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Conservation of minor histocompatibility antigens between human and non-human primates

It is well accepted that minor histocompatibility antigens (mHag) can function as transplantation barriers between HLA-matched individuals Little is known about the molecular nature and evolutionary conservation of mHag It is only very recently that the first human mHag were identified The HLA-A2 1-restricted mHag HA-2 and the HLA-B7-restricted mHag H-Y appeared to be peptides derived from polymorphic self proteins Here we show that the HLA-A2 1-restricted mHag HA-1, HA-2, and the H-Y peptides are conserved between man, chimpanzees and rhesus macaques Human cytotoxic T cell clones specific for the HLA-A2 1-restricted mHag HA-1, HA-2, and H-Y recognized HLA-A2 1 gene-transfected chimpanzee and rhesus macaque cells High-pressure liquid chromatography fractionation of HLA-A2 1-bound peptides isolated from the HLA-A2 1-transfected chimpanzee cells revealed that the chimpanzee HA-1 and HA-2 co-eluted with the human HA-1 and HA-2 Subsequent amino acid sequencing showed that the chimpanzee HA-2 peptide is identical to the human HA-2 peptide Our functional and biochemical results demonstrate that mHag peptides are conserved for over 35 million years

1 Introduction

Disparity for minor histocompatibility antigens (mHag) between HLA-identical individuals can lead to graft-versus-host disease (GVHD) after bone marrow transplantation [1] MHag-specific HLA-restricted T cell clones can be generated of PBMC from patients suffering from GVHD [2 3] Using T cell clones specific for mHag HA-1 HA-2, HA-4, and HA-5, we showed that these non-sex linked mHag segregate as Mendelian traits and independently from each other Each can be considered as the product of a gene with two alleles [4] Population genetic studies revealed that HA 1 and HA-2 appeared frequently (69% and 95%), whereas HA-4 and HA-5 occurred with lower frequencies (16% and 8%) in the healthy population [5] Recently, the first two human mHag have been identified by amino acid sequencing of the HLA-bound peptides that were recognized by the mHag specific T cell clones The non-sex-linked HLA-A2 1-restricted mHag HA-2 most probably originates from a member of the class I myosin protein family [6] and the male-specific HLA-B7 restricted mHag H Y is derived from the Y chromosome-encoded SMCY (selected mouse cDNA on the Y) protein [7] The SMCY gene was shown to be conserved in evolution [8] However until now no information existed on the evolutionary conservation of human non-sex linked mHag In the present study we investigated whether the human non-sex-linked HLA-A2 1 restricted mHag HA-1, HA-2, and HA-4 and the male-specific HLA-A2 1-restricted mHag H Y are evolutionary conserved For this functional studies biochemical purification and amino acid sequence analyses were performed We transfected chimpanzee and rhesus macaque B cell lines with the HLA-A2 1 restriction molecule and used these cells as target cells for recognition by the HLA-A2 1 restricted mHag-specific CTL clones Subsequently the mHag HA-1 and HA-2 peptide were eluted from the transfected HLA-A2 1 chimpanzee cells and showed similar HPLC elution patterns when compared with human HA 1 and HA-2 peptides Sequence analysis of chimpanzee HA-2 revealed an amino acid sequence identical to the human HA-2

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Abbreviation: mHag: Minor histocompatibility antigen

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2 Materials and methods

2.1 Transfection and cell culture

Chimpanzee B cell lines were generated from six unrelated chimpanzees derived from the Biomedical Primate Research Centre colony Theo (chimpanzee, 1, ♂), Pearl (chimpanzee 2, ♀), Debbie (chimpanzee 3, ♀), Brigitte (chimpanzee 4, ♀), Sherry (chimpanzee 5, ♀), Japie (chimpanzee 6, ♂) Two rhesus macaque B cell lines were generated from 2849 (rhesus macaque 1, ♀) and IWM (rhesus macaque 2, ♂), both derived from the Biomedical Primate Research Centre colony The cells were maintained in RPMI 1640 medium containing 10% fetal calf serum and 3 mM L-glutamine Rhesus macaque cell lines were cultured in the presence of 0 6 μg/ml 3′-azido-3′-

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deoxythymidine Cells were transfected with pHEBO A2 1 by electroporation using a Bio-Rad Gene Pulser Cells were routinely shocked at 960 μF, 210 V in the presence of 62 5 μg/ml uncut plasmid DNA Cuvettes of 0 4 cm were used, with about 12 × 10^6 cells in 0 8 ml phosphate-buffered saline Transfected cell lines were maintained in the presence of 125–250 μg/ml hygromycin [9] The HLA-A2 1-allospecific CTL clones 3E7 and 3E5, the HLA-A2 1-restricted HA-1-specific CTL clone 3HA15, the HLA-A2 1-restricted HA-2-specific CTL clone 5H13, the HLA-A2 1-restricted HA-4-specific CTL clones 5G30 and 5Gy8, and the HLA-A2 1-restricted H-Y-specific CTL clone 1R35 were cultured by weekly stimulation with irradiated allogeneic PBMC and B-lymphoblastoid cell line (BLCL) cells in RPMI 1640 medium containing 15 % human serum, 3 mM L-glumtaine, 1 % leukoagglutinin A and 20 U/ml recombinant IL-2 [5]

2.2 ^{51}Cr-release assay

^{51}Cr-labeled target cells (5000) were incubated together with different numbers of HLA-A2 1-allospecific and mHag-specific CTL clones in 200 μl ^{51}Cr release was determined after 4 h at 37 °C The percent specific lysis was calculated as follows percent specific lysis = [(experimental release − spontaneous release)/(maximal release − spontaneous release] × 100 Spontaneous release and the maximal release were the ^{51}Cr release of the target cells in culture medium alone and in culture medium containing 1 % Triton X-100, respectively

2.3 Peptide purification

Peptides were eluted out of purified HLA-A2 1 molecules as described [6, 10] Briefly, HLA-A2 1 molecules were purified from 2 05 × 10^{10} transfected chimpanzee cells by affinity chromatography with BB7 2-coupled CNBr activated Sepharose 4B beads (Pharmacia LKB, Uppsala Sweden) Peptides were eluted from the HLA-A2 1 molecules by acid treatment and separated from the HLA A2 1 heavy chain and β2-microglobulin by filtration over a 10-kDa-cutoff Centricon (Amicon, Lexington, MA) filter Peptides were fractionated using reverse-phase micro HPLC (Smart System, Pharmacia) Buffer A was 0 1 % trifluoroacetic acid (TFA), buffer B was 0 1 % TFA in acetonitrile The gradient consisted of 100 % buffer A (0 to

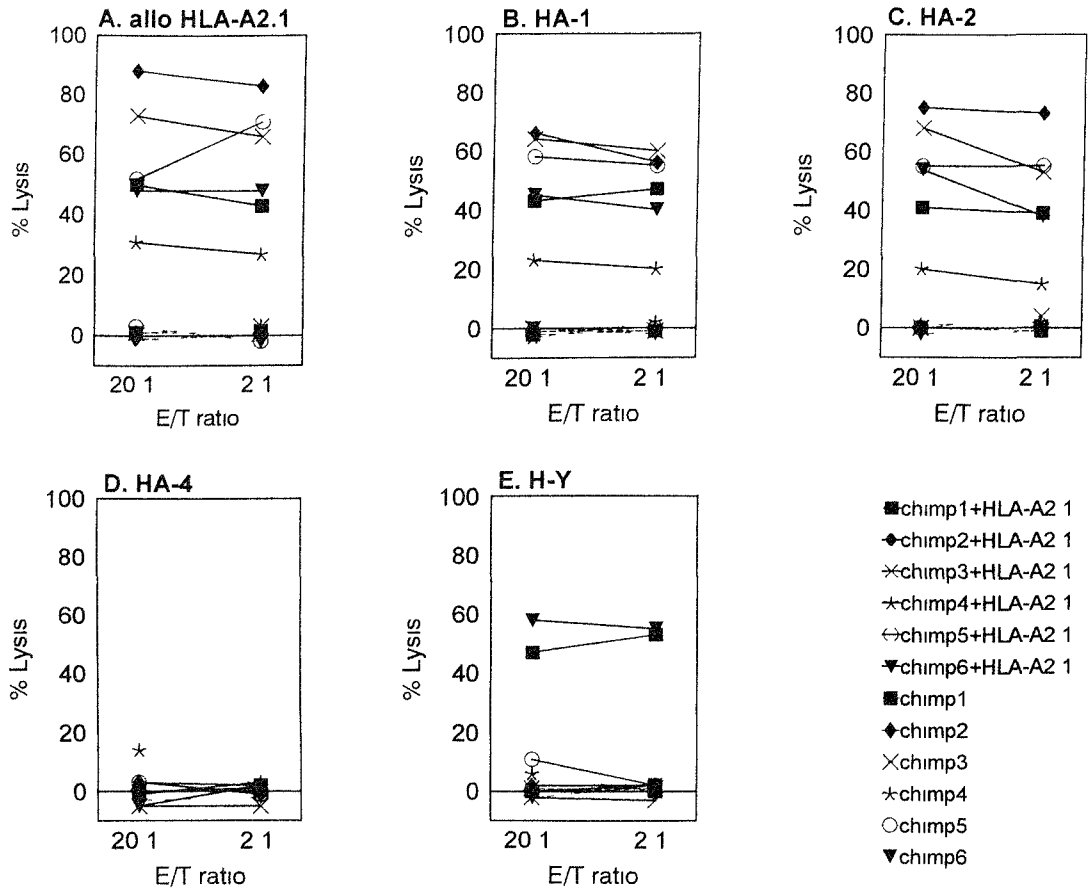

Figure 1 HLA-A2 1-transfected chimpanzee cell lines are lysed by HLA-A2 1-allospecific and by HLA-A2 1-restricted HA 1, HA 2 and H–Y-specific CTL clones (A) Lysis by the HLA A2 1-allospecific CTL clone, (B) lysis by the HLA-A2 1-restricted HA-1-specific CTL clone, (C) lysis by the HLA-A2 1 restricted HA-2 specific CTL clone, (D) lysis by the HLA-A2 1-restricted HA-4-specific CTL clone, and (E) lysis by the HLA-A2 1 restricted H–Y-specific CTL clone Solid lines represent the HLA-A2 1 transfected cell lines, and dotted lines represent the nontransfected cell lines All E/T ratios tested were 20 1 and 2 1 except for chimpanzee 6 For both the HLA A2 1 transfected and the nontransfected chimpanzee 6 cell line, E/T ratios of 13 1 and 1 3 1 were used for the HLA A2 1 allospecific CTL, E/T ratios of 5 1 and 0 5 1 were used for the HA-4-specific CTL and E/T ratios of 11 1 and 1 1 1 were used for the HLA A2 1 restricted H–Y CTL

20 min), 0 to 12 % buffer B (20 to 25 min), and 12 to 50 % buffer B (25 to 80 min) at a flow rate of 100 µl/min. Fractions of 100 µl were collected. In the ^{51}Cr-release assay, 2.5 µl of each fraction was diluted in 25 µl HBSS buffered with 50 mM Hepes. ^{51}Cr-labeled T2 cells (2500) were incubated with the fractions in 50 µl for 30 min at 37 °C. HA-2-specific T cells were added for an E/T ratio of 17:1 in final volume of 150 µl for 4 h at 37 °C.

2.4 Mass spectrometry

Collision-activated dissociation spectra were recorded on a triple-quadrupole mass spectrometer (Finnigan-MAT, San Jose, CA) operating with a two mass-unit window in quadrupole 1.

3 Results

3.1 Recognition of HLA-A2.1-transfected chimpanzee and rhesus macaque B cell lines by human mHag-specific CTL clones

Six unrelated chimpanzee (*Pan troglodytes*) and two unrelated rhesus macaque (*Macaca mulatta*) B cell lines were transfected with the HLA-A2.1 gene and analyzed with HLA-A2.1-restricted mHag-specific CTL clones in a ^{51}Cr-release assay (Figs. 1, 2). All transfected cell lines were recognized by the HLA-A2.1-allospecific CTL clone (Figs. 1A, 2A). Differences in lysis by the HLA-A2.1-allospecific CTL clone was correlated with differences in HLA-A2.1 expression as determined by FACS analysis (data not shown). The HA-1- and HA-2-specific CTL clones lysed all six chimpanzee and two rhesus macaque cell lines, whereas no HA-4 reactivity could be detected (Figs. 1B–D; 2B–D). All HLA-A2.1-transfected male chimpanzee and rhesus macaque cell lines were recognized by the HLA-A2.1-restricted H–Y-specific CTL clone (Figs. 1E, 2E). These results demonstrate that the HA-1, HA-2, and H–Y T cell epitopes are functionally expressed on these cells.

3.2 Chimpanzee HA-1 and HA-2 peptides have similar HPLC retention times as human HA-1 and HA-2

To test the assumption that the endogenously processed mHag HA-1 and HA-2 peptides in non-human primates are identical to the human HA-1 and HA-2, HLA-A2.1-bound peptides were isolated from an HLA-A2.1-transfected chimpanzee cell line as described [6, 10]. After

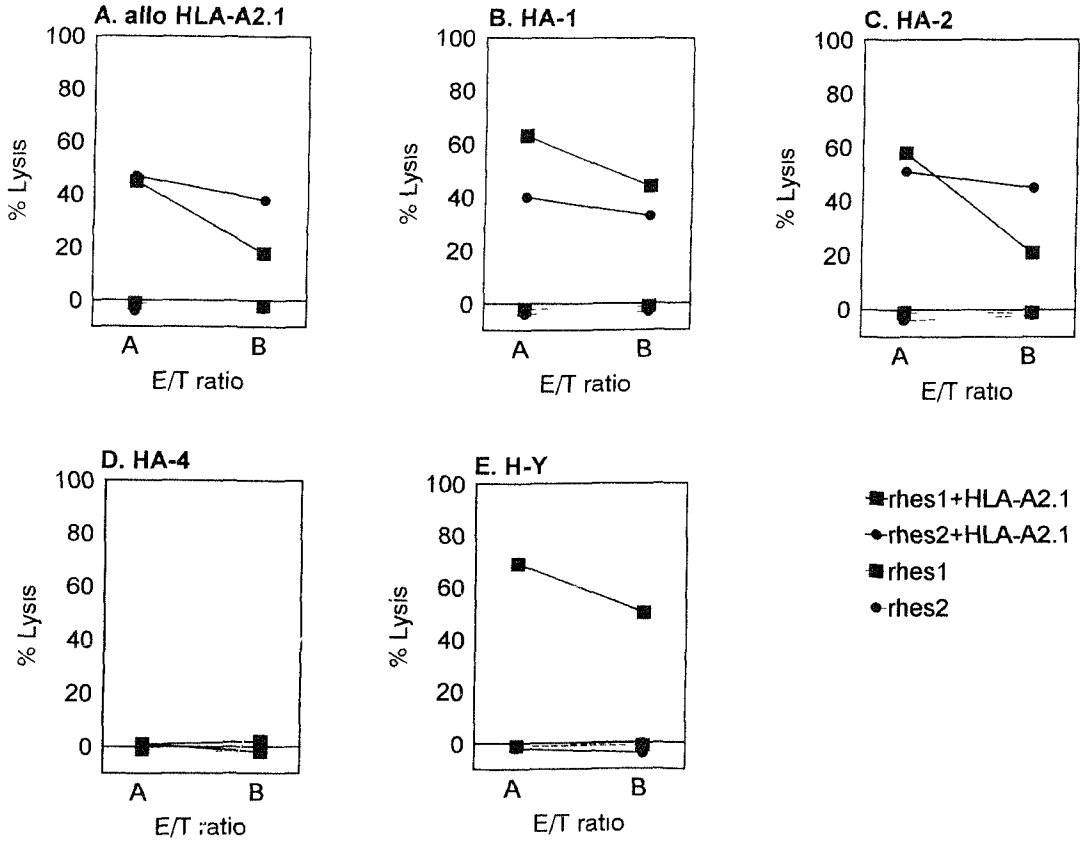

Figure 2 HLA-A2.1-transfected rhesus macaque cell lines are lysed by HLA-A2.1-allospecific and by HLA-A2.1-restricted HA-1, HA-2, and H–Y-specific CTL clones (A) Lysis by the HLA-A2.1-allospecific CTL clone, (B) lysis by the HLA-A2.1-restricted HA-1-specific CTL clone, (C) lysis by the HLA-A2.1-restricted HA-2-specific CTL clone, (D) lysis by the HLA-A2.1-restricted HA-4-specific CTL clone, and (E) lysis by the HLA-A2.1-restricted H–Y-specific CTL clone Solid lines represent the HLA-A2.1-transfected cell lines and dotted lines represent the nontransfected cell lines E/T ratios (A) in (B) were 10:1 and 1:1 for the transfected as well as the nontransfected rhesus macaque 1 cell line for all CTL clones except for the HA-1-specific CTL clone, where E/T ratios of 30:1 and 3:1 were used. For both the transfected as the nontransfected rhesus macaque 2 cell line, E/T ratio A was between 7.1 and 20.1 and ratio B was between 0.7.1 and 2.1 for the different CTL clones

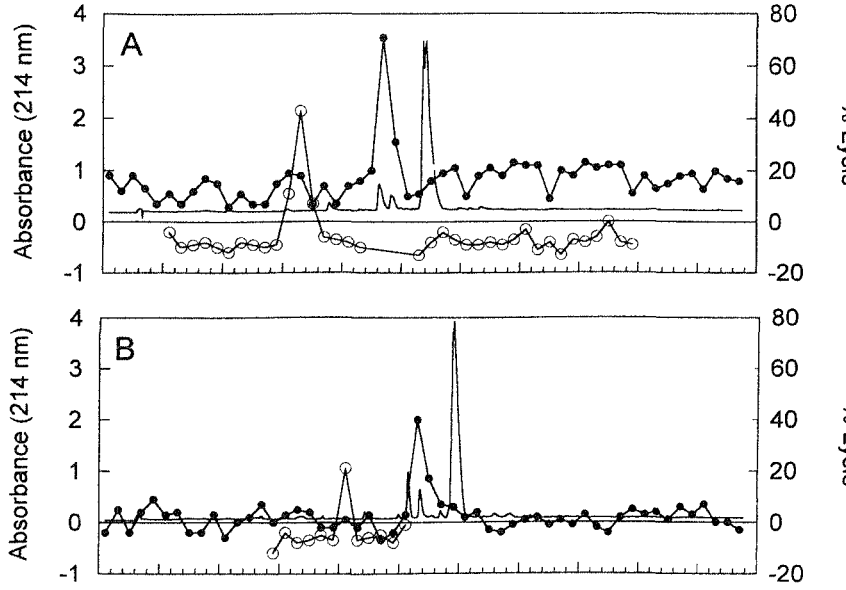

Figure 3 (A) Activity of HPLC fractions of HLA A2 1 eluted chimpanzee peptides in a ^{51}Cr release assay using HA 1 and HA 2 specific CTL clones as effector cells compared with the HPLC elution profile of a reference peptide (B) Activity of HPLC fractions of HLA A2 1 eluted human peptides in a Cr release assay using HA 1 and HA 2 specific CTL clones as effector cells and compared with the HPLC elution profile of a reference peptide For both HA-1 (open circles) and HA 2 (closed circles), one peak of activity was found in the chimpanzee and the human HLA A2 1 eluted peptides Both the chimpanzee and human HA 2 active fractions were found at the same position when compared with the elution profile of the reference peptide (solid line) The human and chimpanzee HA 1 active fractions eluted six and seven fractions earlier when compared with the HA 2 active fractions This shift of one fraction of HA 1 is within the normal range of variation between HPLC runs

HPLC fractionation, the chimpanzee peptide fractions were assayed with the mHag HA-1 and HA 2 specific CTL clones For both HA-1 and HA-2 that were eluted from the transfected chimpanzee cells, one peak of activity was found (Fig 3A) which was also observed for human HLA A2 1 eluted peptides (Fig 3B) When the positions of the HA 1 and HA-2 active fractions were compared to the elution profile of a reference peptide that was run immediately before the eluted peptides, both the chimpanzee HA-1 and HA-2 active fractions eluted approximately at the same position as the human HA 1 and HA 2 active fractions This suggests that the chimpanzee HA 1 and HA-2 peptides have similar biochemical properties as the human HA-1 and HA-2 peptides

3.3 The chimpanzee and human mHag HA-2 peptide sequence is identical

Recently, we identified the human mHag HA 2 as YIGEVLVSV with a mass-to-charge ratio of m/z 978 [6] Collision-activated dissociation (CAD) analysis by tandem mass spectrometry of m/z 978 in the chimpanzee HA-2 positive fraction revealed the sequence YXGEVXVSV (Fig 4A), which is identical to the human HA-2 amino acid sequence (Fig 4B) Since the mass spectrometer we used cannot differentiate between Leu and Ile (represented by X), it remains to be established whether positions 2 and 6 encode Leu or Ile, which is also true for the human mHag HA-2 [6]

4 Discussion

Humans, chimpanzees and rhesus macaques share a common ancestor that lived 35 million years ago [11] The divergence between man and chimpanzees took place around 5 million years ago [12] The functional recognition of the HLA-A2 1 transfected chimpanzee and rhesus macaque cells by the H–Y, HA 1 and HA-2-specific CTL and the biochemical peptide characterization of the non human primate mHag clearly demonstrate that human mHag peptides are conserved for at least 35 million years of primate evolution Thus the mHag proteins leading to these mHag peptides are of functional importance Moreover the mHag peptide identity shows that the processing and presentation machinery in respect to mHag is conserved as well This underlines earlier suggestions that the processing and presentation machinery may be conserved between species in general It has been shown that H 2K transfected human cells could present allopeptides to mouse H $2K^b$ restricted CTL [13] Furthermore lysis was observed of H-$2K^d$/vaccinia virus-transfected rat Syrian hamster, monkey and human cells by mouse H $2K^1$ restricted vaccinia specific CTL [14]

Information on conservation of mHag is scarce except for the mHag H–Y Recently, both a human HLA B7-restricted H–Y antigen and a mouse K^k-restricted H–Y antigen were shown to be derived from the Y chromosome encoded SMCY protein [7, 15] This protein is evolutionarily conserved and expressed in both humans and mice although the H–Y T cell epitopes are not Conservation of an HLA-B27-restricted H–Y antigen has been described in HLA-B27-transgenic mice and rats, however, human cells were not recognized by the rat H–Y-specific HLA B27 restricted CTL [16] Although it remains to be established whether other H–Y mHag, like the HLA-A2 1 restricted H–Y T cell epitope, are also encoded by the SMCY gene, we show here that the HLA-A2 1-restricted H–Y peptide is also conserved in evolution

The phenotype frequencies of mHag HA-1, HA 2, and HA-4 in the HLA-A2 1^+ Caucasian population are 69 % 95 %, and 16 %, respectively [5] All the chimpanzee and rhesus macaque HLA-A2 1-transfected cell lines analyzed in this study expressed HA-1 and HA-2, whereas none expressed HA 4 These results suggest that different phenotype frequencies may exist for the mHag in non-human primates Alternatively, the polymorphisms of the HA 1, HA 2, and the HA 4 loci may have arisen after the divergence between chimpanzee and man However, the number of chimpanzee and rhesus macaque cell lines analyzed in this study is too low to draw any conclusions

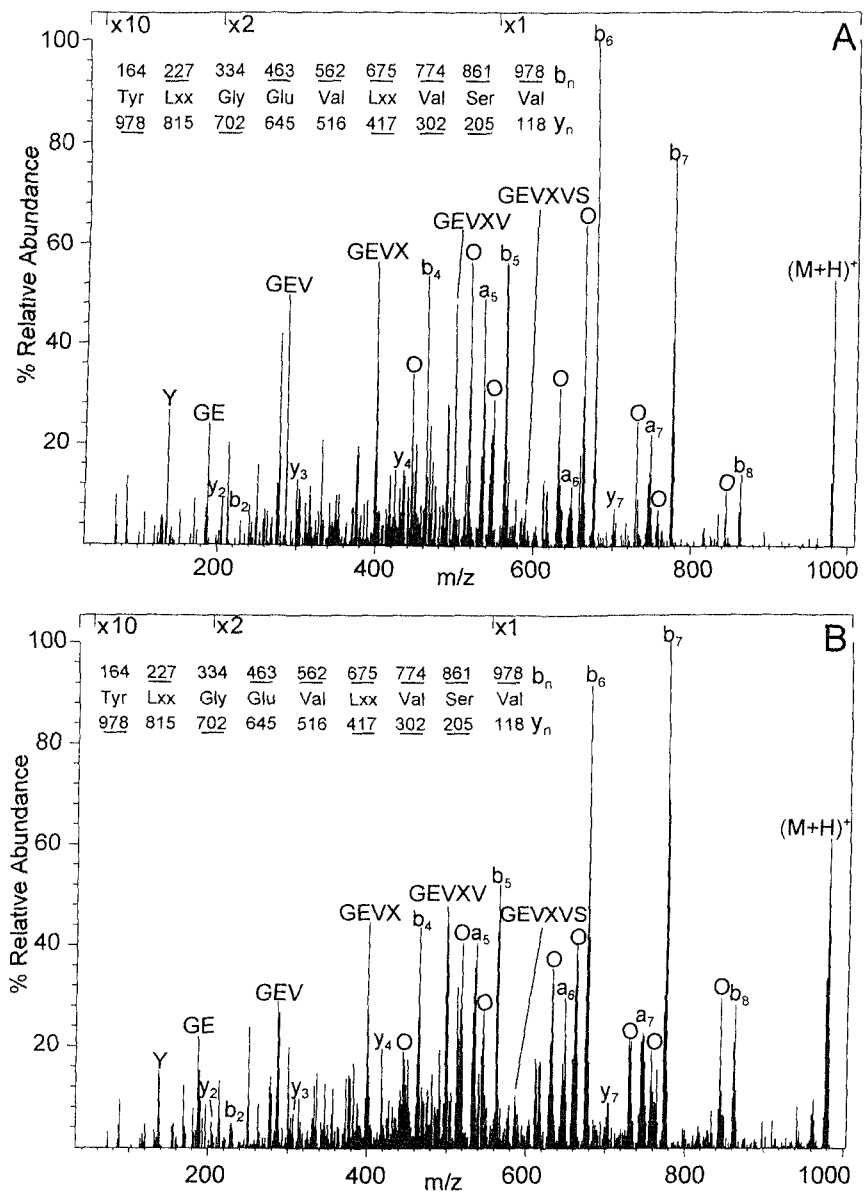

Figure 4 Collision activated dissociation mass spectrum of mHag HA 2 $(M + H)^+$ ions with m/z 978 The predicted mass for fragment ions of type b and y are shown, respectively above and below the deduced amino acid sequence Ions observed in the spectrum are underlined Lxx represents Ile or Leu which have identical masses and cannot be differentiated in this mass spectrometer (A) Spectrum of peptide YXGEVXVSV obtained from chimpanzee cells (B) Spectrum of peptide YXGEVXVSV obtained from human cells

This study is the first demonstration of evolutionary conservation of non-sex-linked human mHag On the one hand, the identity of human and chimpanzee mHag may have implications for xenotransplantation Xenotransplantation of non-human primate tissue can lead to T cell responses to mHag peptides presented via the indirect pathway On the other hand, our results show the possibility to use non-human primates as a model to study bone marrow transplantation-related reactivities such as GVHD and graft-versus-leukemia reactions

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