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Immunology

Alloreactivity studied with mutants of HLA-A2

(histocompatibility/cytotoxic T cells/allogeneic recognition/class I major histocompatibility complex molecules)

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ABSTRACT **Based on the crystal structure of HLA-A2.1 and the recognition of a panel of mutant HLA-A2.1 molecules by a large number of alloreactive cytotoxic T lymphocyte clones, a model to explain alloreactivity is described. In this model recognition of an allogeneic major histocompatibility complex molecule by a self-restricted T-cell receptor occurs as the result of accommodation by the receptor of a few amino acid differences in the major histocompatibility complex molecule—i.e., cross-recognition. Alloreactivity is the result of the presence in the foreign antigen binding site of the allogeneic major histocompatibility complex molecule of unusual self-peptides, reactivity to which could not have been eliminated by negative thymic selection.**

Class I major histocompatibility complex (MHC) molecules (HLA molecules in man) were originally identified as the target structures responsible for the humoral (alloantibody) and cellular (alloreactive cytotoxic T lymphocyte) responses generated during the rejection of transplanted foreign tissue (1). However, the central role of these highly polymorphic cell-surface glycoproteins is to serve as restricting elements in the recognition by T cells of virus-infected, chemically modified, or neoplastic cells (2–5). The regions in contact between the molecules involved in the recognition process (T-cell receptor, class I MHC molecule, peptide, and accessory molecules) are not yet precisely described, and the nature and and biophysical characteristics of these interactions (6) remain to be determined. Identification of specific residues on class I MHC molecules involved in the recognition by human cytotoxic T lymphocytes (CTL) has been facilitated over the last few years by the structural characterization of natural HLA variants initially distinguished by cytotoxicity assays (7) and/or isoelectric focusing (8). In population studies, sequence comparison of these otherwise serologically similar HLA molecules (9), exon shuffling (10, 11), and site-directed mutagenesis (12–15) have all highlighted the importance of polymorphic residues in the α_1 and/or α_2 domains of the HLA molecule in the recognition process. Moreover, the class I MHC molecules were shown to present peptides derived from processed antigens to the receptor of cytotoxic T cells (16, 17). Elucidation of the crystal structure of HLA-A2 revealed a platform of eight antiparallel β strands topped by two α-helices (18, 19). A prominent groove between the helices was identified as the site for binding of foreign peptides.

In the present study, site-directed mutagenesis of HLA-A2.1, coupled with generation of allospecific human CTL clones and cytoxicity assays, has been used to explore the molecular requirements for alloreactivity.

MATERIALS AND METHODS

Cell Lines and HLA-A2.1 Mutants. Lymphoblastoid cell lines used, all typed in the Dana–Farber Cancer Institute, were: JY (HLA-A2.1; -B7; -DR4, 6), M7 (HLA-A2.2F, 3.1; -B35, 53), DK1 (HLA-A2.3, 33; -B40, 44), CLA (HLA-A2.4a, 4; -B8, 35; -DR1, 2), KNE (HLA-A2.4b, 1; -B6, 27; -DR2, 3), MICH (HLA-A2.1, 32; -B27, 15; -C2; -DR5), LB (HLA-Aw68; -B40; -C3; -DR6), IDF (HLA-Aw69, 26; -B38, 18; -DR5), and PGF (HLA-A3; -B7; -DR2). The HLA-A2.1 mutants in the human adherent cell line RD (rhabdomyosarcoma, -A1; -Bw51, 14) have been serologically characterized (20) (Table 1).

Human CTL Clones. CTL clones were generated as described (21, 22). Peripheral blood mononuclear cells from a single volunteer MC (HLA-A3, -Aw30; -B7, 13; -Cw6; -DR4) purified on a Ficoll/Hypaque gradient (lymphocyte separation medium, Bionetics Research Institute) at 2×10^6 cells per ml and the irradiated (10,000 rads; 1 rad = 0.01 Gy) Epstein–Barr-transformed lymphoblastoid cell line JY at 1×10^5 cells per ml were used as responder and stimulator cells, respectively, for primary and secondary mixed lymphocyte culture (MLC). The bulk culture was maintained by stimulation every 1 to 2 weeks with irradiated JY cells. After 5 to 6 weeks in culture, the cells were isolated in a Ficoll/Hypaque gradient and cloned by limiting dilution in 96-well microtiter plates by using 2×10^4 irradiated cells as a feeder layer. Fresh human-conditioned medium containing interleukin 2 was used at 10% for cloning and long-term cultures and added every 3 days. Each clone was subcloned at <1 cell per well to assure clonality. CTL clones were expanded in 16-mm wells. CTL assays were performed in triplicate as described (21, 22) with effector:target ratios of 6:1, 2:1, and 0.7:1. Monoclonal antibodies (mAbs) and their target structures (in parentheses) used in blocking studies were as follows: mAb W6/32 (monomorphic HLA-A, -B, -C), mAb LB3.1 (monomorphic HLA-DR), mAb 4B3 (HLA-A2 plus -A28), mAb PA2.1 (HLA-A2 plus -Aw69), mAb MA2.1 (HLA-A2 plus -B17), mAb ME1 (HLA-B7 plus -B27), mAb TS1/18 or TS1/22 (LFA-1), mAb TS2/18 (LFA-2 = CD2), mAb TS2/9 (LFA-3), mAb OKT3 (CD3), mAb OKT4 (CD4), mAb OKT8 (CD8) and mAb Genox (DQ1).

RESULTS

Generation and Initial Characterization of Human CTL Clones. Human anti-HLA-A2.1 allospecific CTL were generated by stimulating peripheral blood lymphocytes from donor MC (HLA-A3, -Aw30; -B13, 7; -DR4, 6) in primary and secondary cultures with the irradiated Epstein–Barr virus-transformed B lymphoblastoid cell line JY (HLA-A2.1; -B7; -DR4, 6). Because the stimulator cells shared most HLA molecules of the responder, the cellular response was mainly directed towards the HLA-A2.1 antigen; 227 independent CTL clones that lysed JY cells were obtained (Table 2). Repeat testing of these CTL clones with the lymphoblastoid cell lines JY (-A2.1; -B7), MICH (-A2.1; not -B7), and PGF

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Abbreviations: CTL, cytotoxic T lymphocytes; mAb, monoclonal antibody; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture.

Table 1. Mutant HLA-A2.1 genes

HLA cell line	Amino acid change	Orientation
-A2M9	Phe → Tyr	B
-A2M43	Gln → Arg	O
-A2M6263	Gly-Glu → Arg-Asp	↑, *
-A2M6566	Arg-Lys → Gln-Ile	↑, *
-A2M65	Arg → Gln	↑
-A2M66	Lys → Ile	*
-A2M70	His → Gln	*
-A2M74	His → Asp	*
-AwM7074	His-His → Gln-Asp	*, *
-A2M6574	Arg-65 → Gln, Lys-66 → Ile, His-70 → Gln, His-74 → Asp	↑, *, *, *
-A2M7080	His-70 → Gln, His-74 → Asp, Val-76 → Glu, Asp-77 → Ser, Gly-79 → Arg, Thr-80 → Asn	*, *, ↑, *, A, *
-A2M107	Trp → Gly	O
-A2M152	Val → Glu	*
-A2M156	Leu → Ser	*
-A2M43/152	Gln-Val → Arg-Glu	O, *
-AwM43/156	Gln-Lys → Arg-Arg	O, *

The amino acid substitution and orientation of the altered residues in the HLA-A2.1 crystal structure are shown: B, pointing up from the bottom of the groove; *, pointing into the groove from a helix; ↑, pointing upward from the top of a helix; A, pointing away from the top of a helix; and O, outside the groove.

(-B7; not -A2.1) indicated that 174 clones were specific for HLA-A2.1 (as indicated by reactivity with both JY and MICH), whereas seven clones were specific against HLA-B7 (possibly reflecting the presence of a different HLA-B7 subtype expressed by donor and stimulator cells). The remainder of the 227 cloned cells either did not grow or had no lytic activity against HLA-A2- or -B7-typed cell lines and were not studied further. All CTL clones were homogeneously $CD4^-$, $CD8^+$, and $CD3^+$, as determined by analysis on a fluorescence-activated cell sorter with appropriate mAbs.

To define the specificity of the anti-HLA-A2.1 clones further, 56 of them were tested against cell lines expressing previously described structural variants of HLA-A2.1: M7 (-A2.2F), DK1 (-A2.3), CLA (-A2.4a), and KNE (-A2.4b). The heavy chains of these variants are known to differ from the -A2.1 molecule (JY) at the following positions (9): -A2.2F, Arg-43, Leu-95, and Trp-156; -A2.3, Thr-149, Glu-152, and Trp-156; -A2.4a, Tyr-9, and -A2.4b, Cys-99. Based on the reactivity with these cell lines, T-cell clones were classified into four groups (Table 3): group A, 8 clones that recognized all subtypes except -A2.4a; group B, 9 clones that recognized all the HLA-A2 subtypes; group C, 10 clones that recognized all subtypes except -A2.3; and finally, group D, the majority of the clones (twenty-nine), which recognized neither -A2.3 nor -A2.4a. However, their reactivity against cells expressing -Aw68 and -Aw69 (the former differing from -A2.1 by only 13 residues and the latter a natural hybrid of -A2.1 and -Aw68 and differing by only six residues) (23) indicated that these groups were not homogeneous (Table 3).

Table 2. Specificity of human CTL clones on HLA-A2- or HLA-B7-typed cell lines

Target cell line lysed	Clones, no.
JY (-A2.1; -B7)	181
MICH (-A2.1, 32; -B27, 15)	174
PGF (-A3; -B7)	7
Noncytotoxic for the above	39

Responder cells were MC (HLA-A3, w30; -B7, 13; -Cw6; -DR4,6); stimulator cells were JY (HLA-A2.1; -B7; -DR4,6). Total number of T-cell clones obtained was 227. Seven clones did not grow and were not retested.

Table 3. Fine specificity of human CTL clones on cells expressing HLA-A2.1 and its structural variants and mutants

	Natural variants										Site-directed RD mutants																		
	JY-A2.1	MICH-A2.1	PGF-B7	M7-A2.2Y	DK1-A2.3	KNE-A2.4b	CLA-A2.4a	LB-Aw68	IDF-Aw69	RD-mock	RD-A2.1	B7	M9	M43	M6263*	M65*	M66	M6566	M70	M74	M7074	M6574	M7080	M6580	M107	M252	M43-152	M156	M43-156
Group A																													
w1	+	+	−	+	+	+	−	+	−	−	+	−		+		−		−	±	±	−	−	−	−	+		−		
w2	+	+	−	+	+	+	−	−	−	−	+	−	−	+	−	±	−	±	±	+	−	−	−	−	+	+	+	+	−
Group B																													
w41	+	+	−	+	+	+	+	+	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−
w74	+	+	−	+	+	+	+	+	−	−	+	−	+	+	−	−	−	−	+	+	+	−	+	−	+	+	+	+	−
Group C																													
w42	+	+	−	+	−	+	+	−	−	−	+	−	+	+	−	−	−	−	+	+	+	−	±	±	±	−	−	−	−
w44	+	+	−	+	−	+	+	+	+	−	+	−	+	+	−	−	+	±	+	+	+	±	±	−	+	+	+	+	−
Group D																													
w46	+	+	−	+	−	+	−	−	−	−	+	−	−	+			±	±	+	+	+	−	−	−	+	−	−	+	−
w128	+	+	−	+	−	+	−	−	−	−	+	−	−	+	−	+	−	−	+	+	+	−	+	+	+	+	+	−	−
w169	+	+	−	+	−	+	−	+	−	−	+	−	−	+	−	−	±	±	+	+	−	−	−	−	+	−	−	+	−
w193	+	+	−	+	−	+			−	−	+	−	+	−	−			−			+	−	−		+	±	±	±	±
w195	+	+	−	+	−	+			−	−	+	−	+	+	−	+		+			+	−	−		+	−	−	+	±
w219	+	+	−	+	−	+			−	−	+	−	+	+	−	±		−			+	−	±		+	−	−	+	+
w164	+	+	−	+	−	+			−	−	+	−	+	+				+			+	+	+		+	−	−	+	+

+, 60–100% of the lysis of the RD-HLA-A2.1 transfectant; ±, 30–60% of the lysis of the RD-HLA-A2.1 transfectant; −, same as background on RD-mock cells.
*Cytotoxicity assays were performed after induction with human interferon γ because the molecule is not expressed at the surface at normal levels.

Fine Specificity of Anti-HLA-A2 CTL Clones. To characterize the fine specificity of the anti-HLA-A2.1 CTL clones more precisely, a panel of RD cells expressing mutants of HLA-A2.1 was examined as targets (Table 3). All CTL clones analyzed specifically recognized RD-A2.1 and not RD-B7 (although their level of nonspecific killing on the recipient cell line, RD-mock, transfected with pSV2neo, varied with the clone being tested). CTL assays of transfected RD cells were done by using, in each case, a cell sorter-generated population of target cells expressing similar levels of the mutant HLA molecules at their surface, as determined by a panel of HLA-A2-specific mAb (data not shown, cf. ref. 20). These assays were done with three different CTL:target ratios (6:1, 2:1, and 0.7:1). Every experiment with the transfectants included both positive (JY and RD-A2.1 as targets) and negative (RD-mock as target) controls.

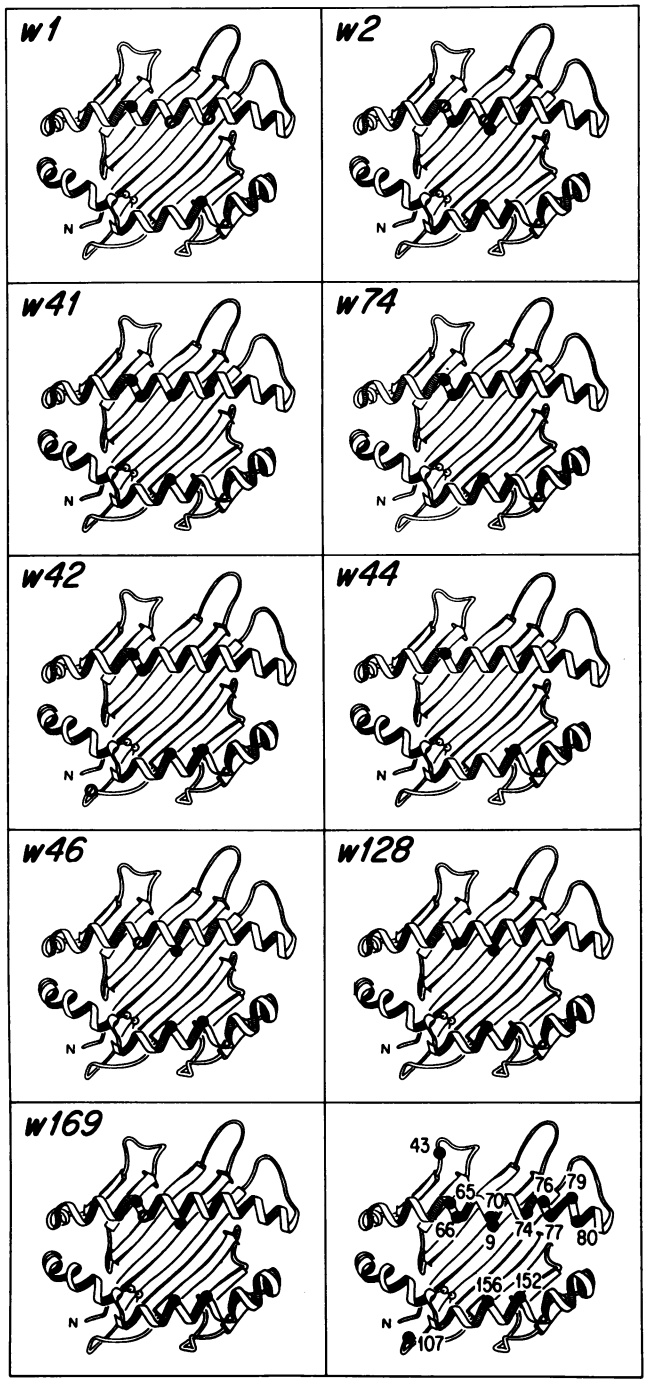

FIG. 1. Pattern of mutations that affected several clones. ●, 0–30% lysis relative to the RD-A2.1 transfectant—i.e., same as background on RD cells; ○, 30–60% lysis. Mutations resulting in 60–100% lysis (no effect) are not shown. Only the single-point mutations were considered in this figure. The positions of all the mutations examined are shown in the lower right panel.

Only some of the clones obtained were examined in detail, including representatives of each of the groups described above. Representative data are shown for one set of clones in Table 3 and Fig. 1, and a statistical summary of all clones examined is shown in Table 4.

Examination of the killing specificity of these clones on the mutant molecules leads to the following observations: (*i*) The repertoire of allogeneic CTL is very large, as evidenced by the fact that no two clones were identical when their specificity was examined on the panel of mutants (Table 3)—i.e., the pattern of mutations that affected recognition by a CTL clone was specific to that clone. This extreme diversity is graphically illustrated for a few clones in Fig. 1. (*ii*) Mutations in single amino acid residues affected allogeneic recognition, just as they affected virus peptide-specific MHC-restricted recognition (24, 25). The effect was even more drastic when multiple substitutions—i.e., cell lines HLA-A2M7080 and HLA-A2M-6580—were introduced. Residues 65 and 76 both point upward from the α_1 helix and are likely to be T-cell receptor contact sites. The fact that *some* clones were not affected by changes at residues 65 or 76 that point *upward* (found in the mutants HLA-A2M65, -A2M6580, and -A2M7080) may suggest that the T-cell receptor does not need to cover both α-helices entirely—i.e., to interact with all residues that point upward, or that some T-cell receptors can accommodate the particular amino acid substitution (although that substitution at residue 76 involves introduction of a negative charge). (*iii*) Allogeneic recognition, just as virus peptide-specific MHC-restricted recognition, involves only those residues that are in or on the groove. In particular, changes in the two residues examined that are outside of the groove, residues 43 and 107, did not affect recognition, whereas each of the other mutations had an effect on at least one CTL clone. These mutations include residue 9 (which points upward into the site from a β strand on the floor) and residue 70 (which points inward from the α_1-helix and interacts with residue 9), as well as residue 74, which had no effect

Table 4. Effects of amino acid substitutions on CTL recognition

Amino acids(s) mutated	Clones analyzed, no.	Results (%)					
		+		±		−	
9	13	9	(69.3)			4	(30.7)
43	28	28	(100)				
6263	10					10	(100)
6566	26	5	(19.2)	3	(11.5)	18	(69.2)
65	10	2	(20)	2	(20)	6	(60)
66	9	1	(11.1)	2	(22.2)	6	(66.6)
70	9	6	(66.6)	2	(22.2)	1	(11.1)
74	9	7	(77.7)	1	(11.1)	1	(11.1)
7074	26	12	(46.1)	2	(7.7)	12	(46.1)
6574	27	2	(7.4)	2	(7.4)	23	(85.2)
7080	26	3	(11.5)	3	(11.5)	20	(76.9)
6580	18	2	(11.1)	3	(16.6)	13	(72.2)
107	15	14	(93.3)	1	(6.6)		
152	14	5	(35.7)	1	(7.1)	8	(57)
43/152	26	7	(26.9)	1	(3.8)	18	(69.2)
156	15	11	(73.3)	1	(6.6)	3	(20)
43/156	16	4	(25)	2	(12.5)	10	(62.5)

+, 60–100% of the lysis of the RD-HLA-A2.1 transfectant; ±, 30–60% of the lysis of the RD-HLA-A2.1 transfectant; −, same as background on RD-mock cells. Mutant 6580 has all of the mutations in 6574 and 7080 (Table 1).

in one virus peptide-specific MHC-restricted CTL recognition study (24) but drastically affected another (25). (*iv*) The most profound effect was found with mutations at both residues 62 and 63; *all* CTL examined failed to recognize this mutant. This result could be due to the effect of the mutation at residue 62, which points upward from the α_1 helix and may be an important T-cell receptor contact residue. (*v*) Changes at residues 152 and 156 that had particularly strong effects in the virus peptide-specific MHC-restricted recognition system affected only some of the clones. The mutation Leu-156 → Ser appeared particularly benign and affected only three clones (20%), as compared with Leu-156 → Arg, which affected 63%.

Cytotoxicity Inhibition Assays. Further analysis of the specificity of the CTL clones was done by analyzing blocking by mAbs (Table 5). As expected, the cytotoxic activity of the clones was not inhibited by anti-CD4 (OKT4), anti-class II monomorphic (LB3.1), or anti-HLA-B7 (ME1) mAbs. However, the monomorphic or polymorphic HLA-A2 reagents—i.e., mAbs W6/32, 4B3, MA2.1, BB7.2, and PA2.1—effectively blocked cytolysis by most of the CTL clones, although heterogeneity in blocking of different clones is evident. The lytic ability of the clones was generally inhibited by OKT8 and OKT3, but the effects of mAbs against LFA-1, CD2 (LFA-2), and LFA-3 accessory molecules again varied with the clone being tested—probably reflecting differences related to the avidity of the effector–target cell interaction.

DISCUSSION

The HLA-A2 serologic specificity is a group of related molecules distinguished by HLA-A2-restricted antiviral, anti-minor histocompatibility antigen, or HLA-A2 allospecific CTL, and by isoelectric focusing (8, 9). Initially, three subtypes (-A2.1, -A2.2, and -A2.3) and later a fourth (-A2.4) were defined with a combined frequency of 11% in the Caucasian population (7, 26). However, the presence of multiple amino acid substitutions in the α chains of some of these variants (-A2.2F, -A2.2Y, and -A2.3) makes it impossible to assess the role of individual residues in CTL recognition. In the present study, recognition of site-specific mutant HLA-A2 molecules, as well as the variants, was examined with a panel of HLA-A2-allospecific CTL clones. This analysis allowed examination of the structural requirements for CTL recognition and lysis of individual clones. By using the HLA-A2 variants as targets, four different patterns of reactivity were observed (Table 3). However, CTL clones within each subgroup were clearly distinguished when tested on targets expressing the natural hybrids LB-Aw68 and IDF-Aw69 or the site-specific HLA-A2 mutants (Table 3 and Fig. 1)—i.e., the clones are extremely heterogeneous in their specificities, and grouping them is artificial.

The amino acid residues altered can be classified into four groups: (*i*) Residues located outside of the putative binding site (residues 43 and 107). These two mutations had no effect on allorecognition; nor did they affect recognition of two distinct influenza virus peptides by appropriate clones (24, 25). (*ii*) Residues on the sides of the helices facing into the site (residues 63, 66, 70, 74, 77, 80, 152, and 156). Changes in these residues all affected allorecognition. Changes in residues 66 (66.6%) and 152 (57%) affected a large number (although not all) of the clones, with smaller percentages for the other residues altered. The conservative change, Ser-156, did not have an important effect on recognition because only 20% of the clones were affected, but the nonconservative substitution of Arg-156 produced a more drastic effect (62.5%). Similar results, suggesting a minor role for position 156, have been reported (27). (*iii*) Residues located in β-pleated sheets that form part of the floor of the site. The mutant at position 9 again affected some clones but not others. However, the variant HLA-A2.4b, which has a change only at position 99, was recognized by all clones examined. Notably this change also had no effect on the recognition of an epitope derived from the type A influenza nucleoprotein (24). (*iv*) Residues on the top face of the α_1-helix-facing solvent (residues 62, 65, and 76) and, in one case, pointing away from the groove (residue 79). It is noteworthy again that these presumed T-cell receptor binding sites affected many, but not all, the clones.

With regard to the recognition of mutants carrying multiple amino acid substitutions, changes in the left part of the α_1-helix (residues 65–74; A2M6574) altered the recognition of 85% of the clones, whereas 77% of them were altered by changes from position 70 to 80. In relation to the domain specificity of the T-cell clones analyzed, some of them were affected more importantly by residues of the α_1 domain (clones w2, w41, w74, and w44), whereas the rest were affected by changes in both α_1 and α_2 domains (compare ref. 28). Only a limited number of changes have been introduced in the second domain of the molecule (residues 99, 107, 152, and 156), and CTL clones affected by the α_2 domain changes exclusively (as measured by reactivity with the HLA-Aw69 molecule) were not observed. The α_2 helix has, in fact, only three positions at which three or more amino acids occur (residues 152 and 156, which point inward, and residue 163, which points inward and upward) as contrasted to the α_1-helix, which contains eleven positions (19). This limited polymorphism of the α_2-helix has a parallel in the class II molecules—i.e., the α chains of -DR (and I-E in the mouse) are completely conserved.

Replacement of most of HLA-A2-specific by HLA-B7 residues in the polymorphic region spanning amino acids 62–80 did not have a major effect on the recognition of the molecule by HLA-A2-specific mAbs (20) or alloantisera (29). Therefore, substitutions with no effect on serologic recognition can abrogate recognition by alloreactive T cells. Thus, as many previous studies suggested, serologic and CTL epitopes are located at different residues in HLA-A2 [as well as H-2 (30)] (with the exception that changes at residues 62–66 affect CTL recognition as well as recognition by mAb MA2.1).

Table 5. Inhibition of cytotoxicity of CTL clones on JY cells with mAbs

	mAb										
Clone	OKT4	OKT8	OKT3	TS1/18	TS2/18	TS2/9	W6/32	PA2.1	MA2.1	4B3	LB.31
w2	−	±	±	+	+	+	+	±	+	+	−
w74	−	−	±	+	±	±	±	±	+	+	−
w128	−	±	±	+	−	−	±	−	−	+	−
w164	−	+	+	+	+	+	+	−	−	+	−
w169	−	+	+	+	+	+	+	+	+	+	−
w172	−	+	+	+	+	+	+	±	+	+	−
w181	−	±	+	+	±	±	+	−	+	+	−
w168	−	+	+	+	+	+	±	−	±	+	−

+, 50–100% inhibition; ±, 20–50% inhibition; and −, no inhibition.

The effects observed, including the very large repertoire of allogeneic CTL, can be explained most readily by a model in which a number of different self-peptides derived from the allogeneic presenting cell are presented by the allogeneic (or mutant) MHC molecule (31, 32) and in which different self-peptides are bound at different specific subsites within the antigen-binding cleft. Such a model is compatible with the size of the cleft and with the biological observation that a very large universe of foreign peptides can be presented to the immune system by a large, but in this sense relatively limited, number of MHC molecules. Each molecule must then be able to present a variety of different peptides. Studies of the recognition of two different peptides from a type A and type B influenza virus strain led to the conclusion that a single peptide, as well as two different peptides, can be bound to HLA-A2 in different ways and possibly in different subsites (24, 25).

Are the data also compatible with a model in which the allogeneic CTL recognizes HLA-A2 molecules that contain no peptide in the antigen-binding cleft? In such a model, the variety of CTL would be explained by specific recognition of different portions of the cleft acting as T-cell epitopes. This model is less attractive, however, because many of the residues analyzed are pointing into the cleft, where they should be less accessible to CTL, and at least one, residue 9, is on the floor of the cleft (although alterations in this residue could affect the conformation of more accessible residues in the α_1 helix). In addition, this model implies no specific recognition of an MHC molecule, and it might be anticipated that altered residues outside the area of the antigen-binding site, such as, for example, residues 43 and 107, could also be targets for allospecific CTL.

The problem, however, of how a *nonself* MHC molecule can be recognized remains. Positive selection in the thymus is believed to ensure that only those T cells bearing receptors for self MHC molecules will reach the periphery. However, the number of polymorphic amino acid residues that point upward from the α-helices and are potential ligands for T-cell receptors is relatively limited—i.e., five (19); in the α_1-helix four of the seven positions that point upward can have three or more different amino acid residues, whereas in the α_2-helix it is only one position out of eleven. Possibly all T-cell receptors do not contact all five of these residues. Moreover, some changes may be tolerated by some T-cell receptors, as is illustrated for residues 65 and 76 in Table 4; this would be an example of cross-recognition. The self peptide in the peptide-binding cleft of an MHC molecule is derived from the allogeneic tissue. For an allogeneic response to occur this peptide must be different from that to which the T cells of the recipient have become tolerant because the individual must have become tolerant to most, if not all, self-peptides bound by self-MHC during negative thymic selection, presumably by clonal deletion. A different set of peptides derived from processed self proteins would have been selected by a different (allogeneic) MHC molecule. In addition, polymorphism for the self-peptide derived from allogeneic tissues may also play a role; polymorphic self proteins may be minor histocompatibility antigens. Finally, the *same* self peptide may be bound in distinct ways by self and allogeneic MHC molecules, presenting different conformations or side chains to T-cell receptors, and, therefore, resulting in different thymic selection. Thus, alloreactivity is the recognition by the host T-cell receptors of a foreign MHC molecule (possibly imperfect recognition) containing either a new set of self peptides or possibly a polymorphic, and therefore foreign, self peptide in its antigen-binding cleft. In this view T-cell epitopes for allorecognition are unusual *self peptides* bound in the foreign peptide-binding site of an allogeneic MHC molecule; they do not exist on the MHC molecule itself.

Similar studies of mutant MHC molecules have been carried out recently by a number of investigators (refs. 33–37 and the references cited therein), although their interpretations are not identical to that in the model presented here.

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