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5′ Regulatory nucleotide sequence of an *HLA-A*0101null* allele

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Abstract We have previously demonstrated an *HLA-A*0101null* allele segregating in a family with the *HLA-B8*, *-Cw7*, *-DR3*, *-DR52*, *-DQ2* haplotype. In the present study the regulatory elements with known transcription enhancement activity of the silenced *HLA-A*0101* allele were analyzed. In the enhancer B element, a T was substituted for a C at position −106, whereas no other alterations were found in the adjacent 5′ section of the *HLA-A*0101null* allele. This substitution was not seen in the enhancer B elements of the corresponding genes involved in normal *HLA-A*0101* membrane expression. Comparison of enhancer B element sequences of classical functional major histocompatibility complex (*MHC*) class I alleles demonstrated a high degree of conservation. In contrast, many *MHC* class I pseudogenes showed mutation in their enhancer B boxes. These results may indicate that the single mutation detected in the enhancer B element plays a pivotal role in the abolishment of membrane expression of the *HLA-A*0101null* allele.

Introduction

Classical human major histocompatibility complex (*MHC*) class I genes encode the HLA-A, -B, and -C molecules, which are expressed on virtually all nucleated cells (David-Watine et al. 1990). The level of MHC class I expression varies between different tissues and cell types and even locus and allele-specific differences have been documented (Neefjes and Ploegh 1988; Hui and Soong 1992). The regulation of MHC class I molecule expression is controlled by a number of DNA sequences located upstream of the transcription initiation site. Most of these nucleotide stretches contain recognition sites for various DNA binding proteins. The interaction between these 5′ regulatory sequences and the various DNA binding proteins governs transcription activity (David-Watine et al. 1990; Halleron et al. 1986). Comparisons of the 5′ non-coding regulatory regions of classical *MHC* class I genes and the rest of the multigene family reveal that considerable nucleotide sequence diversity is mainly restricted to the non-classical genes, whereas the genes that retain classical antigen presenting function display little or no polymorphism (Summers et al. 1993). It has previously been reported that maintenance of a redundant class I locus as a functional unit is determined by conserved promoter elements that regulate responsiveness of the locus during times critical to selection. Mutations in these regulatory elements will eventually result in the extinction of the diverse array of alleles at the locus (Pease and Vallejo 1995a, b).

We have described the existence of an *HLA-A*0101* allele with severely diminished transcription levels, resulting in the absence of detectable HLA-A1 expression (Lardy et al. 1992). We postulated that the down modulated HLA-A1 expression may be due to malfunctioning *cis*-acting regulatory factors. In this study the 5′ regulatory region of the *HLA-A*0101null* allele was analyzed to investigate the nature of the transcription defect.

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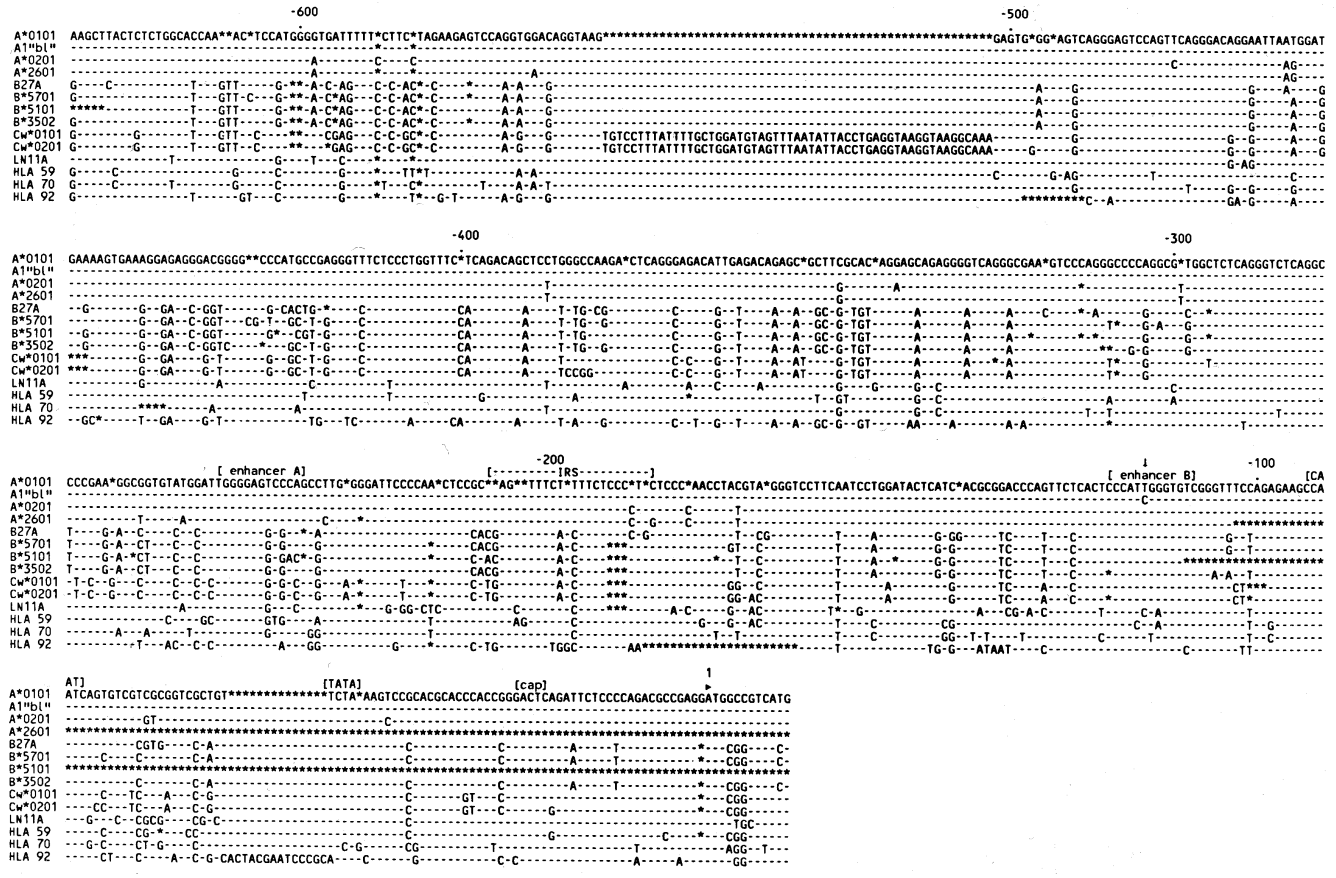
Materials and methods

Cell lines

Epstein-Barr virus (EBV)-transformed B-cell lines with the following serotypes were used: 25–2120) HLA-A1“null”; A2; B8; B41; Cw7; DR3; DR7; DQ2; DQ3, 25–2160) HLA-A1“null”; A25; B8; B35; Cw7; Cw4; R3; DR1; DQ2; DQ1, 25–2230) HLA-A1; A2; B8; B41; Cw7; DR3; DR7; DQ2; DQ3 and 25–640) HLA-A1; B8; Cw7; DR3; DQ2 (the number before each cell line refers to the cell identification).



The EBV B-cell lines with id. 25−2120 and 25−2160 were established from individuals in which no cell membrane expression of the *HLA-A*0101* allele was detected, i.e., HLA-A1"null" (Lardy et al. 1992). The EBV B-cell line with id. 25−2230 was established from a family member with normal cell membrane expression of HLA-A1. Cell line 25−640 was originally described as ws#9023 and was obtained from the homozygous panel cells of the Tenth International Histocompatibility Workshop (IHW).

Polymerase chain reaction (PCR) primers

The nucleotide sequences of the primers used for amplification of the 5′ non- coding regulatory region of *HLA* class I alleles were as follows:

3′A1-primer: 5′TATTCTAGAGCGCCCGCGGCTCCATCT-3′ (pos.+203 to +300)

5′Z-primer: 5′CCCGTCGACCCAGGCGTGGCTCTCAGG-3′ (pos.-305 to -287)

5′Y-primer: 5′GTTGTCGACGACAGCTCCTGGGCCAAG-3′ (pos.-395 to -377)

5′X-primer: 5′GGAGTCGACGATGAAAAGTGAAAGGAG-3′ (pos.-457 to -440)

5′W-primer: 5′CTAGTCGACTCCAGGTGGACAGGTAAG-3′ (pos.-574 to- 556)

The 3′A1 primer was constructed in such a fashion that the 5′ end specifically hybridizes to the *HLA-A*0101* allele. The underlined nucleotide sequence denotes the restriction sites for the restriction enzymes *Xba* I (TCTAGA) or *Sal* I (GTCGAC). The nucleotide sequences of the 5′ non-coding region of *HLA* class I alleles were obtained from Summers and co-workers 1993.

Fig. 1 Alignment of the 5′ non-coding region of several *MHC* class I and class I like genes with the classical *HLA-A*0101* allele. The consensus nucleotide sequence of the *HLA-A*0101* allele was obtained from Summers and co-workers (1993). The nucleotide sequence of the *HLA-A*0101* allele was derived from individuals (25−2230 and 25−640) with normal *HLA-A*0101* cell membrane expression. The *HLA-A1"bl"* nucleotide sequence was derived from individuals positive for the *HLA-A*0101* null allele. Nucleotide sequence positions are numbered from −631 to +1. Nucleotide position +1 corresponds to the first nucleotide position of the transcription initiation codon ATG. *Dashes* indicate identity with the consensus *HLA-A*0101* sequence. An *asterisk* indicates nucleotide sequence not known or introduced to achieve the best alignment. The 5′ non-coding regulatory elements are denoted *above* the corresponding nucleotide sequences

DNA cloning and sequence analysis

Genomic DNA isolation was performed according to the Eleventh International Histocompatibility Workshop (IHW) DNA component protocol (Kimura and Sasazuki 1991). Briefly, EBV-transformed B-cell lines were lysed in a buffer containing 10 mM Tris-HCL (pH 7.6), 10 mM ethylenediaminetetraacetate (pH 8.0), and 50 mM NaCl. Genomic DNA was isolated according to the phenol-chloroform extraction procedure followed by an isopropanol precipitation step. PCR amplification of the 5′ adjacent regulatory region was performed using the following primer combinations: 5′W/3′A1, 5′X/3′A1, 5′Y/3′A1, and 5′Z/3′A1. The PCR products were digested with *Xba* I/*Sal* I and ligated to similarly cut M13mp18/M13mp19 vectors, which were used to transform competent XL1 blue cells. Single-stranded M13mp18/mp19 DNA was isolated and nucleotide sequence of the 5′ regulatory region was determined for both DNA strands by dideoxy termination using the T7 DNA polymerase sequencing system (Promega Corp., Madison, WI). Multiple clones containing the *HLA-A*0101null* allele were sequenced.

Results

Sequence analysis of the 5′ non-coding regulatory region

We have reported an *HLA-A*0101null* allele which segregated in a healthy Caucasoid family (Lardy et al. 1992). Full-length cDNA sequence analysis did not detect any nucleotide mutations in the coding regions or altered splice sites that could account for the abrogation of the expression of this classical *HLA-A*0101* allele. Due to the minute levels of *HLA-A*0101* transcript, we postulated that the *HLA-A*0101null* allele was associated with a malfunctioning *cis*-acting regulatory factor.

In the present study the 5′ non-coding region of the *HLA-A*0101null* allele was analyzed. The cell lines with id. 25−2230 and 25−640, which expresses normal levels of HLA-A1, were used to obtain an unambiquous nucleotide consensus sequence of the 5′ non-coding region of the *HLA-A*0101* allele. The obtained consensus was identical to a previously published nucleotide sequence of the 5′ non-coding region of the *HLA-A*0101* allele (Summers et al. 1993). Sequence analysis of the 5′ regulatory region of the *HLA-A*0101null* allele was performed on multiple clones obtained from independent PCR amplificates of EBV B-cell lines 25−2120 and 25−2160. These EBV B-cell lines were derived from individuals carrying the *HLA-A*0101null* allele. Multiple clones were characterized that revealed a single nucleotide substitution in the enhancer B element. Extended nucleotide sequencing into exons 1 and 2 confirmed that this mutated enhancer B element indeed is linked to the *HLA-A*0101null* allele. Figure 1 demonstrates that in the enhancer B element at position −106, a T was substituted for a C. No further substitutions or deletions were found in any of the other regulatory sequences with known transcription enhancement activity. Furthermore, Figure 1 also demonstrates that the nucleotide sequences of the enhancer B element is highly conserved in *MHC* class I genes that retained their classical antigen presenting function. This is in sharp contrast to the nonfunctional *MHC* class I or class I-like genes with no or an undocumented function such as *HLA-70*, *HLA-92*, *HLA-59*, and *LN11A*.

Discussion

Deleterious nucleotide substitutions resulting in the inactivation of a once functional gene is not a new phenomenon and has been described for several *MHC* loci or alleles. Examples of such events are the *HLA-DQA2* locus, the *I-Ea* alleles in mice, the *HLA-DRB4*0101102N* allele, and the *HLA-AR* locus (Auffray et al. 1987; Begovich et al. 1990; Figueroa et al. 1990; Sutton et al. 1989; Zemmour et al. 1990). More recently an individual homozygous for an *HLA-Anull* allele, *HLA-A*0215N*, was reported. A single nucleotide substitution in exon 4 of the *HLA-A*0207* allele resulted in the premature introduction of a stop codon. As observed in our case, this *HLA-Anull* gene-positive individual is healthy and exhibits no apparent immunological abnormalities (Ishikawa et al. 1995). This is not a surprise, since the *HLA* system contains many *MHC* class I genes that have arisen from several duplication events. As a consequence, the malfunctioning of one *MHC* class I locus has no great effect, since the classical antigen presentation function can be replaced by another highly related locus.

This study represents the analysis of the 5′ non-coding region of a non-expressed classical *HLA-A*0101* allele. Sequence analysis of the promoter region which is linked to the *HLA-A*0101null* allele revealed a single nucleotide substitution in the enhancer B element. At position −106, a T was substituted for a C. This T to C substitution was the only mutation detected in the promoter region, which might explain the abolished cell membrane expression of the *HLA-A*0101null* allele (Lardy et al. 1992).

This finding is in concordance with the findings of Balas and co-workers (1994) in which they described the presence of an *HLA-A*0201* allele with highly diminished cell surface expression segregating in a healthy Spanish family. A 5′-regulatory region nucleotide sequence analysis demonstrated the presence of the same unique point mutation, a T to C substitution, in the enhancer B-inverted CAT box. Although the enhancer B element has been functionally defined in the mouse (Kimura et al. 1986), its functional significance in the regulation of the human *MHC* genes has not been established. The findings reported in the present study and the study performed by Balas and co-workers (1994) do not prove but strongly suggest that the enhancer B element might play a pivotal role in the expression of MHC class I gene products. However, alternative explanations are also feasible. Assays such as site-directed mutagenesis or promoter swopping combined with gene transfection experiments are in progress. These experiments should provide more insight into the exact role of the enhancer B element in gene transcription events.

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