CLASS I MAJOR HISTOCOMPATIBILITY COMPLEX-RESTRICTED T LYMPHOCYTE RECOGNITION OF THE INFLUENZA HEMAGGLUTININ

Overlap between Class I Cytotoxic T Lymphocytes and Antibody Sites

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The influenza A hemagglutinin (HA)[1] plays a major role in initiation of infection and in the pathogenesis of influenza A viruses (1). To escape detection from immune surveillance mechanisms, the influenza HA evolves by continuously accumulating point mutations, i.e., antigenic drift, or by sporadic recombination between distantly related influenza strains, which results in the expression of a serologically distinct HA subtype, i.e., antigenic shift (2, 3). The influenza HA serves as the major target for neutralizing antibodies, and the critical recognition sites for these antibodies have been localized to four or five distinct regions on the three-dimensional structure of the HA (4–6). The influenza HA is also a target antigen for both class I and class II MHC-restricted T lymphocytes (7–12). Class I CTL directed to influenza virus antigens, including the HA, have been shown to play an important role in recovery from viral infection (13–15).

In contrast to antibody, the antigen receptor on T lymphocytes directed to foreign antigens such as viral polypeptides does not recognize free antigen, but rather, foreign antigen in conjunction with products of the MHC (16). It is now clear that class II MHC-restricted T lymphocytes predominantly recognize nonnative processed forms of foreign antigens (17, 18). Recent evidence likewise supports the view that class I T lymphocytes also recognize nonnative forms of antigen on the cell surface (19–21), although the processing and presentation events for the two classes of T lymphocytes occur via different pathways (22). For both class I and class II T lymphocytes the processing events can be bypassed by use of synthetic peptides (23–26).

In our previous studies of class I CTL recognition of the influenza A/JAP/57 HA, we described several distinct patterns of HA recognition, e.g., strain specific and crossreactive, among HA-specific H-2K^d-restricted CTL directed to A/JAP/57 influenza virus of the H2N2 subtype (27). We have mapped these specificities to two immunodominant sites on the A/JAP/57 HA, which can be mimicked by synthetic peptides corresponding to residues 202–221 and 523–545 of the HA (28). In this paper, we have explored the fine specificity of a panel of A/JAP/57 strain-specific

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[1] *Abbreviation used in this paper:* HA, influenza virus hemagglutinin.

class I CTL clones directed to the HA 202–221 site using virus strains with antigenically related HAs. We show that CTL of several different fine specificities map to the HA region spanning residues 202–221. Furthermore, we show that the HA 202–221 immunodominant region can be subdivided into two distinct yet overlapping epitopes and that amino acid substitutions that have occurred via antibody-mediated antigenic drift can also influence class I CTL recognition.

Materials and Methods

Mice. BALB/c ByJ (H-2^d) and $CB6F_1$/J (H-$2^{d\times b}$) mice were purchased from The Jackson Laboratory, Bar Harbor, ME.

Viruses. Influenza H2N2 strains A/JAP/305/57, A/Shanghai/57, A/Singapore/57, A/RI/5^+/57, A/Guiyang/1/57, A/Zhang/4/57, A/AA/23/57, A/Okuda/57, A/England/1/61, and influenza strain B/LEE/40 were grown in the allantoic cavity of 10-d-old chicken embryos. Infectious allantoic fluid was harvested 2 d later and aliquots were stored at −70°C (29). A panel of mAb-selected variants of A/JAP/305/57 and A/Guiyang/1/57 were generously provided by R. Webster (St. Jude Children's Research Hospital, Memphis, TN) and grown as described above (30).

Cell Lines. The P815 (H-2^d) mastocytoma cell line was maintained in DME (Gibco Laboratories, Grand Island, NY) and supplemented with 10% (vol/vol) FCS and 1% (wt/vol) glutamine.

Sequencing of Viral RNA. The viral genome in the region of the 202–221 site of the HA gene was sequenced by primer extension (31). Briefly, influenza virion RNA was isolated by lysis of the virions with an 0.5% SDS extraction buffer, digestion with proteinase K (Sigma Chemical Co., St. Louis, MO), and extraction with phenol/chloroform. A 15-mer oligonucleotide located 40–50 bp upstream of the HA 202–221 region was used to prime the synthesis of DNA complementary to virion RNA in the presence of dideoxynucleoside triphosphates (32) using AMV reverse transcriptase (Life Sciences, Inc., St. Petersburg, FL).

Peptides. Synthetic peptides corresponding to residues 202–221 of the A/JAP/305/57, A/Zhang/4/57, A/GV17, and A/AA/23/57 HA glycoproteins were produced on an automated solid-phase peptide synthesizer from Applied Biosystems (Foster City, CA). All other nested peptides were produced manually on a rapid amino multiple peptide synthesizer system (DuPont Co., Wilmington, DE).

T Cell Clones. The cloned T lymphocytes were derived from BALB/c or BALB/c × C57BL/6 ($CB6F_1$) mice. The procedures developed to establish and maintain them as well as analyses of their viral specificity have been described in detail (27, 33). Briefly, the clones were passaged weekly in the presence of A/JAP/57-infected, irradiated splenocytes in Iscove's modified Dubecco's medium (Gibco Laboratories), 10% (vol/vol) FCS, 1% glutamine, 5×10^{-5} M 2-ME, antibiotics, and supplementation with 10% (vol/vol) culture supernatant of Con A–activated rat splenocytes as a source of IL-2.

Assay for Cell-mediated Cytotoxicity. P815 cells were used as targets in standard ^{51}Cr release assays and the resultant data were analyzed as previously described (29). For assays in which synthetic peptides were used, the peptides were diluted in assay medium to the appropriate concentration and added to the clones and target cells in the 96-well assay plates.

Results

Fine Specificity of HA-specific Class I MHC-restricted CTL for H2N2 Field Strains. We previously analyzed the fine specificity of H-$2K^d$-restricted CTL clones directed to the A/JAP/57 (H2N2) HA and found that three distinct fine specificity patterns are discernible among these clones (27). Least frequent (<10%) are crossreactive CTL clones that recognize a conserved epitope present on HA of both the H1N1 and H2N2 subtypes. At a somewhat higher frequency (10–30%) are CTL clones that recognize an epitope conserved among all HAs of the H2N2 subtype. Both the subtype-specific

and the crossreactive epitopes have been localized to a site within the transmembrane domain of the A/JAP/57 HA corresponding to residues 523–545 of the primary HA translation product (28).

The most frequent pattern (60–80%) is exhibited by CTL clones that recognize the HA of the immunizing strain A/JAP/57 but not the HA of H2N2 strains isolated later during the 1957–1968 intrapandemic period (27). In an attempt to better define the fine specificity of these "strain-specific" CTL clones and the HA epitope(s) recognized, we examined the reactivity of these clones with human influenza field isolates whose HA are serologically highly crossreactive with the A/JAP/57 HA (30). As Table I demonstrates, the HA of four field strains, A/Guiyang/57, A/RI/5$^+$/57, A/Shanghai/57, and A/Singapore/57, are recognized by a panel of K^d-restricted HA-specific CTL clones as efficiently as previously reported for the A/JAP/57 HA. The HAs of two other strains, A/AA/57 and A/Zhang/57, were differentially recognized by the clones. Clone 40-2 recognizes cells infected with A/Zhang/57 but fails to recognize cells infected with A/AA/57. On the other hand, clones 11-1, 14-1, 14-2, 17-2, and 17-4 efficiently recognize the A/AA/57 HA but not target cells expressing the A/Zhang/57 HA. It should be noted that clones 17-2 and 17-4 did show a low level of reactivity on A/Zhang/57-infected targets (see Discussion). These results suggest that there may be two distinguishable epitopes on the A/JAP/57 HA and that these epitopes are differentially present on the A/Zhang/57 and A/AA/57 HAs.

CTL Recognition of an mAb-selected HA Variant. The field strains used in the above analysis were isolated at approximately the same time as the prototype A/JAP/57 virus. Their HAs show minor serological antigenic differences (30) that reflect specific amino acid differences in the HAs and are believed to occur under the selective pressure of neutralizing antibody. Since the class I CTL clones could distinguish between HAs with relatively minor antibody-driven antigenic changes, we considered

TABLE I

Recognition of H2N2 Influenza Field Strains by HA-specific Class I CTL Clones

	Percent specific ^{51}Cr release from target cells*					
Virus strains‡	40-2§	11-1	14-1	14-2	17-2	17-4
Uninfected	8‖	4	11	3	2	4
A/Shanghai/1/57	88	65	62	39	53	84
A/Singapore/202/57	86	74	72	52	62	80
A/RI/5$^+$/57	83	59	56	34	48	68
A/Guiyang/1/57	87	72	74	57	65	81
A/Zhang/4/57	84	6	15	3	11	26
A/AA/23/57	11	72	77	57	68	81

* P815 mastocytoma cells were labeled with ^{51}Cr, infected with the indicated influenza virus strain, and used as targets in a standard 6-h ^{51}Cr release assay (see Materials and Methods).

‡ Indicates virus strain used to infect target cell.

§ Indicates class I CTL clone used to screen infected target cells.

‖ Values are the mean percent specific release from triplicate samples. SEs were <5% of mean values and are omitted. The E/T ratio is 5:1.

the possibility that the antigenic epitope(s) recognized by these CTL clones may overlap with HA site(s) recognized by neutralizing anti-HA antibody. This notion is further supported by the fact that the HA region recognized by these class I CTL clones (HA 202–221) (28) is located within the globular head of the HA near the putative sialic acid binding site of the HA and several residues are exposed to the external milieu (6).

To further explore the possibility of overlap between class I CTL and antibody in HA recognition, we took a large panel of laboratory variants of the A/JAP/57 and A/Guiyang/57 viruses that had been selected for resistance to neutralizing mAbs directed to the A/JAP/57 and A/Guiyang/57 HAs (30) and screened target cells infected with these laboratory variants for recognition by the class I CTL clones. Of the 19 distinct variants examined, 18 are recognized as efficiently as the parental A/JAP/57 and A/Guiyang/57 HAs (not shown). One variant, A/GV17, selected with an mAb against the A/Guiyang/57 virus (30), is differentially recognized by the clones. Table II shows the results of an analysis of the recognition of target cells infected with the A/GV17 virus by several class I CTL clones. Clones 11-1, 14-1, 14-2, 17-2, and 17-4, which do not recognize the A/Zhang/57 HA, also do not recognize the A/GV17 HA. Clone 40-2 and another clone with a similar specificity, 40-1, do recognize the A/GV17 HA. These two clones (40-1, 40-2) also lyse target cells infected with A/Zhang/57, but not A/AA/57 (Table I). The mAb used to generate the A/GV17 variant was tested for its ability to block recognition of A/Guiyang/57-infected cells by the class I CTL clones in a standard ^{51}Cr release assay. No inhibition of lysis of target cells infected with A/Guiyang/57 or A/GV17 by clones 11-1, 14-1, or 14-2 was observed (data not shown).

Single Amino Acid Changes Define Subsites within a CTL Recognition Site. The evidence in Table II suggested that the A/GV17 HA had undergone an antibody-driven selection of a mutational event that resulted in altered recognition by a subset of class

TABLE II

Recognition of mAb-selected Variant A/GV17 by HA-specific Class I CTL Clones

Exp.	CTL clones‡	Percent specific ^{51}Cr release from infected target cells* A/JAP/57	A/Guiyang/57	A/GV17	B/Lee
1	11-1	50§	40	9	1
	14-1	56	48	10	4
	14-2	35	23	2	2
	40-1	67	58	42	13
	40-2	57	40	40	6
2	11-1	47	72	11	7
	17-2	33	65	5	2
	17-4	51	81	11	4
	40-2	70	87	83	7

* As in Table I.

‡ The HA-specific CTL clones listed were tested for cytolytic activity on target cells infected with the indicated influenza strain. Data from two independent experiments are shown.

§ Values are the mean percent specific lysis as noted in Table I. The E/T ratio is 5:1.

I CTL clones. We therefore wanted to determine whether A/GV17 had undergone a mutation within the HA 202–221 site provisionally defined as the region of the HA recognized by these K^d-restricted CTL clones. Also, since the alteration in the A/GV17 HA resulted in a CTL recognition pattern similar to the A/Zhang/57 HA, it was of interest to determine whether a comparable mutation had occurred in the A/Zhang/57 HA. To determine the amino acid sequence of the site in A/Zhang/57 and A/GV17 homologous to the A/JAP/57 HA 202–221 site, we sequenced by oligonucleotide-directed primer extension, the viral RNA of A/Zhang/57, A/GV17, and A/Guiyang/57, along with several other 1957 isolates in the region of the HA gene encompassing HA 202–221. Fig. 1 shows the derived amino acid sequence of these HAs in the region corresponding to the A/JAP/57 HA 202–221. The HAs of A/Guiyang/57, A/Shanghai/57, and A/Singapore/57 strains that are recognized by the CTL clones are identical in sequence to the A/JAP/57 HA in the 202–221 site. On the other hand, both A/GV17 and A/Zhang/57 have a single base change corresponding to a single amino acid substitution at residue 207. In the case of A/GV17, the asparagine at 207 was changed to a lysine, while in the A/Zhang/57, the asparagine at 207 was replaced by an aspartic acid. Furthermore, the A/AA/57 (and two other strains, A/Eng/1/61 and A/Okuda/57, with a similar pattern of recognition; not shown) also varied from the A/JAP/57 sequence at one residue, in this case at position 215 where the glycine has been replaced by a serine. As noted above (Table I), the A/AA/57 HA is not recognized by clones 40-1 and 40-2. It does, however, retain the asparagine at position 207 and is recognized by clones 11-1, 14-1, 14-2, 17-2, and 17-4.

This structural information suggested that the asparagine at position 207 was critical for recognition by one CTL subset (11-1, 14-1, 14-2, 17-2, 17-4), while the glycine at position 215 was essential for the other CTL subset (40-1, 40-2). To directly test this possibility, synthetic peptides corresponding to A/JAP/57 HA 202–221 were synthesized that contained, (*a*) the asparagine→lysine or asparagine→aspartic acid substitutions at position 207 (designated HA 202–221,K207, and HA 202–221,D207, respectively); (*b*) the glycine→serine substitution at position 215 (202–221,S215); or (*c*) the 202–221 site with substitutions at both 207 and 215 (202–221,K207,S215). As Table III shows, while the prototype A/JAP/57 HA 202–221 peptide is efficiently recognized by each of the CTL clones, the asparagine→lysine substitution at 207 abolishes recognition by clones 11-1, 14-1, 14-2, 17-2, and 17-4, but not by CTL clone 40-2. Conversely, the glycine→serine change at position 215 abolishes recognition of the peptide by 40-2 but not by the other clones. Importantly, none of the clones recognize the 202–221,K207,S215 peptide, further implicating the two residues at positions 207 and 215 as critical for CTL recognition. Also noteworthy was the finding

	202 205 210 215 221
A/JAP/305/57	R T L Y Q N V G T Y V S V G T S T L N K
A/Shanghai/1/51	- - - - - - - - - - - - - - - - - - - -
A/Guiyang/1/57	- - - - - - - - - - - - - - - - - - - -
A/Singapore/202/57	- - - - - - - - - - - - - - - - - - - -
A/GV17	- - - - - K - - - - - - - - - - - - -
A/Zhang/4/57	- - - - - D - - - - - - - - - - - - -
A/AA/23/57	- - - - - - - - - - - - S - - - - - -

FIGURE 1. Amino acid sequence of the HA 202–221 region of early H2N2 strains. Viral RNA of the various strains was isolated and sequenced as described in Materials and Methods. The deduced amino acid sequence is shown. Dashes indicate identity with residue in A/JAP/57 HA 202–221.

TABLE III

Recognition of HA 202–221 Region Peptides

Peptide sequence‡	Peptide concentration	Percent specific ^{51}Cr release from target cells*					
		40-2§	11-1	14-1	14-2	17-2	17-4
	μg/ml						
Medium	-	7‖	6	12	6	5	8
202-221	10	83	79	88	81	79	88
(A/JAP/57)	1	83	85	87	74	82	94
	0.1	87	82	64	39	44	72
	0.01	89	24	20	16	8	23
	0.001	39	7	12	7	4	8
202-221,207K	10	85	8	11	5	5	10
(A/GV17)	1	83	5	11	6	5	8
	0.1	85	6	12	8	6	9
	0.01	70	5	14	7	5	9
202-221,215S	10	8	82	89	84	84	83
(A/AA/57)	1	9	88	89	77	83	90
	0.1	10	84	68	46	52	79
	0.01	11	26	22	19	11	28
202-221,207D	10	93	48	18	54	81	87
(A/Zhang/57)	1	92	16	16	36	62	84
	0.1	89	9	15	13	16	36
	0.01	47	7	14	9	9	14
202-221,	10	9	17	18	14	7	18
207K,215S	1	12	9	13	9	6	11

* P815 mastocytoma cells were labeled with ^{51}Cr and assayed for lysis by the CTL clones listed in the presence of the synthetic peptides at the indicated concentration. Assay time was 6 h.

‡ Indicates sequence of the synthetic peptide used to treat the target cells. The designations 207K, 207D, and 215S indicate synthetic HA 202–221 peptides with substitutions of lysine or aspartic acid at position 207 or serine at position 215. The type A influenza strain with the corresponding amino acid substitution in the HA is noted in parentheses.

§ As in Table I.

‖ As in Table I.

that substitution of the asparagine at position 207 to aspartic acid differentially affected CTL recognition. For one clone, 14-1, the asparagine→aspartic acid change at position 207 markedly diminishes recognition of the peptide. For other clones, the primary effect of this substitution is a shift in the peptide dose dependence of target cell recognition. In this regard, it should be noted that the peptide dose dependence of target cell sensitization for clones 17-2 and 17-4 is less dramatically inhibited than that of clones 11-1 and 14-1.

Two Distinct Epitopes Map within HA 202–221. The data in Table III clearly show that within the 202–221 site, residue 207 is critical for clones of which 11-1 is representative, while clones of which 40-2 is representative are unaffected by changes at position 207 but are sensitive to amino acid substitutions at residue 215. These findings are compatible with a model of a single antigenic site spanning residues 207 and 215, in which residues critical for T cell recognition by each subset of clones are

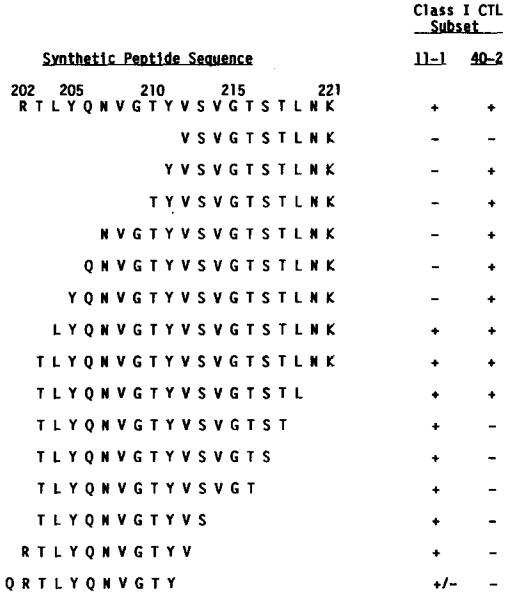

FIGURE 2. Mapping the CTL epitopes within the 202–221 region of the HA. Synthetic peptides were synthesized and tested in standard 6-h ^{51}Cr release assays for their ability to sensitize P815 cells for lysis by the HA-specific class I–restricted CTL clones. Peptide was added at final concentrations ranging from 5×10^{-9} to 5×10^{-6} M and maintained throughout the assay. E/T ratios were 5:1 and 1:1. Peptides were screened for target cell sensitization with 8–10 distinct class I CTL clones representative of the specificities defined by clones 11-1 and 40-2, respectively. (+) Target cell sensitization identical in specificity and efficiency to the HA 202–221 synthetic peptide; (−) no recognition of the peptide-treated target cells by the class I CTL at concentrations up to 5×10^{-6} M; (+/−) intermediate efficiency of target cell sensitization by the peptide.

located at either the NH_2 or COOH terminus of the determinant, while residues essential for MHC binding are located centrally. An alternative would be where two distinguishable CTL epitopes are contained within the HA 202–221 region.

To better define the boundaries of the epitope(s) mimicked by the HA 202–221 peptide, we synthesized a set of nested peptides within the HA 202–221 region and tested their recognition by the CTL clones. As revealed in Fig. 2, the immunodominant region defined by HA 202–221 can be subdivided into two distinguishable sites. CTL clones represented by clone 11-1 recognize the synthetic NH_2-terminal 11-mer RTLYQNVGTYV corresponding to residues 202–212. The CTL clones represented by 40-2 recognize the COOH-terminal 11-mer YVSVGTSTLNK corresponding to residues 211–221. The NH_2-terminal 202–212 site and the COOH-terminal 211–221 site overlap at two residues Y and V at positions 211 and 212.

Discussion

In this report we have examined the fine specificity of H-2K^d-restricted murine CTL clones with a narrow reactivity for the HA of the immunizing A/JAP/57 influenza virus and antigenically closely related HAs of influenza strains of the H2N2 subtype. Using this panel of field strains, we found that the class I CTL clones could be divided into two groups based on their differential recognition of the HAs of the A/Zhang/57 and A/AA/57 viruses. Since all of these clones are directed to an immunodominant region of the A/JAP/57 HA corresponding to HA 202–221, the CTL clones appeared to define two distinct epitopes within the HA 202–221 site. Support for this concept came from sequencing of the HA genes of field strains in the region corresponding to A/JAP/57 HA 202–221. This analysis revealed that a single amino acid substitution at position 207 in the A/Zhang/57 HA and at position 215 in the A/AA/57 resulted in the selective loss of one or the other of these epitopes. That

the HA 202–221 site contains two distinguishable epitopes was directly supported by studies on class I CTL recognition of target cells treated with a nested set of synthetic peptides spanning portions of the HA 202–221 site.

Of interest in these analyses was the finding that an mAb-selected variant virus, A/GV17, had lost one of the two epitopes contained within the HA 202–221 site. Sequencing of the A/GV17 HA gene in this region revealed a single base change resulting in an asparagine→lysine substitution at position 207. The importance of the asparagine at position 207 for CTL clone 11-1 and related clones was confirmed by studies with a synthetic HA 202–221 peptide containing the asparagine→lysine substitution at position 207. The class I CTL clones show comparable reactivity on target cells treated with this peptide or targets infected with the A/GV17 variant virus strain. It is noteworthy that on the three-dimensional crystal structure of the H3 subtype hemagglutinin (6), residues 202–221 of the A/JAP/57 HA correspond to residues 192–211 of the H3 HA. This sequence is located on the outer surface of the HA globular head beginning within an α helix structure preceding β strand number 4 (H3 residues 200–205) and ending in β strand number 5 (H3 residues 210–213). It is also in close proximity to the sialic acid receptor binding site and encompasses portions of two of the major neutralizing antibody sites on the HA, i.e., sites B and D (4). The asparagine at position 207 in the A/JAP/57 HA, which is critical for recognition by one subset of class I CTL clones (represented by 11-1), appears exposed on the surface of the HA within antigenic site B. Likewise, the glycine at position 215, which is essential for recognition by the other subset of CTL clones (represented by 40-2), is in antigenic site D in the interface region formed by HA trimerization.

These data on the recognition of field strains and particularly A/GV17 by these CTL indicate that amino acids implicated in antigen recognition by the T cells are also critical for antibody recognition. The antigenic sites recognized by class I T lymphocytes and neutralizing antibodies, therefore, can directly overlap, as has been noted for influenza HA epitopes (9, 34) recognized by class II T lymphocytes. T lymphocytes and B lymphocytes, however, can recognize unrelated sites on a given protein antigen. This has been well documented for sites recognized by class II T lymphocytes directed to both soluble globular proteins (35–38) and membrane proteins like the influenza HA (9). The fact that the transmembrane domain of the HA is recognized by HA-specific class I–restricted CTL (26) reinforces this view, since the transmembrane domain of the HA is embedded in the lipid bilayer of the virion or infected cell and is probably not accessible to antibody.

The two K^d-restricted epitopes in HA 202–221 can be defined by peptides 202–212 (RTLYQNVGTYV) and 211–221 (YVSVGTSTLNK), respectively. These two regions overlap in two residues, i.e., the tyrosine at position 211 and the valine at position 212. Both residues are required for epitope recognition by either subset of clones. These two epitopes exhibit considerable sequence homology and each contains a five-residue motif characteristic of T cell sites (39). Each epitope also shows modest homology with other antigenic sites recognized in association with H-$2K^d$ (39). These results raise the possibility that a class I T cell site for the H-2^d haplotype has been tandemly duplicated within the A/JAP/57 HA. This may explain the potency of the A/JAP/57 HA over HAs of other subtypes in stimulating class I T lymphocyte responses (T. J. Braciale, unpublished observations).

We found that the asparagine→lysine change at residue 207 abolishes CTL recognition of target cells treated with the synthetic peptide containing this substitution (Table III) and of infected target cells expressing the A/GV17 HA containing this substitution (Table I). On the other hand, while the asparagine→aspartic acid change at position 207 did markedly reduce recognition of target cells infected with A/Zhang/57 virus, the synthetic 202–221 peptide with the aspartic acid substitution at position 207 is recognized by most of the CTL clones (Table III). However, this substitution does lead to a decrease in the efficiency of peptide recognition with a shift in the dose response curve for the 11-1 subset of clones. This finding suggests that the asparagine→aspartic acid change at position 207 (unlike the asparagine→lysine change) leads to an antigenic moiety that binds MHC and interacts with the TCR on the clones to a sufficient extent to permit target cell recognition, albeit with a lower overall efficiency. The failure of most clones to recognize A/Zhang/57-infected cells may well mean that the concentration of processed HA antigen is normally low in an infected cell and that the processed HA antigen moiety containing the asparagine→aspartic acid substitution does not form a sufficient number of complexes to be efficiently recognized. In this connection it is noteworthy that clones 17-2 and 17-4 do show low but significant lysis of A/Zhang/57-infected cells (Table I). These two clones are also the most efficient at recognizing the 202–221 peptide with the asparagine→aspartic acid change at position 207. Thus, their antigen receptors may recognize with sufficient avidity this peptide/MHC complex.

At present we do not know whether the mutations at position 207 and 215 affect peptide binding to MHC and/or the efficiency TCR interaction with this complex. The finding that two different substitutions at 207 differentially affect recognition of this epitope by CTL would suggest that residue 207 may be critical for TCR recognition rather than interaction of the peptide with MHC. Such fine specificity of recognition by class I CTL has been previously reported (40). Furthermore, if HA 202–221 contains a tandem duplication of a K^d binding site, then loss of MHC binding capacity by a mutation within one site, e.g., position 207 in the 202–212 site, should be compensated by peptide binding through residues in the other, 211–221, site. As noted in Table III, the HA 202–221,K207 peptide efficiently binds to K^d, as evidenced by the efficient recognition of this peptide by clone 40-2, yet, no recognition of this peptide by clone 11-1, and related clones, is evident. Experiments are now in progress to directly assess the contribution of individual residues to MHC binding and antigen receptor recognition.

In conclusion, we have analyzed a site on the influenza HA that contains residues recognized by both class I–restricted CTL and antibody. The site has the unusual property of encompassing two distinct epitopes that may represent a tandem duplication of a T cell recognition site. It is not known whether both epitopes are present in the endogenously processed form of the antigen generated in virally infected targets or if these epitopes are generated by separate proteolytic cleavage events. Dissection of this site and the HA antigenic moiety generated during viral infection should provide further insight into the mechanism of antigen processing.

Summary

The influenza hemagglutinin is a critical regulator of disease expression during influenza virus infection and serves as a major target for the host immune response

to this pathogen. In this report, we have analyzed an immunodominant site on the hemagglutinin (residues 202–221) recognized by murine class I MHC-restricted T lymphocytes. This analysis has revealed evidence for the duplication of a T cell recognition site within the region 202–221. We have also identified critical amino acids necessary for class I-restricted T cell recognition within these two epitopes. In addition, we provide evidence that a site on the influenza hemagglutinin recognized by neutralizing antibody directly overlaps with an epitope recognized by class I MHC-restricted CTL.

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