MOST INFLUENZA A VIRUS-SPECIFIC MEMORY CYTOTOXIC T LYMPHOCYTES REACT WITH ANTIGENIC EPITOPES ASSOCIATED WITH INTERNAL VIRUS DETERMINANTS

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Both antibodies and cytotoxic T cells (CTL)[1] play a role in the host defense against influenza A virus infections (1–8). The specificity of anti-influenza A antibodies has been studied in detail. Thus, it has been shown that most anti-influenza A antibodies are directed against the virus surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) (9, 10). Antibodies with this specificity were prevalent in immune sera from influenza A virus-immunized volunteers as well as in a panel of monoclonal antibodies secreted by hybridomas derived from fusions of spleen cells of immunized mice (10). In the elegant studies of Carton and co-workers (11), antigenic epitopes recognized by anti-HA antibodies were localized to defined stretches of amino acids on the tip of the HA molecule.

The specificity of influenza virus-immune T cells is much less clear-cut. CTL stimulated with influenza A virus in bulk cultures distinguish between influenza A and B viruses but show complete cross-reactivity towards all influenza A virus-infected target cells (12, 13). Likewise, Owen et al. (14) found cross-reactivity of CTL in limiting dilution (LD) primed against the influenza A virus, PR8 (H1N1), when tested on target cells infected with PR8 or another influenza A virus, X31 (H3N2). In addition, a number of cytotoxic or proliferating T cell clones were established in long-term tissue culture and analyzed for their fine specificity (15–20). One proliferating human T cell clone recognized antigenic determinants of chemically synthesized peptides of the influenza HA. The response was mapped to one peptide located at the carboxy-terminus of the HA1 molecule (19). A murine, proliferating T cell clone was shown to recognize a segment of nine amino acids that is located in the globular head region of the HA molecule (20). One CTL clone was reported to show exclusive specificity for the virus polymerase P3 (17), while most other CTL clones showed either a characteristic pattern

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[1] *Abbreviations used in this paper:* CAS, Con A-induced supernatant; CML, cell-mediated lympholysis; CTL, cytotoxic T lymphocyte(s); CTL-p, CTL-precursor(s); HA, hemagglutinin; HAU, hemagglutination unit(s); LD, limiting dilution; M, matrix; NA, neuraminidase; PM, probability of monoclonality; SN, supernatant.

of reactivity (15) or cross-reacted on *all* influenza A virus-infected target cells (15, 16).

Long-term T cell clones are highly selected in culture and do not provide any information on the fraction of T cell clones in a normal T cell population that show specificity or cross-reactivity. A correct estimate of the T cell repertoire, however, might be of importance in determining the role of CTL in recurrent infections with different influenza A viruses. To this end, we have recently established (21) a method of evaluating the frequency and specificity of virus-specific short-term CTL clones in LD. Using this method, we have investigated the memory CTL response in C57BL/6 (B6) mice for four influenza A viruses. Surprisingly, our data indicate that viral determinants other than HA or NA are recognized by most memory CTL.

Material and Methods

Mice. Female B6 (H-2^b) mice were purchased from the Zentralinstitut für Versuchstiere, Hannover, Federal Republic of Germany (FRG). The mice were used at 5–8 wk of age.

Medium. All cultures were performed in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with L-glutamine (2 mM final concentration), streptomycin (100 μg/ml), and penicillin (100 U/ml), Hepes buffer (25 mM final concentration), 2-mercaptoethanol (10^{-5} M), and 10% fetal calf serum.

Preparation of Concanavalin A-induced Supernatant (CAS) from Rat Spleen Cells. Spleen cells from Sprague-Dawley rats were used to prepare T cell growth factor containing supernatants (SN) as described previously (21).

Influenza A Viruses. All influenza virus strains were obtained from Dr. J. J. Skehel, World Influenza Centre, National Institute for Medical Research, Mill Hill, London. These strains are described in Table I. All virus strains were grown in embryonated eggs. The titers of virus-containing allantoic fluid are given in hemagglutination units (HAU). Virus aliquots were stored at −70°C.

Immunization of Mice. Mice were inoculated intraperitoneally with 50 HAU of influenza A virus in 100 μl of phosphate-buffered saline. Their spleen cells were used 7 d later as responder cells in LD cultures.

Stimulator Cells for LD Cultures. Spleen cells from normal syngeneic mice were infected with the indicated influenza strain (10 HAU per 10^6 nucleated cells). Infected cells were incubated at 37°C for 90 min, washed extensively, incubated at room temperature for 2 h, and irradiated (2,200 rad) before culturing with the responder cells.

LD Cultures. Graded numbers of responder spleen cells (500–8,000 or 250–2,000 cells/well) from immune mice were plated in 96-well, round-bottom microtiter plates (No. 650101; Greiner, Nürtingen, FRG.). 24 replicates were plated per cell concentration. 10^6 stimulator cells were added to each well. Cells were cultured in 150 μl medium supplemented with 10% CAS. 24 control wells lacked responder cells. All cultures were fed on

TABLE I
Influenza A Virus Strains Used

Virus strains	Serotype		Internal viral proteins derived from:
	Hemagglutinin	Neuraminidase	
A/Hong Kong/31 (X31)	H3	N2	PR8
A2/Aichi/2/68 (Aichi)	H3	N2	Hong Kong
A/Port Chalmers/1/73 (PC)	H3	N2	Hong Kong
A/Puerto Rico/8/34 (PR8)	H1	N1	PR8

day 4 with 50 μl of the same medium. Cytotoxicity assays were carried out on day 7 of LD culture. For priming in vivo and restimulation in LD, the same virus strain was used.

Cytotoxicity Assay. Effector cells were tested for cytotoxic activity on influenza virus-infected or uninfected EL4 (H-2^b) T lymphoma target cells. 2×10^6 EL4 cells were infected with 50 HAU of the particular virus strain in 250 μl medium containing 200 μCi of $Na^{51}CrO_4$ (New England Nuclear, Dreieich, FRG.) and incubated for 90 min at 37°C. Cell-mediated lympholysis (CML) assays were carried out 3 h after infection with virus.

Four replica plates were derived from each LD culture plate. The contents of each microculture was mixed and aliquots of 45 μl were distributed onto four assay plates (No. 650101; Greiner). The contents of the control wells lacking responder cells were split in the same way. All plates were centrifuged (10 min at 100 *g*) and the SN medium was discarded. The cells were then resuspended, 2×10^3 ^{51}Cr-labeled target cells in 200 μl medium were added to each well, and the plates were centrifuged for 5 min at 30 *g*. After 4-h incubation, the plates were centrifuged for 10 min at 100 *g*. 100 μl of the SN was removed and the ^{51}Cr content counted in a gamma counter (Auto-Gamma Scintillation Spectrometer 5260/TT; Packard Instrument Co., Downers Grove, IL). This counter was connected to a teletypewriter (model 546; Packard Instrument Co.). Maximum ^{51}Cr release was determined by counting the ^{51}Cr release in the SN of target cells frozen and thawed five times (high control). Spontaneous ^{51}Cr release was determined in the SN of target cells incubated with the contents of control wells (containing stimulator cells only). Spontaneous ^{51}Cr release was 7–14% of the maximum release from virus-infected target cells and 6–11% from uninfected target cells.

Specificity Analysis. On day 7 of LD culture, four replica plates were derived from a culture plate (see above). Fraction 1 was tested on EL4 target cells infected with the same virus strain used for priming in vivo and stimulation in LD. Fraction 2 was tested on uninfected EL4 target cells. The frequency measured on uninfected target cells was too low to calculate (data not shown) (21). Fractions 3 and 4 were tested on EL4 target cells infected with heterologous virus strains.

All experiments were performed in a crisscross fashion; i.e., homologous and heterologous virus strains were used for priming in vivo, restimulation in LD, and infection of target cells. Thus, proper lysis of all target cells could be assured.

Statistical Analysis. All data were processed using a computer program (21). CML was termed positive when its cpm was greater than that of the low controls plus 3 SD. The maximum likelihood method was used to estimate the CTL precursor (CTL-p) frequencies; the 95% confidence limits of the frequencies were calculated. The chi square value for goodness of fit to the Poisson model and the probability (*P*) corresponding to the chi square value were determined. A *P* value >0.05 indicates that the data from a particular LD assay are consistent with single-hit kinetics of the Poisson model. Only data from LD experiments that gave a *P* value >0.05 for CTL tested on EL4 target cells infected with the homologous virus strains were used in this study. In addition, the probability of monoclonality (PM) was determined for each cell concentration in LD (21). The global test for homogeneity of independent slopes (22) was used to determine whether or not resulting frequencies differed at the significance level of $\alpha = 0.05$.

Results

Frequency and Specificity of X31- and PC-stimulated CTL. Influenza A virus-primed CTL restimulated in bulk cultures against influenza A virus-infected cells show a broad cross-reactivity on all target cells infected with a panel of different influenza A viruses (12, 13). Our experiments confirm these data for B6 cells, the mouse strain used in all LD experiments reported here (data not shown).

We tested whether the extensive cross-reactivity of CTL against influenza A viruses seen with CTL in bulk culture is also seen with CTL in LD. B6 mice were primed in vivo and restimulated in vitro with X31. The frequency and specificity of the CTL in LD was then determined on target cells infected with

X31 or PC virus. These two viruses were chosen because they are both of the H3N2 serotype (see Table I).

The CTL-p frequencies of X31-stimulated cells tested on X31-infected EL4 target cells ranged from 1:604 to 1:7,690. In contrast, the frequencies measured on PC-infected EL4 target cells were >4.9-fold lower (Table II and Fig. 1). Similar results were obtained with CTL stimulated with PC (Table II, Fig. 1). The CTL-p frequencies of cells stimulated with PC and tested on PC-infected EL4 target cells were always significantly higher (>6.3-fold) than the frequencies determined on X31-infected EL4 target cells.

These data agree with the specificity determined for microcultures with CTL that show a high PM (21). As shown in Table III, 80.5% (PM > 0.75) or 75% (PM > 0.85) of X31-stimulated CTL clones were specific for the target cells infected with the homologous virus strain, X31. 85.7 and 100% of PC-stimulated CTL clones (PM > 0.75 and >0.85, respectively) showed specificity for PC-infected EL4 target cells. Thus, although X31 and PC are both of the H3N2 serotype, most CTL clones showed exquisite specificity for their respective stimulating virus. This result is in contrast to the cross-reactivity observed for CTL generated in bulk cultures. Furthermore, it suggests that the surface glycoproteins HA and NA do not play a dominant role in the memory CTL response as opposed to the B cell response.

Although PC and X31 are both of the H3N2 serotype, the amino acid analysis of the HA from these two viruses reveals multiple exchanges (23). To test the appropriately matched viruses, we extended our analysis to the Hong Kong strain Aichi (H3N2), which donated HA and NA to X31 (H3N2) and therefore

TABLE II

*Frequencies of X31- and PC-stimulated CTL-p Measured on EL4 Target Cells Infected with X31 or PC Virus**

Exp.	Stimulating virus	CTL precursor frequency (1/n) (range)		Comparison of frequencies	Ratio of frequencies‡
		EL4 target cells infected with virus strain:			
		X31 (H3N2)	PC (H3N2)		
1	X31 (H3N2)	2,478 (1,671–4,795)	30,352 (19,013–75,206)	D§	12.2
2		7,690 (5,258–14,312)	TL‖	D	—
3		4,941 (3,600–7,874)	TL	D	—
4		604 (458–884)	5,212 (3,811–8,245)	D	8.6
5		1,095 (846–1,550)	5,396 (3,580–10,955)	D	4.9
6	PC (H3N2)	5,683 (4,125–9,130)	895 (682–1301)	D	6.3
7		20,196 (12,880–46,752)	515 (391–756)	D	39.2
8		TL	417 (312–627)	D	—
9		2,854 (2,044–4,729)	366 (279–529)	D	7.8
10		7,547 (4,741–18,488)	1,021 (783–1465)	D	7.4

* LD cultures were carried out as described in Material and Methods.

‡ Ratio of frequency was calculated as (frequency on target cells infected with the homologous virus strain [X31 or PC])/(frequency on target cells infected with the heterologous virus strain [PC or X31]).

§ D, different frequencies according to the global test for homogeneity of independent slopes Ø at the 0.05 level.

‖ TL, too low to calculate.

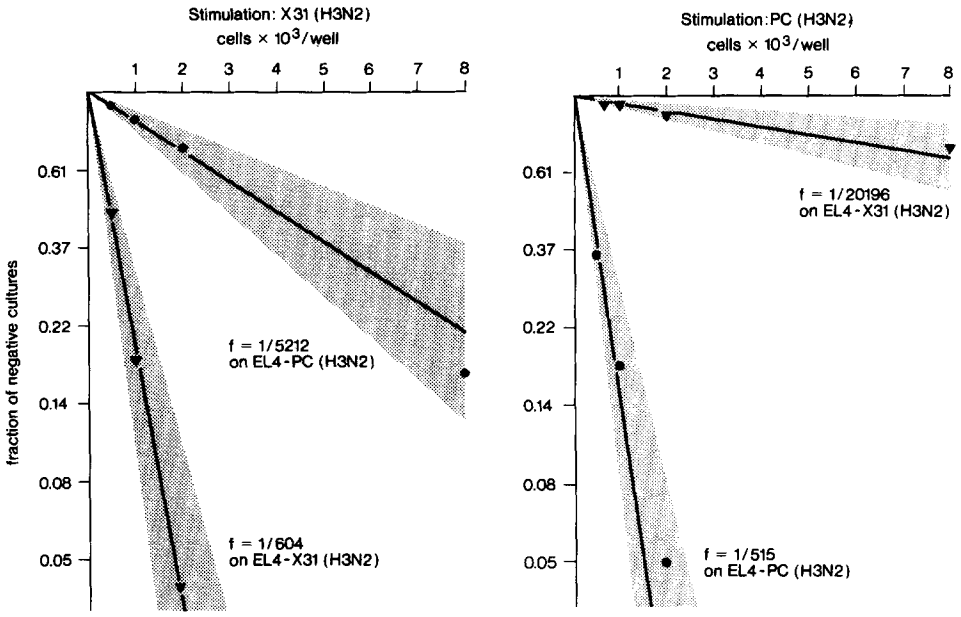

FIGURE 1. Frequencies (f) of X31-stimulated (*left*) and PC-stimulated (*right*) CTL-p in B6 mice tested on X31-infected (▼) and PC-infected (●) EL4 target cells. (Data are from crisscross experiments, Nos. 4 and 7, in Table II.) Cells from virus-primed (X31 and PC) mice were restimulated in LD with their respective virus-infected syngeneic stimulator cells. On day 7 of LD, CML was performed on virus-infected (X31 and PC) and uninfected EL4 target cells (see Material and Methods).

TABLE III

*Specificity of X31- and PC-stimulated LD CTL Clones Tested on EL4 Target Cells Infected with X31 or PC Virus**

Stimulating virus	Pattern of reactivity	^{51}Cr release on target cells:		Probability of monoclonality			
				>0.75		>0.85	
		EL4-X31	EL4-PC	Number of clones	Clones showing pattern of reactivity	Number of clones	Clones showing pattern of reactivity
					%		%
X31 (H3N2)	1	+	−	55	80.5	18	75
	2	+	+	13	19.9	6	25
PC (H3N2)	1	−	+	42	85.7	6	100
	2	+	+	7	14.3	0	0

* Data were pooled from experiments shown in Table II.

carries identical surface glycoproteins.

Frequency and Specificity of X31- and Aichi-stimulated CTL. The results shown in Table IV confirm the above findings. X31 stimulation led to higher CTL-p frequencies on X31-infected than on Aichi-infected EL4 target cells (>3.9-fold) (see Fig. 2). The specificity analysis (Table V, Fig. 3) supports the data of the

TABLE IV

*Frequencies of X31-stimulated CTL-p Measured on EL4 Target Cells Infected with X31 or Aichi Virus**

Exp.	Stimulating virus	CTL precursor frequency (1/n) (range)		Comparison of frequencies	Ratio of frequencies‡
		EL4 target cells infected with virus strain:			
		X31 (H3N2)	Aichi (H3N2)		
1	X31 (H3N2)	1,040 (784–1,545)	4,011 (2,761–7,324)	D§	3.9
2		1,027 (786–1,482)	4,656 (3,152–8,906)	D	4.5
3		6,115 (3,957–13,449)	TL‖	D	—
4		14,742 (9,756–30,153)	TL	D	—
5		7,398 (5,299–12,251)	TL	D	—

* LD cultures were carried out as described in Material and Methods.

‡ Ratio of frequency was calculated as (frequency on target cells infected with the homologous virus strain [X31])/(frequency on target cells infected with the heterologous virus strain [Aichi]).

§ D, different frequencies according to the global test for homogeneity of independent slopes Ø at the 0.05 level.

‖ TL, too low to calculate.

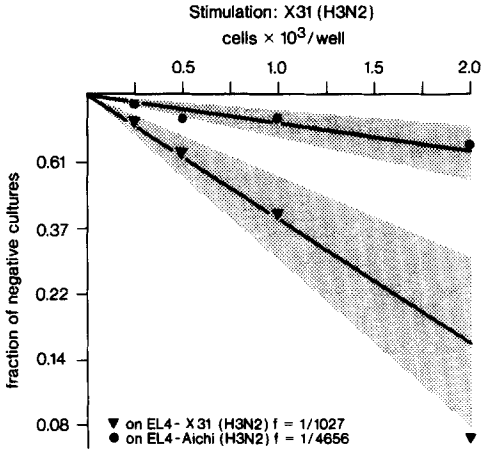

FIGURE 2. Frequencies (f) of X31-stimulated CTL-p in B6 mice tested on X31-infected (▼) and Aichi-infected (●) EL4 target cells. (Data are from experiment 2 in Table IV.) The same method as in Fig. 1 was used.

frequency analysis: 90.7 and 92.9% of the CTL clones (PM > 0.75 and >0.85, respectively) were specific for EL4 target cells infected with the homologous H3N2 virus strain, X31. Thus, only a few X31-stimulated CTL clones (<10%) were able to lyse Aichi-infected target cells. To demonstrate that these latter cells were suitable target cells, all experiments included LD cultures using Aichi-stimulated CTL. Again, Aichi stimulation led to much higher CTL-p frequencies on Aichi-infected (1:4,752 to 1:21,097) than on X31-infected EL4 target cells (too low to calculate). Furthermore, 86 and 97% of the CTL clones showed specificity for EL4 target cells infected with Aichi, and only 14 and 3% of the clones (PM > 0.75 and >0.85, respectively) cross-reacted on X31-infected target cells.

Frequency and Specificity of X31-stimulated CTL Tested on X31- and PR8-infected

TABLE V

*Specificity of X31-stimulated LD CTL Clones Tested on EL4 Target Cells Infected with X31 or Aichi Virus**

Stimulating virus	Pattern of reactivity	^{51}Cr release on target cells:		Probability of monoclonality			
				>0.75		>0.85	
		EL4-X31	EL4-Aichi	Number of clones	Clones showing pattern of reactivity	Number of clones	Clones showing pattern of reactivity
					%		%
X31 (H3N2)	1	+	−	49	90.7	26	92.9
	2	+	+	5	9.3	2	7.1

* Data were pooled from experiments shown in Table IV.

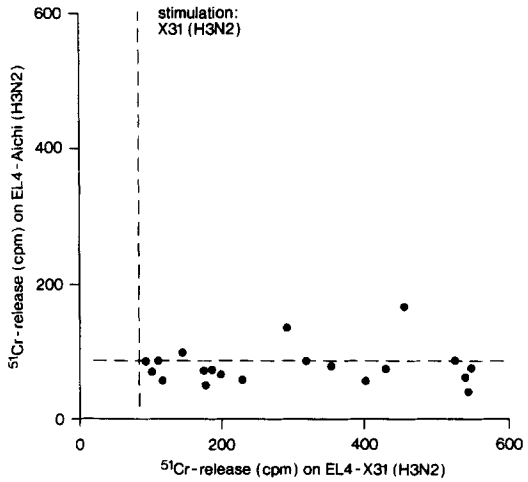

FIGURE 3. Cytotoxic activity of CTL clones (PM > 0.75) stimulated with X31 virus, tested on X31-infected (abscissa) and Aichi-infected (ordinate) EL4 target cells. (Data are from experiments 1 and 2 in Table IV.) The same method as in Fig. 1 was used.

Target Cells. The above experiments again suggest that viral determinants other than HA and NA are the dominant antigenic epitopes recognized by cross-reactive memory CTL. Such antigenic epitopes could be associated with internal virus determinants. We therefore tested X31-stimulated CTL on EL4 target cells infected with PR8. PR8 (H1N1) is serologically different from X31 (H3N2), but shares internal virus components with X31 (Table I) (24). Similar frequencies were obtained for X31-stimulated CTL-p tested on X31- and PR8-infected EL4 target cells (Table VI, Fig. 4). The specificity found for X31-CTL clones supports this result: >80% of the clones (PM > 0.75 and >0.85, respectively) lysed both target cells (Table VII, Fig. 5). These data show that memory CTL cross-reactivity is due to recognition of antigens associated with internal viral determinants.

TABLE VI

*Frequencies of X31-stimulated CTL-p Measured on EL4 Target Cells Infected with X31 or PR8 Virus**

Exp.	Stimulating virus	CTL precursor frequency (1/n) (range)		Comparison of frequencies	Ratio of frequencies‡
		EL4 target cells infected with virus strain:			
		EL4-X31 (H3N2)	EL4-PR8 (H1N1)		
1	X31 (H3N2)	8,095 (5,778–13,517)	8,314 (5,897–14,089)	NSD§	1.03
2		589 (450–854)	611 (467–886)	NSD	1.04
3		1,040 (784–1,545)	1,147 (880–1,647)	NSD	1.1
4		1,172 (893–1,702)	893 (889–1,269)	NSD	0.76
5		1,027 (786–1,482)	1,207 (918–1,761)	NSD	1.18

* LD cultures were carried out as described in Material and Methods.

‡ Ratio of frequency was calculated as (frequency on target cells infected with the homologous virus strain [X31])/(frequency on target cells infected with the heterologous virus strain [PR8]).

§ NSD, not significantly different frequencies according to the global test for homogeneity of independent slopes Ø at the 0.05 level.

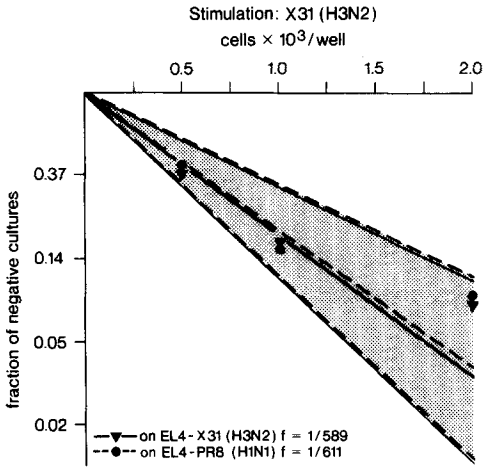

FIGURE 4. Frequencies (f) of X31-stimulated CTL-p in B6 mice tested on X31-infected (▼) and PR8-infected (●) EL4 target cells. (Data are from experiment 2 in Table VI.) The same method as in Fig. 1 was used.

Discussion

The main finding of this paper is that most memory influenza A virus-specific CTL clones react with antigenic epitopes associated with other viral determinants than the influenza A virus surface glycoproteins HA and NA. The CTL reactivity on syngeneic target cells infected with the influenza A virus strains Aichi (H3N2), PR8 (H1N1), or recombinant strain X31 (H3N2) indicates that most antigenic epitopes recognized are associated with internal virus determinants. We used these viral strains in our experiments since X31 and PR8 share the internal, while X31 and Aichi share the external, viral determinants (Table I). In each case in which the stimulating virus shared the internal determinants with the virus infecting the CTL target cells, extensive CTL cross-reactivity was observed. These data agree with those of Owen et al. (14). In contrast, when the internal

TABLE VII

*Specificity of X31-stimulated LD CTL Clones Tested on EL4 Target Cells Infected with X31 or PR8 Virus**

Stimulating virus	Pattern of reactivity	^{51}Cr release on target cells:		Probability of monoclonality			
				>0.75		>0.85	
		EL4-X31	EL4-PR8	Number of clones	Clones showing pattern of reactivity	Number of clones	Clones showing pattern of reactivity
					%		%
X31 (H3N2)	1	+	−	11	19.3	6	18.8
	2	+	+	46	80.7	26	81.2

* Data were pooled from experiments shown in Table VI.

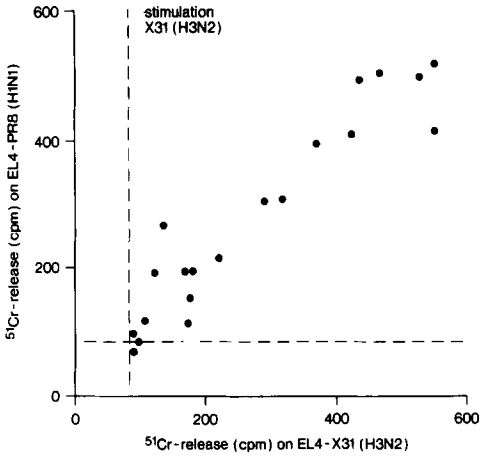

FIGURE 5. Cytotoxic activity of CTL clones (PM > 0.75) stimulated with X31 virus, tested on X31-infected (abscissa) and PR8-infected (ordinate) EL4 target cells. (Data are from experiments 3 and 5 in Table VI.) The same method as in Fig. 1 was used.

virus determinants were different between the priming virus and the virus used to infect the target cells, although there was complete homology of the external virus determinants, HA and NA, we observed almost exclusive CTL specificity for the priming virus. In preliminary experiments, similar results were obtained by intranasal priming. In addition, no differences between the B6 and the BALB/c mouse strain were observed. This specificity of memory CTL clones in LD microcultures is clearly different from the cross-reactivity observed with memory CTL stimulated in bulk cultures and tested on influenza A virus-infected target cells.

A precedent for the reactivity seen in our LD experiments was observed in a few long-term CTL clones against determinants distinct from HA and NA, and associated with internal viral proteins, e.g., the virus polymerase P3 (17) or the viral nucleoprotein (A. Townsend and J. Skehel, personal communication). In addition, long-term CTL clones were found to react with (*a*) target cells infected with the influenza A virus used for selection of such clones from bulk cultures,

(*b*) influenza A virus subtypes, and (*c*) cross-reactive determinants on target cells associated with all influenza A viruses (15, 16). Such long-term CTL clones are highly selected and do not provide any information on the CTL repertoire. Our LD experiments, however, describe the number of CTL of a particular reactivity within the whole T cell population, since selection of clones is minimal.

The reactivity of most memory CTL clones with antigenic epitopes associated with internal virus determinants differs from the reactivity of most anti-influenza A antibodies. Most antiviral antibodies are HA specific (10) and seem to be directed towards sites on the tip of the HA molecule (11, 25). A fraction of CTL clones (<20%) was not directed against antigens associated with internal viral determinants. Such CTL might share specificity with antibodies and correspond to CTL induced in vitro with purified virus HA (26). These CTL, however, represent a minor fraction of the total memory CTL population.

The antigenic epitopes recognized by most memory CTL are associated with internal viral determinants. How could such determinants be generated on the stimulator or target cell membrane? Several possibilities have to be considered: The virus preparations used for priming and infection of stimulator and target cells might contain disrupted virus particles. Internal viral antigens in such preparations might be immunogenic and form stimulator and target cell antigens by adsorption to or integration into the cell membrane (27, 28). *De novo* synthesis of viral proteins not assembled into complete viruses might lead to membrane exposure of internal virus antigens. CTL might recognize these antigens as such or as allosteric determinants of cell surface glycoproteins formed by interaction with these antigens. Alternatively, internal components of the infecting virus might specifically alter cell membrane glycoproteins, e.g., the major histocompatibility molecules, from within the cell, by influencing their synthesis and pattern of glycosylation (29). A further possibility is that antibodies produced during a concomitant B cell response cover determinants on HA and N which could otherwise be recognized by CTL.

Target cell antigens could be expressed in low numbers that are nonetheless sufficient for CTL recognition, but at too low a quantity to allow blocking of CML by monoclonal antibodies. Blocking experiments were indeed unsuccessful with monoclonal antibodies against the influenza A matrix (M) protein. M proteins were expressed on the target cell surface at $<2 \times 10^3$ molecules per cell (30). These experiments failed to determine, however, whether the M protein could function as a suitable target cell antigen. They are therefore inconclusive and do not exclude a role of M protein in CTL recognition.

Our finding of a hitherto unobserved distribution of specificities of influenza A-reactive memory CTL for antigenic epitopes associated with internal virus proteins is based on experiments with a set of four appropriately defined influenza A viruses. This specificity is different from the cross-reactivity of CTL in bulk culture against all influenza A viruses and may be generalized to other influenza A viruses not included in this study. If CTL play a decisive role in defense against or recovery from influenza A infections (7), it remains to be investigated whether heterotypic immunity against all influenza A subtypes, comparable to T cell reactivity in bulk culture, is more relevant than specific responses comparable to our data. If the latter were the rule, T cell immunity

against a new virus infection should mainly be observed in cases where the previous encounter had occurred with a virus with homologous internal virus determinants.

Summary

This paper shows that most murine (C57BL/6) influenza A virus-specific memory cytotoxic T lymphocyte (CTL) clones tested in limiting dilution did not react with the influenza A virus surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). The lysis of syngeneic target cells infected with the influenza A virus strains, Aichi (H3N2), PR8 (H1N1), or recombinant strain X31 (H3N2) indicates that most antigenic epitopes recognized are associated with internal virus determinants. X31 and PR8 share the internal, and X31 and Aichi the external, viral determinants. Extensive CTL cross-reactivity was observed in experiments with target cells infected with virus carrying internal determinants homologous with the priming virus. In contrast, when the internal viral determinants differed between the priming virus and the virus used to infect the target cells, and although HA and NA were homologous, we found almost complete CTL specificity for the priming virus. Thus, the predominant reactivity of influenza A virus-specific CTL differs from that of anti-influenza A antibodies, which are primarily directed towards epitopes on the virus surface glycoproteins. This finding may be relevant for the role of influenza A virus-specific CTL in recurrent infections with different influenza A viruses.

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