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Immunology

Endogenous peptides bound to HLA-A3 possess a specific combination of anchor residues that permit identification of potential antigenic peptides

(major histocompatibility complex class I/β_2-microglobulin/peptide binding motif)

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ABSTRACT **A motif specific to peptides that bind to the human class I major histocompatibility complex molecule HLA-A3 was identified by sequence analysis of HPLC fractions containing endogenous peptides. Twenty-six different sequences were obtained, 19 of which were nonamers. The majority of these endogenous peptide sequences contained Leu at position (P)2, while most sequences contained Tyr or Lys at P9. In addition, Phe was shared by 16 sequences at P3. Synthetic peptides corresponding to endogenous peptide sequences were shown to bind to HLA-A3. The importance of Leu at P2 and Tyr or Lys at P9 ("anchor" residues) for peptide binding to HLA-A3 was demonstrated by the following results: (*i*) peptides GLFGGGGGY, GLFGGGGGK, and GLGGGGFGY, but not GLFGGGGGV, specifically bound to HLA-A3 and (*ii*) six nonapeptides from within the influenza A nucleoprotein, matrix, and polymerase proteins, selected for synthesis based upon their possession of P2 and P9 anchor residues, were shown to bind HLA-A3. In contrast, none of a set of eight peptides that bound to HLA-A2, or six that bound to HLA-B27, bound detectably to HLA-A3. These findings provide a rationale for the design and selection of peptides that can be recognized by HLA-A3-restricted T cells.**

Since it became apparent that the function of class I major histocompatibility complex molecules depends upon the ability to bind small peptides (1), one of the major goals of research in this area has been to establish the specificity of peptide binding for each class I molecule. The isolation of endogenous peptides from five murine and human class I molecules has led to the hypothesis that the endogenous peptide repertoire is largely determined by a limited set of amino acids (anchor residues) at certain positions in the peptides (2–4). Molecular modeling of composite peptide structures in the HLA-A2 and HLA-B27 binding grooves (4–6) indicates that the side chains of anchor residues of the peptides protrude into pockets within the groove. In addition, peptides appear to align in the binding groove with the amino terminus in the A pocket and the carboxyl terminus in the F pocket (5, 7). The exact location of anchor residues within peptides binding to particular class I molecules is most likely a reflection of HLA pocket accessibility and composition.

HLA-A3.1 is an allele found in ≈23% of Caucasians and ≈14% of Blacks (8). A rare natural variant, HLA-A3.2, contains two amino acid substitutions at positions 152 (Glu to Val in pocket E) and 156 (Leu to Gln in pockets D and E) that have significant effects on recognition by virus-reactive and alloreactive cytotoxic T lymphocytes (9). We have isolated endogenous peptides from HLA-A3.1 molecules obtained from a B-lymphoblastoid cell line and from transfected Hmy2.C1R cells. Peptides were also isolated from HLA-A3.1 molecules containing single amino acid substitutions at position 152 or 156, expressed in the Hmy2.C1R cells. Sequence analyses of these peptides revealed a characteristic motif of anchor residues that permits the identification of potential antigenic peptides.

METHODS

Cells. HLA-A3 molecules were isolated from the human plasma cell line Hmy2.C1R transfected with the HLA-A3.1, HLA-A3 L156Q, or HLA-A3 E152V genes (9) and from the Epstein–Barr virus-transformed human B-cell line GB.

Isolation of Peptides from HLA-A3. HLA-A3 complexes were isolated from detergent lysates of 10^{10} cells by immunoaffinity chromatography with the anti-HLA-A3 monoclonal antibody GAP.A3 (10) as described (2). After extensive sequential washing with 10–20 ml each of phosphate-buffered saline containing octyl β-D-thioglucopyranoside (OSGP, Calbiochem) at 15 μg/ml (PBS/OSGP), 0.45 M NaCl/OSGP, and PBS/OSGP, the columns were eluted with 10 ml of 0.1 M NH_4HCO_3/OSGP at pH 11.6. The eluted fractions, containing 100–500 μg (2–9 nmol) of HLA complex, were neutralized immediately upon collection, dried, redissolved, and passed through a Centricon-10 microconcentrator (Amicon) or a C_{18} Sep-Pak cartridge (Waters) to remove the bulk of the intact proteins. The resulting partially purified extracts were fractionated by reversed-phase HPLC (see Fig. 1 legend).

Analysis of Peptide Binding to Class I Heavy Chains. An HLA-A3.1 cDNA clone derived from an HLA-A3.1 B-lymphoblastoid cell line encoding aa 1–278 of HLA-A3.1 was amplified by the polymerase chain reaction using the primers 5′-CAGACCTGGGCGGGATCCCACTCCATGAGGTATTTC-3′ and 5′-GGGGAAGCTTCATTAGGAAGACAGCTCCCATCT-3′ and inserted into the *Escherichia coli* expression plasmid pHN1$^+$ as described (11). HLA complexes were reconstituted by using HLA-A3 heavy chain (0.2 μg) prepared from *E. coli* inclusion bodies, iodinated human β_2-microglobulin (β_2m; 12,000 cpm; specific activity, 0.1–1 × 10^{18} cpm/mol) and peptides (10 μg) in a final volume of 50 μl (11). HLA-A2 and HLA-B27 heavy chains were prepared as described (11). HLA complexes were analyzed by nondenaturing isoelectric focusing (11). The stability of HLA-A3

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Abbreviations: β_2m, β_2-microglobulin; HIV, human immunodeficiency virus.

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complexes was assessed by measuring the rate of dissociation of ^{125}I-β_2m (12). Peptides were synthesized as described (12).

Peptide Sequence Analysis. N-terminal amino acid sequence was analyzed with a model 477A protein sequencer coupled to a model 120A phenylthiohydantoin analyzer (Applied Biosystems, Foster City, CA) according to the manufacturer's program, NORMAL-1.

RESULTS

Separation of Endogenous Peptides. To obtain a source of HLA-A3-associated endogenous peptides, HLA-A3.1 and the mutant molecules HLA-A3 L156Q and HLA-A3 E152V were isolated from detergent lysates of transfected Hmy2.C1R cells or from the HLA-A3.1-positive, Epstein–Barr virus-transformed human B-cell line GB by immunoaffinity chromatography. After isolation of the lower molecular weight material (see *Methods*), the peptides were fractionated by reversed-phase HPLC. A representative HPLC fractionation of HLA-A3 L156Q-associated peptides is shown in Fig. 1. The bulk of the peptides were eluted in the area between 19 and 34 min (fractions 25–45), and an enlarged view of this region is shown in Fig. 1 *Lower*. Seven peaks (indicated by numbers) specific to the HLA-A3-specific eluate were chosen for sequence analysis.

Sequences of the Endogenous Peptides. The results of sequence analyses for 26 peaks are shown in Table 1. Initial yields of phenylthiohydantoin amino acid derivatives were 0.2–20 pmol and repetitive yields were 80–90% of the initial yields (20–90 pmol of peptide would represent 1% occupancy based on the yield of HLA complex from 10^{10} cells). Nineteen of the 26 sequences obtained were 9 aa long, 4 were 10 aa, and 3 sequences yielded information through only six or seven cycles, presumably because of low yield (<0.1 pmol). Each of the primary sequences was unique. In addition to the primary amino acid assignments, in many cases at a given sequence cycle, alternative amino acid residues were present in lower yield, indicating the presence of multiple peptides in many of the HPLC fractions. Comparison of these peptide sequences revealed the presence of characteristic amino acid residues at particular positions in the HLA-A3 binding peptides. Most evident was the presence of Leu or a closely related residue at position P2, predominantly Phe or Tyr at P3, and Tyr or Lys at P9. In addition, hydrophobic residues were observed at P7 and noncharged residues were noted at P6. All classes of amino acids were represented at P4, P5, and P8. Although Gly, Ser, and Lys were observed as predominant residues in most of the peptides at P1, general background associated with automated sequence analysis at cycle 1 often precluded definitive assignments. The proteins that were the sources of the peptides could not be identified in existing protein sequence data bases.¶

Ability of Synthetic Analogs of Endogenous Peptides and a Known HLA-A3 Epitope to Bind to HLA-A3 *in Vitro*. To confirm that these sequences represented peptides that could bind to HLA-A3, peptides pn2, pn4, and pn6 were synthesized (Table 1). pn2 was synthesized as the endogenous decapeptide (pn2a, KLYEKVYTYK) as well as a peptide consisting of the first 9 aa (pn2b, KLYEKVYTY), since the nonamer with Tyr at P9 seemed to have requisite HLA-A3 binding features. Gly was chosen to represent the unidentified residue at P5 of pn6 (GLFPXQFAY). The ability of these peptides to promote HLA-A3 complex formation was investigated with HLA-A3 heavy chains (prepared from *E. coli* inclusion bodies) and iodinated human β_2m. Analysis by nondenaturing isoelectric focusing (Fig. 2*A*, lanes 2–5) showed that all four of these synthetic peptides bound to HLA-A3 as evidenced by the

¶This is not unusual, as the potential sources of only 50% of HLA-A2 endogenous peptides (3) and about 10% of H-2D^d and H-2L^d endogenous peptides have been identified (16).

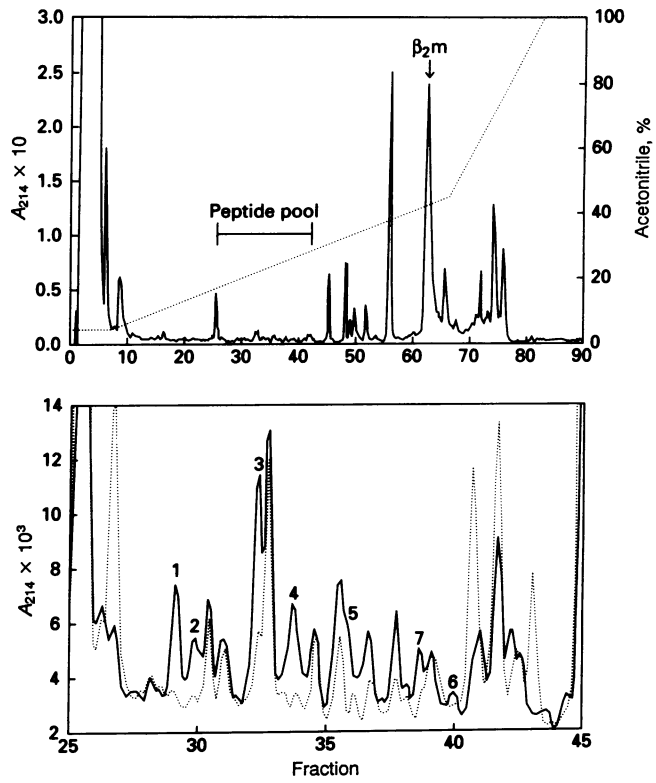

FIG. 1. (*Upper*) Reversed-phase HPLC of immunoaffinity-purified HLA-A3 L156Q-associated peptides. Peptides were separated by reversed-phase HPLC on a C_{18} Nova-Pak column (Waters), using Beckman System Gold instrumentation that included a photodiode-array detector (model 168). The gradient consisted of 0.1% trifluoroacetic acid/acetonitrile, 96:4, for 5 min followed by a linear increase to 40% acetonitrile over 45 min. The flow rate was 1 ml/min and the fraction size was 0.2 ml. Individual fractions were frozen on dry ice and dried by vacuum centrifugation prior to sequence analysis by automated Edman degradation. (*Lower*) Enlarged view of the elution profile for the region between 19 and 34 min. Superimposed upon the profile for the eluate from the anti-HLA-A3-specific Sepharose column (solid line) is the same region of the chromatogram corresponding to material adsorbed onto and eluted from a Gly-Sepharose column (dotted line).

incorporation of free ^{125}I-β_2m into the complex. As expected, the endogenous peptides did not bind to HLA-B27 or HLA-A2 (Fig. 2 *B* and *C*). An HLA-B27-binding peptide (SRYWAIRTR) from influenza A nucleoprotein (aa 383–391) with an Arg at P2 and P9 did not bind to HLA-A3 (Fig. 2*A*, lane 6). A synthetic peptide that represented an endogenous peptide (ILDKKVEKV) that was isolated from an HLA-A2-bearing melanoma cell line by the same methodology as the HLA-A3 endogenous peptides (M.D., unpublished data) did not bind to HLA-A3 (Fig. 2*A*, lane 7) but did bind to HLA-A2 (Fig. 2*C*, lane 2). In addition, a panel of eight HLA-A2-binding peptides as well as six HLA-B27-binding peptides did not bind to HLA-A3 (data not shown). The stability of HLA-A3/endogenous peptide complexes, assessed by measuring the half-time for dissociation of ^{125}I-β_2m (12), ranged from <10 hr to 100 hr ($t_{1/2}$ at 37°C, Table 2).

In some instances, a decapeptide can be the preferred peptide for binding to HLA-A3 even though the nonapeptide contained within it conforms to the peptide binding motif. This has been illustrated for two peptides, pn2a and an HLA-A3-restricted antigenic peptide that had previously been identified from the HIV Nef protein sequence (aa 73–82, QVPLRPMTYK; ref. 13). Fig. 2*A*, lane 8, shows that the 10-residue Nef peptide bound to HLA-A3 ($t_{1/2}$ = 30 hr, Table 2), whereas a 9-residue Nef peptide lacking the carboxyl-

Table 1. HLA-A3 endogenous peptide sequences

Position number										
P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Peptide
Hmy2.C1R HLA-A3.1										
K (S, T)	X	F (A)	K	M	I	L	R	K		pn14
K (A, G)	L	F	K	N (Q, A)	I	L	Y	[K]		pn15
Y (P)	L	X	V	R	X	A	X	[I]	V	pn16
K (S, T)	L (V)	H (Y)	K	Q (Y)	R (V)	A (L, F)	K	S (A, K)		pn17
GB HLA-A3.1										
S (T, K)	L	F (Y, P)	K	Q	V	V	T	K		pn18
K (N, D)	X	F (Y)	V	K	X	L	X	Y		pn19
S (K, A)	L	F	N (Q)	T	H	L	X	K (Y, V)		pn20
T (K, S)	L	A	N (G, K)	D (V)	X	V	V (T, N)	P		pn21
G (S, A)	I (N)	F (Y)	A (H/D)	X	X	X	V	[K]	A	pn22
S (D, Q)	L	Y	X	X	T					pn23
S (K)	L	Y	E (P)	L (H)	X	L				pn24
T	X	F	V	K	X	L	X	Y		pn25
S	L	F	D	H (Y, D)	I (L, D)	L (F, R)	X	K	H	pn26
Hmy2.C1R HLA-A3 L156Q										
K	L (I, V)	F (P)	K (P)	V (E, I)	V	X	N (K)	Y		pn1
K (A, S)	L (F, P)	Y (T, K)	E	K (A)	V (G)	Y (Q, N)	T (L)	Y	K	pn2
K (A)	L (V, M)	F (Y, A)	N (E, K)	I (P, F)	M	V (F, D)	T (R)	Y		pn3
K (S, V)	L (V)	F (A, D)	E (G)	K (G)	V	Y (F)	N	Y		pn4
K	L (I, M)	F	K (I, P)	V	T (I, S)	F (L)	[S]	Y		pn5
G (K, A)	L (V)	F (Y)	P (K)	X	Q	F	A	Y		pn6
S (A, L)	L (V)	F	[E]	[L]	[V]	F	X	Y (M)		pn7
Hmy2.C1R HLA-A3 E152V										
S (H)	L	X	E (Q)	K (F)	[T]	F (V, Y)	[D]	Y		pn8
S (K, R, S)	L (A, F)	H (E)	K	Y	X	[F]	[E]	[Y]		pn9
K (T, W)	M	F	N (Y)	I	T	[V]	T	Y		pn10
K (A, G)	L (V)	F (M)	V	K	V	Y	N	Y		pn11
K (S, T)	I	V (Q)	R	K (Q)	P (M)	G (R)	M (V, L)	A		pn12
S (Q, K)	[L]	F	N	I	T	A				pn13
Motif										
Un*	L	F	Un*	Un*	Hy†	F	Un*	Y/K		
	Hy†	Y			Po‡	Hy†				
		Hy†				Y				

Primary amino acid assignments are given in rows with a corresponding peptide number. Letters in parentheses positioned next to the primary assignments indicate the presence of secondary amino acid residue assignments at these positions. Brackets denote probable amino acid assignments; X denotes unknown residue.

*Unrestricted (no preference for any particular amino acid).

†Hydrophobic amino acid residue.

‡Polar uncharged amino acid residue.

terminal Lys did not (Fig. 2*A*, lane 9). Although both the 9-residue and 10-residue forms of the endogenous peptide pn2 bound to HLA-A3 (Fig. 2*A*, lanes 4 and 5), the 10-residue version of pn2 formed a more stable complex (100 hr vs. 20 hr, Table 2).

Three Non-Glycine Amino Acids Are Sufficient for Stable Binding of Peptide to HLA-A3. As the amino acids at P2 and P9 of the endogenous peptide sequences were the least variable, we hypothesized that P2 and P9 might be anchor positions. To determine whether residues at these hypothetical anchor positions were sufficient for binding of peptides to HLA-A3, we synthesized a "minimal" nonapeptide based upon a poly(Gly) backbone. Gly backbone residues were chosen for these peptides because they allow maximum peptide flexibility and contain no side chains capable of binding in the pockets of the peptide-binding groove. The nonapeptide GLGGGGGGY did not bind to HLA-A3 (Fig. 3, lane 2). Two other peptides, GLFGGGGGY and GLFGGGGGK, each having an additional non-Gly residue, were selected for synthesis based upon the apparent predilection for Phe at P3 (see Table 1). Both of these peptides bound to HLA-A3 (Fig. 3, lanes 3 and 4) but not to HLA-A2 (lanes 9 and 10). The stability of complexes formed with GLFGGGGGK [$(L2F3K9)_9$] was slightly greater than that of complexes formed with GLFGGGGGY [$(L2F3Y9)_9$] (6 hr vs. <1 hr, Table 2). A Gly-backbone peptide, GLFGGGGGV, which contained the HLA-A2 peptide binding motif [$(L2V9)_9$] (2) did not bind to HLA-A3 (Fig. 3, lane 5) but did bind to HLA-A2 (lane 7). Further studies have shown that GLGGGGFGY also binds to HLA-A3, indicating that the requirement for Phe at P3 is not stringent (data not shown).

Peptides from Influenza A Proteins Bind to HLA-A3. The value of the $(L2,Y9/K9)_9$ anchor-residue motif in predicting HLA-A3-binding peptides was investigated by screening the influenza A nucleoprotein (NP), matrix (M1), and RNA polymerase (Pol) protein sequences for nonamer subsequences that contained Leu at P2 and Tyr or Lys at P9. Six of 26 such sequences were synthesized and all 6 bound detectably to HLA-A3 (Fig. 4*A*, lanes 2 and 3, and Fig. 4*B*). Four of them bound especially well.

The importance of the P2 and P9 anchor-residue positions for determining peptide binding specificity was further investigated with the well-studied HLA-A2-specific influenza-A M1 peptide GILGFVFTL (aa 58–66). This peptide, as expected, failed to promote HLA-A3 complex formation (Fig. 4*A*, lane 4). However, a substitution derivative of this peptide with Lys at P9, GILGFVFTK, chosen because it had good matches to the HLA-A3 motif, clearly was able to bind to HLA-A3 (lane 5). The HLA-B27-binding peptide SRYWAIRTR was also converted into a peptide that bound to HLA-A3 by changing the Arg at P2 to Leu (lane 6).

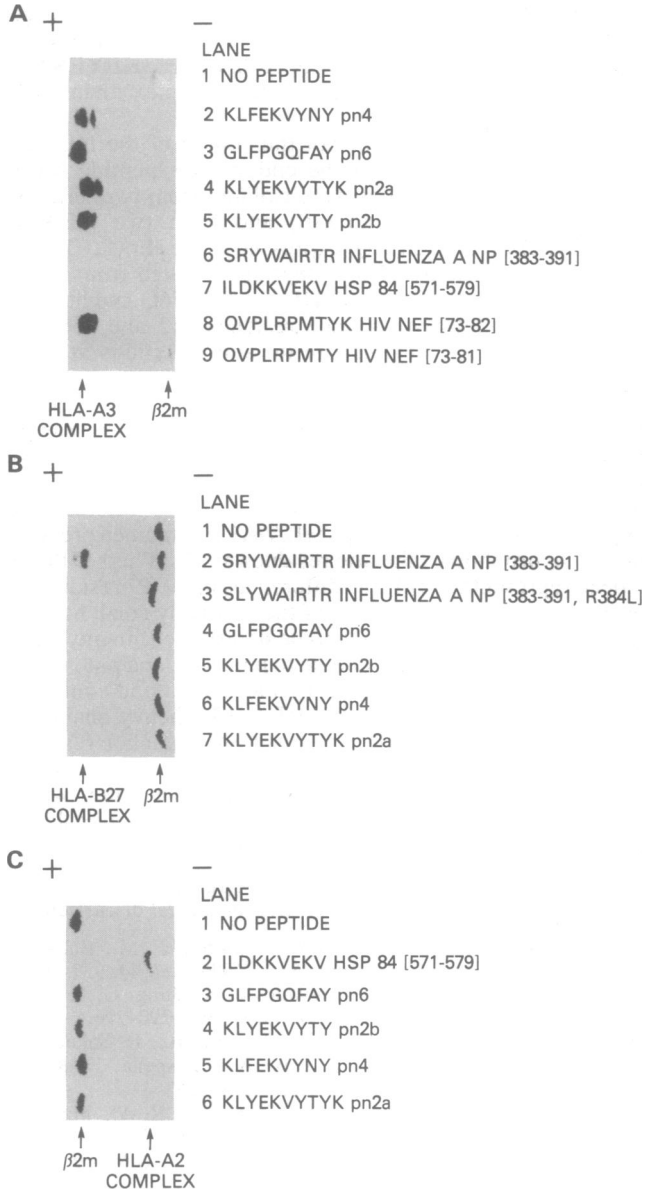

FIG. 2. Nondenaturing isoelectric focusing gel analysis of the ability of peptides to promote HLA-A3/^{125}I-β_2m (*A*), HLA-B27/^{125}I-β_2m (*B*), or HLA-A2/^{125}I-β_2m (*C*) complex formation. Synthetic peptides were produced that corresponded to the sequences of three of the endogenous HLA-A3 peptides (pn2a, pn2b, pn4, pn6) and to that of the HLA-A3 antigenic human immunodeficiency virus (HIV) Nef peptide (aa 73–82). +, Acidic side of the gel (pH 4.0); −, basic side (pH 6.5). The isoelectric focusing position of the complexes is dependent upon the charges present on both the peptides and the HLA heavy chains. The HLA-A3 and HLA-B27 heavy chains have more negative and less positive charges than the HLA-A2 heavy chain; therefore, HLA-A3 and HLA-B27 complexes focus more toward the acidic side of the gel than do HLA-A2 complexes. NP, nucleoprotein; HSP, heat shock protein.

DISCUSSION

Twenty-six amino acid sequences were obtained for endogenous peptides eluted from HLA-A3. The preferred length for these peptides appears to be nine residues, as indicated by 19 of the 26 peptide sequences that were obtained (Table 1). Synthetic peptides corresponding to the sequences of several of the endogenous peptides bound to HLA-A3 *in vitro*, supporting the conclusion that these sequences are indeed representative of peptides that are bound to HLA-A3 *in vivo*.

The P2 and P9 anchor residues are of prime importance for HLA-A3 complex formation as indicated by the limited amino acid variability at these positions for HLA-A3-binding peptides (Table 1). The value of the defined anchor residues at P2 and P9 for predicting peptide binding to HLA-A3 was investigated by screening influenza A protein sequences for segments that contained Leu (P2) followed seven residues later by Tyr or Lys (P9). Six such peptides were selected for synthesis and were shown to be capable of binding to HLA-A3 (Fig. 4). In addition, HLA-A2 and HLA-B27 peptides can be converted to HLA-A3 binding peptides by substituting the appropriate anchor residues at these positions (Fig. 4*A*, lanes 5 and 6); however, the side chains of these anchor residues in themselves do not provide enough interaction for stable binding to the HLA-A3 heavy chain, as indicated by the failure of GLGGGGGGY to promote HLA-A3 complex formation (Fig. 3, lane 2). The ability of GLFGGGGGY and GLFGGGGGK to promote HLA-A3 complex formation emphasizes the potential importance of the P3 side-chain interaction for peptide binding to HLA-A3. However, it is clear that a P3 Phe or Tyr is not necessary for peptides to bind to HLA-A3, as peptides lacking such P3 residues (Fig. 2*A*, lane 8, and Fig. 4*A*, lanes 2 and 5) and GLGGGGFGY (data not shown) also bind. In these cases, other P3 side chains and/or the side chains of other peptide residues such as P6 or P7 may provide interactive forces capable of stabilizing peptide binding.

On the basis of models of peptide binding to HLA-A2 (6) and HLA-B27 (5), it is thought that the side chain of the residue at P2 contacts residues of the B pocket. There are no

Table 2. Half-time of dissociation of β_2m from HLA-A3 complexes formed with various peptides

Sequence	Name	$t_{1/2}$, hr
GLFPGQFAY	pn6	5
KLYEKVYTYK	pn2a	100
KLYEKVYTY	pn2b	20
KLFEKVYNY	pn4	8
QVPLRPMTYK	HIV Nef (73–82)	30
GLFGGGGGY	$(L2F3Y9)_9$	<1
GLFGGGGGK	$(L2F3K9)_9$	6

*Half-time of dissociation of β_2m at 37°C, calculated by linear regression from individual time points. Half-times are accurate to within a factor of 2.

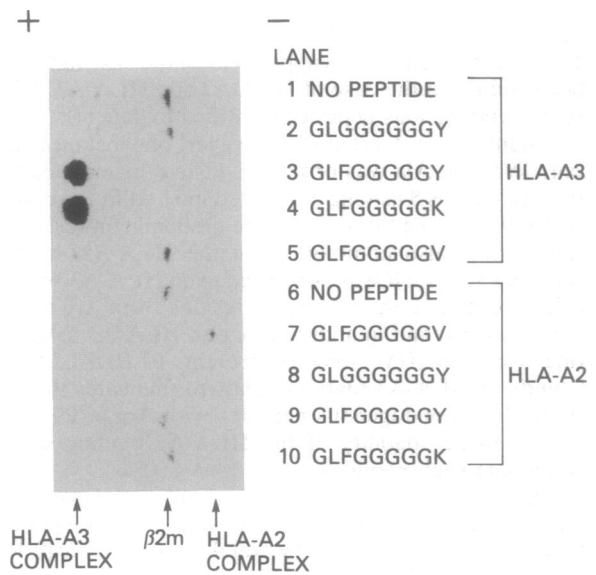

FIG. 3. Determination of residues important for binding to HLA-A3. Poly(Gly)-based peptides which contained the HLA-A3-specific residues at P2 and P9 were tested for binding to HLA-A3 and HLA-A2 as in Fig. 2.

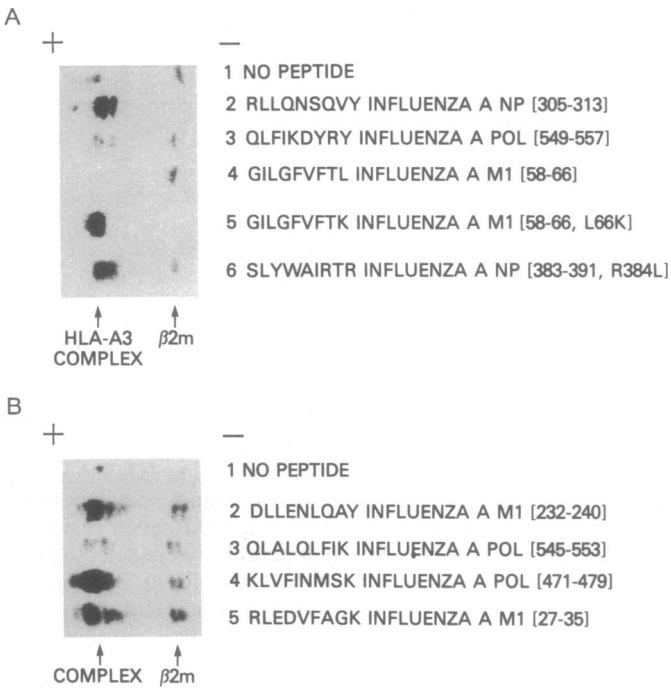

FIG. 4. Predictive value of the HLA-A3 peptide-binding motif. Peptides from influenza A NP, M1 and polymerase (POL) proteins having $(L2,Y/K9)_9$ anchor residues were tested for binding to HLA-A3 (*A* and *B*). Two substitution derivatives of HLA-A2- and HLA-B27-binding peptides with HLA-A3 anchor residues at P2 and P9 were also tested for binding to HLA-A3 (*A*). Peptide GILGFVFTK is related to the HLA-A2-binding influenza-A M1 peptide GILGFVFTL (15), and peptide SLYWAIRTR is related to the HLA-B27-binding influenza-A NP peptide SRYWAIRTR (15).

differences in amino acid residues between HLA-A2 and HLA-A3 in the B pocket that are thought to contribute directly to peptide binding. Therefore, it is not surprising to find that peptides binding to HLA-A2 and HLA-A3 share Leu or a closely related residue as the anchor residue at P2.

The side chain of the residue at P9 in the peptide is thought to bind in the F pocket (5–7). Since the F-pocket residues (Asp^{116}, Thr^{143}, Trp^{147}, Leu^{81}, Tyr^{123}) thought to interact with peptides are identical in HLA-B27 and HLA-A3, one might expect the P9 anchor residues of peptides binding to these molecules to be identical, as appears to be the case for the P2 anchor residues in the case of HLA-A2 and HLA-A3. Of 11 HLA-B27 endogenous peptides identified to date (4), 7 have basic residues (Arg, Lys) at P9; however, one instance each of Leu, Tyr, and Ala was observed. (The remaining endogenous peptide was 8 aa long and ended in His.) In the case of HLA-A3-binding peptides, Tyr is the predominant residue at P9, but Lys is also common. That the HLA-B27-binding peptide SRYWAIRTR was converted to an HLA-A3-binding peptide by changing the P2 anchor residue from Arg to Leu (Fig. 4*A*, lane 6), and in addition that an HLA-A3 antigenic peptide from the HIV envelope protein, RLRDLLLIVTR (14), binds to HLA-A3 (data not shown), indicates that the failure to detect endogenous peptides with Arg at P9 is not due to a general inability of the HLA-A3 binding site to accommodate such peptides.

The majority of the peptides that bind to HLA-A3 are nonamers, but in two cases it is clear that the decapeptide is the preferred sequence. The HIV Nef decapeptide QVPLRPMTYK (aa 73–82) bound to HLA-A3, but the nonapeptide lacking the carboxyl-terminal Lys did not (Fig. 2*A*, lanes 8 and 9). This result is consistent with the prior observation that only the decapeptide was capable of sensitizing target cells for lysis by HLA-A3-restricted, HIV-specific cytotoxic T cells (16). The second example is that pn2a (KLYEKVYTYK) formed a 5-fold more stable complex than pn2b (KLYEKVYTY) (Table 2).

To determine whether or not the source of the HLA-A3 molecules would influence the endogenous peptide repertoire, endogenous peptides were isolated from two different cell lines (Hmy2.C1R and GB) and from two HLA-A3 mutants (E152V and L156Q) expressed in the Hmy2.C1R cell line. We had anticipated that peptides isolated from HLA-A3.1, HLA-A3 E152V, and HLA-A3 L156Q might have characteristic differences since residues 152 and 156 face toward the peptide binding site (6) and mutations at these sites have been shown to affect recognition by cytotoxic T cells (9). Models of the HLA-A2 (6) and HLA-B27 (5) peptide binding sites indicate that residues 152 and 156 are part of pocket E, which is believed to bind the side chain of P7. We observed that endogenous peptides eluted from HLA-A3.1 mutants contained more Phe or Tyr at P7, whereas the peptides eluted from wild-type HLA-A3.1 contained predominantly hydrophobic aliphatic residues at P7. Whether this is indeed representative of true differences in the P7 residues or whether this is a consequence of the relatively small number of peptides analyzed remains to be determined. In any case, synthetic peptides corresponding to pn2, pn4, and pn6, which were isolated from the HLA-A3 L156 mutant and contained Tyr or Phe at P7, bound to the HLA-A3.1 heavy chain.

In conclusion, a specific combination of anchor residues has been identified from HLA-A3 endogenous peptide sequences. This has allowed the prediction of peptides which are capable of binding to HLA-A3, providing a rationale for the design of HLA-A3-specific peptide-based vaccines.

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