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A subset of HLA-B27 molecules contains peptides much longer than nonamers

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ABSTRACT **An unusual monoclonal antibody (MARB4) directed against HLA-B27 that reacts with only ≈5–20% of the cell surface HLA-B27 was used for large-scale purification of these molecules. Subsequent mass spectrometry of HLA-B27-bound peptides showed that the minor MARB4-reactive population contained peptides primarily from 900 to 4000 Da in size (≈8–33 amino acid residues), whereas the major HLA-B27 population contained peptides in the mass range of 900–1400 Da (≈8–12 amino acid residues). Thus, a subset of HLA-B27 molecules binds to peptides much longer than nonamers. Typical HLA-B27-binding peptides contain arginine in position 2. Further analysis by Edman sequencing of the pooled bound peptides revealed that the major population contained substantial amounts of arginine at positions 1 and 9 (40–50%) and exclusively arginine at position 2, as expected. The minor population of peptides also contained detectable amounts of arginine at these positions, but at the level of only ≈10%; no marked enrichment at any position was observed. These long HLA-B27-bound peptides could represent either intermediates in the formation of nonamers or adventitiously bound peptides. Lastly, in the TAP2 mutant cell line BM36.1 transfected with HLA-B*2705, MARB4-reactive HLA-B27 molecules were absent from the cell surface, indicating that the peptide transporter was required for delivery of the long peptides. Thus, during the folding of class I heavy chains, peptides of diverse lengths are available and participating.**

The peptide-binding cleft of major histocompatibility complex (MHC) class I molecules contains several recesses or pockets (1), one or two of which in each allotype are selective for particular amino acid side chains at peptide residues called anchor positions (2). For example, the crystallographic structure of HLA-B27 displays a nine-residue peptide backbone containing an arginine side chain at position 2 (P2) that extends down into a pocket formed by residues conserved in all HLA-B27 subtypes (including residues E45, T24, and H9). The guanidinium group of the important P2 arginine side chain along with a single water molecule coordinates a planar hydrogen bonding network with these residues that contributes 4 hydrogen bonds out of a total of 22 formed in the binding of the model peptide (RRIKAITLK) (3, 4). Furthermore, characterization of 14 peptides bound to water-soluble HLA-B27 has indicated that these peptides are all nine residues in length (nonamers) and each contains an arginine at P2 (5). This relatively strict size requirement is related to the occurrence of pockets at the two ends of the cleft (sometimes called the A and F pockets) that participate in hydrogen-bonding networks with the charged N and C termini of the peptide.

In this paper the repertoire of peptides bound to HLA-B27 molecules *in vivo* has been examined employing a recently developed monoclonal antibody [mAb (MARB4)] that reacts with only a small subset of the HLA-B27 population and a more typical mAb (ME1) that reacts with all cell surface HLA-B27 molecules.

MATERIALS AND METHODS

Purification of HLA-B27 Molecules and Peptide Analysis. HLA-B27 was immunoaffinity purified from 100 g of the Epstein–Barr virus (EBV)-transformed human B-cell line LG-2 essentially as described (6). Briefly, detergent-soluble material free of cell debris was loaded onto a serial immunoaffinity chromatography tree consisting of Sepharose CL-4b, Sepharose-protein A, Affi-Gel-10-NMS, Sepharose-protein A-MARB4, and Affi-Gel-ME1. Following extensive column washing, the bound HLA-B27 was eluted in 150 mM glycine/0.1% sodium deoxycholate (DOC), pH 11.5, immediately neutralized, and then dialyzed against 10 mM Tris·HCl/0.1% DOC. The yields of purified HLA-B27 were determined using bicinchoninic acid (BCA) assays and the purity was assessed by SDS/PAGE. Bound peptides were extracted and separated by high-performance liquid chromotography as described (7), except that Centricon 3 ultrafiltration devices were used in place of Centricon 10 units because of their lower size exclusion. One-hundred-microliter fractions were collected during the reversed-phase chromatography (RPC) separation and each was concentrated to ≈25 μl. The molecular mass of the extracted peptides was determined by matrix-assisted laser desorption mass spectrometry (Lasermat, Finnigan-MAT, San Jose, CA) essentially as described (7) except that α-cyano-4-hydroxycinnamic acid (10 mg/ml in 50% acetonitrile) instead of sinapinic acid was used as the matrix. One percent of each fraction was used corresponding to 20–100 fmol of peptide.

Pooled sequencing was performed by removing 10 μl (MARB4-B27) or 2.5 μl (ME1-B27) from each concentrated RPC fraction. This mixture (corresponding to 40% or 5% of the total) was sequenced on an Applied Biosystems 477A peptide sequencer. The pmol data presented in each row of Fig. 3 *a* and *b* are the raw sequencing yields multiplied by an Edman sequencing efficiency factor (8).

Construction and Characterization of TAP-Deficient HLA-B27 Transfectants. A genomic clone of HLA-B*2705 (9) was cloned into the *Bam*HI site of the expression vector pTM (10). pTM/B2705 plasmid DNA was introduced into BM36.1

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Abbreviations: mAb, monoclonal antibody; EBV, Epstein–Barr virus; RPC, reversed-phase chromatography; MHC, major histocompatibility complex.

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and BM28.7 by electroporation. Stable HLA-B27-expressing transfectants were obtained following geneticin selection and screening. Cells were grown in Dulbecco's modified Eagle's medium or RPMI 1640 supplemented with antibiotics, 10% fetal calf serum, and, in the case of transfectants, 600 μg of geneticin per ml.

The expression of MARB4-reactive HLA-B27 was also investigated using HLA-B*2705 transfectants of the HLA hemizygous cell line BM28.7 (HLA-A1, -Cw4, -B35, -Bw6; ref. 11) and of the mutant BM36.1, which differs from BM28.7 only in a 2-bp deletion of TAP2 leading to a nonfunctional TAP1/TAP2 complex (12). In addition, the EBV-transformed homozygous HLA typing cell line LG-2 (HLA-A2, -B27, -Cw4) was used as a control.

RESULTS

Characterization of the MARB4 HLA Specificity. The HLA-B27-specific mAb MARB4 (A.R., B.U.-Z., and A.Z., unpublished data) is capable of recognizing only ≈5–20% of the total cell surface HLA-B27 expressed on the surface of human EBV-transformed B cells and reacts with all HLA-B27 subtypes except HLA-B*2703 (Fig. 1). Interestingly, MARB4 does not recognize HLA-B7 molecules, although many other HLA-B27-reactive mAbs do.

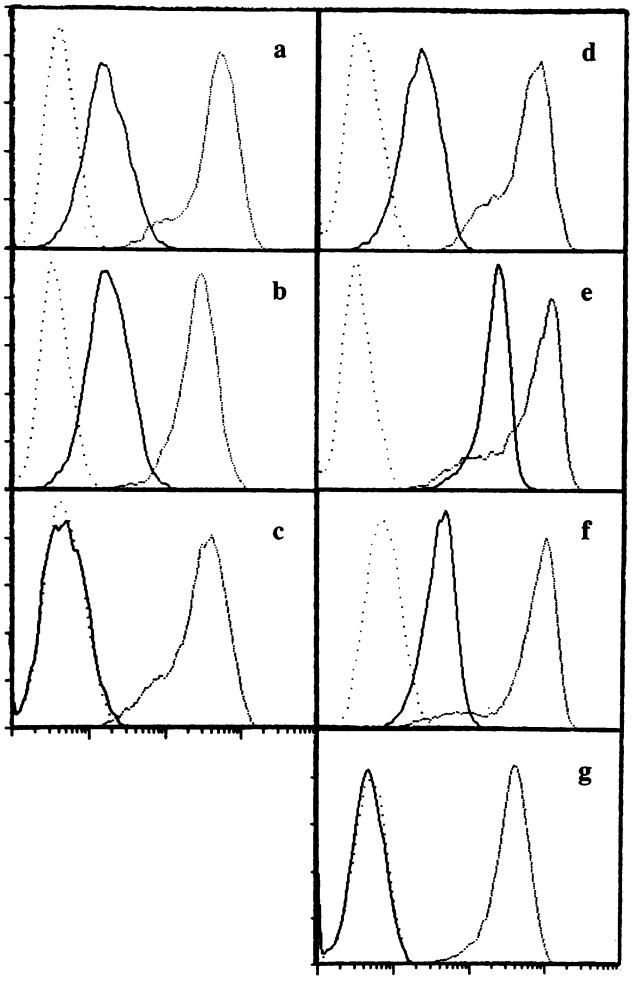

FIG. 1. Analysis of the mAb (MARB4) specificity by flow cytometry. In all panels the lightly dotted line represents the negative control staining (W6/32.HK), the solid line represents the staining with MARB4, and the stippled line represents the staining with ME1 (13). (*a*) HLA-B*2701 (LIH). (*b*) HLA-B*2702 (CHR). (*c*) HLA-B*2703 (LAR). (*d*) HLA-B*2704 (WEWAK). (*e*) HLA-B*2705 (LG-2). (*f*) HLA-B*2706 (PAR). (*g*) HLA-B*0701 (H2LCL). Another HLA-B*2703 cell line (ETO) was tested and gave no reactivity with MARB4 as well but did react with ME1.

Immunoprecipitation followed by isoelectric focusing showed that the heavy chains of the MARB4-reactive subset were identical to those precipitated by several other HLA-B27-specific mAbs and that β_2-microglobulin was present (data not shown). These results rule out the possibility that MARB4 recognizes a novel class I molecule in linkage disequilibrium with HLA-B27.

Analysis of Peptides Bound to HLA-B*2705 Molecules. To investigate whether the MARB4 specificity is dependent on (*i*) a specific peptide or a small family of peptides, (*ii*) empty HLA-B27 molecules, or (*iii*) a conformational subset of HLA-B27 molecules, HLA-B27 was purified from the homozygous human B-cell line LG-2 (HLA-B*2705) on serial MARB4 and ME1 immunoaffinity columns, yielding 450 μg and 730 μg of HLA-B27, respectively. The yield of MARB4-reactive HLA-B27 heterodimers purified from cell lysates was higher than predicted (based on fluorescence-activated cell sorting analysis of cell surface expression, Fig. 1), suggesting that a substantial fraction of MARB4-reactive material failed to reach the cell surface and was retained within the cell. The peptides bound to MARB4-reactive HLA-B27 (MARB4-B27) and to MARB4-depleted ME1-reactive HLA-B27 (ME1-B27) molecules were acid-extracted and separated by RPC (Fig. 2 *a* and *b*). The chromatographic profiles were similar in overall complexity, indicating that MARB4 is not specific for empty HLA-B27 molecules or for those containing a specific or a small subset of peptides and thus must recognize a distinct conformation of HLA-B27 molecules. Further analysis of these peptides by mass spectrometry revealed a striking difference in size distribution (Fig. 2 *c* and *d*). The peptides eluted from MARB4-B27 contained peptides significantly longer than those eluted from ME1-B27. Using an average amino acid mass of 118 Da, the estimated peptide lengths for the predominant MARB4-B27- and ME1-B27-bound peptides were 8–33 and 8–12 residues, respectively. Long peptides have also been found in HLA-A2 and HLA-Aw68 (14). Thus, the dogma that class I MHC molecules only bind peptides 8–12 residues in length does not take account of the full repertoire of naturally bound peptides.

Edman Sequencing of Peptides Extracted from HLA-B*2705 Molecules. To obtain more information about the peptides bound to HLA-B27 molecules, pooled peptide sequencing was carried out after combining aliquots from each individual RPC fraction (Fig. 2 *a* and *b*). The population of peptides extracted from MARB4-B27 contained ≈5–20% arginine at each cycle (up to 25 cycles) but the relative amount of arginine was not selectively enriched at any position (Fig. 3 *a* and *c*). Thus, if arginine residues are involved in the binding of the longer peptides, as seems possible, they are not at a precise distance from the N termini. In contrast, the peptides extracted from ME1-B27 molecules contained substantial arginine at P1 and P9 (40–50%) and exclusively arginine at P2 (Fig. 3 *b* and *c*), whereas the amino acid ratios at other positions were strikingly similar to those of the predominant HLA-B27 peptides previously described (5). Because MARB4 recognizes a subset of the total HLA-B27 population, the individual peptides were in insufficient amounts (subpicomolar) for high-confidence Edman sequencing.

The TAP Complex Is Required for Genesis of MARB4-Reactive HLA-B27 Molecules. To characterize further the source of peptides contributing to the recognition of HLA-B27 by MARB4 (e.g., from the cytosol or endoplasmic reticulum) a cell line expressing a mutated TAP gene was utilized. Specifically, the HLA-B*2705 gene was transfected into the TAP2 mutant cell line BM36.1 and its wild-type parent BM28.7. The TAP2 mutation in BM36.1 is a frameshift alteration immediately 3′ to the putative ATP-binding site of the protein (12). BM36.1 does not present antigen to

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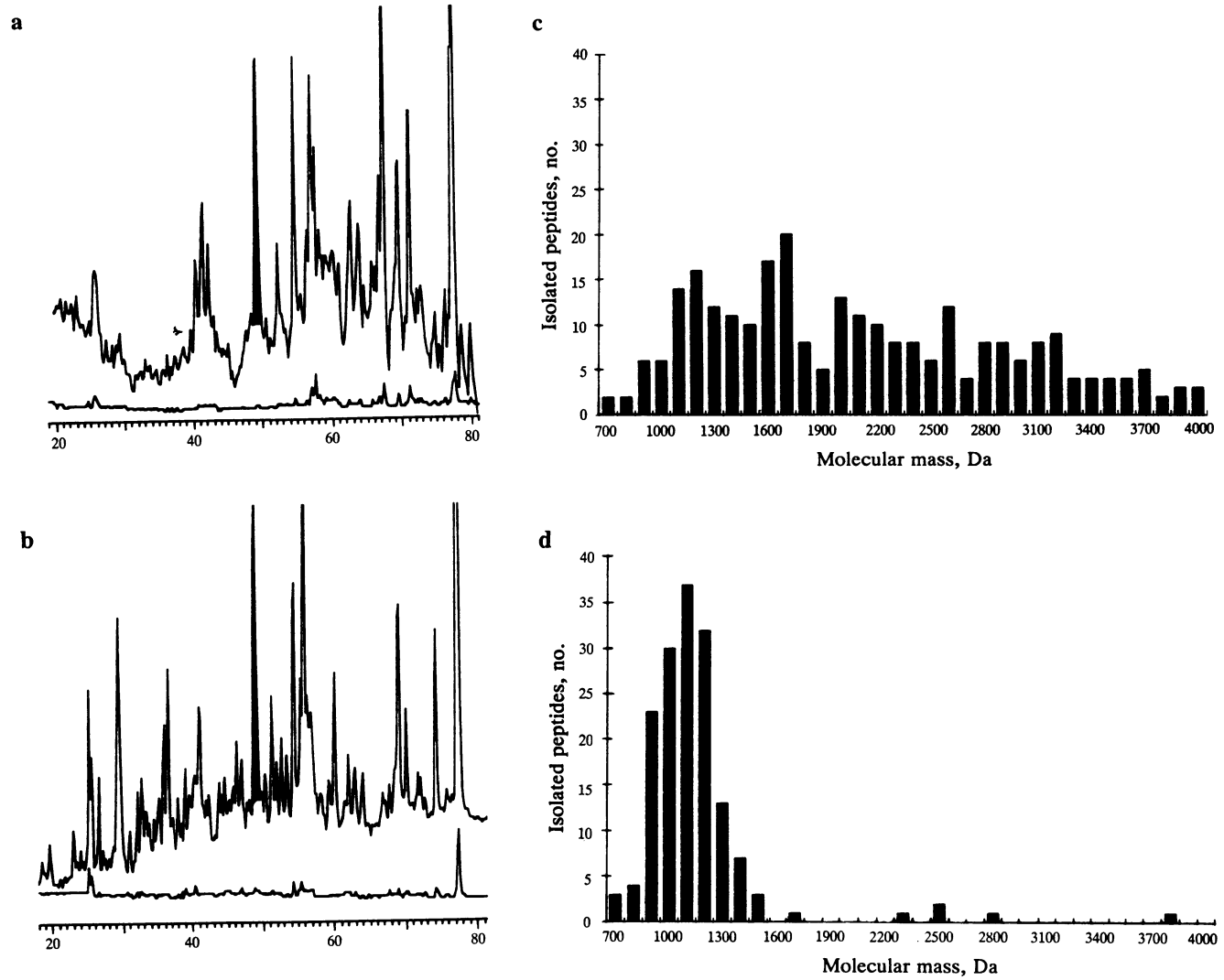

FIG. 2. Analysis of HLA-B27-bound peptide repertoire. (*a* and *b*) Microbore RPC of eluted peptides detected at 210 nm with a full scale absorption of 75 milliatomic units. (*c* and *d*) Size distribution of the eluted peptides determined by matrix-assisted laser desorption mass spectrometry. MARB4-B27-bound peptides are shown in *a* and *c*; ME1-B27-bound peptides are shown in *b* and *d*. Each fraction contains multiple peptides. The blackened peak at 49 min in both chromatograms is a contaminant.

class I-restricted T-cell clones unless a wild-type TAP2 gene is reintroduced. The expression of MARB4-reactive HLA-B27 molecules was examined by cytofluorimetry (Fig. 4). In the absence of a functional TAP2 gene, no MARB4-reactive HLA-B27 was detectable on the surface (Fig. 4*h*, compare to *f* and *g* or the negative controls *i* and *j*). Thus, the source of peptides responsible for generation of surface-expressed MARB4-reactive-HLA-B27 molecules was strictly dependent on a functional TAP complex and must therefore be cytosolic. Thus, the TAP1/TAP2 complex appears capable of transporting peptides of a size considerably larger than that usually found associated with class I MHC molecules. The potential ability of the TAP1/TAP2 complex to transport 23- and 16-residue peptides as well as 8- to 11-residue peptides has been recently demonstrated using these peptides as competitors in an *in vitro* peptide transport assay (18, 19). In fact, in these experiments the two longer peptides competed for interaction with the TAP complex as efficiently and at least in one case more efficiently than some shorter peptides tested. However, another recent publication using a similar reconstitution system but a small number of different test peptides has proposed that the TAP complex does discriminate between peptides on the basis of size (20).

ME1 recognized cell surface HLA-B27 expressed in BM36.1, albeit at reduced levels (Fig. 4*c*, compared to *a* and *b* or the negative controls *d* and *e*), suggesting that the TAP2 component is required for transporting the types of peptides recognized by MARB4 but not all of those recognized by ME1. Some peptides may be produced in or transported to the endoplasmic reticulum independent of the TAP transporter (21, 22). Finally, these molecules could be empty (compare ref. 23).

DISCUSSION

It is difficult to reconcile the binding of these long peptides to HLA-B27 with known mechanisms of tight peptide binding to class I MHC molecules. A maximum of 20% of these long peptides could bind with arginine at P2 in the B pocket and the N terminus deep in the A pocket where a pentagonal hydrogen-bonding network (formed by Y7, Y59, Y171, and a water molecule) accommodates the charged group (3, 4, 24). With the remaining 80% of long peptides, either the N terminus is in the A pocket and no arginine is present in the B pocket or the arginine at P3, P4, or P5, for example, is in the B pocket and the N terminus extends beyond the A pocket. In the latter case, the amide at this position could not

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a

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
D	126.1	151.5	191.3	237.2	228.9	223.6	200.4	128.0	138.8	125.8	102.0	130.4	115.2	97.6	83.3	74.8	59.9	67.9	59.5	57.9	55.2	58.0	52.4	48.0	38.2
N	411.9	268.1	267.5	205.6	142.3	106.5	76.3	51.4	50.8	33.3	28.2	29.1	25.9	22.9	24.4	19.9	17.3	16.9	15.4	15.6	14.7	13.8	12.1	12.0	10.3
S	398.1	204.4	166.2	137.7	125.7	115.9	93.8	65.8	67.6	56.0	55.4	60.7	57.6	51.3	51.4	42.7	27.7	31.0	27.7	29.8	26.0	26.6	24.0	22.5	18.1
Q	161.1	135.5	126.0	93.3	70.5	71.8	59.4	48.4	43.2	37.3	35.9	34.4	29.0	26.3	25.3	25.3	22.1	20.7	19.0	16.9	15.3	15.1	13.5	13.3	10.9
T	130.2	73.2	70.6	72.2	72.9	52.5	60.3	45.9	40.1	35.9	35.9	35.9	33.3	25.9	24.5	19.2	12.6	14.0	13.6	12.6	11.3	10.4	9.2	9.2	7.5
G	425.3	224.4	195.4	161.6	140.2	103.3	82.0	73.3	65.9	54.3	52.0	46.2	43.5	38.5	38.1	34.0	29.4	33.9	33.1	30.6	28.5	27.1	24.2	21.7	19.5
E	141.4	179.8	207.0	205.8	179.2	171.7	142.2	107.7	113.4	89.9	79.0	89.4	79.0	67.9	60.8	53.4	41.7	41.8	37.5	36.2	33.1	31.0	26.8	23.4	18.0
H	28.9	28.6	66.7	88.8	87.6	92.1	75.3	43.4	61.2	46.5	35.8	53.0	52.4	34.2	26.0	23.7	15.2	18.1	15.6	13.3	14.5	13.7	12.7	12.7	9.4
A	432.3	260.9	185.3	148.1	106.2	110.8	95.9	77.2	67.3	59.2	51.8	49.0	42.1	37.1	34.5	30.3	26.3	25.7	25.9	22.5	20.5	19.5	18.0	15.5	15.1
R	192.7	241.2	220.3	249.3	123.7	123.0	118.8	96.5	126.5	100.0	87.5	126.8	119.1	114.5	104.8	111.5	71.5	77.6	70.3	72.5	68.2	70.0	69.5	63.0	43.0
Y	88.0	59.8	52.2	32.6	27.7	26.8	34.7	24.7	26.7	23.5	20.1	19.2	17.6	17.0	14.6	13.3	13.0	11.3	10.1	10.1	8.9	8.0	7.5	6.9	6.0
P	36.2	100.5	112.3	95.0	96.2	92.4	64.5	51.6	53.1	46.4	40.4	43.0	40.6	35.5	32.9	27.8	22.0	22.3	18.0	16.5	15.9	15.7	14.4	12.5	10.3
M	62.1	32.0	29.7	22.2	15.9	15.1	16.3	12.8	10.7	7.7	6.8	5.6	5.1	4.9	4.7	4.0	4.0	4.4	4.0	4.1	3.6	2.9	2.8	2.9	2.5
V	127.9	115.0	79.3	55.3	52.5	47.2	46.9	52.2	50.4	40.6	31.0	31.6	26.1	24.3	19.9	16.5	14.0	13.7	11.8	11.4	10.7	10.4	10.4	8.9	9.3
F	57.1	43.5	44.4	34.4	28.9	28.2	24.6	21.0	20.3	19.3	17.1	14.9	13.8	12.4	10.9	9.8	8.7	8.8	8.0	8.2	7.5	6.9	6.7	6.0	5.8
I	91.9	84.3	47.8	40.1	36.3	36.0	34.5	28.5	25.7	22.2	20.4	17.4	15.4	13.0	12.1	10.6	10.0	9.0	8.9	8.6	8.2	7.6	7.5	7.7	6.8
K	86.4	85.5	83.5	75.8	67.1	74.9	64.9	53.2	53.5	40.2	35.7	38.0	34.4	31.4	27.6	24.1	19.8	23.5	21.3	20.3	18.8	16.3	15.7	14.6	12.7
L	113.6	99.6	90.8	73.0	58.3	50.0	48.1	50.8	42.7	37.2	31.0	27.9	25.9	22.6	21.1	18.5	16.6	16.6	16.9	15.8	16.4	13.4	13.2	12.1	11.3
Total	3111.1	2387.8	2236.3	2028.0	1660.2	1541.7	1339.1	1032.6	1057.9	875.0	766.0	852.2	775.9	677.4	616.7	559.9	431.9	457.3	416.5	402.8	377.4	366.2	340.7	313.1	254.5

b

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
D	6.7	8.4	11.1	13.8	7.8	8.5	7.9	5.9	4.2	3.7	2.2	2.3	1.8	1.7	1.7
N	3.6	1.7	10.0	4.3	4.0	3.7	5.1	7.3	3.1	1.3	1.0	0.7	0.4	0.5	0.6
S	35.0	18.9	17.7	17.7	9.0	7.4	9.6	9.9	4.3	2.7	2.0	1.9	1.9	2.7	1.1
Q	4.3	3.1	6.4	8.3	7.8	9.0	5.2	7.1	2.9	1.7	1.2	0.7	0.7	4.4	0.6
T	5.0	3.3	8.0	8.1	4.4	3.7	10.2	6.1	3.0	2.5	1.3	1.1	0.7	3.1	0.6
G	46.0	18.6	9.9	26.1	12.0	7.0	5.1	8.8	5.5	3.2	2.4	1.9	1.6	2.6	1.6
E	2.7	3.5	3.5	11.3	6.0	4.7	4.7	4.8	2.9	1.8	1.2	1.1	0.9	0.8	0.8
H	4.6	5.9	11.3	8.1	7.1	8.5	6.8	6.0	5.1	4.4	1.6	1.3	0.9	0.7	0.9
A	17.0	7.2	11.8	12.0	4.9	5.4	5.3	5.9	3.0	1.6	1.2	1.1	0.6	0.7	0.7
R	165.1	354.8	75.5	26.9	21.0	29.3	25.6	22.1	32.5	22.8	11.2	7.3	4.9	4.6	4.3
Y	3.5	1.4	7.6	3.4	2.8	4.3	4.3	2.6	2.4	1.8	0.8	0.7	0.5	0.5	0.4
P	1.6	1.7	3.4	10.4	14.9	10.5	8.8	4.9	2.5	1.5	1.0	0.9	0.8	1.0	1.1
M	1.3	0.4	3.4	0.7	1.1	1.0	0.8	0.9	0.9	0.5	0.2	0.1	0.1	0.1	0.0
V	2.8	2.4	7.2	3.3	7.7	6.0	6.5	4.3	2.7	1.2	0.8	0.8	0.6	0.5	0.4
F	2.4	1.5	14.3	5.6	3.6	3.7	2.6	2.0	2.5	1.4	0.9	0.5	0.3	0.4	0.3
I	2.6	1.6	7.1	2.0	4.6	7.5	4.5	2.3	1.1	0.8	0.4	0.3	0.2	0.3	0.2
K	12.3	2.3	3.6	4.6	4.5	4.3	4.7	5.9	4.5	2.5	1.4	0.8	0.6	0.5	0.3
L	1.9	1.7	15.0	5.2	4.1	8.3	4.9	3.4	4.1	2.1	1.2	0.7	0.5	0.5	0.5
Total	318.4	438.3	227.0	171.7	127.4	132.7	122.6	110.3	87.1	57.5	32.1	24.4	17.9	25.5	16.2

c

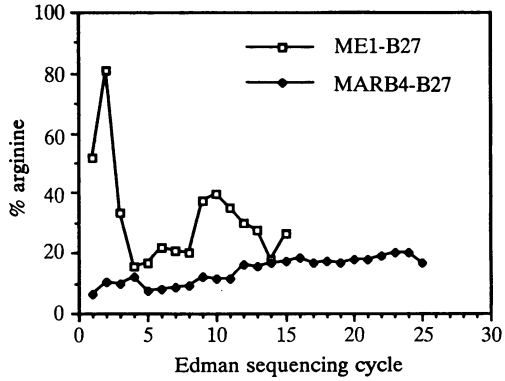

FIG. 3. Pooled sequencing of MARB4-B27 (*a*) and ME1-B27 (*b*) peptides. Every fraction spanning 20–80 min was pooled and sequenced by Edman degradation simultaneously. (*c*) Percent arginine at each sequencing cycle in the ME1-B27 and MARB4-B27 pooled peptide sequences. The data of *a* and *b* were plotted as the relative amount of arginine at each sequencing cycle. The apparent increase above baseline in the percent arginine at sequencing cycles 10–12 in ME1-B27 peptides (as well as the upward slope in MARB4-B27 peptides) is misrepresentative and is the result of arginine sequencing lag. Close analysis of the Edman sequencing yields indicates that few if any of the ME1-B27 extracted peptides were longer than nine residues in length, but the arginine lag results in an apparent percent increase after cycle 9.

participate in the pentagonal hydrogen-bonding network deep in the A pocket nor could this pocket accommodate a peptide bond or additional amino acid(s). The possibility that Y59 is critically involved in the binding of long peptides and/or the formation of the MARB4 epitope is suggested by the observation that the only HLA-B27 subtype that is not recognized by MARB4 is HLA-B*2703. This subtype differs from all of the others only by the substitution of H59 for Y59. This substitution is the only difference between HLA-B*2705 (Fig. 1*e*) and HLA-B*2703 (Fig. 1*c*). Interestingly, HLA-B*2703 is the only subtype that is not associated with the development of ankylosing spondylitis (reviewed in ref. 25).

An important question that remains concerns a possible precursor–product relationship between the "long" and "short" peptides. Is trimming of the long peptides, once bound by class I molecules, the mechanism by which the short peptides are generated (26, 27)? Antigen processing involving the proteasomes and/or other cytosolic proteins may not be strictly tailored to the requirements for tight peptide binding. Given the strict side chain requirements at precise distances from the termini for class I bound peptides ("motifs"), if peptides were cut at intermediate lengths first and then allowed to "position" themselves into a peptide-binding groove before further processing to the precise termini, the efficiency of epitope generation would be enhanced. The fact that the majority of MARB4-reactive HLA-B27 molecules are within the cell is compatible with this view. Indeed, intracellular MARB4-B27 molecules binding long peptides may represent molecules awaiting further peptide cleavage. Alternatively, the peptides bound to MARB4-B27 molecules could represent (*i*) peptides bound to a site outside the cleft (similar to the interaction of superantigens with class II MHC molecules) or (*ii*) acquisition of "inappropriate" peptides such that the conformation required for endoplasmic reticulum egress is not achieved. Efficient release of MHC class I proteins from the endoplasmic reticulum retention/recycling system requires peptide binding, but the types of peptides capable of triggering this release are not well understood (28).

Peptides longer than nonamers can (*i*) sensitize antigen-presenting cells to lysis by class I-restricted cytotoxic T lymphocytes (for example, refs. 29 and 30) and (*ii*) participate in the refolding of class I heavy chain and β_2-microglobulin into the class I HLA complex, although longer peptides tend to have a considerable increase in the k_{off} rate (31, 32). Hence, MARB4-reactive HLA-B27 molecules are likely to be less stable than HLA-B27 molecules containing an "optimal" peptide. Nonetheless, these MHC–peptide complexes were stable enough for isolation under the conditions employed here—i.e., immunoaffinity purification at 4°C with extensive column washing (about 36 hr), pH 11.5 column elution (≈10–20 min) followed by dialysis for 12 hr at neutral pH. In conclusion, the presence, at least transiently, of long peptides bound to MARB4-reactive-HLA-B27 molecules suggests that during peptide selection and protein folding, peptides of diverse lengths are available and participating.

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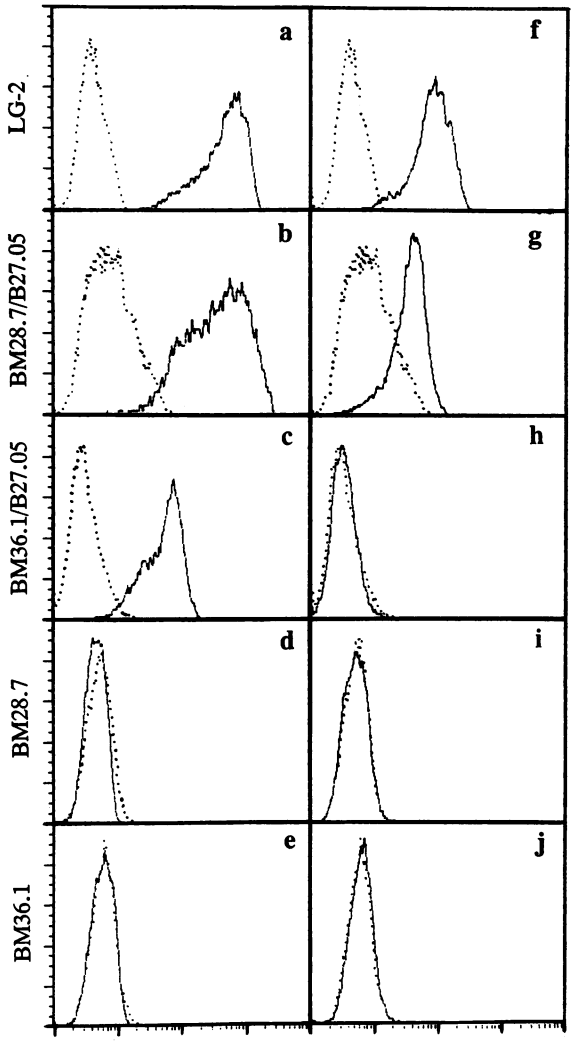

FIG. 4. Functional TAP1/TAP2 complex as a prerequisite for recognition of HLA-B*2705 molecules by MARB4. (*a–e*) Stained with ME1. (*f–j*) Stained with MARB4. Analysis was by cytofluorimetry as detailed (15). The fluorescence-activated cell sorting profiles drawn in solid line represent the relative staining with the indicated specific antibody and those drawn in dotted line represent negative control antibody W6/32.HK (16). Although both MARB4 and ME1 recognize HLA-B27 molecules, only MARB4 does not detect these proteins in the transport-defective mutant BM36.1/B*2705, while it reacts with an HLA-B*2705 subpopulation in the cell line BM28.7/B*2705. In BM36.1, similarly reduced expression of ME1-reactive B27 was observed after transfection with an HLA-B*2705 construct in the episomal expression vector pREP9 (Invitrogen; M.J.H.K. and F.G.C.M.U., unpublished observations). HLA-B27 expression was also observed by fluorescence-activated cell sorting analysis using TÜ48 (17).

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