INVESTIGATIONS INTO THE COMPLEXITY AND POLYMORPHISM OF HLA-D LOCI IN SOUTH AFRICA

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ABSTRACT

The HLA complex is the most polymorphic genetic system known in man. The frequency of the HLA class II antigens have been well studied in Caucasoids but little data is available concerning HLA antigen frequencies in Negroes. In this thesis the class II antigens, excluding HLA-DP, were studied in South African (SA) Negroes (Xhosa), Cape Coloureds (a group of mixed racial origin) and SA Caucasoids using serological, cellular (HTC typing) and Southern blot techniques. The results obtained for the SA Negroes were compared with those previously found in Nigerians and American Negroes. Marked differences in HLA distribution occurred between these groups, which in part may be explained by Khoisan admixture in the SA Negroes. In addition, striking frequency differences were observed between the three SA populations. For example, in the Xhosa the HLA-DR1, DR4, DR7, DRw8, DQw2, DQw3, Dw1 and Dw3 specificities were found at a significantly lower frequency, whereas HLA-DR3, DRw6 and Dw'RSH' were found at a significantly higher frequency compared with the SA Caucasoids. The frequency in the Cape Coloureds was intermediate between those of the Xhosa and Caucasoids. In the SA Negroes and Cape Coloureds, several new specificities were detected such as HLA-DRw18, DR2 LUM(CT), DRw12x6, DRw8x14, Dw'RSH', Dw'JOH' and Dw'BME'. The HLA-DR and DQ haplotypes in significant linkage disequilibrium were

similar in the three groups. However, several haplotypes with unusual DR and DQ combinations such as HLA-DRw17,DQw7; DR9,DQw2 and DR4,DQw5 were present in the SA Negroes and Cape Coloured families. Although some of these unusual haplotypes could be explained in terms of recombination between the common haplotypes, none could be typed using a panel of well defined homozygous typing cells, suggesting that the response observed in mixed lymphocyte culture arises from separate molecular determinants. The data on HLA class II antigen frequencies presented in this thesis is essential for future studies on HLA and disease associations and for establishing population relationships. Knowledge of new HLA class II antigens in the various population groups is also important in renal transplantation as matching for HLA-DR antigens is known to improve graft survival.

ABBREVIATIONS

af	-	antigen frequency
Bf	-	properdin factor B
B-LCL	-	lymphoblastoid B-cell line
Bq	-	Bequerel
^{o}C	-	degree Celcius
cm	-	centimeter
cM	-	centiMorgan
2-D	-	two-dimensional
DMSO	-	dimethyl sulphoxide
EBV	-	Epstein-Barr virus
FCS	-	fetal calf serum
gf	-	gene frequency
GLO	-	glyoxylase I
Gy	-	Gray
HF	-	haplotype frequency
HEPES	-	N-2 hydroxyethylpiperazine-N^{1}-2-ethanesulphonic acid
HL-A	-	Human leukocyte, locus A
IDDM	-	insulin dependent diabetes mellitus
IEF	-	isoelectric focussing
kb	-	kilobase
kDa	-	kiloDalton
mb	-	megabase
MHC	-	major histocompatibility complex
MEM	-	minimum essential medium

MoAb	-	monoclonal antibody
µg	-	microgram
µm	-	micrometer
ml	-	milliliter
MLC	-	mixed lymphocyte culture
NP-40	-	nonidet P40
21-OH	-	21-hydroxylase
PHA	-	phytohemagglutinin
PLT	-	primed lymphocyte typing
RFLP	-	restriction fragment length polymorphism
RPMI 1640	-	Roswell Park Memorial Institute culture medium
SA	-	South African
SDS	-	sodium dodecyl sulphate
spec. act.	-	specific activity
TCM	-	tissue culture medium
TNF	-	tumour necrosis factor
WHO	-	World Health Organization
10 WS	-	Tenth International Histocompatibility Workshop
X^2	-	chi-square
$\triangle$	-	Delta value

CHAPTER 1

INTRODUCTION

1.1 THE HISTORY OF THE HLA SYSTEM

The first evidence for the existence of a histocompatibility system was described in 1936 with the discovery of what was thought to be a new blood group system in the mouse (Gorer 1936). Using inbred strains of mice, it was found that matching for a red cell antigen, also present in fixed tissues, had a major beneficial effect on the survival of a transplantable tumour (Gorer 1937). Snell in 1948 called these antigens, relevant for transplantation, histocompatibility (H) antigens. It was then realized that the H-2 antigens were the most important to match for and they were identified as the major histocompatibility system of the mouse. It was Medawar in 1946, who found that antigens associated with skin transplantation rejection in the rabbit were present on leukocytes. This was later confirmed in man (Friedman et al. 1961). Dausset (1954) and Miescher and Fauconnet (1954) put forward the first evidence in favour of the existence of human leukocyte blood groups when they found white cell antibodies in the serum of patients who had received a large number of blood transfusions. In 1958, van Rood and Payne independently discovered that leukocyte antibodies were produced as a

result of pregnancy (van Rood et al. 1958, Payne and Rolfs 1958). These antibodies were found to be directed against antigens present on leukocytes of the husband and were formed by the mother as a response to foreign fetal antigens. These observations led to the definition of the major histocompatibility complex (MHC) in man, also known as HLA (human leukocyte, locus A). The first leukocyte antigen was detected by Dausset in 1958 and called Mac. The antigen Mac is now known as HLA-A2. The first leukocyte group system was described by van Rood in 1962, when he introduced computer techniques in this field. On the basis of the chi-square values, a computer program helped to select sera with highly similar reaction patterns. He found two groups of antisera (4a and 4b) with contrasting reactions when tested against a large number of lymphocytes, i.e. behaving like the alleles at a single genetic locus. Family studies confirmed this finding and showed that the 4a (now Bw4) and 4b (now Bw6) alleles were inherited in a simple Mendelian autosomal co-dominant fashion (van Rood and van Leeuwen 1963). This system was called Leukocyte Group Four. Payne and co-workers in 1964 defined a different allelic system of leukocyte antigens which they called LA. Further studies indicated that the Four and the LA series were determined by genes at two closely linked loci (Ceppellini et al. 1967, Kissmeyer-Nielsen et al. 1969). The LA locus is now known as the HLA-A locus, while the Four locus is now called the HLA-B locus. The further development of the HLA system has

been greatly facilitated by a series of international collaborative studies started by Amos in 1964. These International Histocompatibility Workshops, as they are called, have involved inter alia the exchange of reagents amongst a large number of participating laboratories. The laboratories worked on combined projects, and the subsequent analyses of the data have each been major turning points in the development of knowledge of every aspect of the HLA system. At the First International Histocompatibility Workshop, the typing methodology used by various laboratories was compared. The microdroplet lymphocytotoxicity test developed by Terasaki and McClelland (1964) was subsequently adopted as the standard method of typing. At the Second International Histocompatibility Workshop organized by van Rood in 1965, the definition of the first described antigen, Mac, became well established. Results of the families studied during the Third International Histocompatibility Workshop organized by Ceppellini in 1967, confirmed the assumption made by Dausset et al. (1965) that most of the leukocyte antigens detected belonged to the same genetic system. The Fourth International Histocompatibility Workshop organized by Terasaki in 1970, fully established the two HLA-A and B loci. During the Fifth International Histocompatibility Workshop organized by Dausset in 1972, more than 50 populations were typed for HLA antigens by 27 laboratories resulting in the determination of the antigen frequencies

among the various populations of the world. Studies by Sandberg et al. (1970) and Thorsby et al. (1970) provided evidence for the existence of a third locus, AJ (now called HLA-C) in the same region as HLA-A and B. This region is now referred to as the class I region. The Sixth Workshop, organized by Kissmeyer-Nielsen in 1975, clarified the definition of HLA-C and revealed that there might be another locus, HLA-D, defined in a mixed lymphocyte culture (MLC). During the Seventh International Histocompatibility Workshop in 1977, organized by Bodmer, a new allelic system, HLA-DR, first described by van Leeuwen et al. in 1973, was defined using serological methods. The genetics of the HLA loci and their specificities were further studied during the Eighth International Histocompatibility Workshop organized by Terasaki in 1980. The Ninth International Histocompatibility Workshop was organized in 1984 under the chairmanship of Albert and Mayr. A new allelic system, HLA-DQ, detected using serological methods, was defined in addition to the HLA-DP system which was defined using primed lymphocyte typing cells. A schematic map of the HLA system on chromosome 6 is given in Figure 1.1. At the Tenth International Histocompatibility Workshop, organized by Dupont in 1987, molecular techniques were included for the first time. Analysis of the molecular data identified and defined the arrangement of many of the genes of the HLA system, as shown in Figure 1.2 and 1.3. HLA-E is a new class I gene located between HLA-A and C (Koller et al.

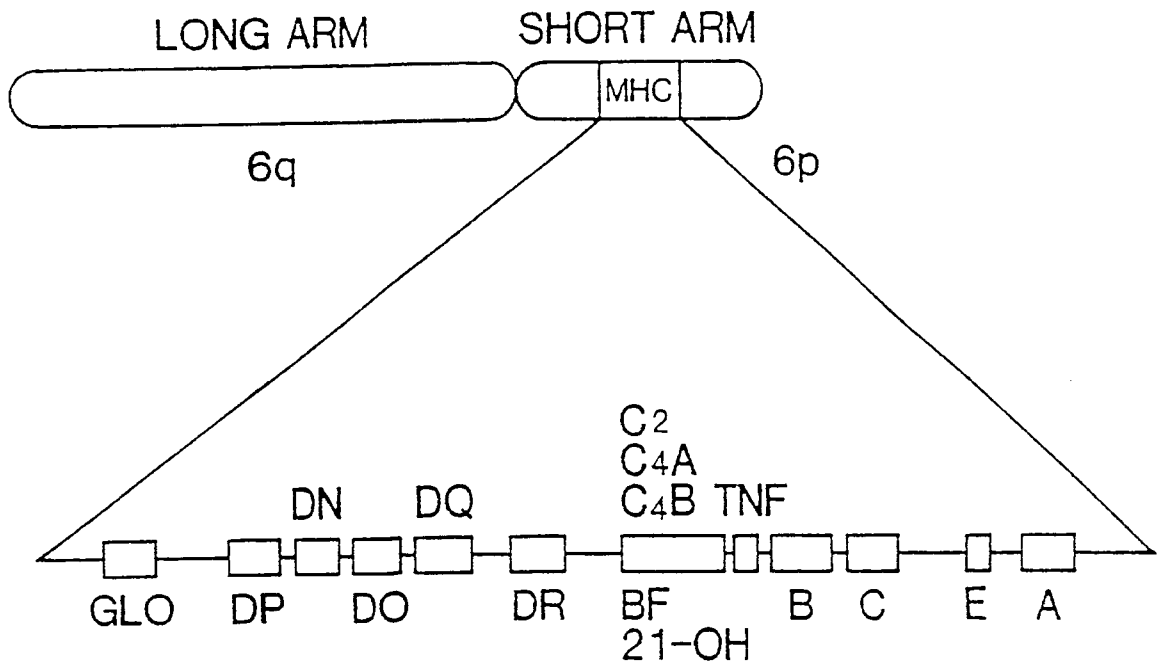

FIGURE 1.1 Map of the MHC region, which in man is known as the HLA region, on the short arm of chromosome 6.

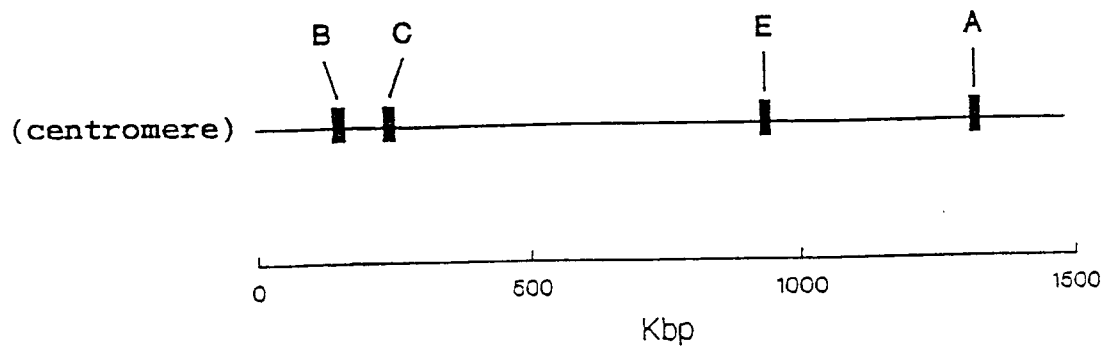

FIGURE 1.2 A map of the HLA class I genes on the short arm of chromosome 6.

Kbp = kilobasepair

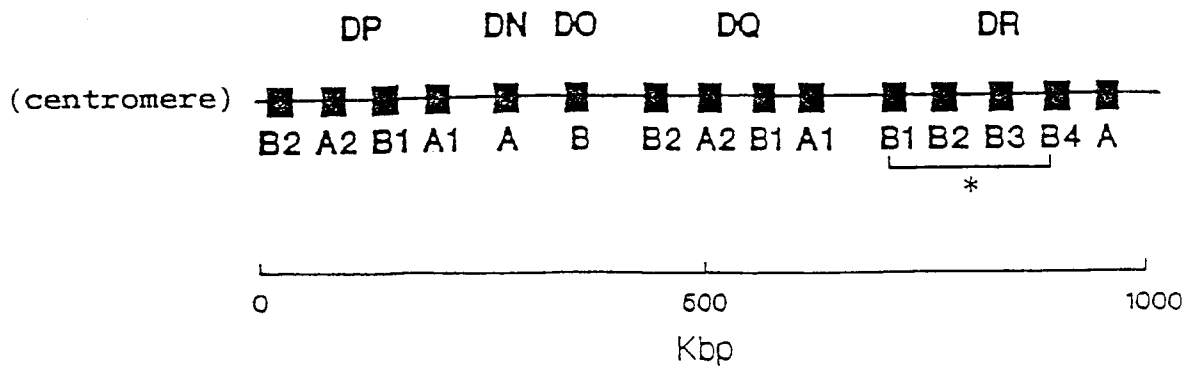

FIGURE 1.3 A map of the HLA class II genes on the short arm of chromosome 6.

* Number of DRB genes depends on haplotype.
Kbp = kilobasepair

1987). The HLA-D region containing five HLA-D loci is also known as the class II region. The loci are HLA-DN, DO, DP, DQ and DR. HLA-DO (formerly known as DOB) and HLA-DN (formerly known as DZ or DO) are new loci in the class II region (Tonnelle et al. 1985, Trowsdale and Kelly 1985). The HLA class II antigens, the subject of this study, will be discussed in more detail in section 1.9.

A complete listing of HLA specificities recognized during the Tenth International Histocompatibility Workshop is given in Table 1.1.

Several components of the serum complement system are coded for by genes in the region between HLA-B and DR, called the class III region. In addition, two steroid 21-hydroxylase genes (21-OHA and 21-OHB) are mapped in this region. Some investigators have suggested that the class III region itself is not part of the MHC but resides on the same chromosome purely by coincidence (Klein, 1983), while others have suggested that its linkage with the MHC is not fortuitous, but could be the result of selective genetic pressure (Campbell, 1987).

Gazit and co-workers in 1980, provided evidence for the existence of a genetic system (HT system) specific to activated T-cells, in close linkage disequilibrium with the HLA system. Subsequently, van Leeuwen and co-workers

TABLE 1.1 Complete listing of recognized HLA specificities (1987)[a].

HLA-A	B	C	D	DR	DQ	DP
A1	B5	Cw1	Dw1	DR1	DQw1	DPw1
A2	B7	Cw2	Dw2	DR2	DQw2	DPw2
A3	B8	Cw3	Dw3	DR3	DQw3	DPw3
A9	B12	Cw4	Dw4	DR4	DQw4	DPw4
A10	B13	Cw5	Dw5	DR5	DQw5(w1)	DPw5
A11	B14	Cw6	Dw6	DRw6	DQw6(w1)	DPw6
Aw19	B15	Cw7	Dw7	DR7	DQw7(w3)	
A23(9)	B16	Cw8	Dw8	DRw8	DQw8(w3)	
A24(9)	B17	Cw9(w3)	Dw9	DR9	DQw9(w3)	
A25(10)	B18	Cw10(w3)	Dw10	DRw10		
A26(10)	B21	Cw11	Dw11(w7)	DRw11(5)		
A28	Bw22		Dw12	DRw12(5)		
A29(w19)	B27		Dw13	DRw13(w6)		
A30(w19)	B35		Dw14	DRw14(w6)		
A31(w19)	B37		Dw15	DRw15(2)		
A32(w19)	B38(16)		Dw16	DRw16(2)		
Aw33(w19)	B39(16)		Dw17(w7)	DRw17(3)		
Aw34(10)	B40		Dw18(w6)	DRw18(3)		
Aw36	Bw41		Dw19(w6)			
Aw43	Bw42		Dw20	DRw52		
Aw66(10)	B44(12)		Dw21			
Aw68(28)	B45(12)		Dw22	DRw53		
Aw69(28)	Bw46		Dw23			
Aw74(w19)	Bw47		Dw24			
	Bw48		Dw25			
	B49(21)		Dw26			
	Bw50(21)					
	B51(5)					
	Bw52(5)					
	Bw53					
	Bw54(w22)					
	Bw55(w22)					
	Bw56(w22)					
	Bw57(17)					
	Bw58(17)					
	Bw59					
	Bw60(40)					
	Bw61(40)					
	Bw62(15)					
	Bw63(15)					
	Bw64(14)					
	Bw65(14)					
	Bw67					
	Bw70					
	Bw71(w70)					
	Bw72(w70)					
	Bw73					
	Bw75(15)					
	Bw76(15)					
	Bw77(15)					
	Bw4					
	Bw6					

a WHO Nomenclature Committee 1988

described another antigenic system designated the T-cell system A (TCA), on subsets of T-cells (van Leeuwen et al. 1980, 1982a). These class I-like products (HT and TCA1) (Gazit et al. 1980, 1984; van Leeuwen et al. 1984, 1985; Fauchet et al. 1986), are possibly the human equivalents of the murine Tla region products expressed on thymocytes and certain leukemia cells (Old et al. 1963) and murine Qa region products detected on subclasses of haemopoietic cells (Flaherty 1981). The function of the HT and TCA systems is still unknown.

1.2 PRESENT DAY CONCEPTS OF HLA AND NOMENCLATURE

The nomenclature has been one of the major problems for non-HLA workers, especially as there have been many changes during the fairly short history of the HLA system. At the Torino Workshop in 1967, the World Health Organization (WHO) Nomenclature Committee was appointed, and in 1968 they recommended the use of the term HL-A system for the set of closely linked genes coding for the leukocyte antigens. As a result of the increasing number of loci defined in the HL-A region, the WHO Nomenclature Committee which met in Aarhus, Denmark, in June 1975, after the Sixth Histocompatibility Workshop, recommended to drop the hyphen of HL-A and call it HLA in order to minimize the number of symbols required to define loci and specificities of this system. In the class I region there are four loci called

HLA-A, B, C and E and in the class II region there are several, named HLA-DN, DO, DP, DQ and DR. The WHO Nomenclature Committee decided to give single letter designations in sequence to class I region loci, excluding D, and prefixed by HLA, whereas the class II region loci are all prefixed with the letter D. The letters, N, O, P, Q and R are used to indicate a subregion of the D region. The officially recognized HLA specificities are designated by a number following the locus symbol e.g. HLA-A1, HLA-A2, HLA-B8 etc. Provisionally identified specificities which have not been accorded full antigen status carry the additional letter "w" inserted between the locus symbol and the specificity number e.g. HLA-Aw43, HLA-Bw42, etc. A list of the currently recognized HLA specificities is given in Table 1.1 (WHO Nomenclature Committee 1988). Numbers between brackets indicate broad "supertypic" specificities or cross reacting specificities. The numbers placed before the brackets indicate "splits" of an antigen, e.g. HLA-A23 and HLA-24 are splits of HLA-A9. Bw4 and Bw6 (previously 4a and 4b) are broad "supertypic" specificities, i.e. each HLA-B molecule either carries the Bw4 or the Bw6 determinant. In addition Bw4 is found on three HLA-A locus molecules (HLA-Aw23, Aw24 and HLA-A32) and Bw6 on HLA-Cw3 (Kostyu et al. 1980, Muller et al. 1982). The diallelism of Bw4 and Bw6 results from their being formed by alternate combinations of amino acid residues in the helical region of the A1 domain (Parham 1988). HLA-DRw52 (previously MT2) and DRw53

(previously MT3) (Park et al. 1980) are members of the HLA-DR subset and are seen in association with different HLA-DR antigens. For example, HLA-DRw52 is usually associated with DR3, DR5, DRw6, DRw11(5), DRw12(5), DRw13(6), DRw14(6), DRw17(3) and DRw18(3), while DRw53 is usually associated with DR4, DR7 and DR9.

The official names for the genes in the HLA region, first described during the Tenth International Histocompatibility Workshop, are given in Figure 1.3. The letters A and B following the letters for the subregions denote the alpha or beta chain genes (or related sequences in the case of pseudogenes). A number is used when there is more than one alpha or beta chain gene (or pseudogene) in a subregion.

1.3 INHERITANCE OF HLA ANTIGENS

The HLA alleles are inherited as codominant traits. A set of HLA genes inherited from one parent is called a haplotype (Ceppellini et al. 1967). Usually, the HLA haplotypes are passed on to the offspring unaltered, but occasionally during meiotic division exchange of chromosomal segments occurs ("crossing-over"), resulting in recombination between the HLA genes. Crossing-over is more likely to occur between genes which are far apart; conversely, the closer two genes are on any particular chromosome the less likelihood there is that crossing-over will occur. Thus the

recombination frequency can be used as a measure of the distance between two genes on a chromosome and is usually expressed as centiMorgans(cM)(1% recombination frequency = 1cM). The map distances have been estimated for HLA-A,B: 0.8 cM (Belvedere et al. 1975, Bijnen et al. 1976); HLA-A,C: 0.7 cM (Staub Nielsen et al. 1975); HLA-B,DR/D: 1 cM (Keuning et al. 1975, Netzel et al. 1975, Thorsby et al. 1975); HLA-DR,DP: 1-3 cM (Shaw et al. 1981, Termijtelen et al. 1982). A more accurate distance between the HLA loci has been established using more modern techniques such as cosmid cloning and pulse-field gradient gel electrophoresis The minimal size of the entire HLA complex has now been established as 2.5 megabase (mb) and the maximum size of the class II region as 1.5 mb (Hardy et al. 1986, Ragoussis et al. 1986).

Figure 1.4 shows the segregation pattern of HLA haplotypes in a family. The father's one haplotype (designated *a*) carries HLA-A1,Cw7,B8,DRw17,DQw2,DPw1 and the other (designated *b*), carries HLA-A2,Cw3,B7,DRw15,DQw6,DPw2. Similarly, one of the mother's haplotypes (designated *c*), carries HLA-A1,Cw6,Bw41,DR1,DQw6,DPw4 and the other (designated *d*), carries HLA-Aw36,Cw2,Bw42,DRw8,DQw4,DPw3. Each child inherits only one haplotype from each parent, i.e. haplotype *a* or *b* from the father and *c* or *d* from the mother. The figure shows all possible combinations of HLA-antigens in the children including an example of a

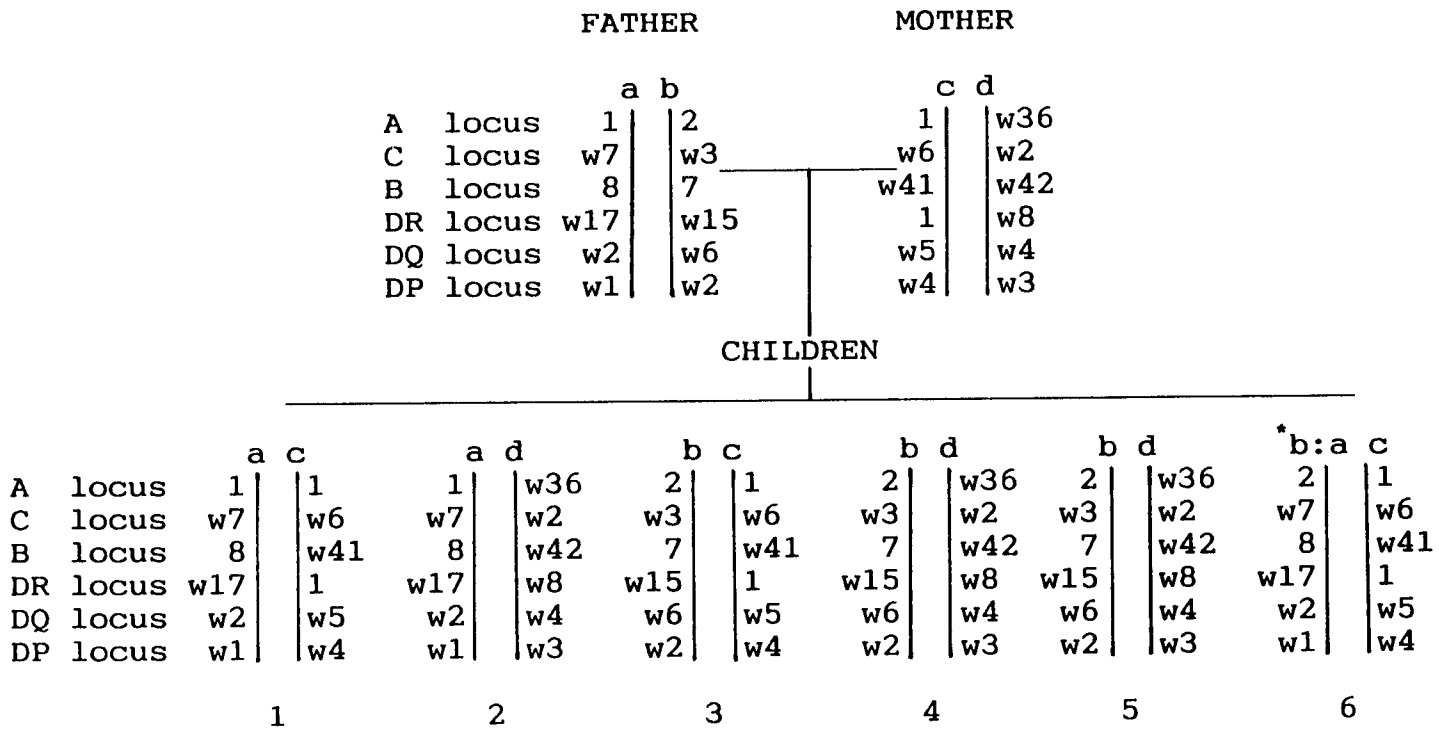

FIGURE 1.4 The segregation of HLA-A, B, C, DR, DQ and DP antigens in a family. By convention the father's haplotypes are designated *a* and *b*, whereas the mother's haplotypes are designated *c* and *d*.

* Recombination between HLA-A2 of the father's b haplotype and HLA-Cw7 of his a haplotype.

recombinant haplotype. In this example, siblings 4 and 5 have inherited the same parental haplotypes, *b* and *d*, thus they are HLA identical. Siblings 1 and 2 differ by one haplotype *c* and *d* and are called haplo-identical. In contrast, the second child (*ad*) has no haplotype in common with the third (*bc*), they are thus HLA non-identical. Siblings 3 and 6 have inherited the same maternal haplotype *c*, but a cross-over has occurred between the HLA-A and HLA-C loci of the father's *a* and *b* haplotypes (see child 6). Thus children 3 and 6 only inherited the same maternal *c* haplotype and the paternal HLA-A locus antigen A2. However, child 6, with the recombinant paternal haplotype (*b:a*), is HLA-C, B DR identical to child 1, differing only at the A locus.

As the HLA system is a very polymorphic system it is a powerful tool in determination of paternity. As shown above, every child must always inherit one haplotype from each parent. This means, for example, that if a child has a haplotype not present in the putative father, the putative father is excluded from being the biological father of the child.

Some combinations of HLA genes tend to occur more frequently together on the same haplotype than would be expected if the genes were randomly distributed in the population. This phenomenon is called gametic association or linkage

disequilibrium. For instance, in SA Negroes HLA-A30 and Bw42 have gene frequencies of 0.182 and 0.113 respectively. The alleles for both antigens would be expected to occur together on one haplotype with a frequency of about 0.182 x 0.113 = 0.021. However, the observed haplotype frequency is 0.058. The difference between the observed haplotype frequency and the expected frequency is a measure of the extent of linkage disequilibrium between the alleles and is expressed as a delta value ($\triangle$). In this example $\triangle$ = 0.058 - 0.021 = 0.037. The explanation for the persistent linkage disequilibrium observed for some pairs of alleles at the HLA loci can best be explained by the action of natural selection (Bodmer and Bodmer 1978). The linkage disequilibrium between alleles of the class II region in South African populations will be discussed in Chapter 3 and 5.

1.4 OTHER MARKERS IN THE HLA REGION

Several components of the serum complement system are coded for by genes in the class III region of HLA. In 1974, Fu and co-workers demonstrated that the locus coding for C2 deficiency, and thus probably also the gene for C2, was closely linked to HLA. At present, three alleles of C2 have been defined. Studies on the frequency of C2 alleles have shown that C2C is the most common variant in Caucasoids and that C2A and C2B are rare variants (Alper 1976). C2C is

also the most common variant found in the SA Negroes and Cape Coloureds (du Toit, unpublished observations).

Allen in 1974 showed that the polymorphism of properdin factor B (BF) was linked to HLA. The BF polymorphism comprises two common alleles (F and S), two less common alleles (F1 and S0.7), and several other rare alleles (Mauff et al. 1978). Molecular mapping suggests a single BF locus, closely linked with the C2 locus and centromeric to the C4 loci (Carrol et al. 1984).

The human Chido and Rodgers "blood group antigens" have been shown to be closely linked to HLA (Middleton et al. 1974, Giles et al. 1976). It is now known that the C4A gene products carry the Rodgers antigenic determinants whereas the C4B gene products carry the Chido determinants (O'Neill et al. 1978b). The human C4A and C4B polymorphism appears to be far more extensive than that of C2 and BF (Rittner and Mauff 1984). In Caucasoids there are four C4A alleles and three C4B alleles that occur at frequencies greater than 0.01, and there are at least 25 to 30 rare variants (Mauff et al. 1983). O'Neill and co-workers found that half-null C4 haplotypes, i.e. either C4A or C4B not expressed, were common in Caucasoids (O'Neill et al. 1978a). Soon afterwards, duplicated C4 genes were recognized and found to be rather common (Nordhagen et al. 1981, Bruun-Petersen et al. 1982, Raum et al. 1984). Two such duplications

*C4A*3,C4A*2* and *C4B*2,C4B*1*, are common in Caucasoids and are part of the extended haplotypes HLA-B35,FC(3,2)O,DR1,DQw1 and HLA-B14,SC2(1,2),DR1, DQw1 respectively, while in the SA Negroes the duplication *C4B*2,C4B*92* is common and is part of the extended haplotype HLA-Bw58,SC3(2,92),DRw12x6,DQw1 (Rittner and Mauff, 1984).

Close linkage between the locus for a gene leading to deficiency of the enzyme 21-hydroxylase and HLA-B was discovered by Dupont et al. (1977). Subsequently, two loci for 21-hydroxylase deficiency have been mapped in the HLA region (Carrol et al. 1985, White et al. 1985, 1986).

The two closely-linked tumour necrosis factor alpha and beta genes (TNFα and TNFβ) have also been shown to be included in the human MHC (Spies et al. 1986). The TNF genes were located 200 kb centromeric of HLA-B and about 350 kb telomeric of the class III cluster (Carrol et al. 1987). The cytokines produced by these genes appear to be capable of altering host physiology in a variety of ways and appear to function as proinflammatory mediators. They have a direct cytotoxic effect on some tumour cells; induce fever, sleep, and synthesis of lymphokines, collagen, and collagenases; activate endothelial cells and macrophages; mediate inflammation, catabolic processes, and septic shock (Dinarello and Mier 1987, Ruddle 1987).

A "disease susceptibility gene" for haemochromatosis is also said to be located in the HLA region, probably between HLA-A and HLA-B (Lamm and Olaisen 1985).

The polymorphic marker, red cell glyoxylase I (GLO) was shown to be linked to HLA (Lewis et al. 1976, Weitkamp and Guttormsen 1976, Meera Khan et al. 1976). The gene coding for GLO is mapped, however, outside the HLA region, between HLA-DP and the centromere, and may be a useful marker when studying extended haplotypes.

1.5 TISSUE DISTRIBUTION OF HLA ANTIGENS

Class I HLA antigens are human cell surface antigens which are found on almost all cells. They are not detectable on neurons (Lampson et al. 1983) or mature trophoblasts (Faulk et al. 1977), and may sometimes be detected on erythrocytes due to solubilised antigens from other tissue (Amos and Ward 1975). Class II HLA antigens are more restricted in their tissue distribution than class I antigens. They are cell surface molecules, expressed on B-lymphocytes, activated T-lymphocytes, macrophages, dendritic cells, vascular endothelial cells, thymic epithelium, enterocytes, renal tubular cells and Langerhans cells (Engleman et al. 1980, Ferrone et al. 1978, Daar et al. 1984, Hirschberg et al. 1980, Bhan et al. 1980, Broathen 1981).

1.6 STRUCTURE AND FUNCTION OF HLA ANTIGENS

The HLA antigens belong to a large family of proteins involved in immune functions, and are composed of domains homologous to those of immunoglobulin (Ig) variable and constant regions. The genes coding for these proteins form the immunoglobulin supergene family, which includes Thy-1, CD1, CD4, CD8, poly Ig receptor, the alpha, beta ,gamma and delta chains of the T-cell receptors, and β_2 microglobulin (Williams and Gagnon 1982, Chien et al. 1984, Saito et al. 1984a,b, Hedrick et al. 1984, Littman et al. 1985, Sukhatme et al. 1985, Maddon et al. 1985, Calabi and Milstein 1986, Hata et al. 1987).

The structure of class I and class II antigens is shown in Figure 1.5. The class I molecules are heterodimeric complexes consisting of a heavy (alpha) chain non-covalently bound to a light chain (β_2 microglobulin). The alpha chain of about 44 kiloDalton (kDa) represents the class I gene product and is glycosylated. The β_2 microglobulin chain is encoded on chromosome 15 and has a molecular weight of about 12 kDa (Goodfellow et al. 1975). HLA class I molecules are involved in the recognition and interaction of cytotoxic T-lymphocytes with their target cells. Zinkernagel and Doherty were the first, in a mouse model, to show that cytotoxic T-cells were able to lyse virus infected target

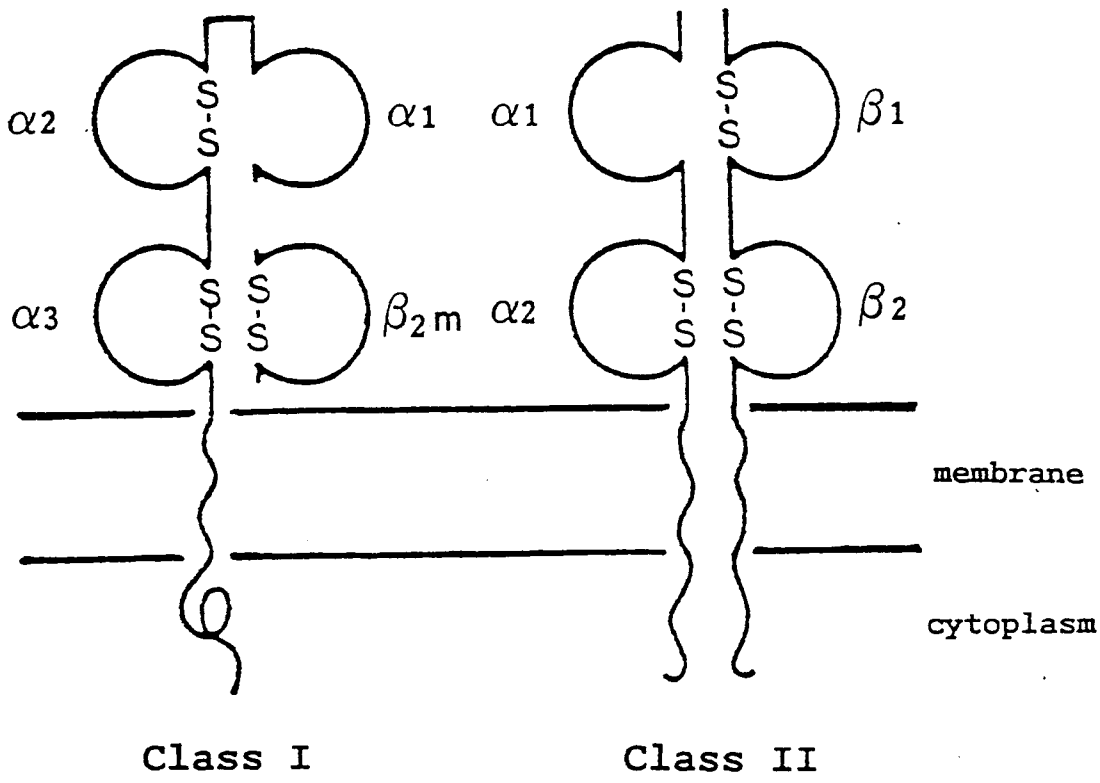

FIGURE 1.5 Schematic representation of MHC class I and class II molecules on the cell surface. S-S indicates disulphide bridges. $\alpha 1$, $\alpha 2$, $\alpha 3$, $\beta 1$ and $\beta 2$ indicate domains corresponding to the exon of the genes and $\beta_2 m$ indicates the β_2-microglobulin chain.

cells only if the foreign antigen on the target cell was presented with self MHC class I antigens (Zinkernagel and Doherty 1974). This phenomenon is called MHC restriction of T-cell activation. The involvement of the class I molecule in T-cell recognition has been shown in several studies, using cytotoxic lymphocytes from donors that have been sensitized against minor histocompatibility antigens, (Goulmy et al. 1977), viral antigens (McMichael 1978, Kreth et al. 1979, Sethi et al. 1980) and haptens (Dickmeiss et al. 1977). A response can also be mounted against the class I antigens of a MHC non-identical individual (allogeneic response). The recognition of a foreign HLA antigen by alloreactive cytotoxic T-cells is thought to be a cross-reaction occurring because the structure of a self MHC molecule plus antigen (self + X) resembles a non-self (allo) MHC molecule (Burakoff et al. 1978). These alloreactions underlie such diverse phenomena as rejection of allografts, graft-versus-host disease in recipients of bone marrow transplants and proliferation of T cells in MLCs. These two properties of T-cell recognition, MHC restriction and alloreactivity, emphasize the bias of the T-cell repertoire towards MHC.

The class II antigens are composed of two different polypeptide chains, a heavy (alpha) chain of about 34 kDa and a light (beta) chain of about 29 kDa (Figure 1.5). These polypeptide chains are both glycosylated and associate non-

covalently to form the class II antigens. Both the alpha and the beta chain genes map within the HLA region on chromosome 6. Although class II antigens are expressed at the cell surface as heterodimers of alpha and beta chains, intracellularly they are found in association with a transmembrane glycoprotein of 31 kDa. This molecule is known as the gamma chain or the invariant chain as a result of its lack of electrophoretic polymorphism (Jones et al. 1978, Charron and McDevitt 1979, Kvist et al. 1982). The function of the invariant chain is unclear but it may facilitate assembly and/or transport of the class II alpha and beta chains to the cell surface (Cresswell 1987).

The class II molecules also function as restriction elements for regulatory T-cells. The regulatory T-cells have the capacity to help (T-helper cell) a cellular or humoral immune response against foreign antigens (Nagy et al. 1981). Processed antigens which are presented on the surface of a class II bearing accessory cell i.e. dendritic cell, macrophage or B-cell (Grey and Chesnut 1985) can be recognized by the regulatory T cells only in association with self MHC class II antigens. The HLA-DP, DQ and DR antigens can all serve as MHC restriction elements for antigen presentation (Bergholtz et al. 1980, Berle and Thorsby 1982, Eckels et al. 1983, Qvigstad et al. 1984, Yasukawa and Zarling 1984, Al-Tawil et al. 1985).

1.7 THE CLINICAL RELEVANCE OF THE HLA SYSTEM

Organ transplantation has been the main reason for the intensity of the research leading to the rapid unraveling of the HLA system. Transplantation studies involving kidney (van Rood 1967) and skin (Amos et al. 1969, Ceppellini et al. 1969) have shown that grafts between HLA identical siblings survive much longer than grafts between HLA non-identical siblings. The exact significance of HLA compatibility in the survival of organs from unrelated donors is more difficult to evaluate, but many studies have shown that matching for HLA antigens improved the survival rates of cadaveric renal grafts (Morris et al. 1968, Patel et al. 1968, Batchelor and Joysey 1969, van Rood et al. 1969).

The discovery of H-2 linked immune response genes and of H-2 associated differences in the susceptibility to oncogenic viruses (Lilly et al. 1964) led to an increasing interest in similar associations with the MHC of man. A possible association between HLA and Hodgkin's disease was suggested by Amiel in 1967. More definite associations between HLA and disease however, were found in 1972, when Russell and co-workers reported the association of HLA-B17 with psoriasis vulgaris and Falchuk and co-workers reported the association of HLA-B8 and coeliac disease. The strongest associations observed are those between HLA-B27 and ankylosing

spondylitis (Brewerton et al. 1973, Schlosstein et al. 1973) and HLA-DR2 and narcolepsy (Juji et al. 1984, 1985; Langdon et al. 1984; Billiard and Seignalet 1985). In most cases, the associations between HLA and disease are strongest with class II antigens (reviewed by Svejgaard et al. 1983, Tiwari and Terasaki 1985). Several mechanisms explaining the associations between HLA antigens and diseases have been proposed but thus far none is supported by clear-cut evidence (reviewed by Tiwari and Terasaki 1985).

Knowledge of the HLA system can also be of use in the management of thrombocytopenic patients. In order to be kept free of bleeding these patients are transfused with random ABO-compatible platelets. However, when this has been done for some time, most patients eventually develop HLA antibodies, and as these are often multispecific, platelets from random donors are no longer effective because they are destroyed by the antibodies (Yankee et al. 1973). In this situation, it becomes necessary to HLA type the patient and to select platelet donors compatible for the HLA-A and B antigens.

1.8 POPULATION DISTRIBUTION OF HLA ANTIGENS

The Fifth International Histocompatibility Workshop held in 1972 was planned specifically to study the HLA antigen

frequencies in various populations of the world. Twenty-seven laboratories typed 54 different populations from many parts of the world with the same set of antisera (Bodmer et al. 1972). The results showed that the frequencies of HLA antigens varied considerably among different population groups, and that many of the HLA antigens which were well defined in Caucasoids were not so in other population groups. Due to the extreme polymorphism of the HLA system, the majority of the HLA antigens occur infrequently in any given population. HLA alleles can be divided into three main categories with respect to their population distribution:

1. Antigens with relatively high frequencies in all populations, i.e. A2, Cw7, DR2 and DQw1.
2. Antigens present in most but absent from one or more populations, for example, A1, A29, A32, B8, B18, Cw5, Cw8 are virtually absent from Orientals, and B39, Bw52 and Cw1 from Negroes.
3. Antigens which are more or less specific to one population, such as A25 in Caucasoids, Bw46 and Bw54 in Orientals and Aw43, Aw36 and Bw42 in Negroes.

Large differences can exist in HLA antigen frequencies between different geographical areas, even if the ethnic origin is the same. For example, the frequency of B8 in European Caucasoids varies from below 10% in parts of the Mediterranean to above 30% in Scotland (Ryder et al. 1978);

the frequency of HLA-DR4 varies from 22.8% in the North-West of France to 9.9% in the South (Cambon-Thomsen and Ohayon 1988). There are several possible explanations for the observed differences such as genetic drift, natural selection and founder effect.

There are also striking differences in the frequencies of HLA haplotypes among different populations. Whereas the haplotype HLA-A1,B8,Cw7,DRw17,DQw2,Dw3 is the commonest haplotype in Caucasoids, HLA-Aw30,Bw42,Cw2,DRw18,Dw'RSH' is the most common SA Negro haplotype. It has been shown that haplotype frequencies for different populations are even more diverse than the single allele frequencies (Bodmer and Bodmer 1978).

The study of the HLA system in different populations is important as it may provide valuable information for anthropological studies and for the understanding of population relationships (Dausset and Colombani 1972, Aizawa 1986). It is also important in the practical application of histocompatibility, such as paternity testing (du Toit 1988), the study of disease associations (reviewed by Tiwari and Terasaki 1985) and in organ transplantation (Opelz 1984). Furthermore population studies are important for the definition of new HLA antigens (Botha et al. 1972, Lawler and Klouda 1972, Sasazuki et al. 1977a, Layrisse et al. 1978, Oudshoorn et al. 1986, 1987b, 1988). Several examples

of new HLA antigens detected in the South African populations will be described in Chapters 3 and 4.

1.9 THE HLA-D REGION

1.9.1 HLA-Dw

Bain and co-workers observed that peripheral blood lymphocytes from unrelated donors when mixed and cultured together for several days, underwent blastoid transformation and mitosis (Bain et al. 1963, Hirschhorn et al. 1963). Hirschhorn et al. (1963) and Bain et al. (1964) demonstrated that no such transformation occurred in mixed lymphocyte cultures (MLC) from monozygotic twins, suggesting that the reactivity in the MLC was genetically determined and might be a reflection of the histocompatibility between two lymphocyte donors. The position of the genes responsible for eliciting MLC reactivity was thought to be in the same chromosomal region as the HLA-A and B loci. Amos and Bach (1968) and others (Sørensen and Kissmeyer-Nielsen 1969; Schellekens et al. 1970) showed that lymphocytes from HLA identical siblings hardly ever show stimulation in MLC. In 1971, Yunis et al. first proposed that the stimulation in MLC in man is mediated by a genetic determinant closely linked to the A and B loci, but distinct from them, and separable by genetic recombination.

In 1972, Eijsvoogel et al. assigned the incompatibility measured in the MLC assay to the HLA-D locus on chromosome 6. In later years it became clear, however, that not all reactivity in the MLC could be attributed to a single locus (Dupont et al. 1973a, Bach 1985, Sterkers et al. 1987). The exact identity of the determinants involved in the stimulation of lymphocytes in an MLC is still unclear, but evidence exists for the involvement of HLA-DR, DQ and DP (Bach 1985).

The MLC test was originally a bilateral one, in other words, cells from both donors reacted against each other if they recognized alloantigens on the other cells. Bach and Vognow in 1966 found a way to overcome this difficulty by prior treatment of the lymphocytes from one of the donors with mitomycin-C; these cells were unable to proliferate but could still stimulate the cells of the other donor. This was called a unilateral or one-way MLC. Sublethal irradiation as described by Kasakura and Lowenstein (1965) is another successful way of inactivating cells. The lymphocytes responsible for the recognition of allo-HLA antigens in the MLC are thymus-derived (T) lymphocytes (Schwarz 1967, Daguillard and Richter 1969). The stimulator cells are predominantly B-lymphocytes although monocytes are also said to be able to stimulate allogeneic lymphocytes (Rode and Gordon 1974).

Polymorphism of HLA-D (Table 1.1) can be detected in a one-way MLC by using irradiated stimulator lymphocytes or mitomycin-C treated lymphocytes which are homozygous for particular HLA-Dw specificities (Dupont et al. 1973b, Jørgensen et al. 1973, Mempel et al. 1973 and van den Tweel et al. 1973). These lymphocytes are usually obtained from the offspring of consanguineous matings. The children of parents who are first cousins have a one in sixteen chance (excluding cross-overs) of inheriting an identical HLA-haplotype from each parent, thus being homozygous for all HLA loci. Such homozygous typing cells (HTCs) are very useful as the integrity of the HLA-Dw type is assured. One of the first HTCs of consanguineous origin, HTC-COX, was identified in our laboratory and is still an important cell in the definition of HLA-Dw3 (du Toit 1978). Non-consanguineous HTCs can also be used but care has to be taken as cells may appear to be homozygous for serologically detected HLA-D region antigens and yet be heterozygous for HLA-Dw. In recent years, it has become clear that cloned populations of T-lymphocytes can be generated which recognize allospecificities similar or identical to those detected by primary or secondary MLC typing. It was therefore decided by the WHO Nomenclature Committee, which met in New York in November 1987, to extend the definition of HLA-Dw specificities to include T-cell defined determinants using either bulk or clonal cellular reagents including either cytotoxic or proliferative T cell clones.

For example the subgroups of the DRB3 gene, Dw24, Dw25 and Dw26, were identified in this manner (Table 1.1). In addition to the HLA-Dw specificities with WHO status there are a number of specificities without as yet "official recognition" (Table 1.2). As will be shown in section 3.1.2 and 3.1.3 we have identified two unique HLA-Dw specificities in the SA Negroes (Dw'BME' and Dw'RSH') as well as a HLA-Dw specificity (Dw'JOH') first defined in the Cape Coloureds (section 3.1.1).

1.9.2 HLA-DR

In 1973, van Leeuwen and co-workers presented evidence that HLA-D related gene products could be recognized by serology. They performed a series of MLC experiments, using as responder cells the lymphocytes from an individual who had produced an HLA antibody and lymphocytes from unrelated HLA-A and B identical donors as stimulator cells. In three of 10 cases tested, serum from the donors of the responder cells inhibited the MLC reaction, indicating that the antibody blocked the determinants responsible for stimulation in the MLC. Fluorescent antibody studies showed that the inhibiting serum recognized determinants largely present on B lymphocytes and monocytes (van Leeuwen et al. 1975) but absent on platelets. The serologically defined B-lymphocyte cell surface antigens detected in this way were designated HLA-DR, which stands for D-related. Thus far 18 HLA-DR antigens, DR1 to DRw18, have been defined (Table 1.1)

TABLE 1.2 HLA-Dw specificities without as yet official recognition by the WHO Nomenclature Committee.

HLA-Dw specificity	Associated DR antigen	References
WH	DR2	Hajek-Rosenmayer et al. 1988
DKT2	DR4	Sasazuki et al. 1980, Kaneko et al. 1980
SHA	DR4	Cohen et al. 1987
DB1	DR7	Dupont et al. 1980
Dw8.1[a]	DRw8	Mickelson et al. 1983
Dw8.2[a] (DB7)	DRw8	Mickelson et al. 1983, Ollier et al. 1984
Dw8.3[a]	DRw8	Mickelson et al. 1983
SHY	DRw10	Amar et al. 1984
DB6	DRw12	Dupont et al. 1972, Jakobsen et al. 1986
HAG	DRw13	Pawelec et al. 1984, Tilanus et al. 1987
RSH	DRw18	Oudshoorn et al. (in press), Chapter 3
BON	DR'Br'	Cambon-Thomsen et al. 1986
JOH	DRw12x6	Chapter 3
BME	DRw12x6	Chapter 3

a Subgroups of HLA-Dw8

(WHO Nomenclature Committee 1988). Although it is generally accepted that the HLA-DR molecules are the major antigens involved in the MLC (which defines HLA-Dw specificities), it is now known that other class II antigens such as HLA-DQ and to a lesser extent HLA-DP can also induce proliferation of T-cells (Bach 1985). The respective contribution of HLA-DR, DQ and DP gene products in the induction of allogeneic response in the primary MLC and, therefore in HLA-Dw typing, is still unclear and thus remains controversial (Jaraquemada et al. 1986, Bach and Reinsmoen 1986, Sterkers et al. 1987). This topic will be discussed briefly in Chapter 3.

The DR subregion contains genes for at least one alpha chain and three beta chains (Figure 1.3). The DR1 haplotype, for instance, contains only one expressed DRB gene, whereas most of the others contain two (Rollini et al. 1985, Spies et al. 1985, Tonnelle et al. 1985). The DRα chain is non-polymorphic whereas the DRβ chains are polymorphic. The DRB1 gene corresponds to the original serologically detected DR type (i.e. DR1, DR2 etc.) while the DRB3 gene corresponds to DRw52 and DRB4 to DRw53. The alpha chain (Lee et al. 1982, Lee and Trowsdale 1983) is always expressed while the number of expressed beta chains is uncertain and may vary between haplotypes (Böhme et al. 1985). The DRB2 gene has been shown to be a pseudogene (Larhammar et al. 1985).

1.9.3 HLA-DQ

Tosi et al. (1978) first recognized that one of the so called "DR supertypic specificities", DC1 (now DQw1), was a determinant present on a molecule distinct from the classical DR antigen. In 1979, Duquesnoy and co-workers found serological evidence for a new set of human class II antigens, which they called MB (Duquesnoy et al. 1979a, Duquesnoy and Marrari 1980). Several other studies showed similar results (Termijtelen et al. 1980a, Goyert et al. 1982). Biochemical studies showed that the alpha and beta chains of DC1 determinants were different to those of the molecules carrying DR determinants (Corte et al. 1981, Shackelford et al. 1981). Nine antigens, DQw1 to DQw9 have been identified (WHO Nomenclature Committee 1988), each of which is in strong linkage disequilibrium with DR antigens. The strong association between DR and DQ as described in the Caucasoid population do not always hold in other populations and will be discussed in Chapter 5.

The DQ region contains two pairs of alpha and beta genes (Auffray et al. 1983, Spielman et al. 1984, Okada et al. 1985)(Figure 1.3). The DQA1 and DQB1 genes are expressed, while there is no evidence, at present, for the expression of DQA2 and DQB2 (Korman et al. 1985). In contrast to HLA-DR, both the HLA-DQα and DQβ chains were shown to be polymorphic (Auffray et al. 1984, Schenning et al. 1984).

1.9.4 HLA-DP

The primed lymphocyte typing (PLT) technique is based on the finding that lymphocytes stimulated in vitro towards allogeneic cells respond with an accelerated proliferation when re-stimulated with the original priming cell. (Andersson and Häyry 1973, Bach et al. 1975, Fradelizi and Dausset 1975, Mawas et al. 1975, Sheehy et al. 1975, Svedmyr 1975, Zier and Bach 1975). Using this technique, a new HLA-linked segregant series, HLA-DP, was discovered (Mawas et al. 1978, Shaw et al. 1980, 1981, Termijtelen et al. 1980b). Five HLA-DP alleles, DPw1 to DPw5, (formerly known as SB1 to SB5) were identified by Shaw and co-workers in 1980, while a sixth allele, DPw6, was identified during the Ninth Workshop (Wank and Schendel 1984). Some of these alleles can be detected with serological reagents (van Leeuwen et al. 1982b). There is little linkage disequilibrium between alleles of the HLA-DP locus and HLA-DQ and DR locus due to a putative recombination hotspot between the HLA-DP and HLA-DQ locus (Bodmer 1984).

The HLA-DP subregion is composed of two alpha (DPA1 and DPA2) and two beta genes (DPB1 and DPB2) (Trowsdale et al. 1984, Servenius et al. 1984)(Figure 1.3). The DPA1 and DPB1 genes are expressed while DPA2 and DPB2 are pseudogenes (Gorski et al. 1984, Kappes et al. 1984, Servenius et al. 1984, Trowsdale et al. 1984). Polymorphism has been

detected in the DP αchain and DPβ chain (Gorski et al. 1984, Kappes et al. 1984, Ando et al. 1986).

1.9.5 HLA-DO and DN

Two genes, DOB and DNA have been mapped in the HLA-D region between DP and DQ (Tonnelle et al. 1985, Trowsdale and Kelly 1985)(Figure 1.3). It has been shown that it is unlikely that these two genes comprise an α/β pair (Tonnelle et al. 1985).

1.10 AIMS AND OUTLINE OF THE STUDY

The aim of this study was to investigate the complexity of the HLA-D region loci and its polymorphism in the SA Negroes and Cape Coloureds, and compare the results with those found in SA Caucasoids. The biological importance of the HLA-D region is apparent from observations on its influence on graft survival, its association with disease susceptibility and its involvement with the regulation of immune response. It is also important to establish reliable frequencies for the HLA class II antigens in the various populations as this data may then be used for comparison with patient groups in HLA and disease studies. New class II antigens and variants as well as atypical HLA-DR,DQ haplotypes were investigated using serological, Southern blot and HTC typing techniques. The HLA-DP antigens were not investigated as they as yet appear to be of lesser biological importance. Matching for

DP antigens has not been convincingly shown to influence the outcome of organ transplantation, although there is some evidence that matching for HLA-DP antigens may reduce acute graft-versus-host-disease after allogeneic bone marrow transplantation (Möller et al. 1984). In addition the HLA-DP antigens have as yet not been shown to be strongly associated with diseases and do not occur in linkage disequilibrium with other D-region antigens.

The results are given in chapters 3 to 5. Chapter 3 deals with a comparative analysis of the HLA-Dw frequencies in the SA Negroes, Cape Coloureds, and SA Caucasoids. New HLA-Dw specificities found in these groups are analyzed. The frequencies of the serologically determined class II antigens, HLA-DR and DQ, were established in three south African population groups and are analyzed in chapter 4. Also in this chapter are the results of further investigation, using a variety of techniques, of the the serologicallly defined HLA-DR variants identified in the SA Negroes and Cape Coloureds. In chapter 5 the frequency of the HLA-DR,DQ haplotypes in the various populations is given. Unusual HLA-DR,DQ haplotypes were studied as they may provide insight into the genetic complexity of the MHC and may be of help in defining the basis for recognition of HLA-Dw epitopes by T-cells.

CHAPTER 2

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 The populations studied

In the course of many decades in the history of South Africa, different ethnic groups settled as separate peoples in certain areas of the land. The respective groups retained their own identities and are still distinguishable in the present populations. There are now a number of distinct population groups living in South Africa: the Negroes, with their many linguistic and geographical subdivisions, the Coloureds, the Asian Indians and the Caucasoids. These separate population groups are distinguished from one another by their different languages, physical appearances and culture. Of these population groups, the three largest groups were selected for inclusion in this study - the Negroes, Coloureds and Caucasoids.

South African Caucasoids

The Caucasoid population of South Africa came from Western European stock. Their ancestral background is mainly Dutch from the first settlers in 1652, with a substantial German and to a lesser extent French addition until 1700. A major British influx occurred in the 19th century followed by a

numerically small influx of Ashkenazim Jews at the beginning of the 20th century. In addition, the SA Caucasoids have a further admixture of approximately 6-7% South East Asian and Southern African genes (Heese 1971, Botha 1972). Evidence of a Southern African indigenous component in the genetic constitution of the SA Caucasoids was also illustrated by the presence of Aw43 in this group (du Toit et al. 1988a). In spite of this admixture, it was shown that the SA Caucasoids were genetically closely related to the Caucasoids tested during the Ninth International Histocompatibility Workshop. This was evident from similar HLA gene and haplotype frequencies in these groups and a low genetic distance value of 0.002 between them (du Toit et al. 1988a). The number of SA Caucasoids at the 1985 Census was 4 576 690. The SA Caucasoids studied were predominantly staff members of the laboratory in Cape Town, the mother and putative father in disputed paternity cases, the healthy parents of all prospective renal and bone marrow transplant patients, and cadaver kidney donors.

Cape Coloureds

The Coloured people of the Cape form an anthropologically distinct population group whose relatively recent origin includes Southern African, Eastern and European elements (Botha 1972). The miscegenation between the Caucasoids and the Khoikhoi, and to a lesser extent between the Caucasoids and the San, at the Cape commenced almost as soon as the

first Caucasoid settlements appeared there (Jenkins et al. 1970, Botha 1972). There were no Negroes at the Cape until the latter part of the 17th century when, over a period of some years, many were imported as slaves from Madagascar, East Africa and from the Gold Coast. The South-East Asian genetic contribution was derived from Malay and Indian slaves introduced in the 17th century from old India (mainly Coromandel, Malabar and Bengal), Ceylon (Sri-Lanka) and from many islands of the Dutch East Indies (present day Indonesia) (Botha et al. 1975). From investigations into the blood group gene frequencies by Botha in 1972, it is conceivable that the Cape Coloured group possesses approximately 34% Western European, 36% Southern African and 30% Asian genes. The 1985 Census figure for the Cape Coloured population is 2 825 094. The Cape Coloureds studied included individuals employed at the laboratory in Cape Town or at a nearby factory, the mother and putative father in disputed paternity cases, the healthy parents of all prospective renal and bone marrow transplant patients and cadaver kidney donors.

South African Negroes

The SA Negroes are believed to be descended from Negro people who lived in East Africa (Dart 1937). They appeared to have journeyed south, down the eastern side of Africa, across the Zambesi river and into South Africa either by way of the Rhodesian highlands or along the coastal plains of

Mozambique in a number of waves estimated between 500 and 1500 A.D. (Goodwin 1937). The names of the various chiefdoms such as Xhosa, Zulu, Sotho etc. are used to describe the subunits within the SA Negro people and all these belong to the South-Eastern linguistic division of Bantu (Jenkins 1972). Of the chiefdoms, the Xhosa were chosen to represent the SA Negroes in this study, as they represent the majority of Negroes living in the Cape area. They also constitute one of the largest Negro chiefdoms in South Africa accounting for approximately 26% of the SA Negroes (Jenkins 1972). Furthermore, preliminary studies have shown little variation in the HLA profiles among the SA Negro chiefdoms (du Toit, unpublished observations). The Xhosa are the southernmost of all of the Bantu-speaking chiefdoms and belong to the Cape Nguni linguistic division of Bantu. They are found mainly in the Ciskei and Transkei and of all the chiefdoms they are presumed to have had the greatest contact with the Khoikhoi and San. The Xhosa individuals tested included normal healthy volunteers employed at Groote Schuur Hospital, the mother and putative father in paternity cases, the healthy parents of all prospective renal and bone marrow transplant patients, cadaver kidney donors, parents of random families and random healthy individuals used for the various International Histocompatibility Workshops and other studies.

2.1.2 Homozygous typing cells (HTCs)

The majority of the HTCs in this laboratory have been found while testing families of patients in need of a live related donor for bone marrow or renal transplantation and in normal healthy families used for the various International Histocompatibility Workshops. The HTCs defined in this laboratory are listed in Table 2.1. The HTCs obtained by exchange with other laboratories are given in Table 2.2.

TABLE 2.1 HLA phenotypes of HTCs defined in this laboratory.

Cell identification	Workshop number[a]	Population Group	HLA					
			A	B	C	DR	DQ	Dw
JGA		C	1,2	37,w56	w1,w6	1	w5	1
ZSI		C	2	7	w7	2	w6	2
JDU		CC	2	7	w7	2	w6	2
COX	10W9022	C	1	8	w7	w17	w2	3
LAMP		C	1	8	w7	w17	w2	3
MITCH		C	1	8	w7	w17	w2	3
OTTO		C	1	8	w7	w17	w2	3
CDT		C	1,24	8	w7	w17	w2	3
BSMI		CC	2,28	7,w70	w3,w7	4	w8	4
MIS		CC	w43	14	w8	4	w8	4
RDA		CC	28,w33	14,44	w7,w8	w11	w7	5
CLIMD		X	2,3	7,w57	w4,w6	w14	w5	9
MST		AJ	3,26	38	w-[b]	4	w8	10
ARU		CC	2,28	14,w47	w6,w8	7	w9	11
RED		AJ	2	w63	w7	4	w8	13
PITOUT	10W9051	C	29	44	w-	7	w2	17
BNI		C	2	49,w57	w6	7	w2	17
OMW	10W9058	X	2	45	w-	w13	w6	18
NSI		X	28,w34	w70	w4	w13	w6	19
RSH	10W9021	Z	30,w68	w42	w2	w18	w4	RSH
BTI		X	30	w42	w2	w18	w4	RSH
PJOH		CC	2,24	35,w60	w4,w7	w12x6	w7	JOH
FJOH		CC	2,24	35,w60	w4,w7	w12x6	w7	JOH
BME		X	28,30	w58	w6	w12x6	w5	BME

a International Histocompatibility Workshop number

b A dash indicates an undetected HLA antigen

C = SA Caucasoid

CC = Cape Coloured

X = Xhosa

AJ = Ashkenazim Jew

Z = Zulu

TABLE 2.2 HLA phenotypes of HTCs obtained from other laboratories.

Cell identification	Workshop Number[a]	Origin	HLA					
			A	B	C	DR	DQ	Dw
WOE	9W0105	Grosse-Wilde	2,29	44	w5	1	w1	1
HEN	9W0101	Grosse-Wilde	3,25	18,35	w4	1	w1	1
EA	10W9081	Thorsby	3	7	nt	2	w1	2
HOF	9W0202	Grosse-Wilde	3,25	7,18	w7	2	w1	2
QBL	10W9020	van Rood	26	18	w5	w17	w2	3
AVL	9W0301	van Rood	1	8	w7	w17	w2	3
LOO81785	10W9018	Dawkins	3,24	18	w5	w17	w2	3
BSM	10W9032	van Rood	2	w62	w3	4	w3	4
GR		Bashir	2,29	44	w5	4	w3	4
ELL		Grosse-Wilde	1,3	8,15	w3	4	w3	4
DHi	9W0503	Festenstein	31	38	nt	w11	w7	5
HVB	9W0504	Bashir	1,32	35,w41	w4	w11	w7	5
ATH		van Rood	1	w60	w-[b]	w11	w7	5
MMR	9W0802	van Rood	3,24	7,18	w7	w8	w4	8
OLG	10W9071	Layrisse	31	w62	w1,w3	w8	w4	8
BAE	9W0807	Grosse-Wilde	2	51	w-	w8	w4	8
HAMBLEY		Bashir	2	44,w60	w3,w5	w8	w3	8
EK	10W9054	Thorsby	2	44	w5	w14	w1	9
CHN	10W9020	Betuel	1,3	8,w41	w-	w14	w1	9
GECL	9W0903	Betuel	28	44,w55	w7,w9	w14	w1	9
MAY		Brautbar	26	38	w-	4	w3	10
GRO	9W1102	Grosse-Wilde	2	w57	w6	7	w3	11
BGE	9W1201	Brautbar	1,2	w52	w-	2	w1	12
JHA	9W1301	Festenstein	31	51	w-	4	w7	13
PEA	9W1302	Grosse-Wilde	31	51	w-	4	w7	13
MT	9W1404	Bashir	31	w60	w3	4	w3	14
KY	9W1402	Bashir	31	w60	w3	4	w3	14
KT3	10W9107	Kashiwagi	24	w55	w1	4	w4	15
KT3b		Kashiwagi	24,26	w55	w1	4	w4	15
AMA	10W9064	Layrisse	2	w62	w9	w14	w7	16
CRI	9W1702	Layrisse	2,31	51,w62	w9	w14	w7	16
HMi	8W157	Festenstein	23	44	nt	7	w2	17
LM	8W303	Bashir	2,29	44	w-	7	w2	17
WDV	10W9062	van Rood	2	39	w-	w13	w1	18
HHK	10W9065	van Rood	3	7	w7	w13	w1	18
ADP		van Rood	1	w60	w-	w13	w1	18
HBS	9W0601	Betuel	2,28	27,39	w2	w13	w1	18
CB	10W9060	Bashir	1	w62	w9	w13	w1	18
KRA	9W0607	Grosse-Wilde	2	w60	w3	w13	w1	19
KT11	9W0610	Kashiwagi	w33	44	w-	w13	w1	19
SLE	10W9059	Hansen	2	w60	w3	w13	w1	19
KLE		Goldman	32	w62	nt	w13	w1	19
FJO		Betuel	2,3	7,39	w7	w16	w5	21
BAS	9W1801	Layrisse	2	51	w-	w16	w7	22
REM	9W1803	Layrisse	2	51	w-	w16	w7	22
KT14	10W9103	Kashiwagi	24,26	52,w62	w-	9	w3	23
C.WONG		Bashir	11	15,w60	w4	9	w3	23
HAG	9W1802	Wernet	2	w41	w7	w13	w7	HAG
HERLUF	9W1503	Svejgaard	2	35,44	w4,w5	w12	w7	DB6
FPA	10W9105	Festenstein	1	35	w4	w11	w1	DBL

a International Histocompatibility Workshop number

b A dash indicates an undetected HLA antigen

nt = not tested

2.2 METHODS

2.2.1 HLA Typing

In most cases, immune complexes formed by human histocompatibility antigens (HLA) and their specific antibodies activate the complement series. When HLA determinants are carried by the cell membrane of a living cell, this activation leads to the killing of the cell. The most common application of this phenomenon is the microlymphocytotoxicity technique (Terasaki and McClelland 1964) used for HLA typing. The microlymphocytotoxicity test was carried out with a purified suspension of blood lymphocytes, which was mixed with allo-antisera to HLA antigens, and complement. If the lymphocytes carried the appropriate antigens the antiserum would combine and in the presence of complement would damage the cell membrane. The resulting increased cell membrane permeability was detected by dye (trypan blue) uptake into the cell. The allo-antisera for the microlymphocytotoxicity test are mainly derived from donors who have become sensitized to HLA antigens due to pregnancy. As most of the available allo-antisera are not monospecific, the assignment of an antigen usually requires reactivity with several antisera containing that specificity. The HLA typing reactions are scored by estimating the percentage of cell death greater than that of the percentage cell death in the negative control wells (using human AB serum instead of antiserum).

Results are recorded according to the following scale:

Score	Interpretation	Percentage dead cells
1	negative	0-10
2	doubtful negative	11-20
4	weak positive	21-50
6	positive	51-75
8	strong positive	76-100
0	not readable or not done	

HLA-A, B and C typing

HLA-A,B and C typing was performed using a modification of the standard NIH microlymphocytotoxicity technique (Terasaki et al. 1974). The test requires 1 microlitre quantities of antisera and cell suspension ($3x10^6$ cells/ml), but both antisera and cell suspension were reduced to 0.5 microlitre quantities. It has been found that using this modification, valuable antisera can be saved without reducing the sensitivity of the test. Approximately 180 class I antisera were used and consisted of local antisera as well as those obtained by exchange with many other laboratories.

HLA-DR and DQ typing

HLA-DR and DQ typing was performed according to the Seventh International Histocompatibility Workshop technique (Bodmer et al. 1978) on B-cells enriched by the nylon wool method

(Danilovs et al. 1980). The test was also modified to use 0.5 microlitre quantities of serum and 0.5 microlitre B-cells ($3x10^6$/ml) instead of the usual 1 microlitre quantities. The approximately 100 antisera used to define the class II antigens were either of local origin or were obtained by exchange with other laboratories.

2.2.2 The mixed lymphocyte culture (MLC) test

Venous blood was collected and added to a 50 ml tube (Greiner, Nürtingen, West Germany) containing thromboliquine (25 IU/ml of blood)(Organon Tecknika, Boxtel, Holland). The amount of blood drawn was calculated assuming a lymphocyte yield of approximately $1x10^6$ per 1 ml of whole blood. When the blood had to be shipped to the laboratory no special precautions were undertaken except that the blood sample was always kept at room temperature and used within 24 hours after it was drawn. The blood was mixed with equal amounts of Eagle's minimum essential medium (MEM) (Gibco Ltd, Paisley, Scotland) and lymphocytes were isolated using the equilibrium density centrifugation method described by Böyum (1968). Eight millilitres of the diluted blood was carefully layered on 4 ml of a Ficoll-sodium iothalamate mixture in a 12 ml tissue culture tube (Sterilin Ltd, Middlesex, England). The separation fluid consisted of ten parts of 32,8% sodium iothalamate solution (Maybaker, Dagenham, England) mixed with twenty-four parts of 9% Ficoll in water (Pharmacia, Uppsala, Sweden), giving a final

density of 1.076 - 1.080. After the blood had been added to the tubes, the tubes were centrifuged at 400xg at the interface, for 20 minutes. The layer of lymphocytes at the interface was removed using a sterile Pasteur pipette and washed twice in Eagle's MEM (150xg for 10 minutes). After the second washing, the cell pellet was resuspended in a known volume of tissue culture medium (TCM) consisting of RPMI 1640 (Gibco Ltd, Paisley, Scotland) supplemented with 50 IU/ml penicillin, 50 µg/ml streptomycin (Gibco Ltd, Paisley, Scotland), 15% heat-inactivated (30 minutes at 56°C) pooled human AB serum and 25mM N-2 hydroxethylpiperazine-N^1-2-ethanesulphonic acid (HEPES) (Sigma, St. Louis, USA). When the cells could not be used directly, they were cryopreserved as described in section 2.2.4 and stored in liquid nitrogen until required. For the one-way MLC, the stimulator cells were either inactivated using X-irradiation (25 Gy from a ^{137}Cs source) or treated with mitomycin C (Kyowa Hakko Kogyo Co Ltd, Japan). Mitomycin C treatment was performed by adding 0.2 ml of mitomycin C (0.25 mg/ml) to 2.0 ml of cell suspension ($10x10^6$- $30x10^6$/per ml). The mixture was incubated at 37°C for 30 minutes in a waterbath. After incubation, the cells were resuspended and washed three times in RPMI 1640 with 5% heat-inactivated AB serum. After the third washing, the cell pellet was resuspended in a known volume of TCM. All HLA-Dw typing tests and checkerboard MLCs were performed using X-irradiated stimulator cells, while family MLCs were

done using mitomycin C treated stimulator cells. The MLCs were performed using a technique which was introduced by Hartzman et al. (1971). Fifty-thousand responder cells were cultured with $5x10^4$ stimulator cells in 0.15 ml of TCM in a 96 well, round-bottom microtitre tray (Nunc, Roskilde, Denmark) in triplicate. The plates were generally laid out as shown in Figure 2.1. For convenience, all stimulators (S1-S3) were placed in vertical columns and responders (R1-R3) were placed in horizontal rows. The identification of the experiment, tray number and date were indicated on the cover of the microtitre tray with a water-proof marker. Autologous combinations (i.e. responder and stimulator cells originating from the same donor) were always included as negative controls in the family MLCs. In addition, lymphocytes from three healthy, unrelated individuals were used as positive controls in the family MLC. All MLCs included a tissue culture medium control, i.e. responder cells in 0.15 ml TCM. The cultures were maintained in a humidified 5.5% CO_2 atmosphere for 5 days, then 55.5 kBq of tritiated thymidine (Amersham, England, TRA 120, specific activity 185 GBq/mM) was added to each well. Sixteen hours later the cultures were harvested with a micro-sample harvester (Skatron, Norway). Cells were collected on glass fibre filters (Flow Laboratories Ltd, Irvine, Scotland) which were then dried for 60 minutes at 120°C. After drying, the filters were transferred to scintillation vials to which 4 ml of scintillation cocktail {Packard Instrument

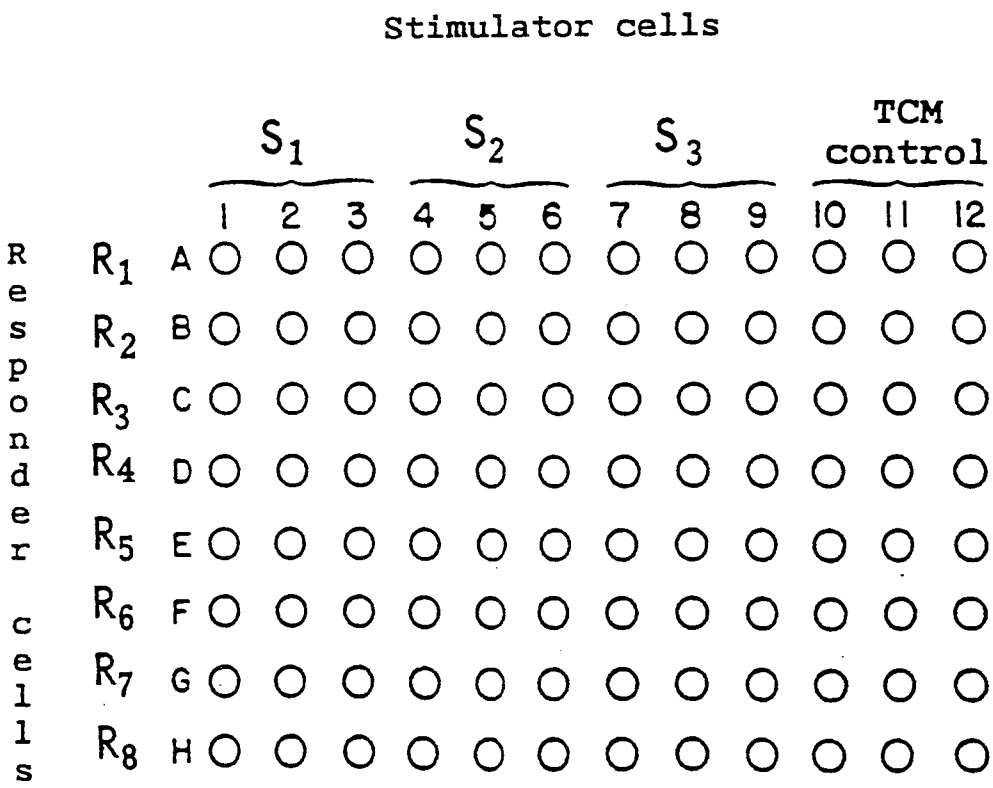

FIGURE 2.1 Schematic representation of a MLC tray, indicating that each experiment was done in triplicate. TCM = tissue culture medium; R1, R2, R3 etc. = responder cells 1, 2, 3 etc.; S1, S2 and S3 = stimulator cells 1, 2 and 3.

(pty) Ltd, Randburg, South Africa} was added. Each vial was counted for one minute in a beta counter {Packard Instrument (pty) Ltd, Randburg, South Africa}.

2.2.3 The HLA-Dw typing test

The lymphocytes used in an HLA-Dw typing test were obtained by the method described in section 2.2.2, cryopreserved and stored in liquid nitrogen prior to use (section 2.2.4). At the time of the test, the cells were thawed and washed according to the method described in section 2.2.4. The responder cells, which are the cells for which the HLA-Dw type has to be established, were resuspended at a concentration of $5x10^4$ lymphocytes per 1 ml of TCM. The stimulator cells were HTCs inactivated by X-irradiation (25 Gy from a ^{137}Cs source). The HTCs used in the HLA-Dw typing tests are listed in Tables 2.1 and 2.2. An MLC test was then set-up as described in section 2.2.2, using a range of 24 to 36 responder cells and 32 to 56 stimulator cells per test.

2.2.4 Cryopreservation of lymphocytes

The isolated lymphocytes were resuspended in RPMI 1640 with 20% heat-inactivated pooled human AB serum at a concentration of $10x10^6$- $20x10^6$ cells per ml. The cell suspension was then cooled to 4°C. A 20% (vol/vol) solution of dimethyl sulphoxide (DMSO)(Merck, Hohenbrunn, West Germany) in RPMI was prepared and cooled to 4°C. An equal

volume of the DMSO solution was added to the cell suspension on ice, mixed and aliquotted immediately into 2 ml cryotubes (Nunc, Roskilde, Denmark). The tubes were wrapped in paper towel and transferred directly to a -80°C freezer. The following day the tubes were transferred to a liquid nitrogen tank for storage. This method was preferred to controlled-rate freezing as it is simple and the average viable cell recovery (70-90%) was excellent.

When the cells were needed, the ampoules were rapidly thawed in a 37°C waterbath. As soon as there was only a small lump of ice left, the cell suspension was transferred to a 12 ml tissue culture tube and placed on ice. The cell suspension was diluted with 10 ml of a 4°C solution of Eagle's MEM, supplemented with 20% Dextrose (BDH Chemicals Ltd, Poole, England) and 10% heat-inactivated pooled human AB serum. This dilution was performed very slowly and continuously over 5 to 10 minutes to produce optimal cell survival (Greene et al. 1975). The cells were then washed twice in Eagle's MEM (10 minutes, 100xg) and resuspended in RPMI 1640. Finally the viability of the cells was assessed by the trypan blue dye-exclusion method (Fluka AG, Buchs, Switzerland).

2.2.5 Primed lymphocyte typing (PLT) cells

Two PLT cells were generated using HLA haplo-identical responder/stimulator cell combinations (section 3.1.1). Ten

million responder lymphocytes were co-cultured with $1x10^7$ X-irradiated (25 Gy) stimulator lymphocytes in 20 ml TCM. The cultures were incubated at 37°C in a humidified 5.5% CO_2 atmosphere. The primed cells were harvested after 10 days, washed twice with Eagle's MEM, frozen and stored in liquid nitrogen (as described in section 2.2.4) until required.

2.2.6 Restimulation of PLT cells

Ten thousand PLT cells were co-cultured with $1x10^5$ X-irradiated (25 Gy) stimulator cells in flat bottom microtitre trays (Flow Laboratories Ltd, Irvine, Scotland) in a total volume of 0.15 ml TCM. Cultures, set up in triplicate, were incubated at 37°C in a humidified 5.5% CO_2 atmosphere. After 48 hours the cultures were labelled with 55.5 kBq of tritiated thymidine (Amersham, England, TRA 120, specific activity 185 GBq/mM) and 16 hours later the cultures were harvested and counted as described for MLCs (section 2.2.2).

2.2.7 Production of human B-lymphoblastoid cell lines (B-LCLs) by Epstein-Barr virus (EBV) transformed lymphocytes

Virus preparation

A Marmoset mycoplasma-free B95.8 cell line (Miller and Lipman 1973) was grown to saturation in RPMI 1640 supplemented with 10% fetal calf serum (FCS)(State Health Laboratory, Cape Town, SA) and 1% penicillin-streptomycin (10,000 units penicillin, 10,000 mg streptomycin/ml)(Gibco

Ltd, Paisley, Scotland). The cells were removed by low speed centrifugation at 100xg for 10 minutes, and the virus-containing supernatant was passed through a 0.45 µm membrane filter (Millex-HA, Millipore Corp., Bedford, England) to ensure removal of viable cells. Filtered aliquots of EBV containing supernatant were stored at -70°C and used without further concentration or purification.

Transformation of lymphocytes by EBV

Either freshly obtained or previously frozen human lymphocytes were used. The lymphocytes were isolated as described in section 2.2.2. Five millilitres EBV supernatant was added to a pellet of approximately $1x10^7$ human lymphocytes. The cells were resuspended and incubated at 37°C in a 5.5% CO_2 incubator, with occasional mixing. After 1-2 hours the cells were spun down at 150xg for 5 minutes and the supernatant removed. The cells were resuspended in RPMI 1640, 10% FCS and 1% penicillin-streptomycin, counted and made up to a concentration of $1x10^6$ cells/ml. One millilitre of cell suspension and 1 ml of 1% phytohemagglutinin (PHA)(Wellcome Reagents Ltd, Beckenham, England) in RPMI 1640, 10% FCS and 1% Penicillin-Streptomycin were added to each well of a 24-well tissue culture tray (Flow Laboratories Inc, Mclean, USA). The PHA was added to prevent the T-cells from maturing into killer cells which can attack the B-LCL via the virally coded lymphocyte defined membrane antigen (Stinchcombe et al.

1985). The cell line was grown for three weeks, feeding as necessary, before transfer to a 25 cm^2 tissue culture flask (Greiner, Nürtingen, West Germany) for further culture. The cells were either stored frozen in liquid nitrogen as described for lymphocytes in section 2.2.4 or used directly.

2.2.8 Genomic HLA-typing by RFLP-analysis

The technique agreed upon for the Tenth International Histocompatibility Workshop was used for DNA preparation, electrophoresis and hybridization (Marcadet et al. 1988).

DNA isolation

Genomic DNA was extracted from peripheral blood lymphocytes or B-LCLs according to the method of Marcadet et al. (1988). Briefly, DNA was prepared from either 300-350x10^6 B-LCLs resuspended in 2 ml RPMI, or from 30 ml anticoagulated whole peripheral blood in 5% Na_2EDTA. Cells were mixed with 13 ml white cell lysis buffer (10 mM Tris-HCl pH 7.6, 10 mM Na_2EDTA pH 8.0, 50 mM NaCl, 0.2% sodium dodecyl sulphate (SDS)(Serva, Heidelberg, West Germany) and 200 ug/ml proteinase K). Lysates were vortexed briefly, and incubated at 42°C for 16-18 hours. Proteins were removed by 2-3 extractions with a 15 ml Tris-saturated mixture of 3 volumes phenol and 1 volume chloroform-isoamylalcohol (24/1), and finally 2 extractions with 15 ml chloroform-isoamylalcohol (24/1). DNA was precipitated from the aqueous layer by the addition of 300 ul 3M NaCl and 15 ml isopropanol. The

isolated DNA was rinsed 3 times with 4 ml 70% ethanol in water. The DNA was assayed spectrophotometrically once a day for three successive days to establish the correct concentration and to ensure there was no protein or RNA contamination. The DNA was stored at a final concentration of 200 ug/ml in TE (1 mM Tris-HCl pH 7.6, 0.1 mM NA_2EDTA pH 8.0) at -80°C prior to use.

Genomic DNA digestion with restriction enzymes

The following restriction enzymes were used to digest DNA: Taq I, Eco RI and Bam HI (Boehringer Mannheim, West Germany or Amersham, Buckinghamshire, England). The digestion was performed as directed by the manufacturers at a concentration of 5 units of enzyme/µg of DNA.

Gel electrophoresis

The restriction fragments were separated on a 0.6% and 0.9% agarose gel (Sigma, Type II, St Louis, USA) by electrophoresis. The 0.9% agarose gel was run for the resolution of small fragments, while the 0.6% gel was run for the resolution of the large fragments. On each gel, molecular weight markers (M1 and M2), as well as a migration marker consisting of linearized plasmid, PAT 153, were loaded. The molecular weight marker, M1, contained a mixture of single digests of phage Lambda DNA, digested with Hind III and Sma I, while M2 contained phage Lambda DNA digested with Kpn I and BstE II. The 0.6% agarose gel was

run for the first half an hour at 30V and for the remaining time (28 to 35 hours) at 40V, while the 0.9% agarose gel was run for 40 to 46 hours at 25V. The electrophoresis was stopped when the PAT 153 band reached 173 mm for the 0.6% agarose gel and 70 mm for the 0.9% agarose gel. After electrophoresis, the track corresponding to PAT 153 was removed, as plasmid contamination of the probes would otherwise strongly hybridize with PAT 153 and thus obscure the bands in the adjoining lane.

Transfer of DNA fragments to Biotrace membranes

The separated DNA fragments were depurinated by soaking the gels in 0.15 N HCl (BDH Ltd, Poole, England) for 10 minutes. The alkaline transfer method as described by Reed and Mann (1985) was used for transfer of DNA fragments to a Biotrace membrane (Gelman Sciences Inc., Ann Arbor, USA).

Hybridization with cDNA probes

The detection of the HLA class II genes was made possible by the use of the Tenth International Histocompatibility Workshop full length cDNA probes for: DRB, DQB, DQA and DPB. Probes were radiolabelled with [α-^{32}P] dCTP (Amersham, England, PB.10205, specific activity $\pm$ 110 TBq/mmol) according to the hexamer method of Feinburg and Vogelstein (1983, 1984). An average specific activity of 1.6-2.6x10^{9} cpm/μg DNA was obtained. Prehybridization of the membranes was carried out for 16 to 18 hours at 42°C in a mixture

containing 50% formamide (BDH, Poole, England), 5% dextran sulphate (Pharmacia, Uppsala, Sweden), 0.1% Denhardt's solution (Maniatis et al. 1982), 1% SDS and 5x SSPE (SSPE 30x: 4.5 M NaCl, 0.3 M NaH_2PO_4, 30 mM EDTA, pH 7.7)(reagents from BDH, Poole, England), and 200 ug/ml salmon sperm DNA (Sigma, St Louis, USA). Hybridization was done at 42°C for 48 hours in the same solution as for pre-hybridization except that 250 ug/ml salmon sperm DNA was used instead of 200 µg/ml. After the radiolabelled probe was added, an approximate concentration of $2.0x10^6$-$2.6x10^6$ cpm/ml of hybridization solution per experiment was obtained. After hybridization, the membranes were washed twice in 2x SSPE for 5 minutes each at room temperature, once in 0.5% SDS, 2x SSPE for 15 minutes at 65°C and once in 0.5x SSPE for 15 minutes at 65°C. The restriction endonuclease fragments were visualized by autoradiography with Kodak XAR.5 film (Kodak, Rochester NY, USA) in a cassette with two Dupont lightning-plus intensifying screens (Dupont, Boston MA, USA) at -70°C for 2-6 days.

2.2.9 Two-dimensional gel electrophoresis

The protocol and equipment designed for the Tenth International Histocompatibility Workshop were used for the isolation and 2-D gel electrophoresis of HLA class II antigens (Knowles and Kienzle 1988). In brief, B-LCLs were metabolically labelled with ^{35}S methionine (New England Nuclear, Boston MA, USA; NEG-009A, specific activity > 29.6

TBq/mmol) extracted with Nonidet P40 (NP-40)(Sigma, St Louis, USA) containing buffer and immunoprecipitated with three monoclonal antibodies (MoAbs): MoAb 7.3.19.1 (Bontrop et al. 1986b), MoAb TAL 1B5 (Adams et al. 1983) and MoAb L 243 (Shackelford et al. 1982). Isoelectric focussing (IEF) was performed using a 4:1 mixture of ampholytes (LKB, Bromma, Sweden) with pH ranges of 6.0-8.0 and 3.5-10.0 respectively. The second dimensional electrophoresis was performed in 12% SDS-polyacrylamide slab gels. Radiolabelled molecular weight and pI markers (Kendrick Laboratories, New York, USA) were included with each sample. The gels were dried and exposed to Kodak XAR.5 film for 28 days at -70°C

2.2.10 Typing for properdin factor B, C2, C4A, C4B and glyoxylase I

Properdin factor B (BF) allotypes were determined by high voltage electrophoresis and subsequent immunofixation (Alper et al. 1972). C2 typing was done by means of isoelectric focussing in polyacrylamide gels (Alper 1976) followed by haemolytic overlay using sheep erythrocytes sensitized with rabbit antibody and C2 deficient normal human serum. C4 typing was performed using neuraminidase treated serum according to the method described by Awdeh and Alper (1980). Glyoxylase I (GLO) was typed using the method of Harris and Hopkinson (1976) with minor modifications to the staining procedures.

The stable four gene unit comprised of alleles of BF, C2, C4A and C4B is called a complotype. An example of a complotype is FC30, whereby F stands for BFF, C for C2C, 3 for C4A*3 and 0 for C4B*Q0.

2.2.11 Data analysis

Expression of MLC reactivity in intra-family MLCs and checkerboard MLCs

The MLC results are expressed as percentage relative response (%RR) calculated as follows, using counts per minute (cpm):

$$\%RR = \frac{\text{median test cpm} - \text{median autologous control cpm}}{\text{reference cpm} - \text{median autologous control cpm}} \times 100$$

The reference cpm is the median cpm of three unrelated individuals.

Expression of MLC reactivity in HLA-Dw typing tests

The method of Ryder et al. (1975) was used to obtain stabilized relative response values (SRR) from the median of triplicate counts. SRR values of 50% or less were considered typing responses. An HLA-Dw specificity was assigned if more than half the HTCs defining a particular specificity gave typing responses. When only two HTCs were available for a certain specificity, this specificity was assigned only when both HTCs gave typing responses. In the case of only one HTC for a particular HLA-Dw specificity,

this specificity was assigned when a typing response was obtained in more than one experiment. All responder cells where none or only one HLA-Dw specificity could be assigned were retested.

Expression of the amount of restimulation by PLT cells

In order to determine the amount of restimulation of PLT cells, for each PLT/stimulator cell combination, the following formula was used:

$$\%RR = \frac{\text{median test cpm - median autologous control cpm}}{\text{reference cpm - median autologous control cpm}} \times 100$$

The reference cpm is the median cpm obtained using the original priming cell as stimulator cell. We defined the relative response of 60% or more as positive, 30-59% as doubtful and below 30% as negative.

Interpretation of Southern blot results

It is known that DRB and DQB probes can cross-hybridize with DQB and DRB genes, and to a lesser extent with DPB genes (Cohen et al. 1985). In most cases, a faint band detected by the DRB probe gave a relatively stronger signal with the DQB probe, and vice versa. By comparing the relative intensity of the signal observed it was in most instances possible to assign the fragments to DRB, DQB or DPB genes. In some cases, however, equal intensity was seen with more than one probe, thus in these cases it was impossible to assign these fragments to DRB, DQB or DPB genes. Only those

fragments which could clearly be assigned to a specific locus or to a haplotype by observing its segregation in a family will be discussed. The molecular weights of DNA fragments were estimated from a standard curve using the known molecular weight markers drawn on semi-logarithmic graph paper. The molecular weight in kilobases (kb) was set out on the Y-axis and the distance (cm) travelled by the DNA fragments on the X-axis. Thus by measuring the distance the unknown DNA fragments travelled, their molecular weight could be obtained directly from the standard curve.

Statistical methods

The gene frequencies (gf) were calculated by Bernstein's formula: $gf = 1 - \sqrt{1-af}$, where af is the antigen frequency. Gene frequencies in the different populations were compared by the chi-square test. The frequency of the "blank" gene(s) was obtained by subtracting the sum total of defined gene frequencies from one. Correlation coefficients (r) were calculated by the formula:

$r = \sqrt{X^2/N}$, where N is the total number of individuals and X^2 the chi-square. Delta values ($\triangle$) as an estimate of linkage disequilibrium and haplotype frequencies were computed from 2x2 tables of the phenotype frequencies using the formula of Mattiuz et al. (1970) and their significance evaluated by the chi-square test. Genetic distance values, based on differences in gene frequencies, was calculated by the method described by Cavalli-Sforza and Bodmer (1971).

CHAPTER 3

HLA-Dw DETERMINANTS IN SOUTH AFRICAN POPULATIONS

Histocompatibility differences among individuals can be defined on the basis of cellular as well as serological reactions. One of the most important cellular tests of histocompatibility is the MLC, which is considered an in vitro model for the recognition phase of allograft rejection. The stimulation in the MLC test is mainly determined by differences in the HLA-Dw determinants of the participating cells. For example, if two cells are identical for the HLA-Dw determinants, they should be mutually nonstimulatory. In fact, this concept forms the basis for cellular typing of the HLA-Dw determinants. When lymphocytes of an individual homozygous for a given HLA-Dw determinant (i.e. HTC) are treated with mitomycin-C or X-irradiated, these cells will not be able to stimulate lymphocytes of another individual whose cells carry the same Dw determinant, whether the individual is heterozygous or homozygous for the determinant. Thus a positive typing result with the HTC typing method is defined as the lack of response of the cell with "unknown" HLA-Dw specificity to stimulation by an HTC. Typing for HLA-Dw determinants using HTCs has, however, various drawbacks: (i) As described above, the assignment of an HLA-Dw specifity is based on the lack of reactivity towards the HTC. However, a nonresponse

may also be induced by specific tolerance or suppression (McMichael and Sasazuki 1977; Engleman et al. 1978a,b; Thomsen et al. 1976, 1977 and 1978). (ii) The threshold for a typing response in an HLA-Dw typing test is set at a level much higher than the response seen with HLA identical siblings, thus proliferative responses to relatively weak stimulating determinants are included in the typing response.

3.1 NEW HLA-Dw SPECIFICITIES IDENTIFIED IN SA NEGROES AND CAPE COLOUREDS

The best chance of finding HTCs is among the offspring of consanguineous marriages. For example, the likelihood of homozygosity in a first cousin marriage is 1 in 16 for each child. The HTCs of consanguineous marriages are very useful as they are "genetically pure" i.e. identical for all determinants involved in MLC reactivity. HTCs can also be found among offspring of non-consanguineous matings in which the parents possess a common HLA-Dw determinant. When a child inherits the haplotypes with this common HLA-Dw determinant from both parents it is then homozygous for that particular HLA-Dw determinant. Several HTCs belonging to the group of recognized HLA-Dw specificities have been detected this way in our laboratory and are listed in Table 2.1. In addition to these, three new HLA-Dw specificities, Dw'JOH', Dw'BME' and Dw'RSH', have been found. The identification and characterization of HTCs defining these new HLA-Dw specificities as well as their frequencies in the

SA Negroes, Cape Coloureds and SA Caucasoids will be discussed below.

3.1.1 HLA-Dw'JOH', a HLA-DRw12x6,DQw7 related HLA-Dw specificity identified in Cape Coloureds

Serological typing of family JOH

Figure 3.1 shows the segregation of the HLA antigens in family JOH. This family was typed with the Tenth International Histocompatibility Workshop sera. From the analyses it became clear that the haplotypes a, b and g of this family did not carry a conventional DR antigen but a new variant which we have named DRw12x6 (section 4.1.3, du Toit and Oudshoorn 1987, du Toit et al. 1988b).

Intra-family MLC

The pedigree of family JOH is shown in Figure 3.1. Individuals III-1 and III-4 inherited the (a) and (g) haplotypes resulting in homozygosity for the HLA-DRw12x6 and DQw7 antigens. In order to see if they were also homozygous for HLA-Dw, an intra-family MLC was done using cells from family members II-2, -3, -4, III-1, -2, -3 and -4 and three unrelated controls. From the results, shown in Table 3.1 it can be seen that cells from individuals III-1 (a/g) and III-4 (a/g) are also homozygous for HLA-Dw, as typing reactions were observed with all family members carrying either the (a) or (g) haplotype. Individual II-2 (b/e) is also typed by the HLA-Dw homozygous cells III-1 (PJOH) and III-4(FJOH), indicating that the haplotype (b) with DRw12x6 present in

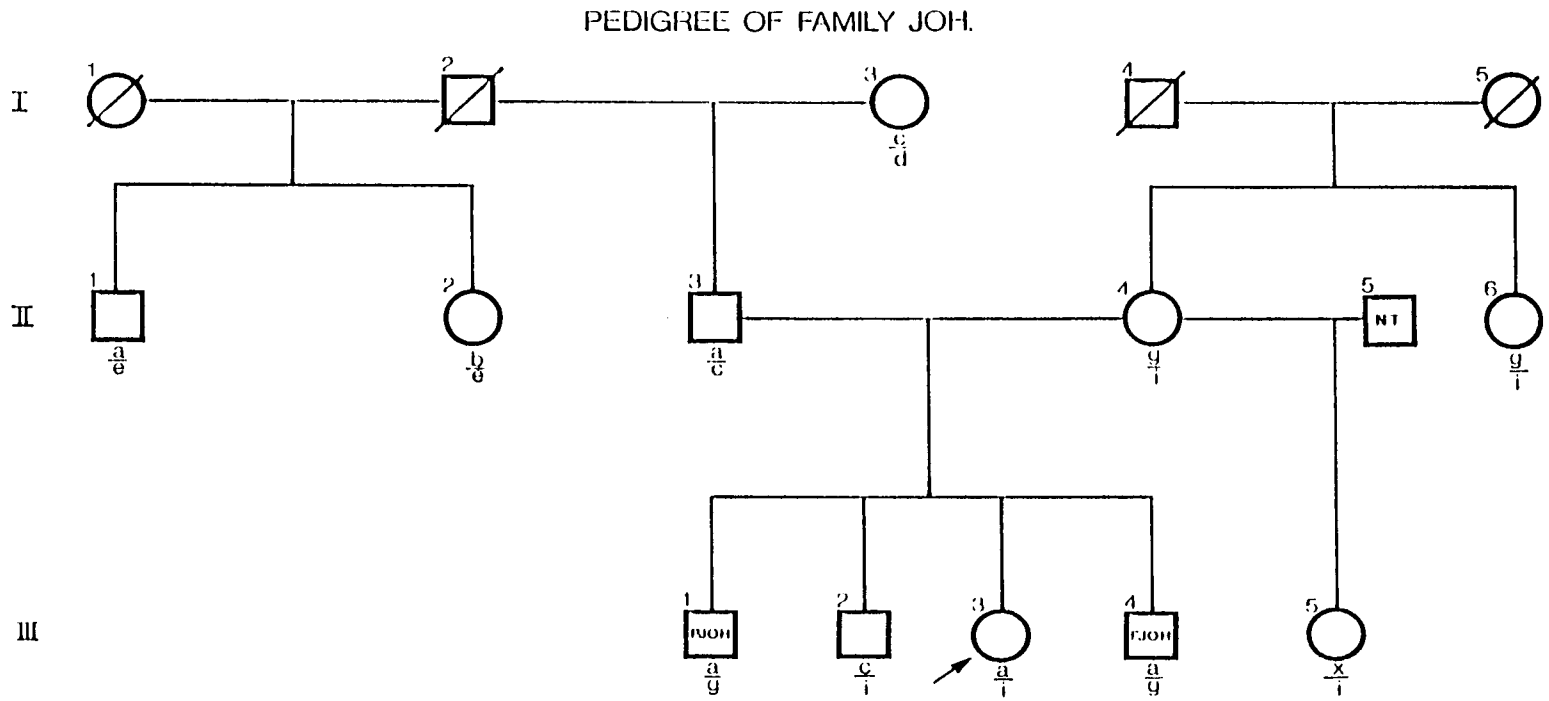

FIGURE 3.1 Pedigree of family JOH. The two individuals III-1 (PJOH) and III-4 (FJOH) were homozygous for the HLA class II region antigens and carried the haplotype DRw12x6,DQw7. Lymphocytes from these individuals were used as homozygous typing cells to define the new HLA-Dw specificity Dw'JOH'.

NT : Not tested
⊠ : Deceased
↗ : Propositus with juvenile chronic arthritis

a	A24,	Cw4,	B35,	DRw12x6,	DQw7, Dw'JOH'
b	A23,	Cw7,	B18,	DRw12x6,	DQw7, Dw'JOH'
c	A23,	Cw4,	Bw70,	DRw8,	DQw7, Dw'blank'
d	A24,	Cw'blank',	B35,	DRw11,	DQw7, Dw5
e	A23,	Cw'blank',	B7,	DRw14,	DQw5, Dw9
g	A2,	Cw7,	Bw60,	DRw12x6,	DQw7, Dw'JOH'
i	A11,	Cw'blank',	Bw62,	DR4,	DQw3, Dw13
x	A1,	Cw7,	B8,	DR3,	DQw2, Dw3

TABLE 3.1 Intra-family MLC of family JOH.

Responder cells		Stimulator cells									
Individuals	HLA haplotype	II-2 b/e	II-3 a/c	II-4 g/i	III-1 a/g	III-2 c/i	III-3 a/i	III-4 a/g	X-1[d]	X-2	X-3
II-2	b/e	1,444 -	93,782[a] 109[b]	63,908 74	26,381 30[c]	95,120 111	73,311 85	24,769 28[c]	74,877 87	99,815 116	85,886 100
II-3	a/c	83,752 86	788 -	100,497 104	19,645 20[c]	107,600 111	103,331 107	19,005 19[c]	97,062 100	118,292 122	76,687 79
II-4	g/i	69,997 62	74,661 66	1,137 -	24,739 21[c]	84,560 75	20,717 18[c]	16,814 14[c]	114,085 102	112,151 100	86,420 77
III-1	a/g	72,864 76	54,196 57	61,080 64	568 -	104,715 110	70,280 74	2,793 2.4[c]	95,233 100	83,852 88	117,274 123
III-2	c/i	85,331 84	87,734 87	58,305 57	97,603 96	907 -	65,851 65	89,143 88	75,427 74	101,217 100	115,938 115
III-3	a/i	68,936 70	81,371 83	26,052 26[c]	35,208 25[c]	74,987 76	1,023 -	30,487 20[c]	68,020 69	85,176 87	97,864 100
III-4	a/g	80,542 78	79,721 77	67,169 65	3,089 2.0[c]	104,854 102	88,844 86	1,083 -	104,854 102	81,975 79	103,052 100
X-1[d]		79,478 81	108,383 111	74,040 76	98,417 101	88,666 91	83,947 86	72,552 74	873 -	98,331 105	96,813 99
X-2		69,684 86	99,627 123	86,280 106	77,614 96	106,742 132	91,808 113	60,481 74	76,783 95	981 -	85,501 105
X-3		54,384 82	67,341 102	68,772 105	65,467 99	69,032 105	67,468 103	58,738 89	66,741 101	64,953 99	2,043 -

a median cpm

b relative response value (%RR)

c MLC reaction was considered negative if RR<30%

d X-1, X-2 and X-3 are unrelated individuals

solid lines indicate typing responses

interrupted lines indicate HLA-Dw identical combinations

individual II-2, carries the same HLA-Dw specificity as haplotypes (a) and (g). A second MLC test with cells II-1(a/e) and II-2(b/e), resulted in non-stimulation between these cells, thus confirming that the HLA-Dw specificity of haplotypes (a) and (b) are identical (results not shown).

HLA-Dw typing of family JOH

In order to define the HLA-Dw specificity associated with HLA-DRw12x6 in family JOH, a Dw typing test was performed using a panel of HTCs that recognize the specificities Dw1 to Dw19 as well as the new specificities: DKT2 (Sasazuki et al. 1980), Dw'DB6' (Dupont et al. 1972, Jakobsen et al. 1986), Dw'HAG' (Pawelec et al. 1984) and two new specificities first defined in this laboratory: Dw'BME' (section 3.1.2) and Dw'RSH' (section 3.1.3). Since the DRw12x6 specificity reacted with DRw12 as well as DRw6 containing sera, the results obtained using HTCs with DRw12, w12x6, w13 and w14 associated Dw specificities were selected and are shown in Table 3.2. For details regarding the HTCs see Tables 2.1 and 2.2. The HTCs, III-1 (PJOH) and III-4 (FJOH) derived from this family were the only HTCs which gave typing responses with haplotypes (a) (II-1, -3, III-1, -3 and -4), (b) (II-2) and (g) (II-4, -6, III-1 and -4), thus indicating the presence of the specificity Dw'JOH' on these haplotypes.

Figure 3.2 shows the SRR values obtained from Dw typing Dw'JOH' positive family members II-3(a/c), II-4(g/i), III-

TABLE 3.2 HLA-Dw typing results of JOH family using DRw12, DRw13, DRw14 and DRw12x6 related HTCs.

				Stimulators (HTCs)																											
Responder cells	HLA haplo-type	HLA-DR	HLA-Dw	Dw DB6		Dw BME		Dw JOH		Dw18						Dw HAG	Dw19						Dw9			Dw16	
				H E R L U F		B M E		P J O H	F J O H	H B S	H H K	J T E D	S C L	W D V	O M W	H A G	E M J	K R A	S L E	K T 1 1	S T E P H	N S I	C L I M D	G E C L	E K	A M A L I A	C R I
I-3	c	d	w8 ,w11 -,w5	-		-		-	-	0	-	0	0	-	-	-	0	-	-	-	0	-	-	-	-	-	0	
II-1	a	e	w12x6,w14 JOH,w9	-		-		+	+	0	-	0	0	-	-	-	0	-	-	-	0	-	+	+	+	-	0	
II-2	b	e	w12x6,w14 JOH,w9	-		-		+	+	0	-	0	0	-	-	-	0	-	-	-	0	-	+	+	+	-	0	
II-3	a	c	w12x6,w8 JOH,-	-		-		+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	
II-4	g	i	w12x6,4 JOH,w13	-		-		+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	
II-6	g	i	w12x6,4 JOH,w13	-		-		+	+	0	-	0	0	-	-	-	0	-	-	-	0	-	-	-	-	-	0	
III-1	a	g	w12x6,w12x6 JOH,JOH	-		-		+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	
III-2	c	i	w8 ,4 -,w13	-		-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
III-3	a	i	w12x6,4 JOH,w13	-		-		+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
III-4	a	g	w12x6,w12x6 JOH,JOH	-		-		+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	
III-5	x	i	3 ,4 3,w13	-		-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Typing score values: + ≤ 50%
- > 50%
0 = not tested

1(a/g), III-3(a/i) and III-4(a/g) with HTCs-Dw16, Dw18, Dw19, Dw'HAG', Dw'BME', Dw'DB6' and Dw'JOH'. The Dw'JOH' positive haplotypes did not show typing responses with HTCs for Dw16, Dw18, Dw19, Dw'HAG', Dw'DB6' or Dw'BME', but a closer look at the SRR values obtained with these HTCs, showed that the mean SRR (63.5 ± 14.7) for the group of HTCs with the Dw19 specificity was much lower than for any of the other typing cells, and approached the cut-off point of 50% used for the definition of a typing response. These results may indicate the sharing of lymphocyte defined (LD) epitopes between the Dw19 and Dw'JOH' defined specificities. The term LD is used for determinants recognized by T-lymphocytes (Bach 1973, 1985). However, the finding of borderline SRR values in the Dw typing of family members with the Dw'JOH' specificity using Dw19 HTCs could also be explained by the action of suppressor cells as originally described by McMichael and Sasazuki (1977).

Checkerboard MLC between DRw8, DRw11, DRw12, DRw12x6, DRw13 and DRw14 associated HLA-Dw specificities

Eighteen homozygous typing cells were tested in a checkerboard MLC (Table 3.3) against each other. As the new variant DRw12x6 was positive with DRw12 sera as well as some DRw6 containing sera, we included DRw12 and DRw6 associated HLA-Dw specificities in this test. Furthermore, on the basis of serological cross-reactions between DRw8, DRw11 and DRw6, we included the DRw8 and DRw11 related Dw specificities. HTCs with the DRw13 related Dw'HAG'

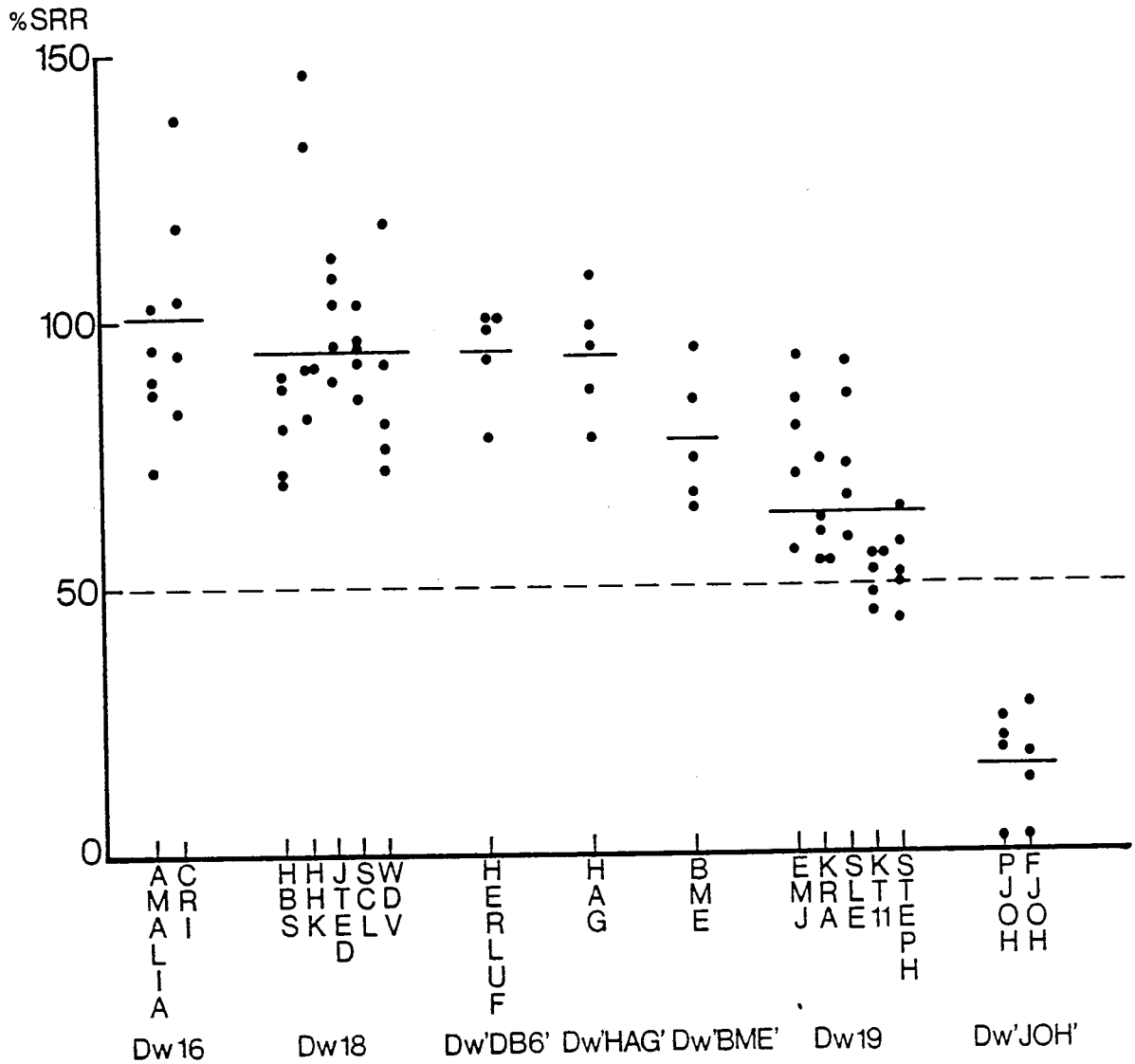

FIGURE 3.2 HLA-Dw typing results of Dw'JOH' positive family members, II-3,II-4, III-1,III-3 and III-4 who were tested with HTCs for the Dw16, Dw18, Dw19, Dw'BME', Dw'HAG', Dw'DB6' and Dw'JOH' specificities. The points indicate the %SRR obtained in the typing experiments. The interrupted line indicates the cut-off point (50% SRR) used for the determination of a typing response. The solid lines indicate mean SRR values obtained from typing Dw'JOH' positive family members with Dw identical HTCs. The mean SRR values with SD in brackets were: 100.6 (16.7) for the Dw16 specificity, 94.3 (18.5) for Dw18, 93.9 (8.4) for Dw'DB6', 93.4 (10.3) for Dw'HAG', 77.4 (12.5) for Dw'BME', 63.5 (14.7) for Dw19 and 16.4 (9.7) for Dw'JOH'.

TABLE 3.3 Checkerboard MLC results between DRw8, DRw11, DRw12, DRw12x6, DRw13 and DRw14 associated HLA-Dw specificities.

Responder HTCs		Stimulator HTCs																			
		JOH		Dw18				Dw19			Dw9			BME	DB6	Dw5		Dw8	
Cell identity	HLA-Dw specificity	PJOH	FJOH	OMW	HHK	WDV	HBS	NSI	KRA	KT11	CLIMD	EK	GECL	BME	HERLUF	ATH	RDA	OLG	BAE
PJOH	Dw'JOH'	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FJOH	Dw'JOH'	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OMW	Dw18	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
HHK	,,	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
WDV	,,	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-
HBS	,,	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
NSI	Dw19	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-
KRA	,,	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-
KT11	,,	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-
CLIMD	Dw9	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
EK	,,	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
GECL	,,	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
BME	Dw'BME'	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
HERLUF	Dw'DB6'	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
ATH	Dw5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
RDA	,,	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
OLG	Dw8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
BAE	,,	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+

Typing score values : + ≤ 50% SRR
- > 50% SRR

specificity and the DRw14 related specificity Dw16 were not included in this test due to insufficient supplies of these HTCs, but Dw typing results of family JOH showed that these HTCs did not type cells with the Dw'JOH' specificity. The results of this experiment clearly indicate that Dw'JOH' is a new specificity as no typing responses were observed with the relevant HTCs. The other HTCs behaved as expected with low reactions between HLA-Dw identical cells.

Frequency of Dw'JOH' in three South African populations

Three hundred and fifty-nine healthy random individuals from three population groups: SA Caucasoids (n=58), Cape Coloureds (n=73) and SA Negroes (n=228) were included in the HLA-Dw typing study. The Dw'JOH' specificity could be assigned to four of the 359 random individuals tested : one SA Caucasoid (af=0.02), two Cape Coloureds (af=0.03) and one SA Negro (af=0.004). The HLA-A, B, C, DR, DQ and Dw typings of these four Dw'JOH' positive cells are given in Table 3.4.

Restimulation of PLT cells by JOH family members and Dw'JOH' positive unrelated individuals

PLT reagents were generated using cells from individual III-2 (c/i) as responder cells and X-irradiated cells from individual II-4(g/i) and individual II-3(a/c) as stimulator cells. The PLT reagent directed against haplotype (a) is referred to as PLT-a, and that against haplotype (g) is referred to as PLT-g.

TABLE 3.4 HLA typing of Dw'JOH' positive cells obtained from random individuals.

No.	Population group	HLA typing
1	SA Caucasoid	A24,A29;B44,Bw62;Cw-,Cw-;DR7,DRw12x6;DQw2,DQw7;Dw7,Dw'JOH'
2	Cape Coloured	A2 ,A28;B7 ,B18 ;Cw7,Cw-;DR4,DRw12x6;DQw7,DQw-;Dw-,Dw'JOH'
3	Cape Coloured	A1 ,A24;B8 ,B35 ;Cw4,Cw7;DR3,DRw12x6;DQw2,DQw7,Dw3,Dw'JOH'
4	SA Negro	A3 ,A29;B8 ,B44 ;Cw7,Cw-;DR3,DRw12x6;DQw2,DQw7;Dw3,Dw'JOH'

In a PLT test with PLT-a and -g as responder cells and cells of family JOH and the four unrelated Dw'JOH' positive cells described above as stimulator cells, restimulation was observed with the random cells and cells from family members with the Dw'JOH' specificity (Table 3.5).

TABLE 3.5 Restimulation of PLT-a and -g by members of the JOH family and Dw'JOH' positive unrelated individuals.

	Stimulators			Responders			
				PLT-a		PLT-g	
Individual	Haplotype	HLA-DR	HLA-Dw	%RR	score	%RR	score
I-3	c/d	w8,5	-,w5	17	-[a]	9	-
II-1	a/e	w12x6,w14	JOH,w9	76	+[b]	83	-
II-2	b/e	w12x6,w14	JOH,w9	76	+	67	+
II-3	a/c	w12x6,w8	JOH,-	100	+	99	+
II-4	g/i	w12x6,4	JOH,w13	127	+	100	+
III-1	a/g	w12x6,w12x6	JOH,JOH	124	+	216	+
III-2	c/i	w8,4	-,w13	0	-	0	-
III-3	a/i	w12x6,4	JOH,w13	82	+	71	+
III-4	a/g	w12x6,w12x6	JOH,JOH	119	+	113	+
III-5	x/i	3,4	w3,w13	24	-	15	-
no.1		w12x6,7	JOH,w7	94	+	78	+
no.2		w12x6,4	JOH,-	104	+	69	+
no.3		w12x6,3	JOH,w3	71	+	82	+
no.4		w12x6,3	JOH,w3	62	+	73	+

a negative when RR value is $\leq$ 30%

b positive when RR value is $>$ 60%

Restimulation of PLT-a and -g cells by random individuals

PLT-a and -g were tested for proliferative response against X-irradiated stimulator cells of 50 random healthy SA

Caucasoids, 50 Cape Coloureds and 50 SA Negroes. There was a significant correlation between the responses of PLT-a and -g in each of the three population groups ($r=0.78$, $p<10^{-6}$) indicating that these PLTs recognize the same LD epitopes. Fourteen of the 150 cells tested were capable of restimulating PLT-a and -g (Table 3.6). The restimulation of the PLT reagents was predominantly (10 of 14) due to cells carrying DRw13,Dw19; DRw12x6,Dw'BME' and DRw12x6,Dw'JOH' haplotypes. These results suggest that LD epitopes are shared between the Dw19 and Dw'JOH' specificities, and also between Dw'BME', and Dw'JOH'.

TABLE 3.6 HLA-DR and Dw haplotypes of 14 random cells capable of re-stimulating PLT-a and -g.

Haplotype	Number of cells tested[a]	Number of cells restimulated by PLT-a and g
DRw12x6, Dw'JOH'	2	2
DRw12x6, Dw'BME'	5	4
DRw13, Dw19	5	4
DRw13, Dw6	6	1
DRw13, Dw'blank'	8	1
DR5, Dw'blank'	11	2
Total	37	14

a Only the haplotypes of the group of cells capable of re-stimulating the PLTs are given, the haplotypes of the remaining 113 cells are not shown.

The results did not provide information on the possible restimulation of PLT-a and -g by cells with the Dw16, Dw'HAG' or Dw'DB6' specificities as the 150 individuals used

were not typed for these specificities. In order to address this question, however, we subsequently tested the PLTs for proliferative responses against X-irradiated cells of HTC-HAG, Amalia(Dw16) and Herluf(DB6). None of these HTCs were capable of restimulating the PLT reagents.

In *summary*, the data presented in this study demonstrate a unique HLA-Dw specificity, Dw'JOH', related to DRw12x6,DQw7. This new HLA-Dw specificity had a low frequency in three of the SA populations studied i.e. SA Caucasoids, Cape Coloureds and SA Negroes. Furthermore, the study provided some evidence that the Dw'JOH' specificity may share one or more LD epitopes with the Dw19 and Dw'BME' specificities. The Dw'JOH' specificity was further investigated (section 4.1.3) in the hope that a combination of serological, cellular and molecular approaches would further elucidate the intricate relationships between the Dw'JOH', Dw'BME' and Dw19 specificities.

3.3.2 HLA-Dw'BME', a HLA-DRw12x6,DQw5 related HLA-Dw specificity identified in SA Negroes

The donor of the HTC-Dw'BME' is a member of a normal healthy SA Negro (Xhosa) family (ME). The segregation of the HLA antigens in this family is shown in Figure 3.3. No problems were encountered with the definition of the HLA antigens in this family except for the HLA-DR antigens on haplotypes (a)

PEDIGREE OF FAMILY ME

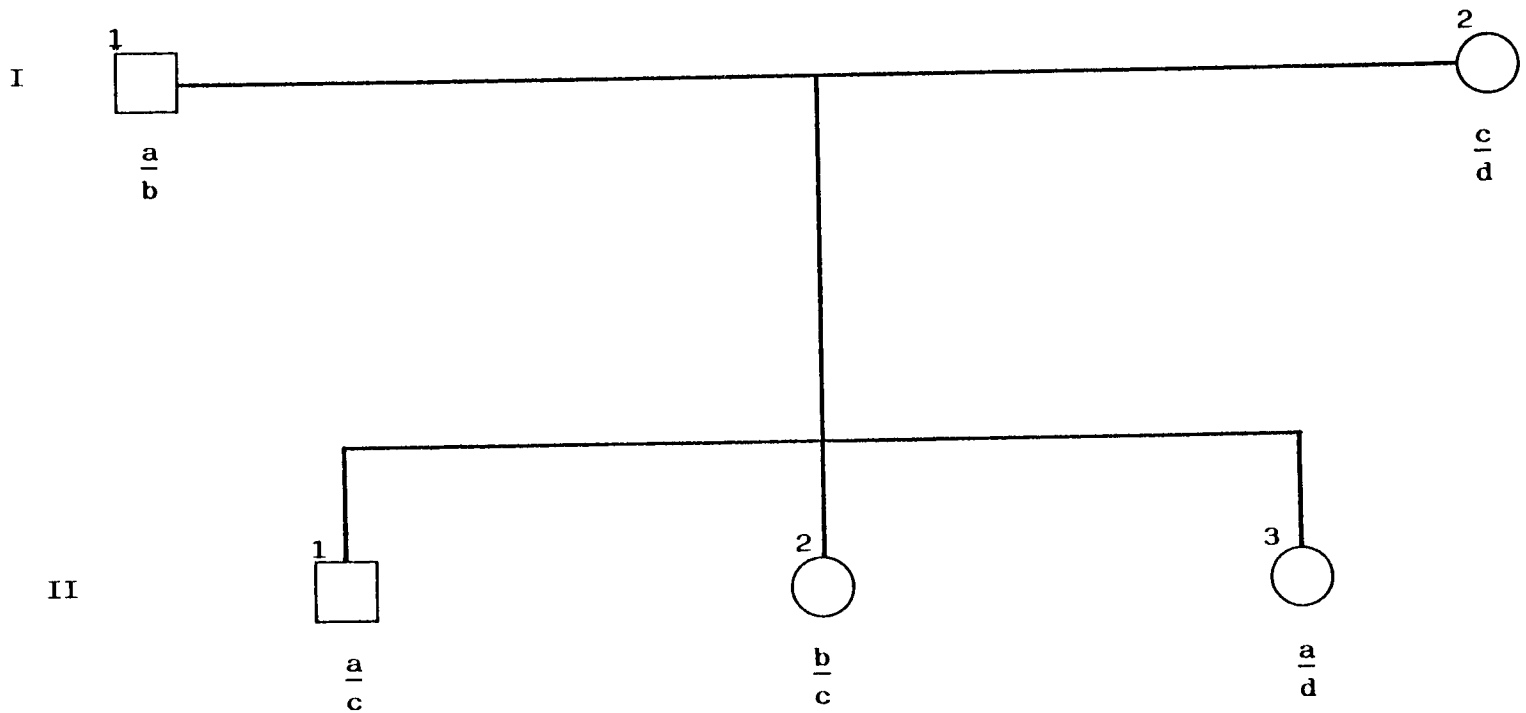

FIGURE 3.3 The pedigree of family ME. Individual I-1 was homozygous for the HLA class II region antigens and carried the haplotype DRw12x6,DQw5. Lymphocytes from this individual were used to define the new HLA-Dw specificity Dw'BME'.

a : Aw68, Cw6, Bw58, BF*S, C4A*3, C4B*2,92[a], DRw12x6, DQw5, Dw'BME'
b : A30, Cw6, Bw58, BF*S, C4A*3, C4B*2,92[a], DRw12x6, DQw5, Dw'BME'
c : Aw33, Cw7, B8, BF*F, C4A*2, C4B*1, DRw17 , DQw2, Dw blank
d : Aw68, Cw10, Bw22, BF*F, C4A*3, C4B*1, DRw15 , DQw6, Dw2

a C4B duplication (Mauff et al. 1984).

and (b). A new HLA-DR specificity, HLA-DRw12x6, was assigned to these haplotypes (section 4.1.3, du Toit and Oudshoorn 1987, du Toit et al. 1988b). Individual I-1 (BME) appeared to be homozygous for all sixth chromosome markers tested except HLA-A (Figure 3.3). The intra-family MLC (Table 3.7) showed that individual I-1 (BME) was also homozygous for the HLA-Dw determinants as cells of this individual typed his offspring. HLA-Dw identity of haplotypes (a) and (b) is further proven by non-stimulation seen between the cells II-1(a/c) and II-2(b/c) (Table 3.7). A checkerboard MLC performed with DRw8, DRw11, DRw12, DRw12x6, DRw13 and DRw14 related Dw specificities is shown in Table 3.3 of section 3.1.1. HTC-Dw'BME' reacted strongly both as a responder and stimulator with all relevant HTCs suggesting that HLA-Dw'BME' is a new HLA-Dw specificity.

The frequency of Dw'BME' was established in 53 random healthy SA Caucasoids, 72 Cape Coloureds and 236 SA Negroes (Table 3.8). The HLA-Dw'BME' antigen frequency in the SA Negroes (af=0.07) was significantly higher when compared to the antigen frequency of the Cape Coloureds (af=0.01). This specificity was not found in the SA Caucasoids. The families of the random individuals with Dw'BME' were tested in order to establish genotypes. In five of the nine families tested, the Dw'BME' specificity occurred together with A30,Bw58,Cw6,SC3(2,92),DRw12x6,DQw5 on the same haplotype, in three with

TABLE 3.7 Intra-family MLC of family ME.

Responder cells		Stimulator cells							
Individual	HLA haplotype	I-1 a/b	I-2 c/d	II-1 a/c	II-2 b/c	II-3 a/d	X-1[d]	X-2	X-3
I-1	a/b	991 -	50,563 98	44,112 85	27,315 52	20,097 38	51,621 100	84,548 165	47,668 92
I-2	c/d	45,296[a] 109[b]	858 -	20,853 49	18,985 44	68,451 93	39,119 165	41,798 100	61,613 148
II-1	a/c	8,987 17[c]	29,442 62	1,098 -	4,637 7.8[c]	31,229 66	34,647 74	46,488 100	63,789 138
II-2	b/c	6,798 14[c]	15,821 36	5,670 12[c]	811 -	71,123 170	79,494 190	39,980 95	42,229 100
II-3	a/d	4,483 6.8[c]	46,461 81	19,724 34	40,982 71	643 -	57,269 100	30,401 53	71,069 124
X-1[d]		42,406 94	37,814 83	40,175 89	27,112 59	51,316 114	1,124 -	34,528 76	55,672 124
X-2		66,321 136	51,969 106	33,742 68	38,689 79	41,416 84	36,138 73	650 -	61,821 127
X-3		38,498 73	47,229 90	55,278 105	29,372 55	67,210 128	57,017 109	48,157 91	987 -

a medium cpm

b relative response value (%RR)

c MLC reaction was considered negative if %RR< 30%

d X-1, X-2 and X-3 are unrelated individuals

A28,Bw58,Cw6,SC3(2,92),DRw12x6,DQw5, and once with A29,B44,Cw7,FC31,DRw12x6,DQw5.

TABLE 3.8 HLA-Dw'BME' frequency in South African populations.

Population group	Antigen frequency	Gene frequency
SA Caucasoids (n=53)	0	0
Cape Coloureds (n=72)	0.01	0.01
SA Negroes (n=236)	0.07	0.04

In *summary*, the data presented in this study demonstrate that the HLA-Dw determinant defined by HTC-BME is a unique specificity. HLA-Dw'BME' appears to be a Negro specificity as it was absent in the SA Caucasoids and was found at a significantly higher frequency in the SA Negroes compared to Cape Coloureds. Family studies showed that eight of nine Dw'BME' positive haplotypes also carried the antigens Bw58,Cw6,SC3(2,92),DRw12x6,DQw5 indicating that they represent a typical allelic combination in the SA Negro population. In section 4.1.3, the serological and RFLP data of HTC-BME will be discussed.

3.1.3 HLA-Dw 'RSH', a HLA-DRw18,DQw4 related HLA-Dw specificity identified in SA Negroes

Characterization of HLA-Dw'RSH'

The phenotype of HTC-RSH, a cell of Zulu origin, was : HLA-A30,w68;Bw42;Cw2;FC10,DRw18;DQw4;GLO1. The HLA genotype of RSH could unfortunately not be established as no family members could be traced. Although HTC-RSH was HLA-Dw typed using 56 well-defined HTCs, no recognized HLA-Dw specificity could be assigned. As this cell appeared to be homozygous for all the antigens tested, with the exception of HLA-A, it was evaluated in a MLC with 50 unrelated SA Negro cells (Table 3.9). Typing responses were obtained for eight of the ten cells carrying the haplotype HLA-DRw18,DQw4 which is present in RSH. The remaining two DRw18,DQw4 cells, BCE and STS, which were negative for Dw'RSH', were also negative for Dw3, the only other Dw specificity known to be associated with a subgroup of DR3 i.e. DRw17. These cells typed as HLA-Dw17,Dw'blank' (BCE) and HLA-Dw'blank',Dw'blank' (STS) (Table 3.9). No typing responses were observed for the remaining forty cells negative for DRw18,DQw4. RSH was further investigated in a checkerboard MLC (Table 3.10), using HTCs with the specificity Dw3. Six non-DR3 HTCs were included as controls. HTC-RSH stimulated and responded strongly to all these HTCs, indicating that Dw'RSH' is a unique Dw specificity.

TABLE 3.9 Panel of 50 unrelated SA Negroes typed for HLA-Dw.

Responder cells	HLA				HTC-RSH	
	B	DR	DQ	Dw	%SRR	Score
EKW	w42,w58	w18,w13	w1,w4	RSH,18	18	+
IME	w42,w72	w18,w15	w1,w4	RSH,-	21	+
JND	7,w71	w18,w11	w4,w7	RSH,-	8	+
ETU	w42,w45	w18,1	w1,w4	RSH,-	33	+
RMB	w42,-	w18,-	w4,-	RSH,-	24	+
JSI	w42,w72	w18,w13	w1,w4	RSH,19	38	+
EKL	13,w58	w18,w12x6[a]	w1,w4	RSH,BME	19	+
YWA	w42,44	w18,4	w4,w7	RSH,4	12	+
BCE	w42,7	w18,7	w2,w4	17,-	74	-
STS	w42,44	w18,-	w1,w4	-,-	55	-
ETA	w58,w72	w13,w17	w1,w2	18,-	105	-
GSI	w58,w72	w13,-	w1,-	19,-	77	-
JDA	w42,w58	w11,w17	w2,w7	3,-	92	-
NGI	w58,44	w11,w15	w1,w7	-,-	85	-
SGI	8,w58	w10,w12x6	w1,-	BME,-	117	-
TMA	w58,w65	w13,w14	w1,-	9,-	89	-
MAP	w64,49	7,w11	w2,w7	17,-	97	-
PME	8,w22	w17,w15	w1,w2	2,-	135	-
WMD	7,45	7,w14	w1,w2	9,17	102	-
JMW	45,w57	4,w13	w1,w3	4,18	78	-
MAX	w58,w64	7,w11	w1,w2	5,7	94	-
HAP	18,w71	w11,w13	w1,-	19,-	115	-
IMD	w57,w58	w14,-	w1,-	9,-	99	-
MST	44,w58	w12x6,w13	w1,-	BME,6	120	-
ANG	7,w71	w17,w11	w2,w7	-,-	62	-
VGU	w42,w72	w10,w11	w1,-	-,-	101	-
AST	7,w72	w15,w13	w1,-	2,6	94	-
EMW	45,w53	w8,w13	w1,w7	18,-	67	-
JDO	w58,w72	4,w6	w1,w3	4,6	110	-
VVU	44,45	w13,-	w1,-	19,-	78	-
KKO	7,44	w15,w13	w1,-	2,18	99	-
KGA	18,w58	9,w12x6	w1,w2	BME,-	155	-
DPA	44,w71	w6,w10	w1,-	18,-	53	-
JTA	w58,-	7,4	w2,w3	4,17	103	-
VDL	44,w58	7,w11	w1,w2	-,-	116	-
MDA	8,w71	4,-	w3,-	4,14	98	-
MMA	44,w58	4,-	w3,-	4,14	95	-
EVU	w42,w58	4,w8	w3,w4	-,-	73	-
FSI	w53,w58	4,w8	w3,w4	8,-	61	-
LNO	w42,7	w8,9	w2,w7	-,-	96	-
CMA	45,w53	1,w8	w1,w7	-,-	96	-
LGX	8,w65	w17,-	w1,w2	3,-	134	-
JGW	44,w71	w17,w11	w2,w7	-,-	77	-
EJA	18,w71	1,4	w1,w3	4,-	93	-
WMT	7,44	w17,w10	w1,w2	3,-	94	-
LMX	7,18	7,w11	w2,w7	5,7	111	-
GXD	w42,49	w17,w11	w2,w7	5,-	92	-
WNO	35,w58	4,7	w2,w3	7,10	85	-
TSH	8,w58	w15,w10	w1,-	2,-	117	-
DME	8,w72	4,w11	w7,-	5,-	94	-

\+ < 50% SRR (typing response)

\- > 50% SRR (no typing response)

a New HLA-DR specificity defined in this laboratory (section 4.1.3)

TABLE 3.10 Checkerboard MLC results between DR3 associated HLA-Dw specificities and non-DR3 controls.

Responder HTCs		Stimulator HTCs												
HLA-Dw specificities[a]		RSH	Dw3						Dw1	Dw2	Dw4	Dw5	Dw17	Dw18
		RSH	COX	OTTO	MITCH	LAMP	CDT	QBL	JGA	ZSI	BSMI	RDA	PITOUT	OMW
RSH	Dw'RSH'	+	-	-	-	-	-	-	-	-	-	-	-	-
COX	Dw3	-	+	+	+	-	+	-	-	-	-	-	-	-
OTTO	Dw3	-	-	+	-	+	+	-	-	-	-	-	-	-
MITCH	Dw3	-	+	-	+	+	+	+	-	-	-	-	-	-
LAMP	Dw3	-	+	-	+	+	-	+	-	-	-	-	-	-
CDT	Dw3	-	-	+	+	+	+	-	-	-	-	-	-	-
QBL	Dw3	-	+	+	+	-	+	+	-	-	-	-	-	-
JGA	Dw1	-	-	-	-	-	-	-	+	-	-	-	-	-
ZSI	Dw2	-	-	-	-	-	-	-	-	+	-	-	-	-
BSMI	Dw4	-	-	-	-	-	-	-	-	-	+	-	-	-
RDA	Dw5	-	-	-	-	-	-	-	-	-	-	+	-	-
PITOUT	Dw17	-	-	-	-	-	-	-	-	-	-	-	+	-
OMW	Dw18	-	-	-	-	-	-	-	-	-	-	-	-	+

Typing score values : + ≤ 50% SRR
- > 50% SRR

a All local HTCs except HTC-QBL (van Rood)

Family studies

The segregation of HLA-Dw'RSH' was investigated in two informative families with the haplotype DRw18,DQw4. Figure 3.4 shows the segregation of the HLA haplotypes in family 10WS 161. The paternal haplotype (b) carried Dw'RSH' and it was present in two of his offspring, II-2 and II-3. The

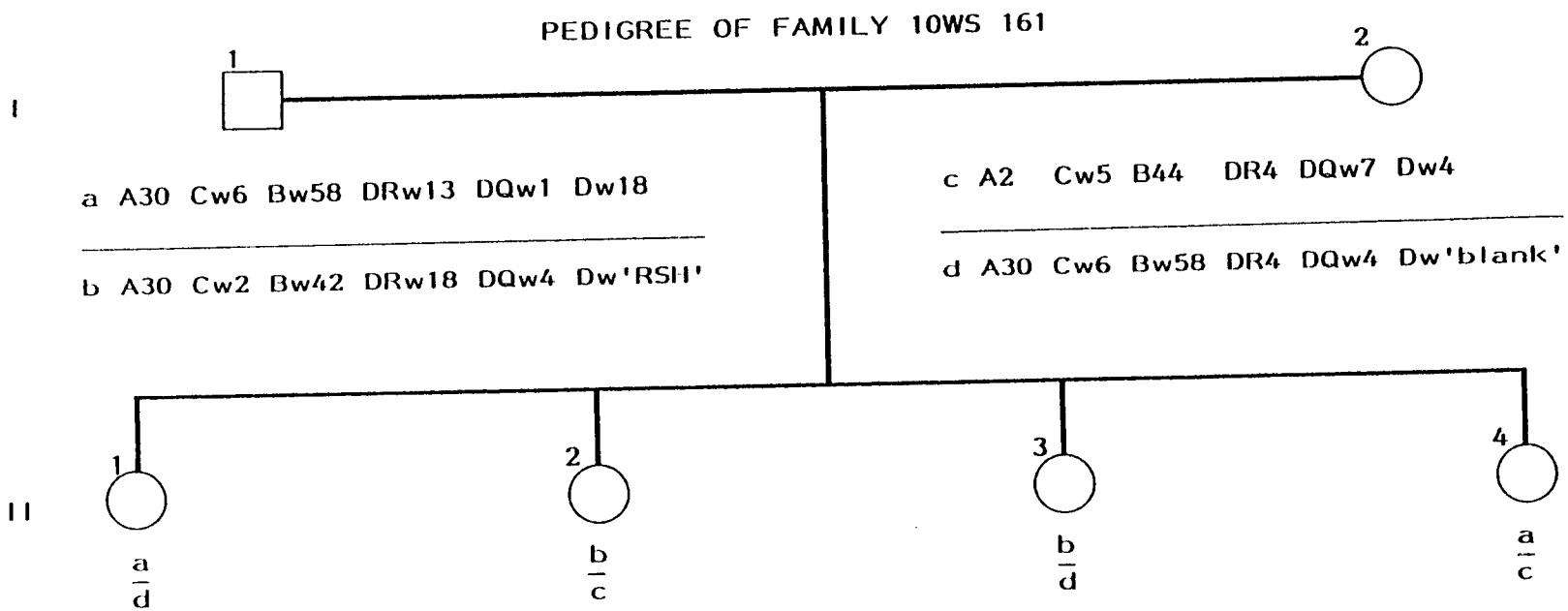

FIGURE 3.4 Segregation of HLA-Dw'RSH' in family 10WS 161.

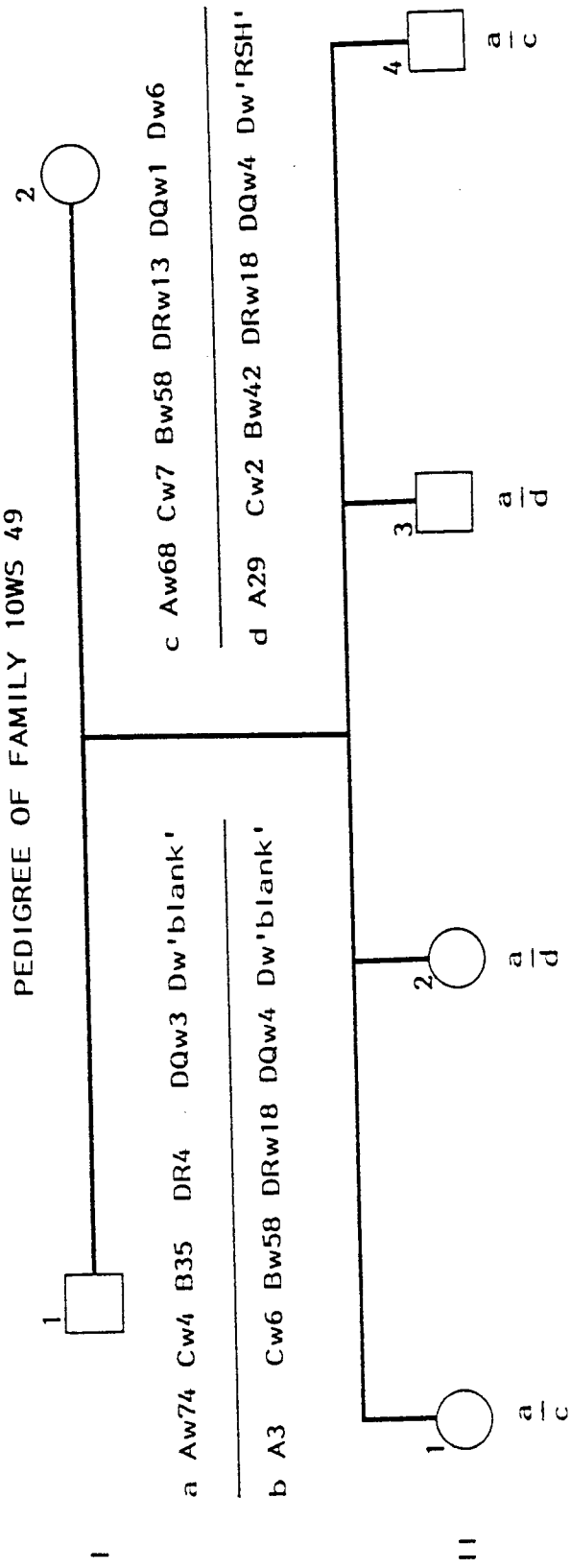

FIGURE 3.5 Segregation of HLA-Dw'RSH' in family 10WS 49.

HLA haplotypes of family 10WS 49 are shown in Figure 3.5. In this family the maternal haplotype (d) carried the DRw18,DQw4,Dw'RSH' specificities and was transmitted to two of her four children, II-2 and II-3. The paternal haplotype (b) also carried the DRw18,DQw4 antigens but no Dw specificity could be assigned to this haplotype. These families demonstrate that HLA-Dw'RSH' is a distinct genetic determinant which segregates characteristically with the haplotype DRw18,DQw4.

Population studies

The HLA-Dw'RSH' frequency was determined in a group of 368 random healthy individuals. The antigen and gene frequencies of HLA-Dw'RSH' in the SA Negroes, Cape Coloureds and SA Caucasoids are presented in Table 3.11, along with the Dw3 frequencies for comparison. A significantly higher frequency of Dw'RSH' ($p<0.001$) was seen in the SA Negroes compared to the Cape Coloureds. HLA-Dw'RSH' was absent in the sample of SA Caucasoids studied. The Dw3 frequency was significantly decreased in the SA Negroes ($p<0.001$) compared to SA Caucasoids, while no significant difference was observed between the SA Negroes and the Cape Coloureds.

HLA-Dw'RSH' was in significantly associated with DR3 and Bw42 in SA Negroes ($p<0.001$) and Cape Coloureds ($p<0.01$). HLA-Dw'RSH' was not in linkage disequilibrium with any of the HLA-DQ antigens tested (DQw1, DQw2 and DQw3). No

information on the association of Dw'RSH' with the subgroups of DR3 (DRw17 and DRw18) and the newly defined DQw4 specificity could be established from this group of 368 individuals as they were HLA typed before these new specificities were defined. However, in the panel of 50 Negro cells tested with the HTC-RSH (Table 3.9), cells with Dw'RSH' were always positive for DRw18 and DQw4.

TABLE 3.11 Antigen and gene frequencies of DR3 associated HLA-Dw specificities in South African populations.

Population	HLA-Dw'RSH'		HLA-Dw3	
	af	gf	af	gf
SA Negroes (n=236)	0.20	0.10	0.09	0.05
Cape Coloured (n=72)	0.03	0.01	0.14	0.07
SA Caucasoids (n=60)	0	0	0.27	0.14

af = antigen frequency
gf = gene frequency

In *summary*, this study describes a new HLA-Dw specificity, Dw'RSH'. HLA-Dw'RSH' is associated with DRw18(3) and is clearly different from the DRw17(3) associated specificity Dw3. Striking variation in the frequency of Dw'RSH' among the different SA populations was observed. Whereas HLA-Dw'RSH' is the most common HLA-Dw specificity seen in the SA Negroes, it was significantly less common in the Cape Coloureds and absent in the Caucasoids tested. These results indicate that Dw'RSH' is probably of Negro origin.

(Xhosa). From the absence of Dw'RSH' on the DRw18,DQw4 positive haplotype (b) of family 49 (Figure 3.5) however, it is evident that there are as yet unidentified HLA-Dw specificity(ies) associated with DRw18,DQw4. Significant linkage disequilibrium of Dw'RSH' with Bw42 and DR3 was seen in the SA Negroes and Cape Coloureds. Both family and limited population studies suggest that Dw'RSH' is part of the haplotype Bw42,DRw18,DQw4 which is seen as commonly in the SA Negroes as the B8,DRw17,DQw2,Dw3 haplotype is seen in the Caucasoids.

Comparison of the RFLP data of HTC-RSH and HTC-COX (DRw17,DQw2,Dw3) showed differences in the pattern associated with the DRB1 gene in these cells (section 4.1.2). A difference in the DRB1 genes of DRw17,DQw2 and DRw18,DQw4 cells was also found in the DNA sequencing studies of Hurley et al. (unpublished observations). The differences in the DRB1 chains of the DR3(DRw18),DQw4,Dw'RSH' cells and the DR3(DRw17),DQw2,Dw3 cells may possibly explain the difference in Dw typing between these cells.

3.2 A STUDY OF THE HLA-Dw DETERMINANTS AND THEIR RELATIONSHIP TO DR AND DQ ANTIGENS IN THREE SOUTH AFRICAN POPULATION GROUPS: SA NEGROES (XHOSA), CAPE COLOUREDS AND SA CAUCASOIDS

The use of HTCs has made it possible to define at least 23 different HLA-Dw specificities (WHO Nomenclature Committee 1988). In addition to these, there are several HLA-Dw specificities which have not yet been officially recognized by the WHO Nomenclature Committee (Table 1.2). As described above we have found three new HLA-Dw specificities Dw'BME', Dw'JOH' and Dw'RSH' which fall in this latter category. Investigations of HLA-Dw specificities in populations have largely focused on the studies of Caucasoids (Dupont et al. 1980, Termijtelen 1981, Thomsen et al. 1982), Japanese (Sasazuki et al. 1980) and both North and South American Indians (Layrisse et al. 1976, 1978, 1979; Troup et al. 1978). Extensive data on the HLA-Dw characteristics of African Negroes are not available. The only two studies are one of Nigerians by Okoye et al. (1985) and one by our own group (Oudshoorn et al. 1986).

In this section a comparative study of the HLA-Dw determinants and their relationship to HLA-DR and DQ in the three major population groups living in the Cape, the SA Negroes (Xhosa), Cape Coloureds and SA Caucasoids, will be described. The Xhosa as well as the Nigerians studied by

Okoye et al. (1985) and used for comparison, belong to the Bantu-speaking Negroes (Greenberg 1963). Although the Nigerians are believed to be a relatively homogeneous group (Okoye et al. 1985), there was some population mixing with Islamic peoples during the 11th century (Okoye et al. 1985). The Xhosa were also compared to a group of American Negroes studied by Johnson (1986) for the Third Asia-Oceania Workshop. Most of the American Negroes were brought from the West Coast of Africa to America as slaves (Curtin 1969). The proportion of Caucasoid genes in the American Negroes was estimated to be between 10% and 20%, based on the Duffy blood group system (Reed 1969) and in addition to Caucasoid admixture they also have a large proportion of (approximately 27%) of American Indian genes (Foster 1935).

The Xhosa, Cape Coloureds and SA Caucasoids were HLA-Dw typed with 56 HTCs for the specificities Dw1 to Dw9, Dw13 to Dw15, Dw17 to Dw19, DKT2, Dw'BME', Dw'JOH' and Dw'RSH' (Tables 2.1 and 2.2). In addition to these specificities, the SA Negroes were typed for HLA-Dw10. No HTCs for the specificities Dw11 to Dw12, Dw16, and Dw20 to Dw23 were available for the HLA-Dw frequency determinations. The individuals were also typed for all the recognized HLA-A,B,C,DR and DQ specificities.

Table 3.12 demonstrates the HLA-Dw gene frequencies for the specificities Dw1 to Dw8 in our Caucasoid panel, as well as

in Danish Caucasoids (Thomsen et al. 1982), Dutch Caucasoids (Termijtelen 1981) and Caucasoids tested as part of the Eighth International Histocompatibility Workshop (Dupont et al. 1980). There were no significant differences in the gene frequencies of the HLA-Dw determinants in the SA Caucasoid panel compared to the three other groups.

The antigen and gene frequencies of the HLA-Dw determinants in SA Caucasoids, Cape Coloureds, and Xhosa are given in Table 3.13.

TABLE 3.12 HLA-Dw gene frequencies in SA Caucasoids and three other Caucasoid groups.

HLA-Dw specificity	SA Caucasoids (n=53)	Danish Caucasoids[a] (n=389)	Dutch Caucasoids[b] (n=130)	Eighth International Workshop[c] Caucasoids (n=292)
Dw1	0.089	0.111	0.097	0.069
Dw2	0.110	0.146	0.141	0.075
Dw3	0.142	0.160	0.168	0.082
Dw4	0.068	0.134	0.072	0.053
Dw5	0.099	0.060	0.059	0.056
Dw6	0.097[d]	0.072	0.097	0.103
Dw7	0.110[e]	0.093	0.101	0.103
Dw8	0.010	0.037	0.034	0.031

a Thomsen et al. 1982
b Termijtelen 1981
c Dupont et al. 1980
d HLA-Dw6 was defined by typing reactions with Dw18 and/or Dw19 HTCs
e HLA-Dw7 was defined by typing reactions with Dw17 HTCs

The sum of the gene frequencies detected in the Caucasoid group is 0.781, in the Cape Coloureds 0.696 and in the Xhosa

TABLE 3.13 The frequency of HLA-Dw determinants in three SA population groups.

	SA Caucasoids (n=53)		Cape Coloureds (n=57)		SA Negroes (n=85)		Nigerians[a] (n=103)	American Negroes[b] (n=59)
HLA-Dw	af	gf	af	gf	af	gf	af	af
Dw1	0.170	0.089	0.018	0.009[c]	0.024	0.012[d]	0.069	0.068
Dw2	0.208	0.110	0.228	0.121	0.094	0.048	0.256	0.169
Dw3	0.264	0.142	0.175	0.092	0.071	0.036[e]	0.158	0.017
Dw4	0.132	0.068	0.193	0.102	0.082	0.042	0	0.034
Dw5	0.189	0.099	0.175	0.092	0.112	0.061	0.208	0.034
Dw6[f]	0.057	0.029	0.018	0.009	0.047	0.024	0	0
Dw7[g]	0.208	0.110	0.211	0.112	0.153	0.080	0.119	0.119
Dw8	0.019	0.010	0.018	0.009	0	0	0.019	0.017
Dw9	0.019	0.010	0.035	0.018	0.047	0.024	0.029	0.017
Dw10	nt	nt	nt	nt	0.012	0.006	0	0
Dw13	0.038	0.019	0.053	0.027	0	0	0.039	nt
Dw14	0.038	0.019	0	0	0.024	0.012	nt	0.017
Dw15	0	0	0.018	0.009	0	0	0	nt
Dw18	0.113	0.058	0.088	0.045	0.165	0.086	0.071	0.085
Dw19	0.019	0.010	0.035	0.018	0.071	0.036	0	0.068
DKT2	0	0	0.018	0.009	0	0	0	nt
Dw'BME'	0	0	0	0	0.082	0.042	nt	nt
Dw'JOH'	0.019	0.010	0.018	0.009	0	0	nt	nt
Dw'RSH'	0	0	0.035	0.018	0.224	0.119[h]	nt	nt
BLANK		0.219		0.304		0.373		

nt = not tested

a Okoye et al. 1985

b Johnson 1986

c Significant decrease of Dw1 gene frequency in the Cape Coloured compared to the Caucasoid (X^2=7.71;p<0.01).

d Significant decrease of Dw1 gene frequency in the Xhosa compared to the Caucasoids (X^2=9.52;p<0.01).

e Significant decrease of Dw3 gene frequency in the Xhosa compared to the Caucasoids (X^2=9.87;p<0.01).

f HLA-Dw6 was assigned when HTCs for the specificities Dw18 and Dw19 both gave typing responses.

g HLA-Dw7 was assigned when HTCs for the specificity Dw17 gave typing responses. Due to the lack of Dw11 HTCs and the problem of cross-reactivity between Dw11 and Dw17 we were unable to identify the Dw7 splits.

h Significant increase of Dw'RSH' gene frequency in the Xhosa compared to the Caucasoids (X^2=13.74;p<0.001) and the Cape Coloureds (X^2=9.61;p<0.01).

0.627. Thus, 37% of the HLA-Dw specificities in the Xhosa were not detected which is approximately twice as much as in the SA Caucasoid group (22%). The Cape Coloureds behave as an intermediate group in this respect as 30% of their HLA-Dw specificities were not detected.

When Xhosa and Cape Coloured cells were typed with Caucasoid HTCs, 40 to 60% of the typings were "borderline" (SRR 40-50%), as opposed to 15 to 30% in the Caucasoid group. The only exception to this was the clear typing reactions seen in the Xhosa, Cape Coloureds, and the SA Caucasoids when HTCs with the Dw2 specificity were used. When Caucasoids were typed with Cape Coloured HTCs carrying the specificities Dw2, Dw4, Dw5, and Dw'JOH' and the SA Negro HTCs Dw9, Dw18 and Dw19 unequivocal typing responses were again only seen with HTC-Dw2.

The HLA-Dw frequencies (Table 3.13) and the association of the HLA-Dw determinants with HLA-DR (Table 3.14) and HLA-DQ antigens (Table 3.15) in the different population groups are discussed below. The HLA-Dw'BME' and Dw'JOH' specificities were excluded from Table 3.14 as the majority of individuals were not tested for the newly defined antigen DRw12x6 found in association with these specificities (section 4.1.3).

TABLE 3.14 Association between HLA-Dw and DR.

HLA Dw	HLA DR	Population group	++	+-	-+	--	Delta	Chi-square X^2	Correlation coefficient r
Dw1	DR1	C[a]	9	0	3	41	0.078	37.040[f]	0.836
		CC[b]	1	0	2	54	0.009	18.321[f]	0.567
		N[c]	2	0	7	76	0.011	17.296[f]	0.451
Dw2	DR2	C	11	0	1	41	0.097	47.427[f]	0.946
		CC	13	0	7	37	0.098	31.156[f]	0.739
		N	8	0	4	73	0.045	53.723[f]	0.795
Dw3	DR3	C	14	0	1	38	0.120	48.198[f]	0.954
		CC	10	0	2	45	0.082	45.479[f]	0.893
		N	6	0	28	51	0.028	9.684[e]	0.338
Dw'RSH'	DR3	C	0	0	15	38	0	0	0
		CC	2	0	10	45	0.016	7.773[e]	0.369
		N	19	0	15	51	0.092	36.705[f]	0.657
Dw4	DR4	C	7	0	5	41	0.039	27.556[f]	0.721
		CC	11	0	6	40	0.085	32.072[f]	0.750
		N	7	0	6	72	0.039	42.249[f]	0.715
Dw13	DR4	C	2	0	10	41	0.017	7.101[e]	0.366
		CC	3	0	14	40	0.022	7.451[e]	0.362
		N	0	0	13	72	0	0	0
Dw14	DR4	C	2	0	10	41	0.017	7.101[e]	0.366
		CC	0	0	17	40	0	0	0
		N	2	0	11	72	0.011	11.344[f]	0.365
Dw15	DR4	C	0	0	12	41	0	0	0
		CC	1	0	16	40	0.007	2.395	0.205
		N	0	0	13	72	0	0	0
DKT2	DR4	C	0	0	12	41	0	0	0
		CC	1	0	16	40	0.007	2.395	0.205
		N	0	0	13	72	0	0	0
Dw5	DR5	C	10	0	4	39	0.076	34.336[f]	0.805
		CC	10	0	7	40	0.078	28.536[f]	0.708
		N	10	0	13	62	0.052	30.551[f]	0.600
Dw7	DR7	C	11	0	2	40	0.095	42.711[f]	0.898
		CC	12	0	2	43	0.097	46.686[f]	0.905
		N	13	0	2	70	0.072	71.620[f]	0.918
Dw8	DRw8	C	1	0	2	50	0.009	16.987[f]	0.556
		CC	1	0	1	55	0.009	27.991[f]	0.701
		N	0	0	1	84	0	0	0
Dw6	DRw13	C	3	0	8	42	0.026	12.142[f]	0.479
		CC	1	0	10	46	0.008	4.257[d]	0.273
		N	4	0	35	46	0.018	4.951[d]	0.241
Dw18	DRw13	C	6	0	5	42	0.052	25.834[f]	0.698
		CC	5	0	6	46	0.040	22.920[f]	0.634
		N	13	1	26	45	0.055	14.895[f]	0.419
Dw19	DRw13	C	1	0	10	42	0.008	3.892[d]	0.271
		CC	2	0	9	46	0.016	8.668[e]	0.390
		N	5	1	34	45	0.018	3.647	0.207
Dw9	DRw14	C	1	0	0	52	0	53.000[f]	1.000
		CC	2	0	0	55	0	57.000[f]	1.000
		N	4	0	1	80	0.023	67.161[f]	0.889

a = SA Caucasoid
b = Cape Coloured
c = SA Negro
d = $p<0.05$
e = $p<0.01$
f = $p<0.001$

TABLE 3.15 Association between HLA-Dw and DQ.

HLA Dw	HLA DQ	Population group	++	+-	-+	--	Delta	Chi-square X^2	Correlation coefficient r
Dw1	DQw1	C[a]	9	0	19	25	0.061	9.679[e]	0.427
		CC[b]	1	0	32	24	0.006	0.740	0.114
		N[c]	2	0	58	25	0.006	0.853	0.100
Dw2	DQw1	C	11	0	17	25	0.075	12.394[f]	0.484
		CC	13	0	20	24	0.079	12.248[f]	0.464
		N	8	0	52	25	0.026	3.680	0.208
Dw3	DQw2	C	14	0	10	29	0.105	22.990[f]	0.659
		CC	10	0	8	39	0.019	26.277[f]	0.679
		N	6	0	14	65	0.031	20.981[f]	0.497
Dw4	DQw3	C	7	0	20	26	0.048	7.767[e]	0.383
		CC	11	0	28	18	0.057	6.291[d]	0.332
		N	7	0	31	47	0.031	9.435[e]	0.333
Dw5	DQw3	C	10	0	17	26	0.070	11.869[f]	0.473
		CC	10	0	29	18	0.052	5.597[d]	0.313
		N	9	0	29	46	0.045	12.347[f]	0.381
Dw6	DQw1	C	3	0	25	25	0.020	2.839	0.232
		CC	1	0	32	24	0.006	0.740	0.114
		N	4	0	56	25	0.013	1.749	0.143
Dw7	DQw2	C	10	1	14	28	0.068	11.662[f]	0.469
		CC	9	3	9	36	0.060	13.264[f]	0.482
		N	11	2	9	63	0.056	31.828[f]	0.612
Dw7	DQw3	C	6	5	21	21	0.006	0.072	0.037
		CC	7	5	32	13	-0.022	0.716	0.112
		N	8	5	30	42	0.019	1.759	0.144
Dw9	DQw1	C	1	0	27	25	0.007	0.910	0.131
		CC	2	0	31	24	0.011	1.507	0.163
		N	4	0	56	25	0.013	1.749	0.143
Dw13	DQw3	C	2	0	25	26	0.013	2.002	0.194
		CC	3	0	36	18	0.015	1.462	0.160
		N	0	0	38	47	0	0	0
Dw14	DQw3	C	2	0	25	26	0.013	2.002	0.194
		CC	0	0	39	18	0	0	0
		N	2	0	36	47	0.004	1.252	0.121
Dw18	DQw1	C	6	0	22	25	0.040	6.041[d]	0.338
		CC	5	0	28	24	0.029	3.986[d]	0.264
		N	14	0	45	27	0.047	6.984[e]	0.287
Dw19	DQw1	C	1	0	27	25	0.006	0.910	0.131
		CC	2	0	31	24	0.011	1.507	0.163
		N	6	0	54	25	0.020	2.690	0.178
DKT2	DQw3	C	0	0	27	26	0	0	0
		CC	1	0	38	18	0.005	0.470	0.091
		N	0	0	38	47	0	0	0
Dw'BME'	DQw1	C	0	0	28	25	0	0	0
		CC	0	0	33	24	0	0	0
		N	7	0	53	25	0.023	3.178	0.193
Dw'JOH'	DQw3	C	1	0	26	26	0.007	0.982	0.136
		CC	1	0	38	18	0.005	0.470	0.091
		N	0	0	38	47	0	0	0

a = SA Caucasoid
b = Cape Coloured
c = SA Negro
d = $p<0.05$
e = $p<0.01$
f = $p<0.001$

Dw1

The frequency of the HLA-Dw1 specificity (Table 3.13) is significantly decreased in the Cape Coloureds (X^2=7.71, p<0.01) and in the Xhosa (X^2=9.52, p<0.01) compared to the Caucasoids. Good correlation of Dw1 with DR1 was observed in the Caucasoid group only (r=0.836) (Table 3.14). The linkage disequilibrium between B35,DR1,Dw1 as seen in the Caucasoids was absent in the Cape Coloureds and Xhosa. In the latter two groups, DR1 is in linkage disequilibrium with HLA-B45 ($\triangle$ = 0.007, p<0.001 Cape Coloureds; $\triangle$ = 0.021, p<0.001 Xhosa) but not with Dw1. In all three groups tested, Dw1 was always seen in association with DQw1 (Table 3.15).

Dw2

The Dw2 frequency (Table 3.13) was high in the three population groups tested and good correlation with DR2 was seen in all three (Table 3.14). All Dw2 cells were positive for DQw1 (Table 3.15).

Dw3/Dw'RSH'

HLA-Dw3 was found to have a relatively high frequency in SA Caucasoids (gf=0.142) and Cape Coloureds (gf=0.092) compared to other HLA-Dw specificities as shown in Table 3.13 and was associated with HLA-DQw2. In both these groups excellent correlation with DR3 was observed (r=0.954; r=0.893,

respectively) (Table 3.14). The frequency of Dw3 (Table 3.13) was, however, much lower in the Xhosa (gf=0.036) and showed only limited correlation (Table 3.14) with DR3 (r=0.338). This was not surprising as the majority of the HLA-DR3 positive individuals consisted of the DR3 subgroup DRw18 which is not associated with Dw3 (section 3.1.3 and 4.1.2). This weak association between HLA-DR3 and Dw3 seen in the Xhosa was in accordance with the data published by Duquesnoy et al. (1979b) on Northern American Negroes (r=0.33). The HLA-Dw'RSH' specificity is the most frequent Dw specificity seen in the Xhosa (gf=0.119) and correlated better than Dw3 with the DR3 antigen (r=0.657). It was also present in the Cape Coloureds (gf=0.018) but absent in the SA Caucasoids. HLA-Dw'RSH' was always seen in association with DR3. In a collaborative study with Hurley and co-workers on the Dw specificities associated with DR3 in American Negroes, the presence of Dw'RSH' was also established in this group. HTC-RSH gave clearer typing reactions in the SA Negroes than American Negroes, mean RR=18% compared to mean RR=32% (C. Hurley, personal communication). This indicates some differences in the American Negro Dw'RSH' bearing haplotype compared to that of SA Negroes.

Dw4/Dw10/Dw13/Dw14/Dw15/DKT2

The HLA-Dw specificities included in the DR4-associated cluster are Dw4, Dw10, Dw13, Dw14, Dw15, and DKT2

(Jaraquemada et al. 1983). The SA Caucasoids and Cape Coloureds were typed for al these specificities with the exception of Dw10, while the Xhosa were typed for all DR4 related HLA-Dw specificities. In all three population groups DR4 was most frequently seen in association with Dw4 as shown in Table 3.14. Dw13 which was not found in the Xhosa, had a low frequency in the Caucasoids (gf=0.019) and Cape Coloureds (gf=0.027) (Table 3.13) and it was significantly correlated with DR4 in both groups (Table 3.14). In the Cape Coloured and Caucasoid population groups no Dw13 positive, DR4 negative individuals were found, unlike the findings of Jaraquemada et al. (1983) in Shanghai Chinese and Nigerians. The Dw14 (previously LD40) frequency was low in the Caucasoids (gf=0.019) and the Xhosa (gf=0.015) and absent in the Cape Coloureds (Table 3.13). All Dw14 positive cells were also positive for DR4. Only one individual (Cape Malay) was found to be positive for Dw15 (previously DYT), a specificity originally identified in the Japanese population by Sasazuki et al. (1977b). From family studies it could be seen that in this individual Dw15 occurred together with DQ'blank' on the same haplotype. The observation that Dw15 is associated with DQ'blank' and not with DQw3 as seen in all other DR4 associated HLA-Dw specificities, was first mentioned at the Ninth International Histocompatibility Workshop by L. Gebuhrer, M. Honeyman, and I. Schreuder. This DQ'blank' specificity has subsequently been identified as DQw4 (Endo et al. 1987).

One individual (Cape Coloured) was found to be positive for DKT2, and was also positive for DR4,DQw3. The DKT2 specificity which was originally identified in the Japanese population, has been shown to be cross-reactive with Dw13 in the Caucasoids (Jaraquemada et al. 1983, Reinsmoen and Bach 1982). Neither the Dw13 cells (Cape Coloured and Caucasoid) nor the one DKT2 positive cell (Cape Coloured) showed such cross-reactivity. HLA-Dw10 had a low frequency in the Xhosa (gf=0.006) and was not tested for in the other two groups. All Dw10 cells were positive for DR4 and DQw3.

Dw5

In all three populations DR5 was mainly associated with DQw3, but DR5 was also seen in association with DQw1 in the Xhosa and to a lesser extent in the Cape Coloureds and SA Caucasoids (section 5.1). However, all Dw5 cells found in these three population groups were positive for DR5 (Table 3.14) as well as DQw3 (Table 3.15)

Dw6/Dw18/Dw19

The HLA-Dw6 specificity is split into Dw18 (previously 6a) and Dw19 (previously 6b) (Termijtelen et al. 1984). The broad specificity Dw6, as well as the splits Dw18 and Dw19, were found in the three groups tested and in all but two cells these specificities were associated with DRw13 (Table 3.14) and DQw1 (Table 3.15). The two cells with the discrepant Dw/DR association will be discussed below. The

HLA-Dw18 specificity was more frequently seen in the three South African populations than the Dw6 subgroup, Dw19 which is known to occur more commonly in Orientals (Mickelson et al. 1986).

Dw7

The HLA-Dw7 specificity is split into Dw11 and Dw17. Due to the lack of Dw11 typing cells and the problem of cross-reactivity between Dw11 and Dw17 we were unable to identify the Dw7 splits. The frequency of Dw7 was high in Cape Coloureds (gf=0.112), Caucasoids (gf=0.110), and Xhosa (gf=0.080) as shown in Table 3.13. In all three groups the typical association of Dw7 with DR7 (Table 3.14) and either DQw2 or DQw3 (Table 3.15) was seen.

Dw8

The HLA-Dw8 specificity was absent in the Negroes tested and present in one Caucasoid and one Cape Coloured individual both DRw8,DQ'blank'. A total of four DRw8 positive, Dw8 negative cells were identified in the three population groups. Two of these cells (Caucasoid) were DQ'blank' while the other two (one Xhosa, one Cape Coloured) were DQw3 positive.

Dw9

The Dw9 specificity was shown to be associated with DRw14(w6) (previously 6.9, 6.3, 6x, 901) (Gebuhrer et al.

1983b, Schreuder and Parlevliet 1983). In all three populations tested, cells typed as Dw9 were positive for DRw14 (Table 3.14) and DQw1 (Table 3.15).

Dw'JOH'/Dw'BME'

Two new HLA-DRw12x6 related HLA-Dw specificities Dw'JOH' and Dw'BME' were identified by us (section 3.1.1 and 3.1.2). HLA-Dw'BME', identified in the Xhosa, is associated with DRw12x6,DQw5(1), while Dw'JOH', originally identified in the Cape Coloureds, is associated with DRw12x6,DQw7(3). The Dw'BME' specificity which was found at a gene frequency of 0.042 in the Xhosa (Table 3.13), was not found in the Caucasoids and Cape Coloureds. Subsequently, it was found in one of 72 Cape Coloured individuals (section 3.1.2). The specificity Dw'JOH' was present in one Caucasoid and one Cape Coloured cell. Both these cells were positive for DRw12x6 (Table 3.14) as well as DQw3 (Table 3.15).

When comparing the HLA-Dw frequencies of the SA Negroes (Xhosa) to those of the other Negro groups, marked similarities as well as differences were observed (Table 3.13). The most striking difference was the absence of Dw4 and Dw19 and the higher frequency of Dw2 and Dw5 in the Nigerians when compared to the Xhosa. The American Negroes had a much lower frequency of HLA-Dw3, Dw4, Dw5, Dw18 and a higher frequency of Dw7 than the Xhosa (Table 3.13).

As described above and shown in Table 3.14, the HLA-Dw specificities for the SA Negroes are almost all included within the corresponding HLA-DR specificities. However, in two cells exceptions to the usual HLA-Dw/DR associations were observed. In the one cell Dw18 and in the other cell Dw19 was present without the expected HLA-DRw6 antigen being detected. In both these an "undetected" or "blank" HLA-DR antigen was involved. Thus these HLA Dw/DR discrepancies were either due to a "missed" DR antigen or a new DR antigen for which no antisera are yet available. A "missed" HLA-DR antigen may be the more likely explanation for these cells. This assumption is based on a previous study in which we analyzed the DR typings done before and after the Ninth International Histocompatibility Workshop separately in order to evaluate possible changes in HLA-DR distribution (du Toit et al. 1988a). In this study, the only notable change was an increase in the DRw6 gene frequency and a simultaneous drop in the DR'blank' frequency, indicating that previously some DRw6 antigens were missed. Confirming this possibility by repeating the DR typing of these cells was not possibly as both individuals could not be retraced. In the Nigerian panel relatively more discrepant HLA-Dw/DR typings were observed compared to the SA Negroes. These findings have not been substantiated by repeat typings or family studies. As shown in Table 3.15 no discrepant DR,DQ association was observed in this study. Thus typing reactions were in general only observed when both HLA-DR and

HLA-DQ antigens were identical in the test cell and the HTC as illustrated by the following examples:

1. DR3,DQw3 cells were not typed by HTCs Dw3 (DR3,DQw2). From further studies we know that both the DR3,DQw3 and DR3,DQw2 cells carry the same DR3 split, namely DRw17(section 5.2.1)

2. DR5,DQw1 cells were not typed by HTCs Dw5 (DR5,DQw3).

3. DR4,DQ'blank' cells were not typed by HTCs Dw4, Dw13, Dw14, or DKT2 which are all positive for DR4 and DQw3. The DR4,DQ'blank' cell was, however, typed by HTCs Dw15 (DR4,DQ'blank').

4. DRw8,DQw3 cells were not typed by HTCs Dw8 (DRw8,DQ'blank').

5. HTC-BME (DRw12x6,DQw1) and HTC-JOH (DRw12x6,DQw3) did not type the same cells.

In *summary*, the gene frequencies of HLA-Dw determinants in the SA Caucasoids do not differ significantly from those of the European Caucasoids (Table 3.12). This is in accordance with studies on HLA-A,B,C,DR and DQ frequencies which showed

similar gene frequencies in SA Caucasoids to those found in European Caucasoids (du Toit et al. 1988a).

The majority of the HLA-Dw specificities occur in all three population groups tested. However, when the frequencies were compared differences were seen between the Xhosa, Cape Coloureds and SA Caucasoids. In the Cape Coloureds and Xhosa, the frequency of HLA-Dw1 was significantly decreased compared to the Caucasoids. The classic linkage disequilibrium between B35 and DR1/Dw1 seen in the Caucasoids was absent in Cape Coloureds and Xhosa. However, in these two groups, DR1 was in linkage disequilibrium with B45 (Cape Coloureds, $\triangle$ =0.007, $p<0.001$; Xhosa, $\triangle$ = 0.021, $p<0.001$) but not with Dw1. A marked decrease of the HLA-DR3 related specificity HLA-Dw3 (gf=0.036) and an increase of HLA-Dw'RSH' (gf=0.119) was seen in the Xhosa compared to the other two groups studied. The frequency of Dw'blank' in the Xhosa (gf=0.373) and the Cape Coloureds (gf=0.304) is still high despite the new HLA-Dw specificities detected in these groups, whereas the Dw'blank' frequency in SA Caucasoids (gf=0.219) is comparable to the European Caucasoids. This high frequency of Dw'blank' in the Xhosa and Cape Coloureds suggests the existence of even more unidentified HLA-Dw specificities in these populations than in the Caucasoids.

The comparison of HLA-Dw frequencies in the SA Negroes with those in the Nigerians revealed similarities as well as

differences. In both these groups the specificities HLA-Dw8, Dw9, Dw10, Dw13, Dw15 and DKT2 were either absent or present at very low frequencies. However, the Nigerian group differed from the SA Negroes by the absence of Dw4 and Dw19 and a relatively higher frequency of HLA-Dw2 and Dw5. Differences in the HLA-Dw frequencies seen between the SA Negroes and Nigerians were to be expected, as the groups were separated from each other many centuries ago. Random genetic drift, natural selection as well as the admixture of Khoisan genes to the SA Negroes have probably resulted in the differences between their HLA-Dw frequencies and those of the Nigerians.

The HLA-Dw profile in the SA Negroes was different to that of the American Negroes. This was evident from the lower frequency of HLA-Dw3, Dw4, Dw5 and Dw18 and the higher frequency of Dw7 in the American Negroes when compared to the SA Negroes. Similarities were however seen for the HLA-Dw8, Dw9, Dw10 and Dw14 specificities, which were either rare or absent in both groups. It should be noted that HLA-Dw'RSH' hitherto only identified in SA Negroes, has now been identified in the American Negroes (C.Hurley personal communications). The considerable amount of admixture from different groups (i.e. 10% to 20% Caucasoid admixture and approximately 27% American Indian admixture) in the American Negroes (Reed 1969, Foster 1935) is probably the main reason

for the differences in HLA-Dw frequencies seen between the SA Negroes and American Negroes.

The HLA-Dw, DR and DQ data showed that if a certain HLA-Dw specificity was associated with a particular HLA-DR and DQ antigen, this association almost always remained a fixed entity in the different population groups.

CHAPTER 4

HLA-DR AND DQ ANTIGENS IN SOUTH AFRICAN POPULATIONS

The first serological description of human HLA-DR antigens was reported by van Leeuwen et al. (1973) and a general agreement on the polymorphism of the HLA-DR antigens came after the Seventh International Workshop held in Oxford in 1977. Soon afterwards, certain antisera were found to react in a supertypic fashion and these were believed to define cross-reactive groups of HLA-DR antigens. However, Tosi et al. (1978), first recognized that one of the supertypic specificities, DC1, now referred to as HLA-DQw1, was a determinant present on a molecule distinct from the classical HLA-DR antigen. This antigen was shown to belong to a polymorphic system of antigens called the DQ system in the Ninth International Histocompatibility Workshop held in Munich in 1984. There are now 18 HLA-DR antigens, DR1 to DRw18, and 9 HLA-DQ antigens, DQw1 to DQw9, that have been identified (WHO Nomenclature Committee 1988).

A study of the HLA-DR and DQ antigens is important as these antigens are involved in various immune phenomena. They play a major role in cell-cell interactions and antigen presentation to regulator T-cells (Schwartz 1985) and can function as targets for cytotoxic T-cells (Feighery and

Stastny 1979). Furthermore, these antigens seem to be involved in disease susceptibility, particularly those with an immunological component (Svejgaard et al. 1983). Matching for HLA antigens between donor and recipient has a significantly beneficial effect on survival of renal grafts. It has been shown recently (Opelz 1988) that matching for the splits of HLA class I antigens resulted in a better renal transplant outcome and that this effect was potentiated by matching for HLA-DR. From this it was concluded that typing for HLA-DR splits will probably further improve the correlation of HLA matching with graft survival, thus providing further impetus to the detailed study of additional polymorphisms at the HLA loci.

4.1 NEW HLA-DR SPECIFICITIES IDENTIFIED IN SOUTH AFRICAN NEGROES AND CAPE COLOUREDS

The HLA-DR antigens in the Caucasoids are relatively well studied and one can expect very few new HLA antigens to be found in this group, but new HLA-DR antigens can still be found in the less studied populations such as African Negroes and Cape Coloureds. The classical serological method is still the most important way of identifying HLA antigens, but in addition to this method, the HLA antigens and genes can be studied by a variety of methods such as cellular techniques (typing with HTCs, PLTs and T-cell clones), immunochemical techniques (one- and two dimensional

electrophoresis), as well as molecular techniques (Southern blotting and DNA sequencing). In this study we have used the serological, Southern blot, two dimensional gel electrophoresis (DR3 subgroups only), as well as HTC typing techniques for the identification of HLA-DR variants in the SA Negroes and Cape Coloureds. The HLA-DQ antigens are at present still ill-defined in all populations due to lack of good monospecific HLA-DQ antisera. More information on possible new HLA-DQ antigens or splits might however be found by using RFLP analysis as is evident from the data in section 4.1.3 and 5.2.3. The local names of the 10 WS sera and MoAbs used are listed in the Appendix.

4.1.1 HLA-DR2 LUM(CT), a new Negro subgroup of HLA-DR2

The division of DR2 into two entities, one long and one short, was first mentioned by Richiardi et al. (1978) and during the Eighth Workshop, Vassali et al. (1980) and Betuel et al. (1980), confirmed this division. During the Ninth International Histocompatibility Workshop, HLA-DR2 was divided into two serological subgroups, DR2 long and DR2 short (Gebuhrer et al. 1983a). This finding was confirmed in our laboratory (Oudshoorn et al. 1983) and also by Honeyman et al. (1984). Two additional variants of DR2 observed during the Ninth International Histocompatibility Workshop were DR2 LUM(CT) which was identified in SA Negroes in our laboratory (Oudshoorn et al. 1983), and FT31 which was observed in Chinese by Honeyman et al. (1984). However,

this group have since reported that FT31 is more likely to be a DQw1 subgroup than a DR2 subgroup (Doran 1986). During the Tenth International Histocompatibility Workshop the DR2 splits DR2 long and DR2 short were accepted and named DRw15 and DRw16 (WHO Nomenclature Committee 1988). At this Workshop Mervart et al. (1987) reported yet another DR2 variant called DR2.3. The data of the present study shows the identification of the new HLA-DR2 subgroup, DR2 LUM(CT), by serological methods using the Tenth International Histocompatibility Workshop sera, by Southern blot analysis and HLA-Dw typing. The new HLA-DR2 subgroup will be compared to the two officially recognized HLA-DR2 subgroups, DRw15 and DRw16.

Serology

One SA Caucasoid, 5 Cape Coloured and 5 SA Negro (Xhosa) families, as well as 16 SA Caucasoid, 14 Cape Coloured and 12 SA Negro (Xhosa) unrelated individuals with HLA-DR2 were typed using the Tenth International Histocompatibility Workshop (10WS) sera.

The conventional DR2 specificity now known as DRw15 was clearly defined by positive reactions with the 10WS DR2 sera as shown in Table 4.1. HLA-DRw15 was identified in all three population groups: SA Negroes (Xhosa), Cape Coloureds and SA Caucasoids (Table 4.2). The DR2 subgroup HLA-DRw16 was defined by positive reactions with all the 10WS DR2

sera, with exception of the sera 1031 and 1032 (Table 4.1). The serological reaction pattern for HLA-DRw16 was found in one Cape Coloured family (KEM) and in one unrelated Cape Coloured individual (Table 4.2). HLA-DR2 LUM(CT) could be identified by positive reactions with 10WS sera 1030, 1025, 1027, 1031 and 1032 and negative reactions with 10WS sera 1029, 1024, 1026 and 1023. HLA-DR2 LUM(CT) was found in one Cape Coloured family (SLI) and four unrelated SA Negroes (Table 4.1).

TABLE 4.1 Serum reaction patterns with 10WS DR2 sera.

	10WS sera DR2								
	1030	1025	1027	1031	1032	1029	1024	1026	1023
HLA-DR									
DRw15	+	+	+	+	+	+	+	+	+
DRw16	+	+	+	-	-	+	+	+	+
DR2 LUM(CT)	+	+	+	+	+	-	-	-	-

\+ a positive serum reaction
\- a negative serum reaction

The segregation of HLA-DRw15 in family DAN, HLA-DRw16 in family KEM and HLA-DR2 LUM(CT) in family SLI is shown in Table 4.3. In all three Cape Coloured families the reaction pattern for the respective DR2 haplotypes segregated clearly.

In the families tested, DRw15, DRw16 and DR2 LUM(CT) segregated with HLA-DQw1. However, due to the lack of good sera for the HLA-DQw1 splits, DQw5 and DQw6, they could not be assigned to the HLA-DR2 haplotypes.

TABLE 4.2 Number of unrelated DR2 positive individuals[a] tested with 10WS sera in three SA populations studied.

DR2 subgroup	SA Negro (n=17)	Cape Coloured (n=21)	SA Caucasoid (n=17)
DRw15	13	18	17
DRw16	0	2	0
DR2 LUM(CT)	4	1	0

a Only one DR2 haplotype per family was included.

HLA-Dw typing

Family DAN with HLA-DRw15, family KEM with HLA-DRw16 and family SLI with HLA-DR2 LUM(CT) were typed using 59 HTCs with the HLA-Dw specificities HLA-Dw1 to Dw19, Dw21 to Dw23, Dw'BME', Dw'JOH' and Dw'RSH'. No HTC was available for the HLA-DR1 associated specificity HLA-Dw20. The HLA-Dw typing results using HTCs with HLA-DRw15 (Dw2 and Dw12) and HLA-DRw16 (Dw21 and Dw22) related specificities are shown in Table 4.4. The HLA-DRw15 positive members of family DAN were typed as HLA-Dw2 and the HLA-DRw16 cells of family KEM

TABLE 4.3 Segregation of DRw15, DRw16 and DR2 LUM(CT) in families.

					10 WS sera DR2									
					1	1	1	1	1	1	1	1	1	
					0	0	0	0	0	0	0	0	0	
					3	2	2	3	3	2	2	2	2	
		DR	DQ	Dw	0	5	7	1	2	9	4	6	3	
Family DAN														
Mother	c	w15	w1	w2	8[a]	8	8	8	8	8	6	8	6	
	d	w13	w1	w18										
Child 1	b	1	w1	blank	6	8	6	4	6	8	4	8	4	
	c	w15	w1	w2										
Child 2	b	1	w1	blank	6	8	4	6	6	6	6	4	6	
	c	w15	w1	w2										
Child 3	a	4	w3	w4	1	1	1	2	1	1	1	1	1	
	d	w13	w1	w18										
Child 4	b	1	w1	blank	1	1	1	1	1	1	1	2	1	
	d	w13	w1	w18										
Family KEM														
Father	a	7	w3	w11	1	1	1	1	1	1	1	1	1	
	b	w11	w7	w5										
Mother	c	w16	w1	w21	8	8	6	1	4	8	4	8	8	
	d	w10	w1	blank										
Child 1	a	7	w3	w11	8	8	8	1	1	8	8	8	6	
	c	w16	w1	w21										
Child 2	a	7	w3	w11	1	1	4	1	1	1	1	1	1	
	d	w10	w1	blank										
Child 3	b	w11	w7	w5	2	1	1	1	1	1	1	1	1	
	d	w10	w1	blank										
Family SLI														
Mother	c	7	w2	blank	1	1	1	1	1	1	1	1	1	
	d	w18	w4	RSH										
Child 1	a	2LUM	w1	blank	8	8	6	8	8	1	1	4	1	
	c	7	w2	blank										
Child 2	a	2LUM	w1	blank	8	8	6	8	8	1	1	1	4	
	d	w18	w4	RSH										
Child 3	b	4	w3	w4	1	2	1	1	1	1	1	2	1	
	d	w18	w4	RSH										
Grand-child[b]	a	2LUM	w1	blank	8	8	8	8	8	1	1	1	2	
	e	w17	w2	w3										

a The results are scored as follows: 1 = negative; 2 = doubtful negative; 4 = weak positive; 6 = positive; 8 = strong positive

b Offspring of child 3

TABLE 4.4 HLA-Dw typing results (%RR) of family DAN, KEM and SLI using HLA-DRw15 and DRw16 related HTCs.

				DRw15					DRw16		
				Dw2			Dw12		Dw21	Dw22	
Responder cells	HLA haplo-types	HLA-DR	HLA-Dw	Z S I	J D U	H O F	B G E	T O K	F J O	B A S	R E M
Family DAN											
Mother	cd	**w15**,w13	w2,w18	**4**	**18**	**15**	63	nt	75	99	95
Child 1	bc	1,**w15**	blank,w2	**14**	**17**	**26**	71	nt	89	119	102
Child 2	bc	1,**w15**	blank,w2	**16**	**9**	**19**	64	nt	105	87	93
Child 3	ad	4,w13	w4,w18	101	141	83	89	nt	94	115	132
Child 4	bd	1,w13	blank,w18	119	99	114	113	nt	99	87	117
Family KEM											
Father	ab	7,w11	w11,w5	106	111	108	84	93	80	132	92
Mother	cd	**w16**,w10	w21,blank	97	102	77	75	87	**17**	43	55
Child 1	ac	7,**w16**	w11,w21	89	98	104	71	111	**16**	35	51
Child 2	ad	7,w10	w11,blank	112	132	84	87	81	118	112	112
Child 3	bd	w11,w10	w5,blank	99	87	77	80	100	91	77	100
Family SLI											
Mother	cd	7,w18	blank,RSH	109	85	94	124	nt	96	121	112
Child 1	ac	**2LUM**,7	blank,blank	53	64	69	97	nt	135	65	105
Child 2	ad	**2LUM**,w18	blank,RSH	53	66	47	73	nt	81	92	65
Child 3	bd	4,w18	w4,RSH	117	111	99	92	nt	73	66	96
Grand-child	ae	**2LUM**,w17	blank,w3	49	54	64	81	nt	80	108	73

nt = not tested

Typing response = ≮50% RR

as Dw21. In addition, HTCs with the HLA-DRw16,Dw22 specificity gave borderline typing reactions with the HLA-DRw16,Dw21 haplotype in family KEM, indicating some shared stimulatory determinants between these specificities. Although no HLA-Dw specificity could be assigned to the HLA-DR2 LUM(CT) cells, shared stimulatory determinants with Dw2 positive cells was evident from the borderline typing reactions with HTCs with the Dw2 specificity.

RFLP analysis

Southern blot analysis was performed on the two Cape Coloured families, SLI {DR2 LUM(CT)} and KEM (DRw16). The HLA-DRw15 specificity was represented by two cell lines JDU and ZSI, homozygous for HLA-A2,B7,Cw7,DRw15,DQw6,Dw2.

The results of restriction endonuclease fragment patterns for DNA isolated from family KEM and SLI, digested with Taq I and Eco RI, and hybridized to the DRB cDNA probe are shown in Figures 4.1 and 4.2. A summary of the DRB results with these restriction endonucleases, showing distinction between DRw15, DRw16 and DR2 LUM(CT) is given in Table 4.5. The RFLP pattern observed after digestion of DNA from the DRw15 cell lines with Taq I showed an 11.5 and a 1.3 kb fragment which was not present in either family KEM (DRw16) or SLI {DR2 LUM(CT)}. A 1.6 kb fragment segregated with the DRw15 as well as the DR2 LUM(CT) haplotypes but was absent in the DRw16 haplotype. A 14.1 and 1.5 kb kb Taq I fragment

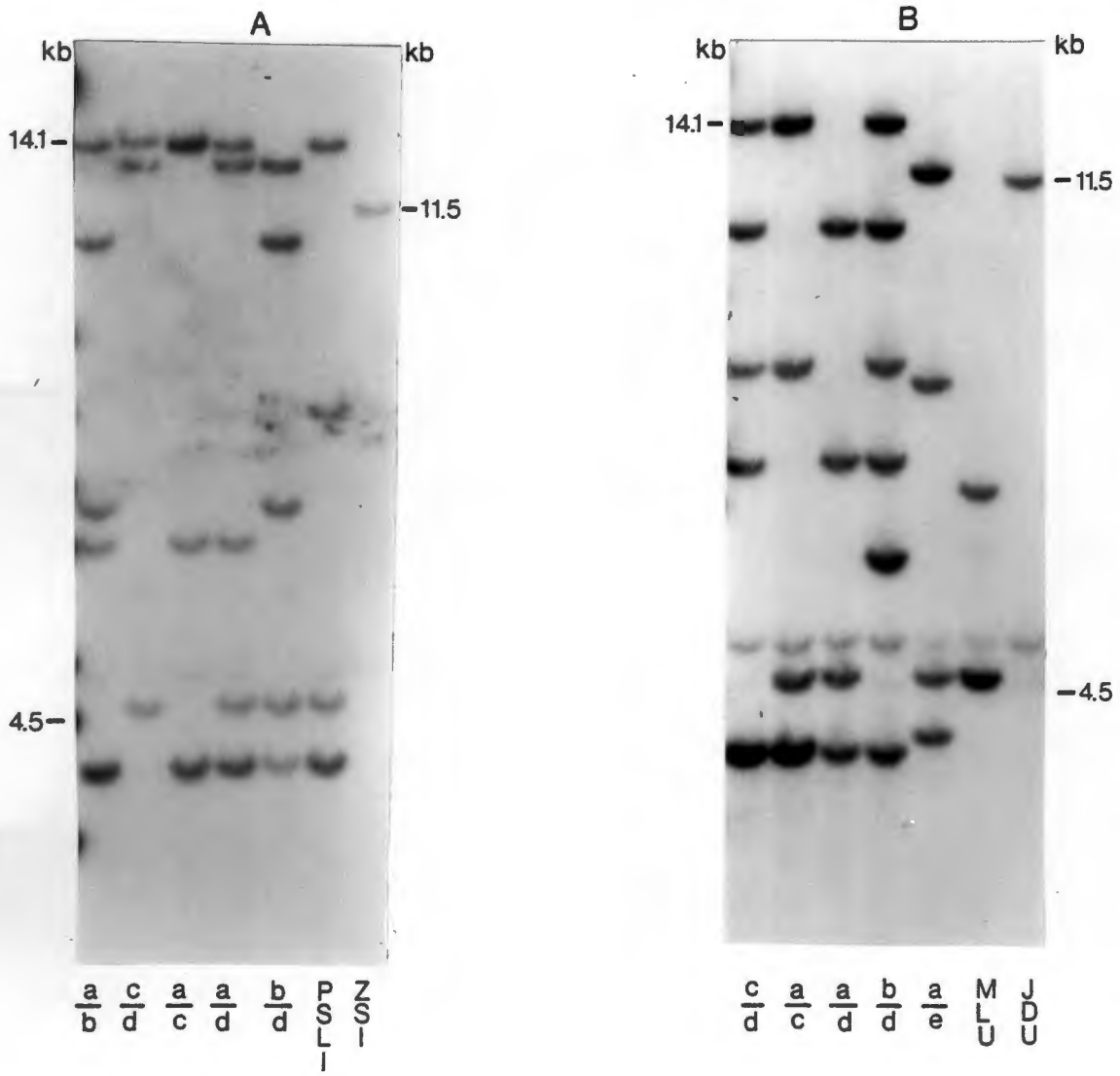

FIGURE 4.1 Hybridization of the DRB probe to DNA digested with Taq I and separated on a 0.6% agarose gel.

A. DNA obtained from family KEM (DRw16), a DR2 LUM(CT),DR7 positive member (PSLI) of family SLI and a homozygous cell line ZSI (DRw15).

Haplotypes:

a	A29,	B7,	Cw'blank',	DR7,	DQw3, Dw11
b	A2,	Bw72,	Cw2,	DRw11,	DQw7, Dw5
c	Aw68,	B44,	Cw7,	DRw16,	DQw5, Dw21
d	Aw34,	Bw75,	Cw4,	DRw10,	DQw5, Dw'blank'

B. DNA obtained from family SLI {DR2 LUM(CT)}, an unrelated individual (MLU) with DR1, DR2 LUM(CT) and a homozygous cell line JDU (DRw15).

Haplotypes:

a	A32,	B27,	Cw2,	DR2 LUM(CT),	DQw6,	Dw'blank'
b	Aw43,	Bw71,	Cw4,	DR4,	DQw3,	Dw4
c	Aw43,	Bw64,	Cw8,	DR7,	DQw2,	Dw'blank'
d	A30,	B7,	Cw2,	DRw18,	DQw4,	Dw'RSH'
e	A23,	B7,	Cw4,	DRw17,	DQw2,	Dw3

The 11.5 kb fragment is specific for DRw15, whereas the 14.1 kb fragment is shared by the DRw16 and DRw53 cells and the 4.5 kb fragment is present in DR2 LUM(CT), DR1 and DRw10 cells. The smaller fragments are not shown in this figure.

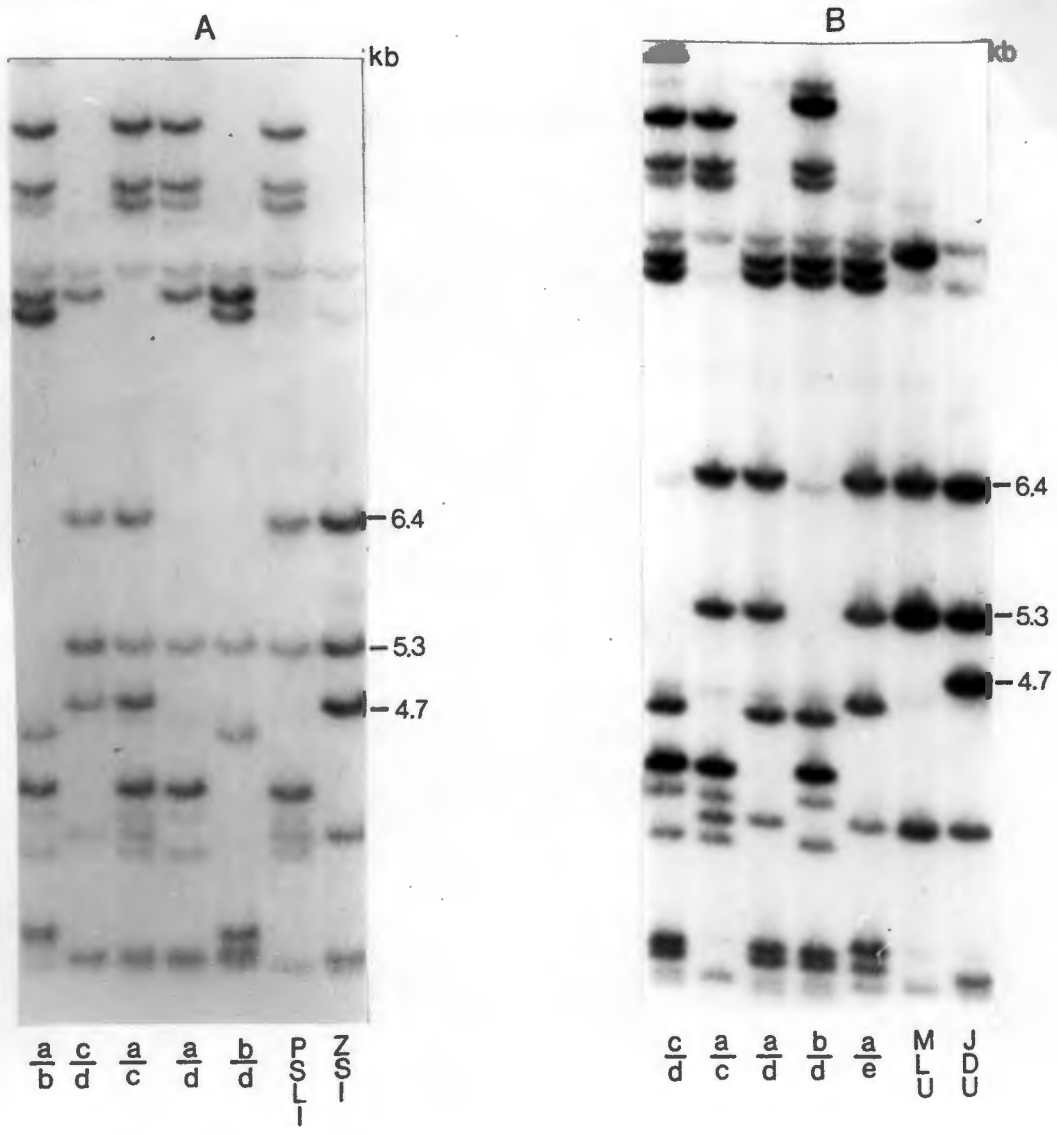

FIGURE 4.2 Hybridization of the DRB probe to DNA digested with Eco RI and separated on a 0.6% agarose gel.

A. DNA obtained from family KEM (DRw16), a DR2 LUM(CT),DR7 positive member (PSLI) of family SLI and a homozygous cell line ZSI (DRw15).

Haplotypes:

a	A29,	B7,	Cw'blank',	DR7,	DQw3, Dw11
b	A2,	Bw72,	Cw2,	DRw11,	DQw7, Dw5
c	Aw68,	B44,	Cw7,	DRw16,	DQw5, Dw21
d	Aw34,	Bw75,	Cw4,	DRw10,	DQw5, Dw'blank'

B. DNA obtained from family SLI {DR2 LUM(CT)}, an unrelated individual (MLU) with DR1, DR2 LUM(CT) and a homozygous cell line JDU (DRw15).

Haplotypes:

a	A32,	B27,	Cw2,	DR2 LUM(CT),	DQw6, Dw'blank'
b	Aw43,	Bw71,	Cw4,	DR4,	DQw3, Dw4
c	Aw43,	Bw64,	Cw8,	DR7,	DQw2, Dw'blank'
d	A30,	B7,	Cw2,	DRw18,	DQw4, Dw'RSH'
e	A23,	B7,	Cw4,	DRw17,	DQw2, Dw3

The 6.4 kb fragment segregated with the three DR2 splits, DRw15, DRw16 and DR2 LUM(CT). The 5.3 kb fragment is shared by the three DR2 splits, DR1 and DRw10. The 4.7 kb fragment is present in DRw15 and DRw16 cells but absent in DR2 LUM(CT) cells.

TABLE 4.5 Summary of DRB RFLPs among HLA-DR2 positive cells.

		Taq I(kb)						Eco RI(kb)		
HLA-DR	HLA-DQ	14.1[a]	11.5	4.5[b]	1.6	1.5	1.3	6.4	5.3[b]	4.7
w15	w6	-	+	-	+	-	+	+	+	+
w16	w5	+	-	-	-	+	-	+	+	+
2 LUM(CT)	w6	-	-	+	+	-	-	+	+	-

+ indicates fragment present

- indicates fragment absent

a co-segregation with DRw53

b co-segregation with DR1 and DRw10

segregated with HLA-DRw16 in family KEM. The 14.1 kb fragment also segregated with HLA-DRw53 on haplotype (a) of family KEM and haplotypes (b) and (c) of family SLI, but did not segregate with DRw15 or DR2 LUM(CT). A 4.5 kb Taq I fragment segregated with DR2 LUM(CT) on haplotype (a) of family SLI (Figure 4.1B). The 4.5 kb Taq I fragment also segregated with DRw10 on haplotype (d) of family KEM (Figure 4.1A), but was not seen with the DRw16 positive individuals of family KEM (haplotype c) nor with the DRw15 positive cell lines (ZSI and JDU) (Figure 4.1). Two Eco RI fragments (6.4 and 5.3 kb) segregated with all three DR2 subgroups (Figure 4.2) and were thus not informative for the distinction between these subgroups. It should be noted that the 5.3 kb Eco RI fragment also segregated with DRw10 on haplotype (d) of family KEM and with DR1 (data not shown). A 4.7 kb Eco RI fragment segregated with the DRw16 haplotype (c) in family KEM and with the DRw15 cell lines JDU and ZSI, but not with HLA-DR2 LUM(CT).

Results obtained using the DQB cDNA probe are summarized in Table 4.6. The RFLPs seen for the DQw1 antigen associated with the DR2 subgroups, DRw15 and DR2 LUM(CT), were identical whereas the RFLP observed for the DQw1 antigen associated with DRw16 was clearly different. By comparing this data to that of Bidwell (1988), it can be seen that the RFLP pattern for the DQw1 antigen associated with DRw15 and

DR2 LUM(CT) correlated with DQw6 and that of the DRw16 haplotype with DQw5.

TABLE 4.6 Summary of DQB RFLPs among HLA-DR2 positive cells.

		Taq I(kb)			Eco RI(kb)		
HLA-DR	DQ	5.5	3.0	2.6	12.2	9.1	2.3
w15	w6	-	+	+	-	+	+
2 LUM(CT)	w6	-	+	+	-	+	+
w16	w5	+	-	-	+	-	-

+ indicates fragment present
- indicates fragment absent

Although both the DQA1 and DQA2 RFLPs are detected with the DQA probe their respective RFLPs could easily be distinguished. The DQA2 series is di-allelic (Festenstein et al. 1986), with a 2.2 kb Taq I and a 1.35 kb Eco RI

TABLE 4.7 DQA RFLP patterns associated with the DQA2 gene obtained using the restriction endonucleases Taq I and Eco RI.

	Taq I (kb)		Eco RI (kb)	
	2.2	2.1	2.0	1.35
Pattern 1	+	-	-	+
Pattern 2	-	+	+	-

fragment (pattern 1) corresponding to the one allele and with a 2.1 kb Taq I and 2.0 kb Eco RI (pattern 2) to the other allele (Table 4.7).

As shown in Table 4.8 the DQA2 pattern 1 was present in the DRw15 haplotypes studied here as well as the DR2 LUM(CT) haplotype of family SLI, whereas the the DQA2 pattern 2 was obtained for the DRw16 haplotype of family KEM. The remaining 6.2 kb Taq I and 15.0 kb Eco RI fragments probably corresponded to the DQA1 gene. These fragments were present in all three DR2 subgroups, thus showing identical RFLPs associated with the DQA1 gene.

TABLE 4.8 Summary of DQA RFLPs among HLA-DR2 positive cells.

		Taq I (kb)			Eco RI (kb)		
HLA-DR	DQ	6.2	2.2	2.1	15.0	2.0	1.35
w15	w6	+	+	-	+	-	+
2LUM(CT)	w6	+	+	-	+	-	+
w16	w5	+	-	+	+	+	-

+ indicates fragment present
- indicates fragment absent

In *summary*, in this section we have described the polymorphism among the DR2 haplotypes using serological, cellular and DNA analysis.

In a previous study (Oudshoorn et al. 1983), we reported a new DR2 subgroup, DR2 LUM(CT). Our results, using the Tenth International Histocompatibily Workshop sera confirmed the previous suggestion of heterogeneity of the HLA-DR2 antigen. As shown in Table 4.2 there is considerable variation in the distribution of the DR2 subgroups in the three populations tested. HLA-DRw15 was the only DR2 subgroup seen in the SA Caucasoids, and was also the most frequent DR2 subgroup in the Cape Coloureds and SA Negroes. HLA-DRw16 was seen in only two of 21 Cape Coloureds with DR2, while DR2 LUM(CT), virtually confined to SA Negroes, was seen in 4 unrelated individuals. Although no HLA-Dw specificity could be assigned to the HLA-DR2 LUM(CT) haplotypes, borderline typing results were seen with HTCs with the HLA-Dw2 specificity indicating shared stimulatory determinants. A possible sharing of stimulatory determinants was also seen between cells with the Dw21 and Dw22 specificities, in accordance with studies by Reinsmoen et al. (1984) and Freidel et al. (1984). Following digestion with Taq I and hybridization with a DRB probe, different RFLPs were seen for each DR2 subgroup. The 4.5 kb Taq I fragment shown to segregate with DR2 LUM(CT) also segregated with DR1 and DRw10, indicating sequence homology between these alleles.

Digests of Eco RI showed similar RFLP patterns for DRw15 and DRw16. This RFLP pattern was, however, different to that observed for DR2 LUM(CT). The DQB RFLP patterns obtained for the HLA-DRw15 and DR2 LUM(CT) specificities correlated with the DQw1 split DQw6, whereas that of HLA-DRw16 correlated with the DQw1 split DQw5. The RFLP pattern associated with the DQA1 gene was the same for the three DR2 subgroups. An identical DQA2 pattern, pattern 1, was seen for the DRw15 homozygous cell lines and the DR2 LUM(CT) haplotype whereas the DRw16 haplotype was shown to have the DQA2 pattern 2. An additional HLA-DR2 LUM(CT) haplotype was found in a Cape Coloured family described in section 5.2.4. This haplotype was identical to the DR2 LUM(CT) haplotype presented in this section, with the exception of its RFLP results corresponding to the DQA2 gene. The DQA RFLP results of the DR2 LUM(CT) haplotype studied in section 5.2.4 showed that this haplotype had the DQA2 pattern 2 instead of pattern 1.

4.1.2 HLA-DRw18, a new Negro subgroup of HLA-DR3

The HLA-DR3 antigen was first described by the Leiden group in 1973 and 1975 (van Leeuwen et al. 1973, van Rood et al. 1975) and was clearly defined serologically at subsequent International Histocompatibility Workshops (Bodmer et al. 1978, Curtoni and Borelli 1980, Fuller et al. 1984). During the Tenth International Histocompatibility Workshop, the existence of subgroups of DR3 i.e. DRw17 (previously DR3.1)

and DRw18 (previously DR3.2) became evident (Oudshoorn et al. 1987b). In this section, we report the identification and characterization of the HLA-DR3 subgroups, HLA-DRw17 and DRw18, using serological, Southern blot and 2-D gel electrophoresis methods. The Dw specificities associated with the HLA-DR3 subgroups have been discussed in section 3.1.3 and will not be discussed here.

Serology

Six SA Negro, 4 Cape Coloured and 2 SA Caucasoid families as well as 39 unrelated SA Negroes, 9 Cape Coloureds and 7 SA Caucasoids positive for HLA-DR3 were included in this study. These subjects were selected for HLA typing during the Tenth International Histocompatibility Workshop for various reasons such as possible new antigens, inclusion in several "study groups" and for Southern blot studies.

Analysis of the serological data revealed two patterns of reaction (Table 4.9). The first pattern defines the conventional DR3 specificity DRw17 and can be identified by positive reactions with Tenth International Histocompatibility Workshop (10WS) sera 1039, 1040, 1043, 1037, 1034, 1036, 1038 and 10WS MoAb 3023. Pattern 2 defines the DR3 variant, DRw18, and is characterized by variably positive reactions with 10WS sera 1039, 1040, 1043, 1037 and negative reactions with 10 WS sera 1034, 1036, 1038 and MoAb 3023.

TABLE 4.9 Serological patterns with 10 WS DR3 sera.

	10 WS DR3 sera								
	1	1	1	1	1	1	1	3	
	0	0	0	0	0	0	0	0	
	3	4	4	3	3	3	3	2	
DR3 splits	9	0	3	7	4	6	8	3	
DRw17	+	+	+	+	+	+	+	+	pattern 1
DRw18	+	+	+	+	-	-	-	-	pattern 2

+ = positive serum reaction
- = negative serum reaction

All of these antisera behaved as monospecific DR3 reagents in our hands, except for 10WS 1040, which contained DRw52 antibodies, and the MoAb 3023, which recognized DR1 and DRw8. Table 4.10 shows the segregation of DRw17 and DRw18 in two Cape Coloured families (PLA and SLI). HLA-DRw17 was present on the paternal haplotype (a) of family PLA and was inherited by two of his children (child 1 and 2). HLA-DRw17 was also present in one individual of family SLI (Table 4.10). The maternal (d) haplotype in this family carried the HLA-DRw18 antigen and this haplotype was passed on to two of her children (child 2 and 3).

The number of unrelated DR3 positive individuals, including parents, with DRw17 and DRw18 found in this study is shown in Table 4.11. The DRw18 specificity was seen in 30 of 46 SA Negroes (65%), 3 of 12 Cape Coloureds (25%) and was

TABLE 4.10 Segregation patterns for HLA-DRw17 and DRw18 in families.

	Haplo-types	HLA-DR	DQ	10WS DR3 sera							
				1	1	1	1	1	1	1	3
				0	0	0	0	0	0	0	0
				3	4	4	3	3	3	3	2
				9	0	3	7	4	6	8	3
Family PLA											
Father	a	**w17**	**w2**	**8**[a]	**8**	**8**	**8**	**8**	**8**	**6**	**8**
	b	7	w3								
Mother	c	w15	w6	1	1	1	1	1	1	1	1
	d	w8x14	w5								
Child 1	a	**w17**	**w2**	**8**	**8**	**8**	**6**	**6**	**4**	**8**	**6**
	d	w8x14	w5								
Child 2	a	**w17**	**w2**	**8**	**8**	**8**	**8**	**8**	**8**	**4**	**6**
	c	w15	w6								
Child 3	b	7	w3	2	1	1	1	1	1	1	1
	c	w15	w6								
Child 4	b	7	w3	1	1	1	1	1	1	1	1
	d	w8x14	w5								
Family SLI											
Mother	c	7	w2	**8**	**8**	**8**	**8**	1	1	2	2
	d	**w18**	**w4**								
Child 1	a	2LUM(CT)	w6	1	1	1	1	1	1	1	1
	c	7	w2								
Child 2	b	4	w3	**8**	**8**	**8**	**8**	1	1	1	2
	d	**w18**	**w4**								
Child 3	a	2LUM(CT)	w6	**8**	**8**	**8**	**8**	2	2	1	1
	d	**w18**	**w4**								
Grand-child[b]	a	2LUM(CT)	w6	**8**	**8**	**8**	**8**	**6**	**8**	**6**	**8**
	e	**w17**	**w2**								

a The results are scored as follows: 1 = negative; 2 = doubtful negative; 4 = weak positive; 6 = positive; 8 = strong positive

b The grandchild is an offspring of child 3

absent in the nine SA Caucasoids tested. HLA-DRw18 was seen with Bw42 and DQw4 in 18 of 30 SA Negroes and 3 of 3 Cape Coloureds. HLA-DRw17 was associated with B8 and DQw2 in 7 of 9 SA Caucasoids, 7 of 9 Cape Coloureds, and 4 of 16 SA Negroes. HLA-DRw17 was also associated with Bw71 and DQw2 in 7 of 16 SA Negroes. Since this sample was not drawn at random, the delta values as an estimate of linkage disequilibrium could not be calculated.

TABLE 4.11 Number of unrelated individuals with DRw17 and DRw18 in the three South African (SA) population groups.

Population	DRw17 (n=37)	DRw18 (n=33)
SA Negroes	16	30
Cape Coloureds	12	3
SA Caucasoids	9	0

RFLP analysis

The HTCs used are listed in Table 4.12 and were selected so that the two HLA-DR3 haplotypes most commonly seen in Caucasoids (B8,DR3,DQw2; B18,DR3,DQw2) as well as the most common SA Negro DR3 haplotype (Bw42,DR3,DQw4) would be represented in this study. The HTC-RSH is of Zulu origin, HTC-BTI of Xhosa origin, while HTC-COX, MITCH and LOO81785 are of Caucasoid origin. The HTCs were transformed using Epstein-Barr virus (section 2.2.7) and the lymphoblastoid

cell lines obtained were used as a source of DNA for Southern blotting.

TABLE 4.12 HLA-DR3 positive homozygous cell lines.

Cell line	10WS number	HLA typing						
		A	B	C	DR	DQ	Dw	Dw[a]
COX	10W 9022	1	8	w7	w17	w2	3	24
MITCH	-	1	8	w7	w17	w2	3	nt
LOO81785	10W 9018	3,24	18	w5	w17	w2	3	25
RSH	10W 9021	30,w68	w42	w2	w18	w4	RSH	24
BTI	-	30	w42	w2	w18	w4	RSH	nt

nt = not tested

a Alleles of the DRB3 gene (DRw52)

Autoradiographs obtained using a DRB probe with DNA from the HTCs cleaved with endonucleases Taq I and Eco RI are depicted in Figure 4.3 A and B respectively. The two locally defined DRw18 cell lines, RSH and BTI, showed identical hybridization patterns and could easily be distinguished from the DRw17 cell lines. The HLA-DRw18 specificity was associated with two Taq I fragments of 6.2 and 4.1 kb, as well as a 4.4 kb Eco RI fragment, while DRw17 was associated with two different Taq I fragments of 7.1 and 4.2 kb, together with a 4.5 kb Eco RI fragment (Figure 4.3). The 4.2 kb Taq I and 4.5 kb Eco RI fragments of cell line LOO81785 (DRw17) migrated slightly further than expected and this observation was confirmed by repeat testing. The reason for this discrepancy is still unclear. In order to

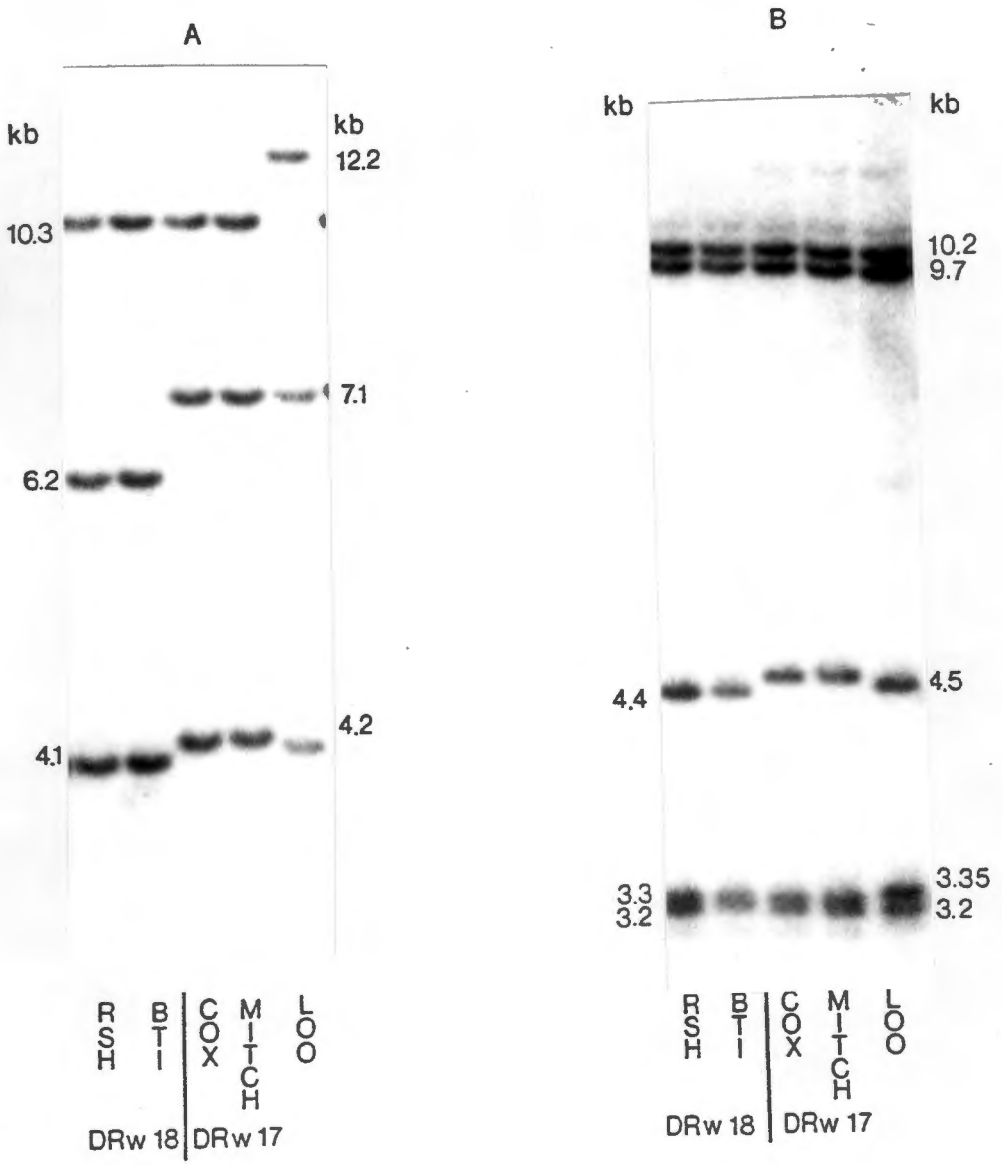

FIGURE 4.3 Autoradiographs obtained after hybridization of DRB cDNA probe with DNA digested with (**A**) Taq I and (**B**) Eco RI, from DRw17 and DRw18 homozygous cell lines. The fragment sizes are indicated adjacent to the corresponding bands.
LOO = cell line LO081785.

clarify this discrepancy the cell line LO081785 will be tested together with another B18,DRw17,DQw2 homozygous cell line QBL to see if the apparent discrepancy is specific for B18,DRw17,DQw2 cell lines or merely for cell line LO081785. Furthermore, the Dw24 positive cell lines COX (DRw17) and RSH (DRw18) possessed an additional 10.3 kb Taq I fragment, whereas the Dw25 positive cell line LO081785 (DRw17) has an additional 12.2 kb Taq I fragment. It is likely that the fragments which distinguish DRw17 from DRw18 are associated with the respective DRB1 genes, while the fragments which distinguish between the Dw24 and Dw25 positive cell lines are associated with the DRB3 genes of these haplotypes, as has been previously suggested (Bontrop et al. 1986b). In addition analysis of numerous HLA-DRw52 positive cells showed that the 10.2, 9.7, 3.2, 3.3 and 3.35 kb Eco RI fragments were associated with the DRB3 gene. The 9.7 kb Eco RI fragment was present in all DRw52 cells, whereas the 10.2 kb fragment was present in all DRw52 cells with the exception of DRw8 cells. The 3.2, 3.3 and 3.35 kb fragments appeared to demonstrate further polymorphism of the DRB3 gene which could not be correlated with the T-cell defined DRw52 polymorphism (i.e. Dw24, Dw25 and Dw26). The hybridization pattern observed for the DRw18 specificity using the Taq I enzyme appeared to be identical to that observed for DRw13,DQw3 (Bidwell 1988).

The segregation of the hybridization pattern defining DRw18 was studied in 10WS family SLI (Figure 4.4). The mother and two children (C2 and C3) carried DRw18. It is evident from the results obtained using a DRB probe that the inheritance of DRw18 correlated with the 6.2 and 4.1 kb Taq I fragments and the 4.4 kb Eco RI fragment as shown in the cell lines. The 10.3 kb Taq I fragment which segregated with this haplotype would indicate its association with the DRB3 allele Dw24. This association between DRw18 and the Dw24 RFLP pattern was also seen in the three other families and three unrelated individuals studied. The hybridization pattern observed for the DRw17 specificity, seen only in the grandchild of this family, was identical to that observed for cell line LOO81785, suggesting that this haplotype carried the Dw25 specificity.

The results obtained using the DQB probe are summarized in Table 4.13. The haplotype DRw18,DQw4 showed an identical hybridization pattern in the cell lines RSH and BTI and in the haplotype (d) of family SLI. This pattern was clearly different to that observed for the DRw17,DQw2 haplotypes in cell lines COX, MITCH and LOO81785 and in family SLI (haplotype e).

Table 4.14 shows the results obtained with the DQA probe. The DQA2 pattern 1 (2.2 kb Taq I and 1.35 Eco RI fragments)(Table 4.7) was observed for the B8,DRw17,DQw2

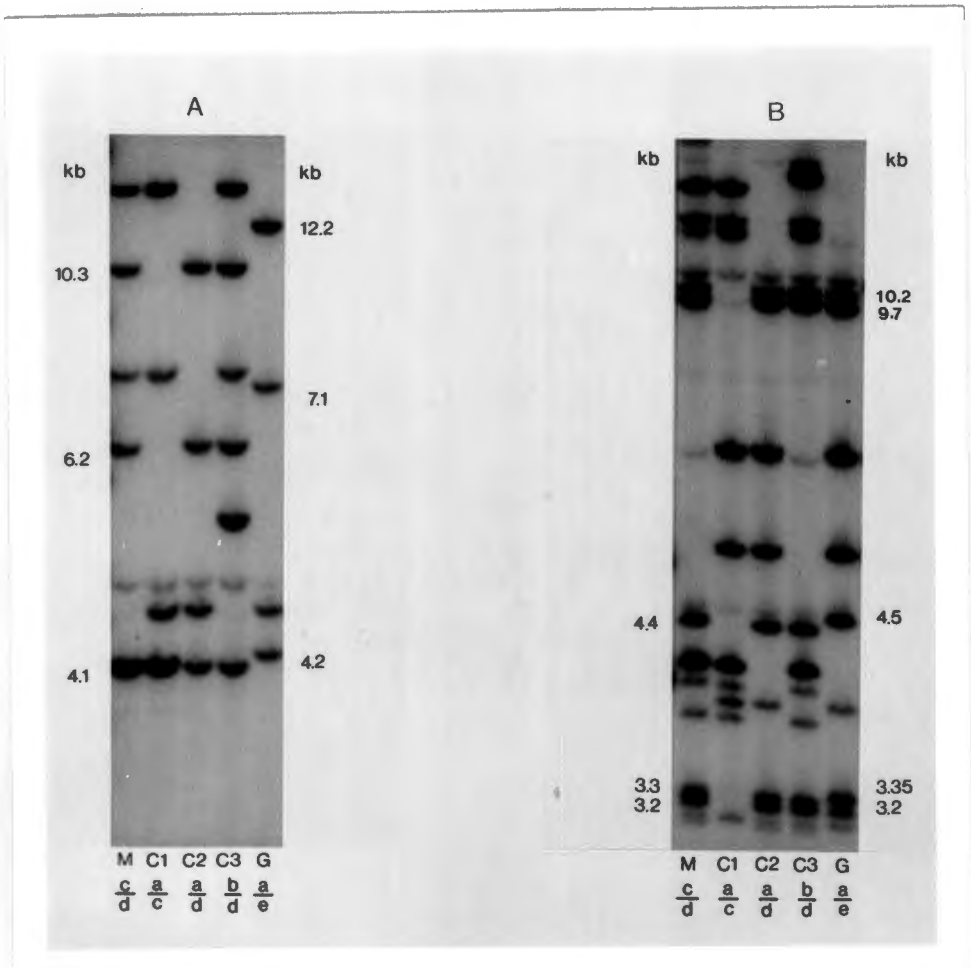

FIGURE 4.4 Autoradiographs obtained after hybridization of DRB cDNA probe with DNA digested with (**A**) Taq I and (**B**) Eco RI, from family 10WS 138.
Fragment sizes in kilobases are indicated adjacent to the corresponding bands. M = mother, C1-3 = children and G = grandchild.

Haplotypes:

a	A32,	B27,	Cw2,	DR2 LUM(CT),	DQw6,	Dw'blank'
b	Aw43,	Bw71,	Cw4,	DR4,	DQw3,	Dw4
c	Aw43,	Bw64,	Cw8,	DR7,	DQw2,	Dw'blank'
d	A30,	B7,	Cw2,	DRw18,	DQw4,	Dw'RSH'
e	A23,	B7,	Cw4,	DRw17,	DQw2,	Dw3

TABLE 4.13 Summary of DQB RFLPs in HLA-DRw17 and HLA-DRw18 homozygous cell lines and DRw17 and DRw18 haplotypes of family SLI and PLA.

Cell identification	HLA-DR	DQ	Dw[a]	Taq I (kb)			Eco RI (kb)	
				4.6	2.4	1.9	19.5	12.5
COX	w17	w2	24	+	-	-	-	+
MITCH	w17	w2	nt	+	-	-	-	+
LO081785	w17	w2	25	+	-	-	-	+
Haplotype (e)(SLI)	w17	w2	nt	+	-	-	-	+
RSH	w18	w4	24	-	+	+	+	-
BTI	w18	w4	nt	-	+	+	+	-
Haplotype (d)(SLI)	w18	w4	nt	-	+	+	+	-

a = alleles of the DRB3 (DRw52) gene

\+ indicates fragment present

\- indicates fragment absent

nt = not tested

TABLE 4.14 Summary of DQA RFLPs in HLA-DRw17 and HLA-DRw18 homozygous cell lines and DRw17 and DRw18 haplotypes of family SLI and PLA.

Cell identification	HLA-DR	DQ	Dw[a]	Taq I (kb)				Eco RI (kb)		
				6.2	4.7	2.2	2.1	12.1	2.0	1.35
COX	w17	w2	24	-	+	+	-	+	-	+
MITCH	w17	w2	nt	-	+	+	-	+	-	+
LOO81785	w17	w2	25	-	+	-	+	+	+	-
Haplotype (e)(SLI)	w17	w2	nt	-	+	-	+	+	+	-
RSH	w18	w4	24	+	-	-	+	+	+	-
BTI	w18	w4	nt	+	-	-	+	+	+	-
Haplotype (d)(SLI)	w18	w4	nt	+	-	+	-	+	-	+

a = alleles of the DRB3 (DRw52) gene

\+ indicates fragment present

\- indicates fragment absent

nt = not tested

cell lines and the DRw18,DQw4 haplotype (d) of family SLI. DQA2 pattern 2 (2.1 kb Taq I and 2.0 Eco RI fragments)(Table 4.7) was seen for the non-B8,DRw17,DQw2 haplotypes and the DRw18,DQw4 homozygous cell lines. The DRw18,DQw4 haplotypes were thus shown to be heterozygous for the RFLP pattern corresponding to the DQA2 gene. Both the B8,DRw17,DQw2 and non-B8,DRw17,DQw2 haplotypes showed the 4.7 kb Taq I fragment corresponding to the DQA1 gene, while a 6.2 kb Taq I fragment was present in the DRw18,DQw4 haplotypes. The fragment corresponding with the DQA1 gene obtained using the restriction endonuclease Eco RI (12.1 kb) was identical for all DR3 haplotypes tested.

2-D gel analysis

Lymphoblastoid cell lines of three HTCs COX, LOO81785 and RSH (Table 4.12) were used as a source of HLA class II antigens for 2-D gel electrophoresis. These cell lines are representative of the three different HLA-DR3 haplotypes studied. Three different MoAbs were used: MoAb 7.3.19.1 (van Rood, Leiden), MoAb TAL-1B5 (Bodmer, London) and MoAb L243 (Bach, Minnisota). MoAb 7.3.19.1 isolates two DR molecules from HLA-DR3 homozygous cell lines (Bontrop et al. 1986b), MoAb TAL-1B5 binds to epitopes on the alpha chain subunits of class II molecules, but also precipitates the beta chains under normal conditions (Adams et al. 1983) and MoAb L243 is directed against DR molecules (Shackelford et al. 1982).

The autoradiographs shown in Figure 4.5 were selected since they best demonstrated the two sets of beta chain spots observed. Up to four different spots were seen for each beta chain, due to the sequential glycosylation which occurs during biosynthesis of the polypeptide. The beta chain which has been labelled DRB1 in Figure 4.5 migrated to a similar position in all three cell lines. The other beta chain, labelled DRB3, was poorly resolved in the cell lines COX and RSH but could still be clearly identified at the basic end of the gel, while in the cell line LOO81785 this beta chain migrated to a more acidic position. From a knowledge of the DRw52 T-cell defined specificities assigned to these cells, it was possible to conclude that the beta chain spots labelled DRB3 corresponded to the DRB3 (DRw52) gene product, while the spots labelled DRB1 corresponded to the DRB1 gene product. These findings are also in agreement with previous reports (Bontrop et al. 1986b, Dasgupta et al. 1988). It was thus not possible to distinguish between the DRB1 gene products of the DRw17 and DRw18 specificities using 2-D gel electrophoresis.

In *summary*, the results in the present study demonstrated that HLA-DR3 could be subdivided into two serologically distinct specificities, DRw17 and DRw18. In addition, we demonstrated that these specificities can be identified by Southern blot analysis using the restriction endonucleases

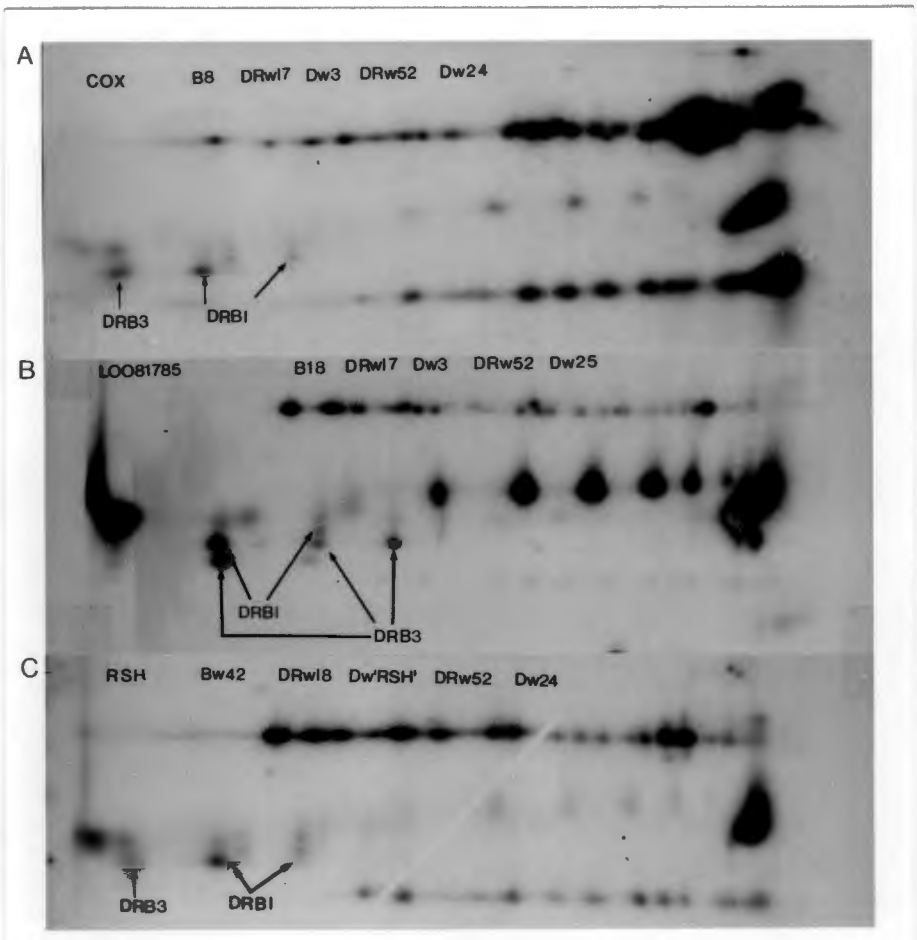

FIGURE 4.5 Two-dimensional gel patterns of three HLA-DR3 homozygous cell lines COX, LOO81785 and RSH. **A)** The HLA-DR molecules isolated from cell line COX by immunoprecipitation with MoAb 7.3.19.1. **B)** The HLA-DR molecules isolated from cell line LOO81785 by immunoprecipitation with MoAb TAL-1B5. **C)** The HLA-DR molecules isolated from cell line RSH by immunoprecipitation with MoAb L243. The arrows labelled DRB1 and DRB3 indicate the DRB1 and DRB3 gene products respectively.

Taq I and Eco RI. HLA-DRw18 was associated with the DRB Taq I fragments of 6.2 and 4.1 kb and an Eco RI fragment of 4.4 kb, whereas DRw17 could be identified by the presence of the DRB Taq I fragments of 7.1 and 4.2 kb and an Eco RI fragment of 4.5 kb. These RFLPs thus correlated with the presence of the DRB1 allelic products DRw17 and DRw18. The RFLP pattern observed for DRw18 was also shown to segregate in the family SLI. In contrast, the 2-D gel electrophoresis patterns could not distinguish between the DRB1 gene products of the HLA-DRw17 and DRw18 HTCs investigated. cDNA sequencing data reported by Hurley et al. (unpublished observations), may provide an explanation for these 2-D gel patterns. The predicted amino acid sequence of the HLA-DRw18 beta chain differs from that of HLA-DRw17 by four substitutions in the first domain, resulting in negligible molecular weight and pI differences between these two polypeptide chains, thus explaining the identical position of the DRW17 and DRw18 gene products on the autoradiographs.

Using a combination of 2-D gel electrophoresis, Southern blot analysis and cellular recognition assays, Bontrop et al. (1986b) showed that one of the two DR beta chains in the B8,DR3 positive cells differed from that of B18,DR3 positive cells. These differences in DR beta chains were subsequently shown to correlate with the DRB3 alleles Dw24 (LB-Q4) present in B8,DR3 cells and Dw25 (LB-Q1) present in B18,DR3 cells (Termijtelen et al. 1987a,b). The cell lines

COX, RSH and LO081785 were typed in the Tenth International Histocompatibility Workshop for the HLA-Dw (T-cell defined) specificities Dw24 and Dw25, which are coded for by the DRB3 (DRw52) gene (Table 4.12). Both COX and RSH were Dw24 positive, whereas the Dw25 determinant was present on LO081785. The results of the current study suggest that the presence of the 10.3 kb Taq I fragment are correlated with the DRB3 allele Dw24, while the 12.2 kb Taq I fragment correlated with the DRB3 allele Dw25. Although MITCH and BTI have not been tested for these determinants, the Southern blot results would indicate that they are both Dw24 positive. This is in agreement with previous reports describing the association of Dw24 with both of the haplotypes B8,DRw17 (Termijtelen et al. 1987a) and Bw42,DRw18 (Hurley unpublished observations).

The results obtained using the DQB probe revealed hybridization patterns characteristic for the different serologically defined HLA-DQ specificities of the cell lines tested.

The DQA2 RFLP pattern 1 (Table 4.7) was observed for the B8,DRw17,DQw2 haplotypes while the DQA2 pattern 2 (Table 4.7) was seen for the non-B8,DRw17,DQw2 haplotypes. Both RFLP patterns were seen among the DRw18,DQw4 haplotypes, thus showing DQA2 heterogeneity associated with the DRw18,DQw4 haplotype. The RFLP pattern obtained for the

DRw18,DQw4 haplotypes and corresponding to the DQA1 gene was identical to that of DRw12x6,DQw7 (section 4.1.3), DRw8,DQw4 (section 4.1.4) and DRw8,DQw7 (data not shown) haplotypes. The DQA1 RFLP pattern seen for the DRw17,DQw2 haplotype was identical to the only other DRw17 haplotype DRw17,DQw7 (section 5.2.1).

Our results strongly suggests that DRw18 is the most common DR3 subgroup in SA Negroes and as expected, DRw18 was also seen in the Cape Coloureds, a group which has a significant genetic contribution derived from the SA Negroes, but was absent in the Caucasoids studied. The HLA-DR3 subgroup DRw17, however, was observed in all three populations. HLA-DRw18 forms part of the most characteristic SA Negro haplotype, HLA-A30,Bw42,Cw2,DRw18,DQw4,Dw'RSH', while DRw17 is most often seen with the Caucasoid haplotype HLA-A1,B8,Cw7,DRw17,DQw2,Dw3. The association of DRw18 with DQw4 and Dw'RSH' is in keeping with a previous study (Oudshoorn et al. 1986) where it was noted that the majority of SA Negro cells positive for DR3 were negative for DQw2 as well as Dw3 both of which are specificities having a very good correlation with DR3 in Caucasoids and Cape Coloureds. At that time the commonest SA Negro HLA-DR3 haplotype was associated with Dw'blank',DQ'blank', but these blank alleles have subsequently been identified as Dw'RSH' (section 3.1.3) and DQw4.

The observed heterogeneity of DR3 may have important implications for the study of HLA and disease in Negroid populations. In a previous study of insulin dependent diabetes mellitus (IDDM) in SA Negroes, no association could be demonstrated with DR3 (Orren et al. 1985), in contrast to the findings in Caucasoids and North American Negroes (Tiwari and Terasaki 1985). This finding may be explained on the basis that IDDM is associated with DRw17 rather than DRw18, which is almost certainly the more common DR3 split seen in SA Negroes. Other diseases which have been found to be associated with HLA-DR3, such as systemic lupus erythematosis, Grave's disease and dermatitis herpetiformis may also require reassessment in SA Negroes and Cape Coloureds in the light of these findings.

4.1.3 HLA-DRw12x6, a possible new subgroup of HLA-DRw12

The existence of splits of HLA-DR5 was first mentioned during the Eighth Histocompatibility Workshop (Engelfriet et al. 1980) although they were not officially accepted at that time. During the Ninth International Histocompatibility Workshop two splits of DR5 were recognized, DRw11 and DRw12 (Bétuel et al. 1984a). HLA-DRw11 was characterized by positive reactions with DR5 and DR5+w13+w8 sera whereas DRw12 was negative for the DR5 sera but positive for the DR5+w13+w8 sera. The definition of DRw12 during the Tenth International Histocompatibility Workshop was greatly facilitated by the availability of a DRw12 specific Moab (Dupont 1988).

Serology

HLA-DRw12 could be assigned on the basis of a positive reaction with the 10WS MoAb 9999 or 9050 or 3086 (supplied as a hidden triplicate to the participants of the Tenth International Histocompatibility Workshop). Both 10WS 9999 as well as 9050 worked well in our hands but serum 3086 gave unsatisfactory results (probably due to a dilution problem) and has thus been excluded from the analysis. The HLA-DRw12 antigen is usually found together with HLA-DQw7 on the same haplotype (Bétuel et al. 1984a). This HLA-DRw12,DQw7 haplotype was shown to segregate in the family CLO (Table 4.15). The DRw12 specific MoAbs 9999 and 9050 reacted strongly with the DRw12 haplotypes. The assignment of HLA-

TABLE 4.15 Segregation pattern of the DRw12,DQw7 haplotype in family CLO.

				DRw11	DRw12	DRw11 +13	DRw13 +14 +3	DRw13 +14 +11	DRw14	DQw1	DQw5	DQw3	DQw7	DRw52
				1131V	99	11V	11	11	1M9M	131	1	333	333	111 3[a]
				0101R	90	11R	11	11	1E0E	101	1	111	111	111 0
				90327	95	322	53	32	5262	595	3	111	122	959 6
				62691	90	360	15	23	0601	329	6	124	901	829 3
				2		0			3 6					
				0		8			5 0					
				0					0 4					
Father	a	DRw12	DQw7	1111[b]	46	110	11	11	1010	101	1	880	888	864 1
	b	DR7	DQw2											
Mother	c	DR7	DQw2	1111	11	110	11	11	1010	606	2	110	111	111 1
	d	DRw10	DQw5											
Child 1	a	DRw12	DQw7	1111	88	221	11	11	1010	102	2	860	888	866 1
	c	DR7	DQw2											
Child 2	b	DR7	DQw2	1111	11	110	11	11	0010	804	6	120	111	111 1
	d	DRw10	DQw5											
Child 3	b	DR7	DQw2	1111	11	110	11	11	0010	804	4	110	112	111 1
	d	DRw10	DQw5											
Child 4	a	DRw12	DQw7	1111	86	110	11	11	0010	804	4	680	888	686 1
	d	DRw10	DQw5											

a negative for DRw8, DRw12 and DRw8x14 positive individuals

b the results are score as follows: 0 = not tested; 1 = negative; 2 = doubtful negative; 4 = weak positive; 6 = positive; 8 = strong positive

DQw3 split DQw7 was based on positive reaction with the broadly reacting DQw3 MoAbs, 10WS 3111, 3112 and 3114 as well as the HLA-DQw7 specific MoAbs, 10WS 3119, 3120 and 3121.

In several families with cells reacting strongly with the DRw12 specific MoAb a different segregation pattern emerged as shown in the two examples, family JOH and BME, in Table 4.16. The reaction pattern for haplotypes (a) and (c) in family JOH was primarily that seen with cells carrying the "classic" DRw12 antigen. In addition there was a DRw6 component, which was evident from the weak positive reactions with DRw13+DRw14 containing sera, as shown in Table 4.16. This new reaction pattern was called **DRw12x6** and is associated with a new HLA-Dw specificity Dw'JOH' which was described in section 3.1.1. In this family DRw12x6 was linked to DQw7 and children 1 and 4 were homozygous for this variant. The pattern observed for DRw12x6 was also seen linked with HLA-DQw5 in several families. The assignment of the HLA-DQw1 split, HLA-DQw5, was somewhat difficult as only one DQw5 specific serum, 10WS 1136, was available and its reactions were not always reliable. Further confirmation of the presence of DQw5 on these haplotypes came from RFLP studies as will be shown later in this section. Table 4.16 shows the segregation pattern for the DRw12x6,DQw5 haplotype in family MEM. The only difference between the DRw12x6 reaction pattern in the

TABLE 4.16 Serum reaction pattern of DRw12x6,DQw7 and DRw12x6,DQw5 haplotypes.

				DRw11	DRw12	DRw11 +13	DRw13 +14 +3	DRw13 +14 +11	DRw14	DQw1	DQw5	DQw3	DQw7	DRw52
				1131V	99	11V	11	11	1M9M[b]	131	1	333	333	111 3[a]
				0101R	90	11R	11	11	1E0E	101	1	111	111	111 0
				90327	95	322	53	32	5262	595	3	111	122	959 6
				62691	90	360	15	23	0601	329	6	124	901	829 3
				2		0			3 6					
				0		8			5 0					
				0					0 4					
Family JOH														
Father	a	DRw12x6	DQw7	11161[c]	88	111	68	64	1111	111	4	888	808	888 1
	b	DRw8	DQw7											
Mother	c	DRw12x6	DQw7	11111	88	111	16	22	1111	111	1	888	888	888 1
	d	DR4	DQw3											
Child 1	a	DRw12x6	DQw7	11111	88	111	88	64	1111	111	6	888	888	888 1
	c	DRw12x6	DQw7											
Child 2	b	DRw8	DQw7	11101	11	111	11	11	1111	111	1	888	888	882 1
	d	DR4	DQw3											
Child 3	a	DRw12x6	DQw7	11110	88	111	14	48	1111	111	1	888	808	888 6
	d	DR4	DQw3											
Child 4	a	DRw12x6	DQw7	12111	88	111	28	86	1111	111	1	888	888	888 1
	c	DRw12x6	DQw7											
Family MEM														
Father	a	DRw12x6	DQw5	12121	88	221	11	18	1111	888	8	111	111	888 1
	b	DRw12x6	DQw5											
Mother	c	DRw17	DQw2	11161	12	111	88[d]	11	1118[d]	888	1	111	111	888 0
	d	DRw15	DQw6											
Child 1	a	DRw12x6	DQw5	11141	86	144	86[d]	48	1118[d]	844	2	111	111	888 8
	c	DRw17	DQw2											
Child 2	b	DRw12x6	DQw5	11111	88	142	88[d]	88	1118[d]	844	8	111	111	888 8
	c	DRw17	DQw2											
Child 3	a	DRw12x6	DQw5	11011	88	141	81	48	1121	888	1	011	114	888 0
	d	DRw15	DQw6											

a negative for DRw8, DRw12 and DRw8x14 positive individuals

b plus DR3

c the results are score as follows: 0 = not tested; 1 = negative; 2 = doubtful negative; 4 = weak positive; 6 = positive; 8 = strong positive

d positive due to DRw17(3) on haplotype (c)

DRw12x6,DQw5 families, and that described for the DRw12x6,DQw7 family JOH was a negative reaction with 10WS serum 1135, with haplotype DRw12x6,DQw5 (Table 4.17). As the DRw12x6,DQw7 and DRw12x6,DQw5 haplotypes carry different HLA-DQ antigens, the differences in reaction with serum 1135 was initially thought to be due to DQ differences but the subsequent serum analysis did not show any anti-DQ reactivity in serum 10WS 1135. This negative reaction with 10WS serum 1135 was obscured by the presence of DR3 in mother and children 1 and 2 in family MEM, but clearly shown by negative reactions in the father and child 3 in Table 4.16 as well as a number of other DRw12x6,DQw5 positive families. The father of family MEM is homozygous for DRw12x6,DQw5 and his cells have been shown to define a new HLA-Dw specificity, Dw'BME' which has been described in section 3.1.2. A summary of the reaction patterns for the DRw12 and DRw12x6 haplotypes is given in Table 4.17. The summary was compiled from the serum reactions obtained from the family data shown here, as well as data from other families and unrelated individuals (data not shown).

The number of unrelated individuals, including parents, with DRw12x6,DQw7 and DRw12x6,DQw5 found in this study is shown in Table 4.18. The DRw12x6,DQw5 reaction pattern appeared to be mainly a Negro haplotype, while the haplotype DRw12x6,DQw7 was only seen in the Cape Coloureds.

TABLE 4.17 Serum reaction patterns for DRw12,DQw7; DRw12x6,DQw7; DRw12x6,DQw5; DRw13,DQw6 and DRw14,DQw5.

		DRw11	DRw12	DRw11 +13	DRw13 +14 +3	DRw13 +14 +11	DRw14	DQw1	DQw5	DQw3	DQw7	DRw52
		1131V	99	11V	11	11	1M9M	131	1	333	333	111 3[a]
		0101R	90	11R	11	11	1E0E	101	1	111	111	111 0
		90327	95	322	53	32	5262	595	3	111	122	959 6
		62691	90	360	15	23	0601	329	6	124	901	829 3
		2		0			3 6					
		0		8			5 0					
Haplotype		0					0 4					
DRw12	DQw7	-----	++	---	--	--	----	---	-	+++	+++	+++ -
DRw12x6	DQw7	-----	++	---	w+	ww	----	---	-	+++	+++	+++ -
DRw12x6	DQw5	-----	++	---	w-	w+	----	+++	w	---	---	+++ -
DRw13	DQw6	-----	--	+w+	++	++	----	+++	-	---	---	+++ +
DRw14	DQw5	-----	--	---	++	++	++++	+++	+	---	---	+++ +

a negative for DRw8, DRw12, DRw12x6 and DRw8x14 positive individuals

+ denotes a positive serum reaction; - denotes a negative serum reaction; w denotes a doubtful serum reaction

TABLE 4.18 Unrelated individuals with the DRw12x6,DQw5 and DRw12x6,DQw7 haplotypes.

	DRw12x6,DQw5 (n=6)	DRw12x6,DQw7 (n=2)
SA Negroes	5	0
Cape Coloureds	1	2
SA Caucasoids	0	0

RFLP analysis

Cells with the HLA-DRw12x6 reaction pattern were further investigated at a genomic level using the Southern blot technique. The HTCs used are listed in Table 4.19.

TABLE 4.19 HLA typing of HTCs used in this study.

		HLA typing						
HTC	WS number[a]	A	B	C	DR	DQ	Dw	Dw[b]
BME	-	28,30	w58	w6	w12x6	w5	BME	nt
JOH	-	2,24	35,w60	w4,w7	w12x6	w7	JOH	nt
OMW	10W 9058	2	45	blank	w13	w6	w18	24
NSI	-	28,34	w70	w4	w13	w6	w19	nt
HERLUF	9W 1503	2	35,44	w4,w5	w12	w7	DB6	25
CLIMD	-	2,3	7,w57	w4,w6	w14	w5	w9	nt

nt = not tested

a International Histocompatibility Workshop number

b Alleles of the DRB3 (DRw52) gene

The HTC-JOH is of Cape Coloured origin, HTC-Herluf of European Caucasoid origin, while HTC-BME, OMW, NSI and CLIMD are of Xhosa origin. The HTCs were transformed using the Epstein-Barr virus (section 2.2.7) and the lymphoblatoid cell lines thus obtained were used as a source of DNA. Three restriction endonucleases were used to digest the DNA: Taq I, Bam HI and Eco RI. The results using the DRB probe with DNA from the HTCs (except HTC-JOH) digested with Taq I are shown in Figure 4.6. A summary of the DRB results with the three restriction endonucleases used is given in Table 4.20. The DRB RFLP patterns of both DRw12x6 haplotypes were distinguished from the haplotypes bearing the DRw12, DRw13 (Dw18 and Dw19), and DRw14 specificities by the presence of a 4.2 kb Taq I fragment. The two DRw12x6 haplotypes appeared to have identical DRB RFLP patterns, except for the presence of the 12.2 kb Taq I fragment in the DRw12x6,DQw5 haplotype as opposed to the presence of a 10.3 kb Taq I fragment in the DRw12x6,DQw7 haplotype. These RFLP differences are likely to be due to DRB3 differences as the 12.2 kb Taq I is known to correlate with the DRB3 allele Dw25, whereas the 10.3 kb fragment correlates with Dw24 and Dw26 (section 4.1.2). The restriction endonucleases Bam HI and Eco RI revealed no distinctive RFLP pattern correlating with the DRw12x6 specificity.

The results obtained using the DQB probe are summarized in Table 4.21. The DQB pattern seen for the DQw7 antigen

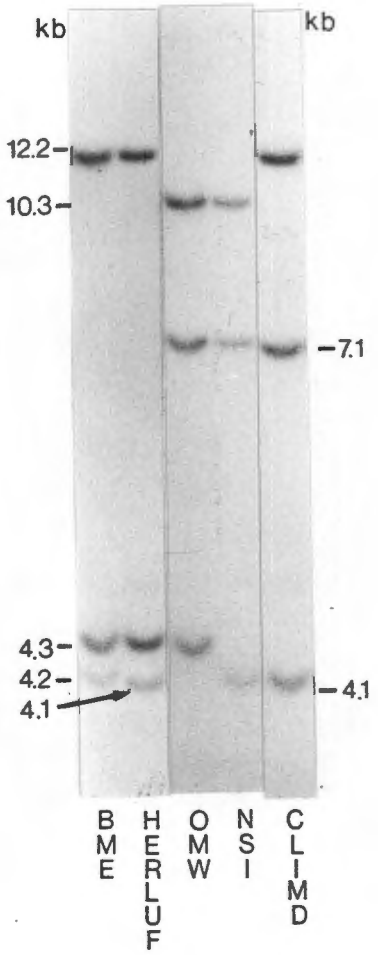

FIGURE 4.6 Autoradiographs obtained after hybridization of the DRB cDNA probe with DNA digested with Taq I. DNA was obtained from homozygous cell lines BME (DRw12x6), HERLUF (DRw12), DRw13,Dw18 (OMW), DRw13,Dw19 (NSI) and DRw14 (CLIMD). The 4.2 kb band was present in the DRw12x6 homozygous cell line only, whereas a 4.1 kb band was seen with the DRw12, DRw14 and DRw13(Dw18) cell lines.

TABLE 4.20 Summary of DRB RFLPs in HLA-DRw12x6, DRw12, DRw13 and DRw14 homozygous cell lines.

Cell identification	HLA-DR	DQ	Dw	Dw[a]	Taq I (kb)						Bam HI (kb)		Eco RI (kb)			
					12.2	10.3	7.1	4.3	4.2	4.1	12.5	4.0	10.2	9.7	4.8	4.4
BME	w12x6	w5	BME	nt	+	-	-	+	+	-	+	-	+	+	-	+
JOH	w12x6	w7	JOH	nt	-	+	-	+	+	-	+	-	+	+	-	+
HERLUF	w12	w7	DB6	25	+	-	-	+	-	+	+	-	+	+	-	+
OMW	w13	w6	w18	24	-	+	+	-	-	+	+	+	+	+	-	+
NSI	w13	w6	w19	nt	-	+	+	+	-	-	+	-	+	+	+	-
CLIMD	w14	w5	w9	nt	+	-	+	-	-	+	+	-	+	+	-	+

a = alleles of the DRB3 (DRw52) gene

+ indicates fragment present

- indicates fragment absent

nt = not tested

TABLE 4.21 Summary of DQB RFLPs in HLA-DRw12x6, DRw12, DRw13 and DRw14 homozygous cell lines.

Cell identification	HLA-DR	DQ	Dw	Taq I (kb)				Bam HI (kb)						Eco RI (kb)			
				5.5	4.6	3.0	2.6	6.7	6.6	6.0	3.7	3.1	3.0	12.5	12.2	9.1	2.3
BME	w12x6	w5	BME	+	-	-	-	-	-	+	-	+	-	-	+	-	-
JOH	w12x6	w7	JOH	-	+	-	-	+	-	-	+	-	-	+	-	-	-
HERLUF	w12	w7	DB6	-	+	-	-	+	-	-	+	-	-	+	-	-	-
OMW	w13	w6	w18	-	-	+	+	-	+	-	-	-	+	-	-	+	+
NSI	w13	w6	w19	+	-	-	-	-	+	-	-	-	-	-	-	+	+
CLIMD	w14	w5	w9	+	-	-	-	-	+	+	-	+	+	-	+	+	+

+ indicates fragment present
- indicates fragment absent

associated with the DRw12x6,DQw7 haplotype was identical to that of the DRw12,DQw7 homozygous cell line Herluf and other DQw7 cells tested such as DRw11,DQw7 (data not shown); DR4,DQw7 (data not shown) and DRw17,DQw7 (section 5.2.1). The DQB pattern observed for the DRw12x6,DQw5 homozygous cell line was identical to the pattern seen for the DQw5 antigen associated with DR1 (data not shown), DRw10 (section 4.1.1), DRw16 (section 4.1.1) and DRw8x14 (section 4.1.4). The DQB patterns seen with the DRw13(Dw18),DQw6 cell was identical to that of DRw15,DQw6 (section 4.1.1) and DRw11,DQw6 (data not shown) cells, whereas the DQB pattern of DRw13(Dw19) as well as of DRw14(Dw9) homozygous cell lines appeared to be unique indicating further heterogeneity of DQw1 at the DNA level.

Table 4.22 shows the result obtained using the DQA probe. The cell line Herluf (DRw12,DQw7) appeared to have both patterns (pattern 1 and 2)(Table 4.7) associated with the DQA2 gene and is thus heterozygous at the DQA2 locus. The DQA2 RFLP pattern 2 was observed for the homozygous cell line JOH (DRw12x6,DQw7) and CLIMD (DRw14,DQw5), while the remaining cell lines all showed pattern 1 (Table 4.22). The homozygous cell line BME (DRw12x6,DQw5) showed an identical DQA1 RFLP pattern to that of the DQw5 positive haplotypes DRw14,DQw5 (Table 4.22), DRw8x14,DQw5 (section 4.1.4), DR1,DQw5 and DRw10,DQw5 (data not shown). Although the homozygous cell lines JOH (DRw12x6) and Herluf (DRw12) were

TABLE 4.22 Summary of DQA RFLPs in HLA-DRw12x6, DRw12, DRw13 and DRw14 homozygous cell lines.

Cell identification	HLA-DR	DQ	Dw	Taq I (kb)							Bam HI (kb)		Eco RI (kb)			
				7.0	6.5	6.2	4.8	2.7	2.2	2.1	29.5	11.5	15.0	12.1	2.0	1.35
BME	w12x6	w5	BME	-	-	-	-	+	+	-	-	+	+	-	-	+
JOH	w12x6	w7	JOH	-	-	+	-	-	-	+	-	-	-	+	+	-
HERLUF	w12	w7	DB6	-	-	-	+	-	+	+	-	-	-	+	+	+
OMW	w13	w6	w18	+	-	-	-	-	+	-	-	+	+	-	-	+
NSI	w13	w6	w19	-	+	-	-	-	+	-	+	-	+	-	-	+
CLIMD	w14	w5	w9	-	-	-	-	+	-	+	-	+	+	-	+	-

+ indicates fragment present

- indicates fragment absent

shown to have a serological identical DQ antigen, DQw7, differences in the RFLP pattern associated with the DQA1 gene were observed. The DQA1 RFLP pattern of JOH was shared with the DRw18,DQw4 (section 4.1.2), DRw8,DQw7 and DRw8,DQw4 haplotypes (data not shown), whereas that of Herluf was identical to DRw11,DQw7 haplotypes (data not shown).

In *summary*, the data in this section has shown heterogeneity of the DRw12 haplotypes. Serological analysis showed that some DRw12 positive cells gave extra reactions with DRw6 containing sera and we have called this unusual reaction pattern DRw12x6. This pattern was shown to be associated with two different DQ antigens, DQw5 and DQw7. The HLA-DRw12x6,DQw5 haplotype was found mainly in Negroes, while DRw12x6,DQw7 was only seen in the Cape Coloureds. Heterogeneity of the DRw12 haplotype was also evident from cellular studies (section 3.1.1 and 3.1.2), the DRw12x6,DQw7 haplotype was associated with Dw'JOH', the DRw12x6,DQw5 haplotype with Dw'BME' and the "classic" DRw12,DQw7 haplotype with DB6 (Jakobsen et al. 1986). Southern blot analysis of the different DRw12 haplotypes revealed identical DRB1 RFLP patterns for the DRw12x6,DQw5 and DRw12x6,DQw7 homozygous cell lines, both of which could be distinguished from the DRB1 RFLP patterns of DRw12, DRw13 and DRw14. The two DRw12x6 haplotypes, however, carried different alleles of the DRB3 gene. The DQB probe showed identical hybridization patterns for DRw12x6,DQw7 and

DRw12,DQw7 as well as all other DQw7 haplotypes tested. As expected the RFLP pattern for the DQw5 positive DRw12x6 haplotype was different from the afore mentioned pattern but identical to other DQw5 haplotypes tested such as DR1,DQw5 DRw10,DQw5, DRw16,DQw5 and DRw8x14,DQw5. Results obtained with the DQA probe showed that the RFLP patterns associated with the DQA1 gene for the DQw7 positive haplotypes, DRw12x6,DQW7 and DRw12,DQw7 were different indicating a possible different DQA1 gene product present in these cell lines. The DQA1 RFLP result of the DRw12x6,DQw5 homozygous cell line was shown to be identical to other DQw5 positive haplotypes (DRw14, DRw8x14, DR1 and DRw10).

In conclusion the data presented here and in sections 3.1.1 and 3.1.2 has shown that the DRw12 haplotypes carry different DQ, Dw, DRB3 and possibly DRB1 alleles which result in heterogeneous haplotypes within the DRw12 specificity.

4.1.4 HLA-DRw8x14, a new subgroup of HLA-DRw6

The antigen **HLA-DRw6** was first defined during the Seventh International Histocompatibility Workshop using two sets of sera, the DR2+DRw6 and DR3+DRw6 sera (Bodmer et al. 1978). The definition of DRw6 was still not clear after the Eighth International Histocompatibility Workshop, where the assignment of DRw6 was mostly based on positive reactions with DR3+DRw6 sera. It was, however, established that DRw6

was always associated with DRw52 and nearly always with DQw1. Furthermore, several subgroups of DRw6 were described by different laboratories (Jeannet 1980, Sekiguchi et al. 1980, Kreisler et al. 1980, Bodmer et al. 1980). In a re-analysis of the DRw6 complex, including the HLA-Dw specificities of the cells, Schreuder and co-workers divided it into several subgroups (Schreuder et al. 1983). During the Ninth International Histocompatibility Workshop the existence of subgroups of DRw6, i.e. **DRw13** and **DRw14** was formally acknowledged by the WHO Nomenclature Committee, but difficulties in the distinction of DRw12 from DRw6 positive, DQw1 negative specificities remained (Schreuder et al. 1984b).

HLA-DRw8 was first described during the Seventh International Histocompatibility Workshop, where this antigen was known as WIA8 (Bodmer et al. 1978). Due to lack of good sera many problems were encountered with the assignment of this antigen and it was therefore not officially recognized by the WHO Nomenclature Committee. The definition of DRw8 in the Eighth International Histocompatibility Workshop was based on five multispecific sera with relatively low r values for DRw8 (0.40-0.58) (Dausset 1980). Due to difficulties in the distinction of DRw8 from DR5 and DRw6 related specificities, the DR5 and DRw6 related serum reactions were included in the Ninth International Histocompatibility Workshop analysis of HLA-

DRw8 (Bétuel et al. 1984b). During this Workshop the definition of DRw8 was based on positive reactions with the group of DRw8 and DR5+w8+w13 sera. HLA-DRw8 was mainly found in linkage disequilibrium with DQ'blank' (now identified as DQw4), but was also found together with DQw1 and DQw3. The existence of a possible **HLA-DRw8 variant** in the Japanese was suggested during the Eighth International Histocompatibility Workshop (Nakata et al. 1980) and the Second Asia-Oceania Histocompatibility Workshop (Doran et al. 1983). Further evidence for the existence of a Japanese HLA-DRw8 variant was supplied by analysis of the serological data obtained during the Ninth International Histocompatibility Workshop (Betuel et al. 1984b) and the Third Asia-Oceania Histocompatibility Workshop (Yagita et al. 1986).

Several HLA-DRw8 positive cells typed locally for the Tenth International Histocompatibility Workshop showed additional reactions with HLA-DRw14 containing sera and were assigned the specificity **HLA-DRw8x14** (du Toit and Oudshoorn 1987, du Toit et al. 1988b). Serological and Southern blot analysis of HLA-DRw8x14 and the "classic" DRw8 and DRw6 (DRw14 and DRw13) antigens will be described here.

Serology

We studied a total of 326 individuals with the Tenth International Histocompatibility Workshop antisera. This

group included 23 families (12 SA Negro, 9 Cape Coloured and 2 SA Caucasoid) and 195 unrelated normal individuals (87 SA Negro, 50 Cape Coloured and 58 SA Caucasoid). Before the serological reaction pattern of the new variant DRw8x14 can be described the serological patterns for the "classic" DRw8 and DRw14 antigens need to be defined as described below. As HLA-DRw14 is not easily distinguished from the other DRw6 subgroup, DRw13, a description of the reaction pattern for DRw13 will also be given. To confirm the reaction patterns obtained for the various specificities, family studies were done.

In our families **DRw8** was seen in two haplotypes: DRw8,DQw4 and DRw8,DQw7 (a split of DQw3). Both haplotypes reacted equally well with the 10WS DRw8 sera as shown in Table 4.23. The segregation pattern of DRw8 is shown in a SA Negro family MWA where DRw8 was linked to HLA-DQw7 (Table 4.24). HLA-DRw8 could be assigned to fifteen unrelated cells (data not shown).

HLA-DRw14 could be assigned on the basis of positive reactions with the DRw14 sera 10WS 1150, ME21604, 9060 and ME26350, as well as the group of DRw11+w13+w14 and DRw13+w14 containing sera. All the DRw14 cells were negative with the DRw11+w13 sera shown in Table 4.23. HLA-DRw14 could be assigned in two families and in both these families HLA-DRw14 was seen linked to HLA-DQw1. The assignment of the

TABLE 4.23 Tenth Workshop serum reaction patterns for HLA-DRw8, DRw13, DRw14 and DRw8x14 haplotypes.

Haplotype		DRw8	DRw11 +13 +3	DRw13 +14 +11	DRw13 +14	DRw14	DQw1	DQw5	DQw4	DQw3	DQw7	DRw52	
		111	11V	11	11	1MM9	131	1	31	333	333	111	3[a]
		000	11R	11	11	1EE0	101	1	10	111	111	111	0
		889	322	53	32	5226	595	3	08	111	122	959	6
		571	360	15	23	0160	329	6	13	124	901	829	3
			0			63							
			8			05							
						40							
w8	w4	+++	---	--	-w	----	---	-	++	---	---	+++	-
w8	w7	+++	---	ww	--	----	---	-	--	+++	+++	+++	-
w14	w5	---	---	++	++	++++	+++	+	--	---	---	+++	+
w13	w6	---	+w+	++	++	----	+++	-	--	---	---	+++	+
w8x14	w5	+++	---	+-	+w	WWWW	+++	+	--	---	---	+++	-

a negative for DRw8, DRw12, DRw12x6 and DRw8x14 positive individuals

+ denotes a positive serum reaction; - denotes a negative serum reaction; w denotes a doubtful serum reaction

TABLE 4.24 Segregation of the serum reaction pattern for HLA-DRw8 and DRw13 in family MWA.

				DRw8	DRw11 +13	DRw13 +14 +3	DRw13 +14 +11	DRw14	DQw1	DQw5	DQw4	DQw3	DQw7	DRw52
				111	11V	11	11	1MM9	131	1	31	333	333	111 3[a]
				000	11R	11	11	1EE0	101	1	10	111	111	111 0
				889	322	53	32	5226	595	3	08	111	122	959 6
				571	360	15	23	0160	329	6	13	124	901	829 3
					0			63						
					8			05						
								40						
Father	a	DRw13	DQw6	111[b]	426	24	44	1214	486	1	11	888	111	888 8
	b	DRw4	DQw3											
Mother	c	DRw13	DQw6	448	214	44	84	1011	644	1	11	888	848	888 8
	d	DRw8	DQw7											
Child 1	a	DRw13	DQw6	111	828	86	62	2011	682	1	11	811	111	888 8
	c	DRw13	DQw6											
Child 2	a	DRw13	DQw6	888	848	64	82	1201	686	1	11	886	888	888 8
	d	DRw8	DQw7											
Child 3	b	DR4	DQw3	111	848	42	68	1111	888	4	11	888	111	888 8
	c	DRw13	DQw6											

a negative for DRw8, DRw12, DRw12x6 and DRw8x14 positive individuals

b the results are score as follows: 0 = not tested; 1 = negative; 2 = doubtful negative; 4 = weak positive; 6 = positive; 8 = strong positive

DQw1 split, DQw5 to these haplotypes was based on the Taq I RFLP results as discussed below. In addition four unrelated cells were found to carry the HLA-DRw14 antigen. Clear segregation of the serological pattern for HLA-DRw14 was seen in both families. The segregation pattern is illustrated in family MDI shown in Table 4.25.

HLA-DRw13 was assigned on the basis of weak positive reactions with the group of DR3+w11+w13, DRw11+w13+w14 and DRw13+w14 containing sera (Table 4.23). HLA-DRw13 was seen in five families and 43 unrelated individuals. In the families HLA-DRw13 was linked to DQw1. The assignment of the DQw1 split, DQw6 to these haplotypes was based on RFLP studies described below. Segregation of HLA-DRw13 is shown in family MWA (Table 4.24).

All the haplotypes included in this analysis were **DRw52** positive as shown in Table 4.23. One of the DRw52 sera 10WS 3063 was confirmed to be positive for DRw13, DRw14 and DRw11 haplotypes and negative for DRw8 and DRw12 haplotypes as reported by Kennedy et al. (1987).

In two families the above segregation patterns could not be applied without resulting in the assignment of "triplets". In order to avoid the assignment of "triplets" and explain the segregation of HLA-DRw8 and DRw14 on the same haplotype we had to postulate a new HLA-DR specificity, i.e. **HLA-**

TABLE 4.25 Segregation of the serum reaction pattern for DRw14 in family MDI.

				DRw8	DRw11 +13	DRw13 +14 +3	DRw13 +14 +11	DRw14	DQw1	DQw5	DRw52
				111	11V	11	11	1MM9	131	1	111 3[a]
				000	11R	11	11	1EE0	101	1	111 0
				889	322	53	32	5226	595	3	959 6
				571	360	15	23	0160	329	6	829 3
					0			63			
					8			05			
								40			
Mother	c	DRw14	DQw5	411[b]	111	44	88	2886	888	8	888 8
	d	DRw14	DQw5								
Child 1	a	DR7	DQw2	111	141	68	48	4888	682	8	868 8
	c	DRw14	DQw5								
Child 2	b	DRw14	DQw5	111	111	48	88	4886	886	6	688 8
	c	DRw14	DQw5								
Child 3	b	DRw14	DQw5	111	111	68	88	0081	868	4	888 8
	d	DRw14	DQw5								
Child 4	b	DRw14	DQw5	111	111	84	68	6646	868	6	688 8
	c	DRw14	DQw5								
Child 5	b	DRw14	DQw5	112	111	84	88	4864	868	4	888 8
	d	DRw14	DQw5								

a negative for DRw8, DRw12, DRw12x6 and DRw8x14 positive individuals

b the results are score as follows: 1 = negative; 2 = doubtful negative; 4 = weak positive; 6 = positive;

TABLE 4.26 Segregation of HLA-DRw8x14 in family PLA.

				DRw8	DRw11 +13	DRw13 +14 +3	DRw13 +14 +11	DRw14	DQw1	DQw5	DRw52	
				111	11V	11	11	1MM9	131	1	111	3[a]
				000	11R	11	11	1EE0	101	1	111	0
				889	320	53	32	5226	595	3	959	6
				571	360	15	23	0160	329	6	829	3
					8			63				
					9			05				
								40				
Father	a	DR7	DQw3	111[b]	210	26[c]	22	1002	111	1	888	8
	b	DRw17	DQw2									
Mother	c	DRw15	DQw6	664	110	81	68	2001	886	6	888	1
	d	DRw8x14	DQw5									
Child 1	a	DR7	DQw3	111	110	21	12	2004	868	1	111	1
	c	DRw15	DQw6									
Child 2	b	DRw17	DQw2	111	110	88[c]	11	1002	888	1	888	8
	c	DRw15	DQw6									
Child 3	b	DRw17	DQw2	886	110	86[c]	64	4004	888	8	888	8[c]
	d	DRw8x14	DQw5									
Child 4	a	DR7	DQw3	886	120	82	61	6006	888	8	888	1
	d	DRw8x14	DQw5									

a negative for DRw8, DRw12, DRw12x6 and DRw8x14 positive individuals

b the results are score as follows: 1 = negative; 2 = doubtful negative; 4 = weak positive; 6 = positive; 8 = strong positive

c positive due to DRw17 on haplotype (b)

DRw8x14. The serum reaction pattern for this specificity is shown in Table 4.23. Cells with the DRw8x14 specificity reacted with the 10WS DRw8 sera as well as most of the DRw14 containing sera. Like HLA-DRw8, but unlike DRw14, cells with the DRw8x14 specificity did not react with the DRw52 MoAb 3063. Two families with the DRw8x14 antigen were tested and in both DRw8x14 segregated with DQw1. The DQw1 split, DQw5 was assigned to these haplotypes based on RFLP studies. The segregation of HLA-DRw8x14 in one of these families (PLA) is shown in Table 4.26.

HLA-Dw typing

Two Cape Coloured families, PLA and LAN, with HLA-DRw8x14 were HLA-Dw typed using 59 HTCs. HTCs of all known HLA-Dw specificities were included except for the DR1 associated Dw specificity, Dw20. No typing response for the HLA-DRw8x14 haplotypes were observed with any of the HTCs, suggesting that the haplotype carries a new but unidentified HLA-Dw specificity.

RFLP analysis

The Southern blot technique was applied to study the HLA-DR variant, HLA-DRw8x14, at a genomic level. Cells from families (PLA and LAN) with DRw8x14 were used for Southern blot analysis as well as homozygous cell lines for HLA-DRw8,Dw8 (OLGA), DRw13,Dw18 (OMW), DRw13,Dw19 (NSI) and DRw14,Dw9 (CLIMD). Unfortunately no homozygous cell line

for DRw14,Dw16 was available for the Southern blot analysis. The DNA isolated from peripheral blood lymphocytes of family PLA and LAN and from B-LCLs of the HTCs was digested with the endonucleases Taq I and Bam HI.

The results obtained using a DRB probe and DNA digested with Taq I and Bam HI are summarized in Table 4.27. The RFLP patterns for two of the three DRw8x14 haplotypes, haplotype (d) of family PLA and haplotype (a) of family LAN, were identical to each other ("pattern A") and to the DRw14,Dw9 homozygous cell line CLIMD (Table 4.27). The RFLP pattern, "pattern B", for the remaining DRw8x14 haplotype, haplotype (c) of family LAN was different to pattern A, but was identical to that of the DRw13,Dw18 homozygous cell line OMW (Table 4.27). It remains a question whether the differences between the RFLP patterns A and B were due to DRB1 and/or DRB3 gene differences. We do however know that the 12.2 kb Taq I fragment correlates with the DRB3 allele Dw25 as shown in section 4.1.2 and this fragment was present in the RFLP pattern A, whereas the 10.3 kb fragment known to correlate with the DRB3 alleles, Dw24 and Dw26 (section 4.1.4), was present in the RFLP pattern B. The remaining fragments (Table 4.27), probably associated with the DRB1 gene, were identical for all three DRw8x14 haplotypes as well as the DRw14,Dw9 and DRw13,Dw18 homozygous cell lines. Whether the Bam HI RFLP differences between the RFLP patterns A and B reflect differences in the DRB1 or DRB3 genes remains

TABLE 4.27 Summary of DRB RFLP among DRw8x14, DRw8, DRw13 and DRw14 positive cells.

Cell Identification	HLA-DR	HLA-Dw	Taq I (kb)						Bam HI (kb)		
			12.2	10.3	8.7	7.1	4.3	4.1	12.5	4.0	
Haplotype (d)(PLA)	w8x14	blank	+	-	-	+	-	+	+	-	pattern A
Haplotype (a)(LAN)	w8x14	blank	+	-	-	+	-	+	+	-	
HTC-CLIMD	w14	w9	+	-	-	+	-	+	+	-	
Haplotype (c)(LAN)	w8x14	blank	-	+	-	+	-	+	+	+	pattern B
HTC-OMW	w13	w18	-	+	-	+	-	+	+	+	
HTC-NSI	w13	w19	-	+	-	+	+	-	+	-	
HTC-OLGA	w8	w8	-	-	+	-	-	-	-	-	

+ indicates fragment present

- indicates fragment absent

uncertain. It should be noted that the DRw8 specific 8.7 kb Taq I fragment was absent in the DRw8x14 haplotypes, demonstrating that the DRw8x14 specificity bears no resemblance to DRw8 at a genomic level.

The results obtained using the DQB probe are summarized in Table 4.28. An identical RFLP pattern was observed for the three DRw8x14,DQw5 haplotypes and this RFLP pattern was similar to those observed for other DQw5 haplotypes such as DR1,DQw5 (data not shown), DR4,DQw5 (section 5.2.2), DRw10,DQw5 (section 4.1.1) and DRw16,DQw5 (section 4.1.1). The DRw14,DQw5 homozygous cell line CLIMD of SA Negro origin appeared to have a unique DQB RFLP pattern (Table 4.28).

Table 4.29 shows the results obtained using the DQA probe. The DRw8x14,DQw5; DRw8,DQw4 and DRw14,DQw5 haplotypes tested all showed the DQA2 pattern 2 (Table 4.7)(2.1 kb Taq I and 2.0 kb Eco RI fragment). The RFLP pattern corresponding to the DQA1 gene for the DRw8x14 haplotypes (2.7 kb Taq I and 11.5 kb Bam HI fragments) was identical to that of DRw14,DQw5 (Table 4.29), DRw12x6,DQw5 (section 4.2.3), DR1,DQw5 and DRw10,DQw5 haplotypes (data not shown).

In *summary*, in this section we have described a new serological variant of HLA-DRw14, which we have named HLA-DRw8x14.

TABLE 4.28 Summary of DQB RFLP among DRw8x14, DRw8, DRw13 and DRw14 positive cells.

Cell Identification	HLA-DR	DQ	Taq I (kb)					Bam HI (kb)				
			5.5	3.0	2.6	2.4	1.9	11.0	6.6	6.0	3.1	3.0
Haplotype (d)(PLA)	w8x14	w5	+	-	-	-	-	-	-	+	+	-
Haplotype (a)(LAN)	w8x14	w5	+	-	-	-	-	-	-	+	+	-
Haplotype (c)(LAN)	w8x14	w5	+	-	-	-	-	-	-	+	+	-
HTC-OLGA	w8	w4	-	-	-	+	+	+	-	-	-	-
HTC-CLIMD	w14	w5	+	-	-	-	-	-	+	+	+	+
HTC-OMW	w13	w6	-	+	+	-	-	-	+	-	-	+
HTC-NSI	w13	w6	+	-	-	-	-	-	+	-	-	-

+ indicates fragment present
- indicates fragment absent

TABLE 4.29 Summary of DQA RFLP among DRw8x14, DRw8, DRw13 and DRw14 positive cells.

Cell Identification	HLA-DR	DQ	Taq I (kb)						Bam HI (kb)	
			7.0	6.5	6.2	2.7	2.2	2.1	29.5	11.5
Haplotype (d)(PLA)	w8x14	w5	-	-	-	+	-	+	-	+
Haplotype (a)(LAN)	w8x14	w5	-	-	-	+	-	+	-	+
Haplotype (c)(LAN)	w8x14	w5	-	-	-	+	-	+	-	+
HTC-OLGA	w8	w4	-	-	+	-	-	+	-	-
HTC-CLIMD	w14	w5	-	-	-	+	-	+	-	+
HTC-OMW	w13	w6	+	-	-	-	+	-	-	+
HTC-NSI	w13	w6	-	+	-	-	+	-	+	-

+ indicates fragment present

- indicates fragment absent

Cells with this new variant were characterized by positive reactions with DRw8 as well as DRw14 sera. MoAb 3063, said to react against the common DR3 and DRw6 part of the DRβ1 chain, gave no reactions with DRw8x14 cells indicating that they are like DRw8 in the first hypervariable region. The HLA-DRw8x14 specificity was only seen in the Cape Coloureds and could be identical to the rare DRw8I variant seen in Asian Indians by Awad and Festenstein (1987). HLA-DRw8x14 is however different to the Japanese HLA-DRw8 variant (Nakata et al. 1980, Doran 1983, Betuel et al. 1984b and Yagita et al. 1986), which did not react with DRw14 sera (Bétuel et al. 1984b). HTC typing of DRw8x14 positive cells showed that no recognized HLA-Dw specificity was associated with DRw8x14, although, reservation with respect to Dw20 has to be made, since the DRw8x14 cells were not typed for Dw20. Southern blot analysis using a DRB probe and DNA digested with Taq I and Bam HI showed that the RFLP pattern for two of the three DRw8x14 haplotypes was identical to that of a DRw14 homozygous cell line and that the RFLP pattern for the third DRw8x14 haplotype was identical to DRw13,Dw18. From data obtained using the Taq I restriction enzyme, it would appear that the observed heterogeneity in the DRw8x14 haplotypes may be due to differences in the DRB3 genes rather than DRB1 genes. The absence of the DRw8 specific 8.7 kb Taq I fragment in the DRw8x14 haplotypes suggest that this variant is related to DRw14 rather than DRw8. It is still possible that both DRw8 as well as DRw14 antigens are

present on the same haplotype as has previously been shown for DR1 and DR2 (Bontrop et al. 1986a). To clarify this further studies such as 2-D gel electrophoresis and DNA sequencing should be done. The DQB RFLP pattern observed for the DRw14x8,DQw5 haplotypes was identical to all DQw5 haplotypes tested with the exception of the DRw14,DQw5 haplotype. The DQA RFLP pattern seen using the DQA probe and DNA digested with Taq I and Bam HI were identical for the DRw8x14,DQw5; DRw14,DQw5; DRw12x6,DQw5; DR1,DQw5 and DRw10,DQw5 haplotypes.

4.2 THE FREQUENCIES OF HLA-DR AND DQ ANTIGENS IN SA NEGROES (XHOSA), CAPE COLOUREDS AND SA CAUCASOIDS

In this section we describe a comparative study on the HLA-DR and DQ antigen frequencies in the three major South African populations: SA Negroes, Cape Coloureds and SA Caucasoids. The findings reported in this study were similar to those reported by du Toit et al. (1988a). The Xhosa were chosen to represent the SA Negroes in this study, for the reasons described under materials in section 2.1.1. We compare the findings of the HLA-DR and DQ frequencies in the SA Negroes to those of the Nigerians, the only other recently studied African Negro group, (Okoye et al. 1985) and the American Negroes studied by Johnson (1986). Of the various studies on the class II antigen frequencies in American Negroes, the study by Johnson (1986) was chosen for comparison in this study as it was the most recently reported study on American Negroes. The HLA-DR frequencies of American Negroes studied by Johnson (1986) did not differ significantly from those studied by others (Duquesnoy et al. 1979b, Baur and Danilovs 1980). In addition the extended haplotypes present in the South African populations were investigated as accumulating evidence would suggest that interactions of specific allelic combinations may have some overall biological function. For example, extended haplotype matching rather than individual alleles increases transplant survival (Wilton et al. 1985). Furthermore some

of the most stable extended haplotypes are strongly associated with increased disease susceptibility (Dawkins et al. 1983).

The associations of HLA-DR and HLA-DQ antigens in the population groups will not be discussed in this section, as they will be discussed in detail in section 5.1.

Table 4.30 shows the antigen and gene frequency distributions in the SA Negroes (Xhosa), the Cape Coloureds and SA Caucasoids for the HLA-DR and DQ antigens. The HLA-DR results presented here were those obtained since 1980, when the number and quality of the antisera to determine all the DR1 to DRw10 specificities became adequate. The DR locus polymorphism was investigated in 435 Xhosa, 641 Cape Coloureds and 467 SA Caucasoids, who were tested for the presence of the HLA-DR antigens, HLA-DR1 to DRw10. We were unable to determine reliable frequencies for the splits of DR2 (DRw15 and DRw16), DR3 (DRw17 and DRw18), DR5 (DRw11 and DRw12,) and DRw6 (DRw13 and DRw14), due to the lack of good antisera and have therefore not included these results in Table 4.30. The HLA-DRw52, DRw53 and DQ results were those obtained since the Ninth International Histocompatibility Workshop, when these systems were formally defined. The DRw52 and DRw53 antigens were tested in 433 Xhosa, 637 Cape Coloureds and 466 SA Caucasoids. The HLA-DQ antigens were identified in 362 Xhosa, 395 Cape Coloureds and 281 SA

TABLE 4.30 Antigen and gene frequencies of HLA-DR and DQ antigens in the Xhosa, Cape Coloureds, SA Caucasoids, Nigerians and American Negroes.

HLA Antigen	SA Negroes (Xhosa) (n=435)		Cape Coloureds (n=641)		SA Caucasoids (n=467)		Nigerians[a] (n=103)		American Negroes[b] (n=92)	
	af	gf	af	gf	af	gf	af	gf	af	gf
DR1	0.092	0.047	0.115	0.060	0.203	0.108	0.117	0.060	0.163	0.085
DR2	0.206	0.109	0.347	0.192	0.272	0.147	0.388	0.218	0.293	0.159
DR3	0.432	0.246	0.200	0.106	0.206	0.109	0.126	0.065	0.250	0.134
DR4	0.125	0.064	0.214	0.114	0.294	0.160	0.010	0.005	0.108	0.056
DR5	0.275	0.148	0.283	0.153	0.234	0.125	0.320	0.176	0.228[d]	0.121
DRw6	0.349	0.193	0.214	0.114	0.163	0.085	0.415	0.235[c]	0.391	0.209
DR7	0.157	0.082	0.258	0.139	0.230	0.122	0.175	0.092	0.239	0.128
DRw8	0.025	0.013	0.053	0.027	0.062	0.032	0.155	0.081	0.120	0.062
DR9	0.014	0.007	0.019	0.009	0.026	0.013	0.029	0.015	0.033	0.017
DRw10	0.051	0.026	0.024	0.012	0.030	0.015	0	0	0.054	0.027
Blank		0.065		0.076		0.085		0.054		0.002
	(n=433)		(n=637)		(n=466)		(n=103)		(n=92)	
	af	gf	af	gf	af	gf	af	gf	af	gf
DRw52	0.829	0.587	0.639	0.399	0.570	0.344	0.772	0.522	0.770	0.520
DRw53	0.254	0.136	0.336	0.185	0.369	0.205	0.193	0.102	0.283	0.153
	(n=362)		(n=395)		(n=281)		(n=103)		(n=92)	
	af	gf	af	gf	af	gf	af	gf	af	gf
DQw1	0.693	0.446	0.661	0.418	0.634	0.395	0.786	0.538	0.750	0.500
DQw2	0.247	0.133	0.286	0.155	0.324	0.178	nt	nt	0.380	0.213
DQw3	0.383	0.215	0.458	0.264	0.482	0.280	0.261	0.141	0.370	0.206
Blank		0.206		0.163		0.147		0.322		0.081

a Okoye et al. 1985

b Johnson 1986

c DRw6 was assigned to cells which were negative for DR3, w8 and w11 and were positive for DRw52

d DRw13+DRw13v+DRw14

af = antigen frequency

gf = gene frequency

Caucasoids, who were tested for the presence of HLA-DQw1, DQw2 and DQw3. The frequencies for the newly defined antigen DQw4 as well as the splits of DQw1 (DQw5 and DQw6) and DQw3 (DQw7, DQw8 and DQw9) were not determined in this group. All individuals in this study were also typed for the HLA-A, B and C locus antigens.

HLA-DR antigens

HLA-DR1

The frequency of HLA-DR1 in the SA Caucasoids was significantly higher ($p<0.001$) than in the Xhosa and Cape Coloureds. The frequency for DR1 seen in the Xhosa (gf=0.047) and Nigerians (gf=0.060) were similar, but the frequency in the American Negroes (gf=0.085) was significantly higher ($p<0.05$) than in the Xhosa.

HLA-DR2

HLA-DR2 is the most common DR antigen in the Cape Coloureds (Table 4.30) as well as in the Chinese (Jaraquemada et al. 1984) and the second most common DR antigen in the Nigerians (Okoye et al. 1985). During the Tenth International Histocompatibility Workshop the definition of the two DR2 splits became well established and they were given official names by the WHO Nomenclature Committee; HLA-DRw15 (previously known as HLA-DR2 long) and HLA-DRw16 (previously known as HLA-DR2 short). From the combined Ninth and Tenth Histocompatibility Workshop data on DR2 positive individuals

tested by us, it was seen that DRw16 occurred in 4 of the 28 Cape Coloureds (14%), in 1 of 22 SA Caucasoids (4.5%) and was not seen in the 24 SA Negroes (Oudshoorn et al. 1987a). The fact that DRw16 has its highest frequency in Cape Coloureds may be due to their mixed South-East Asian ancestry rather than their Western European or SA Negro genetic background. In addition, a further HLA-DR2 split, DR2 LUM(CT) was detected (section 4.1.1) and observed predominantly in the SA Negroes (Oudshoorn et al. 1983, Oudshoorn et al. 1987a, 1988). The definition of this new Negro specificity was described in section 4.1.1. HLA-DR2 LUM(CT) was observed in 6 of the 24 (25%) unrelated SA Negroes tested in the Ninth and Tenth International Histocompatibility Workshops, in 1 of the 28(4%) DR2 positive Cape Coloureds and was absent in the 22 DR2 positive SA Caucasoids (Oudshoorn et al. 1987a).

HLA-DR3

The HLA-DR antigen with the highest frequency in the Xhosa is DR3 with a gene frequency of 0.246, which is much higher than the DR3 frequencies reported in any of the population groups studied during the Eighth and Ninth International Histocompatibility Workshops (Baur and Danilovs 1980, Baur et al. 1984), as well as in the American Negroes (Johnson 1986) and Nigerians (Okoye et al. 1985). Using the Tenth International Histocompatibility Workshop allo- and monoclonal antibodies HLA-DR3 was split into two subgroups,

HLA-DRw17 (previously DR3.1) and HLA-DRw18 (previously DR3.2) (Oudshoorn et al. 1987b). Of the HLA-DR3 positive individuals studied in this Workshop, HLA-DRw18 was seen in 30 of 46 SA Negroes (65%) and in 3 of 12 Cape Coloureds (25%) and was absent in the SA Caucasoids studied (section 4.1.2). HLA-DRw18 has also been detected in the American Negroes (Hurley et al. 1988).

HLA-DR4

HLA-DR4, although relatively less common in the Xhosa (gf=0.064) than in the Cape Coloureds (gf=0.114) and SA Caucasoids (gf=0.160), was still present in an appreciable number of individuals. The frequency of DR4 in the Xhosa was similar to the frequency found in the American Negroes but was significantly higher ($p<0.001$) compared to the frequency in the Nigerians in whom this antigen was virtually absent.

HLA-DR5

The gene frequency of HLA-DR5 was similar in all three South African populations tested and ranged from 0.148 in the Xhosa to 0.125 in the SA Caucasoids. Using the Tenth International Histocompatibility Workshop antisera we have found a possible new Negro variant (HLA-DRw12x6) related to HLA-DRw12(5) (section 4.1.3). Due to the lack of DRw12 sera, monospecific DRw6 sera, and the strong association of DRw12x6 with DQw1 (often a basis for defining DRw6),

individuals with this specificity were typed as DRw6 before the Tenth International Histocompatibility Workshop. Thus the HLA-DR5 frequencies in the Xhosa and Cape Coloureds as shown in Table 4.30 are somewhat underestimated, while those seen for DRw6 are probably overestimated.

HLA-DRw6

The frequency of HLA-DRw6 in the Xhosa was significantly higher ($p<0.001$) than in the Cape Coloureds and SA Caucasoids. The frequency of DRw6 in the Xhosa (gf= 0.193) was similar to the frequency found in the Nigerians (gf=0.235) and the American Negroes (gf=0.209). Analysis of our Tenth International Histocompatibility Workshop data has shown that the HLA-DRw6 serology is still complex. HLA-DRw8x14, a new variant of HLA-DRw14(6), was reported by us (du Toit and Oudshoorn 1987, du Toit et al. 1988b) and described in section 4.1.4. This specificity was seen in two Cape Coloured families and may be identical to the rare DRw8I variant seen in Asian Indians (Awad and Festenstein 1987). As mentioned above the HLA-DRw6 frequencies for the Cape Coloureds and Xhosa are still somewhat doubtful. New HLA-DRw6 variants have also been reported for the American Negroes (Johnson 1986).

HLA-DR7

HLA-DR7 was clearly defined in all three South African populations and appeared to be serologically homogeneous.

Similar frequencies for DR7 were seen in the SA Caucasoids (gf=0.122) and Cape Coloureds (gf=0.139), but the frequency in the Xhosa (gf=0.082) was significantly lower than in the SA Caucasoids ($p<0.01$) and Cape Coloureds ($p<0.001$). The frequency of DR7 was similar in the Xhosa (gf=0.082) and the Nigerians (gf=0.092), and slightly higher in the American Negroes (gf=0.128).

HLA-DRw8, DR9 and DRw10

The HLA-DR antigens, DRw8, DR9 and DRw10, were found to have a low frequency in all three South African population groups tested. The frequency for DRw8 was significantly lower in the Xhosa when compared with the SA Caucasoids ($p<0.01$), Cape Coloureds ($p<0.05$), Nigerians ($p<0.001$) and American Negroes ($p<0.001$). There was no evidence of heterogeneity of DRw8, as described during the Third Asia-Oceania Histocompatibility Workshop by Yagita et al. (1986) in the Japanese. Similar low frequencies for HLA-DR9 were seen in the three South African populations tested as well as the Nigerians and American Negroes. The frequency for HLA-DRw10 in the Xhosa (gf=0.026) was similar to that found in the American Negroes (gf=0.027), but was significantly lower ($p<0.02$) in Nigerians, in whom this antigen was not detected.

HLA-DRw52 and DRw53

The HLA-DR specificities DRw52 and DRw53 (Table 4.30) were associated with the same HLA-DR antigens in all three groups tested. HLA-DRw52 includes DR3, DR5, DRw6 and DRw8, and HLA-DRw53 includes DR4, DR7 and DR9. There was a significant increase in frequency of DRw52 in the Xhosa compared to the SA Caucasoids ($p<0.001$) and Cape Coloureds ($p<0.001$). This was largely due to an increased frequency of DR3 in the Xhosa. The frequency of DRw53 was lower in the Xhosa when compared with the SA Caucasoids ($p<0.001$) and Cape Coloureds ($p<0.01$). This can be attributed to the decreased frequencies of DR4 and DR7 in the Xhosa. The frequencies of HLA-DRw52 and DRw53 in the Xhosa were similar to those observed for the Nigerians and American Negroes.

HLA-DQ antigens

The frequency of HLA-DQw1 did not differ significantly in the three South African population groups tested. The frequency of DQw1 in the Xhosa was similar to that of the Nigerians and American Negroes. The frequency of HLA-DQw2 was significantly decreased in the Xhosa when compared with SA Caucasoids ($p<0.05$) and American Negroes ($p<0.02$)(Table 4.30). This low DQw2 frequency in the Xhosa, in spite of a high frequency of DR3, was not surprising as DR3 was seen together with DQw4 more often than with DQw2 in this group (section 4.1.2). HLA-DQw2 was not tested in the Nigerians. HLA-DQw3 was significantly decreased in the Xhosa when

compared with the SA Caucasoids ($p<0.02$) and Cape Coloureds ($p<0.05$). The frequency of DQw3 in the Xhosa was significantly increased ($p<0.05$) compared with the Nigerians but similar to that of American Negroes.

HLA-B,DR haplotypes

In Table 4.31, a comparison of the HLA-B,DR haplotypes with a significant delta value ($p<0.001$) for linkage disequilibrium in one or more of the three South African populations is shown. Only two HLA-B,DR haplotypes, B7,DR2 and B8,DR3 were in strong positive linkage disequilibrium in all three populations. The HLA-B,DR haplotype with the highest delta value and haplotype frequency in the Xhosa was Bw42,DR3 which is part of the SA Negro haplotype A30,Bw42,Cw2,DR3(DRw18),DQw4,Dw'RSH'. The Bw42,DR3 haplotype was also common in the Cape Coloureds (Table 4.31).

The most common HLA-B,DR haplotypes in significant linkage disequilibrium in the Nigerians were Bw53,DRw8; B18,DR3 and Te74,DR1 and these were different to those observed in the Xhosa (Table 4.31). The antigen Te74 is a rare variant of HLA-B15 identified in Negroes and first described in the report of the 31st International cell exchange. The Xhosa were not tested for the presence of Te74. No HLA-B,DR haplotypes were given in the studies on American Negroes reported by Johnson (1986) and Duquesnoy et al. (1979b).

TABLE 4.31 HLA-B,DR haplotypes with a significant delta value ($p<0.001$) in one or more of the three South African populations.

Haplotype		South African Negroes(Xhosa) (n=435) Delta	HF	Chi-square	Cape Coloureds (n=549) Delta	HF	Chi-square	South African Caucasoids (n=365) Delta	HF	Chi-square
B35	DR1	.0002	.0012	0	.0048	.0079	4.5[b]	.0292	.0381	46.8[a]
B45	DR1	.0183	.0173	56.1[a]	.0072	.0077	18.3[a]	0	.0008	0
Bw56	DR1	0	0	0	-.0002	0	0.2	.0037	.0019	12.6[a]
B7	DR2	.0361	.0462	62.9[a]	.0201	.0401	16.3[a]	.0489	.0702	58.1[a]
B51	DR2	.0054	.0044	11.9[a]	.0013	.0061	0.3	-.0031	.0036	0.8
Bw53	DR2	-.0027	0	2.6	-.0001	.0021	0	-.0007	0	0.7
B8	DR3	.0245	.0357	18.0[a]	.0284	.0303	108.5[a]	.0645	.0659	224.8[a]
Bw42	DR3	.0547	.0809	57.4[a]	.0138	.0161	41.5[a]	.0005	.0001	0.3
Bw62	DR4	-.0008	0	0.7	.0132	.0148	22.9[a]	.0242	.0349	24.5[a]
B13	DR7	.0029	.0029	3.6	.0035	.0055	2.8	.0133	.0142	29.7[a]
B14	DR7	-.0005	.0021	0	.0006	.0053	0.1	.0095	.0140	12.3[a]
Bw57	DR7	.0034	.0076	1.3	.0234	.0277	53.3[a]	.0088	.0110	11.4[a]
Bw58	DRw8	.0126	.0250	5.6[b]	-.0011	.0012	0.4	.0002	.0004	0
Bw63	DRw8	-.0003	0	0.2	0	.0004	0	.0037	.0028	15.5[a]
B7	DRw10	.0039	.0056	2.9	.0001	.0012	0	.0114	.0105	26.8[a]

HF = haplotype frequency

a $p<0.001$

b $p<0.05$

However, HLA-B,DR haplotypes were reported for the American Negroes studied during the Eighth International Histocompatibility Workshop (Baur and Danilovs 1980). The Bw42,DR3 haplotype found in positive linkage disequilibrium in the Xhosa was also found in significant positive linkage disequilibrium in the American Negroes. In addition, the haplotypes B7,DR1; B44,DR5; Bw57,DR7 and Bw58,DR2 were found in linkage disequilibrium in the American Negroes.

Extended haplotypes

Examination of the positive delta values as well as the haplotypes derived from family studies (section 5.1) suggest the existence of the following common extended haplotypes among the SA Negroes:

A30,Cw2,Bw42,Bw6,FC10,DRw18,DRw52,DQw4;

A3,Cw6,Bw58,Bw4,SC3Q0,DRw18,DRw52,DQw4;

A29,Cw7,B44,Bw4,FC511,DRw11,DRw52,DQw6;

A2,Cw-,B45,Bw6,FC31,DRw13,DRw52,DQw6;

A2,Cw7,Bw58,Bw4,FC31,DRw11,DRw52,DQw7;

A30,Cw6,Bw58,Bw4,SC3(2,92),DRw12x6,DRw52,DQw5.

The extended haplotypes found in the SA Caucasoids were identical to those of other Caucasoids (Dawkins et al. 1983, Awdeh et al. 1985, Alper et al. 1986) These were:

A1,Cw7,B8,Bw6,SC01,DRw17,DRw52,DQw2;

A3,Cw7,B7,Bw6,SC31,DRw15,DQw6;

A2,Cw7,B7,Bw6,SC31,DRw15,DQw6;

A2,Cw5,B44,Bw4,SC30,DR4,DRw53,DQw8;

A29,Cw'blank',B44,Bw4,FC31,DR7,DRw53,DQw2.

In the Cape Coloureds the same extended haplotypes found in the SA Negroes and SA Caucasoids were seen. No unique extended haplotype was seen in this group.

Genetic Distances

Pairwise genetic distances were calculated using the gene frequencies for the HLA-A, B, C, DR and DQ loci, by the method of Cavalli-Sforza and Bodmer (1971) The genetic distance values between SA Negroes (Xhosa), Cape Coloureds and SA Caucasoids are shown in Table 4.32. The numerical

TABLE 4.32 Genetic distances between SA Negroes (Xhosa), SA Caucasoids and Cape Coloureds.

HLA	Xhosa versus SA Caucasoids	Xhosa versus Cape Coloureds	Cape Coloureds versus SA Caucasoids
HLA-A	0.043	0.015	0.014
HLA-B	0.032	0.011	0.009
HLA-C	0.029	0.006	0.011
HLA-DR	0.020	0.015	0.004
HLA-DQ	0.009	0.004	0.001
A+B	0.035	0.012	0.011
A+B+C	0.035	0.012	0.011
A+B+C+DR	0.032	0.012	0.010
A+B+C+DR+DQ	0.032	0.012	0.010

results varied somewhat for the individual HLA loci but the cumulative values for the various combinations of the HLA loci were similar. The relatively low values obtained between Xhosa and Cape Coloureds (0.012) as well as between Cape Coloureds and SA Caucasoids (0.010) suggest only a limited genetic dissimilarity between the Cape Coloureds and SA Caucasoids and between the Xhosa and the Cape Coloureds. These results therefore confirm that the Cape Coloureds are genetically intermediate between the SA Negroes and the SA Caucasoids. The genetic distance value obtained for the Xhosa versus the SA Caucasoids (0.032) is in line with that reported between major populations in a previous study by us (Nurse et al. 1975).

Table 4.33 shows the genetic distances between the Xhosa, Cape Coloureds, Nigerians and American Negroes. The genetic distance of the Xhosa from the Nigerians was 0.023, which is numerically about half the distance observed between two major populations (0.050) (Nurse et al. 1975). As shown in Table 4.33, the genetic distance of the Xhosa from American Negroes was 0.017, which is similar to the distance between the Nigerians and American Negroes (0.017) and the Zambians and American Negroes (0.016) (Nurse et al. 1975).

In *summary*, this study provides a detailed description of the HLA-DR and DQ polymorphism in the three main populations resident in Cape Town. The HLA-DR and DQ frequencies in the

TABLE 4.33 Genetic distances between the Xhosa, Cape Coloureds, Nigerians and American Negroes.

HLA	Xhosa vs Nigerians	Cape Coloureds vs Nigerians	Xhosa vs American Negroes	Cape Coloured vs American Negroes	Nigerians vs American Negroes
HLA-A	0.023	0.035	0.018	0.013	0.015
HLA-B	0.020	0.024	0.012	0.011	0.017
HLA-C	0.028	0.020	0.025	0.013	0.017
HLA-DR	0.035	0.027	0.020	0.019	0.024
HLA-DQ	0.012	0.027	0.026	0.017	0.007
A+B	0.021	0.028	0.014	0.012	0.016
A+B+C	0.022	0.027	0.016	0.012	0.016
A+B+C+DR	0.024	0.027	0.016	0.013	0.017
A+B+C+DR+DQ	0.023	0.027	0.017	0.013	0.017

Xhosa were found to be different from those of the Caucasoids and Cape Coloureds. The HLA-DR1, DR4, DR7, DRw8 as well as the HLA-DQw2 and DQw3 antigens were found at a significantly lower frequency, whereas HLA-DR3 and DRw6 were found at a significantly higher frequency in the Xhosa compared with the SA Caucasoids. The HLA-DR3 antigen was the most frequently occurring in the Xhosa and studies have shown that HLA-DRw18 is the predominant HLA-DR3 split in this group (section 4.3.2). Of the existing DR2 subgroups, the classic HLA-DR2 subgroup, DRw15, occurred most frequently in all three population groups tested. Of the remaining two subgroups, HLA-DRw16 was seen more often in the Cape Coloureds and HLA-DR2 LUM(CT) in the Xhosa. The Cape Coloureds, whose genetic constitution is a result of early gene mixing at the Cape, now have HLA gene frequencies that are completely different from the original groups. In some instances, the phenotype frequencies were closer to the SA Caucasoids. For example, HLA-DR7 was present in 23.0% of SA Caucasoids and 25.8% of Cape Coloureds, but in only 15.7% of the Xhosa. In other instances, the frequencies in the Cape Coloureds approximated the Xhosa frequencies, such as in the case of HLA-DR1 which was present in 11.5% of Cape Coloureds, 9.2% of Xhosa but was much higher in the SA Caucasoids where it was present in 20.3%.

The genetic distance values between the Xhosa and Cape Coloureds and the SA Caucasoids and Cape Coloureds were of

the same order of magnitude, indicating that the Cape Coloureds are genetically intermediate between the SA Caucasoids and SA Negroes and thus provide a classic example of a hybrid race of recent origin. Only two HLA-B,DR haplotypes, B7,DR2 and B8,DR3 were in significant association in all three populations tested. These two haplotypes are, however, not commonly observed in other populations such as the Nigerians, American Negroes, Japanese (Baur and Danilovs 1980) and Chinese (Tait 1987). The extended haplotypes found in the SA Negroes differed from those found in the SA Caucasoids. Several of the extended haplotypes seen in the Caucasoids are strongly associated with increased disease susceptibility (Dawkins et al. 1983, Alper et al. 1986). As yet no information on the SA Negro extended haplotypes and disease susceptibility is available.

There were considerable differences in the frequencies of the HLA-DR and DQ antigens in the Xhosa compared with the Nigerians. The gene frequencies of HLA-DR3 and DR4 were lower and those for HLA-DR2, DRw8, DR9 and DQw3 were higher in the Nigerians compared with the Xhosa. The relatively high genetic distance (0.023) between the Xhosa and Nigerians would indicate that the southern African Bantu-speaking Negroes diverged from the other Bantu-speaking Negroes at a relatively early time, in keeping with the probable routes of mankind's migration suggested by Cavalli-

Sforza and Bodmer (1971). Large differences were also seen when the frequencies for the HLA-DR and DQ antigens were compared with those in the American Negroes. The American Negroes differed from the Xhosa for the frequencies of HLA-DR3 ($p<0.02$) and DRw8 ($p<0.001$) which were significantly lower and the frequencies of HLA-DR1 ($p<0.05$) and DQw2 ($p<0.02$) which were significantly higher in the American Negroes. The difference in gene frequencies is not surprising as the American Negroes have a large proportion of Caucasoid and American Indian admixture. Furthermore, as they mainly originated from the West Coast of Africa, they are unlikely to have any Khoisan admixture as was shown for the SA Negroes (Jenkins et al. 1970, du Toit et al. 1988a).

CHAPTER 5

THE ASSOCIATION OF HLA-DR AND HLA-DQ ANTIGENS IN SOUTH AFRICAN POPULATIONS

The HLA-D region is said to play a crucial role in the pathogenesis of many diseases (Korman et al. 1985, Strominger 1986). Certain HLA-DR antigens have been shown to be present at increased frequency in patients with autoimmmune and other disorders compared with random individuals from the same population (reviewed in Svejgaard et al. 1983 and in Tiwari and Terasaki 1985). The HLA-DR genes may be directly involved in disease susceptibility, or they may merely be in linkage with the "true" genes. There is now evidence that some disease susceptibilities may be related to HLA-DQ specificities, either as primary associations (Tosi et al. 1983) or in combination with particular HLA-DR antigens (Festenstein et al. 1986). It is therefore important to establish reliable frequencies for HLA-DR and DQ antigens (see section 4.2) and HLA-DR,DQ associations in the various populations as these data can then be used for comparison with patient groups.

5.1 A COMPARISON OF THE ASSOCIATION OF HLA-DR AND HLA-DQ ANTIGENS IN SA NEGROES, CAPE COLOUREDS AND SA CAUCASOIDS

In this section an analysis of the HLA-DR,DQ associations obtained from random individuals of three South African population groups as well as linkage data obtained from families will be given.

The HLA-DQ and DR loci are closely linked on chromosome 6 thus ensuring a marked correlation between HLA-DR and DQ specificities. Strong associations of DQw1 with DR1, DR2, DRw6 and DRw10, of DQw2 with DR3 and DR7, and of DQw3 with DR4, DR5, and to a lesser extent with DR7, have been well documented in the Caucasoid populations (Baur et al. 1984, Awad et al. 1987 and Dupont 1988). Studies of various non-Caucasoid populations have shown that different DR,DQ associations from those observed in Caucasoids exist (Oudshoorn et al. 1984, Ikeda et al. 1986, Layrisse et al. 1988).

Table 5.1 shows the the HLA-DR,DQ associations in the SA Negroes (Xhosa), Cape Coloureds and SA Caucasoids. The HLA-DR,DQ haplotypes with significant delta values ($p<0.01$) were the same in all three population groups tested, with the exception of HLA-DRw10,DQw1 which was not found in significant association in the Cape Coloureds.

TABLE 5.1 HLA-DR,DQ haplotypes with significant delta values ($p<0.01$) for linkage disequilibrium in one or more of the three South African populations.

Haplotype		South African Negroes(Xhosa) (n=362)			Cape Coloureds (n=395)			South African Caucasoids (n=281)		
		Delta	HF	Chi-square	Delta	HF	Chi-square	Delta	HF	Chi-square
DR1	DQw1	.0258	.0375	16.1[a]	.0222	.0340	12.7[a]	.0494	.0687	27.1[a]
DR2	DQw1	.0523	.0865	31.0[a]	.1051	.1799	87.0[a]	.0786	.1322	48.9[a]
DR3	DQw2	.0325	.0653	14.9[a]	.0294	.0362	24.2[a]	.0727	.0837	87.6[a]
DR4	DQw3	.0360	.0471	46.0[a]	.0576	.0705	67.4[a]	.0865	.1226	64.4[a]
DR5	DQw3	.0766	.0999	92.3[a]	.0879	.1187	106.7[a]	.0678	.1028	50.3[a]
DRw6	DQw1	.1049	.2017	74.7[a]	.0807	.1334	67.3[a]	.0663	.1106	39.2[a]
DR7	DQw2	.0499	.0564	96.2[a]	.0852	.1055	173.8[a]	.0560	.0701	55.9[a]
DRw10	DQw1	.0124	.0174	7.4[b]	.0052	.0070	3.7	.0109	.0163	6.0[c]

HF = haplotype frequency

a $p<0.001$

b $p<0.01$

c $p<0.05$

Family studies

The HLA-DR,DQ haplotypes were studied in a total of 180 families, consisting of 42 SA Negro, 93 Cape Coloured and 45 SA Caucasoid families. The 42 SA Negro families were predominantly Xhosa. The families studied consisted of random families tested during the Ninth International Histocompatibility Workshop and families of prospective renal and bone-marrow transplant patients (Table 5.2). Only those haplotypes shown to segregate in a family were counted and analyzed (Table 5.3). As only the parental haplotypes were included in this analysis, the data obtained should approximate that produced by random individuals, assuming the families were a random sample. However, this may not be the case as there is some evidence for the association of HLA with leukemia and aplastic anemia (Reviewed by Tiwari and Terasaki 1985).

TABLE 5.2 Families tested for the HLA-DR,DQ haplotype study.

Population	Family of bone-marrow transplant patient (n=74)	Family of renal trans-plant patient (n=83)	Random healthy family (n=23)
SA Negro	9	18	15
Cape Coloured	35	52	6
SA Caucasoid	30	13	2

n = number of families tested

TABLE 5.3 HLA-DR,DQ haplotypes in the SA Negroes, Cape Coloureds and SA Caucasoids.

HLA-DR	HLA-DQ	SA Caucasoids (n=150)	Cape Coloureds (n=287)	SA Negroes (n=149)
DR1	DQw1	10.0[a]	4.5	4.7
DR2	DQw1	16.0	22.2	10.1
DR3	DQw2	10.0	8.0	10.1
DR3	DQ-	0	1.4	12.8
DR3	DQw3	0	0.3	2.0
DR4	DQw3	15.3	9.8	3.4
DR4	DQw1	0	0.3	0
DR4	DQ-	0	1.0	0
DR5	DQw3	12.0	12.5	12.8
DR5	DQw1	0.7	1.7	5.4
DRw6	DQw1	13.3	17.4	20.8
DR7	DQw2	10.2	12.2	10.1
DR7	DQw3	2.7	1.0	0.7
DRw8	DQ-	4.0	1.0	0
DRw8	DQw3	0.7	1.4	2.0
DR9	DQw3	2.7	1.0	1.3
DR9	DQw2	0	0	0.7
DRw10	DQw1	1.3	3.1	3.4
DR-	DQw3	0	0.3	0
DR-	DQ-	1.3	0.7	0

n = number of haplotypes investigated

a expressed as percentage of total number of haplotypes

HLA-DR1, DR2, DRw6 and DRw10

HLA-DR1, DR2, DRw6 and DRw10 were seen only with DQw1. The haplotypes DR2,DQw3 and DRw6(DRw14),DQw3 observed in American Indians (Layrisse et al. 1988) and DRw6(DRw13),DQw3 observed in Caucasoids (Schreuder et al. 1984b) were not seen in this group.

HLA-DR3

The haplotype HLA-DR3,DQw2 was present in all three populations, while HLA-DR3,DQ'blank' and HLA-DR3,DQw3 were seen only in the SA Negroes and Cape Coloureds. From studies described in section 4.1.2 it is likely that the majority, if not all of the DR3 antigens linked with DQw2, are the DRw17 split of DR3, while those linked with DQ'blank' are, in fact, DRw18. The DQ'blank' mentioned is most likely the newly defined DQw4 antigen (Endo et al. 1987) for which this group was not tested. The existence of the haplotype HLA-DR3,DQw3 was first reported by us in SA Negroes (Oudshoorn et al. 1984) and it has to our knowledge not been seen in any other population. In this study the HLA-DR3,DQw3 haplotype was seen in the SA Negroes and Cape Coloureds (Table 5.3). A Xhosa family carrying this rare haplotype will be discussed in more detail in section 5.2.1.

HLA-DR4

HLA-DR4 was linked to HLA-DQw3 in virtually all DR4 containing haplotypes. There were however, two rare haplotypes DR4,DQw1 and DR4,DQ'blank' (probably DR4,DQw4). The haplotype DR4,DQ'blank' is commonly seen in Mongoloids such as the Japanese and the Australian Aborigines (Ikeda et al. 1986). The DR4,DQ'blank' haplotypes in this study were only observed in the Cape Coloureds, illustrating their Mongoloid admixture. To our knowledge, this is the first time that the haplotype DR4,DQw1 has been observed. The

Cape Coloured family with this unusual haplotype was studied further using serological, cellular and Southern blot analysis (section 5.2.2).

HLA-DR5

The HLA-DR5,DQw3 haplotype was frequently found in the SA Negroes, Cape Coloureds and SA Caucasoids. HLA-DR5 was also found with DQw1 in the SA Negroes (5.4%), Cape Coloureds (1.7%) and SA Caucasoids (0.7%). This haplotype was also present in the Nigerians (Okoye et al. 1985).

HLA-DR7

More than 90% of the DR7 haplotypes in the SA Negroes and Cape Coloureds carried the DQw2 antigen, while the others carried DQw3. In the SA Caucasoids 15 (79%) of the DR7 haplotypes carried DQw2 and four (19%) DQw3. The figures for the SA Caucasoids were similar to those of British Caucasoids, where 72% of DR7 haplotypes were found to be associated with DQw2 and 18% with DQw3 (Awad et al. 1987).

HLA-DRw8

Two different HLA-DRw8 haplotypes, HLA-DRw8,DQw3 and HLA-DRw8,DQ'blank' (probably DRw8,DQw4) were found in this study. Both haplotypes were seen in the SA Caucasoids and Cape Coloureds, whereas the HLA-DRw8,DQ'blank' haplotype was the only DRw8 bearing haplotype observed in the SA Negroes. HLA-DRw8,DQw1, observed in Australian Aborigines and

Orientals, was not seen in this group (Termijtelen et al. 1980a, Serjeantson et al. 1982, Bétuel et al. 1984b).

HLA-DR9

In the SA Caucasoids and Cape Coloureds, DR9 segregated with DQw3. However, in the SA Negroes DR9 was seen linked with DQw3 and DQw2. The haplotype HLA-DR9,DQw2 was first reported during the Ninth Histocompatibility Workshop by Nunez and Stastny (1983) where it was seen in an American Negro family. The haplotype DR9,DQw2 will be discussed further in section 5.2.3.

DR'blank'

In two Caucasoid and two Cape Coloured haplotypes no HLA-DR or DQ antigens could be detected. Whether this was due to a problem with the expression of class II antigens in these families or due to lack of appropriate antisera is not clear. Molecular and biochemical studies planned for the future may clarify this. One DR'blank',DQw3 haplotype was seen in a Cape Coloured family.

In *summary*, HLA-DR,DQ haplotypes with significant delta values were the same in the three populations tested, with the exception of DRw10,DQw1 which was not significantly associated in the Cape Coloureds. Family data showed that in all three populations, HLA-DR1, DR2, DRw6 and DRw10 were seen linked to one DQ antigen only, namely DQw1. Two or

more different DR,DQ haplotypes were seen for the remaining HLA-DR antigens, DR3, DR4, DR5, DR7, DRw8 and DR9. The predominantly Negro haplotypes were: DR3,DQ'blank', DR3,DQw3; DR5,DQw1 and DR9,DQw2. The haplotypes DR4,DQ'blank', DR4,DQw1 and DR'blank',DQw3 were found exclusively in the Cape Coloureds. The presence of the DR4,DQ'blank' haplotype in the Cape Coloureds may be attributed to their Mongoloid admixture, but the origin of DR4,DQw1 and DR'blank',DQw3 is unclear. The unusual DR,DQ haplotypes, DR5,DQw1; DRw8,DQw3 and DR'blank',DQ'blank', found in the Caucasoids represented 2.7% of the haplotypes tested in this group.

5.2 UNUSUAL HLA-DR,DQ HAPLOTYPES IN SA NEGROES AND CAPE COLOURED FAMILIES

In section 5.2 some unusual HLA-DR,DQ haplotypes will be analyzed using serological, cellular and Southern blot techniques.

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. unpublished observations). These unusual associations also provide valuable information for geneticists and anthropologists in the characterization of human populations and their genetic relationships. They may provide valuable insight into the mechanisms such as gene conversion, homologous crossing-over, gene duplication or mutation, by which the HLA region has evolved. Furthermore cells with unusual DR,DQ associations can be helpful in detecting DR (or DQ) antibodies in supposedly monospecific DQ (or DR) antisera (Oudshoorn et al. 1984, Schreuder et al. 1984a). Four unusual HLA-DR,DQ haplotypes were studied to see if the antigens constituting the unusual HLA-DR,DQ haplotype were different to the "classic" DR and DQ antigens. The HLA-DR,DQ haplotypes were investigated using serological, cellular (HTC) and Southern blot analysis. HLA typing was performed using the Tenth International Histocompatibility workshop sera and HTC typing was done using 59 HTCs for the

specificities, Dw1-Dw19, Dw21-Dw23. No HTCs were available for HLA-Dw20, a DR1 associated Dw specificity. Southern blot analysis was performed as described in section 2.2.8, using the Tenth International Histocompatibility Workshop DRB, DQB and DQA probes.

5.2.1 The HLA-DRw17,DQw7 haplotype

Serology

Table 5.4 shows the HLA typing results of a SA Negro (Xhosa) family TAB. Using the serological definitions for the HLA-DR3 subgroups, DRw17 and DRw18 (section 4.1.2), it could be seen that there were two different HLA-DR3 haplotypes in this family. The classic Negro HLA-DR3 haplotype, DRw18,DQw4,Dw'RSH', was present in the mother (haplotype c) and was inherited by three of her children 2, 3 and 4. The father's (a) haplotype carried the DR3 subgroup DRw17. As shown in Table 5.4, the DQw2 sera gave negative reactions with this haplotype, whereas positive reactions were obtained with the "broad" DQw3 sera and sera for the DQw3 subgroup DQw7, thus indicating that DRw17 was linked to DQw7 instead of DQw2. The HLA-DRw17,DQw7 haplotype was inherited by the children 1 and 2.

HLA-Dw typing

The HLA-Dw typing results of family TAB with the relevant (i.e. the DR3 and DRw13 related) HTCs are depicted in Table 5.5. The HTC-RSH (Dw'RSH') gave typing responses with the

DRw18,DQw4 haplotype (c) in the mother and children 2, 3 and 4, the DRw13,DQw6 haplotype (b) was typed by HTCs with the Dw18 specificity and the DRw13,DQw6 haplotype (d) was typed by HTCs with the Dw19 specificity. However, no typing responses were observed for the DRw17,DQw7 haplotype (a) indicating that this haplotype carried an unknown HLA-Dw specificity.

TABLE 5.4 Segregation patterns for HLA-DRw17,DQw7 and DRw18,DQw4 in family TAB.

				10 WS sera				
				DR3		DQw2	DQw3	DQw7	DQw4
				1111	1113	1133	111333	333	113
				0000	0000	1111	111111	111	001
				3443	3332	6700	778111	122	890
	HLA-DR		DQ	9037	4683	9357	694124	901	321
Father	a	w17	w7	**8886**	**6846**[a]	1101	**686648**	**486**	110
	b	w13	w6						
Mother	c	w18	w4	8886	2142	1111	111111	210	468
	d	w13	w6						
Child 1	a	w17	w7	**8888**	**8868**	4201	**888068**	**800**	110
	d	w13	w6						
Child 2	a	w17	w7	**4464**	**6646**	2201	**266668**	**664**	460
	c	w18	w4						
Child 3	b	w13	w6	8886	1114	1101	111111	112	660
	c	w18	w4						
Child 4	b	w13	w6	8688	1241	1100	111011	000	860
	c	w18	w4						

a The results are scored as follows: 0 = not tested 1 = negative; 2 = doubtful negative; 4 = weak positive; 6 = positive; 8 = strong positive

TABLE 5.5 HLA Dw typing results for family TAB.

Responder cells	HLA-DR	DQ	Dw	Stimulator cells (HTCs) Dw'RSH' RSH	Dw3 COX	Dw3 LAMP	Dw3 QBL	Dw3 OTTO	Dw3 CDT	Dw18 OMW	Dw18 HHK	Dw18 CB	Dw19 NSI	Dw19 KRA	Dw19 KT11
Father	a w17	w7	blank	102	60	86	52	46	64	22	34	48	81	66	101
	b w13	w6	w18												
Mother	c w18	w4	RSH	23	62	88	64	71	118	49	71	56	39	41	52
	d w13	w6	w19												
Child 1	a w17	w7	blank	109	73	66	75	51	83	64	116	77	28	32	49
	d w13	w6	w19												
Child 2	a w17	w7	blank	29	49	73	68	63	76	95	131	69	111	98	100
	c w18	w4	RSH												
Child 3	b w13	w6	w18	34	92	78	107	63	67	14	39	45	80	50	112
	c w18	w4	RSH												
Child 4	b w13	w6	w18	21	134	97	81	75	111	20	35	36	66	74	97
	c w18	w4	RSH												

RR‹50% = typing response

RFLP analysis

Cells of family TAB as well as seven HTCs (Table 5.6) were included in this part of the study. The HTCs were selected so that the two most common Caucasoid HLA-DR3 haplotypes, B8,DRw17,DQw2,Dw3 and B18,DRw17,DQw2,Dw3, as well as the most common SA Negro DR3 haplotype, Bw42,DRw18,DQw4,Dw'RSH', were represented. In addition HTCs with the HLA-DRw13 haplotypes, DRw13,DQw6,Dw18 and DRw13,DQw6,Dw19 present in family TAB were included.

TABLE 5.6 Homozygous typing cells used in this study.

Cell identity	Workshop Number	HLA A	B	C	DR	DQ	Dw	Dw[a]
RSH	10W9021	30,w68	w42	w2	w18	w4	RSH	w24
BTI		30	w42	w2	w18	w4	RSH	nt
COX	10W9022	1	8	w7	w17	w2	w3	w24
MITCH		1	8	w7	w17	w2	w3	nt
LOO81785	10W9018	3,24	18	w5	w17	w2	w3	w25
OMW	10W9058	2	45	w-	w13	w6	w18	w24
NSI		28,34	w70	w4	w13	w6	w19	nt

a = alleles of the DRB3(DRw52) gene
nt = not tested

The results of the RFLP patterns for DNA isolated from family TAB and the HTCs, digested with Taq I and Eco RI, and hybridized to the DRB cDNA probe are shown in Figures 5.1A and B. A summary of these results is given in Table 5.7.

The DRw17,DQw7 haplotype (a) of family TAB has the DRB1 RFLP pattern associated with DRw17 and the DRB3 pattern

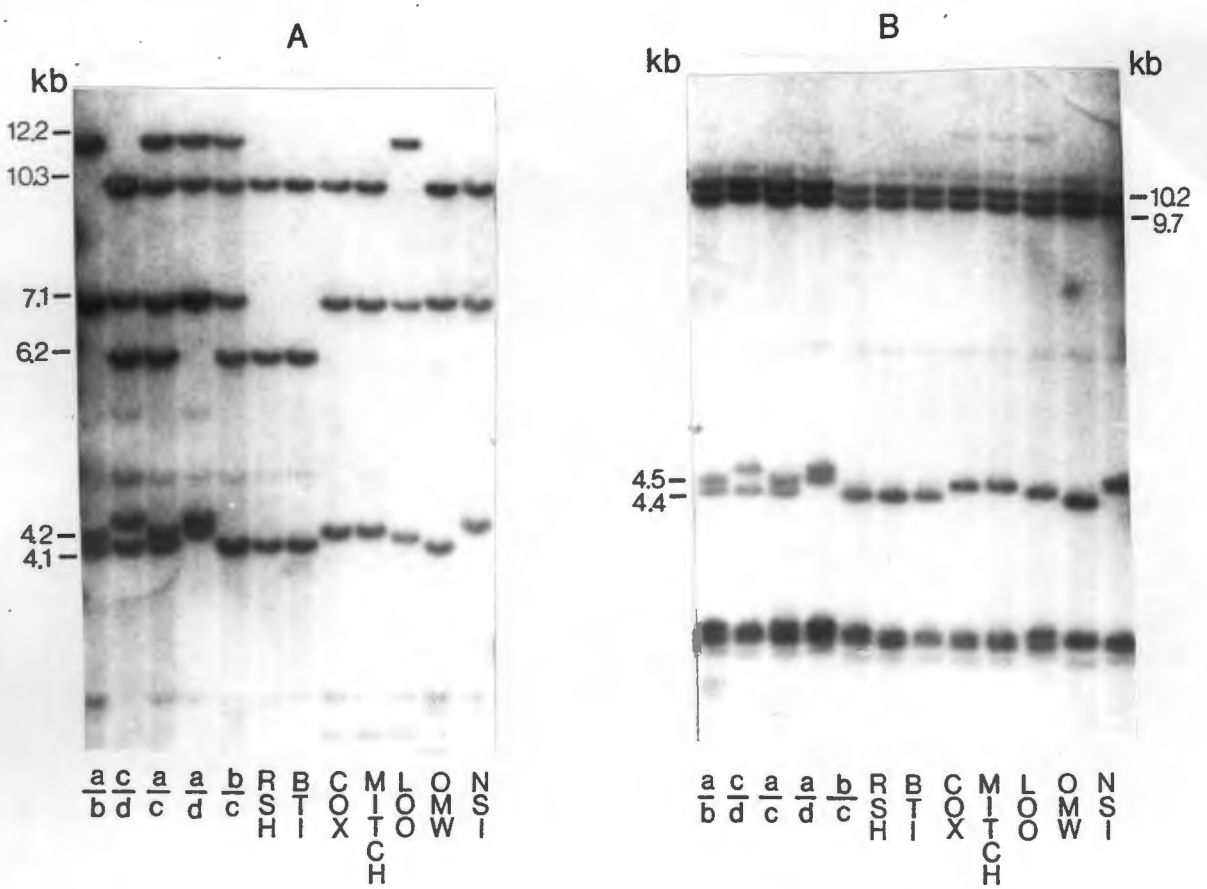

FIGURE 5.1 Hybridization of the DRB cDNA probe to DNA digested with (A) Taq I and (B) Eco RI. DNA was obtained from family TAB (DRw17,DQw7) and the homozygous cell lines RSH (DRw18), BTI (DRw18), COX (DRw17), MITCH (DRw17), LOO81785 (DRw17), OMW (DRw13,Dw18) and NSI (DRw13,Dw19).

Haplotypes:

a Aw68, Bw72, Cw6, DRw17, DQw7, Dw'blank'
b A3, Bw58, Cw6, DRw13, DQw6, Dw18
c Aw34, B8, Cw7, DRw18, DQw4, Dw'RSH'
d A30, Bw71, Cw2, DRw13, DQw6, Dw19

The following fragments segregated with the DRw17 haplotypes: the 7.1 kb Taq I fragment (also with DRw13,Dw18 and DRw13,Dw19), the 4.2 kb Taq I fragment, and the 4.5 kb Eco RI fragment. Fragments segregating with the DRw18 haplotypes were the 6.2 kb Taq I fragment, the 4.1 kb Taq I fragment (also with DRw13,Dw18) and the 4.4 kb Eco RI (also with DRw13,Dw18). The 12.2 kb Taq I fragment is associated with Dw25, whereas the 10.3 kb Taq I fragment is associated with Dw24 and Dw26.

TABLE 5.7 Summary of DRB RFLPs in family TAB and HTCs tested.

					Taq I (kb)							Eco RI (kb)				
	DR	DQ	Dw	Dw[a]	12.2	10.3	7.1	6.2	4.3	4.2	4.1	10.2	9.7	4.8	4.5	4.4
Family TAB																
Haplotype(a)	w17	w7	blank	nt	+	-	+	-	-	+	-	+	+	-	+	-
Haplotype(b)	w13	w6	w18	nt	+	-	+	-	-	-	+	+	+	-	-	+
Haplotype(c)	w18	w4	RSH	nt	-	+	-	+	-	-	+	+	+	-	-	+
Haplotype(d)	w13	w6	w19	nt	-	+	+	-	+	-	-	+	+	+	-	-
HTCs																
RSH	w18	w4	RSH	w24	-	+	-	+	-	-	+	+	+	-	-	+
BTI	w18	w4	RSH	nt	-	+	-	+	-	-	+	+	+	-	-	+
COX	w17	w2	w3	w24	-	+	+	-	-	+	-	+	+	-	+	-
MITCH	w17	w2	w3	nt	-	+	+	-	-	+	-	+	+	-	+	-
LOO81785	w17	w2	w3	w25	+	-	+	-	-	+	-	+	+	-	+	-
OMW	w13	w6	w18	w24	-	+	+	-	-	-	+	+	+	-	-	+
NSI	w13	w6	w19	nt	-	+	+	-	+	-	-	+	+	+	-	-

a = alleles of the DRB3(DRw52) gene
+ indicates fragment present
- indicates fragment absent
nt = not tested

TABLE 5.8 Summary of DQB RFLPs of family TAB and the HTCs tested.

					Taq I (kb)						Eco RI (kb)				
	DR	DQ	Dw	Dw[a]	5.5	4.6	3.0	2.6	2.4	1.9	19.5	12.5	9.1	6.5	2.3
Family TAB															
Haplotype(a)	w17	w7	blank	nt	-	+	-	-	-	-	-	+	-	-	-
Haplotype(b)	w13	w6	w18	nt	-	-	+	+	-	-	-	-	+	-	+
Haplotype(c)	w18	w4	RSH	nt	-	-	-	-	+	+	+	-	-	-	-
Haplotype(d)	w13	w6	w19	nt	+	-	-	-	-	-	-	-	+	-	+
HTCs															
RSH	w18	w4	RSH	w24	-	-	-	-	+	+	+	-	-	-	-
BTI	w18	w4	RSH	nt	-	-	-	-	+	+	+	-	-	-	-
COX	w17	w2	w3	w24	-	+	-	-	-	-	-	+	-	+	-
MITCH	w17	w2	w3	nt	-	+	-	-	-	-	-	+	-	+	-
LOO81785	w17	w2	w3	w25	-	+	-	-	-	-	-	+	-	+	-
OMW	w13	w6	w18	w24	-	-	+	+	-	-	-	-	+	-	+
NSI	w13	w6	w19	nt	+	-	-	-	-	-	-	-	+	-	+

a = alleles of the DRB3(DRw52) gene

\+ indicates fragment present

\- indicates fragment absent

TABLE 5.9 Summary of DQA RFLPs in family TAB and HTCs tested.

					Taq I (kb)						Eco RI (kb)			
	DR	DQ	Dw	Dw[a]	7.0	6.5	6.2	4.7	2.2	2.1	15.0	12.1	2.0	1.35
Family TAB														
Haplotype(a)	w17	w7	blank	nt	-	-	-	+	-	+	-	+	+	-
Haplotype(b)	w13	w6	w18	nt	+	-	-	-	-	+	+	-	+	-
Haplotype(c)	w18	w4	RSH	nt	-	-	+	-	-	+	-	+	+	-
Haplotype(d)	w13	w6	w19	nt	-	+	-	-	+	-	+	-	-	+
HTCs														
RSH	w18	w4	RSH	w24	-	-	+	-	-	+	-	+	+	-
BTI	w18	w4	RSH	nt	-	-	+	-	-	+	-	+	+	-
COX	w17	w2	w3	w24	-	-	-	+	+	-	-	+	-	+
MITCH	w17	w2	w3	nt	-	-	-	+	+	-	-	+	-	+
LOO81785	w17	w2	w3	w25	-	-	-	+	-	+	-	+	+	-
OMW	w13	w6	w18	w24	+	-	-	-	+	-	+	-	-	+
NSI	w13	w6	w19	nt	-	+	-	-	+	-	+	-	-	+

a = alleles of the DRB3(DRw52) gene

+ indicates fragment present

- indicates fragment absent

associated with HLA-Dw25. The DQB hybridization pattern seen for the DRw17,DQw7 haplotype and shown in Table 5.8 was identical to other DQw7 haplotypes (section 4.1.3). It differed from the pattern observed for the DQw2 homozygous cell lines COX, MITCH and LOO81785 by the absence of the 6.5 kb Eco RI fragment, which is a fairly weak band specific for DQw2 and can be useful in the distinction of DQw7 from DQw2 as shown here. The DQA RFLP pattern of the DRw17,DQw7 haplotype was identical to the DRw17,DQw2 bearing haplotypes (Table 5.9) and differed from DQw7 haplotypes (section 4.1.3). The DRB, DQB and DQA RFLP patterns of the DRw18,DQw4 haplotype (c) (Tables 5.7, 5.8, 5.9) were identical to the patterns described previously for the DRw18,DQw4 haplotypes (section 4.1.2).

In *summary*, in this section a family with the unusual haplotype DRw17,DQw7 was described. Serological analysis showed no differences in the HLA-DR antigen of the DRw17,DQw7 haplotype when compared to the DR antigen of the DRw17,DQw2 haplotype, nor was any serological difference observed for the DQ antigen of the DRw17,DQw7 haplotype when compared to other DQw7 haplotypes. The DRB and DQA RFLP results of haplotype DRw17,DQw7 were identical to HTC-LOO81785 (B18,DRw17,DQw2,Dw3,Dw25) whereas the DQB RFLP pattern was identical to DQw7 haplotypes. These results are consistent with the sequence data of Todd et al. (1987) showing that the serological DQw7 determinant resides on the

DQ beta chain. Cellular typing tests showed that the HLA-Dw3 frequently seen in association with DRw17,DQw2 was not present on the DRw17,DQw7 haplotype nor was any other known HLA-Dw specificity detected.

5.2.2 The HLA-DR4,DQw5 haplotype

Serology

Table 5.10 shows the HLA typing results of a Cape Coloured family HER. In this family the classic DR4,DQw8 haplotype was present in the mother, whereas the father carried the unusual haplotype HLA-DR4,DQw1. HLA-DRw53 was present on both DR4 haplotypes in this family (Table 5.10). The DR4,DQw1 haplotype was passed on to two of his children (child 3 and 4). Clear segregation of the serological reaction patterns for the DR4,DQw1 haplotype can be seen in Table 5.10. Using the serological technique it could not be established which DQw1 subgroup, DQw5 or DQw6, was present on this haplotype due to the lack of good sera for these subgroups. However, DQw5 could be assigned to this DR4 haplotype based on the Southern blot data. The father's other haplotype carried the DRw16 and DQw5 antigens. This haplotype which has an unusual RFLP pattern will be discussed in section 5.2.4.

HLA-Dw typing

The family HER was HLA-Dw typed using 59 HTCs with the specificity, Dw1 to Dw19, Dw21 and Dw23. The results

TABLE 5.10 Segregation of the DR4,DQw8 and DR4,DQw5 haplotypes in family HER.

				10 WS sera					
				DR4	DRw53	DQw1	DQw5	DQw3	DQw7
				111111111111	11111	11111113	1	111333	333
				000000000011	22222	11111110	1	111111	111
				444544545599	11011	55556610	3	778111	122
		HLA-DR	HLA-DQ	847156093614	26953	93542132	6	694124	901
Father	a	w16	w5	888228686881[a]	64844	86888888	6	112111	111
	b	4	w5						
Mother	c	4	w8	426214264244	16644	46188884	1	468468	111
	d	2LUM(CT)	w6						
Child 1	a	w16	w5	111111111111	11111	66888868	1	124111	211
	d	2LUM(CT)	w6						
Child 2	a	w16	w5	111111111111	11111	86888888	4	111111	111
	d	2LUM(CT)	w6						
Child 3	b	4	w5	648848888888	68866	48168888	6	688888	111
	c	4	w8						
Child 4	b	4	w5	468648686686	46664	48664868	4	466888	111
	c	4	w8						

a The results are scored as follows: 1 = negative; 2 = doubtful negative; 4 = weak positive; 6 = positive; 8 = strong positive

TABLE 5.11 HLA-Dw typing results of family HER using DR4 related HTCs.

					Stimulator cells (HTCs)											
					Dw4			Dw10		Dw13		Dw14		DKT2	Dw15	
Responder cells		HLA-DR	DQ	Dw	BSMI	MIS	GR	MST	MAY	RED	JHA	MT	KY	KT13	KT3	
Father	a	w16	w5	blank	85	86	102	65	77	95	94	70	69	65	100	
	b	4	w5	blank												
Mother	c	4	w8	w4	30	31	27	102	101	101	100	104	91	117	81	
	d	2LUM(CT)	w6	blank												
Child 1	a	w16	w5	blank	101	86	88	100	111	75	73	95	76	105	136	
	d	2LUM(CT)	w6	blank												
Child 2	a	w16	w5	blank	147	124	79	69	99	103	79	77	84	69	97	
	d	2LUM(CT)	w6	blank												
Child 3	b	4	w5	blank	21	35	28	82	53	67	101	75	112	62	77	
	c	4	w8	w4												
Child 4	b	4	w5	blank	29	24	34	74	103	85	103	60	99	95	80	
	c	4	w8	w4												

RR‹50% = typing response

obtained with DR4 related HTCs (Dw4, Dw10, Dw13, Dw14, Dw15 and DKT2) are shown in Table 5.11, while those obtained with the DR2 related HTCs (Dw2, Dw12, Dw21 and Dw22) are shown in section 5.2.4. The HLA-DR4,DQw8 haplotype in this family was typed with all three HTCs with the Dw4 specificity, however, none of the 59 HTCs gave typing responses with the HLA-DR4,DQw5 haplotype.

RFLP analysis

Genomic DNA from family HER and HLA-DR4 positive homozygous B-lymphoblastoid cell lines were digested with the restriction endonucleases Taq I and Eco RI and hybridized with a DRB, DQB and DQA probe according to the method described in section 2.2.8. The homozygous cell lines used are listed in Table 5.12 and include all recognized DR4 associated Dw specificities except Dw14 and Dw15.

TABLE 5.12 List of HLA-DR4 positive cells used.

Cell identity	HLA A	B	C	DR	DQ	Dw
MIS	w43	14	w8	4	w8	w4
MST	3,26	38	w-	4	w8	w10
RED	2	w63	w7	4	w8	w13
Haplotype (a)(SAD)	24	w62	w7	4	w4	w15

To compensate for the lack of Dw15 positive homozygous cell lines, we included a heterozygous individual (SAD) with Dw15. The mother of this individual was also included to

allow assignment of the various fragments to the respective haplotypes. No HLA-DR4,Dw14 cell was available for inclusion in this test. An autoradiograph obtained using a the DRB probe with DNA digested with Taq I is shown in Figure 5.2, and the results obtained with Taq I and Eco RI are summarized in Table 5.13 Two distinct patterns for HLA-DR4 emerged, the one pattern was specific for the HLA-Dw4 homozygous cell line MIS and haplotype (c) of family HER, the other pattern was seen in the DR4,non-Dw4 cells, including the DR4,DQw5 haplotype of family HER. The fragments said to be unique for HLA-DRw53, i.e. the 2.8 kb Taq I fragment and the 12.8 kb, 4.2 kb, 4.0 kb and 3.7 kb Eco RI fragments (Cohen et al. 1988) were present in all the DR4 haplotypes tested.

The results obtained using the DQB probe and DNA digested with Taq I are shown in Figure 5.3 and the results obtained with Taq I and Eco RI are summarized Table 5.14. The 5.5 kb Taq I and 12.2 kb Eco RI fragment correlating with DQw5 (section 4.1.1) segregated with the DR4,DQw5 and DRw16,DQw5 haplotype of family HER. A 2.4 kb and 1.9 kb Taq I fragment and 19.5 kb Eco RI fragment was seen for the DR4,DQw8 positive cell lines, the DR4,DQw4 haplotype of family SAD and the DR4,DQw8 haplotype (c) of family HER.

The RFLP results using the DQA probe and DNA digested with Taq I are shown in Figure 5.4 and Table 5.15. Included in

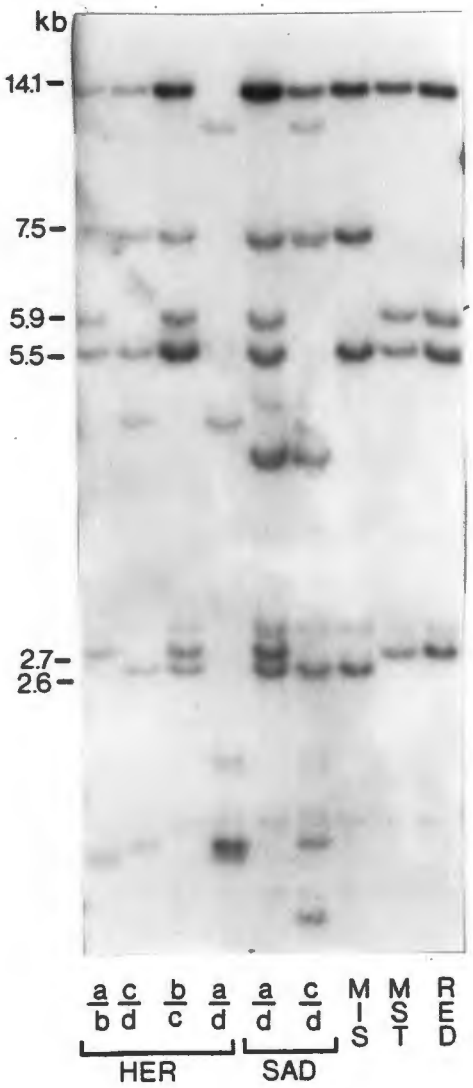

FIGURE 5.2 Hybridization of the DRB cDNA probe to DNA digested with Taq I. DNA was obtained from family HER (DR4,DQw5), family SAD (DR4,Dw15) and the HLA-DR4 homozygous cell lines MIS (Dw4), MST (Dw10), RED (Dw13).

Haplotypes family HER:

a	Aw33,	B44,	Cw7,	DRw16,	DQw5, Dw'blank'
b	A24,	Bw60,	Cw'blank',	DR4,	DQw5, Dw'blank'
c	A32,	Bw63,	Cw'blank',	DR4,	DQw8, Dw4
d	A29,	Bw58,	Cw6,	DR2 LUM(CT),	DQw6, Dw'blank'

Haplotypes family SAD:

a	A24,	Bw62,	Cw7,	DR4,	DQw4, Dw15
c	A3,	B7,	Cw7,	DRw15,	DQw6, Dw2
d	A29,	B13,	Cw6,	DR9,	DQw2, Dw'blank'

The 5.5 kb fragment segregated with all DR4 haplotypes and the 14.1 kb fragment with all the DR4 haplotypes as well as the DR9,DQw2 haplotype (d) of family SAD. The 7.5 and 2.6 kb fragments segregated with DR4,DQw8,Dw4 haplotypes and the DR9,DQw2 haplotype only. The 5.9 and 2.7 kb fragments segregated with the remaining DR4 haplotypes, including the DR4,DQw5 haplotype (b) of family HER. The 2.8 kb fragment (Table 5.13), which gives a faint hybridization signal, was not seen consistently in all individuals tested.

TABLE 5.13 Summary of DRB RFLPs in family HER, family SAD and HLA-DR4 positive homozygous cell lines.

Cell identification	HLA-DR	DQ	Dw	Taq I (kb)							Eco RI (kb)						
				14.1	7.5	5.9	5.5	2.8	2.7	2.6	16.8	15.5	13.2	12.8	4.2	4.0	3.7
Haplotype (c)(HER)	4	w8	w4	+	+	-	+	+	-	+	+	-	+	+	+	+	+
MIS	4	w8	w4	+	+	-	+	+	-	+	+	-	+	+	+	+	+
Haplotype (b)(HER)	4	w5	blank	+	-	+	+	+	+	-	-	+	+	+	+	+	+
Haplotype (a)(SAD)	4	w4	w15	+	-	+	+	+	+	-	-	+	+	+	+	+	+
MST	4	w8	w10	+	-	+	+	+	+	-	-	+	+	+	+	+	+
RED	4	w8	w13	+	-	+	+	+	+	-	-	+	+	+	+	+	+

+ indicates fragment present

- indicates fragment absent

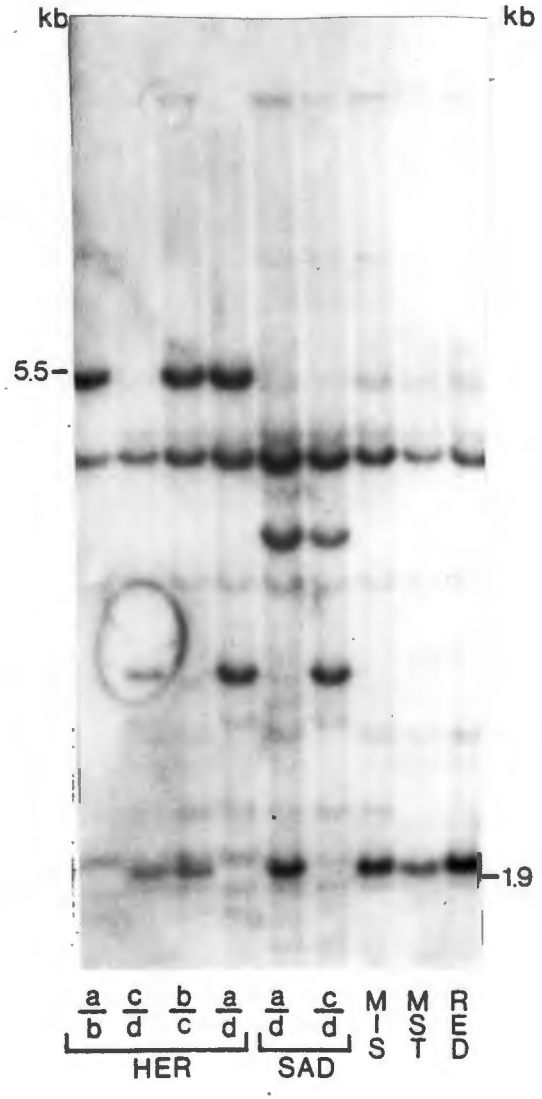

FIGURE 5.3 Hybridization of the DQB cDNA probe to DNA digested with Taq I. DNA was obtained from family HER (DR4,DQw5), family SAD (DR4,DQw4,Dw15) and the HLA-DR4,DQw8 homozygous cell lines MIS (Dw4), MST (Dw10), RED (Dw13).

Haplotypes of family HER:

a	Aw33,	B44,	Cw7,	DRw16,	DQw5, Dw'blank'
b	A24,	Bw60,	Cw'blank',	DR4,	DQw5, Dw'blank'
c	A32,	Bw63,	Cw'blank',	DR4,	DQw8, Dw4
d	A29,	Bw58,	Cw6,	DR2 LUM(CT),	DQw6, Dw'blank'

Haplotypes family SAD:

a	A24,	Bw62,	Cw7,	DR4,	DQw4, Dw15
c	A3,	B7,	Cw7,	DRw15,	DQw6, Dw2
d	A29,	B13,	Cw6,	DR9,	DQw2, Dw'blank'

The 5.5 kb fragment segregated with DQw5 on haplotypes (a) and (b) of family HER. The 1.9 kb fragment segregated with DQw8 and DQw4. The 2.4 kb fragment (Table 5.14), which gives a faint hybridization signal, was not seen consistently in all individuals tested.

TABLE 5.14 Summary of the DQB RFLPS in family HER, family SAD and DR4 positive homozygous cell lines.

Cell identification	HLA-DR	DQ	Dw	Taq I (kb)			Eco RI (kb)	
				5.5	2.4	1.9	19.5	12.2
Haplotype (a)(HER)	w16	w5	blank	+	-	-	-	+
Haplotype (b)(HER)	4	w5	blank	+	-	-	-	+
Haplotype (c)(HER)	4	w8	w4	-	+	+	+	-
Haplotype (a)(SAD)	4	w4	w15	-	+	+	+	-
MIS	4	w8	w4	-	+	+	+	-
MST	4	w8	w10	-	+	+	+	-
RED	4	w8	w13	-	+	+	+	-

+ indicates fragment present

- indicates fragment absent

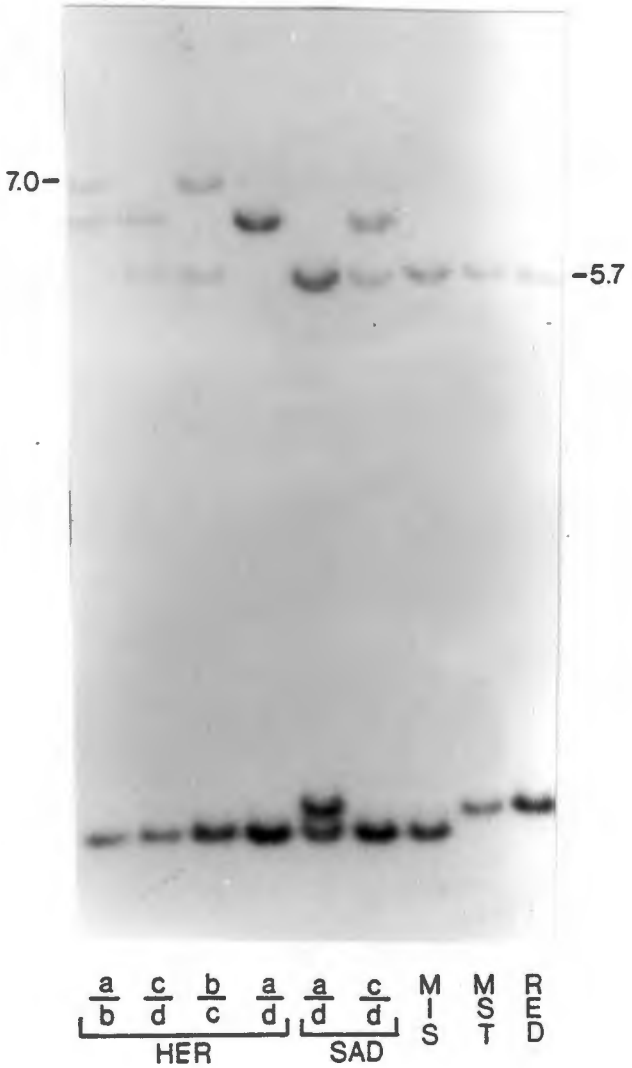

FIGURE 5.4 Hybridization of the DQA cDNA probe to DNA digested with Taq I. DNA was obtained from family HER (DR4,DQw5), family SAD (DR4,DQw4,Dw15) and the HLA-DR4,DQw8 homozygous cell lines MIS (Dw4), MST (Dw10), RED (Dw13).

Haplotypes family HER:

a	Aw33,	B44,	Cw7,	DRw16,	DQw5, Dw'blank'
b	A24,	Bw60,	Cw'blank',	DR4,	DQw5, Dw'blank'
c	A32,	Bw63,	Cw'blank',	DR4,	DQw8, Dw4
d	A29,	Bw58,	Cw6,	DR2 LUM(CT),	DQw6, Dw'blank'

Haplotypes family SAD:

a	A24,	Bw62,	Cw7,	DR4,	DQw4, Dw15
c	A3,	B7,	Cw7,	DRw15,	DQw6, Dw2
d	A29,	B13,	Cw6,	DR9,	DQw2, Dw'blank'

The 7.0 kb fragment segregated with the DR4,DQw5 haplotype (b) of family HER, whereas the 5.7 kb fragment was present in the remaining DR4 haplotypes and the DR9,DQw2 haplotype (d) of family SAD. The fragments associated with the DQA2 gene are seen at the bottom of the figure.

TABLE 5.15 Summary of the DQA RFLPs in family HER, family SAD and DR4 positive homozygous cell lines.

Cell identification	HLA-DR	DQ	Dw	Taq I (kb)				Eco RI (kb)			
				7.0	5.7	2.2	2.1	15.0	6.5	2.0	1.35
Haplotype (b)(HER)	4	w5	blank	+	-	-	+	+	-	+	-
Haplotype (c)(HER)	4	w8	w4	-	+	-	+	-	+	+	-
Haplotype (d)(SAD)	9	w2	blank	-	+	-	+	-	+	+	-
Haplotype (a)(SAD)	4	w4	w15	-	+	+	-	-	+	-	+
MIS	4	w8	w4	-	+	-	+	-	+	+	-
MST	4	w8	w10	-	+	+	-	-	+	-	+
RED	4	w8	w13	-	+	+	-	-	+	-	+

+ indicates fragment present

- indicates fragment absent

this Table are the DQA results obtained with Eco RI. All the DRw53 haplotypes investigated throughout the study such as DR7,DQw2 and DR7,DQw9 (data not shown); DR9,DQw9 and DR9,DQw2 (section 5.2.3); DR4,DQw4 and DR4,DQw8 (Table 5.15) had the same RFLP pattern associated with the DQA1 gene (5.7 kb Taq I and 6.5 kb Eco RI), with the exception of unusual the DR4,DQw5,DRw53 haplotype (Table 5.15). This haplotype had a similar DQA1 RFLP pattern (7.0 kb TaqI and 15.0 kb Eco RI) to that of the DRw13,Dw18,DQw6 haplotypes (section 4.1.3).

In *summary*, in this section we presented a Cape Coloured family with the unusual DR,DQ haplotype HLA-DR4,DQw5. The serological reaction patterns for the HLA-DR4 and DQw5 antigens of this haplotype were similar to the "classic" DR4 and DQw5 antigens. The HLA-Dw determinant of the HLA-DR4,DQw5 haplotype remains undetermined as none of the 59 HTCs used gave typing responses with this haplotype. The DRB RFLP pattern obtained for the DR4,DQw5 haplotype, using the restriction endonucleases Taq I and Eco RI, was identical to the DR4,Dw10; DR4,Dw13 and DR4,Dw15 haplotypes but differed from the DR4,Dw4 haplotype. The DQB RFLP pattern for the DR4,DQw5 haplotype did not differ from other DQw5 haplotypes, while the RFLP pattern associated with the DQA1 gene was identical to that of DRw13,Dw18,DQw6 haplotypes.

5.2.3 The HLA-DR9,DQw2 haplotype

Serology

The HLA typing of two families with DR9 are shown in Table 5.16. The haplotype DR9,DQw2 was seen in the SA Negro family GAI consisting only of mother and daughter, whereas the conventional DR9,DQw9 haplotype was present in a Cape Coloured family DANI. An additional DR9,DQw2 haplotype was observed in a Cape Coloured family SAD (section 5.2.2). As this family was not typed with the Tenth International Histocompatibility Workshop sera it was excluded from Table 5.16.

HLA-Dw typing

An HLA-Dw typing test was performed to establish the HLA-Dw specificity associated with the HLA-DR9,DQw2 haplotype. The HTCs (HTC-KT14 and HTC-C.WONG) with the HLA-Dw23 specificity, the only known HLA-Dw specificity associated with HLA-DR9, did not give typing reactions with the HLA-DR9,DQw2 haplotypes of families GAI and SAD, nor were typing responses observed for this haplotype with any of the other HTCs.

RFLP analysis

The results using a DRB probe with DNA from families GAI and DANI cleaved with restriction endonucleases Taq I, Eco RI and Bam HI are shown in Table 5.17. The results obtained for the DR9,DQw2 haplotype of family SAD (section 5.2.2) were included in this Table. The RFLP pattern for the

TABLE 5.16 Segregation of DR9,DQw2 in family GAI and DR9,DQw9 in family DANI.

	HLA-DR	DQ	10 WS sera DR9	DQw2	DQw3	DQw7
			1111111	1133	111333	333
			0000000	1111	111111	111
			6656666	6700	778111	122
			2380476	9357	694124	901
Family GAI						
Mother	c 9	w2	**8884868**[a]	**8688**	112111	111
	d w18	w4				
Child	a w12x6	w5	**8888686**	**8888**	211011	111
	c 9	**w2**				
Family DANI						
Father	a w17	w2	1111411	4888	886886	861
	b w11	w7				
Mother	**c 9**	**w9**	**8828688**	1111	**884061**	111
	d w13	w6				
Child 1	a w17	w2	1111111	2886	111011	111
	d w13	w6				
Child 2	b w11	w7	**8888486**	1111	**888886**	886
	c 9	**w9**				
Child 3	a w17	w2	**8848686**	6888	**884621**	111
	c 9	**w9**				
Child 4	b w11	w7	1111211	1111	884818	884
	d w13	w1				

a The results are scored as follows: 0 = not tested 1 = negative; 2 = doubtful negative; 4 = weak positive; 6 = positive; 8 = strong positive

TABLE 5.17 Summary of DRB RFLPs of DR9 positive haplotypes.

			Taq I (kb)								Eco RI (kb)							Bam HI (kb)		
Haplotype	HLA-DR	DQ	14.1	10.3	7.5	5.9	4.0	2.8	2.7	2.6	15.5	14.5	13.2	12.8	4.2	4.0	3.7	7.0	4.8	4.0
Haplotype (c)(GAI)	9	w2	+	-	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+
Haplotype (d)(SAD)	9	w2	+	-	+	-	+	+	-	+	+	-	+	+	+	+	+	nt	nt	nt
Haplotype (c)(DANI)	9	w9	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+

nt = not tested

+ indicates fragment present

- indicates fragment absent

DR9,DQw2 haplotypes of families GAI and SAD were identical and clearly different to that of DR9,DQw9 of family DANI for the restriction endonucleases Taq I and Eco RI. Identical RFLP patterns were observed for these haplotypes when using the restriction endonuclease Bam HI.

Table 5.18 shows the results obtained using the DQB probe. The two other known DQw2 positive haplotypes, DRw17,DQw2 and DR7,DQw2, are shown for comparison. Data for the DRw17,DQw2 haplotype (HTC-COX) was derived from a previous study (section 4.1.2) and the DR7,DQw2 haplotype (HTC-PITOUT) was studied by this laboratory for the Tenth International Histocompatibility Workshop. As shown in Table 5.18 the pattern for each of the DQw2 positive haplotypes studied was different. A difference in the DQB RFLP pattern for the DRw17 and DR7 haplotypes was previously reported by Bidwell (1988).

As shown in Table 5.19, identical DQA RFLP patterns were observed for the DR9,DQw2 and DR9,DQw9 haplotypes. This pattern was identical to other DRw53 haplotypes with the exception of the unusual DR4,DRw53,DQw5 haplotype described in section 5.2.2.

In *summary*, in this section we have described an unusual Negro haplotype, DR9,DQw2. From the limited serological data presented here, there appeared to be no difference in

TABLE 5.18 Summary of DQB RFLPs of DR9 positive haplotypes.

			Taq I (kb)					Eco RI (kb)		Bam HI (kb)		
Haplotype	HLA-DR	DQ	6.7	4.6	3.8	2.4	1.9	12.5	13.5	11.0	3.9	3.2
Haplotype (c)(GAI)	9	w2	-	-	+	-	-	+	-	-	+	+
Haplotype (d)(SAD)	9	w2	-	-	+	-	-	+	-	nt	nt	nt
Haplotype (c)(DANI)	9	w9	-	-	-	+	+	-	+	+	-	-
COX	w17	w2	-	+	-	-	-	+	-	-	+	-
PITOUT	7	w2	+	-	-	-	-	+	-	-	+	+

nt = not tested

+ indicates fragment present

- indicates fragment absent

TABLE 5.19 Summary of DQA RFLPs among HLA-DR9 positive haplotypes.

			Taq I (kb)			Eco RI (kb)			Bam HI (kb)
Haplotype	HLA-DR	DQ	5.7	2.2	2.1	6.5	2.0	1.35	8.1
Haplotype (c)(GAI)	9	w2	+	-	+	+	+	-	+
Haplotype (d)(SAD)	9	w2	+	-	+	+	+	-	nt
Haplotype (c)(DANI)	9	w9	+	-	+	+	+	-	+

nt = not tested

+ indicates fragment present

- indicates fragment absent

the serological reaction patterns for the DR9 and DQw2 antigens when compared to similar antigens on other haplotypes such as DR9,DQw9, DRw17,DQw2 and DR7,DQw2. However, the Southern blot data showed a different DRB pattern for the DR9 antigen of the DR9,DQw2 haplotype compared to the DR9 antigen on the DR9,DQw9 haplotype and a different DQB pattern compared to DQw2 antigen of the DRw17,DQw2 and DR7,DQw2 haplotypes. Identical DQA patterns were observed for the two DR9 positive haplotypes, DR9,DQw2 and DR9,DQw9, and this pattern was identical to other DRw53 haplotypes with the exception of the DR4,DQw5 haplotype. Like the other unusual haplotypes presented, no HLA-Dw specificity has as yet been identified for this haplotype.

5.2.4 An unusual HLA-DR,DQ haplotype based on RFLP analysis

Serology

The HLA typing results of family HER using DR2 and DQw1 sera and MoAbs are shown in Table 5.20. Two HLA-DR2 positive haplotypes were present in this family, the paternal haplotype (a) and the maternal haplotype (d). Based on the reaction patterns for the DR2 subgroups described in section 4.1.1 and the results shown in Table 5.20 we assigned the DR2 subgroup DR2 LUM(CT) to the maternal haplotype (d) and DRw16 to the paternal haplotype (a). The serological assignment of DRw16 to haplotype (a) was based on the negative reactions seen with 10WS sera 1031 and 1032 and positive reactions seen with the other DR2 sera as described

TABLE 5.20 HLA typing results of family HER using DR2 and DQw1 sera.

		HLA-DR	DQ	10 WS sera DR2			DQw1	DQw5
				111	11	1111	11111113	1
				000	00	0000	11111110	1
				322	33	2222	55556610	3
				057	12	9463	93542132	6
Father	a	w16	w5	868[a]	11	8862	86888888	6
	b	4	w5					
Mother	c	4	w8	666	84	1112	46188884	1
	d	2LUM(CT)	w6					
Child 1	a	w16	w5	868	84	6444	66888868	1[b]
	d	2LUM(CT)	w6					
Child 2	a	w16	w5	868	86	8466	86888888	4
	d	2LUM(CT)	w6					
Child 3	b	4	w5	111	11	1121	48168888	6
	c	4	w8					
Child 4	b	4	w5	121	11	1111	48664868	4
	c	4	w8					

a The results are scored as follows: 1 = negative; 2 = doubtful negative; 4 = weak positive; 6 = positive; 8 = strong positive

b False negative result

in section 4.1.1. None of the children who inherited haplotype (a) of the father were informative for the assignment of DRw16 as they also inherited the DR2 LUM(CT) positive haplotype (d) of the mother, resulting in positive reactions with sera 1031 and 1032. The DRw16 positive individuals of family HER all typed as DQw1 (Table 5.20), and whether they carried the DQw5 or DQw6 subgroup of DQw1 could not be established due to the lack of good sera for these subgroups. However, based on the Southern blot data shown below, DQw5 could be assigned to the DRw16 haplotype and DQw6 to the DR2 LUM(CT) haplotype.

HLA-Dw typing

The HLA-Dw typing results obtained using HLA-DR2 related HTCs are shown in Table 5.21. It should be noted that the DRw16,DQw5 haplotype of family HER was not typed by the DRw16 associated HTCs (Dw21 and Dw22). The DRw16,DQw5 haplotype tested here was also not typed by any of the other HTCs used. Although no HLA-Dw specificity could be assigned to either of the DR2 haplotypes, borderline typing reactions were seen for the DR2 LUM(CT) haplotypes using HTCs with the Dw2 specificity. This was in accordance with the HLA-Dw typing results obtained for the DR2 LUM(CT) haplotype described in section 4.1.1.

TABLE 5.21 HLA-Dw typing results of family HER using DR2 related HTCs.

Responder cells		HLA-DR	DQ	Dw	Stimulator cells (HTCs) Dw2 ZSI	Dw2 JDU	Dw2 HOF	Dw12 BGE	Dw21 FJO	Dw22 BAS	Dw22 REM
Father	a	w16	w5	blank	104	77	105	110	79	53	69
	b	4	w5	blank							
Mother	c	4	w8	w4	55	61	75	78	112	121	92
	d	2LUM(CT)	w6	blank							
Child 1	a	w16	w5	blank	51	44	55	93	72	92	63
	d	2LUM(CT)	w6	blank							
Child 2	a	w16	w5	blank	39	53	52	75	69	56	59
	d	2LUM(CT)	w6	blank							
Child 3	b	4	w5	blank	97	95	94	89	103	111	85
	c	4	w8	w4							
Child 4	b	4	w5	blank	106	105	124	100	72	102	76
	c	4	w8	w4							

RR<50% = typing response

RFLP analysis

The RFLP patterns for DNA isolated from family HER (DRw16,Dw'blank') and family SAD (DRw15,Dw2), digested with Taq I and hybridized to the DRB cDNA probe are shown in Figure 5.5. A summary of these results as well as the results obtained with the endonuclease Eco RI are given in Table 5.22. Included in this Table is the data described in section 4.1.1 for the DR2 subgroups, DRw15, DRw16 and DR2 LUM(CT). The RFLP results obtained for the DR2 LUM(CT) haplotype (d) is consistent with the DR2 LUM(CT) haplotype of family SLI. The RFLP results observed for the serologically defined DRw16 antigen on the paternal haplotype (a) shared fragments which are characteristic of both the DRw15 and DRw16 haplotypes (Table 5.22). The normal DRw16 haplotype, as seen in family KEM, had the 14.1 kb and 1.5 kb Taq I fragment present. The DRw16 haplotype of family HER, however, lacked the 14.1 kb fragment, but had the 1.5 kb Taq I fragment characteristically seen in the normal DRw16 haplotype. Furthermore, the DRw16 haplotype in family HER also shared the 11.5 kb Taq I fragment with the DRw15 haplotypes, but lacked the 1.6 kb and 1.3 kb Taq I fragments which are characteristic of the DRw15 haplotype.

The results obtained using the DQB probe are shown in Figure 5.6A and B and summarized in Table 5.23. The RFLP pattern seen for the DQ antigen on the DRw16 haplotype (a) was identical to that of the DRw16,DQw5 haplotype (c) of family

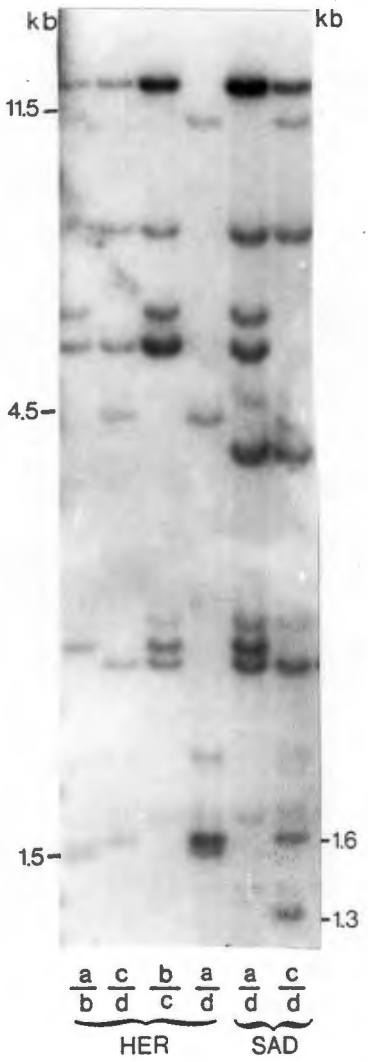

FIGURE 5.5 Hybridization of the DRB cDNA probe to DNA digested with Taq I. DNA was obtained from family HER (DRw16,DQw5) and family SAD (DRw15,DQw6).

Haplotypes family HER:

a	Aw33,	B44,	Cw7,	DRw16,	DQw5, Dw'blank'
b	A24,	Bw60,	Cw'blank',	DR4,	DQw5, Dw'blank'
c	A32,	Bw63,	Cw'blank',	DR4,	DQw8, Dw4
d	A29,	Bw58,	Cw6,	DR2 LUM(CT),	DQw6, Dw'blank'

Haplotypes family SAD:

a	A24,	Bw62,	Cw7,	DR4,	DQw4, Dw15
c	A3,	B7,	Cw7,	DRw15,	DQw6, Dw2
d	A29,	B13,	Cw6,	DR9,	DQw2, Dw'blank'

The 11.5 kb, which is specific for DRw15 (see section 4.1.1), and the 1.5 kb fragment which is seen with DRw16, segregated with the DRw16,DQw5 haplotype (a). It is important to note that the 14.1 kb fragment seen in normal DRw16 haplotypes was absent in the DRw16 haplotype of family HER. The DRw15 positive haplotype (c) of family SAD had the 11.5, 1.6 and 1.3 kb fragments. The 4.5 kb and 1.6 kb fragments segregated with DR2 LUM(CT) on haplotype (d).

TABLE 5.22 Summary of the DRB RFLPS in family HER.

Cell identification	HLA-DR	DQ	Dw	Taq I (kb)						Eco RI (kb)		
				14.1[a]	11.5	4.5[b]	1.6	1.5	1.3	6.4	5.3[b]	4.7
Haplotype (d)(HER)	2LUM(CT)	w6	blank	-	-	+	+	-	-	+	+	-
Haplotype (a)(SLI)	2LUM(CT)	w6	blank	-	-	+	+	-	-	+	+	-
Haplotype (a)(HER)	w16	w5	blank	-	+	-	-	+	-	+	+	+
Haplotype (c)(SAD)	w15	w6	w2	-	+	-	+	-	+	+	+	+
ZSI	w15	w6	w2	-	+	-	+	-	+	+	+	+
JDU	w15	w6	w2	-	+	-	+	-	+	+	+	+
Haplotype (c)(KEM)	w16	w5	w21	+	-	-	-	+	-	+	+	+

+ indicates fragment present

- indicates fragment absent

a co-segregation with DRw53

b co-segregation with DR1 and DRw10

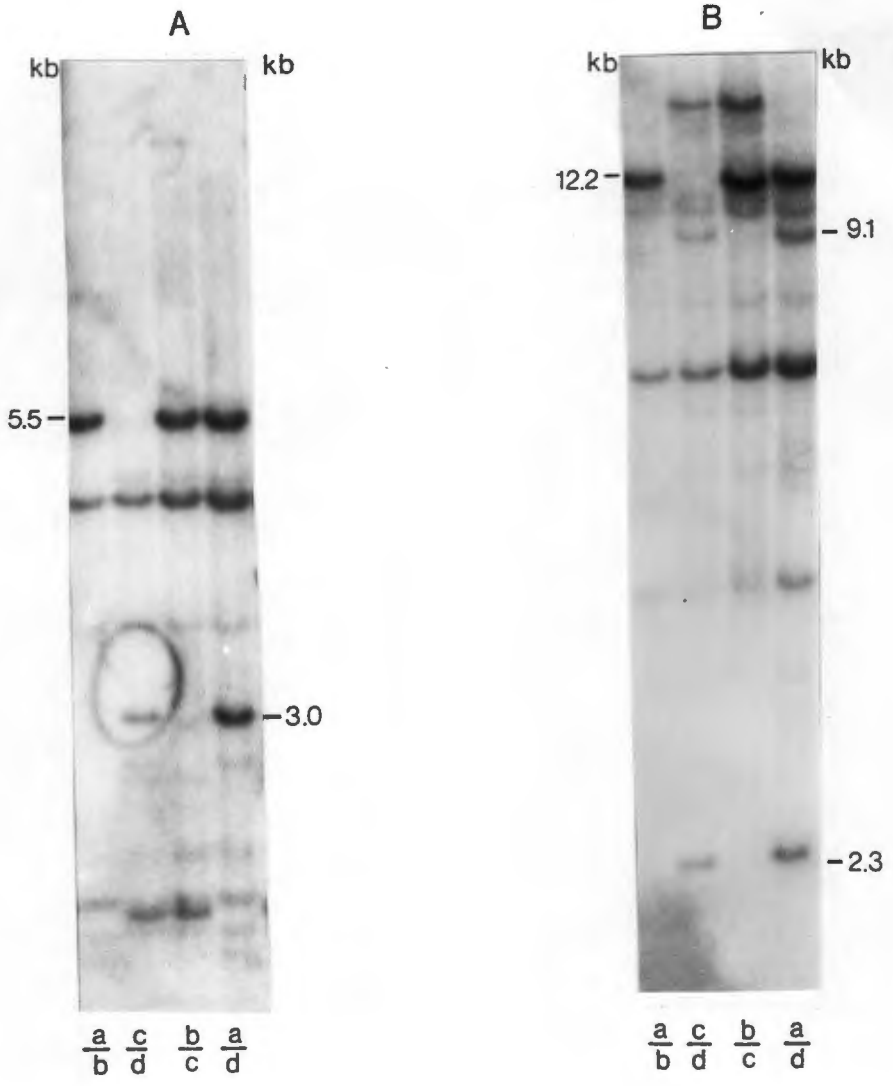

FIGURE 5.6 Hybridization of the DQB cDNA probe to DNA digested with (A) Taq I and (B) Eco RI. DNA was obtained from family HER (DRw16,DQw5).

Haplotypes:

a	Aw33,	B44,	Cw7,	DRw16,	DQw5, Dw'blank'
b	A24,	Bw60,	Cw'blank',	DR4,	DQw5, Dw'blank'
c	A32,	Bw63,	Cw'blank',	DR4,	DQw8, Dw4
d	A29,	Bw58,	Cw6,	DR2 LUM(CT),	DQw6, Dw'blank'

The 5.5 kb Taq I fragment and the 12.2 kb Eco RI fragment segregated with DQw5 on the DRw16,DQw5 haplotype (a) and the DR4,DQw5 haplotype (b). The 3.0 kb Taq I fragment and the 9.1 and 2.3 kb Eco RI fragments segregated with DQw6 on haplotype (d). The 2.6 kb Taq I fragment (Table 5.23), which gives a faint hybridization signal, was not seen consistently in all individuals tested.

TABLE 5.23 Summary of DQB RFLPs in family HER.

Cell identification	HLA-DR	DQ	Dw	Taq I (kb)			Eco RI (kb)		
				5.5	3.0	2.6	12.2	9.1	2.3
Haplotype (a)(HER)	w16	w5	blank	+	-	-	+	-	-
Haplotype (d)(HER)	2LUM(CT)	w6	blank	-	+	+	-	+	+
Haplotype (c)(SAD)	w15	w6	w2	-	+	+	-	+	+
ZSI	w15	w6	w2	-	+	+	-	+	+
JDU	w15	w6	w2	-	+	+	-	+	+
Haplotype (c)(KEM)	w16	w5	w21	+	-	-	+	-	-
Haplotype (a)(SLI)	2LUM(CT)	w6	blank	-	+	+	-	+	+

+ indicates fragment present

- indicates fragment absent

KEM (Table 5.23) and of other DQw5 haplotypes such as DR4,DQw5 (Figure 5.6), DRw12x6,DQw5 (section 4.1.3), DRw8x14,DQw5 (section 4.1.4), DR1,DQw5 and DRw10,DQw5 (data not shown). The pattern observed for the DR2 LUM(CT),DQw6 haplotype corresponded to the pattern observed for the DQw6 haplotypes, DRw13(Dw18),DQw6 (section 4.1.3) and DRw15,DQw6 (Table 5.23) but differed to that of the DRw13(Dw19),DQw6 haplotype studied in section 4.1.3.

Table 5.24 shows the results obtained using the DQA probe. The DQA2 pattern 2 (2.1 kb Taq I and 2.0 kb Eco RI fragments) was observed for the DRw16,DQw5 and DR2 LUM(CT),DQw6 haplotype of family HER as well as the DRw16,DQw5 haplotype of family KEM. The remaining haplotypes in Table 5.24 showed the DQA2 pattern 1 (2.2 kb Taq I and 1.35 kb Eco RI). The RFLP pattern corresponding to the DQA1 gene (6.2 kb Taq I and 15.0 kb Eco RI fragments) was identical for all DR2 haplotypes studied.

In *summary*, in this section a Cape Coloured family with two DR2,DQw1 haplotypes was presented. Serological and Southern blot data of the mother's (d) haplotype showed that this haplotype carried the antigens DR2 LUM(CT) and DQw6. The serological data of the father's (a) haplotype corresponded with the DR2 subgroup DRw16, whereas the DRB RFLP results showed fragments typical of both the normal DRw15 and DRw16 specificities, indicating the occurrence of a "hybrid"

TABLE 5.24 Summary of the DQA RFLPs in family HER.

Cell identification	HLA-DR	DQ	Dw	Taq I (kb)			Eco RI (kb)		
				6.2	2.2	2.1	15.0	2.0	1.35
Haplotype (a)(HER)	w16	w5	blank	+	-	+	+	+	-
Haplotype (d)(HER)	2LUM(CT)	w6	blank	+	-	+	+	+	-
Haplotype (c)(SAD)	w15	w6	w2	+	+	-	+	-	+
ZSI	w15	w6	w2	+	+	-	+	-	+
JDU	w15	w6	w2	+	+	-	+	-	+
Haplotype (c)(KEM)	w16	w5	w21	+	-	+	+	+	-
Haplotype (a)(SLI)	2LUM(CT)	w6	blank	+	+	-	+	-	+

+ indicates fragment present

- indicates fragment absent

haplotype. None of the DRw16 or DRw15 associated HTCs, nor any of the other HTCs used, gave typing responses with the DRw16,DQw5 haplotype of family HER indicating possible class II region differences between this haplotype and those of the HTCs. The results obtained using the DQB probe were identical to that previously observed for DQw5 haplotypes and that of the DQA probe identical to the patterns observed for DR2 haplotypes such as DRw15,DQw6; DR2 LUM(CT),DQw6 and DRw16,DQw5.

CHAPTER 6

DISCUSSION

This study provides a detailed description of the HLA class II polymorphism in three South African populations, and is important in several areas of HLA research such as estimation of population relationships, the understanding of the generation of HLA class II antigen polymorphism, the unravelling of the relationships of class II antigen products inducing the T-lymphocyte response in the primary MLC, organ transplantation and disease studies.

All the recognized HLA-DR and DQ antigens, DR1 to DRw10 and DQw1 to DQw3 and the majority of recognized Dw specificities, were present in the SA Negroes (Xhosa), Cape Coloureds and SA Caucasoids, although striking variations in frequencies were seen. In the Xhosa the HLA-DR1, DR4, DR7, DRw8, DQw2, DQw3, Dw1 and Dw3 antigens were found at a significantly lower frequency, whereas HLA-DR3, DRw6 and Dw'RSH' were found at a significantly higher frequency compared with the SA Caucasoids. From this study we know that HLA-DR3 is heterogeneous in the SA Negroes. The HLA-DR3 subgroup, HLA-DRw18, and the Dw specificity, Dw'RSH', associated with DRw18, may be regarded as the most important specificities identified in this study as they are the first examples of high frequency class II determinants restricted

to the Negroes and those with Negroid admixture (i.e SA Negroes, Cape Coloureds and American Negroes). Approximately 65% of DR3 positive SA Negroes carried DRw18, whereas DRw17 was the only subgroup observed in the SA Caucasoids. Using these percentages and the established HLA-DR3 frequencies in the respective groups, the DRw17 and DRw18 gene frequencies were estimated to be 0.09 and 0.16 respectively in the Negroes, and DRw17 was estimated to occur with a gene frequency of 0.11 in the SA Caucasoids. As the DRw17 frequencies in these groups were similar, the difference in DR3 frequency between the Xhosa and SA Caucasoids was almost certainly due to the presence of DRw18 in the Xhosa and its absence in the SA Caucasoids.

The Cape Coloureds, whose genetic constitution is a result of early gene mixing at the Cape, now have HLA gene frequencies that are completely different from the original groups. In some instances, the phenotype frequencies were closer to those of the Caucasoids. For example, HLA-DR7 was present in 23.0% of SA Caucasoids and 25.8% of Cape Coloureds, but only in 15.7% of the Xhosa. In other instances, the frequencies in the Cape Coloureds approximated the Xhosa frequencies, as was the case with HLA-DR1 which was present in 11.5% of Cape Coloureds and 9.2% of Xhosa, but was present in 20.3% of SA Caucasoids. The South-East Asian contribution to the Cape Coloured genome is illustrated by the presence of the DR4,DQw4

haplotype and the DRw14 variant, DRw8x14 which is presumably identical to the DRw8I seen in Asian Indians (Awad and Festenstein 1987). Using various blood group systems, May and du Toit (unpublished observations) have shown that the Cape Coloureds and the Coloureds living in the Johannesburg area are a fairly homogeneous group. Thus the HLA gene frequencies established for the Cape Coloureds in this study may also be used as normal control frequencies for the Coloureds living in the Johannesburg area for whom no HLA class II frequencies have been documented.

Gene frequency data established in different populations can be used to determine genetic distances between populations. The genetic distance values were calculated using the gene frequencies for HLA-A,B,C,DR and DQ loci. The largest genetic distance value (0.032) was observed between the Xhosa and the SA Caucasoids and was comparable to that found between the major populations of the world (0.05) (Nurse et al. 1975). The genetic distance values between the Xhosa and Cape Coloureds (0.012) and the SA Caucasoids and Cape Coloureds (0.010) were of the same order of magnitude, indicating that the Cape Coloureds are genetically intermediate between the SA Caucasoids and SA Negroes and thus provide a classic example of a hybrid population group of recent origin. The relatively large genetic distance (0.023) observed between the Xhosa and the Nigerians indicates that although they are said to have originated

from the same Bantu-speaking group (Greenberg 1963), they are now genetically quite distinct, thus providing evidence that the SA Negroes diverged from the other Bantu-speaking Negroes at a relatively early time. Although both the Cape Coloureds and American Negroes have a substantial admixture of Negroid and Caucasoid genes, they are genetically dissimilar as is shown by the genetic distance value of 0.013 between these hybrid groups. This is not surprising as the American Negroes originated from Negroes living along the West Coast of Africa (Curtin 1969), whereas the Cape Coloureds originated predominantly from Southern African Negroes (du Toit et al. 1988a). Furthermore, the American Negroes have a considerable proportion (27%) of American Indian genes (Foster 1935, Johnson 1986) whereas the Cape Coloureds possess South-East Asian as well as Khoisan genes (Botha 1972, du Toit et al. 1988a).

The HLA-DR,DQ haplotypes with significant delta values were virtually the same in the SA Caucasoids, Cape Coloureds and Xhosa and identical to those observed in Orientals (Baur et al. 1984), indicating that these haplotypes were present before the divergence of the populations. The alleles have probably stayed together in the populations for a very long time due to the fact that the loci are so closely linked. However, some selective advantage cannot be excluded. Although recombinations do occur in the HLA-DR,DQ region as shown below, the recombination fraction may be too low for

the DR,DQ association to dissipate and lead to linkage equilibrium. Family data has shown that various less common HLA-DR,DQ haplotypes such as DR9,DQw2; DR3,DQw3 and the more common haplotype DR3,DQ'blank' (presumably DRw18,DQw4) were restricted to the Negroid population. These population restricted haplotypes may be of use in determining admixture between populations.

The data presented in this thesis as summarized in Table 6.1 may be used to gain insight into the mechanisms that generate polymorphisms during the course of evolution. For example, a Negro DR2 variant, DR2 LUM(CT), was identified using serological and Southern blot analysis. The DR2 LUM(CT),DQw6 haplotype was similar to the DRw15,DQw6 haplotype in that it had identical DQA and DQB RFLP patterns, and shared some DRB Taq I and Eco RI RFLPs and some LD determinants. This indicated that the DR2 LUM(CT) haplotype may have arisen from the DRw15,DQw6 haplotype. Sequence data of the DR2 LUM(CT) haplotype may provide information about the genetic mechanisms which contributed to the change of the DRw15,DQw6 haplotype into the DR2 LUM(CT),DQw6 haplotype. The fact that HLA-DR2 LUM(CT) has as yet only been found in the SA Negroes and Cape Coloureds and not in the other Negroid populations studied during the Ninth and Tenth International Histocompatibility Workshops may indicate Khoisan rather than Negro origin, as has been observed for HLA-Aw43 (du Toit et al. 1988a). Further HLA

TABLE 6.1 Summary of serological, cellular and Southern blot results of HLA-DR, DQ haplotypes described in this thesis.

HAPLOTYPE	Number of haplotypes tested per population			SEROLOGICAL TYPING			HLA-Dw TYPING	DNA TYPING[a]					
	N	CC	C	DR	DRw52 DRw53	DQ	Dw	DQA2 1	DQA2 2[b]	DQB1	DQA1	DRB[c]	DRB3[d] 10.3kb (Dw24/26)	DRB3[d] 12.2kb (Dw25)
DRw15,DQw1	3	3	2	w15	-	w1	w2	7	1	DQw6	DR2,DQw1 DRw11,DQw6 DRw13(Dw19),DQw6	DRw15	-	-
DR2LUM(CT),DQw1	1	2	0	2 LUM(CT)	-	w1	blank	2	1	,,	,,	DR2 LUM(CT)	-	-
DRw16,DQw1	0	1	0	w16	-	w1	w21	0	1	DQw5	,,	DRw16	-	-
	0	1	0	w16	-	w1	blank	0	1	,,	,,	DRw15/w16	-	-
DRw17,DQw2	3	3	3	w17	w52	w2	w3	4	5	DQw2(DRw17)	DRw17	DRw17	3	6
DRw17,DQw7	1	0	0	w17	w52	w7	blank	0	1	DQw7	,,	,,	0	1
DRw18,DQw4	9	2	0	w18	w52	w4	RSH	1	10	DQw4/DQw8	DRw18,DQw4 DRw8,DQw4 DRw8,DQw7 DRw12x6,DQw7	DRw18	11	0
DR4,DQw3	1	4	0	4	w53	w3	w4	2	3	DQw4/DQw8	DRw53	DR4A[e]	-	-
	0	0	2	4	w53	w3	w10	2	0	,,	,,	DR4B[e]	-	-
	0	1	2	4	w53	w3	w13	3	0	,,	,,	DR4B	-	-
DR4,DQw4	0	2	0	4	w53	w4	w15	2	0	,,	,,	DR4B	-	-
DR4,DQw1	0	1	0	4	w53	w1	blank	0	1	DQw5	DR4,DQw5 DRw13(Dw18),DQw6	DR4B	-	-
DR9,DQw9	0	1	0	9	w53	w9	w9	0	1	DQw9	DRw53	DR9,DQw9	-	-
DR9,DQw2	1	1	0	9	w53	w2	blank	0	2	DQw2(DR9)	DRw53	DR9,DQw2	-	-

HAPLOTYPE	Number of haplotypes tested per population			SEROLOGICAL TYPING			HLA-Dw TYPING	DNA TYPING[a]					
	N	CC	C	DR	DRw52 DRw53	DQ	Dw	DQA2 1	DQA2 2[b]	DQB1	DQA1	DRB[c]	DRB3[d] 10.3kb (Dw24/26)	DRB3[d] 12.2kb (Dw25)
DRw12,DQw7	0	3	2	w12	w52	w7	DB6	2	3	DQw7	DRw12,DQw7 DRw11,DQw7	DRw12	3	2
DRw12x6,DQw7	0	2	0	w12x6	w52	w7	JOH	0	2	,,	DRw12x6,DQw7 DRw18,DQw4 DRw8,DQw7 DRw8,DQw4	DRw12x6	2	0
DRw12x6,DQw5	5	0	0	w12x6	w52	w1	BME	1	4	DQw5	DQw5	DRw12x6	0	5
DRw13,DQw1	5	1	2	w13	w52	w1	w18	6	2	DQw6	DRw13(Dw18),DQw6 DR4,DQw5	DRw13(Dw18)	6	2
	3	0	2	w13	w52	w1	w19	5	0	DQw6V[f]	DRw13(Dw19),DQw6 DR2,DQw1 DRw11,DQw6	DRw13(Dw19)	5	0
DRw14,DQw1	2	0	0	w14	w52	w1	w9	0	2	DQw5V[f]	DQw5	DRw14 DRw8x14	0	2
DRw8x14,DQw5	0	2	0	w8x14	w52	w1	blank	0	2	DQw5	,,	,,	0	2
	0	1	0	w8x14	w52	w1	blank	0	1	,,	,,	DRw8x14 DRw13(Dw18)	1	0
DRw8,DQw4	1	1	2	w8	w52	w4	w8	0	4	DQw4/DQw8	DRw8,DQw4 DRw8,DQw7 DRw12x6,DQw7 DRw18,DQw4	DRw8	0	0
DRw8,DQw7	4	1	0	blank	w52	w7	w8	1	4	DQw7	,,	,,	0	0

N = SA Negro; CC = Cape Coloured; C = SA Caucasoid

a The Southern blot results were obtained using the restriction endonucleases Taq I, Eco RI and Bam HI.

b DQA2 pattern 1 was defined by the presence of a 2.2 kb Taq I and 1.35 kb Eco RI fragment, whereas DQA2 pattern 2 was characterized by the presence of a 2.1 kb Taq I and 2.0 kb Eco RI fragment (see section 4.1.1 Table 4.7). The number of haplotypes with the respective DQA2 patterns are given.

c Includes all DRB genes with the exception of DRB3.

d The 12.2 kb Taq I fragment was shown to be associated with the presence of the T-cell clone defined DRB3 allele Dw25, whereas the 10.3 kb Taq I fragment was associated with Dw24 and Dw26. The number of haplotypes with the respective fragments are given.

e Two DRB RFLP patterns were found with the DR4 positive cells and the different patterns are indicated with the letters A and B (see section 5.2.2).

f DQw5V and DQw6V indicate DQw5 and DQw6 variant RFLP patterns (see sections 4.1.3 and 4.1.4).

studies in the Khoisan would be required to confirm this suggestion. Apart from the new DR2 subgroup, DR2 LUM(CT), a new HLA-DR2 haplotype was observed in a Cape Coloured family HER. The DR2 haplotype of the father corresponded with the serologically defined DR2 subgroup DRw16, whereas the DRB RFLP results showed fragments typical of both the "classic" DRw15 and DRw16 specificities, indicating the occurrence of a "hybrid" haplotype. The DQA and DQB RFLP patterns, however, were identical to those of the classic DRw16,DQw5 haplotype. The DR2 haplotype probably has two expressed DRB genes, the nomenclature of which have not been clearly defined (Lee et al. 1987, Liu et al. 1988). It is thought that the one gene may be supertypic to the various HLA-Dw subgroups associated with DR2, is relatively non-polymorphic and it is referred to in this thesis as the DRB2 gene. The other gene which is probably more polymorphic and varies with the Dw subtypes (Lee et al. 1987) is referred to as the DRB1 gene. The paradoxical results in family HER whereby the father's (a) haplotype types as a DRw16 serologically, but as a "hybrid" of DRw15 and DRw16 using Southern blotting is best explained as follows. The RFLP pattern observed for the smaller fragments was identical in both the classic DRw16,Dw21 haplotype and the DRw16,Dw'blank' haplotype of family HER (i.e. the presence of a 1.5 kb Taq I fragment and the absence of a 1.6 and 1.3 kb Taq I fragment). These fragments may be associated with the DRB1 gene as the serological subgroups of the DR2 specificities are probably

defined by the more polymorphic DRB1 gene. The RFLP pattern for the larger Taq I fragments seen in the DRw16 haplotype of family HER was identical to that of DRw15,Dw2 haplotypes (i.e. the presence of a 11.5 kb Taq I fragment and the absence of a 14.1 and 4.5 kb fragment). It is thus conceivable that these fragments are associated with the DRB2 gene. If these suppositions are correct the DRw16,DQw5 haplotype of family HER may have arisen from a homologous crossover between the DRB1 and DRB2 genes of a DRw16,DQw5 haplotype and a DRw15,DQw6 donor haplotype as shown in Figure 6.1. DNA sequencing of this haplotype would be useful in order to test the hypothesis described above. Another example of possible recombinational events having given rise to an unusual haplotype, with the proposed site of recombination occurring within the DQ sub-region, rather than within the DR sub-region was identified. The DRw17 allele of the DRw17,DQw7 haplotype showed the same DRB Taq I and Eco RI RFLP patterns as the DRw17 allele linked with DQw2 and Dw25. The DQw7 allele of the DRw17,DQw7 haplotype shared the same DQB Taq I and Eco RI RFLPs as the DQw7 allele of other DQw7 haplotypes, whereas the DQA RFLP pattern was identical to those of the DRw17,DQw2 haplotypes. Thus the DRw17,DQw7 haplotype may have arisen from a homologous crossover between the DQB1 and DQA1 genes of a DRw17,DQw2 haplotype and a DQw7 haplotype (Figure 6.2) similar to that described for the DR4,DQw7 haplotype (Holbeck and Nepom 1988). However, not all unusual HLA-

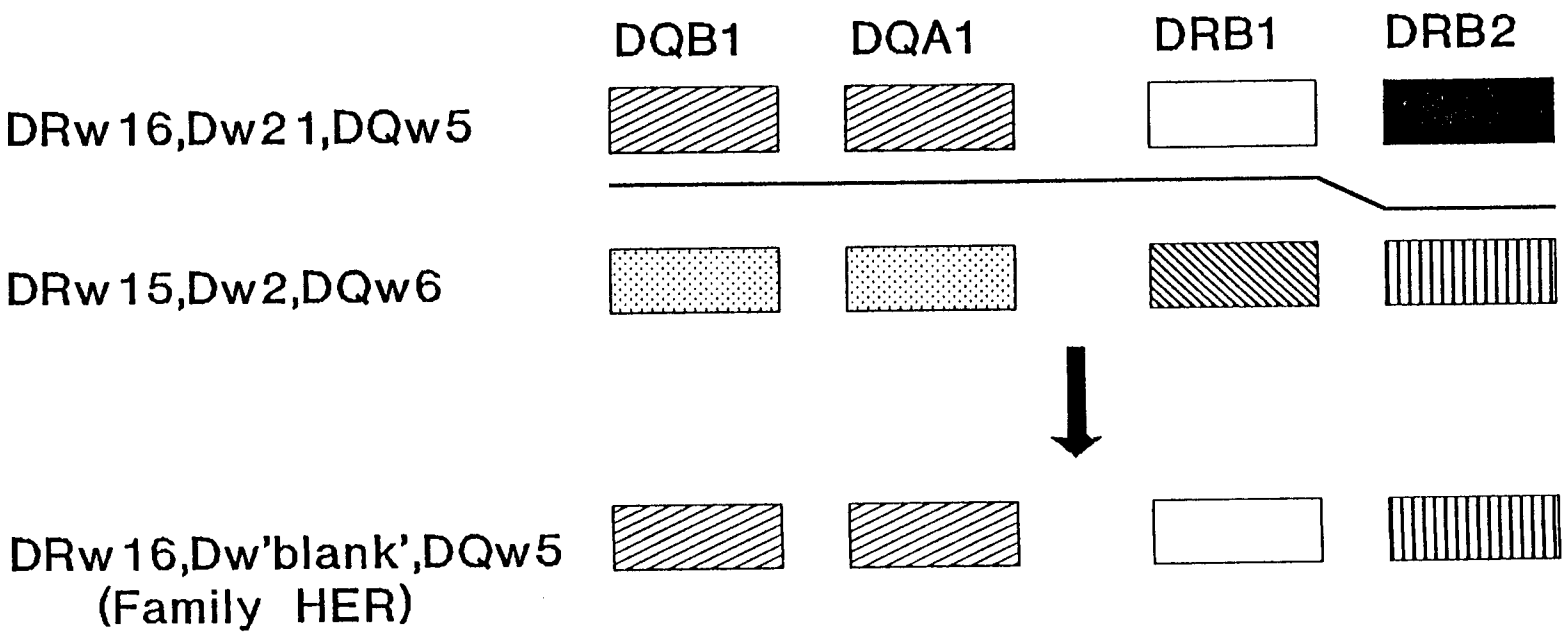

FIGURE 6.1 Schematic representation of the proposed hypothesis for the generation of the DRw16,Dw'blank',DQw5 haplotype (a) of family HER (see text for details).

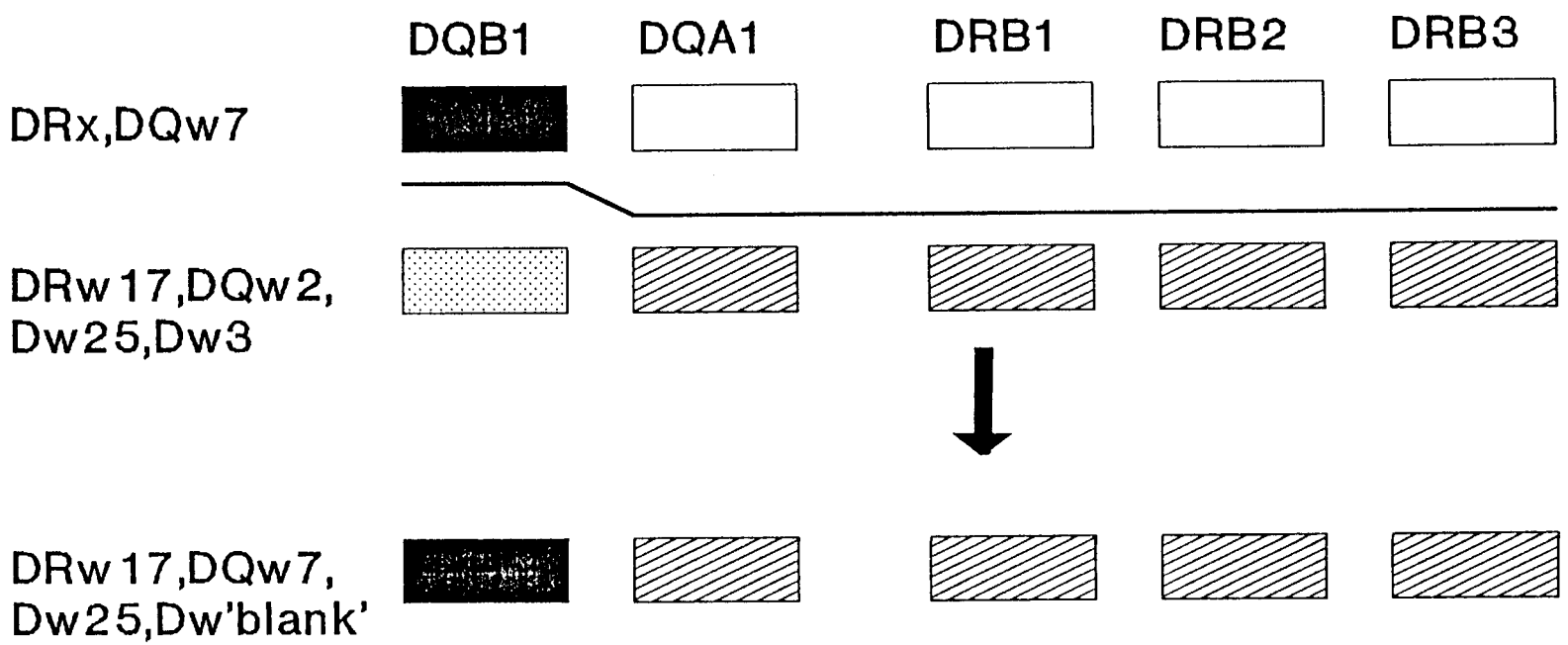

FIGURE 6.2 Schematic representation of the proposed crossover resulting in the generation of the DRw17,DQw7,Dw25,Dw'blank' haplotype (see text for details.

DR,DQ haplotypes have arisen from a single homologous crossing over event. This is best exemplified by the DR9,DQw2 haplotype observed in the SA Negro population. Both DRB as well as DQB RFLP patterns of this haplotype appeared to be unique and this diversity may have been generated by a variety of mechanisms which have been proposed to underlie the class II gene polymorphisms. These include gene conversion (Gregersen et al. 1986, Gorski and Mach 1986), point mutation (Rask et al. 1985), gene duplication (Rollini et al. 1985) and reciprocal recombination (Wu et al. 1986, Hurley et al. unpublished observations).

The origin of the stimulatory determinant in the MLC remains a controversial issue. There is one school of thought that asserts that the determinants are located on a single molecular structure (Jaraquemada et al. 1986), whereas others suggest that the stimulating determinants are located on several molecules (Bach 1985, Sterkers et al. 1987). Since we identified several unusual HLA-DR,DQ haplotypes we were able to compare the Dw typing results of these with the typing results of the "classic" HLA-DR,DQ combinations. This showed that in practically all cases a non-response in an MLC was obtained only if both the HLA-DR and DQ antigens of the cells in culture were compatible supporting the second notion mentioned above. Sequencing such unusual HLA-DR,DQ haplotypes and comparing their sequences with their

"classic" DR counterparts may be valuable in elucidating the molecular definition of HLA-Dw.

Since the introduction during the latter part of the 1970's of serological typing for DR antigens prior to cadaver kidney transplantation, many reports have proposed that DR compatibility is of overriding importance for kidney graft survival (reviewed by Morris and Ting 1982). However, since HLA-DQ antigens occur in strong linkage disequilibrium with HLA-DR, this data could equally well be used to support the importance of DQ compatibility for graft survival, especially as it is now known that HLA-DQ polymorphism is not as limited as was first suggested (Dupont 1988) and that HLA-DQ antigens are important in T-cell recognition (Zeevi et al. 1982, 1987; Yasukawa and Zarling 1984). Studies have shown that HLA-DQ may contribute to the T-cell response in a primary MLC (Carlsson et al. 1984, Reinsmoen and Bach 1986, Nakatsuji et al. 1986) and it is conceivable that matching for these antigens in addition to HLA-DR may be of importance in organ transplantation. This would be particularly so in those countries with a variety of population groups, such as South Africa, since the unusual DR,DQ haplotypes observed in this study were shown to be largely population specific.

It has been shown recently (Opelz 1988) that matching for the "splits" of HLA class I antigens resulted in a better

renal transplant outcome and that this effect was potentiated by matching for HLA-DR antigens. From this it was concluded that typing for HLA-DR splits will probably further improve the correlation of HLA matching with graft survival. Thus the investigation and definition of new class II antigens or splits are of importance to organ transplantation. The identification of splits is of particular importance in South Africa as they were shown to be more or less population specific. This can be clarified using HLA-DR3 as an example. We have shown that in the SA Negroes HLA-DRw18 was the predominant HLA-DR3 split, whereas HLA-DRw17 was the only DR3 split seen in the SA Caucasoids, and that cells with these antigens stimulated each other in an MLC. Since the MLC may be seen as the in vitro model for the recognition phase of allograft rejection, our data may have relevance when matching for HLA-DR in organ transplantation. If, for example, a SA Negro recipient with HLA-DR3 is transplanted with an organ from a SA Caucasoid donor with HLA-DR3, this apparent HLA-DR match could well be a mismatch.

The analysis of the frequency of HLA class II antigens and their subgroups in various populations has also contributed to the understanding of the relationship between HLA and disease. For example, in a previous study of insulin dependent diabetes mellitus (IDDM) in SA Negroes, no association could be demonstrated with HLA-DR3 (Orren et al.

1985), in contrast to the findings in Caucasoids and North American Negroes (Tiwari and Terasaki 1985). This finding may be explained on the basis that IDDM is associated with DRw17 in the Caucasoids rather than with DRw18, which is the more common DR3 split seen in SA Negroes. More recently, it has been shown that the DQ locus may be responsible for susceptibility or resistance to IDDM (Todd et al. 1987). Since DRw17 and DRw18 are in linkage disequilibrium with different DQ alleles (DQw2 and DQw4 respectively), the absence of an association between DR3 and IDDM in SA Negroes could also be explained on this basis. Furthermore, in a study on systemic lupus erythematosus in the Cape Coloured population by our group (Klemp et al. 1988), HLA-DR2 was found at an increased frequency in the patients. Reanalysis of this data may be important to see whether the association is with one of the DR2 subgroups, DRw15, DRw16 or DR2 LUM(CT) or with the "broad" DR2 antigen. These observations emphasize the importance of the detailed definition of the HLA antigens observed in the populations of South Africa.

In conclusion, the data on HLA class II antigen frequencies presented in this thesis is essential for future studies of HLA and disease associations and for establishing population relationships. Knowledge of new HLA class II antigens in the various population groups is also important in renal transplantation, as matching for HLA-DR antigens is known to improve graft survival. The recent trend towards using

unrelated live donors in bone marrow transplantation also emphasizes the need for a detailed knowledge of the polymorphism of HLA in the indigenous Southern African populations. The data presented in this thesis serves as a guideline for the selection of those alleles which require DNA sequencing for the ultimate understanding of the generation of class II polymorphism.

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APPENDIX

List of 10 WS sera used.

10 WS number	Region	Laboratory	Local name
1023	NCY	MRV	9839.1
1024	NCY	MRV	9887.2
1025	US6	BRN	CCB1010.2
1026	FRA	FAU	COTTAIS
1027	FRA	FAU	PACAULT
1029	NCY	YUN	ZIAKIS
1030	US2	BAC	OLSON.T.79
1031	NCY	MRV	19315
1032	US6	BRN	CC440.2
1034	NCY	MRV	11552
1036	UK1	FES	ALLEN
1037	SAF	HAM	N1164
1038	US8	RUB	NYBC002
1039	US6	BRN	CC434.1
1040	SAF	HAM	N1165
1043	UK1	DRK	WILLIAMS.773
1044	NCY	MRV	5094.1
1045	NCY	MRV	9671
1046	NCY	GOE	GOEKEN
1047	UK1	FES	HUGHES
1048	NCY	MRV	7407.7
1049	UK1	AST	DB2932
1050	US8	RUB	NYBC001
1051	US6	BRN	CCB.1064.4
1053	ANZ	DAW	R5.6308
1056	JAP	JUJ	1.1908
1058	NCY	MRV	9254
1060	FRA	FAU	BRULE
1062	US1	HJH	252C
1063	JAP	NAI	BI1014
1064	FRA	PRR	VEL1557
1066	FRA	FAU	ANTIN
1067	FRA	FAU	JEGU
1083	US1	THP	BRAUN
1087	US7	HAN	SEA1114
1091	US2	MOO	OWEN
1092	UK1	DRK	DANDO.1068
1096	US5	SWE	GRACE
1102	GER	GOL	16979
1113	US6	BRN	CC437.1
1123	BEN	BER	MSD8520

List of 10 WS sera used (continued).

10 WS number	Region	Laboratory	Local name
1126	US7	HAN	SEA1175
1129	BEN	BER	MSD8519
1132	ANZ	BSH	CALTHORPE
1133	BEN	ROO	62008
1135	BEN	BER	MSD6
1136	JAP	TKB	NS2220
1150	US2	BAC	REYNOLDS
1151	FRA	JEA	DROZ
1152	UK1	JOY	HAYES
1153	ANZ	DAW	ALDERSON.P7.987
1154	ANZ	BSH	DRAYTON
1155	BEN	BER	MSD14
1159	US4	STA	CJO
1161	UK1	MOR	LANGRIDGE.29
1162	UK1	MOR	EDWARDS
1169	IT1	MTT	F102406
1173	FRA	FAU	CHEVRIER
1176	NCY	MRV	21631
1179	ANZ	DAW	BUNTER.N/4.R5.8
1184	US5	TER	TER.DQ3C
1191	US2	MBC	BC.JO.WAUK
1194	ANZ	DAW	R5.8410
1198	NCY	MRV	26201
1199	UK1	FES	COOK
1209	FRA	FAU	BOUINIER
1212	NCY	MRV	24784
1213	IT2	FER	FE200
1215	GER	GOL	17782
1216	US8	JLE	H181

List of 10 WS MoAbs used.

10 WS number	Region	Laboratory	Local name
3023	FRA	DDC	CHE41.2
3036	US7	GSC	GSP88.2
3063	UK1	BOD	8.1
3092	UK1	BOD	4.1
3101	JAP	AIZ	HU46
3107	IT2	GAN	XIII358
3111	JAP	AIZ	HU18
3112	JAP	JUJ	PLM12
3114	US7	GSC	GS100.1
3119	JAP	JUJ	A10/13
3120	JAP	JUJ	PLM2
3121	JAP	AIZ	HU23
9050	US5	TER	Q0044
9999	US5	TER	Q0044