Recognition of an Immunoglobulin V_H Epitope by Influenza Virus–specific Class I Major Histocompatibility Complex–restricted Cytolytic T Lymphocytes

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Summary

There are two immunogenic sites on the type A influenza A/Japan/57 (H2N2) hemagglutinin (HA) that can be recognized by class I major histocompatibility complex (MHC), H-2K^d-restricted cytolytic T lymphocytes (CTLs). One of these sites encompasses two distinct partially overlapping epitopes, which span HA residues 204-212 and 210-219. During the analysis of the fine specificity of CTL clones directed to the HA 210-219 epitope, we found that one clone 40-2 also recognized the myeloma cell line P3x63-Ag8. P3x63-Ag8 is derived from the MOPC 21 myeloma and expresses an immunoglobulin (Ig) heavy chain variable region (V_H) gene which is a member of the murine 7183 V_H gene family. Recognition was specific for the endogenously processed MOPC 21 heavy chain in association with the K^d molecules, since the SP2/0 derivative of P3x63-Ag8, which does not make a functional Ig H chain, is not recognized. The V_H epitope recognized by clone 40-2 could be mapped to a 10 amino acid peptide spanning MOPC 21 V_H residues 49-58. Cross-reactivity for the V_H gene product was also demonstrable in some heterogeneous populations of CTL generated in response to influenza virus infection. These results represent the first demonstration of cross-reactivity for an endogenously processed product of a self-Ig by the CTL directed to a foreign antigen and raise the possibility that the Ig V_H expression may regulate the CD8$^+$ T cell response to foreign antigens.

More than 30 years ago, it was found that T lymphocytes, unlike B lymphocytes, fail to discriminate between native and denatured protein antigens (1). Crucial observations over the last decade have led to the generally accepted belief that protein antigens must be processed by APCs into peptide fragments for recognition by T lymphocytes (2–4). These fragments associate with MHC molecules primarily in intracellular compartments. The MHC molecules then present the antigenic fragments to T lymphocytes at the surface of APCs. Evidence now suggests that antigenic peptides recognized by CD8$^+$ lymphocytes may be chiefly generated in the cytosol by proteolytic processing of peptides. The peptides are then translocated into the endoplasmic reticulum (ER)[1] by MHC-linked transporter proteins (5–10). These peptides bind to class I MHC molecules and are believed to facilitate the stable assembly of class I MHC H chains and β_2-microglobulin (8, 11). Extensive sequence analysis of antigenic peptides isolated from the clefts of class I MHC molecules demonstrated that the endogenous antigenic peptide lengths are typically 8–10 amino acids, though longer peptides have also been found (12–16). More importantly, allele-specific motifs have been identified for the epitopes recognized in association with particular class I MHC alleles.

Even though the potential T cell repertoire is very diverse, and the MHC molecules can bind a plethora of peptide fragments, the number of T cell epitopes for a given MHC haplotype is surprisingly limited in a particular antigen (17–19). It has been shown that the expression of an allele-specific MHC binding motif, though necessary, is not sufficient to render a site immunogenic (20). Besides good binding ability to specific MHC alleles, a protein antigen needs to be fragmented properly in the cytosol. The resultant peptide epitopes need to be translocated into the ER where they can complex with class I MHC molecules. In addition, the responding individual must have T lymphocytes with antigen receptors capable of recognizing the peptide–MHC complex.

[1] *Abbreviations used in this paper:* ER, endoplasmic reticulum; HA, hemagglutinin; RT, room temperature.

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Since self-peptides can bind MHC molecules (21–23), the formation of self-peptide–MHC complexes in the thymus and/or the periphery can potentially regulate the T cells available to react to foreign antigens through positive and/or negative selection mechanisms. Ig V region gene products are a particularly rich source of self-peptides because of their great structural diversity. They have been proposed as donors of self-peptides which can regulate the T lymphocyte repertoire (24–28).

Influenza virus hemagglutinin (HA) is a target antigen for $CD8^+$ CTLs (29). The influenza strain A/Japan/57 contains two immunodominant sites that are recognized by $CD8^+$ CTLs in association with the H-$2K^d$ molecules (18, 30). One of these sites encompasses two distinct partially overlapping subsites recognized by the different subsets of K^d-restricted CTLs (30). These overlapping sites span HA residues 204-212 (LYQNVGTYV) and 210-219 (TYVSVGTSTL) (30, and Hahn, C. S., Y. S. Hahn, V. L. Braciale, C. M. Rice, and T. Braciale, manuscript submitted for publication). The magnitude of the CTL responses of H-2^d haplotype mice to these overlapping subsites is different (Myers-Powell, B. A., V. L. Braciale, and T. J. Braciale, manuscript in preparation). The NH_2-terminal nonamer epitope (JHA 204-212) is immunodominant, eliciting vigorous CTL responses in A/Japan/57–immunized BALB/c mice. The CTL responses to the COOH-terminal decamer epitope (JHA 210-219) are weak and variable among the immunized mice.

During the analysis of the specificity of JHA 210-219–specific CTL clones on different target cells, we unexpectedly found that a JHA 210-219–reactive CTL clone 40-2 recognized uninfected P3x63-Ag8 myeloma cells. This cell line expresses the murine MOPC 21 Ig V_H gene product. In this report, we demonstrate that the HA-specific CTL clone 40-2 recognizes an endogenously processed site within the MOPC 21 V_H which can be mapped to residues 49-58 in the CDR2 of the H chain. This cross-reactivity is also seen in short term cultures of heterogeneous CTLs specific for the A/Japan/57 HA. The significance of these findings for CTL repertoire development and the control of immunogenicity to foreign proteins by Ig V_H regions is discussed.

Materials and Methods

Mice. BALB/cAnNTac (H-2^d) were purchased from Taconic Laboratory Animals and Services (Germantown, NY).

Virus Stocks. Influenza virus strains A/Japan/57 (H2N2 subtype) was grown in the allantoic cavity of 10-d-old chicken embryo and stored as infectious allantoic fluid at −70°C (31).

Cell Lines and CTL Clones. The P815 (H-2^d) mastocytoma cells were maintained in DME (GIBCO BRL, Gaithersburg, MD) supplemented with 10% (vol/vol) FCS and 1% (wt/vol) glutamine. P3x63-Ag8 and SP2/0 myeloma cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained as suggested (32). H17-L19, 17-L7-ZR1, IC3-3A4-1, and H35-C6-5 hybridomas were generously provided by Dr. W. Gerhard (The Wistar Institute of Anatomy and Biology, Philadelphia, PA), and maintained as described elsewhere (32). CTL clones 14-7, 40-2, and NI 12 were isolated CTL precursors primed with influenza A/Japan/57 virus and established as previously described (18, 30). They were passed weekly in the presence of irradiated influenza A/Japan/57–infected splenocytes and a source of exogenous T cell growth factor.

Peptides. Synthetic oligopeptides were produced either on a rapid amide multipeptide synthesis system (RaMPs®, NEN-DuPont, Boston, MA) or an automated Solid Phase Peptide Synthesizer from Applied Biosystems, Inc. (Foster City, CA). HPLC analysis showed that peptides were >95% pure. Stock solutions of synthetic peptides were dissolved in 100% DMSO at concentrations of 5 mg/ml, and diluted to the desired concentrations in MEM immediately before use in cytotoxicity assays. Control experiments have shown that DMSO at concentrations used in the assays has no effect on target viability or CTL function.

Bulk CTL Populations. Mice were primed intraperitoneally with $\sim 10^3$ hemagglutinin units of infectious A/Japan/57 virus. 3 wk later, splenocytes were removed and restimulated in vitro with irradiated A/Japan/57–infected splenocytes. The third passage was done by using JHA 210-219 peptide pulsed splenocytes. Cultures were assayed for cytolytic activity after 5–7 d of incubation at 37°C.

CTL-mediated Cytolysis Assay. The P815 (H-2^d) cells were used as target cells in a ^{51}Cr-release assay as previously described (30). ^{51}Cr-release assay was also used to assess peptide competition in 4-h cytotoxicity assays. Competing peptides at appropriate concentrations were incubated with labeled P815 for 15 min at room temperature (RT) in 96-well microtiter plates before clone 14-7–specific peptide JHA 523-545 was added. After another 15 min at RT, clone 14-7 cells were added at an E/T ratio of 5 to 1.

Results

Lysis of P3x63-Ag8 Myeloma by a HA-specific Class I MHC–restricted CTL Clone. CTL clone 40-2 is a H-$2K^d$-restricted $CD8^+$ clonal T cell line derived from BALB/c mice immunized with infectious A/Japan/57 virus. It recognizes a site in the A/Japan/57 HA that can be mapped to residues 210-219 (JHA210-219). During the analysis of the fine specificity of this influenza-specific clone, we unexpectedly found that clone 40-2 lysed the uninfected BALB/c myeloma cell line P3x63-Ag8 (P3) (Fig. 1). This drug-resistant hybridoma fusion partner is derived from the MOPC 21 myeloma (33). Recognition of the uninfected myeloma cell by clone 40-2

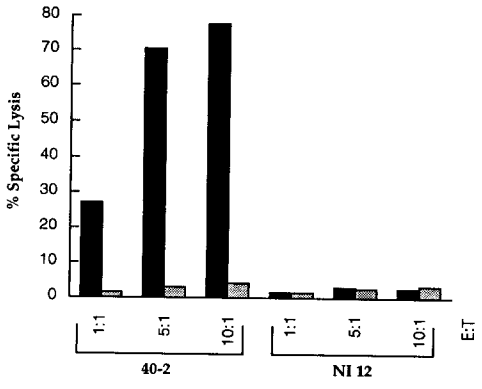

Figure 1. Lysis of myeloma P3x63-Ag8 by the A/Japan/57 HA 210-219–specific CTL clone 40-2. ^{51}Cr-labeled P3x63-Ag8 (■) or SP2/0 (▨) myeloma cells were incubated with JHA 210-219–specific CTL clone 40-2 or NI 12 for 4.5 h at 37°C. Different E/T ratios were used as shown. The percent specific cytolytic activity from quadruplicate samples was calculated. Spontaneous lysis has been subtracted from the values shown.

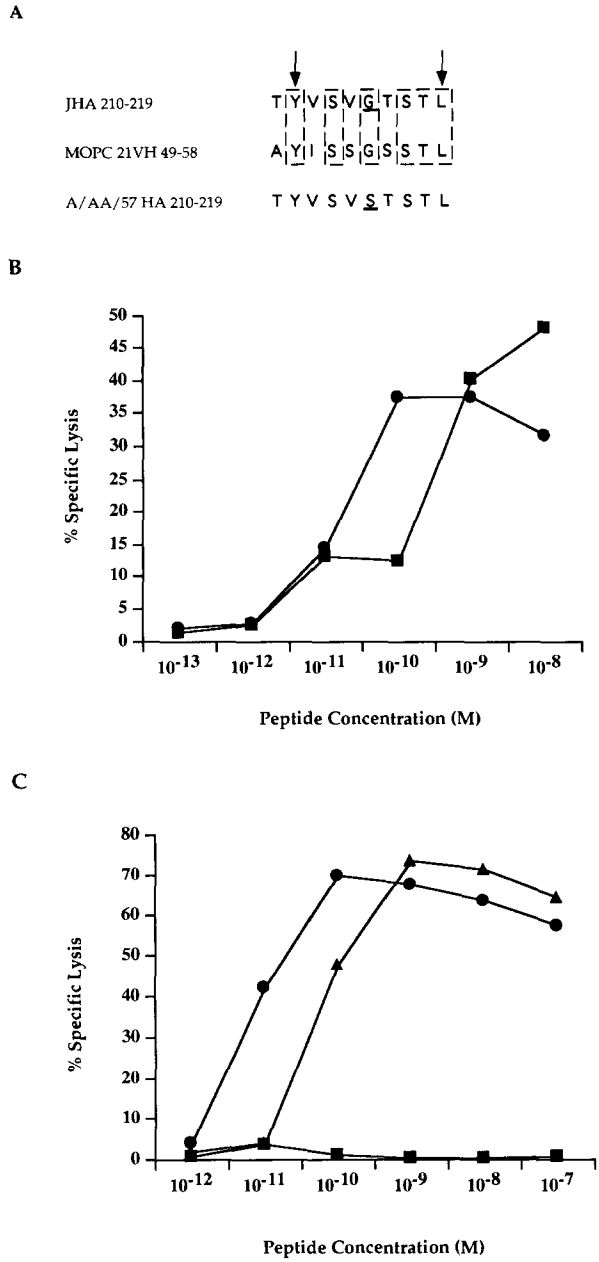

Figure 2. Recognition of the MOPC 21 V_H 49-58 peptide by CTL clone 40-2. (*A*) Amino acid sequences of JHA 210-219 homologous peptides in MOPC 21 V_H and A/AA/57 HA. Those residues framed by dashed lines are the residues conserved between JHA 210-219 and MOPC 21 V_H 49-58. Residue 215 is different between JHA 210-219 and AHA 210-219 (*underlined*). Arrows highlight residues believed to be critical for K^d binding. (*B* and *C*) ^{51}Cr-labeled P815 target cells were incubated alone or in the presence of synthetic peptides representing JHA 210-219 (—●—), MOPC 21 V_H 49-58 (—■—), or AHA 210-219 (—▲—). JHA 210-219–specific CTL clone (*B*) 40-2 or (*C*) NI 12 was added to targets and incubation continued for 4 h at 37°C. The E/T ratio for each assay was 5:1. The percent specific cytolytic activity from quadruplicate samples was calculated. Nonspecific killing was 2.6% by clone 40-2, 0.5% by clone NI 12.

was very efficient with lysis demonstrable over a range of effector to target ratios. The lysis of the P3 myeloma was not nonspecific. The H-2K^d-restricted clone NI-12 did not lyse P3 cells (Fig. 1). This CTL clone, like 40-2, is directed to the JHA 210-219 site but differs in fine specificity from clone 40-2. Clone 40-2 recognizes the JHA 210-219 site but not the corresponding site in the A/AA/57 virus (AHA 210-219) which differs from the JHA 210-219 by one residue (Ser for Gly at position 215) (Fig. 2 *A*) (30). Clone NI 12 recognizes both JHA 210-219 and AHA 210-219 with comparable efficiency.

Whereas clone 40-2 lysed uninfected P3 cells, it did not lyse the SP2/0 myeloma cell line (Fig. 1). This cell line is a variant of P3 which does not express the MOPC 21 V_H gene (34). SP2/0 was however readily lysed by clone 40-2 after pulsing with the decamer synthetic peptide corresponding to the JHA 210-210 epitope (data not shown).

Crossrecognition of a MOPC 21 V_H Peptide by CTL Clone 40-2. The observation of lysis by clone 40-2 of the uninfected P3 cell line but not the SP2/0 cell line raised the possibility that clone 40-2 recognized, in a cross-reactive manner, a processed epitope derived from the MOPC 21 V_H gene product. The decamer epitope in the A/Japan/57 HA recognized by clone 40-2 contains a Tyr residue at position 2 and Thr and Leu residues at position 9 and 10. This is a motif defined by Healy et al. (20) and Romero et al. (35) to be critical for peptide binding to K^d. An inspection of the MOPC 21 V_H amino acid sequence (33) revealed that this K^d binding motif was contained within a 10 amino acid segment of the MOPC 21 V_H. This segment encompasses residues 49–58 and partially overlaps the CDR2 region of the MOPC 21 V_H (Fig. 3). The V_H 49-58 sequence has 6 of 10 residues in common with JHA 210-219. This includes, in addition to the Y50 at position two and the T57 and L58 at positions 9 and 10 in the sequence, S52, G54, and S56, corresponding to S213, G215, and S217, in the JHA 210-219 sequence (Fig. 2 *A*).

To determine if the cross-reactivity of clone 40-2 for P3 cells could be due to recognition of the MOPC 21 V_H 49-58 site, we made a synthetic peptide corresponding to the MOPC 21 V_H 49-58 site and tested clone 40-2 for recognition of H-2K^d-bearing P815 cells treated with the V_H 49-58 peptide. As Fig. 2 *B* shows, clone 40-2 recognized the V_H 49-58 peptide at peptide concentration down to 10^{-12}–10^{-11} M with an efficiency similar to that of the JHA 210-219 peptide. In contrast, clone NI 12 recognizes P815 target cells treated either with the JHA 210-219 peptide or the AHA 210-219 peptide with a similar efficiency, but does not recognize target cells pulsed with the V_H 49-58 peptide (Fig. 2 *C*). This suggests that treatment of cells with the V_H 49-58 peptide does not render cells susceptible to nonspecific lysis.

The efficient recognition of the V_H 49-58 peptide implied that this peptide would also bind to the H-2K^d molecule on cell surfaces. We examined the V_H 49-58 peptide binding to K^d in a peptide competition assay. In this assay, the capacity of the V_H 49-58 and JHA 210-219 peptides to inhibit binding and subsequent CTL recognition of a K^d binding

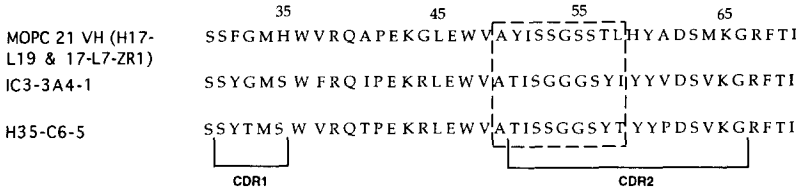

Figure 3. Sequences of four hybridoma cell lines using different members of the 7183 V_H gene family (32). The regions homologous to JHA 210-219 are framed by dashed lines.

peptide corresponding to an epitope in the transmembrane domain of the A/Japan/57 HA was analyzed. As Fig. 4 shows, both the JHA 210-219 and V_H 49-58 peptides inhibited recognition of the transmembrane peptide by the CTL clone with a comparable efficiency. The synthetic JHA 129-145 peptide which does not bind K^d failed to compete.

Recognition of MOPC 21 V_H Expressing Hybridoma Cell Lines by Clone 40-2. Our analysis with the synthetic V_H 49-58 peptide supported the view that an endogenously processed fragment of the MOPC 21 V_H was recognized on P3 cells by the influenza-specific CTL clone 40-2. The MOPC 21 V_H gene is a germline member of the 7183 murine V_H gene family (36). If clone 40-2 does recognize a site in the MOPC 21 V_H encompassing residues 49-58, we anticipate that other H-2K^d haplotype hybridomas expressing the MOPC 21 V_H gene would be recognized by clone 40-2. Also, hybridomas utilizing other members of the 7183 Ig V_H family with sequence difference in the 49-58 region should not be recognized by clone 40-2. As Fig. 5 shows, this is indeed the case. Two independently derived hybridomas H17-L19 and 17-L7-ZR1 (32) express the MOPC 21 V_H gene and are lysed by clone 40-2. Two other hybridomas, IC-3A4-1 and H35-C6-5 (32), use two other members of the 7183 V_H gene family. These V_H genes are highly homologous to the MOPC 21 V_H gene but do not contain the critical Tyr/Thr/Leu motif in the 49-58 region necessary for binding to K^d (Fig. 3). These two hybridomas are not recognized by clone 40-2. Again, clone NI 12 does not lyse any of the hybridoma targets.

Recognition of the Processed MOPC 21 V_H Gene Product by Heterogeneous Populations of Influenza-specific CTLs. Clones 40-2 and NI 12 were independently derived from splenocyte cultures of BALB/c mice infected in vivo with A/Japan/57 virus. Bulk cultures of immune splenocytes were then restimulated with infectious virus in vitro for three to four passages before cloning on A/Japan/57 infected splenocyte feeders. Because clone 40-2 has been maintained in long-term in vitro cultures, it was of interest to assess if the cross-reactivity of JHA 210-219–reactive CTLs for the P3 myeloma and the V_H 49-58 peptide was demonstrable among virus-specific CTL in short-term in vitro cultures. To address this, we examined JHA 210-219–specific T cells in short-term culture for reactivity toward P3 and V_H 49-58. Splenocytes from each of five A/Japan/57–infected BALB/c mice were restimulated in vitro with infectious A/Japan/57 virus and individually tested for reactivity on several K^d-restricted epitopes and for cross-reactivity on P3 cells.

Although the K^d-restricted CTL responses to the immunodominant influenza nucleoprotein (NP) 147-158 epitope and the JHA 204-212 epitope were readily demonstrable, the response to the JHA 210-219 epitope was weak and variable for each mouse (i.e., ~20% of the response to the NP 147-158 site) (data not shown). To amplify the response to the JHA 210-219 epitope, virus-immune splenocytes were restimulated in vitro with the JHA 210-219 peptide. 7 d after exposure to the JHA 210-219 peptide, individual cultures were

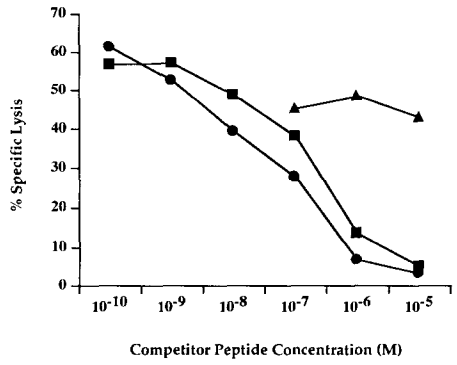

Figure 4. Inhibition of CTL-mediated lysis of JHA 523-545–sensitized targets by JHA 210-219 and MOPC 21 V_H 49-58 peptides. ^{51}Cr-labeled P815 target cells were incubated 15 min at RT with JHA 210-219 (—●—) or MOPC 21 V_H 49-58 (—■—) peptides at various concentrations. 1.0 μg/ml JHA 523-545 peptide was then added and incubation at RT continued for another 15 min before the addition of JHA 523-545–specific CTL clone 14-7. The non-K^d binding peptide JHA 129-145 (—▲—) was used as a control. The assay time was 4.5 h, and the E/T ratio was 5:1. The percent specific cytolytic activity from quadruplicate samples was calculated.

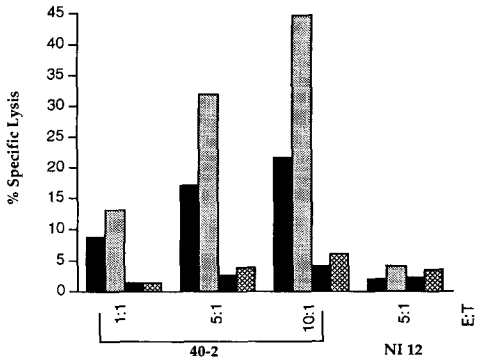

Figure 5. Lysis of MOPC 21 V_H–expressing hybridoma cells by CTL clone 40-2. ^{51}Cr-labeled H17-L19 (■), 17-L7-ZR1 (▨), IC5-3A4-1 (▩), or H35-C6-5 (▩) hybridoma cells were incubated with CTL clone 40-2 or NI 12 for 5.5 h at 37°C. Different E/T ratios were used as shown. The percent specific cytolytic activity form quadruplicate samples was calculated. Spontaneous lysis has been subtracted from the values shown.

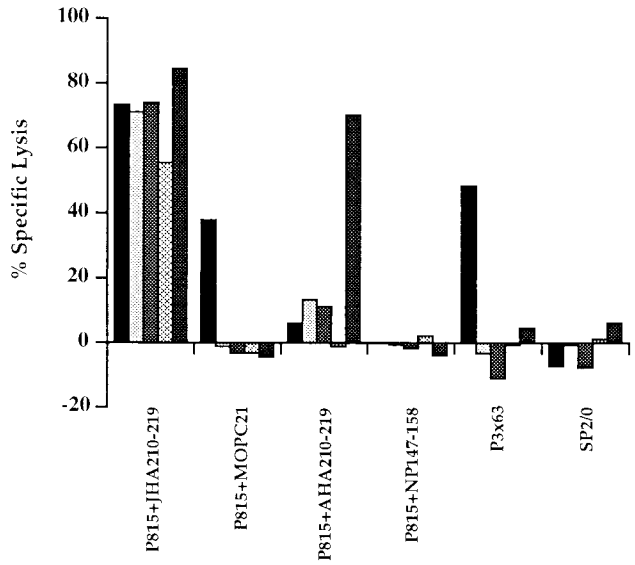

Figure 6. Specificity of A/Japan/57-immune cytolytic T lymphocytes. Five BALB/c mice were primed with A/Japan/57 virus. Immune splenocytes from each animal were individually stimulated in vitro 3 wk later with A/Japan/57-infected splenocytes. After two passages, the individual bulk cultures were restimulated with peptide JHA 210-219-pulsed splenocytes. The activated CTLs were used in a standard 6-h cytotoxicity assay with ^{51}Cr-labeled P815 target cells and 0.01 μg/ml peptide, or ^{51}Cr-labeled myeloma P3x63-Ag8 or SP2/0. The E/T ratio was 10:1. The percent specific cytolytic activity from quadruplicate samples was calculated. Spontaneous lysis has been subtracted from the values shown. The five bar grafts represent the response of the individual mice on each target cell group.

tested for reactivity on peptide-treated P815 cells and P3 myeloma cells. As Fig. 6 shows, cultures derived from all five animals recognize JHA 210-219 pulsed target cells as expected. In vitro stimulation with JHA 210-219 peptide enriches for JHA 210-219-reactive CTLs, and the reactivity to the NP 147-158 epitope is lost. Of particular interest is the finding that only one culture in five bulk cultures shows reactivity to the P3 myeloma cell and to target cells treated with the MOPC 21 V_H 49-58 peptide. This reactivity pattern in heterogeneous cultures is characteristic of specificity of the clone 40-2. Likewise, only one culture among the others showed reactivity for the AHA 210-219 peptide, which is characteristic of the NI 12 clonal specificity. Importantly, the bulk culture from the A/Japan/57-immune individual which recognized the MOPC V_H 49-58 peptide was also the only CTL population that lysed uninfected P3 myeloma cells. Thus cross-reactivity for the MOPC 21 V_H was also demonstrable in short-term bulk cultures of specific CTL from an influenza virus-immunized mouse.

Discussion

In this report, we have examined the structural basis for the unexpected cross-reactivity exhibited by a class I MHC-restricted influenza HA-specific CTL clone 40-2 for the P3x63-Ag8 myeloma cell line. We found that recognition and lysis of the myeloma cell line requires expression of the MOPC 21 V_H gene since the MOPC 21 V_H nonexpressor SP2/0 is not recognized by clone 40-2. The MOPC 21 V_H has a 10-amino acid segment spanning CDR2 residues 49-58 which contains a sequence motif critical for peptide binding to the K^d molecule (35). Clone 40-2 recognizes target cells treated with a synthetic decamer peptide corresponding to the MOPC 21 V_H residues 49-58. This K^d binding peptide is recognized as efficiently by clone 40-2 as the JHA 210-219 peptide. The MOPC 21 V_H gene is a member of the murine 7183 Ig V_H gene family. Hybridomas expressing other 7183 Ig V_H members that are highly homologous to the MOPC 21 V_H but lack this K^d binding motif in the CDR2 region were not recognized by clone 40-2. Importantly, cross-reactivity of JHA 210-219-reactive T cells for the MOPC 21 V_H gene product was not only seen with the 40-2 clone but also seen with a heterogeneous population of JHA 210-219-reactive CTLs derived from short-term cultures of influenza virus-immune splenocyte CTL precursors. Thus, this cross-reactivity is not the unique property of a long-term CTL clone, but rather demonstrable in virus-immune T cell populations. Taken together, these data strongly suggest that some influenza virus-specific $CD8^+$ CTL can specifically recognize an endogenously processed fragment of a murine Ig V_H gene product.

Unlike clone 40-2, clone NI-12 does not recognize the MOPC 21 V_H 49-58 site, but does recognize the HA 210-219 site of both the A/Japan/57 and the A/AA/57 virus. The JHA 210-219 and AHA 210-219 sites differ by one residue (a Gly → Ser substitution at position six in the peptide). As discussed below, this residue may be critical for 40-2 recognition of the peptide-MHC complex, since clone 40-2 does not recognize A/AA/57 virus or the AHA 210-219 peptide. It is also noteworthy that the CTL specificities comparable to the 40-2 and the NI-12 clonal specificities are represented as dominant specificities in bulk populations of HA 210-219-reactive CTLs from different individual mice.

Earlier studies by Romero and co-workers (20, 35) have implicated the Tyr at position two and the Thr/Leu at positions 9 and 10 as critical for decamer peptide binding to the K^d molecule. We have modeled the JHA 210-219 peptide into a three-dimensional crystal structure of the K^d molecule based on the HLA-A2 coordinates (37). In our preliminary computer modeling, the Ser, Val, Gly, and Thr at positions 4, 5, 6, and 9 of JHA 210-219 are solvent accessible and represent potential TCR contact residues. The JHA 210-219 site and the MOPC 21 V_H 49-58 site have identical residues at positions 4, 6, and 9. It is likely that one or several of these residues is critical for peptide recognition by the 40-2 TCR. Consistent with this view is our finding that the AHA 210-219 site which differs by only one residue, a Gly → Ser change at position 6, is not recognized by clone 40-2. Clone NI-12, on the other hand, recognizes both viral peptides but not the MOPC 21 V_H 49-58 peptide. These data suggest that the difference in the amino acids occupying positions accessible to the TCR could account for the variability in the recognition of these related decamer peptides.

The cross-reactivity of virus-specific CTLs for an Ig V_H site raises the possibility that the CTL repertoire to foreign

antigens might be regulated at the level of Ig V_H expression. The K^d-restricted CTL response to the JHA 210-219 site is weak relative to the vigorous K^d-restricted CTL responses to the JHA 204-212 site and the NP 147-158 site. More than 20 H-2^d haplotype mice of different strains have been examined by us and show a consistent pattern of weak responsiveness to the JHA 210-219 site. (Myers-Powell, B. A., and S. Tykodi, unpublished data). This is not due to defective processing of the JHA 210-219 site, as mice immunized with a viral vector expressing the preprocessed 210-219 site also mount a weak response (Myers-Powell, B. A., manuscript in preparation). Rather, this weak response may reflect a limited repertoire of CTL precursors available to respond to the JHA 210-219 site. Consistent with this view is the apparent limited heterogeneity in the response to the JHA 210-219 site in bulk cultures from individual mice reported here. As shown in Fig. 6, CTLs from one animal exhibits a fine specificity similar to clone 40-2 with recognition of the JHA 210-219 epitope and the MOPC 21 V_H site, but not the AHA 210-219 epitope. Another individual shows the fine specificity of clone NI-12 with recognition of the JHA 210-219 and AHA 210-219 epitopes, but not MOPC 21 V_H reactivity. Three other mice recognize the JHA 210-219 site exclusively.

A limited repertoire diversity to a K^d-restricted CTL epitope was also noted by J.-L. Casanova et al. (38). In that instance, the authors suggested that the structural homology of that epitope for a self-peptide derived from the K^d molecule itself could result in T lymphocyte deletion in the thymus and account for the limited heterogeneity in the response to that epitope. Thus, an intriguing explanation for the limited response to JHA 210-219 is that the expression of the MOPC 21 V_H gene regulates the response to this viral epitope. B cells and their Ig gene products have been proposed as important regulators of T cell responsiveness, and evidence for recognition of Ig gene products by T cells has been reported (22–27). The simplest mechanism to explain the weak response to JHA 210-219 and the variable cross-reactivity for MOPC 21 V_H 49-58 is that the expression of the MOPC 21 V_H gene product by B cell results in cross-tolerance to the JHA 210-219 epitope. Therefore, in four of the five mice examined, expression of the MOPC 21 V_H results in deletion or anergy of the majority of JHA 210-219–reactive T cells including all CTL precursors that cross-react with the MOPC 21 V_H. In one of five individuals, the MOPC 21 V_H gene product is not expressed or not expressed at a sufficient level to completely eliminate all of the MOPC 21 V_H–reactive $CD8^+$ clones.

At present we cannot exclude a positive selection model where the expression of the MOPC 21 V_H gene is required to amplify the clonal repertoire to the JHA 210-219 epitope. In this connection, it is noteworthy that MOPC 21 gene is a member of the 7183 murine V_H gene family. This family is the closest one to the D/J gene segment and may rearrange early in B cell development (36, 39). Thus, the gene family and its individual members could provide an important positive selective pressure in the development of the T cell repertoire to foreign antigen (26, 27). A detailed analysis of MOPC 21 V_H gene expression and JHA 210-219 responsiveness in individual mice is ongoing and should distinguish these models for CTL repertoire regulation.

This report provides, we believe, the first demonstration of cross-reactivity by $CD8^+$ CTL directed to a foreign antigen site for an endogenously processed Ig V_H gene product. This cross-reactivity, which is detectable both at the clonal level and in bulk cultures, seems more than fortuitous. Rather, it may reflect an ongoing process of T lymphocyte repertoire selection and molding by Ig V_H gene products. The germline Ig V_H genes are an obvious source of diverse peptides that could regulate T lymphocyte selection in the thymus or regulate the state of activation or anergy of mature T lymphocyte precursors in the periphery. We do not as yet know if the T lymphocyte repertoire molding by Ig gene products is solely at the level of responses to an individual antigenic epitope or if it can effect the CTL response to a microorganism. Understanding the influence of Ig gene products on T lymphocyte function and repertoire development will be important in understanding the regulation of T lymphocyte responses to foreign antigens during infections and to self-proteins during autoimmune diseases.

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