface, thereby making them available for endosomal antigen processing.

Collectively these data indicate that endogenously synthesized secreted, membrane-bound, and intracellular self-antigens sequestered in the ER can be available for presentation by class II molecules. A threshold intracellular concentration of self-antigen appears necessary for endogenous presentation by class II molecules, and this concentration is higher for ER-trapped antigen. This notion has important implications for self-tolerance in the T-cell repertoire and the development of autoimmunity. The preference of class II molecules for presentation of antigens that intersect the distal vacuolar compartment (endosomes) could serve to limit the extent of antigenic competition for class II occupancy by endogenously derived antigens (38, 39). Nevertheless, the ability of some sequestered endogenous antigens to be presented by class II molecules, despite their low abundance, may facilitate T-cell self-tolerance by exploiting the greater ligand-sensitivity of developing T cells compared with mature T cells (40).

We are grateful to P. Allen and L. Glimcher for gifts of T hybridomas, to H. Pelham and P. Munro for plasmids encoding ERHEL and sHEL, to L. Martin and A. Romeo for assistance with flow cytometry, to D. Hewish for assistance with confocal laser microscopy, and to F. R. Carbone, R. L. Boyd, and D. Kanost for critical reading of the manuscript. This work was supported in part by grants from the National Health and Medical Research Council of Australia.

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