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The HA-2 Minor Histocompatibility Antigen Is Derived from a Diallelic Gene Encoding a Novel Human Class I Myosin Protein

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Human minor histocompatibility Ags (mHag) present significant barriers to successful bone marrow transplantation. However, the structure of human mHag and the basis for antigenic disparities are still largely unknown. Here we report the identification of the gene encoding the human mHag HA-2 as a previously unknown member of the class I myosin family, which we have designated MYO1G. The gene is located on the short arm of chromosome 7. Expression of this gene is limited to cells of hemopoietic origin, in keeping with the previously defined tissue expression of the HA-2 Ag. RT-PCR amplification of MYO1G from different individuals led to the identification of two genetic variants, designated MYO1G^{V} and MYO1G^{M}. The former encodes the peptide sequence previously shown to be the HA-2 epitope (YIGEVLVSV), whereas the latter shows a single amino acid change in this peptide (YIGEVLVS*M*). This change has only a modest effect on peptide binding to the class I MHC-restricted element HLA-A*0201, and a minimal impact on recognition by T cells when added exogenously to target cells. Nonetheless, as detected using either T cells or mass spectrometry, this amino acid change results in a failure of the latter peptide to be presented at the surface of cells that express MYO1G^{M} endogenously. These studies have thus identified a new mHag-encoding gene, and thereby provide additional information about both the genetic origins of human mHag as well as the underlying basis of an Ag-positive vs Ag-negative state. *The Journal of Immunology,* **2001, 167: 3223–3230.**

Minor histocompatibility Ags (mHag)[3] provoke transplantation immunity and function as targets of graft-versus-host disease (GVHD) in bone marrow transplant (BMT) in HLA-identical donor/recipient pairs (reviewed in Ref. 1). This necessitates life-long pharmacological immunosuppression of organ and BMT recipients. Although >50 different mHag loci have been defined among inbred strains of mice (2), the number in humans is still unknown. The genetics and tissue distribution of several human mHag have been studied by using T cells isolated from patients suffering from GVHD after BMT (3–5). mHag have been shown to be peptides derived from cellular proteins that are presented by class I MHC molecules and recognized by MHC-restricted T cells (6, 7). However, because of the difficulty in identifying such peptides, the chemical structures of mHag in both humans and mice remain largely unknown.

We previously used a combination of peptide fractionation, T cell Ag reconstitution, and mass spectrometry to identify the first amino sequence of an autosomally encoded human mHag, known as HA-2 (7). This Ag is restricted by HLA-A*0201, present in 95% of the HLA-A*0201^{+} population, and expressed only on cells of hemopoietic origin (3, 5). The peptide sequence of HA-2 (YIGEVLVSV) did not yield an exact match in genetic databases available at the time, although it was highly homologous to a sequence present in several genes of the class I myosin family, one of the classes of nonfilament-forming myosins thought to play a role in intracellular transport and locomotion (8). The nearest human match, YIGSVLISV, was present in MYO1E and MYO1F (9). However, the ubiquitous tissue expression of these class I myosins was inconsistent with the possibility that either was an allelic homolog of HA-2. Failure to identify the gene encoding HA-2 has stood as an impediment to understanding the basis for its antigenicity and to the development of molecular methods to screen for its presence or absence.

In the present study, we relied on newly available genetic information from the Human Genome Project to identify a candidate genomic DNA sequence encoding the HA-2 epitope. Using this information, we have established that this candidate sequence does encode HA-2, and that it represents a new member of the class I human unconventional myosin family that is expressed only in cells of hemopoietic origin. We have also defined its nonantigenic

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[3] Abbreviations used in this paper: mHag, minor histocompatibility Ag; GVHD, graft-versus-host disease; BMT, bone marrow transplant; EBV-BLCL, EBV-transformed B lymphoblastoid cell line; MS/MS, tandem mass spectrometry; amu, atomic mass unit; *m/z*, mass-to-charge; ER, endoplasmic reticulum; FTMS, Fourier transform ion cyclotron resonance mass spectrometer.

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allelic homolog and established the basis for its differential antigenicity.

Materials and Methods

Cell lines and culture

The origin and maintenance of the HA-2-specific $CD8^+$ CTL clone 5H17 has been previously described (5). The HLA-A*0201^+ EBV-transformed B lymphoblastoid cell lines (EBV-BLCL) Rp (HLA-A*0101, A*0201, B8, B27), JY (HLA-A*0201, B*0702 homozygous), Blk (HLA-A*0201, B44 homozygous), and Maja (HLA-A2, B35, C4) have all been described previously (7). C1R-A2 and JK-A2 are HLA-A*0201^+ stable transfectants of C1R (10) and Jurkat (11), respectively. Other transformed cell lines used and their tissues of origin are described in the text and the figure legends. All transformed cell lines were cultured in RPMI 1640 containing 5% FBS, 4 mM HEPES, 0.125% SerXtend (Irvine Scientific, Santa Ana, CA), and 3 mM L-glutamine. To maintain the expression of the *HLA-A*0201* gene in the C1R and Jurkat transfectants, this medium was supplemented with either 200 μg/ml Hygromycin (Mediatech, Herndon, VA) or 300 μg/ml G418, respectively.

Melanocytes, epidermal keratinocytes, HUVEC, and proximal tubular epithelial cells were all isolated and cultured as described elsewhere (3, 12, 13). PBMC were isolated by Ficoll-Isopaque density centrifugation of whole donor blood, washed twice with PBS, and used immediately.

PCR amplification and cloning of the HA-2 coding sequence

Poly(A)$^+$ mRNA was prepared from 1×10^7 cells using the QuickPrep mRNA Purification kit (Amersham Pharmacia Biotech, Piscataway, NJ). Alternatively, total RNA was prepared from patient PBMC and normal tissue samples with the RNAzol method (Cinaa/Biotecx Laboratories, Houston, TX). First-strand cDNA was synthesized using an oligo(dT) primer as per the manufacturer's instructions (First Strand cDNA Synthesis kit; MBI Fermentas, Hanover, MD). Amplifications were performed on cDNA using 0.5 μM the forward and reverse primers shown in Fig. 2 (Research Genetics, Huntsville, AL) in 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 2.5 U *Taq* polymerase in 1× PCR buffer (all obtained from Life Technologies, Rockville, MD). Cycle parameters were: initial denaturation at 94°C for 2 min; 36 cycles of denaturation at 94°C for 1 min, annealing at 60.5°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. PCR products were gel purified and isolated with the Wizard PCR-Preps DNA purification system (Promega, Madison, WI). Purified fragments were cloned into the pT-Adv plasmid using the AdvanTAge cloning kit (Clontech Laboratories, Palo Alto, CA), and the inserts were sequenced at the University of Virginia Biomolecular Core Facility using M13 forward and reverse primers. At least seven individual clones were sequenced and analyzed bidirectionally for each sample.

PCR amplification for RFLP and tissue-specific expression analyses

For RFLP analysis, PCR fragments corresponding to bases 1–206 in Fig. 2 were amplified from cDNA using the forward primer 5′-AAGCTTTTCGAGAAGGGCCGCATCTA-3′ and the reverse primer 5′-GAATTCGAGATGACGATGCAGGTGTC-3′ under the conditions described above. Samples were digested at 37°C for 12 h with either *Hsp*92 II (Promega) or *Nla*III (New England Biolabs, Beverly, MA), and analyzed on a 4% agarose gel. The $MYO1G^V$ derived sequence was not digested under these conditions and ran as a fragment of 218 bp, whereas the $MYO1G^M$ sequence gave fragments of 163 and 55 bp. For tissue-specific expression, PCR fragments corresponding to bases 1–334 in Fig. 2 were amplified from cDNA using the forward primer 5′-AAGCTTTTCGAGAAGGGCCGCATCTA-3′ and the reverse primer 5′-GAATTCCACAGGTGGACTTGAGCAGC-3′. Primers specific for glucose-6-phosphate dehydrogenase (Stratagene, La Jolla, CA) were used to amplify a 358-bp positive control fragment. The conditions used were as above except that the $MgCl_2$ concentration was adjusted to 1.0 mM, and the annealing temperatures used were 66.2°C and 61.6°C for MYO1G and glucose-6-phosphate dehydrogenase, respectively.

Fluorescence in situ hybridization

Three cloned PCR fragments representing bases 1–1246, 978–1397, and 1215–1900 were isolated from bacterial cultures with the Wizard Plus Minipreps DNA Purification System (Promega), and inserts were excised by digestion of the pT-Adv plasmid with *Bst*XI. The products were separated by gel purification, and the excised inserts were isolated using the Wizard PCR Preps DNA Purification System (Promega). The fragments were labeled with biotin-14-dATP using the BioNick labeling system (Roche Molecular Biochemicals, Indianapolis, IN), and standard methodology was used for hybridization and detection of this probe (14).

Synthetic peptides

Peptides were synthesized on an AMS 422 multiple peptide synthesizer (Gilson, Middleton, WI) using solid-phase FMOC chemistry and Wang resins. Sequences of all synthetic peptide structures were confirmed by tandem mass spectrometry (MS/MS).

Cytotoxicity assays

^{51}Cr release assays were conducted as previously described (7).

Class I MHC peptide affinity binding assays

The HLA-A*0201 peptide binding assay was conducted as previously described (15) using the iodinated indicator peptide FLPSDYFPSV.

*Preparation of HLA-A*0201-associated peptides*

HLA-A*0201 molecules were immunoaffinity purified from JY (HLA-A*0201, B*0702 homozygous) or JK-A2 (HLA-A*0201) cells using the mAb BB7.2 (16), and their associated peptides were extracted as previously described (7, 17). Iodoacetamide was omitted from the protease inhibitor mixture to avoid potential amidocarboxy methylation of free cysteine residues. Peptides were separated from class I heavy chains and β_2-microglobulin by elution in 10% acetic acid and passage through a 5-kDa-cutoff filter. Peptides were fractionated using a HAISIL C18 column (2.1 × 40 mm, 5-μm particles, 300-Å pore size; Higgins, Winter Park, FL) on an Applied Biosystems 130A HPLC (Foster City, CA) using a gradient of acetonitrile (HPLC grade; Mallinckrodt, Paris, KY) with trifluoroacetic acid (HPLC grade; Applied Biosystems) as a modifying agent. Those fractions expected to contain the synthetic peptide of interest (either YIGEV LVSV or YIGSVLISV) were established by separating a mixture of synthetic peptides including $HA2^V$ and $HA2^M$ in a parallel HPLC fractionation experiment.

*Targeted MS/MS analysis of HLA-A*0201-associated peptides*

Data-dependent MS/MS analysis was performed using an LCQ mass spectrometer (Thermo-Finnigan, San Jose, CA) equipped with sheathless nanoflow HPLC electrospray ionization as previously described (18). MS/MS data were acquired only on masses within 3-atomic-mass-unit (amu) windows centered on either 978.6 amu (the +1 mass of YIGEVLVSV) or 1010.6 amu (the +1 mass of YIGEVLVSM). Dynamic exclusion was not enabled for these experiments, such that multiple MS/MS spectra of the same mass were taken, increasing the quality of the data for masses of interest. The identity and amount of these peptides were established by comparison of fragmentation spectra with those of synthetic peptides.

*Fourier transform mass spectrometry of HLA-A*0201-associated peptides*

Mass spectrometric data were acquired on a home-built Fourier transform ion cyclotron resonance mass spectrometer (FTMS) (19) equipped with a nano-HPLC microelectrospray ionization source. Nano-HPLC columns were 50 μm inside diameter fused silica packed with ~8 cm of 5-μm diameter reversed phase beads. An integrated microESI emitter tip (~1-μm diameter) was located a few millimeters from the column bed. Samples were loaded onto the column and eluted into the FTMS using a gradient of 0–60% B in 32 min and 60–100% B in the next 3 min, where solvent A is 0.1 M acetic acid (Sigma, St. Louis, MO) in NANOpure water (Barnstead, Dubuque, IA), and solvent B is 0.1 M acetic acid in 70% acetonitrile. Full scan mass spectra, over a mass-to-charge (m/z) range $300 \leq m/z \leq 2500$, were acquired at a rate of 1 scan/s.

Streptolysin O peptide transport assay

In vitro assays of TAP-mediated peptide transport were performed as previously described (20), with modifications. T1 cells (1×10^6/sample) were permeabilized on ice for 15 min with streptolysin O (15 U/ml; Murex, Norcross, GA) and incubated for 5 min at 37°C with 100 ng of the reporter peptide TVNKTERAY (21) (radiolabeled with $Na^{125}I$ using the chloramine T method; Ref. 22), 10 μl of 100 mM ATP, and indicated dilutions of competitor peptides. The reporter peptide contains an N-linked glycosylation site (Asn-X-Thr/Ser), and will become glycosylated after translocation by TAP into the endoplasmic reticulum (ER). Glycosylated reporter peptide was isolated using Con A Sepharose (Pharmacia Biotech, Uppsala, Sweden), eluted with 0.2 M methyl α-D-mannopyranoside (Sigma), and quantitated on a gamma counter. Reporter peptide transport in TAP-negative T2 cells was assessed as a negative control. Samples were tested in

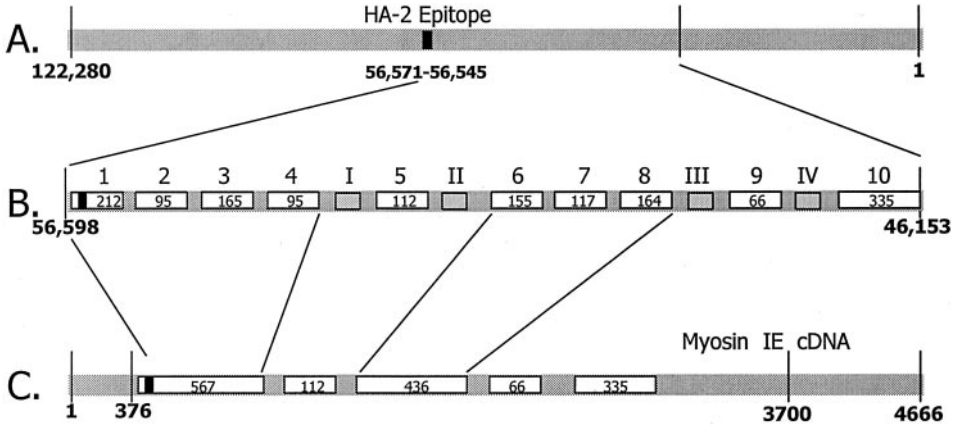

FIGURE 1. Identification of MYO1E-related gene encoding HA-2. *A*, Location of HA-2 coding sequence in GenBank AC004847 genomic contig. Reverse numbering indicates that HA-2 is encoded on the complementary strand. *B*, Exon organization of gene encoding HA-2. Exons numbered 1 through 10 were originally predicted based on homology to MYO1E and confirmed by RT-PCR amplification. Exons numbered I-IV were identified in RT-PCR products. Numbers in boxes refer to nucleotide length. *C*, Superposition of exons 1–10 onto the MYO1E cDNA sequence.

duplicate except for T2 negative control and T1 cells with no inhibitor, tested in triplicate.

Results

Identification of the gene encoding HA-2

The peptide sequence of HA-2 (YIGEVLVSV) was used to search the high throughput genomic sequencing database, and a precise match was identified in nucleotides 56,571–56,545 of GenBank accession no. AC004847 (Fig. 1*A*).[4] Additionally, the protein sequence encoded by nucleotides 56,598–56,388 of AC004847 was 44% identical and 67% homologous to amino acids 42–111 of MYO1E, which had previously been identified as containing a peptide that matched HA-2 at 7/9 residues (7, 9). We next searched AC004847 using 50- to 200-bp overlapping stretches of the MYO1E cDNA sequence and low stringency parameters, and regions of homology were further delimited based on putative splice sites. This analysis led to the identification of 10 putative exons each with amino acid sequence homology of greater than 40% to the corresponding region of MYO1E (labeled 1–10 in Fig. 1*B*). The apposition of these exons defined a putative partial cDNA corresponding to amino acids 42–214, 260–285, 358–497, 513–533, and 575–647 of MYO1E (Fig. 1*C*). Therefore, we hypothesized that AC004847 contained a previously unidentified unconventional myosin that was the source of the HA-2 mHag.

To establish the validity of this hypothesis, RT-PCR was performed on poly(A) mRNA isolated from the HA-2$^+$ cell lines Rp and C1R-A2 using primers that would selectively amplify overlapping segments of this putative message (Fig. 2). Each primer combination generated a product of the expected size (data not shown), which was then sequenced. This analysis established 1900 bp of a cDNA sequence that encompassed the 10 predicted exons, as well as four additional exons (labeled I-IV in Fig. 1*B*). The amplified cDNA that was sequenced reproducibly differed from the AC004847-derived sequence by three nucleotides (one each in exons 8, 9, and 10) of 1900, none of which occurred within the region encoding the HA-2 epitope. We think it most likely that these three nucleotides represent sequencing errors in AC004847.

In Fig. 2, the HA-2 epitope is encoded by nucleotides 25–51, which lie entirely within a single exon, and represents amino acids 9–17 of the corresponding coding sequence. The translated cDNA sequence also includes three sequence elements that are conserved in all or most myosins: the ATP-binding sequence GESGAGKT (23) at amino acids 70–77; the consensus sequence EAFGNART (9) at amino acids 114–121; and the actin-binding motif PFYVR CIKPNE (23) at amino acids 564–574. It should also be noted that the 43 nucleotides at the 3′ end of the sequence in Fig. 2 may represent one of two alternatively spliced transcripts. This sequence is identical with that of a putative exon that matches to nucleotides 46,192–46,150 of AC004847. However, three human expressed sequence tags (GenBank accession nos. AA310652, AW340433, and AA824566) match our cDNA sequence for 60–250 nucleotides to the 5′ side of this sequence, but are then juxtaposed to AC004847 residues 45,807–45,598. Nonetheless, the results of this analysis established convincingly that a cDNA encoding an unconventional myosin and containing the HA-2 sequence was expressed in HA-2$^+$ cells.

The gene encoding HA-2 encodes a novel human class I myosin located on chromosome 7

By comparison with the sequences of other human unconventional myosins, the 617 amino acids encoded by the cDNA in Fig. 2 represent ~89% of the head region of the protein. To determine the relationship of the HA-2-encoding myosin to other known human unconventional myosins, we constructed an unrooted phylogenetic tree, once using that region spanned by the known HA-2 sequence and once using the same spanned region, but with gaps produced in HA-2 by insertions in other sequences removed. Both analyses gave similar results, and the results of the latter alignment are shown in Fig. 3. This analysis demonstrates that this new gene falls definitively within the MYO1 clade, and is most closely related to MYO1C. In accordance with the nomenclature in this field, we propose that this new gene be named MYO1G.

We also established the chromosomal location of this gene by fluorescence in situ hybridization using a pooled probe consisting of three segments covering the entire sequence shown in Fig. 2. Ninety one percent of 22 metaphase spreads showed hybridization to chromosome 7 band p12p13, with no evidence for additional loci on other chromosomes (data not shown). These findings establish MYO1G as the first human myosin, as well as the first mHag-encoding gene, to be localized to this chromosome.

MYO1G exists in two allelic forms

All of the RT-PCR-amplified molecular clones derived from the cell lines Rp and C1R-A2 contained precisely the same sequence in the interval encoding the HA-2 epitope. To determine the basis for differential expression of this minor Ag, we RT-PCR amplified and sequenced the region of MYO1G known to encode the HA-2 epitope from several additional cell lines. Approximately 50% of the molecular clones from the HA-2$^+$ cell lines JY, Blk, and Maja, and all of the clones derived from the HA-2$^-$ cell line JK-A2,

[4] The reverse numbering indicates that the HA-2 epitope is encoded on the complementary (reverse) strand of AC004847.

contained a single G-to-A transition at nucleotide 49 of the sequence in Fig. 2. This was the only variation observed in over 100 independent clones from 14 cell lines of hemopoietic origin. This change alters the sequence of the HA-2 epitope from YIGEVL VS*V* to YIGEVLVS*M*. We designated the sequence YIGEVLVS*V* as HA-2^{V} and YIGEVLVS*M* as HA-2^{M}, and the respective genes as MYO1G^{V} and MYO1G^{M}.

To confirm the genotypes inferred from direct sequencing, we took advantage of the fact that the restriction endonucleases Hsp92II and *Nla*III specifically recognize the sequence CATG, which is present only in HA-2^{M}. In keeping with this, the RT-PCR product from C1R-A2 (HA-$2^{V/V}$ by sequencing) representing nucleotides 1–206 of the sequence in Fig. 2, was not cut by either *Hsp*92II or *Nla*III, whereas that obtained from JK-A2 (HA-$2^{M/M}$ by sequencing) was completely cut into two fragments of 163 and 55 bp by both enzymes (Fig. 4, *top*). Digestion of the RT-PCR products from Blk, JY, and Maja (HA-$2^{V/M}$ by sequencing) resulted in a mixture of both cleaved and uncleaved fragments. These results established definitively that the HA-2^{+} cell lines Rp and C1R-A2 are homozygous for HA-2^{V}, whereas JY, Blk, and Maja are heterozygous. The HA-2^{-} cell line JK-A2 is homozygous for HA-2^{M}.

To confirm that MYO1G^{V} and MYO1G^{M} represent two alleles of the same gene, we performed genotyping experiments on material derived from family "VR", which had previously been typed for HA-2 expression using the CTL clone 5H17 (Fig. 4, *bottom*). Four family members whose lymphoid cells were recognized by 5H17 CTL were found to be heterozygous or homozygous for HA-2^{V}, whereas two that were phenotypically HA-2^{-} were homozygous for HA-2^{M}. Similar results were seen with a second, unrelated family (data not shown). This genealogy also confirmed the autosomal, Mendelian inheritance of the *HA-2* gene, as both parents are heterozygous at this locus and produced offspring in all three allelic combinations.

Tissue-specific expression of the HA-2 *gene*

HA-2^{+} cells of hemopoietic origin are well recognized by 5H17 CTL, but cells derived from other tissue sources are not (3). Thus, we examined whether MYO1G expression was restricted to hemopoietic cells. We used RT-PCR to amplify nucleotides 1–334 of the sequence in Fig. 2 (the primer pair used does not discriminate between MYO1G^{V} and MYO1G^{M}). Strong amplification of the HA-2-specific fragment was consistently seen in lymphocytic lines of both B cell (Rp, C1R-A2, JY, Blk, and 18LCL) and T cell (Jurkat) origin (Fig. 5). Interestingly, however, MYO1G was not expressed in K562 erythroleukemia cells, which represent erythroblasts, and had not been assessed for HA-2 expression using CTL because they are class I MHC negative. Conversely, no amplification of the HA-2-specific fragment was observed in melanoma (18 Mel, DM93), embryonal kidney (293K), melanocyte (MC292, MC293), proximal tubule epithelial cells (PTEC29), HUVEC (HUVE59, HUVE62), and two samples of epidermal keratinocytes (KC25, KC45). Very weak signals were seen in lung carcinoma (VBT2), ovarian carcinoma (COV413), colorectal carcinoma (SW480), osteosarcoma (Tk-), fibroblasts (GM0568), and


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   1 F  E  K  G  R  I  Y  T  Y  I  G  E  V  L  V  S  V  N  P  Y  Q  E  L  P  L  Y  G  P  E  A  I  A  R  Y  Q
   1 TTCGAGAAGGGCCGCATCTACACCTACATCGGTGAGGTGCTGGTGTCCGTGAACCCCTACCAGGAGCTGCCCCTGTATGGGCCTGAGGCCATCGCCAGGTACCAG
  36 G  R  E  L  Y  E  R  P  P  H  L  Y  A  V  A  N  A  A  Y  K  A  M  K  H  R  S  R  D  T  C  I  V  I  S ¦G
 106 GGCCGTGAGCTCTATGAGCGGCCACCCCATCTCTATGCTGTGGCCAACGCCGCCTACAAGGCAATGAAGCACCGGTCCAGGGACACCTGCATCGTCATCTCAGGG
  71 E  S  G  A  G  K  T  E  A  S  K  H  I  M  Q  Y  I  A  A  V  T  N  P  S  Q  R  A  E  V  E  R ¦V  K  D  V
 211 GAGAGTGGGGCAGGGAAGACAGAAGCCAGTAAGCACATCATGCAGTACATCGCTGCTGTCACCAATCCAAGCCAGAGGGCTGAGGTGGAGAGGGTCAAGGACGTG
 106 L  L  K  S  T  C  V  L  E  A  F  G  N  A  R  T  N  R  N  H  N  S  S  R  F  G  K  Y  M  D  I  N  F  D  F
 316 CTGCTCAAGTCCACCTGTGTGCTGGAGGCCTTTGGCAATGCCCGCACCAACCGCAATCACAACTCCAGCCGCTTTGGCAAGTACATGGACATCAACTTTGACTTC
 141 K  G  D  P  I  G  G  H  I  H  S  Y  L  L  E  K ¦S  R  V  L  K  Q  H  V  G  E  R  N  F  H  A  F  Y  Q ¦L
 421 AAGGGGGACCCGATCGGAGGACACATCCACAGCTACCTACTGGAGAAGTCTCGGGTCCTCAAGCAGCACGTGGGTGAAAGAAACTTCCACGCCTTCTACCAATTG
 176 L  R  G  S  E  D  K  Q  L  H  E  L  H  L  E  R  N  P  A  V  Y  N  F  T  H  Q  G  A  G  L  N  M  T  V  H
 526 CTGAGAGGCAGTGAGGACAAGCAGCTGCATGAACTGCACTTGGAGAGAAACCCTGCTGTATACAATTTCACACCAGGGAGCAGGACTCAACATGACTGTGCAC
 211 S ¦A  L  D  S  D  E  Q  S  H  Q  A  V  T  E  A  M  R  V  I  G  F  S  P  E  E  V  E  S  V  H  R  I  L  A
 631 AGTGCCTTGGACAGTGATGAGCAGAGCCACCAGGCAGTGACCGAGGCCATGAGGGTCATCGGCTTCAGTCCTGAAGAGGTGGAGTCTGTGCATCGCATCCTGGCT
 246 A  I  L  H  L ¦G  N  I  E  F  V  E  T  E  E  G  G  L  Q  K  E  G  L  A  V  A  E  E  A  L  V  D  H  V  A
 736 GCCATATTGCACCTGGGAAACATCGAGTTTGTGGAGACGGAGGAGGGTGGGCTGCAGAAGGAGGGCCTGGCAGTGGCCGAGGAGGCACTGGTGGACCATGTGGCT
 281 E  L  T  A  T  P  R  D  L  V  L  R  S  L  L  A  R  T  V  A  S  G  G  R  E  L  I  E  K  G  H  T  A  A  E
 841 GAGCTGACGGCCACACCCCGGGACCTCGTGCTCCGCTCCCTGCTGGCTCGCACAGTTGCCTCGGGAGGCAGGGAACTCATAGAGAAGGGCCACACTGCAGCTGAG
 316 A  S  Y  A  R  D  A  C  A  K ¦A  V  Y  Q  R  L  F  E  W  V  V  N  R  I  N  S  V  M  E  L  R  G  R  D  P
 946 GCCAGCTATGCCCGGGATGCCTGTGCCAAGGCAGTGTACCAGCGGCTGTTTGAGTGGGTGGTGAACAGGATCAACAGTGTCATGGAACTCCGGGGCCGGGATCCT
 351 R  R  D  G  K  D  T  V  I  G  V  L  D  I  Y  G  F  E  V  F  P  V  N  S ¦F  E  Q  F  C  I  N  Y  C  N  E
1051 CGGCGTGATGGCAAGGACACAGTCATTGGCGTGCTGGACATCTATGGCTTCGAGGTGTTTCCCGTCAACAGTTTCGAGCAGTTCTGCATCAACTACTGCAATGAG
 386 K  L  Q  Q  L  F  I  Q  L  I  L  K  Q  E  Q  E  E  Y  E  R  E  G  I  T  W  Q  S ¦V  E  Y  F  N  N  A  T
1156 AAGCTGCAGCAGCTATTCATCCAGCTCATCCTGAAGCAGGAACAGGAAGAGTACGAGCGCGAGGGCATCACCTGGCAGAGCGTTGAGTATTTCAACAACGCCACC
 421 I  V  D  L  V  E  R  P  H  R  G  I  L  A  V  L  D  E  A  C  S  S  A  G  T  I  T  D  R  I  F  L  Q  T  L
1261 ATTGTGGATCTGGTGGAGCGGCCCCACCGTGGCATCCTGGCCGTGCTGGACGAGGCCTGCAGCTCTGCTGGCACCATCACTGACCGAATCTTCCTGCAGACCCTG
 456 D  T  H  H  R  H  H  L  H  Y  T  S  R  Q ¦L  C  P  T  D  K  T  M  E  F  G  R  D  F  R  I  K  H  Y  A  G
1366 GACACGCACCACCGCCATCACCTACACTACACCAGCCGCCAGCTCTGCCCCACAGACAAGACCATGGAGTTTGGCCGAGACTTCCGGATCAAGCACTATGCAGGG
 491 D  V  T ¦Y  S  V  E  G  F  I  D  K  N  R  D  F  L  F  Q  D  F  K  R  L  L  Y  N ¦S  T  D  P  T  L  R  A
1471 GACGTCACGTACTCCGTGGAAGGCTTCATCGACAAGAACAGAGATTTCCTCTTCCAGGACTTCAAGCGGCTGCTGTACAACAGCACGGACCCCACTCTACGGGCC
 526 M  W  P  D  G  Q  Q  D  I  T  E  V  T  K  R  P  L  T  A  G  T  L  F  K  N  S  M  V  A  L  V  E  N  L  A
1576 ATGTGGCCGGACGGGCAGCAGGACATCACAGAGGTGACCAAGCGCCCCCTGACGGCTGGCACACTCTTCAAGAACTCCATGGTGGCCCTGGTGGAGAACCTTGCC
 561 S  K ¦E  P  F  Y  V  R  C  I  K  P  N  E  D  K  V  A  G  K  L  D  E  N  H  C  R  H  Q  V  A  Y  L  G  L
1681 TCCAAGGAGCCCTTCTACGTCCGCTGCATCAAGCCCAATGAGGACAAGGTAGCTGGGAAGCTGGATGAGAACCACTGTCGCCACCAGGTCGCATACCTGGGGCTG
 596 L  E  N  V  R  V  R  R  A  G  F  A  S  R  Q  P  Y  S  R  F  L  L  R  Y  W  H  L  T  P  I  T  P  W  A  I
1786 CTGGAGAATGTGAGGGTCCGCAGGGCTGGCTTCGCTTCCCGCCAGCCCTACTCTCGATTCCTGCTCAGGTACTGGCACCTGACACCCATCACTCCATGGGCCATA
 631 V  P  V
1891 GTCCCTGTGT
```

FIGURE 2. Nucleotide and amino acid sequence of the gene encoding HA-2. Arrows indicate primers used in RT-PCR. The HA-2 epitope is bold and italicized, and the allelically variable residue at position 9 of the epitope is underlined. The myosin consensus sequences GESGAGKT, EAFGNART, and PFYVRCIKPNE are bolded. Exons are separated by broken vertical bars in the amino acid sequence. The 3′ region in bold represents potential alternate splicing as discussed in *Results*.

two other samples of epidermal keratinocytes (KC8, KC42). However, because HA-2-specific CTL fail to recognize these nonhemopoietic cells (3), this low level of gene expression does not result in peptide presentation at the cell surface. Thus, high-level expression of MYO1G is restricted to hemopoietic cells, consistent with the expression of the HA-2 epitope.

HA-2-specific CTL recognize both forms of the HA-2 peptide similarly

To more clearly define the basis for the HA-2^+ and HA-2^- phenotypes, we compared the recognition of HA-2^V and HA-2^M by the HA-2-specific CTL clone 5H17. Surprisingly, when these peptides were exogenously pulsed onto T2 (HA-2^-, HLA-A*0201^+) target cells, we found that both were recognized at a similar peptide concentration (Fig. 6). The difference in recognition between the two allelic forms of the peptide ranged from 2- to 10-fold in five independent experiments (data not shown). This suggested that differences in the endogenous processing and presentation of these two peptides, rather than T cell specificity, accounted for the failure of cells that expressed MYO1G^M only to be recognized by 5H17 CTL.

HA-2 peptide presentation at the cell surface

The cell surface density of peptide achieved under the short-term pulsing conditions used to make CTL targets in the above experiments minimize differences in peptide affinities for class I MHC molecules that might be important during peptide binding in the ER (24). Accordingly, we performed quantitative, cell-free peptide binding assays to determine the affinity of the HA-2^V and HA-2^M peptides for HLA-A*0201. HA-2^V half-maximally inhibited the binding of an iodinated indicator peptide (IC_{50}) at a concentration of 4.4 nM, whereas comparable inhibition by HA-2^M required ~15 times as much peptide (Table I). Thus, substitution of Met for Val at position 9 of HA-2 reduces peptide binding significantly, but this appeared unlikely to be large enough to account for the difference in recognition of HA-2^+ and HA-2^- cells.

To assess the expression of HA-2^V and HA-2^M directly, HLA-A*0201 molecules were immunoaffinity purified from either JY (MYO1G$^{V/M}$ heterozygous) or JK-A2 (MYO1G^M homozygous) cells and separated by HPLC. Fractions that could have contained either HA-2^V or HA-2^M peptides were identified based on the elution position of synthetic peptides in parallel HPLC runs, and these fractions were analyzed by mass spectrometry. Next, peptide ions of 978.6 and 1010.5 amu, corresponding to the masses of the +1 ions of HA-2^V and HA-2^M, respectively, were targeted for MS/MS analysis. The identification of HA-2^V was established by comparison of the fragmentation spectrum of a naturally processed peptide with that of synthetic HA-2^V. By comparing the magnitudes of the ion current of several fragment ions from naturally processed HA-2^V with those of a known quantity of synthetic material, we calculated that HA-2^V was present in the JY peptide sample in an amount corresponding to ~54 copies/cell (Fig. 7). Although JY also expresses mRNA encoding HA-2^M (Fig. 4), a similar analysis of fragmentation spectra established that this peptide was not detected at a level above 0.2 copies/cell (data not shown). The absence of the HA-2^M peptide was also established by analysis of the JK-A2 extract using a FTMS, which is ~10× more sensitive than the LCQ. A single mass with an *m/z* of 1010.52^{+1} and an appropriate elution time for HA-2^M was detected in a single HPLC fraction of this sample (Fig. 8). However, when synthetic HA-2^M was added to an aliquot of this sample and a second analysis performed, the endogenous peptide eluted almost a full half-minute before synthetic HA-2^M. No peptide of the

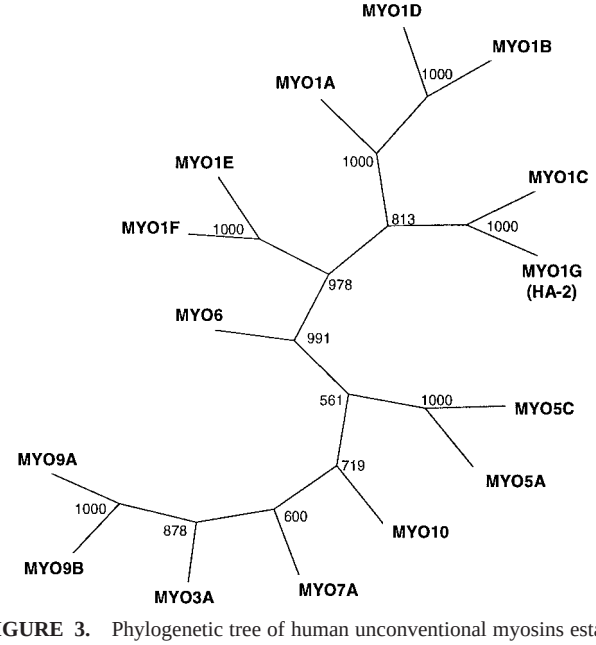

FIGURE 3. Phylogenetic tree of human unconventional myosins establishes HA-2 encoding gene as MYO1G. The unrooted tree was constructed using the PHYLIP suite of phylogenetic analysis programs (http://evolution.genetics.washington.edu/phylip.html) using the MYO1G amino acid sequence of Fig. 2 and the amino acid sequences of the head regions of the known human unconventional myosins. The sequence for MYO5B was omitted because only a small portion of the COOH-terminal head region is currently reported. The confidence scores of 1000 bootstrap analyses are shown at each node.

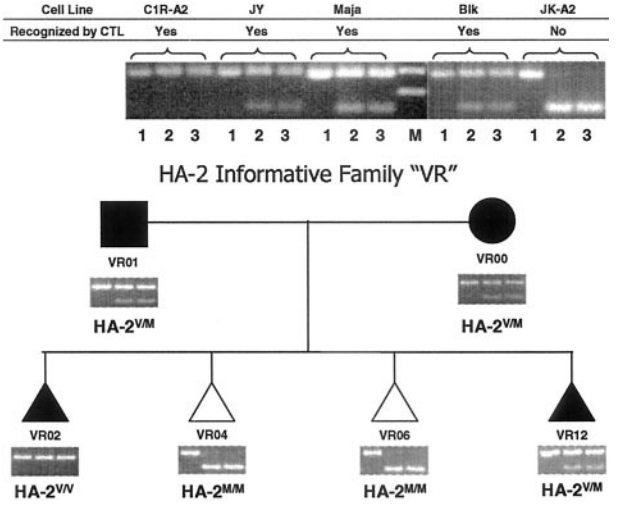

FIGURE 4. RFLP analysis of allelic polymorphism in the sequence encoding HA-2. Primers were used to RT-PCR amplify nucleotides 1–206 of the DNA sequence in Fig. 2 from the indicated samples. The amplification products were digested with *Hsp*92II or *Nla*III, which will cut the sequence encoding HA-2^M, but not that encoding HA-2^V. In all cases where digestion was observed, an additional expected 55-bp fragment was also seen (data not shown). *Top*, For the indicated B-lymphoblastoid cell lines, *lanes 1*, *2*, and *3* correspond to undigested, *Hsp*92II-digested, and *Nla*III-digested material, respectively. The m.w. standards (M) are 234 and 194 bp. All material was run on a single gel, with the band-containing regions aligned for clarity. *Bottom*, For the indicated samples from a family previously typed for HA-2 expression with CTL clone 5H17, *left*, *middle*, and *right lanes* correspond to undigested, Hsp92II-digested, and *Nla*III-digested material, respectively. Filled symbols indicate positive recognition by 5H17 and open symbols indicate no recognition.

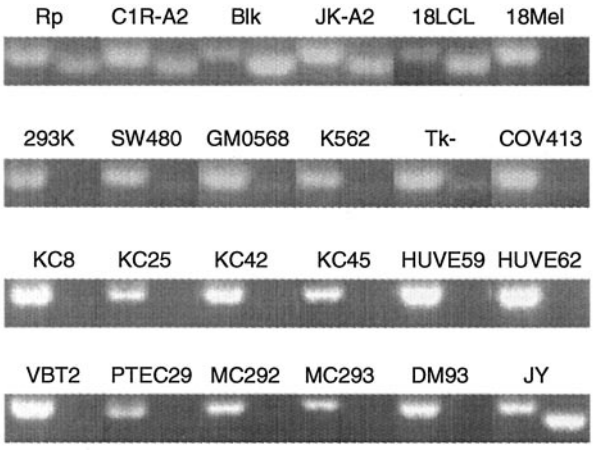

FIGURE 5. Tissue-specific expression of HA-2. RNA from each of the indicated samples was amplified by RT-PCR using primers for either G6PD (*left lane*) or MYO1G (*right lane*). Samples and their tissue of origin are as follows: Rp, C1R-A2, Blk, JY, 18LCL – EBV-BLCL; JK-A2 – T cell leukemia; 18 Mel, DM93 – melanoma; 293K – adenovirus-transformed primary embryonal kidney; SW480 – colorectal carcinoma; Tk^- – 143bTk^- osteosarcoma; GM0568 – normal fibroblast; K562 – erythroleukemia; COV413 – ovarian carcinoma; KC8, KC25, KC42, KC45 – epidermal keratinocytes; HUVE59, HUVE62 – HUVEC; VBT2 – lung carcinoma; PTEC29 – proximal tubular epithelial cells; MC292, MC293 –melanocytes.

appropriate mass was detected at this elution position in the original sample. In these experiments, we also did not detect appropriate masses at the elution position of HA-2 M with an oxidized Met residue (25) (data not shown). Based on the amount of peptidic material loaded and an instrument detection limit of ~6 amol, the detection limit in these experiments is ~0.04 copies/cell. We conclude that HA-2^M peptide was present at less than this level in the JK-A2 extract, despite the expression of mRNA encoding HA-2^M in this cell.

To gain additional insight into the failure of HA-2^M to be presented at the cell surface, we compared the ability of HA-2^V and HA-2^M to inhibit TAP-dependent transport of the radiolabeled reporter peptide TVNKTERAY in streptolysin O-permeabilized T1 cells. Somewhat surprisingly, we found that HA-2^M was transported more efficiently than HA-2^V (Fig. 9). Although it is possible that the actual substrates for TAP transport may correspond to precursors peptides rather than the mature 9 mer epitope, these data are consistent with the hypothesis that factors other than TAP

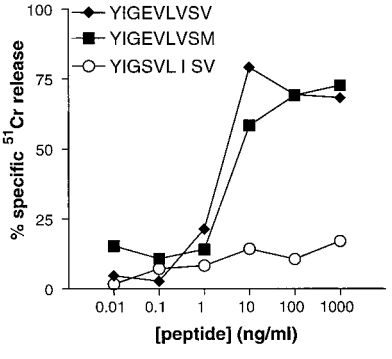

FIGURE 6. CTL recognition of HA-2^V and HA-2^M peptides. The indicated MYO1 peptides were pulsed onto ^{51}Cr-labeled EBV-LCL targets, and their ability to be recognized by the HA-2-specific CTL clone 5H17 at an E:T ratio of 10:1 was assessed by a standard ^{51}Cr-release assay. Data are representative of five independent experiments.

Table I. *Binding of HA-2-associated peptides to HLA-A*0201*[a]

Peptide	Origin	IC_{50}
YIGEVLVSV	MYO1G^V	4.5
YIGEVLVSM	MYO1G^M	66
YIGSVLISV	MYO1E and MYO1F	6.8

[a] Peptide binding affinity for HLA-A*0201 was determined by identifying the concentration necessary to inhibit the binding of a radioiodinated indicator peptide by 50% (IC_{50}) as described in *Materials and Methods*.

transport determine the differential expression of the HA-2^V and HA-2^M peptides in association with HLA-A*0201.

Discussion

In this study, we have identified 1900 bp of sequence representing the gene from which the previously identified mHag HA-2 is derived. This identification confirmed our previous hypothesis that HA-2 originates from a class I myosin protein. Based on a phylogenetic analysis of the known portion of the HA-2-encoding gene compared with the same region of the other 14 human unconventional myosins, we have classified this gene as a new member of the myosin I family, and have named it MYO1G. Although it is relatively homologous to myosin IC, it is the first class I myosin whose expression is largely confined to hemopoietic tissues. It is also the first class I myosin whose gene has been localized to chromosome 7. Our assignment of MYO1G to 7p12p13 is also supported by the identification of a chromosome 7-derived human expressed sequence tag (GenBank accession no. AA078664) that matches nucleotides 375–595 of our sequence at 218 of 221 possible residues (98.6% identity), and by the fact that AC004847 was recently reported to be derived from chromosome 7-enriched material (http://www.nhgri.nih.gov/DIR/GTB/CHR7). This identification process illustrates the impact of information provided by the Human Genome Project to augment the rapid identification of new genes and proteins.

The results of our work also provide an understanding of how HA-2 is expressed as a mHag. By analyzing the sequence of MYO1G in cells from different individuals, we identified only a single polymorphism that encoded either a valine or a methionine at position 9 of the HA-2 peptide sequence. The inheritance of this polymorphism establishes two alleles of MYO1G. Furthermore, there was complete concordance between the presence MYO1G^V

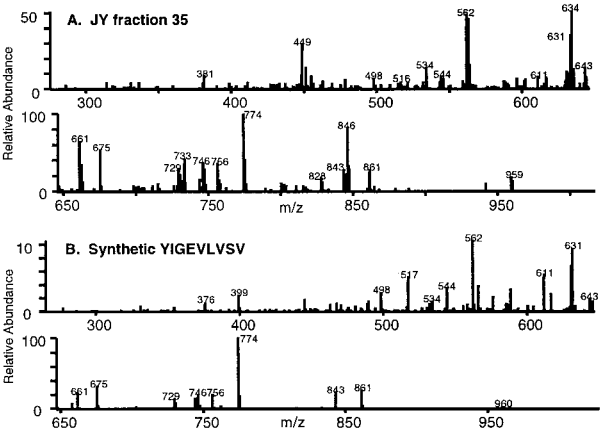

FIGURE 7. MS/MS analysis of HA-2^V peptide in extracts of HLA-A*0201 from JY (MYO1G$^{V/M}$) cells. *A*, MS/MS spectrum of ions of 978.6^{+1} detected in an aliquot of JY first-dimension fraction 35 that had been loaded onto a microcapillary HPLC column and eluted into the LCQ mass spectrometer. *B*, MS/MS spectrum of synthetic HA-2^V peptide.

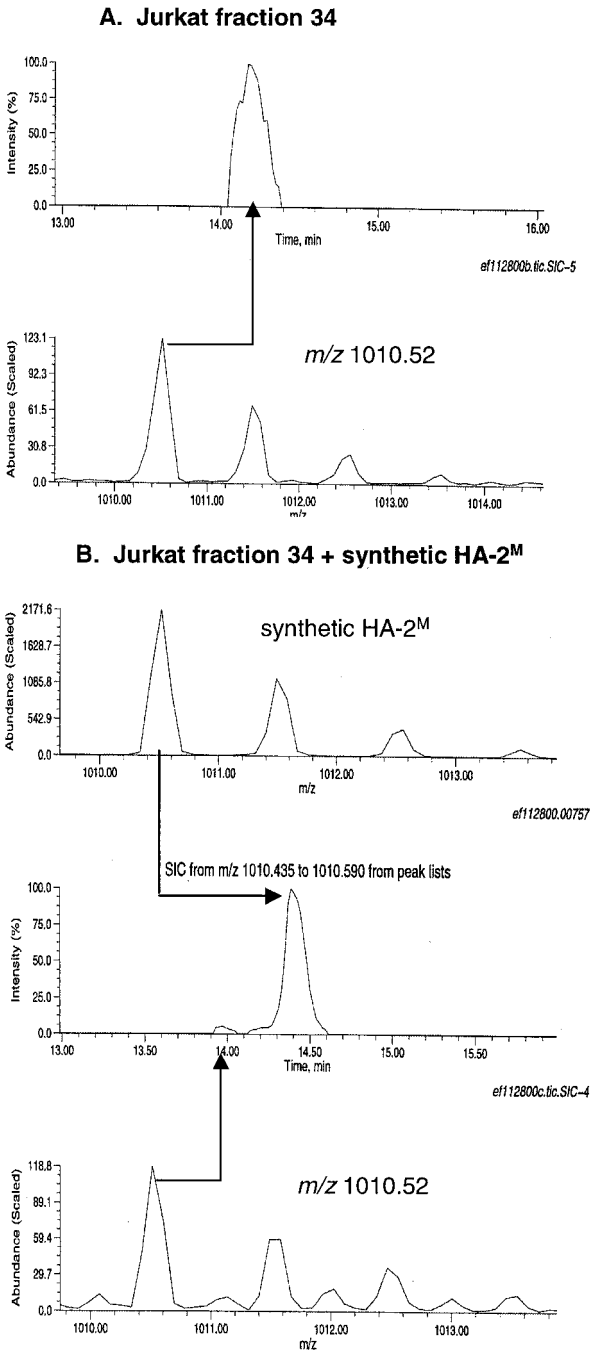

FIGURE 8. Fourier Transform mass spectrometry analysis of HA-2^M peptides in extracts of HLA-A*0201 from JK-A2 (MYO1$^{M/M}$) cells. *A, top,* detection of ions with *m/z* of between 1010.451 and 1010.561 in Jurkat first-dimension fraction 34 (1×10^8 cell equivalents) eluting from microcapillary HPLC column during the time interval of 13–16 min; *bottom,* Fourier Transform mass spectrum of ions in *top panel* establishing that they have an *m/z* of 1010.52^{+1}. This is based on the precise base peak mass of 1010.52, together with a series of masses that progressively increase by 1 amu and decrease in intensity, and which represent peptides containing 1 or more ^{13}C atoms in place of ^{12}C. *B, middle,* detection of ions with *m/z* of 1010.435–1010.590 in an aliquot of Jurkat first-dimension fraction 34 (1×10^8 cell equivalents) that had been spiked with 114 fmol of synthetic HA-2^M peptide eluting from microcapillary HPLC column; *top,* Fourier Transform mass spectrum of ions eluting in the major peak at 14.4 min as seen in the *middle panel.* The abundance of this peak and its precise mass establish that this peak represents synthetic HA-2^M peptide; *bottom,* Fourier Transform mass spectrum of ions eluting in minor peak at 13.95 min in *middle panel* establishing that this peak represents the same material detected in *A.*

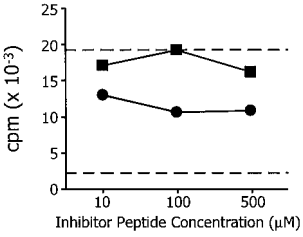

FIGURE 9. In vitro TAP transport of HA-2^V and HA-2^M. T1 cells were permeabilized with streptolysin O (15 U/ml) and incubated with radioiodinated reporter peptide TVNKTERAY plus the indicated dilution of test peptides. Reporter peptide transport in TAP-negative T2 cells was assessed as a negative control. Samples were tested in duplicate except for T2 negative control and T1 cells with no inhibitor, tested in triplicate.

and detection of HA-2 by CTL, as well as the homozygous presence of MYO1G^M and the lack of detection of HA-2. Despite the differences in peptide structure, both HA-2^V and HA-2^M are recognized similarly by HA-2-specific CTL when the synthetic peptides are exogenously pulsed onto HLA-A*0201^+ target cells. In addition, the equilibrium binding of HA-2^M to HLA-A*0201 is only ~12-fold lower than that of HA-2^V. Based on the detection of HA-2^V at ~54 copies/cell on JY (HA-$2^{V/M}$) and assuming that equilibrium binding conditions exist in the ER, we would expect that HA-2^M should be detected at roughly 4–8 copies/cell and that such cells should be relatively well-recognized by HA-2-specific CTL. However, we found no HA-2^M above a level of 0.04 copies/cell in the material derived from either JY or JK-A2 (HA-$2^{M/M}$).

One possible explanation for the failure of HA-2^M to be presented is that it is transported into the ER relatively poorly by TAP. In fact, we have recently shown this to be the basis for differential expression of another human mHag, HA-8 (26). However, the V-to-M change is relatively conservative, and Met at P9 is not contraindicated for successful TAP transport (27–29). Furthermore, direct analysis of the ability of these two peptides to be transported by TAP was not consistent with this explanation. A second possibility is that MYO1G^V and MYO1G^M are processed differently by the proteasome, such that substitution of an M for a V results in either creation or loss of a cleavage site. Although the specific sites recognized by proteasomes are less well defined than are the binding motifs for TAP and HA-A*0201, this amino acid change is rather minor, and it is not immediately evident that such a switch would have an effect on proteasomal cleavage (30). Finally, it should be noted that our analysis of polymorphism encompasses only the 24 bp immediately upstream of the HA-2 sequence. We have not defined the complete 5′ end of MYO1G or the sequence of any relevant control sequences. Thus, it is possible that as yet unrecognized differences in MYO1G^M influence proteolytic cleavage efficiency or proper translation of the gene. In addition, our RT-PCR conditions were not quantitative, such that there might be unrecognized differences in the levels of transcription of the two alleles. However, in this regard it should be noted that we were able to detect very low levels of transcription of MYO1G in a few cell lines, yet these were not recognized by HA-2-specific CTL. Further experimentation will be required to fully resolve this issue.

In keeping with previous work on HA-2 expression (5), we found significant transcription of MYO1G only in cells of hemopoietic origin. Such highly restricted tissue expression suggests great promise for immunotherapy. In addition to those derived posttransplant from the original patient, HA-2-specific T cells have now been generated ex vivo and shown to efficiently lyse EBV-BLCL and malignantly transformed leukemic blasts, while ignoring cells of nonhemopoietic origin (31). Thus, they represent excellent potential adoptive immunotherapy reagents in conjunction

with BMT for hemologic malignancies. It is significant that we failed to detect expression of MYO1G in K562 erythroleukemia cells. The identity of the gene in the present work should enable a more thorough examination of the tissues in which it is expressed.

HA-2 is also expressed in nonmalignant leukocytes (PBL, thymocytes, monocytes, dendritic cells) and should thereby present a target on those cells that mediate autoimmune disease in patients with the proper haplotype. Although not yet common practice, studies in both mice and humans have suggested the potential of BMT as a curative treatment for various autoimmune diseases (32–37). Therefore, it is feasible that HA-2-specific T cells could also be used to treat these diseases in the same way that they might be used to treat leukemia. Finally, the facts that the HA-2 epitope is so prevalent (95% in the HLA-A*0201$^+$ population), and that disparity for this minor H Ag has not been shown to have a positive correlation with the incidence of GVHD, only reinforce the potential therapeutic value of T cells directed against this Ag.

References

1. Goulmy, E. 1997. Human minor histocompatibility antigens: new concepts for marrow transplantation and adoptive immunotherapy. *Immunol. Rev. 157:125.*
2. Doolittle, D. P., M. T. Davisson, J. N. Guidi, and M. C. Green. 1996. Catalog of mutant genes and polymorphic loci, In *Genetic Variants and Strains of the Laboratory Mouse,* 3rd ed. M. F. Lyon, S. Rastan, and S. D. M. Brown, eds. Oxford University Press, New York, p. 17.
3. de Bueger, M., J. J. Rood, A. Bakker, F. van der Woude, and E. Goulmy. 1992. Tissue distribution of human minor histocompatibility antigens: ubiquitous versus restricted tissue distribution indicates heterogeneity among human cytotoxic T lymphocyte defined non-MHC antigens. *J. Immunol. 149:1788.*
4. de Bueger, M., A. Bakker, J. J. van Rood, and E. Goulmy. 1991. Minor histocompatibility antigens, defined by graft-vs.-host disease-derived cytotoxic T lymphocytes, show variable expression on human skin cells. *Eur. J. Immunol. 21: 2839.*
5. van Els, C., J. D'Amaro, J. Pool, A. Bakker, P. J. van den Elsen, J. J. van Rood, and E. Goulmy. 1992. Immunogenetics of human minor histocompatibility antigens: their polymorphism and immunodominance. *Immunogenetics 35:161.*
6. Wallny, H. J., and H. G. Rammensee. 1990. Identification of classical minor histocompatibility antigen as cell-derived peptide. *Nature 343:275.*
7. den Haan, J. M., N. E. Sherman, E. Blokland, E. Huczko, F. Koning, J. W. Drijfhout, J. C. Skipper, J. Shabanowitz, D. F. Hunt, V. H. Engelhard, and E. Goulmy. 1995. Identification of a graft versus host disease-associated human minor histocompatibility antigen. *Science 268:1476.*
8. Mermall, V., P. L. Post, and M. S. Mooseker. 1998. Unconventional myosins in cell movement, membrane traffic, and signal transduction. *Science 279:527.*
9. Bement, W. M., J. A. Wirth, and M. S. Mooseker. 1994. Cloning and mRNA expression of human unconventional myosin-IC: a homologue of amoeboid myosins-I with a single IQ motif and an SH3 domain. *J. Mol. Biol. 243:356.*
10. Hogan, K. T., C. Clayberger, A.-X. T. Le, S. F. Walk, J. P. Ridge, P. Parham, A. M. Krensky, and V. H. Engelhard. 1988. Cytotoxic T lymphocyte-defined epitope differences between HLA-A2.1 and HLA-A2.2 map to two distinct regions of the molecule. *J. Immunol. 141:4005.*
11. Irwin, M. J., W. R. Heath, and L. A. Sherman. 1989. Species-restricted interactions between CD8 and the α3 domain of class I influence the magnitude of the xenogeneic response. *J. Exp. Med. 170:1091.*
12. Miltenburg, A. M., M. E. Meijer-Paape, M. R. Daha, and L. C. Paul. 1987. Endothelial cell lysis induced by lymphokine-activated human peripheral blood mononuclear cells. *Eur. J. Immunol. 17:1383.*
13. Detrisac, C. J., M. A. Sens, A. J. Garvin, S. S. Spicer, and D. A. Sens. 1984. Tissue culture of human kidney epithelial cells of proximal tubule origin. *Kidney Int. 25:383.*
14. Pettenati, M. J., P. N. Rao, R. Hodge, and G. Brewer. 1998. Gene mapping by fluorescence in situ hybridization (FISH). In *Nucleic Acid Hybridization: Essential Techniques.* J. Ross, ed. Wiley, New York, p. 81.
15. Ruppert, J., J. Sidney, E. Celis, R. T. Kubo, H. M. Grey, and A. Sette. 1993. Prominent role of secondary anchor residues in peptide binding to A2.1 molecules. *Cell 74:929.*
16. Parham, P., and F. M. Brodsky. 1981. Partial purification and some properties of BB7.2: a cytotoxic monoclonal antibody with specificity for HLA-A2 and a variant of HLA-A28. *Hum. Immunol. 3:277.*
17. den Haan, J. M., L. Meadows, W. Wang, J. Pool, E. Blokland, T. L. Bishop, C. Reinhardus, J. Shabanowitz, R. Offringa, D. F. Hunt, et al. 1998. The minor histocompatibility antigen HA-1: a diallelic gene with a single amino acid polymorphism. *Science 279:1054.*
18. Shabanowitz, J., R. E. Settlage, J. A. Marto, R. E. Christian, F. M. White, P. S. Russo, S. E. Martin, and D. F. Hunt. 1999. Sequencing the primordial soup. In *Mass Spectrometry in Biology and Medicine.* A. L. Burlingame, S. A. Carr, and M. A. Baldwin, eds. Humana Press, Totowa, NJ.
19. Martin, S. E., J. Shabanowitz, D. F. Hunt, and J. A. Marto. 2000. Sub-femtomole MS and MS/MS peptide sequence analysis using nano-HPLC micro-ESI Fourier transform ion cyclotron resonance mass spectrometry. *Anal. Chem. 72:4266.*
20 Yellen-Shaw, A. J., C. E. Laughlin, R. M. Metrione, and L. C. Eisenlohr. 1997. Murine transporter associated with antigen presentation (TAP) preferences influence class I-restricted T cell responses. *J. Exp. Med. 186:1655.*
21. Neisig, A., J. Roelse, A. J. Sijts, F. Ossendorp, M. C. Feltkamp, W. M. Kast, C. J. Melief, and J. J. Neefjes. 1995. Major differences in transporter associated with antigen presentation (TAP)-dependent translocation of MHC class I-presentable peptides and the effect of flanking sequences. *J. Immunol. 154:1273.*
22. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochem. J. 89:114.*
23. Bement, W. M., T. Hasson, J. A. Wirth, R. E. Cheney, and M. S. Mooseker. 1994. Identification and overlapping expression of multiple unconventional myosin genes in vertebrate cell types. *Proc. Natl. Acad. Sci. USA 91:11767.*
24. Bullock, T. N. J., T. A. Colella, and V. H. Engelhard. 2000. The density of peptides displayed by dendritic cells affects immune responses to human tyrosinase and gp100 in HLA-A2 transgenic mice. *J. Immunol. 164:2354.*
25. Pierce, R. A., E. D. Field, J. M. den Haan, J. A. Caldwell, F. M. White, J. A. Marto, W. Wang, L. M. Frost, E. Blokland, C. Reinhardus, et al. 1999. Cutting edge: the HLA-A*0101-restricted HY minor histocompatibility antigen originates from DFFRY and contains a cysteinylated cysteine residue as identified by a novel mass spectrometric technique. *J. Immunol. 163:6360.*
26. Brickner, A. G., E. H. Warren, J. A. Caldwell, Y. Akatsuka, T. N. Golovina, A. L. Zarling, J. Shabanowitz, L. C. Eisenlohr, D. F. Hunt, V. H. Engelhard, and S. R. Riddell. 2001. The immunogenicity of a new human minor histocompatibility antigen results from differential antigen processing. *J. Exp. Med. 193:195.*
27. Momburg, F., J. Roelse, J. C. Howard, G. W. Butcher, G. J. Hammerling, and J. J. Neefjes. 1994. Selectivity of MHC-encoded peptide transporters from human, mouse and rat. *Nature 367:648.*
28. van Endert, P. M., D. Riganelli, G. Greco, K. Fleischhauer, J. Sidney, A. Sette, and J.-F. Bach. 1995. The peptide-binding motif for the human transporter associated with antigen processing. *J. Exp. Med. 182:1883.*
29. Uebel, S., W. Kraas, S. Kienle, K.-H. Wiesmuller, G. Jung, and R. Tampe. 1997. Recognition principle of the TAP transporter disclosed by combinatorial peptide libraries. *Proc. Natl. Acad. Sci. USA 94:8976.*
30. Uebel, S., and R. Tampe. 1999. Specificity of the proteasome and the TAP transporter. *Curr. Opin. Immunol. 11:203.*
31. Mutis, T., R. Verdijk, E. Schrama, B. Esendam, A. Brand, and E. Goulmy. 1999. Feasibility of immunotherapy of relapsed leukemia with ex vivo- generated cytotoxic T lymphocytes specific for hematopoietic system-restricted minor histocompatibility antigens. *Blood 93:2336.*
32. Wang, B., Y. Yamamoto, N. S. El Badri, and R. A. Good. 1999. Effective treatment of autoimmune disease and progressive renal disease by mixed bone-marrow transplantation that establishes a stable mixed chimerism in BXSB recipient mice. *Proc. Natl. Acad. Sci. USA 96:3012.*
33. De Stefano, P., M. Zecca, G. Giorgiani, C. Perotti, E. Giraldi, and F. Locatelli. 1999. Resolution of immune haemolytic anaemia with allogeneic bone marrow transplantation after an unsuccessful autograft. *Br. J. Haematol. 106:1063.*
34. Snowden, J. A., W. N. Patton, J. L. O'Donnell, E. E. Hannah, and D. N. Hart. 1997. Prolonged remission of longstanding systemic lupus erythematosus after autologous bone marrow transplant for non-Hodgkin's lymphoma. *Bone Marrow Transplant. 19:1247.*
35. Brodsky, R. A., and B. D. Smith. 1999. Bone marrow transplantation for autoimmune diseases. *Curr. Opin. Oncol. 11:83.*
36. Marmont, A. M. 1998. Stem cell transplantation for severe autoimmune diseases: progress and problems. *Haematologica 83:733.*
37. Marmont, A. M. 1997. Stem cell transplantation for severe autoimmune disorders, with special reference to rheumatic diseases. *J. Rheumatol. Suppl. 48:13.*