Brief Report

Recombination in human leukocyte antigen region in two Asian Indian families

Mahendra N. Mishra, Ajay Sharma[1]

Department of Pathology, Dr. Lal Path Labs Pvt Ltd, Rohini, New Delhi, [1]Department of Clinical Haematology and Internal Medicine, Army Hospital Research and Referral, Delhi Cantt., India

BACKGROUND: Recombination (crossing over) may generate novel haplotypes that can be beneficial to a population against recently introduced pathogens. It may lead to the generation of new alleles.
SETTINGS AND DESIGN: A prospective study at a tertiary care centre.
AIM: To report two rare cases of crossing over in HLA region.
MATERIALS AND METHODS: Tissue-typing was done by sequence specific primers (SSP) for DR locus and by both SSP and serology for Class I which was reconfirmed on fresh samples.
RESULTS: In one patient crossing over had taken place in the region of A locus resulting in inheritance of A*01 instead of expected A*11. In second family crossing over had taken place in region of DRB1 locus and the sibling inherited DRB1*08 instead of DRB1*10.
CONCLUSIONS: Possibility of recombination must be considered when interpreting implausible tissue-typing results of families worked up for BMT.

Key words: AML, human leukocyte antigen, recombination, sequence specific primers

Introduction

The Human Major Histocompatibility Complex (MHC) contains genes encoding highly polymorphic cell surface glycoproteins, which mediate both cell-mediated and humoral immune responses. Mechanisms for generating polymorphism within genes of the MHC include recombination (crossing over), gene conversion, and, to a lesser extent, mutation.[1]

Meiotic recombination does not appear to occur randomly across chromosomes, but rather seems to be restricted to specific regions. A striking example of this phenomenon is illustrated by the HLA class II region. No recombination within the 100 kb encompassing the DRB1-DQA1-DQB1 loci has been reported, whereas the random association of TAP1 with TAP2 alleles alleles suggests the presence of a hotspot for recombination within the 15 kb separating the closest variant sites of these two loci.[2] While doing a literature search we came across few recent reports of crossing over of human leukocyte antigen (HLA) alleles and none where the studies were based on simple haplotype analysis. More reports were on recombination in the class II loci than on class I loci.[3,4]

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Materials and Methods

Tissue-typing was performed at Command Hospital (SC), Pune for members of 60 families to select fully matched HLA matched donors for possible allogenic bone marrow transplantation (BMT). Two of the samples on haplotype construction showed slight deviations from expected haplotypes and HLA typing was again performed on fresh samples of the patient and family members. Both patients had acute myeloblastic leukemia. Smears made from peripheral blood showed few blasts as samples for DNA extraction were collected

Address for correspondence: Dr. Mahendra Narain Mishra, Pathology, Dr. Lal Path Labs Pvt Ltd, Block E, Sector 18, Rohini, New Delhi – 110 085, India. E-mail: mnmishra@hotmail.com

when patients were in remission. One was a 27-year-old lady (patient 1) and the second was sibling of a six-year-old boy (patient 2).

Genomic DNA was extracted from whole blood using Qiagen (Germany) kits by column based methods. Tissue-typing was done by serology or low resolution DNA typing for Class I and by sequence specific primers (SSP) for DR locus for the patient, two siblings, and both parents. DNA typing by SSP (Innotrain, Germany) was repeated on fresh sample using a second commercial kit, (Olerup, Austria) when we could not explain the antigens by simple Mendelian inheritance.

Results

Results of tissue–typing by SSP are shown in Table 1. Interpretation of tissue-typing results was done by software and manually using tables provided with the kit insert. Both cases of crossing over had taken place in the paternal chromosomes. This was first suspected when the HLA typing results did not correspond to that predicted by simple haplotype inheritance from parents, so the results of tissue-typing were confirmed by testing fresh samples from the patient and sibling whose tissue-typing results were aberrant, using a different commercial kit. The paternal and maternal haplotypes are represented as (a, b) and (c, d), respectively. In one patient crossing over had taken place in the region of paternal A locus and the inherited haplotype was (b/a, c). The patient had inherited A*01 instead of expected A*11. In the second family, the crossing over had taken place in the region of DR locus in one sibling of the patient who inherited DRB1 *08 instead of expected DRB1 *10 and the inherited haplotype was (b/a, c).

Table 1: Results of tissue-typing of two families with recombination

Relation (Haplotype)	Method	A	B	C	DRB1, others
Patient 1 b/a, c	SSP	*01,*01	*40,*52	*12,*15	*04. *11, DRB3
Sibling 1A a, d	SSP CDC	*01, *11 01, 11	*07,*49 07,49, w4, w6	*07,*15	*15, *15, DRB5
Sibling1 B a, c	SSP CDC	*01, *01 01, -	*07,*40 07, -,w6	*15, *15	*11, *15, DRB3, 5
Mother 1 c, d	SSP (c) (d)	*01 *11	*40 *49	*15 *07	*11, DRB3 *15, DRB5
Father 1 a, b	SSP (a) (b)	*01 *11	*07 *52	*15 *12	*15, DRB5 *04, DRB4
Patient 2 a c	SSP	*02,*31	*51,*18	*14,*15	*04, *10
Sibling 2A b/a,c	SSP	*02, *31	*51,*18	*14,*15	*04, *08
Sibling 2B a, d	SSP	*02, *31	*51,*15	*01, *14	*10, *15, DRB 5
Mother 2 c, d	SSP (c) (d)	*02 *02	*18 *15	*15 *01	*04, DRB4 *15, DRB5
Father 2 a, b	SSP (a) (b)	*31 *01	*51 *53	*14 *02	*10 *08

Discussion

The incidence of recombination in paternal or maternal chromosomes is 1%.[5] One study reported unusually high incidence of recombination - 9% in 18 families of juvenile diabetes mellitus, which could probably be attributed to the juvenile diabetes mellitus gene itself.[6] Meiotic recombination in Class II region has been reported to be 0.74%[3] and accepted frequency for intra HLA recombination is 1.6 %.[4] Studies of recombination and linkage disequilibrium can provide insight into the selective pressures on those DR-DQ and DR-DQ-DP haplotypes that may be beneficial in mediating an effective immune response against relatively old, common pathogens. The frequency of recombination between DR-DQ and DP is 0.74%, which is within the expected range; given 1% recombination per megabase of DNA per meiosis.[7,8] The strong linkage disequilibrium among loci in the HLA class II region (DR, DQ, and DP) indicates that they are likely to interact synergistically in their response to foreign agents. Crossing over may sometimes result in new alleles. Mytilineos *et al*., in two studies reported discovery of new alleles as a result of double crossing over.[9,10] However, in the present study we did not come across any new alleles.

Haplotypic analysis enabled us to confirm the crossing-over in the region of A and DR loci in the patient and in the sibling of the second patient with AML. This is not to be confused with uniparental disomy, which has also been reported in AML patients – a condition which gets reversed with treatment and may be seen if the peripheral blood contains many blasts.[11] We had excluded this by performing the test when the patient was having very few blasts in peripheral blood due to chemotherapy and obtained similar results. In both cases crossing over had taken place in the paternal 6p.

Based in this study, we recommend that one must be careful in the interpretation of the results of HLA typing between donors and recipients of bone marrow. Complementary investigations should be performed for studying genetic abnormalities, which could be involved with recombination.

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