JOURNAL OF VIROLOGY, Mar. 1986, p. 786–791 Vol. 57, No. 3
0022-538X/86/030786-06$02.00/0
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Recognition of Cloned Influenza Virus Hemagglutinin Gene Products by Cytotoxic T Lymphocytes

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Received 18 June 1985/Accepted 15 November 1985

The influenza A virus hemagglutinin (HA) is an integral membrane glycoprotein expressed in large quantities on infected cell surfaces and is known to serve as a target antigen for influenza virus-specific cytotoxic T lymhocytes (CTL). Despite the fact that HAs derived from different influenza A virus subtypes are serologically non-cross-reactive, the HA has been implicated by previous experiments to be a target antigen for the subset of T cells capable of lysing cells infected with any human influenza A subtype (cross-reactive CTL). To directly determine whether the HA is recognized by cross-reactive CTL, we used vaccinia virus recombinants containing DNA copies of the PR8 (A/Puerto Rico/8/34) (H1N1) or JAP (A/JAP/305) (H2N2) HA genes. When these viruses were used to stimulate HA-specific CTL and to sensitize target cells for lysis by HA-specific CTL, we found no evidence for HA recognition by cross-reactive CTL aside from a relatively small degree of cross-reactivity between H1 and H2 HAs. Results of unlabeled target inhibition studies were consistent with the conclusion that the HA is, at most, only a minor target antigen for cross-reactive CTL.

The influenza A virus hemagglutinin (HA) is a trimeric glycoprotein expressed in large quantities on the surface of infected cells (approximately 10^6 trimers per cell) and mature virions (approximately 10^3 trimers per virion). The HA consists of two disulfide-linked polypeptide chains, termed HA1 and HA2. Antigenic variation in the HA1 subunit is largely responsible for the well-known ability of influenza A viruses to escape existing host immunity and cause epidemic and pandemic disease. Consequently, the antigenicity of the HA has been intensively studied, and a number of antigenic sites recognized by antibodies have been identified on the three-dimensional structure of the molecule (5, 23). In contrast, the antigenic sites involved in T-cell recognition of the HA are poorly characterized. This is particularly true for the subset of T cells which mediates destruction of histocompatible target cells. Such cytotoxic T lymphocytes (CTL) are a prominent feature of both animal and human immune responses to influenza, and the available evidence suggests they might play a role in limiting viral replication and dissemination (12, 13, 24). Early studies have indicated that anti-influenza CTL can be divided into two major categories: those specific for immunization against closely related strains (specific) and those able to lyse cells infected with any influenza A virus (cross-reactive) (7, 28). The possibility that some or all of the cross-reactive CTL recognize the HA has been a major interest to investigators, since anti-HA serum antibodies elicited in response to influenza A viruses of different subtypes are not cross-reactive (human type A influenza viruses are grouped into three subtypes, designated H1N1, H2N2, and H3N2. H1, H2, and H3 and N1 and N2 each designate non-cross-reactive serotypes of the HA and neuraminidase molecules, respectively).

With the availability of cloned HA genes in suitable expression vectors, it is now possible to examine CTL recognition of cells expressing the HA in the absence of other influenza virus proteins and to determine directly whether cells expressing the isolated HA are recognized by cross-reactive CTL. We have recently shown that H2-VAC, a recombinant vaccinia virus (VAC) containing the HA gene of A/JAP/305 (H2N2) (JAP), is able to stimulate murine CTL which recognize JAP-infected cells and that H2-VAC cells are able to serve as targets for JAP-induced CTL (2). Inoculation of mice with H2-VAC primed them for a secondary CTL response when the splenocytes were stimulated in vitro with JAP-infected autologous splenocytes. Significantly, JAP-infected target cells were lysed by these CTL far more efficiently than were cells infected with influenza A viruses of other subtypes, which suggested that the HA was not a major target antigen for cross-reactive CTL. In the present report, we have used a H1-VAC, recombinant VAC containing the PR8 (A/Puerto Rico/8/34 [H1N1]) HA gene in addition to H2-VAC to carefully examine the recognition of the HA by cross-reactive CTL.

MATERIALS AND METHODS

Mice. Eight- to 12-week-old male BALB/c mice were purchased from the Jackson Laboratory, Bar Harbor, Maine.

Viruses. Influenza virus strains PR8, NT60 (A/Northern Territory/60 [H3N2]), HK (A/Hong Kong/68 [H3N2]), JAP (A/Japan/305/57 [H2N2]), and B/Lee (B/Lee/40) were grown in the allantoic cavity of 10-day-old embryonated chicken eggs and stored as infectious allantoic fluid at −70°C.

The construction of a VAC recombinant that expresses the HA gene of A/JAP/305/57, referred to as H2-VAC (2), was described previously (17). A new VAC recombinant, H1-VAC, expressing the HA gene of influenza A/PR/8/34, was constructed in a similar manner (14) and will be described in more detail elsewhere. The HA genes of H1-VAC and H2-VAC are regulated by the same VAC promoter.

VAC and the recombinants H1-VAC and H2-VAC were grown in BSC cells for 48 h, sonicated, and stored frozen at −70°C as crude virus stocks in a balanced salt solution containing 0.1% bovine serum albumin (8).

Medium. Iscoved modified Dulbecco modified Eagle medium (IMDM; GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (Hyclone, Logan, Utah) was used for all in vitro cultures.

Immunization. Mice were immunized intraperitoneally

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TABLE 1. Cross-reactive CTL fail ro recognize cells expressing the cloned H2 HA gene product

Effectors (In vivo primary, in vitro secondary)	% Specific ^{51}Cr release from virus-infected target cells[a]									
	JAP		B/Lee		H2-VAC		VAC		H2-VAC plus JAP	
	7:1	2:1	7:1	2:1	7:1	2:1	7:1	2:1	7:1	2:1
NT60 (H3), NT60 (H3)	70	45	5	3	4	1	7	4	64	50
NT60 (H3), WSN (H1)	67	42	3	3	0	0	8	5	60	52
NT60(H3), PR8 (H1)	77	54	8	5	5	3	11	5	67	42
PR8 (H1), NT60 (H1)	76	50	5	3	2	0	5	6	66	49
PR8 (H1), PR8 (H1)	56	39	13	7	9	1	8	5	60	41
JAP (H2), JAP (H2)	84	66	1	2	58	60	9	4	71	66
VAC, VAC	6	5	4	4	65	51	56	44	63	62

[a] Cytotoxicity assays were performed with effector/target ratios of 7:1 and 2:1. Spontaneous release values: JAP, 28%; B/Lee, 9%; H2-VAC, 12%; VAC, 15%; H2-VAC plus JAP, 34%.

with 100 to 300 hemagglutinating units of PR8, JAP, or NT60 virus. Intravenous inoculations of 1×10^8 to 3×10^8 PFU were used for VAC, H1-VAC, and H2-VAC.

Secondary cultures. Splenocytes from immunized mice were used as in vitro responders 2 or more weeks after in vivo priming. Stimulator cells for in vitro secondary responses were obtained from autologous splenocyte populations. Stimulator cells (25×10^6 to 50×10^6) were infected with 2×10^9 egg infectious doses of influenza virus or 1×10^8 to 3×10^8 PFU of VAC. Secondary cultures of 25×10^6 stimulator cells and 50×10^6 responder cells were incubated in 45 ml of IMDM at 37°C in a 10% CO_2 environment. After 6 days, the effectors were harvested and assayed for specific cytotoxicity.

Cytotoxic assay. Cytotoxicity was assayed by using a ^{51}Cr release assay. Targets were prepared by using 10^7 P815 mastocytoma cells infected with 1×10^9 to 3×10^9 egg infectious doses of influenza virus or 1×10^8 to 3×10^8 PFU of VAC. After 1 h of incubation at 37°C, the targets were washed and incubated for 6 h at 37°C in 10 μl of IMDM before ^{51}Cr labeling. After incubation with 250 μCi of sodium chromate, cells were washed twice and diluted to 10^5 cells per ml in IMDM, and 100 μl was added to each well of a 96-well round-bottom microtiter plate. Effector cells, also in 100 μl of IMDM, were added at ratios of 20:1, 6.6:1, 2.2:1, and 0.7:1. Four hours after incubation at 37°C under an atmosphere of 10% CO_2, 100 μl of supernatant was harvested to determine the concentration of ^{51}Cr released. All samples were counted in a rackgamma counter (LKB Instruments, Inc., Rockville, Md.), and percent specific release was calculated as: [(experimental release − spontaneous release)/(total release − spontaneous release)] × 100. Release was measured in counts per minute.

All assays were performed in triplicate. Cold target inhibition assays were performed as described above with the exception that unlabeled target cells were added to the ^{51}Cr-labeled targets at ratios of 50:1, 25:1, 12.5:1, and 6.25:1.

Antibody binding assay. Antibody binding assays were performed on viable PR8- and H1-VAC-infected cells with the monoclonal antibody H37-312 (18) iodinated with ^{125}I by the chloramine T method. The fraction of labeled antibody retaining antigen-binding activity was determined by using viral immunoadsorbant bound to polyvinyl 96-well plates. Target cells' used which had not been labeled with ^{51}Cr which were used in a parallel CTL assay were incubated with dilutions of iodinated antibody for 3 h at 4°C and washed twice before quantitation of bound antibody with an LKB rackgamma counter. The percentage of specifically bound antibody was determined by comparison with uninfected cells or cells infected with H2-VAC. Scatchard analysis of data (9, 16) and indirect immunofluorescence (26) were performed as described previously.

RESULTS

Recognition of H1-VAC- and H2-VAC-infected cells by influenza-specific CTL. To determine whether the HA can serve as a target antigen for cross-reactive CTL, the latter were generated by a variety of immunization protocols and tested for their ability to lyse H2-VAC-infected cells (Table 1). While in all cases, JAP-infected cells were efficiently lysed by cross-reactive CTL, in no case was there significant lysis of H2-VAC-infected cells. The inability of cross-reactive CTL to lyse H2-VAC-infected cells was not due to interference from processes related to vaccinia infection of these cells, since cells coinfected with H2-VAC and JAP were efficiently lysed by both cross-reactive CTL and VAC-specific CTL. The efficient lysis of H2-VAC-infected cells by anti-JAP CTL makes it unlikely that the lack of recognition by cross-reactive CTL was due to either low levels of expression of the HA on H2-VAC-infected cells or to the failure of the HA to assume the proper conformation. Nevertheless, these possibilities were examined in detail by using H1-VAC, a recombinant VAC containing the PR8 HA gene.

The structural integrity of the HA expressed on the surface of H1-VAC-infected cells was assessed by using a panel of 18 HA-specific monoclonal antibodies. The antibodies included in this panel define unique epitopes in the four major antigenic sites of the PR8 HA. Each of these antibodies was found to bind to viable H1-VAC-infected cells, as determined by indirect immunofluorescence (not shown). This finding indicates that the HA expressed on the surface of H1-VAC-infected cells is structurally highly similar, if not identical, to the HA produced during PR8 infection. To quantitate the amount of HA expressed on H1-VAC- or PR8-infected cell surfaces, binding assays were performed on viable cells by using a radioiodinated anti-HA monoclonal antibody. Initial experiments documented that cells infected with a high multiplicity of PR8 expressed approximately five times the amount of HA (3×10^6 molecules per cell) that H1-VAC-infected cells expressed. In additional experiments, it was found that the amount of HA expressed on PR8-infected cell surfaces was proportional to the multiplicity of infection. Based on these experiments, it was possible to choose a PR8 multiplicity of infection such that roughly equivalent amounts of HA were expressed on PR8- and H1-VAC-infected cell surfaces. Cells infected under these conditions were then analyzed in antibody binding and ^{51}Cr

TABLE 2. Failure of cross-reactive CTL to recognize cloned H1 HA is not due to low levels of antigen on H1-VAC-infected cell surfaces

Effectors (In vivo primary, in vitro secondary)	% Specific ^{51}Cr release from virus-infected target cells[a]													
	PR8 (H1)		H1-VAC		JAP (H2)		H2-VAC		H2-VAC plus JAP		None[b]		VAC	
	7:1	2:1	7:1	2:1	7:1	2:1	7:1	2:1	7:1	3:1	7:1	2:1	7:1	3:1
PR8, PR8	63	49	41	18	70	46	14	5	59	37	9	5	6	2
JAP, JAP	62	53	19	18	85	76	61	47	82	70	9	6	6	5
NT60, JAP	68	60	7	4	80	68	8	5	83	75	11	7	7	4
JAP, NT60	62	51	12	6	77	66	8	4	81	67	11	8	8	4

[a] Cytotoxicity assays were performed with effector/target cell ratios of 7:1 and 3:1. Spontaneous release values were all less than 20% of total release.
[b] Uninfected target cells were used.

release assays performed in parallel. Scatchard analysis of the antibody binding data (not shown) revealed that antibody bound to PR8- and H1-VAC-infected cells with nearly identical avidity (H1-VAC K_a, 1.9×10^9 M^{-1}; PR8 K_a, 1.7×10^9 M^{-1}) and that H1-VAC-infected cells expressed slightly more HA (7.1×10^5 molecules per cell) than did PR8-infected cells (6.1×10^5 molecules per cell). The nearly identical binding constants provide additional evidence for the structural integrity of the HA produced by H1-VAC-infected cells. Despite the greater expression of HA on cells infected with H1-VAC than on those infected with PR8, only the latter were lysed by two cross-reactive CTL populations (NT60 [primary]-JAP [secondary], JAP [primary]-NT60 [secondary]) (Table 2). The H1-VAC-infected cells were, however, specifically lysed by CTL generated by PR8 priming and stimulation. As would be expected due to the stimulation of CTL specific for other viral components, anti-PR8 CTL demonstrated more lytic activity against cells infected with PR8, or even with JAP, than against cells infected with H1-VAC. Although cross-reactive CTL induced by priming and stimulation with heterologous viruses did not lyse H1-VAC-infected cells, a small amount of specific lysis was detected with CTL primed and stimulated with JAP. This finding was repeated in three of four additional experiments, but the degree of lysis above control values was never as high as that seen in Table 2.

The failure of cross-reactive CTL to recognize the HA was further demonstrated by using unlabeled cells as inhibitors. While unlabeled JAP-infected cells efficiently inhibited the lysis of labeled JAP-infected cells by cross-reactive CTL, inhibition by H2-VAC-infected cells was not greater than that observed with VAC-infected cells (Fig. 1A). However, the same unlabeled H2-VAC-infected cells efficiently inhibited lysis of labeled H2-VAC-infected cells by JAP-specific CTL (Fig. 1B).

These experiments indicate that the vast majority of cross-reactive CTL induced by in vivo priming and in vitro stimulation of BALB/c splenocytes do not recognize the HA. It does appear, however, that relatively infrequent clones do exist which react with both H2 and H1 HAs.

Priming of cross-reactive CTL by H2-VAC. CTL recognition of the HA was further examined in a number of experiments in which the ability of H2-VAC to prime for a secondary in vitro cross-reactive CTL response was tested. Incubation of splenocytes derived from H2-VAC-infected mice with autologous JAP-infected splenocytes consistently (12 of 12 experiments) resulted in the generation of CTL which lysed JAP-infected cells. In most of these experiments (8 of 12), significant cytotoxicity (defined as twice the specific release observed against B/Lee-infected cells) also was observed against PR8-infected cells, and in slightly less than half of these experiments (5 of 12), target cells infected with viruses (HK, NT60) containing H3 HAs were specifically recognized (one of these experiments was the source of Fig. 3 in reference 2). Significant lysis of HK-infected cells was not observed in the absence of specific recognition of PR8-infected cells.

The specificity of cross-reactive CTL sporadically detectable in H2-VAC-primed JAP-stimulated cultures is illustrated by the experiment shown in Fig. 2A. PR8-infected cells were lysed much less efficiently than were JAP-infected targets, and lysis of HK-infected cells was barely significant. Importantly, cells infected with H2-VAC or H1-VAC were often lysed at equal or higher levels than were cells infected with JAP or PR8, respectively. This demonstrates that most, if not all, of these CTL recognize the HA and provides further evidence for the existence of H1-H2 cross-reactive CTL.

Cross-reactivity between PR8 and JAP HAs was also tested by stimulating H1-VAC-primed splenocytes with JAP. In three of three experiments, these cells had a greater cytotoxic activity for cells infected with PR8 than for JAP-infected cells. This original antigenic sin phenomenon was almost certainly due to stimulation of H1-H2 cross-reactive CTL (Fig. 2B), since H1-VAC-infected cells were lysed at levels almost identical to those observed with PR8-infected cells.

DISCUSSION

In this study, we have examined recognition of the cloned H1 and H2 HA gene products by cross-reactive CTL. Related experiments have been performed by two other groups of investigators, both of whom examined recognition of cloned HA gene products expressed on transfected L cells (H-2^k) by secondarily stimulated in vitro splenocyte cultures. Townsend et al. failed to detect lysis of cells expressing the PR8 HA by CTL primed and stimulated by an influenza A virus containing the H3 HA, and they detected low levels of lysis by JAP-stimulated CTL (20). The transfected targets used by these authors expressed about one-third as much cell surface HA as did the PR8-infected control targets. Braciale et al. observed sporadic lysis of cells expressing the JAP HA by WSN (A/WSN/33 [H1N1])-stimulated CTL (3). They also found that 12 of 13 cloned cross-reactive CTL cell lines failed to lyse these targets and that the one apparently HA-specific, fully cross-reactive line lysed transfected cells only at a low efficiency compared with either lysis of influenza A virus-infected cells or lysis of the transfected targets by subtype-specific anti-HA CTL cell lines. Since the transfected targets expressed roughly 1% of

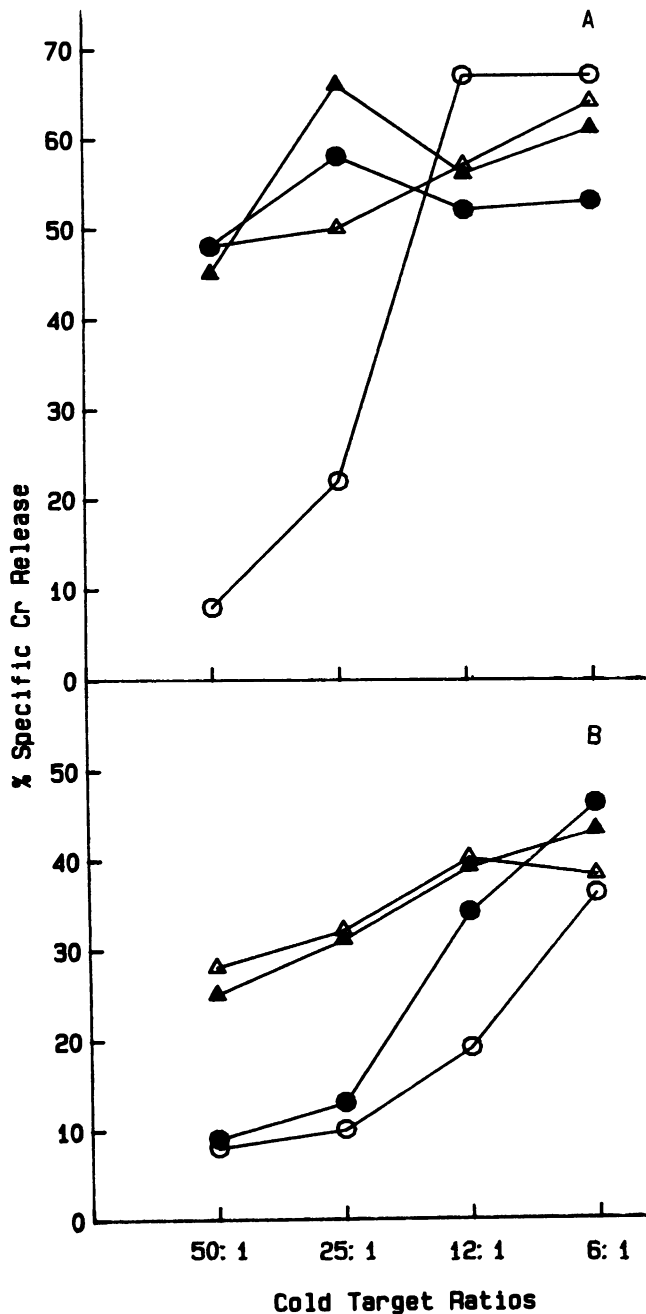

FIG. 1. Unlabeled cells expressing the cloned H2 HA fail to inhibit cross-reactive CTL. Unlabeled cells infected with H2-VAC (●), VAC (▲), JAP (○), and B/Lee (△) were coincubated with ^{51}Cr-labeled target cells at the indicated ratios in cytotoxicity assays. (A) ^{51}Cr-labeled JAP-infected targets, PR8 in vitro-stimulated splenocytes derived from NT60-primed mice. Effector/labeled target ratio, 7:1. (B) ^{51}Cr-labeled H2-VAC-infected targets, JAP in vitro-stimulated splenocytes derived from JAP-primed mice. Effector/labeled target ratio, 3:1.

the amount of HA expressed on infected cell surfaces, the authors suggested that the poor recognition of HA by cross-reactive relative to specific CTL may be due to a lower avidity of these cells for the HA.

In the present study, we have eliminated the problem of low levels of HA expression on target cell surfaces by use of recombinant VACs and have shown that the HA expressed by cells infected with one of these viruses is antigenically indistinguishable from the HA produced by influenza virus-infected cells. Despite this, cross-reactive CTL populations induced by priming and stimulating with various influenza A viruses in a number of permutations failed to lyse cells expressing the H1 or H2 HA. As did Townsend et al., we found that CTL primed and stimulated with JAP lysed cells expressing the H1 HA to a slight extent. Further evidence for a moderate degree of H1-H2 cross-reaction was found in

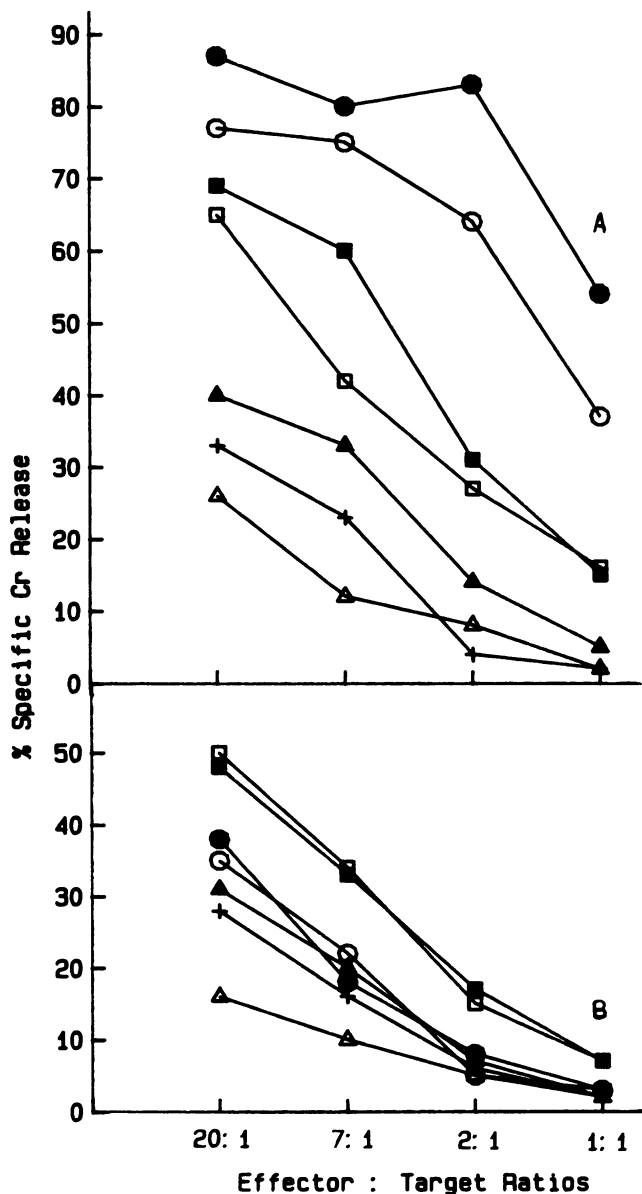

FIG. 2. (A) H2-VAC stimulation of splenocytes derived from JAP-primed mice. Cytotoxicity assays were performed by using the effector/target cell ratios indicated. Symbols (spontaneous release value): ■, H1-VAC (15%); ●, H2-VAC (18%); ▲, VAC (15%); □, PR8 (22%); ○, JAP (33%); +, HK (22%); △, B/Lee (14%). (B) JAP stimulation of splenocytes derived from H1-VAC-primed mice. Cytotoxicity assays were performed by using the effector/target cell ratios indicated. Spontaneous release values were all less than 12% of total release.

experiments in which the ability of H1-VAC and H2-VAC to prime for cross-reactive CTL responses was tested. Using H1-VAC-infected targets, we clearly showed that cross-reactive CTL induced by H2-VAC priming and JAP stimulation did indeed recognize the HA. In a number of experiments, these same effectors were occasionally found to exhibit a small degree of specific lysis against cells expressing an H3 HA. In the absence of target cells expressing a cloned H3 gene product, it is uncertain whether this slight degree of lysis was due to the presence of H2-H3 HA cross-reactive CTL or to CTL specific for some other viral component. Two lines of evidence are consistent with the former possibility. First, the H2-VAC-primed JAP-stimulated CTL recognized cells expressing the cloned HA gene just as well as they recognized cells infected with the homologous influenza virus. Second, these same effector cells have not been found to recognize recombinant VAC-infected target cells expressing the influenza A virus nucleotprotein (unpublished results), an antigen which we (25) and others (20) have shown to be a major target antigen for cross-reactive CTL.

Although the existence of cross-reactive anti-HA CTL must ultimately be proven at the clonal level, it should be noted that the pattern of HA cross-reactivity observed is consistent with the evolutionary relationship of the three human HAs. Thus, the H1-H2 HAs are among the most closely related of 13 known HA subtypes (58 and 79% homologous in HA1 and HA2 subunits, respectively), while the H1-H3 (35 and 53% homologous in HA1 and HA2) and H2-H3 (36 and 50% homologous in HA1 and HA2) HAs represent the least closely related HAs (22). While it does appear that H1-H2 and perhaps H2-H3 cross-reactive CTL clones do exist, it must be emphasized that, due to (i) the small numbers of these clones, (ii) their low affinities for one or both HAs, or (iii) both, the response represents only a minor portion of the cross-reactive CTL activity detected in secondary in vitro cultures. This is in marked contrast to the considerable cross-reactive anti-NP CTL response (20, 25). Based on these results, the HA is at best only a marginal candidate for inclusion in vaccines designed to prime recipients for a cross-reactive CTL response upon influenza A virus challenge.

CTL recognition of the HA is still of major immunological interest, since the HA provides a unique opportunity to compare the recognition of a structurally defined, non-self protein antigen by B cells with that by both helper and cytotoxic T cells. In this context, it is interesting to speculate as to which portion of the HA may be recognized by H1-H2 cross-reactive CTL. Although a weak cross-reaction between H1 and H2 viruses in hemagglutination inhibition tests has been reported (6), which presumably would be due to antibodies recognizing the HA1 subunit, more direct evidence for cross-reactive anti-HA1 antibodies has yet to be presented. In our own experience, none of over 200 anti-H1-HA1 monoclonal antibodies has been found to cross-react with the H2 HA. In contrast, the single monoclonal antibody we have produced which recognizes the PR8 HA2 subunit strongly cross-reacts with the JAP-HA2 (J. Yewdell and W. Gerhard, manuscript in preparation). This finding would be consistent with the higher amino acid homology between HA2 subunits, and with several reports that anti-HA2 antisera cross-react between heterosubtype HA2s (10, 15) (although it should be duly noted that a number of laboratories [1, 4] have failed to detect cross-reactive anti-HA2 antibodies by using both antisera and monoclonal antibodies in carefully performed studies). Further support for preferential cross-reactive recognition of the HA2 by immune cells comes from a recent report by Katz et al. (11), who found that Lyt 1^+2^- T cells, presumably T helper cells derived from virus-primed mice, proliferated more vigorously to isolated heterosubtypic HA2 chains than HA1 chains. Thus, it would seem that both B and T helper cells are more likely to cross-reactively recognize the HA2 subunit than the HA1 subunit. The relevance of these findings to CTL is uncertain, however, since the form of antigen recognized on target cells by CTL is unknown. This includes not only whether CTL recognize altered self determinants (27) on class I major histocompatibility complex determinants but also whether CTL recognize native or processed forms of foreign antigens. While CTL do not appear to recognize the forms of processed antigen recognized by T helper cells (produced by cycling of antigen through cellular endosomes and lysosomes), it is possible that CTL target antigens are produced by other cellular pathways which process endogenously produced cellular proteins. This difference between T helper cells and CTL has precluded the type of fine specificity analysis that has recently been performed for T helper cells by determining the stimulatory capacity of oligopeptides. While some success has been achieved by using antigenic fragments for in vitro stimulation of CTL dervied from virus-primed animals, this approach is limited by two factors. First, unlike studies of T helper cell specificity, in which the relevant parameters of recognition are directly measured (proliferation or IL-2 production), stimulation of cytotoxic activity by antigen provides only indirect evidence for CTL recognition of the same fragment on the target cell. Second, unlike stimulation of T helper cells by oligopeptides, CTL stimulation appears to require the retention of at least some of the antigenic structure present in the intact protein. McFarland et al. (R. I. McFarland, B. Dietzschold, and H. Koprowski, Mol. Immunol., in press) obtained stimulation of rabies-specific CTL by using cyanogen bromide fragments of G protein which maintained reactivity with neutralizing monoclonal antibodies. Reduction and alkylation of the fragment resulted in concomitant loss, antibody reactivity and CTL stimulation. Similarly, Wabuke-Bunoti et al. (21) reported stimulation of anti-influenza CTL by using a synthetic peptide corresponding to amino acid residues 181 through 204 in the JAP HA1 subunit which maintained reactivity with monoclonal antibodies recognizing the intact HA molecule.

An alternative approach to CTL fine specificity analysis is the use of genetically engineered target cells expressing nominal antigen fragments. This method has been successfully applied to the study of CTL recognition of SV-40 T antigen (19) and, recently, to influenza virus NP (19a). It offers the attractive feature of providing a direct measure of CTL recognition of nominal antigen fragments. While it may eventually be limited to the study of only a few nominal antigens by the apparent requirement of CTL to recognize native protein structure, it could provide significant information regarding the cellular events involved in the creation of CTL target structures.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grants AI-14162, AI-20338, and NS-11036 from the National Institutes of Health.

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