THE GENERATION OF CLASS II POLYMORPHISM BY RECOMBINATION

Machteld Oudshoorn, Ernette D du Toit and Rob W Martell

Provincial Laboratory for Tissue Immunology, Cape Town, South Africa

INTRODUCTION

One of the most striking features of the HLA gene products is their high degree of polymorphism. The HLA class II polymorphism varies from locus to locus, e.g. at present there are 18 serological defined antigens at the DR locus and only half that number at the DQ locus (Bodmer et al. 1989). Within a polymorphic gene certain exons are more polymorphic than others, e.g.the gene segments encoding the first domains of DRB, DQA and DQB chains contain most of the sequence variation. Furthermore, even within the first domain, the polymorphism is concentrated in areas called allelic hypervariable regions (Bell et al. 1987). Various mechanisms have been implicated as being responsible for the generation of HLA polymorphism such as homologous and non-homologous crossing-over, point mutation and gene conversion.

Another striking characteristic of the class II region is the strong linkage disequilibrium between DQ and DR antigens and which varies from population to population. For example, Caucasoids who type serologically as HLA-DRw17 almost exclusively type as DQw2. In contrast, South African (SA) blacks with DRw17 can type as DQw2 or DQw7.

Several unusual HLA-DR,DQ haplotypes were found in SA blacks and individuals of mixed ancestry (Cape Coloureds) (du Toit et al. 1988) and studied by serological, cellular and DNA typing techniques to see if they may have arisen as a result of a recombination event.

MATERIALS AND METHODS

Three families with unusual HLA-DR,DQ haplotypes were studied, two of SA black origin and one of mixed ancestry (Cape Coloured). In addition, 25 homozygous typing cells (HTCs) were used in the RFLP analysis.

HLA-A, B and C typing was performed by the NIH complement-dependent microlymphocytotoxicity technique (Terasaki et al. 1974). HLA-DR and DQ typings were performed by the technique agreed upon for the Seventh International Histocompatibility Workshop. All families were typed with the Tenth International Histocompatibility Workshop (10WS) sera.

Advances in Forensic Haemogenetics 3
Edited by H. F. Polesky and W. R. Mayr

HLA-Dw typing was performed in duplicate by the method described previously (Oudshoorn et al. 1986). Fifty-six HTCs were used to define the specificities HLA-Dw1 to Dw11, Dw13 to Dw15, Dw17 to Dw19 as well as the specificity DKT2 and three locally defined specificities, Dw'RSH' (Oudshoorn et al. 1989), Dw'BME' and Dw'JOH' (Oudshoorn et al. 1986).

DNA was isolated from peripheral blood lymphocytes, digested with the restriction enzyme TaqI and used for Southern blotting by the method described for the 10WS (Marcadet et al. 1989).

RESULTS

The unusual haplotypes DRw17,DQw7; DRw16(HER),DQw5 and DRw14,DQw1(MDI) were analysed.

The serological patterns observed for the antigens constituting the unusual haplotypes did not differ from those of the "classic" haplotypes. HLA-Dw typing tests performed with the unusual haplotypes revealed no typing responses for any of the unusual haplotypes, indicating that these haplotypes carry unknown HLA-Dw specificities. The DRB and DQA RFLP results of the rare *DRw17,DQw7* haplotype were identical to common DRw17,DQw2,Dw3,Dw25(DRw52b) haplotypes, whereas the DQB RFLP pattern was identical to other DQw7 haplotypes. The *DRw14,DQw1(MDI)* haplotype showed DRB and DQA RFLPs identical to the "classic" DRw14,DQw5 haplotypes, but the DQB RFLP pattern was identical to DQw6 instead of DQw5, which is the allele commonly in linkage disequilibrium with DRw14. The DRB RFLP results of the *DRw16(HER),DQw5* haplotype showed fragments typical of both the normal DRw15 and DRw16 specificities. The results obtained using the DQB probe were identical to other DQw5 haplotypes and that of the DQA probe identical to the patterns observed for DR2 haplotypes such as DRw15,DQw6 and DRw16,DQw5.

CONCLUSION

Population differences in DR,DQ associations have been important in establishing the genetic organization of the HLA-D region (Oudshoorn et al. 1984; Hurley et al. 1989). These ethnically different DR,DQ haplotypes may also provide valuable insight into mechanisms such as homologous and non-homologous crossing-over, point mutation and gene conversion, by which the HLA region has evolved. Three unusual HLA-DR,DQ haplotypes were found in SA blacks and individuals of mixed ancestry. The origin of these haplotypes, DRw17,DQw7; DRw16(HER),DQw5 and DRw14,DQw1(MDI) could be explained by a single cross-over event.

The unusual DRw16(HER),DQw5,Dw"blank" haplotype which types serologically as DRw16 but shows a "hybrid" DRw15/DRw16 on DNA typing, may have arisen from a homologous cross-over between the two expressed DRB genes, DRB1 and "DRB2" (DRB2 was used to

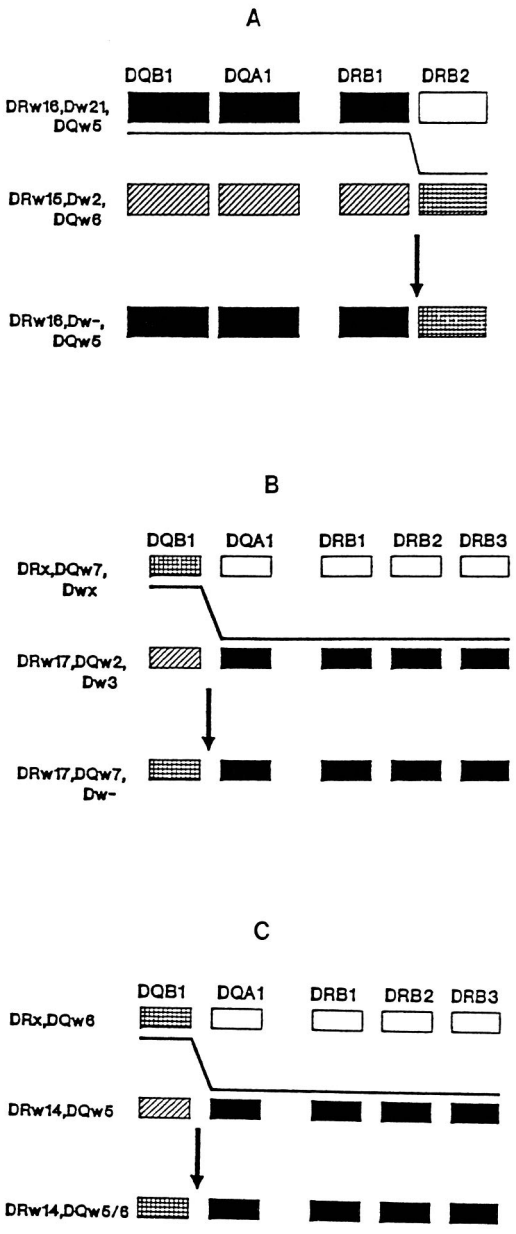

Fig. 1. Schematic diagram illustrating the probable site of recombination to generate (A) the DRw16(HER),DQw5,Dw"blank", (B) the DRw17,DQw7,Dw"blank" and (C) the DRw14,DQw1(MDI) haplotypes.

indicate the second expressed DRB gene in this paper) as shown in Fig. 1A. The results obtained for the DRw17,DQw7 haplotype indicate that this haplotype may have originated from a homologous cross-over between the DQB1 and DQA1 genes of a DRw17,DQw2 and a DQw7 haplotype (Fig. 1B) similar to that described for the DR4,DQw7 haplotype (Holbeck and Nepom 1988). The DQB RFLP pattern of the DRw17,DQw7 haplotype was identical to that of DQw7 haplotypes, whereas its DQA RFLP pattern was identical to that of DRw17,DQw2 haplotypes indicating that the serological DQw7 determinant resides on the DQB chain rather than the DQA chain. Fig. 1C shows the proposed recombination event for the generation of the DRw14,DQw1(MDI) haplotype indicating that this haplotype could also have originated from a cross-over between the DQA1 and DQB1 genes.

This study showed that recombinational events may have occurred between the DRB genes as well as the DQA and DQB genes during evolution and that they may be important elements in generating class II haplotype diversity. Of particular importance are the recombinational events between the DQA and DQB genes as they might result in new specificities, thus enhancing the degree of class II polymorphism.

REFERENCES

Bell JI, Denney D, Foster L, Belt T, Todd JA, McDevitt HO (1987) Allelic variation in the DR subregion of the human major histocompatibility complex. Proc Natl Acad Sci USA 84:6234-6238

Bodmer WF, Albert E, Bodmer JG, Dupont B, Mach B, Mayr WR, Sasazuki T, Schreuder GMTh, Svejgaard A, Terasaki PI (1989) Nomenclature for factors of the HLA system, 1987. In: Dupont B (ed) Immunobiology of HLA, vol I. Springer-Verlag, New York, p 72

Du Toit ED, Macgregor KJ, Taljaard DG, Oudshoorn M (1988) HLA-A, B, C, DR and DQ polymorphisms in three South African population groups: South African Negroes, Cape Coloureds and South African Caucasoids. Tissue Antigens 31:109-125

Holbeck SL, Nepom GT (1988) Molecular analysis of DQβ 3.1 genes. Hum Immunol 21:183-192

Hurley CK, Gregersen PK, Gorski J, Steiner N, Robbins FM, Hartzman R, Johnson AH, Silver J (1989) The DR3(w18),DQw4 haplotype differs from DR3(w17),DQw2 haplotypes at multiple class II loci. Hum Immunol 25:37-50

Marcadet A, O'Connell P, Cohen D (1989) Standardized Southern Blot Workshop technique. In: Dupont B (ed) Immunobiology of HLA, vol I. Springer-Verlag, New York, p 72

Oudshoorn M, Schreuder GMT, Campbell EM, du Toit ED (1984) Segregation of DQ and DR: "Exceptions to the rule". In: Albert ED, Baur MP, Mayr WR (eds) Histocompatibility Testing 1984. Springer-Verlag, Berlin, p419

Oudshoorn M, du Toit ED, Taljaard DG, MacGregor KJ (1986) A study of the HLA-Dw determinants and their relationship to DR and DQ antigens in three South African population groups: South African Caucasoids, South African Negroes, and Cape Coloureds. Hum Immunol 17:273-287

Oudshoorn M, du Toit ED, Taljaard DG (1989) HLA-Dw'RSH': a new HLA-Dw specificity associated with HLA-DRw18(3). Tissue Antigens 33:367-374

Terasaki PI, McClelland JD, Park MS, McCurdy B (1974) Microdroplet lymphocyte cytotoxicity test. In: Ray JG, Hare DB, Pedersen PD, Kayhoe DE (eds). Manual of tissue typing techniques. Bethesda, NIH, p67