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Immunology

Class I major histocompatibility complex-restricted cytolytic T lymphocytes recognize a limited number of sites on the influenza hemagglutinin

(epitopes/immunodominance/recombinant vaccinia virus/peptides)

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ABSTRACT **Two distinct regions of the influenza A/JAP/305/57 hemagglutinin molecule are identifiable as sites recognized by murine class I major histocompatibility complex (MHC) (H-2^d)-restricted cytolytic T lymphocytes (CTL) generated in response to immunization with infectious type A influenza virus. Each of these sites can be mimicked by a synthetic oligopeptide of ≈20 amino acids. Data presented herein indicate that these two sites define the dominant immunogenic epitopes on the hemagglutinin recognized by H-$2K^d$-restricted CTL. These same sites are not efficiently recognized by hemagglutinin-specific class I MHC-restricted CTL of several unrelated MHC haplotypes. These observations show that even for a large complex glycoprotein molecule like the influenza hemagglutinin, only a limited number of class I CTL recognition sites are generated in the infected cell and that the subset of immunogenic epitopes is dependent on the MHC haplotype of the responding individual. These parameters need to be considered in the design of synthetic and recombinant vaccines.**

The influenza hemagglutinin (HA) is the major membrane-associated glycoprotein produced in cells as a result of influenza infection (1, 2). The molecule has served as a prototype for the study of intracellular protein assembly and transport (3). It plays an essential role in the initiation of influenza infection and in the pathogenicity of influenza A viruses (4). The influenza HA is also an important target for immune recognition. Several distinct regions on the mature HA molecule have been identified that are critical recognition sites for neutralizing antibody. These sites have been precisely localized to specific amino acid residues on the three-dimensional structure of HA (5–7). Furthermore, HA is a target antigen for T lymphocytes (8–10) including class I major histocompatibility complex (MHC)-restricted cytolytic T lymphocytes (CTL) (11–13), which have been shown to play a positive role in recovery from influenza infection (14–16).

Recently several lines of evidence have emerged that support the hypothesis that class I MHC-restricted CTL recognize nonnative (processed) forms of influenza HA and other viral antigens expressed on the surface of virally infected cells (17–19). Antigen processing has been shown (20) to be a critical step in the presentation of protein antigens to class II MHC-restricted helper T lymphocytes. The class II MHC-linked genetic control of helper T-lymphocyte responsiveness to protein antigens is believed, in part at least, to reflect the direct interaction of processed antigens with specific class II MHC gene products (21, 22). Accordingly, both the phenomenon of MHC restriction and the apparent immunodominance of certain sites on protein antigens recognized by class II MHC-restricted helper T lymphocytes may be dependent as well on the interaction of processed antigen with class II MHC molecules. In the case of class I MHC-restricted CTL, specific regions on viral (17–19) and cellular polypeptides (23, 24) have been identified as CTL recognition sites, but it is not known whether a limited number or a large number of distinct sites on a given viral antigen are available for recognition by class I MHC-restricted CTL. Here we examine the question of immunodominance of specific antigenic sites on a viral polypeptide in antigen recognition by murine CTL and the influence of MHC haplotype on the immunodominance of antigenic sites.

MATERIALS AND METHODS

Mice. BALB/cByJ (H-2^d) and CBA/J (H-2^k) mice were purchased from The Jackson Laboratory.

Viruses. Influenza virus strains A/JAP/305/57 (H2N2 subtype) and B/LEE were grown in the allantoic cavity of 10-day-old chicken embryos. Infectious allantoic fluid was harvested 2 days later, and aliquots were stored at −70°C (25). Vaccinia viruses were grown on CV-1 monkey kidney cell monolayers (American Type Culture Collection) infected with a multiplicity of infection of 0.01 and incubated at 37°C. On day 2, the CV-1 cells were pelleted, resuspended in a small volume, and subjected to three cycles of freezing/thawing to release the virus particles from the cells. Preparations were plaqued on CV-1 monolayers to obtain titers. The vaccinia virus recombinant carrying the A/JAP/305/57 HA gene, designated VV(HA) (26), was obtained from B. Moss (National Institutes of Health, Bethesda, MD). The vaccinia virus recombinants encoding the internal HA deletion mutants [designated VV(HAΔXba) and VV(HAΔRI)] were generated in our laboratory by insertion at the vaccinia virus thymidine kinase (*tk*) locus using standard techniques (27).

Cell Lines. The P815 (H-2^d) mastocytoma cell line was maintained in Dulbecco's modified Eagle's (DME) medium (GIBCO) and supplemented with 10% (vol/vol) fetal bovine serum and 1% (wt/vol) glutamine. The LK35.2 (H-$2^{k \times d}$) cell line is an A20.2J × B10.BR B-cell hybridoma obtained from P. Marrack (The National Jewish Center for Immunology and Respiratory Medicine, Denver) and maintained in DME medium supplemented with 10% fetal bovine serum, 1% glutamine, 1% nonessential amino acids, sodium pyruvate, and 50 μM 2-mercaptoethanol. The A20-1.11 (H-2^d) Ia^+ B-cell lymphoma was passaged as described for LK35.2 cells.

Peptides. Synthetic oligopeptides corresponding to residues 126–145, 202–221, 299–321, 316–340, and 523–545 of the A/JAP/305/57 HA molecule were produced on an automated

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Abbreviations: MHC, major histocompatibility complex; CTL, cytolytic T lymphocytes; HA, hemagglutinin.

solid-phase peptide synthesizer from Applied Biosystems (Foster City, CA).

Plasmids. Plasmid pSC11 (28), which contains the β-galactosidase gene linked to the vaccinia virus early promoter P11K and to the P7.5K promoter in opposite orientations was obtained from B. Moss. Plasmid pJHB16 (29), which contains the wild-type A/JAP/305/57 HA gene, was obtained from M.-J. Gething (The Howard Hughes Medical Institute, University of Texas Health Science Center, Dallas).

T-Cell Clones. The cloned T lymphocytes used in this assay were derived from BALB/c or BALB/c × C57BL/6 F_1 ($CB6F_1$) mice. The procedures developed to establish and maintain them and an analysis of their viral specificity have been described in detail elsewhere (30, 31). Briefly, the clones were passaged weekly in the presence of A/JAP/305/57-infected irradiated splenocytes and a source of exogenous T-cell growth factor.

Bulk CTL Populations. Mice were inoculated by intravenous tail-vein injection with ≈5 × 10^7 infectious doses of A/JAP/305/57 virus. Three or more weeks later, spleens from groups of 2 or 3 mice were removed and restimulated *in vitro* with infectious A/JAP/305/57 virus in the form of virally infected irradiated syngeneic splenocytes (26, 27). After 5–7 days of culture at 37°C, activated effector cells were tested for *in vitro* cytotoxicity.

Assay for Cell-Mediated Cytotoxicity. P815 or LK35.2 cells were used as targets in standard ^{51}Cr-release assays as described (25). Cytotoxicity assays were carried out for 6 hr, and the resultant data were analyzed as described in detail (25). For assays in which synthetic peptides were utilized, the peptides were diluted in assay medium to the appropriate concentrations and added to the clones and target cells in the 96-well microtiter plates.

Generation of HA Gene Deletion Constructs. Plasmid pJHB16 contains the wild-type A/JAP/305/57 HA gene on a 1.9-kilobase (kb) *Hin*dIII/*Bam*HI fragment. The HA gene contains two *Eco*RI and two *Xba* I restriction sites (see Fig. 1*B*), which yield in-frame deletions. To generate the HA *Eco*RI deletion mutant (HAΔRI), the 1.9-kb *Hin*dIII/*Bam*HI fragment of pJHB16 was introduced into the *Hin*cII site of pUC13, which lacked an *Eco*RI site in its polylinker. The resulting plasmid was digested with *Eco*RI, liberating a small 500-base-pair (bp) DNA fragment (corresponding to nucleotides 764–1264 of the HA gene) and religating the isolated large fragment. The resulting 1.4-kb *Hin*dIII/*Bam*HI insert encoding the *Eco*RI deletion mutant was cloned into the *Sma* I site of plasmid pSC11. The HAΔRI construction encodes a mutant protein consisting of 398 amino acids and lacking amino acids 248–409 of the primary HA translation product. The HA *Xba* I deletion mutant (HAΔXba) was generated by digesting the original pJHB16 plasmid with *Xba* I to liberate the internal *Xba* I fragment corresponding to nucleotides 145–1327 of the HA gene and religating the isolated large fragment. An 800-bp *Hin*dIII/*Bam*HI insert encoding the HAΔXba deletion mutant was isolated and inserted into the *Sma* I site of pSC11. The HAΔXba construction encodes a mutant protein consisting of 166 amino acids and lacking HA residues 35–428.

RESULTS AND DISCUSSION

Recognition of HA Sites by Cloned Class I MHC-Restricted CTL. We have analyzed CTL recognition of influenza HA in mice of the *H-2^d* MHC haplotype, using cloned CTL populations directed to the prototype HA derived from the A/JAP/305/57 (H2N2 subtype) virus. As part of our analysis of the fine specificity of CTL recognition, we have identified two sites on the A/JAP/305/57 HA molecule that are recognized by *H-2K^d*-restricted CTL. One site is located in the hydrophobic transmembrane anchor domain of HA and can be mimicked by a short synthetic peptide corresponding to residues 523–545 of the HA protein (Fig. 1*A*). This site was

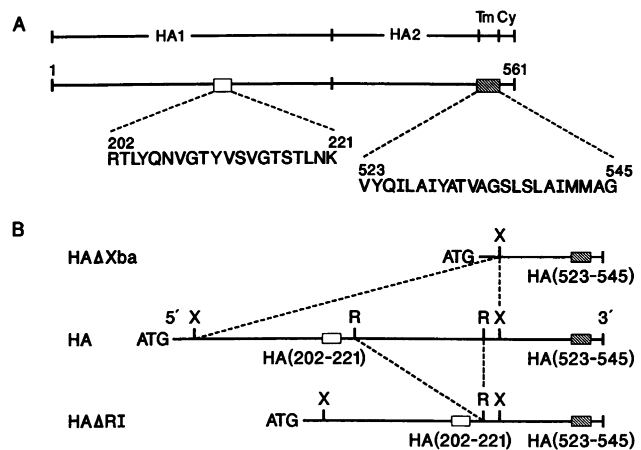

FIG. 1. Structure of the influenza gene for HA and HA gene deletion mutant constructions. (*A*) Sites on the A/JAP/305/57 HA recognized by *H-2K^d*-restricted murine CTL (32, 33). Numbers refer to the derived amino acid sequence of the primary HA translation product. (*B*) Strategy for the construction of HA deletion mutants utilizing restriction sites yielding in-frame deletions. Restriction enzyme sites: R, *Eco*RI; X, *Xba* I.

originally identified by the failure of several HA-specific class I CTL clones to recognize target cells expressing a truncated HA gene lacking the portion of the gene encoding the transmembrane anchor domain of the HA (32). The second site is located in the HA1 portion of the molecule and can be mimicked by a synthetic peptide encompassing residues 202–221 of HA (Fig. 1*A*). In the studies defining these two sites, we used two *H-2K^d*-restricted CTL clones with different fine specificity of target cell recognition, which were representative of the two major fine specificities of HA-specific CTL (25, 31). Clone 14-7, which is subtype specific and recognizes the HA of H2N2 subtype influenza virus strains with comparable efficiency, is directed to the HA-(523–545) site within the highly conserved anchor region of the HA molecule. The other clone 11-1, which has a reactivity for the HA of the A/JAP/305/57 strain (25, 31), recognizes the N-terminal HA1 site, HA-(202–221) (33). Since these two clones reflect the major antigenic fine specificities of HA-specific CTL in the *H-2^d* haplotype, we wanted to know if any other class I CTL clones directed to HA recognized one or the other of these sites. Therefore, we screened a large panel of HA-specific *H-2^d*-restricted class I CTL clones for their ability to lyse target cells treated with either the HA-(523–545) peptide or the HA-(202–221) peptide. We found that all of the clones mapped to either the N-terminal HA1 site or the C-terminal anchor site with the majority of clones directed to the site located in the N-terminal HA1 site (Table 1, experiments 1 and 2). No class I CTL clone recognized target cells treated with another HA peptide, encompassing residues 126–145 of the A/JAP/305/57 HA, which defines a site recognized by human class II MHC-restricted T lymphocytes (T.J.B. and L. Brown, unpublished observations) (Table 1, experiment 2). Also, an *H-2^d*-restricted class I CTL clone, 14-13, which is directed to the A/JAP/305/57 nucleocapsid protein (25, 31, 34, 35), failed to recognize target cells treated with either of these antigenic peptides. More than 30 *H-2^d*-restricted HA-specific CTL of differing fine specificity were screened, and all could be mapped to the HA1 site or the anchor site.

Limited Site Recognition by Heterogeneous CTL Populations. Reactivity of a large panel of independently isolated CTL clones with these two HA sites suggested to us that these two sites are important sites for *H-2K^d*-restricted class I CTL directed to the influenza HA. If so, then these sites likewise should be recognized by heterogeneous populations of HA-specific CTL generated in responses to infectious

Table 1. Recognition of synthetic HA peptides and mutant HA gene products by class I MHC-restricted CTL clones directed to the A/JAP/305/57 HA

	% Specific ^{51}Cr release from target cells								
	Exp. 1			Exp. 2			Exp. 3		
CTL clone	Un-infected	A/JAP/305/57 virus	HA-(523–545) peptide	A/JAP/305/57 virus	HA-(202–221) peptide	HA-(126–145) peptide	VV(HA)	VV(HAΔRI)	VV(HAΔXba)
A-4	2	32	30	70	1	2	63	69	64
C-1	6	67	68	80	7	9	66	69	65
14-7	3	46	49	80	1	2	58	62	61
E-2	4	57	4	76	78	5	—	—	—
40-1	9	59	9	82	72	11	—	—	—
40-2	1	54	1	75	68	6	28	36	1
40-4	2	56	2	76	63	14	—	—	—
11-1	4	57	3	85	85	2	65	68	0
17-2	4	55	1	75	80	1	—	—	—
17-4	2	48	1	48	79	1	—	—	—
14-1	4	62	6	85	87	4	56	60	1
14-13	15	61	13	77	2	2	0	1	1

CTL-to-target cell ratios are 5:1 in 6-hr ^{51}Cr-release assays. Target cells are either A20-1.11 (experiment 1) or P815 cells (experiments 2 and 3) treated with the indicated peptide or infected with recombinant vaccinia viruses expressing the HAΔRI or HAΔXba mutant HA genes (Fig. 1*B*). Peptide concentrations ranged from 400 to 600 nM. Positive control targets are infected with either the A/JAP/305/57 virus or a recombinant vaccinia vector expressing the full-length HA [VV(HA)]. Construction of recombinant vaccinia vectors expressing the HAΔRI mutant gene [VV(HAΔRI)] and the HAΔXba mutant gene [VV(HAΔXba)] is as described.

influenza virus. Target cells treated with the HA-(202–221) peptide were efficiently recognized by a heterogeneous population of BALB/c (*H-2^d*)-restricted CTL stimulated in response to infectious A/JAP/305/57 virus in short-term *in vitro* culture (Fig. 2). This heterogeneous CTL population also recognized target cells treated with the HA-(523–545) peptide with similar efficiency, but these anti-viral CTL failed to recognize target cells treated with 20-mer amino acid peptides from two other regions of the A/JAP/305/57 HA corresponding to residues 299–321 and 316–340 (Fig. 2).

In contrast to the results in the *H-2^d* haplotype, influenza-specific CTL from CBA/J (*H-2^k* haplotype) mice did not recognize histocompatible target cells treated with either the HA-(523–545) or the HA-(202–221) peptide. In this mouse strain, the influenza HA is an important class I CTL target antigen (11–13) (accounting for ≈30% of the total CTL response in our hands), and target cells expressing only the influenza HA gene product are efficiently lysed by heterogeneous influenza-specific CTL populations from CBA/J mice

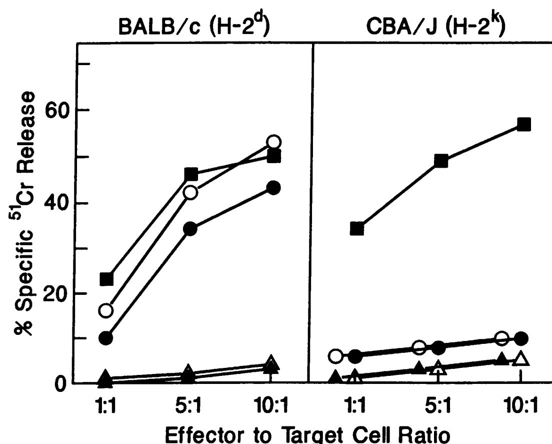

FIG. 2. Recognition of HA peptides in ^{51}Cr-release cytotoxicity assay by BALB/c and CBA/J bulk populations. Target cells were ^{51}Cr-labeled LK-35.2 (*H-2$^{k \times d}$*) cells infected with A/JAP/305/57 virus (■) at a multiplicity of infection of ≈50 infectious doses per cell as described (25, 31) or LK-35.2 cells maintained in the presence (at 10 μg/ml) of synthetic peptide corresponding to the HA-(202–221) site (○), to the HA-(523–545) site (●), or to two unrelated regions, HA-(299–321) (△) and HA-(316–340) (▲).

(see Fig. 4). Similarly heterogeneous populations of A/JAP/305/57-specific CTL from C57BL/6 (*H-2^b*) mice also fail to recognize histocompatible EL4 (*H-2^b*) target cells treated with the HA-(202–221) or the HA-(523–545) peptides (not shown).

The efficient recognition of the HA-(202–221) and HA-(523–545) sites by both clonal and unselected heterogeneous populations of *H-2^d* haplotype-restricted class I T lymphocytes suggested to us that these two sites on the HA molecule are immunodominant sites recognized by *H-2^d*-restricted class I CTL. To further test the contribution of the HA-(202–221) and HA-(523–545) sites to the overall response to the HA molecule, we examined the capacity of unlabeled target cells treated with either the HA-(202–221) peptide or the HA-(523–545) peptide or with a mixture of both peptides, to compete with radiolabeled target cells for recognition and lysis of radiolabeled target cells by HA-specific class I CTL. For this analysis we used, as a source of HA-specific CTL, heterogeneous populations of cytotoxic effectors generated in response to immunization with infectious A/JAP/305/57 virus because infectious virus should stimulate the full range of HA-specific CTL. However, the CTL response to infectious influenza virus is heterogeneous and there are, in addition to HA-specific CTL, CTL directed to the nucleocapsid protein and other influenza viral gene products (34, 35). Therefore, it was necessary to limit the analysis to the CTL subpopulation directed to the HA. To do so we used radiolabeled target cells expressing only the influenza HA gene product as a result of infection with a recombinant vaccinia virus vector containing the influenza A/JAP/305/57 HA gene (26) rather than influenza virus-infected target cells that would display all potential viral target antigens. P815 (*H-2^d*) target cells treated with either the HA-(523–545) or HA-(202–221) peptides were able to partially inhibit lysis of HA-expressing labeled target cells (Fig. 3). In contrast, competitor cells treated with a mixture of the two peptides completely inhibited lysis of labeled target cells by heterogeneous populations of class I CTL and did so even more efficiently than did competitor cells expressing the full-length HA as a result of influenza infection. Complete inhibition of lysis by competitor cells treated with the mixture of peptides is the expected outcome if all of the HA-specific CTL generated in response to infectious influenza virus were restricted to the sites defined by these two peptides.

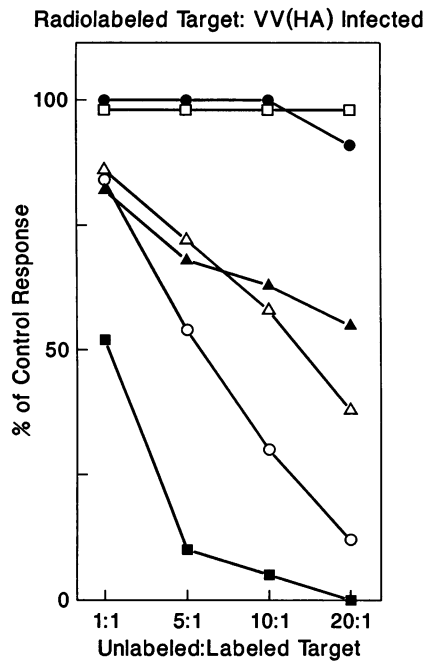

FIG. 3. Inhibition of CTL-mediated lysis of HA-expressing radiolabeled target cells by peptide-treated unlabeled competitor cells. P815 (H-2^d) target cells were ^{51}Cr-labeled and infected with a recombinant vaccinia virus vector expressing the gene for the full-length influenza A/JAP/305/57 HA [VV(HA)]. These radiolabeled cells were mixed with unlabeled P815 competitor cells infected with A/JAP/305/57 virus (○) or influenza B/LEE (●) or treated with a 10 μg/ml solution of either the HA-(126–145) peptide (□) or the HA-(202–221) peptide (△), the HA-(523–545) peptide (▲), or a mixture of the HA-(202–221) and HA-(523–545) peptides, each at a final concentration of 10 μg/ml (■). Peptide-treated unlabeled competitor cells were incubated with peptide solutions at 37°C for 4 hr and then washed free of unbound peptide before addition of competitor cells to the assays. For these assays 1×10^4 VV(HA)-infected ^{51}Cr-labeled P815 targets were cultured with 5×10^4 A/JAP/305/57-immune BALB/c CTL effector cells for 6 hr at 37°C in the presence of increasing ratios of unlabeled peptide-treated or infected competitor. Control lysis of VV(HA)-infected ^{51}Cr-labeled targets by the CTL effector cells in the absence of inhibitor cells was 67.5%.

Recognition of HA Gene Deletion Mutants by Class I CTL. The results with both CTL clones and heterogeneous populations of CTL indicated that the sites on the HA defined by these two synthetic peptides represent the primary regions of the A/JAP/305/57 HA recognized by H-2^d-restricted class I CTL. Therefore, we wanted to know if the generation of these two sites *de novo* in an infected cell requires expression of the full-length HA gene product and concomitantly whether the formation of one site in the infected cell is dependent on the presence of the other site. To examine these questions, we expressed in P815 target cells two deletion mutants of the A/JAP/305/57 HA using recombinant vaccinia virus expression vectors. One of these mutants (HAΔRI) was generated by deletion of a 0.5-kb *Eco*RI fragment from the HA gene (Fig. 1*B*). The resultant in-frame deletion mutant retains both the HA-(202–221) and the HA-(523–545) sites but lacks the coding sequence for 167 amino acids from residues 227 to 394 (*Eco*RI segment) of HA. P815 target cells expressing this HA deletion mutant are efficiently recognized by both HA-(202–221)- and HA-(523–545)-specific CTL clones (Table 1, experiment 3). The second mutant (HAΔXba) was generated by an in-frame deletion of a 1.1-kb *Xba* I fragment that spans the HA coding sequence from residues 20 thru 413, including the N-terminal HA-(202–221) site (Fig. 1*B*). When expressed in histocompatible P815 target cells by using a recombinant vaccinia expression vector, the HAΔXba mutant, which retains the HA-(523–545) anchor site, is efficiently recognized by anchor-specific CTL clones but not by CTL clones directed to the deleted HA-(202–221) site (Table 1, experiment 3). These results indicate that the full-length HA gene product need not be expressed in the target cell for generation of the antigenic sites recognized by HA-specific CTL and that these antigenic sites can be expressed independently.

As a follow up to the analyses of recognition of the HAΔRI and HAΔXba mutants by long-term cloned CTL, we tested whether these deletion forms of the HA molecule are efficiently recognized by heterogeneous populations of HA-specific CTL generated in response to infectious influenza virus. Both the HAΔRI and HAΔXba were efficiently recognized by heterogeneous populations of H-2^d-restricted CTL from BALB/c mice when expressed in histocompatible target cells by using recombinant vaccinia virus vectors (Fig. 4). The lower efficiency of lysis of target cells expressing the HAΔXba mutant gene compared with targets expressing the HAΔRI mutant gene or the full-length HA gene likely reflects the absence of the HA-(202–221) site from this construction with the concomitant loss of recognition of the HAΔXba targets by the HA-(202–221)-specific CTL subset within the heterogeneous CTL populations.

In contrast to the results with CTL of BALB/c (H-2^d) origin, CTL from H-2^k haplotype recognize histocompatible target cells expressing the full-length HA (Fig. 4) but fail to recognize histocompatible target cells expressing either the HAΔXba or the HAΔRI deletion mutants. The lack of recognition of the HAΔRI construction by H-2^k haplotype-restricted CTL supports the view that H-2^k haplotype-restricted CTL recognize regions of the HA molecule other than the HA-(202–221) and HA-(523–545) sites and implicates a site (or sites) within the deleted *Eco*RI segment as the region of the A/JAP/305/57 HA recognized by H-2^k haplotype-restricted CTL. In this connection it should be noted that a single hybrid cell line that expresses both H-2^d and H-2^k class I MHC products, LK-35.2, was used as the target cell for the analysis of both BALB/c and CBA/J recognition of the HA deletion mutants. Therefore, the failure of CBA/J CTL to lyse cells expressing the HAΔRI or the HAΔXba deletion mutant genes is not due to lack of expression of the mutant

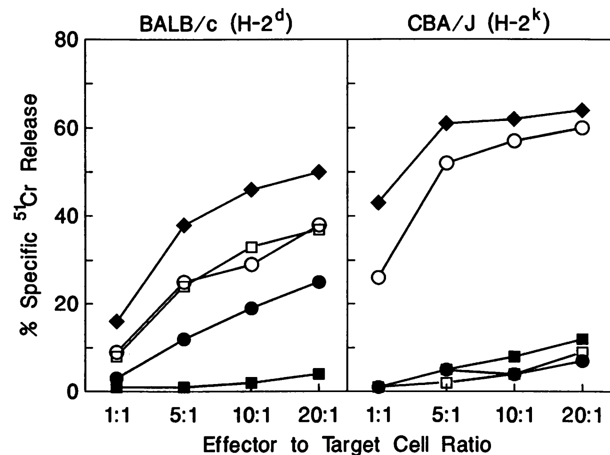

FIG. 4. Recognition of HA deletion mutants by bulk CTL populations. LK-35.2 (H-$2^{k \times d}$) cells were ^{51}Cr-labeled and infected with A/JAP/305/57 virus (◆), B/LEE virus (■), or recombinant vaccinia virus vectors expressing the full-length A/JAP/305/57 HA [VV(HA)] (○), the HA *Eco*RI deletion mutant (VVHAΔRI) (□), or the HA *Xba* I deletion mutant (VVHAΔXba) (●). CTL effector cells were generated by *in vitro* restimulation of A/JAP/305/57 virus immune CTL precursor cells from A/JAP/305/57 immune BALB/c (H-2^d) (*Left*) and CBA/J (H-2^k) (*Right*) mice.

HA genes in the target cells expressing the $H\text{-}2^k$ haplotype class I MHC products.

Our data indicates that only a limited number of sites on the influenza HA glycoprotein are immunodominant for recognition by class I MHC-restricted CTL and that the location of these sites is dependent upon the haplotype of the class I MHC molecule seen in association with the HA antigenic epitope. That a nonnative (processed) form of HA is recognized by class I CTL is evidenced by several findings. First, the location of one of the antigenic sites at positions 523–545 within the hydrophobic transmembrane anchor domain of HA is incompatible with recognition of this site on the HA molecule in its native form. Also, the finding that the product of a mutant HA gene—i.e., HAΔXbaI deletion gene, which encodes a polypeptide containing less than one-third of the amino acids present in the native glycoprotein—is still efficiently recognized by class I CTL argues against presentation of a native HA structure and favors recognition of a processed, possibly fragmented form of HA.

At present the basis for the dominance of two stretches of ≈20 amino acids at the N- and C-terminal portions of the 561-amino acid primary A/JAP/305/57 translation product is not yet clear. One attractive explanation for both the immunodominance of certain sites and the haplotype preference in site recognition by CTL is that class I MHC products of different haplotypes directly bind and present to CTL processed forms of HA in a haplotype-dependent fashion. Such direct interactions between processed antigens and MHC molecules have been directly quantitated for class II MHC molecules and synthetic peptides (21, 22). The recent crystallographic data on the structure of a human class I MHC molecule are also consistent with the view that class I MHC molecules bind and present processed antigen to the antigen receptor on T lymphocytes (36). Homology to some amino acid sequence motifs proposed to be characteristic of T-lymphocyte recognition sites on protein antigens was detected in the two immunodominant $H\text{-}2^d$ haplotype sites (37, 38). This panel of clones with differing fine specificity of influenza HA recognition should be analyzed for the purpose of distinguishing which residues participate in MHC binding versus antigen receptor recognition (39).

Results previously reported by Taylor *et al.* (40) for the influenza nucleoprotein similarly suggest that T-cell epitopes for a given haplotype are dictated in part by the class I MHC interaction with the specific determinant. Our results extend such observations to the HA molecule, which elicits both neutralizing antibodies and cell-mediated immune responses. Epitopes located either at a serologically polymorphic region [HA-(202–221)] or a conserved region [HA-(523–545)] of the HA molecule, while immunodominant for $H\text{-}2K^d$, are not immunogenic areas in a different haplotype. A similar inference can be drawn from recent work of Oldstone and co-workers (41, 42), who have examined the response of class I MHC-restricted CTL to lymphocytic choriomeningitis virus polypeptides. One implication of these results is that, if there are similarities (motifs) shared among the various T-cell epitopes that would relate to MHC interaction, then these motifs should be distinct for individual MHC molecules.

Finally, our results raise important issues concerning strategies for the design of vaccines utilizing glycoprotein antigens like the influenza HA, which are major neutralizing antigens. Our results suggest that not only may there be a limited number of antigenic sites on such molecules recognized by class I CTL but also the location of these sites will be strongly influenced by the MHC type of the individual responding to the vaccine. In addition, our data imply that the sites recognized by antibody and class I CTL can be distinctly different. These issues must be taken into account in the design of synthetic vaccines for influenza, human immunodeficiency virus, and other important human pathogens.

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1. Lazarowitz, S. G., Compans, R. W. & Choppin, P. W. (1971) *Virology* **46**, 830.
2. Skehel, J. J. (1972) *Virology* **49**, 23.
3. Gething, M.-J., McCammon, K. & Sambrook, J. (1986) *Cell* **46**, 939–950.
4. Webster, R. G. & Rott, R. (1987) *Cell* **50**, 665–666.
5. Wiley, D. C., Wilson, I. A. & Skehel, J. J. (1981) *Nature (London)* **289**, 373–381.
6. Webster, R. G. & Laver, W. G. (1980) *Virology* **104**, 139–156.
7. Caton, A. J., Brownlee, G. G., Yewdell, J. W. & Gerhard, W. (1982) *Cell* **31**, 417–431.
8. Lamb, J. R., Eckels, D. D., Lake, P., Woody, J. N. & Green, N. (1982) *Nature (London)* **300**, 66–68.
9. Hurwitz, J. L., Heber-Katz, E., Hackett, C. J. & Gerhard, W. (1984) *J. Immunol.* **133**, 3371–3375.
10. Anders, E. M., Katz, J. M., Jackson, D. C. & White, D. O. (1984) *J. Immunol.* **127**, 669–672.
11. Braciale, T. J., Braciale, V. L., Henkel, T. J., Sambrook, J. & Gething, M.-J. (1984) *J. Exp. Med.* **159**, 341–354.
12. Townsend, A. R. M., McMichael, A. J., Carter, N. P., Huddlestone, J. A. & Brownlee, G. G. (1984) *Cell* **39**, 13–25.
13. Bennink, J. R., Yewdell, J. W., Smith, G. L., Moller, C. & Moss, B. (1984) *Nature (London)* **311**, 578–580.
14. Yap, K. L., Ada, G. L. & McKenzie, I. F. C. (1978) *Nature (London)* **273**, 238–240.
15. Lin, Y. L. & Askonas, B. L. (1981) *J. Exp. Med.* **154**, 223.
16. Lukacher, A. E., Braciale, V. L. & Braciale, T. J. (1984) *J. Exp. Med.* **160**, 814–822.
17. Wabuke-Bunoti, M., Fan, D. P. & Braciale, T. J. (1981) *J. Immunol.* **127**, 1122–1125.
18. Townsend, A. R. M., Rothbard, J., Gotch, F. M., Bahadur, G., Wraith, D. & McMichael, A. J. (1986) *Cell* **44**, 959–968.
19. Gotch, F. M., Rothbard, J., Howland, K., Townsend, A. & McMichael, A. (1987) *Nature (London)* **326**, 881–882.
20. Unanue, E. R. (1984) *Annu. Rev. Immunol.* **2**, 395–428.
21. Babbit, B. P., Allen, P. M., Matsueda, G., Haber, E. & Unanue, E. R. (1985) *Nature (London)* **317**, 359–361.
22. Buus, S., Sette, A., Colon, S. M., Miles, C. & Grey, H. M. (1987) *Science* **235**, 1353–1357.
23. Maryanski, J. L., Pala, P., Corradin, G., Jordan, B. R. & Cerrotini, J. C. (1986) *Nature (London)* **324**, 578–579.
24. Clayberger, C., Parham, P., Rothbard, J., Ludwig, D. S., Schoolnik, G. K. & Krensky, A. M. (1988) *Nature (London)* **330**, 763–765.
25. Braciale, T. J. (1977) *Cell Immunol.* **33**, 423–429.
26. Smith, G. L., Murphy, B. R. & Moss, B. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7155–7159.
27. Mackett, M., Smith, G. L. & Moss, B. (1985) in *DNA Cloning Vol. II A Practical Approach*, ed. Glover, D. M. (IRL, Oxford), pp. 191–211.
28. Chakrabarti, S., Brechling, K. & Moss, B. (1985) *Mol. Cell. Biol.* **5**, 3403–3409.
29. Gething, M.-J. & Sambrook, J. (1981) *Nature (London)* **293**, 620–625.
30. Braciale, T. J., Andrew, M. E. & Braciale, V. L. (1981) *J. Exp. Med.* **153**, 910–923.
31. Braciale, T. J., Henkel, T. J., Lukacher, A. E. & Braciale, V. L. (1986) *J. Immunol.* **137**, 995–1002.
32. Braciale, T. J., Braciale, V. L., Winkler, M., Stroynowski, I., Hood, L., Sambrook, J. & Gething, M.-J. (1987) *J. Exp. Med.* **166**, 678–692.
33. Wabuke-Bunoti, M. & Fan, D. P. (1984) *J. Immunol.* **133**, 2194–2201.
34. Townsend, A. R. M., Gotch, F. M. & Davey, J. (1985) *Cell* **42**, 457–467.
35. Yewdell, J. W., Bennink, J. R., Smith, G. L. & Moss, B. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1785–1789.
36. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennet, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329**, 506–512.
37. DeLisi, C. & Berzofsky, J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7048–7052.
38. Rothbard, J. B. & Taylor, W. R. (1988) *EMBO J.* **7**, 93–100.
39. Allen, P. M., Matsueda, G. R., Evans, R. J., Dunbar, J. B., Jr., Marshall, G. R. & Unanue, E. R. (1987) *Nature (London)* **327**, 713–715.
40. Taylor, P. M., Davey, J., Howland, K., Rothbard, J. B. & Askonas, B. A. (1987) *Immunogenetics* **26**, 267–272.
41. Whitton, J. L., Southern, P. J. & Oldstone, M. B. A. (1988) *Virology* **162**, 321–327.
42. Whitton, J. L., Gebhard, J. R., Lewicki, H., Tishon, A. & Oldstone, M. B. A. (1988) *J. Virol.* **62**, 687–695.