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-A21-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-A21-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-A21-restricted mHag HA-1, HA-2, and H-Y recognized HLA-A21 gene-transfected chimpanzee and rhesus macaque cells. High-pressure liquid chromatography fractionation of HLA-A21-bound peptides isolated from the HLA-A21-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 from 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 mHags have been identified by amino acid sequencing of the HLA-bound mHag peptides that were recognized by the mHag specific T cell clones. The non-sex-linked HLA-A21-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-A21-restricted mHag HA-1, HA-2, and HA-4 and the male-specific HLA-A21-restricted mHag H-Y are evolutionary conserved. For this functional studies into the biochemical purification and amino acid sequence analyses were performed. We transfected chimpanzee and rhesus macaque B cell lines with the HLA-A21 restriction molecule and used these cells as target cells for recognition by the HLA-A21-restricted mHag-specific CTL clones. Subsequently the mHag HA-1 and HA-2 peptide were eluted from the transfected HLA-A21 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.

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′,5′-azido-3′...
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 S3A35, the HLA-A2 1-restricted HA-2-specific CTL clone S913, the HLA-A2 1-restricted HA-4-specific CTL clones S5G30 and S5G8, 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-glutamine, 1% leukosarcomin A and 20 U/ml recombinant IL-2 [5].

2.2 51Cr-release assay

51Cr-labeled target cells (5000) were incubated together with different numbers of HLA-A2 1-allospecific and mHag-specific CTL clones in 200 μl. 51Cr 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 51Cr 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 × 10^9 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 100% buffer B in 60 min) at a flow rate of 0.5 ml/min.

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 13:1 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 11:1 were used for the HLA-A2 1-restricted H-Y CTL.
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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 3Cr-release assay, 2.5 μl of each fraction was diluted in 25 μl HBSS buffered with 50 mM Hepes. 3Cr-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 3Cr-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](image_url)
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 YXGEXVSV (Fig 4A), which is identical to the human HA-1 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. More over, 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 transduced human cells could present allopeptides to mouse H 2K restricted CTL [13]. Furthermore, lysis was observed of H 2K-vaccinia virus-transfected rat Syrian hamster, monkey and human cells by mouse H 2K 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-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 risen 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.
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 activities such as GVHD and graft-versus-leukemia reactions.

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5 References

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