5' Regulatory nucleotide sequence of an HLA-A*0101 null allele
Lardy, NM; Otting, N.; van der Horst, A.R.; dr. Bontrop, R.E.; de Waal, L.P.

Published in:
Immunogenetics

DOI:
10.1007/s002510050254

Link to publication

Citation for published version (APA):
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.

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; DR4; DR3; 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 Histocompat-
ibility 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'-TATCCTCTAGAAGGGGCTCCCTCCATCTCT-3' (pos.+203 to +300)
- **5' Z-primer:** 5'-CCCCTGCAGACCCCGCCGCTCCTCAGG-3' (pos.-305 to -287)
- **5' Y-primer:** 5'-GTTGTCGACGACGCTCTGGGCAAG-3' (pos.-395 to -377)
- **5' X-primer:** 5'-GGAGTCGACGTGAAAAAGTGAAGGAG-3' (pos.-457 to -440)
- **5' W-primer:** 5'-CTAGTCGACCTAGGGGATGGTGAGAAG-3' (pos.-574 to -556)

The 5'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 XbaI (TCTAGA) or SalI (GTCGAC). The nucleotide
sequences of the 5' non-coding region of HLA class I alleles were obtained from Summers and co-workers 1993.

**DNA cloning and sequence analysis**

Genomic DNA was isolated 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, and 5' Y/3' A1. The PCR products were digested with XbaI and SalI 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 (Pro-
mega 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 Caucasian 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 unambiguous 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. 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.

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).

References


