EXTENSIVE DIVERSITY IN THE RECOGNITION OF INFLUENZA VIRUS HEMAGGLUTININ BY MURINE T HELPER CLONES

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An essential feature of the immune system is its ability to recognize and respond to a diverse spectrum of foreign antigens encountered in the environment. Two kinds of antigen specific receptors are used, the Ig receptor on B cells and the antigen-specific receptor on T cells. In contrast to Ig, which binds antigen directly, the T cell antigen receptor binds antigen only in association with self MHC-encoded gene products (1, 2). Despite this functional difference between T and B cell antigen recognition, recent molecular studies (3–6) on the T cell antigen receptor have shown considerable structural homology with Ig and similar mechanisms for the generation of antibody and T cell receptor diversity.

At the cellular level, the diversity of Ig molecules generated at the DNA level is reflected by the ability of B cells to recognize an almost unlimited number of antigenic determinants (7–9). However, Th cells are thought to have a much more restricted repertoire (10). Studies with T cell clones specific for myoglobin (11, 12), lysozyme (13), and cytochrome c (14) have suggested that Th cells recognize a limited number of immunodominant carrier epitopes. The demonstration of T cell determinants distinct from antibody-combining sites (10, 15, 16) has been explained by the T cell requirement for antigen processing (17), which allows recognition of linear or unexposed determinants, in contrast to direct binding to exposed conformational determinants by B cells. However, in view of the extensive germline and combinatorial diversity demonstrated in genes coding for the variable region of the T cell receptor, it is difficult to explain the apparent limited number of T cell specificities so far demonstrated from studies of T cell clones specific for an individual antigen. The majority of studies with T cell clones have focused on synthetic antigens or conserved globular proteins. The use of an infectious agent such as influenza virus, which permits priming by infection rather than by the usual footpad route using adjuvant, may reveal a somewhat different pattern of T cell recognition.

The immune response to influenza virus involves a T cell–dependent production of neutralizing antibodies directed against the hemagglutinin (HA)[1] surface glycoprotein (18, 19). The HA molecule undergoes continuous antigenic variation as a result of point mutations in the virus genome (antigenic drift) which

[1] *Abbreviations used in this paper:* HA, hemagglutinin; sup, supernatant.

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amounts to three or four amino acid substitutions per year (20, 21). The antibody recognition of HA has been well studied (22, 23), and extensive diversity in mAb specificities have been shown. Anti-HA antibodies are HA subtype–specific, and recognize determinants on five distinct sites found in variable regions of the molecule (20–23). In contrast, studies with cloned populations of human (24, 25) or murine (26, 27) T cells have suggested that T cell recognition is more limited, and major epitopes have been identified in regions of the HA molecule conserved within a subtype.

We have examined the fine specificity of a large panel of HA-specific, class II–restricted T cell clones established from individual influenza virus–primed mice. Evidence is presented which suggests extensive diversity in the T cell recognition of HA, and includes specificities for determinants in variable antibody-combining regions of the molecule.

Materials and Methods

Mice. CBA/Ca (H-2^k) mice were bred under specific pathogen–free conditions at the National Institutes for Medical Research and used at 2–3 mo of age.

Viruses. A/X31 is a recombinant virus between A/Aichi/2/68 (A/Aichi) and A/PR/8/34 (PR8) with Hong Kong glycoproteins (H3N2) and PR8 internal components (28). A/Jap/305/57 (A/Jap) and A/USSR/8/57 (A/USSR) are of H2N2 and H1N1 subtypes, respectively. Natural variant viruses are all of the H3N2 subtype and were isolated from major influenza outbreaks between 1968 and 1984. A/duck/Ukraine/63 (duck/63) is an avian variant virus (H3N8). The amino acid sequences of HA1 from variant viruses were deduced from the nucleotide sequences of the RNA genes (our unpublished results) or were taken from published data (21, 22). R19 is a mutant virus of X31 selected for its ability to replicate in Mardin Darby canine kidney cells in the absence of exogenous trypsin, and has a His → Arg substitution at position 17 of HA1 (29). All viruses were grown in the allantoic cavity of embryonated hen eggs and stored at −70°C. Virus titers were determined by hemagglutination assay and expressed as HAU/ml.

Viral Fragments. The HA glycoprotein was solubilized from purified X31 by bromelain digestion as previously described (30). Fragments of X31 were prepared by tryptic digestion after pH5 treatment of the virus (31, 32). The products obtained were separated into aggregate and soluble fractions by sucrose density gradient centrifugation. The soluble fraction designated tops contains a monomeric glycopeptide consisting of residues 28–328 of HA1. The aggregate contains the 27-residue amino-terminal glycopeptide of HA1 disulfide linked to HA2 and the remainder of the virus.

Synthesis of Peptide 48–68. Peptide 48–68 was synthesized according to the sequence of HA1 residues 48–68 of X31, using the solid-phase method developed by Merrifield (33). The peptide was cleaved from the resin using trifluoroacetic acid containing 5% anisole at 20°C for 55 min. The purity of the peptide was confirmed by amino acid analysis of acid hydrolysates, and by analytical HPLC.

Synthetic Peptides of X47. Peptides of HA synthesized according to the amino acid sequence of A/X47 (recombinant containing VIC/3/75 HA [H3N2]) were a kind gift from R. A. Lerner, Research Institute of Scripps Clinic, La Jolla, CA. (34).

Establishment and Maintenance of HA-specific Th Clones. T cell clones were established from spleens of individual immune mice. CBA mice were immunized by intranasal infection with 2.2 HAU X31 influenza virus and boosted in vivo with virus or purified HA. Spleen cells were cultured at 2×10^6 cells/ml for 4 d with 50 HAU/ml of X31. These cultures were either cloned or restimulated with X31 (50 HAU/ml) and feeder cells (2×10^6 cells/ml; irradiated syngeneic spleen cells) to establish T cell lines. Cloning was performed on 4-d spleen cell cultures or on T cell lines by limiting dilution at 1 cell/well in 200 μl volumes in 96-well plates in the presence of feeder cells (5×10^5 cells/ml), X31 (50 HAU/ml), and 10% IL-2-containing supernatant (IL-2-sup; prepared

from Con A–activated rat spleen cells). A further 25 μl of IL-2-sup was added 5 d later. After 10–12 d, clones were restimulated and expanded in 24-well plates and eventually in 25 cm^3 flasks. Clones were maintained by restimulation at an initial concentration of 10^5 cells/ml with virus (50 HAU/ml) and feeder cells (2×10^6 cells/ml) every 8–10 d, with IL-2-sup and feeders added 4 d after antigen stimulation. The antigen specificity of clones was tested using a proliferation assay. HA-specific clones were selected for further study and were recloned at 0.5 cells/well.

Proliferation Assay. T cell lines and clones were rested for 8–10 d after stimulation with virus before assaying. T cells (2×10^4) were cultured with antigen (viruses, viral fragments, or HA peptides) in the presence of 4×10^5 feeder cells as APC in 200 μl volumes in flat-bottomed 96-well microtiter plates. Cultures were incubated for 2 or 3 d, and pulsed for the last 4–6 h with 0.5 μCi [^{3}H]thymidine. Cells were harvested onto filter paper using an automatic cell harvester, and [^{3}H]thymidine incorporation was measured by liquid scintillation counting. Results are mean cpm for triplicate cultures from two to six tests.

Results

Isolation of HA-specific T Cell Clones. Proliferative T cell clones were established by limiting dilution of bulk cultures or T cell lines derived from spleen cells of individual CBA mice primed by infection with X31 influenza virus. A total of 42 clones were generated from 11 distinct donors. Each of the clones was of the helper phenotype (L3T4$^+$, Lyt-2$^-$) and was MHC class II–restricted, since it proliferated in the presence of CBA (H-2^k), and B10 AQR (H-2 K^q, I-a^k, D^d), but not BALB/c (H-2^d) APC (data not shown).

Clones were screened for antigen specificity by proliferation in the presence of viral antigens and syngeneic APC. From the 42 clones maintained in vitro by stimulation with X31 (A/Aichi glycoproteins and PR8 internal proteins) four clones failed to respond to A/Aichi, the parental virus of the X31 recombinant. These clones probably recognize internal components of X31. A further five clones responded to A/Aichi and to purified HANA (hemagglutinin and neuraminidase) but not to purified HA, and therefore probably recognize the neuraminidase glycoprotein (data not shown; to be described in detail elsewhere). The remaining 33 clones were specific for the HA surface glycoprotein and were recloned and tested for fine specificity.

The number of HA-specific clones examined from individual mice and the frequency of distinct antigen specificities are summarized in Table I. Clones with identical specificity and derived from an individual donor were found for mouse 9 (two distinct specificities from four clones) and mouse 18 (one distinct specificity from five clones). In contrast to the clones from the other nine mice, which were generated from 4-d bulk cultures, these clones were generated from established cell lines, and since they may have arisen by cell duplication in culture, will be now treated as one clone. Therefore, a total of 27 distinct HA-specific T cell clones from 11 individual donors are described in the detailed specificity analysis.

Specificity for Viral Subtypes and HA Fragments. The results in Table II show that all of the HA-specific clones were subtype specific, since they failed to respond to A/JAP (H2N2) and A/USSR (H1N1). A parental T cell line 3.2 was crossreactive, and recognized A/JAP and A/USSR. The region of the HA molecule recognized by the T cell clone was examined by testing their proliferative responses to tryptic fragments of X31. Tryptic cleavage of X31, after pH

TABLE I

Frequency of Distinct Specificities among HA-specific T Cell Clones Examined from Individual Mice

Mouse number	Number of HA-specific clones examined	Number of distinct specificities*
1	4	4
2	1	1
3	4	4
4	5	5
5	2	2
6	1	1
8	2	2
9	4	2
12	2	2
13	3	3
18	5	1

* Specificities of individual clones were established by the proliferative responses to variant viruses and synthetic peptides of HA1 (detailed in Fig. 1 and Table III).

5 treatment, yielded a soluble monomeric polypeptide of HA1 28–328 (tops), and the remainder of the virion, including HA1 1–27 and the HA2 polypeptide (aggregate). The majority (20 of 27) of HA-specific clones recognized tops, but did not respond to aggregate. The remaining HA-specific clones failed to respond to either tops or aggregate (Table II), whereas the clones directed against neuraminidase or internal proteins recognize aggregate only (data not shown). The crossreactive line 3.2 responded to tops and aggregate.

Fine Specificity for Natural Variant Viruses. The recognition by HA-specific T cell clones of natural variant viruses isolated from major influenza outbreaks between 1968 and 1984 revealed a broad range of specificities. The specificity patterns, together with the response to the X31 mutant virus R19 are grouped and summarized in Fig. 1. A total of 12 distinct specificity groupings were evident, varying from recognition of all variants (group 1) to recognition of only variants isolated from 1968 to 1972 (group 7). A major group (group 3), which failed to recognize TX/77, BK/77, or CN/84 consisted of eight clones from seven different mice. Another group of four clones (group 11) failed to recognize ENG/72; this group is part of the family of seven clones that failed to recognize either tryptic fragments or mutant virus R19. The remaining three clones in this family either recognized all variants (clone 5.41 and 3.F3) or failed to recognize ENG/72 and CN/84 (clone 9.9).

The proliferative response of each of the clones to the natural variant viruses measured by [^{3}H]thymidine incorporation was ~50–200% of X31 response, except for clone 1.22 where the response to ENG/72, HAN/73, and PC/73 was consistently between 15–40% of the X31 value. Five subclones with identical specificities all showed this same diminished but not completely negative response over a range of virus concentrations (data not shown).

Recognition of Synthetic Peptide of HA. In an attempt to analyze the antigenic determinants recognized by the HA-specific T cell clones, their specificity was tested against synthetic peptides corresponding to different regions of the HA

TABLE II

Specificity of HA-specific T Cell Clones for Influenza Virus A Strain Subtypes and Viral Fragments

Clone	[^{3}H]Thymidine incorporation (cpm × 10^{-2})							
	X31 (H3)	A/Aichi (H3)	A/Jap (H2)	A/USSR (H1)	BHA (H3)	Tops	Aggregate	Medium
1.14	165	233	3	2	138	171	1	7
1.22	269	313	2	2	153	251	3	3
1.3	751	788	2	2	386	337	8	2
1.9	81	80	2	2	57	119	2	2
3.B9	670	641	3	3	740	720	4	3
3.F10	438	705	1	1	544	608	4	1
4.2	74	111	2	2	127	121	2	2
4.3	104	73	2	2	125	134	1	4
4.4	95	100	3	2	54	46	4	2
4.7	164	152	2	2	145	173	12	2
5.2	372	459	5	4	411	366	6	1
6.1	35	30	1	3	58	32	2	2
8.1	25	20	1	3	34	25	2	2
8.3	53	36	2	2	47	27	2	2
9.5	37	57	7	5	38	33	6	5
12.3	310	482	5	4	261	238	8	5
12.4	224	216	1	1	132	180	11	1
13.1	134	147	1	1	114	248	3	1
13.10	1,194	1,213	5	5	1,261	1,127	26	7
18.44	325	302	3	2	340	345	6	4
2.P2	244	235	1	1	182	3	5	1
3.E1	313	275	2	7	260	4	11	2
3.F3	269	270	1	1	330	2	14	3
4.99	320	323	3	2	556	4	14	4
5.41	604	629	5	4	749	7	17	3
9.9	62	25	1	5	39	3	4	4
13.46	319	404	3	2	245	11	13	4
Line 3.2	966	1,020	784	910	370	360	660	15

Results are means of two to four proliferative responses to 5 HAU/ml of whole virus or to 1 μg/ml of BHA or tryptic fragments (tops and aggregate); SE < 25%.

molecule. Since the panel of peptides were synthesized according to the sequence of X47 (recombinant virus of VIC/75), only those clones that recognize VIC/75 were tested. Six out of eight clones from specificity group 3 responded to peptide 7, which corresponds to residue 53–87 of HA1 (designated group 3a; Table III). These clones did not recognize peptide 5, which corresponds to the eight *C*-terminal residues (53–60) of peptide 7. The remaining clones tested from specificity groups 1, 2, 3b, 8, 10, and 11 failed to recognize peptide 7 or any of the other peptides tested (Table III).

Mapping of Antigenic Determinants Recognized by Clones from Group 3a. A peptide corresponding to residues 48–68 of HA1, which overlaps with the sequence of peptide 7, was synthesized according to the sequence of X31 (since HA1 of X47 has an insertion following position 8 of X31, residues 53–87 of

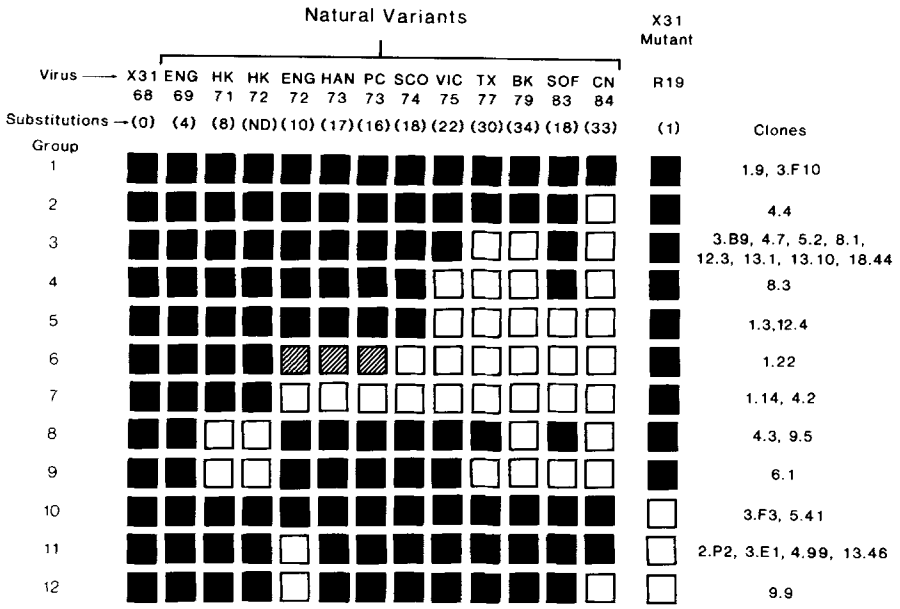

FIGURE 1. Specificity of T cell clones for mutant and variant viruses. The proliferative responses of individual HA-specific T cell clones was tested against natural H3N2 isolates from 1968 to 1984, each with an increasing number of amino acid substitutions in the HA1 polypeptide, and against an X31 mutant virus R19 with single amino acid substitution at position 17 of HA1. Clones with identical specificity patterns were grouped and are shown as: positive (>50%, ■), weak (5–50%, ▨) or negative (<5%, □) responses, with respect to X31, with the optimum concentration of virus in the range 0.2–200 HAU/ml.

X47 [peptide 7] correspond to 52–86 of X31). Each of the clones that recognized peptide 7 also responded to peptide 48–68 (Table III and Fig. 2). A comparison of the amino acid sequence of the natural variant viruses from the region spanned by the peptides shows amino acid substitutions at seven different positions (Fig. 3). Substitution at positions 63, 78, and 83 are conserved from VIC/75 to CN/84, and residue 50 is outside the area of peptide 7. However, residue changes at position 53 (Asn to Asp), 54 (Asn to Ser), and 62 (Ile to Lys) are only found in the natural variants TX/77, BK/79, and CN/84, which are not recognized by the T cell clones (Figs. 1 and 2). An avian variant virus, duck/63, has an Ile to Arg substitution at position 63 (Asp to Asn in variants from VIC/75 to CN/84). All of the clones in specificity group 3a were almost completely unresponsive to duck/63 (Fig. 2, data shown for clone 18.44), this suggests that residue 62 may be involved in the recognition epitope of these clones.

Discussion

The significant finding of this study is that murine HA-specific Th clones established from individual CBA donors primed by infection with X31 influenza virus (H3N2) exhibit exquisite specificity in the recognition of natural virus variants, thereby suggesting a positive role for the T cell in immune pressure for antigenic drift. Furthermore, their pattern of reactivity against natural variants, mutant viruses, and synthetic peptides of HA reveal a hitherto unrecognized diversity in the class II–restricted T cell recognition of a globular protein antigen.

TABLE III

Recognition of Synthetic Peptides of HA1 by HA-specific T Cell Clones

Specificity group	Clone	Proliferative response to peptide											
		2 (1–36)	3 (15–53)	5 (53–60)	7 (53–87)	10 (76–111)	12 (130–151)	16 (140–176)	17 (174–196)	20 (306–329)	23 (267–300)	48–68	BHA
1	1.9	1	1	2	1	2	1	2	2	1	1	1	560
	3.F10	4	4	3	4	1	2	5	4	4	4	4	61
2	4.4	2	3	2	4	2	2	3	3	2	2	4	156
3a	3.B9	3	5	4	80	3	4	4	3	4	5	153	110
	4.7	2	3	3	109	2	3	4	3	2	2	147	141
	5.2	3	3	5	241	3	2	4	4	3	2	365	339
	12.3	3	2	4	196	2	2	2	3	2	4	646	188
	13.10	7	7	7	621	8	8	8	7	8	7	503	596
	18.44	3	6	7	384	4	4	5	4	4	6	361	290
3b	8.1	4	4	3	4	2	3	2	2	4	2	2	47
	13.1	2	2	4	6	5	2	4	3	4	5	6	175
8	4.3	4	4	3	6	4	6	4	3	2	4	4	81
10	3.F3	1	4	5	4	4	1	2	3	2	3	2	334
	5.41	7	8	8	5	6	5	6	5	6	7	6	803
11	2.P2	4	3	4	2	5	4	3	4	4	2	5	125
	3.E1	2	2	3	5	2	3	3	3	2	2	2	350
	4.99	5	3	2	3	3	2	6	3	2	2	2	556
	13.46	2	2	3	2	2	2	2	3	2	2	2	250

Data are mean [^{3}H]thymidine incorporations (cpm × 10^{-2}) for two or three assays with the optimum concentration of peptide in the range 0.1–10.0 μg/ml. Peptide 2–23 was synthesized according to the sequence of X47, and peptide 48–68 was synthesized according to the sequence of X31.

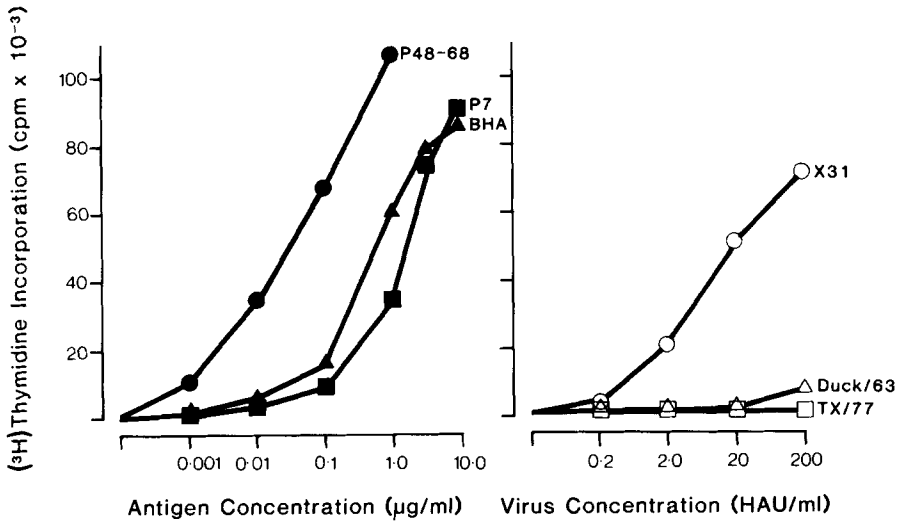

FIGURE 2. The response of clone 18.44 to synthetic peptides and variant viruses. Results are mean [^{3}H]thymidine incorporation in cpm for triplicate cultures, with SE <10%. Similar response curves were found for the other five clones from group 3a (clones 3.B9, 4.7, 5.2, 12.3, and 13.10; data not shown). P48–68 and P7 are synthetic peptides that correspond to residues 48–68 of X31 and 53–87 of X47 respectively.

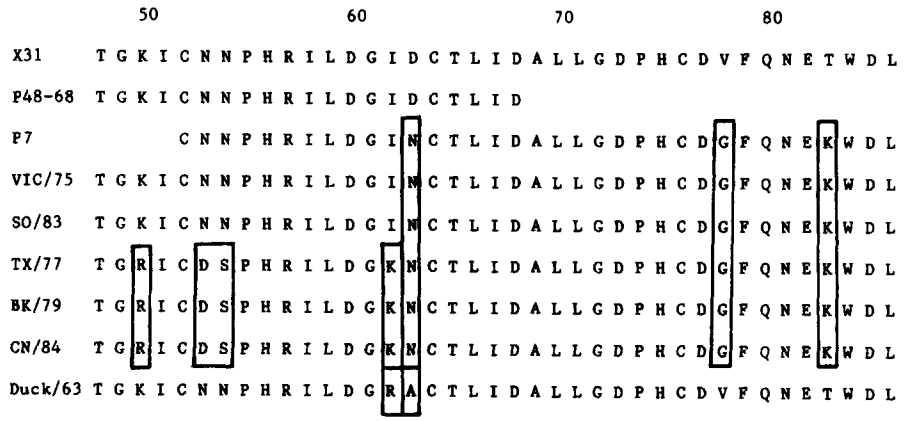

FIGURE 3. Amino acid sequence of variant virus from the region of HA1 spanned by two overlapping synthetic peptides, P7 and P48–68 (see Fig. 1). Boxed areas indicate amino acid substitutions from X31.

Previous studies (22, 23, 32) with mAb against influenza virus HA have shown extensive diversity in the B cell repertoire: an examination of 125 mAb derived from individual BALB/c mice immunized with PR8 (H1N1) influenza virus revealed 104 distinct specificities for 51 related viruses (23). A similar diversity was found in the B cell repertoire of BALB/c mice for HA of H3N2 viruses (22, 32). In contrast, studies on T cell recognition of influenza virus HA have led to the conclusion that the Th repertoire is more limited. In man, HA-specific T cell clones recognized an immunodominant epitope close to the *C*-terminus of HA1 (25). A study of eight murine T cell hybridomas specific for PR8 (H1N1) influenza virus showed the presence of three major Th cell determinants (27). However, there is evidence from studies with polyclonal T cell populations that the repertoire may not be so restricted: Lamb and coworkers (24) showed that PBL or a T cell line responded to 12 overlapping peptides corresponding to almost the complete sequence of HA1. Similarly, Atassi and Kurasaki (35) have shown that lymph node T cells from X31-primed BALB/c mice responded to 6 out of 12 synthetic peptides of HA.

The present investigation with class II–restricted T cell clones reveals a potential for extensive diversity in the T cell repertoire for influenza virus HA. A panel of HA-specific T cell clones has established a minimum of 12 specificity groups, distinguished by their reactivity with H3N2 variant viruses. One further subgroup was defined on the basis of recognition of a synthetic peptide of HA. The Th cell specificity for natural variant viruses isolated from major virus epidemics between 1968 and 1984 varied from recognition of all isolates (group 1) to only those from 1968 to 1972 (group 7). One major specificity group (group 3) consisted of eight distinct clones from seven different mice. Another major group (group 11) included four clones from four individual mice, and was part of a family of seven distinct clones, which failed to recognize mutant virus R19 or tryptic fragment of X31.

The demonstration of extensive diversity in the Th cell recognition of a globular protein antigen may not necessarily be surprising in view of extensive

homology at the genomic level in structure and mechanism(s) for the generation of diversity for the T cell receptor and Igs (3–7). Although somatic mutation has not so far been demonstrated for T cells, multiple gene segments and in particular a large number of joining segments in the gene coding for the α chain suggest the potential for extensive combinatorial diversity in the T cell receptor (6).

Diversity has previously been demonstrated in the class I–restricted T cell repertoire for alloantigen: cytotoxic T cell clones recognized 23 distinct antigenic determinants on the H-2K^b molecule (36). However, reports with class II–restricted T cell clones specific for conserved globular proteins have suggested that T cells recognize a limited number of immunodominant epitopes (10–14). For example, myoglobin-specific H-2^d T cells showed a predominant response to an epitope centering on Glu_{109} and His_{116} (11). Conclusions of limited diversity in the repertoire of class II–restricted T cells were derived from studies on T cell clones established by the procedure of Kimoto, Fathman, and Frelinger (37, 38). In the present investigation, T cell clones were generated from mice immunized by infection, and the majority of clones were established from 4-d bulk cultures of immune spleen cells. When cloning was performed on established T cell lines (mice 9 and 18), a high number of repeat specificities was noted among the HA-specific clones examined. In contrast, each of the clones established from 4-d bulk cultures had a distinct specificity. A tentative conclusion from this observation is the possibility of dominant clonotypes being selected by in vitro culture.

A major T cell epitope of the HA molecule has been defined in this study: six out of eight clones from specificity group 3 failed to recognize natural variants TX/77, BK/79, or CN/84, but responded to two overlapping peptides corresponding to residues 53–87 of VIC/75 (peptide 7) and residues 48–68 of X31. From a comparison of the amino acid sequences of the peptides and the corresponding regions of the natural variants (Fig. 3), it is evident that substitutions at residues 62 (Ile to Lys), 53 (Asn to Asp), and 54 (Asn to Ser) are unique to variant viruses TX/77, BK/79, and CN/84. However, these clones failed to recognize peptide 5, and showed a diminished but not completely negative response to the avian variant virus duck/63, which has an Ile to Arg substitution at position 62. This confirms the role of Ile_{62} in recognition of HA by these clones. However, the failure to recognize peptide 5, corresponding to the eight *N*-terminal amino acids of peptide 7 (53–60), does not contradict the suggestion that residues 53 and 54 may also be involved, since a minimum length of 8–10 residues may be necessary for the integrity of a T cell epitope or for antigen processing/presentation (26, 39). We may, therefore, conclude that Ile_{62}, and possibly Asn_{53} and Asn_{54}, are critical for recognition of HA by a major population of class II–restricted T cells.

A family of seven clones, which includes specificity groups 10–12, failed to respond to tryptic fragments of X31 or to an X31 mutant virus R19. R19 has a single amino acid substitution (His to Arg) at position 17 of HA1, which is known to affect the conformational stability of the HA molecule (29). The recognition site of four of these clones, which failed to recognize ENG/72, has previously been mapped, tentatively, to a conformational determinant in the region of residue 208 (40). One further clone (9.9) from this family failed to respond to

variant viruses ENG/72 and CN/84. Examination of the amino acid sequences of HA1 for these viruses suggests that residues Arg_{208} and Ile_{213}, both in the interface of the HA trimer, are critical for recognition by clone 9.9.

It was not possible to map definitively determinants recognized by the remaining clones. Further examination of their reactivity against synthetic peptides corresponding to the variable regions of X31 may establish the fine specificity of these clones. However, 12 clones from 6 different specificity groups failed to respond to a range of overlapping synthetic peptides, the sequences of which correspond to large areas of the HA molecule of VIC/75. Each of these clones recognized the native VIC/75 virus; therefore it is possible that antigen conformation may play a role in the recognition of HA by a further proportion of T cell clones.

It is interesting to note that the T cell epitopes defined in this study do map to variable regions of the HA molecule that include antibody recognition sites. First, peptide 48–68 (Fig. 2) includes part of antibody sites C and E. Second, the interface antibody-binding response (site D) is implicated in the recognition by clones from groups 11 and 12. Third, the majority of clones respond to trypsin cleaved tops (HA1, 28–328), which includes the major antibody-combining sites of the HA molecule. This finding contrasts with previous reports (25, 41) on the recognition of HA by Th cells, which conclude that class II–restricted T cells recognize epitopes from regions of HA distinct from antibody-combining sites or conserved within HA subtypes. However, our results are supported by a report (27) on HA-specific murine T cell hybridomas suggesting that a certain degree of structural overlap does exist between T and B cell determinants. Several T cell hybridomas failed to recognize mAb-selected mutant viruses differing from the parental PR8 virus (H1N1) by one or more amino acid substitutions in the HA1 polypeptide. However, a proportion of these clones failed to distinguish natural variant viruses of the H1 subtype isolated between 1931 and 1957 (27, 42).

The majority of HA-specific Th clones described here can discriminate between natural variant viruses within the H3N2 subtype, suggesting that the amino acid changes occurring during antigenic drift, which are known to affect anti-HA antibody binding, also affect Th cell recognition. Therefore it appears that Th cells may play a role in the selective pressures involved in antigenic variation in the HA molecule. In conclusion, we have shown that H-2^k class II–restricted T cell clones are specific for the immunizing HA subtype (H3N2), and recognize a diverse spectrum of epitopes, the majority of which are located on variable regions of the HA1 polypeptide. The Th repertoire may shed new light on the role of Th cells in protective immunity to influenza viruses, and may therefore be instrumental in directing antigenic variation in influenza.

Summary

A panel of H-2^k class II–restricted Th clones were established from individual CBA mice primed by infection with X31 influenza virus. 27 clones, which showed specific recognition of the HA surface glycoprotein, were all H3N2 subtype specific, in contrast to a T cell line which was crossreactive and which may have other specificities. 20 distinct HA-specific clones recognized a tryptic cleavage

fragment of X31 consisting of residue 28–328 of HA1 (tops) which includes all the Ab-combining regions of the HA molecule. Seven other HA-specific clones failed to respond to either tops or to aggregate (the remainder of the virus after tryptic cleavage of tops). The specificity of these clones has been mapped, tentatively, to a conformational determinant in the interface antibody-binding region of the HA trimer. Analysis of the fine specificity of the HA-specific clones against a panel of H3N2 natural variant viruses isolated from major virus epidemics from 1968 to 1984 revealed a hitherto unrecognized diversity in T cell recognition of a HA. A total of 12 specificity groupings were evident, and varied from groups of clones that recognized all natural variants to one clone that responded only to isolates from 1968 to 1972. Six out of eight clones from a major specificity group, which failed to recognize variants TX/77, BK/79, or CN/84, responded to two overlapping peptides (48–68 and 53–87), corresponding to a region of HA1 that includes part of two antibody combining sites. An examination of the amino acid sequences of natural variant viruses from this region of HA revealed that residues Asn_{53} and Asn_{54} and/or Ile_{62} were critical for recognition by these clones. We conclude that recognition of HA by Th cells is not restricted to a limited number of epitopes in the conserved regions of the molecule, but is extremely diverse and includes specificities that map to variable antibody-combining regions of the molecule. In addition, the sensitivity of the T cell clones to the amino acid substitutions occurring in HA1 of natural variant viruses suggests that Th may play a role in the immune pressure for antigenic variation in the HA molecule.

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