8 Duffy Blood Group System

8.1 Introduction

Fy^a and Fy^b, the products of *FY*A* and *FY*B*, give rise to three phenotypes in white people: Fy(a+b–), Fy(a+b+), and Fy(a–b+). Another allele, *FY*X*, produces a weak Fy^b antigen. In people of African origin the most common Duffy phenotype is Fy(a–b–), the result of homozygosity for an allele that is silent in erythroid cells (*FY*Null*) . Fy(a–b–) is extremely rare in other races.

Fy3, Fy5, and Fy6, are high frequency antigens in white and Asian people, polymorphic in African Americans, and private antigens in parts of West Africa. Fy3 and Fy6 are expressed on red cells of all Duffy phenotypes apart from Fy(a–b–). Fy3 is defined by alloantibodies occasionally made by Fy(a–b–) individuals, Fy6 is defined by murine monoclonal antibodies. Fy5 resembles Fy3, but is not present on Fy3-positive Rh_{null} cells. The antigens of the Duffy system are listed in Table 8.1.

Duffy antigens are located on a glycoprotein of apparent MW 35–50 kDa, a chemokine receptor of the G protein-coupled family (Sections 8.2 and 8.7). Fy^a and Fy^b result from a Gly42Asp substitution within the Duffy glycoprotein (Section 8.3.2). Duffy glycoprotein is present on endothelial cells of post-capillary venules and on other cells throughout the body (Section 8.6).

The Fy(a–b–) phenotype common in people of African origin is caused by homozygosity for a mutation within an erythroid-specific, GATA-1 transcription-factor binding site upstream of the coding region of the Duffy gene (Section 8.4.1). This mutation prevents expression of the Duffy glycoprotein on red cells, but not on other cells.

Individuals with the Fy(a–b–) phenotype are resistant to infection by the malarial parasite *Plasmodium vivax* and Fy(a–b–) red cells are refractory to invasion by *P. vivax in vitro*. Interaction between the Duffy glycoprotein and receptors on *P. vivax* merozoites are essential, but not sufficient, for red cell invasion (Section 8.8).

Duffy glycoprotein is a binding protein for chemokines and often referred to as Duffy antigen receptor for chemokines (DARC) (Section 8.7).

The Duffy (*FY* or *DARC*) locus is on chromosome 1q21-q22.

8.2 DARC, the Duffy glycoprotein, and the gene that encodes it

Moore *et al.* [1] found by immunoprecipitation that Fy^a was associated with components of apparent MW 39.5, 64, and 88 kDa. Immunoblotting with anti-Fy^a or -Fy6 revealed a broad band with an intense region of apparent MW 35–50 kDa [2–5], which inhibited anti-Fy^a after electroelution from the gel [2]. DARC aggregates readily *in vitro* [2,6], and may exist in the membrane in oligomeric form [6,7].

DARC is *N*-glycosylated, but has no, or very little, *O*-glycosylation. Treatment of red cells with the *N*-glycanase endo F prior to solubilisation and immunoblotting with anti-Fy^a resulted in a dramatic reduction in apparent

Human Blood Groups, Third Edition. Geoff Daniels.

© 2013 Geoff Daniels. Published 2013 by Blackwell Publishing Ltd.

Table 8.1 Antigens of the Duffy system.

No.	Name	Relative frequency		Comments
		White people	Black people	
FY1	Fy^a	Polymorphic	Polymorphic	Antithetical to Fy^b (FY2), Gly42
FY2	Fy^b	Polymorphic	Polymorphic	Antithetical to Fy^a (FY1), Asp42
FY3	Fy3	High	Polymorphic	Absent from Fy(a–b–) cells
FY4	Fy4	Low	Polymorphic	Possibly antithetical to Fy3. Obsolete
FY5	Fy5	High	Polymorphic	Absent from Fy(a–b–) and Rh_{null} cells
FY6	Fy6	High	Polymorphic	Very similar to Fy3. Defined by monoclonal antibodies

MW and sharpening of the band on the blot [3]. Similar results were obtained by treatment of purified DARC or of tryptic peptide derived from it [5,8]. Variation in the degree of *N*-glycosylation may account for the range of MW.

From an internal peptide sequence obtained from DARC purified with anti-Fy6, Chaudhuri *et al.* [6,9] constructed degenerate oligonucleotide primers and amplified, by PCR, a segment of cDNA derived from Fy(a–b+) individuals. This amplified product was then used to isolate cDNA clones from a human bone marrow library. An open reading frame of 1267 bp, predicting a 338 amino acid polypeptide of 35 733 Da, was identified. Hydropathy analysis supported by *in silico* modelling suggested that the mature protein has seven membrane-spanning α-helices, an extracellular N-terminus, and a cytoplasmic C-terminus [10–12] (Figure 8.1). This arrangement is characteristic of the G protein-coupled superfamily of receptors, which includes chemokine receptors [13–15]. The 65 amino acid extracellular domain contains three asparagine *N*-glycosylation sites, at residues 16 (Asn-Ser-Ser), 27 (Asn-Ser-Ser), and 33 (Asn-Asp-Ser). Site directed mutation experiments revealed that all three sites are used for sugar attachment [16].

DARC appears to be part of the junctional or 4.1R protein complex containing band 3, glycophorin C, Rh, Kell, and Xk, and attached to the spectrin–actin junction through protein 4.1R and p55 (see Section 10.7) [17].

The first *FY* cDNA to be cloned was encoded by a single exon [18–20]. Subsequently, Iwamoto *et al.* [21]

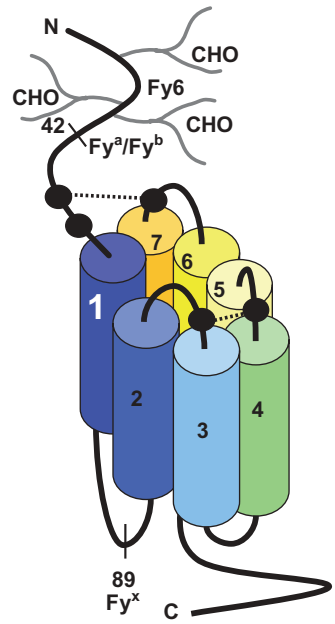

Figure 8.1 Three-dimensional model of DARC (adapted from [37], with permission from John Wiley & Sons). An extracellular N-terminal domain of 61 amino acids, containing three *N*-glycosylation sites (CHO) and the site of the Fy^a/Fy^b polymorphism at position 42, is followed by seven membrane-spanning domains and a cytoplasmic C-terminal domain. The first cytoplasmic loop contains the site of the amino acid substitution responsible for the Fy^x phenotype. There are five extracellular cysteine residues (•). Dotted lines represent the likely positions of disulphide bridges between cysteine residues in the N-terminal domain and the third loop and between the first and second loops.

showed that the predominant transcript in all tissues represented two exons separated by a 479 bp intron, the first exon encoding the seven N-terminal amino acids of DARC, including the translation-initiating methionine codon. Furthermore, the sequence of the minor transcript that encodes the nine N-terminal amino acid residues is part of the intron of the predominant, spliced transcript. The N-terminal sequence of the majority of DARC molecules is, therefore, MGNCLHRAEL [21] and not MASSGYVLQAEL [18], and the former protein is two amino acids shorter than the latter. The major erythroid transcription start point is 34 bp upstream of the first methionine codon in erythroid cells, but 82 bp upstream in lung and kidney [21]. In this chapter, nucleotides and amino acids will be numbered from the translation-initiating methionine residue of the major form of the glycoprotein.

The 5′ upstream region of *FY* contains no TATA or CAAT boxes, but it does contain several transcription-factor binding site motifs, including those for Sp1 and GATA [19,20].

In 1968, *FY* was the first human gene to be assigned to an autosome when Donahue *et al.* [22] showed *FY* to be linked to a visible deformity on the long arm of chromosome 1. More precise assignment was achieved by *in situ* hybridisation with *FY* cDNA [23].

Genes homologous to *FY* have been cloned from non-human primates, cow, pig, rabbit, and mouse [18,24].

8.3 Fy^a and Fy^b (FY1 and FY2)

Anti-Fy^a was discovered in the serum of a transfused haemophiliac, Mr Duffy, by Cutbush *et al.* in 1950 [25,26]. Only one year elapsed before Ikin *et al.* [27] identified the antithetical antibody, anti-Fy^b. Fy^x is the name given by Chown *et al.* [28] for a quantitative variant of Fy^b.

8.3.1 Frequency of Fy^a and Fy^b

Fy^a and Fy^b are useful anthropological markers. An extensive study of 2182 white Canadians, in which anti-Fy^b capable of detecting Fy^x was included, provided the following phenotype and deduced gene frequencies: Fy(a+b–) 0.1823; Fy(a+b+) 0.4735; Fy(a+b+^{w}) 0.0136; Fy(a–b+) 0.3302; Fy(a–b+^{w}) 0.0004; Fy(a–b–) 0; *FY*A* 0.425; *FY*B* 0.557; *FY*X* 0.016; *FY*Null* 0.002 [28,29]. The frequencies of Fy^a and Fy^b in African Americans and in Africans are variable, but low compared with Europeans because of the high frequency of the silent allele *FY*Null*. As in Northern Europeans, *FY*B* is more common than *FY*A*. In eastern Asia, *FY*B* has a far lower frequency than *FY*A* [30–35] (Tables 8.2 and 8.3).

8.3.2 The molecular basis of the Fy^a/Fy^b polymorphism

Sequencing of PCR products derived from reticulocyte cDNA and representing the entire coding sequence for the DARC revealed a SNP at position 125 encoding an amino acid substitution representing the Fy^a/Fy^b polymorphism: Fy^a, Gly42; Fy^b, Asp42 [18,19,36,37] (Figure 8.2). This was confirmed by expressing the appropriate cDNA clones in simian COS-7 cells and detecting Fy^a or Fy^b by flow cytometry [36]. The *FY*A* sequence creates a *Ban*I restriction site.

An Ala100Thr (G298A) polymorphism in the second membrane-spanning domain does not appear to affect antigenic expression [9,10,37,38]. In Swedish donors, the allele encoding Thr100 was detected in 22% of

Table 8.2 Genotype and allele frequencies in black South African, white Swedish, and Han Chinese populations, determined by molecular analysis of the Duffy genes of about 100 individuals from each population [32,33].

Phenotype	Genotype %				Allele %			
		Black	White	Chinese		Black	White	Chinese
Fy(a+b–)	*FY*A/A*	0	21	89	*FY*A*	3	41	94
	*FY*A/Null*	4	0	0	*FY*B*	17.5	59	6
Fy(a+b+)	*FY*A/B*	2	40	10	*FY*Null*	79.5	0	0
Fy(a–b+)	*FY*B/B*	2	39	1				
	*FY*B/Null*	29	0	0				
Fy(a–b–)	*FY*Null/Null*	63	0	0				

*FY*X* cannot be distinguished from *FY*B* by this method.

Table 8.3 Duffy genotype frequencies on four populations of American blood donors, obtained by testing on the BeadChip array [34].

		*FY*A/B*			*GATA1* mutation†			*FY*X*†		
Ethnic group	No. tested	*A/A*	*A/B*	*B/B*	*AB/AB*	*AB/Null*	*Null/Null*	*AB/AB*	*AB/X*	*X/X*
Caucasians	1243	0.18	0.43	0.39	0.97	0.02	0.01	0.98	0.02	0
African	690	0.04	0.19	0.77	0.12	0.33	0.55	0.99	0.01	0
Hispanic	119	0.15	0.45	0.40	0.68	0.26	0.06	0.99	0.01	0
Asian	51	0.69	0.25	0.06	0.98	0.02	0	1.00	0	0

†*AB* represents the alleles encoding Fy^a or Fy^b; *Null* is erythroid-silent.

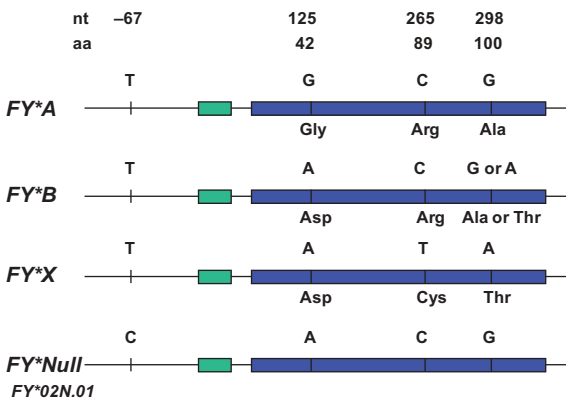

Figure 8.2 The four common Duffy alleles, showing nucleotide (nt) differences in the promoter regions and in exon 2 (blue) and encoded amino acid (aa) substitutions. Numbering from the major translation-initiating methionine residue [21].

Fy(a–b+), 9% of Fy(a+b+), but in 0% of Fy(a+b–); none of 100 black South Africans had Thr100 [39]. Other alleles in African Americans are listed in [40].

DARC homologues are present in non-human primates, with amino acid sequence homology of 99% between human and chimpanzee and 93–94% between human and squirrel, rhesus, and dourocouli monkeys [18]. All non-human primates have Duffy genes encoding Asp42, suggesting that *FY*B* is the ancestral allele [18,40,41].

8.3.3 Effects of enzymes on Fy^a and Fy^b

Fy^a and Fy^b are very sensitive to most proteolytic enzymes: they are completely destroyed by treatment of the red cells with papain, ficin, bromelin, pronase, or chymotrypsin, but trypsin does not abolish Fy^a and Fy^b activity. Use of impure preparations of trypsin containing chymotrypsin probably accounts for some early reports that Duffy antigens are trypsin-sensitive. Sialidase treatment of red cells does not affect the activity of Duffy antigens.

8.3.4 Fy^x

Fy^x behaves as a weak Fy^b antigen; there is no anti-Fy^x. Some anti-Fy^b sera react weakly with red cells of *FY*A/X* individuals, whereas others, particularly directly agglutinating anti-Fy^b, do not agglutinate those cells. The presence of Fy^x may be confirmed serologically by adsorption and elution of anti-Fy^b [28,42]. It is not possible to distinguish *FY*B/X* from *FY*B/B* serologically.

Fy^x has mostly been described in white populations, where it is not especially rare. Eleven Fy(a+b+^{w}) and one Fy(a–b+^{w}) were found among 1108 white people [29]. Fy^x has also been detected in a Canadian Cree kindred [43]. Several probable *FY*X* homozygotes have been reported, two of whom were originally thought to be Fy(a–b–) [29,42,44,45].

In addition to Fy^b, there is a very marked depression of Fy3, Fy5, and Fy6 in *FY*X* homozygotes [37–39, 43,44,46,47]. Flow cytometric analysis produced the following estimations of Fy6 site numbers per red cell: Fy(a–b+) (*FY*B/B*) 2200–2400; Fy(a–b+^{w}) (*FY*X/Null*) 150; Fy(a–b+^{w}) (*FY*X/X*) 250 [47]. Reduced levels of Fy6 binding associated with Fy^x were also detected by immunoblotting of DARC isolated from the red cell membrane [38,47]. This suggests that Fy^x represents reduced levels of DARC in the membrane rather than an altered Fy^b determinant or conformational changes affecting several

determinants and *FY*X* (also known as *FY*02M.01*) should be considered a mod allele.

*FY*X* and *FY*B* have the same coding sequence apart from 265C>T encoding Arg89Cys in the first cytoplasmic loop of DARC [39,41,47,48] (Figures 8.1 and 8.2). The replacement of a positively charged arginine residue by a neutral cysteine results in protein instability and might compromise the insertion of the molecule in the red cell membrane. Mammalian cells transfected with *FY* cDNA constructs in which the Cys89 codon had been introduced by site-directed mutagenesis had substantially reduced expression of Fy^b, Fy3, and Fy6, compared with cells transfected with normal *FY*B* cDNA [38,47] or with *FY*B* cDNA in which Arg89 had been replaced with positively charged lysine [49]. The *FY*X* mutation abolishes an *Aci*I restriction site. *FY*X* (*FY*02M.01*) encodes threonine at the site of the Ala100Thr polymorphism (Figure 8.2 and Section 8.3.2) [39,45]. Two Brazilians, one Caucasian, and one African, with extremely weak Fy^b, Fy3, and Fy6 expression, were heterozygous for *FY*Null* and an *FY*X* allele (*FY*02M.02*) containing an additional SNP, 145G>T encoding Ala49Ser [50].

*FY*X*, as determined by the presence of the Arg89Cys mutation, has an allele frequency of 0.025, 0.015, and 0.010 in Caucasian donors from Sweden, Austria, and the United States, respectively, and 0.005 in African Americans, but was not found in 100 black South Africans [34,39,48] (Tables 8.2 and 8.3). Fy^x phenotype, however, is associated with some degree of genetic heterogeneity. In some individuals with the Fy^x phenotype no change from the *FY*B* allele was detected in the coding region of the gene [37] and Fy^x has also been associated with a single base deletion in an Sp1 transcription-factor binding site upstream of the transcription start position [51].

8.3.5 Anti-Fy^a

The original anti-Fy^a of Mr Duffy was reported in 1950 [25,26]. Anti-Fy^a is estimated to be three times less frequent than anti-K [52]. Of 1778 alloimmunised Dutch patients, 9% of the antibodies were anti-Fy^a and 0.9% anti-Fy^b [53]. In Japan, however, where Fy(a–) phenotype is relatively uncommon, no anti-Fy^a was detected in 3554 patients with irregular blood group antibodies, but 4% of the antibodies were anti-Fy^b [54]. There is substantial evidence, from several different centres in the United States, that Fy^a is less immunogenic in black people than in white people [52,55–57], though one survey disputes this [58]. Anti-Fy^a often accompanies or precedes anti-Fy3 in Fy(a–b–) black people [59–62].

Some anti-Fy^a may have been stimulated by pregnancy, but most arise from blood transfusion. 'Naturally occurring' anti-Fy^a are very rare [63,64]. Anti-Fy^a may occur alone, but is often found in mixtures of antibodies. Duffy antibodies are not commonly detected in the first 6 months following transfusion, but, relative to other antibodies, are more common after 6 months and even more so after 5 years [53]. Anti-Fy^a are usually IgG, mostly IgG1 [65,66]. They generally react best by an antiglobulin test, but rarely anti-Fy^a may be directly agglutinating [67,68]. About 50% of anti-Fy^a activate complement up to the C3 stage [52].

Anti-Fy^a has been incriminated in immediate and delayed HTRs [69]. Though generally mild, a few immediate reactions have been fatal [70,71] and antigen-negative red cells should be selected for transfusion to patients with Duffy antibodies. The majority of radiolabelled Fy(a+) red cells injected into a patient with anti-Fy^a were eliminated within 10 minutes [72]. Anti-Fy^a in donor blood was responsible for a transfusion reaction in a Fy(a+b+) patient [73]. HDFN caused by anti-Fy^a is usually mild, but is occasionally severe. In a survey of 68 pregnancies in which the mother has anti-Fy^a, three resulted in a severely anaemic fetus, two requiring intrauterine transfusion [74]. A drop in the titre of anti-Fy^a in a D– Fy(a–b+) pregnant woman from 4096 to 256 following three injections of anti-D immunoglobulin could have resulted from an immunosuppressive effect of the prophylactic treatment [75].

There is a clear association between production of anti-Fy^a and *HLA-DRB1*04* [76,77], although an additional association with *HLA-DRB1*15* is also reported [77]. Duffy antigens could be minor histocompatibility antigens: Duffy mismatched renal grafts had significantly more chronic lesions compared with Duffy matched grafts [78].

8.3.6 Anti-Fy^b

Anti-Fy^b is a relatively rare antibody usually found only in mixtures of red cell antibodies. It has been stimulated by pregnancy and transfusion, and by intrauterine transfusion in the mother [79]; 'naturally occurring' anti-Fy^b has been found [80]. Often consisting entirely of IgG1 [65,66], Fy^b antibodies generally react best by an antiglobulin test, but directly agglutinating examples are known [28]. Some anti-Fy^b bind complement.

Anti-Fy^b has been responsible for immediate and delayed HTRs [69], two of which were reported to have been fatal [81,82]. One delayed reaction occurred in a man with no prior history of transfusion [83]. The single

reported case of HDFN caused by anti-Fy^b was treated by phototherapy and two transfusions [84].

Autoantibodies mimicking anti-Fy^b in Fy(a+b–) patients have been described, one responsible for AIHA [85,86]. The antibodies reacted more strongly with Fy(a–b+) and Fy(a+b+) cells than with Fy(a+b–) cells, and not at all with Fy(a–b–) cells. In one case, pure anti-Fy^b specificity was subsequently found [85].

8.3.7 Monoclonal anti-Fy^a and Fy^b

Murine monoclonal anti-Fy^a and -Fy^b have been produced [87], including anti-Fy^a from lymphocytes derived from transgenic mice expressing Fy^b and immunised with a transfected human embryonic kidney cell line expressing Fy^a [88]. One monoclonal IgM anti-Fy^b directly agglutinated Fy(b+) red cells and reacted weakly with Fy(a–b+^{w}) (*FY*X/X*) cells [87].

8.4 Fy(a–b–) phenotype; Fy3, Fy5, and Fy6 antigens

8.4.1 Fy(a–b–) in people of African origin

The Fy(a–b–) phenotype came to light when Sanger *et al.* [89] found that red cells of nearly 70% of African American blood donors failed to react with both anti-Fy^a and -Fy^b. A silent allele, recessive to *FY*A* and *FY*B*, was postulated in order to account for this null-phenotype. Fy(a–b–) has a frequency of about 63% in black New Yorkers, West Indians [90], and South Africans [32], but the frequency is higher in Africa, approaching fixation in parts of the continent [30,35] (Tables 8.2 and 8.3). All of 1168 donors from rural Gambia were Fy(a–b–) [91].

Although Fy(a–b–) red cells lack DARC [4,6], it is expressed in endothelial cells lining post-capillary venules of soft tissues and splenic sinusoids from black Fy(a–b–) individuals [92]. Duffy mRNA was not detected in the bone marrow of Fy(a–b–) individuals, but was present in their lung, spleen, and colon [18]. The coding sequence of the common erythroid-silent allele (*FY*Null* or, more precisely, *FY*02N.01*) is identical to that of an *FY*B* allele [9,18,19,36,37,92], but a T>C change is present in the promoter region of the gene, 33 bp upstream of the erythroid transcription start point and 67 bp upstream of the major translation start codon (position –67), introducing a *Sty*I restriction site [20,93]. This mutation is within a GATA consensus sequence (CTTATCT to CTTACCT), disrupting binding of the erythroid-specific GATA-1 transcription factor and preventing expression of the gene in erythroid cells, but not in other cells. A human erythroid cell line (HEL) and human microvascular endothelial cells transfected with a construct consisting of the promoter region of the *FY*B* allele and the reporter gene chloramphenicol acetyltransferase (CAT) had high levels of CAT activity. Transfection with a construct containing the –67T>C GATA mutation abolished CAT activity in the HEL cells, but not in the endothelial cells [20,93]. Transgenic mice with the human *FY*Null* allele expressed no DARC on their red cells, but did express it in non-erythroid tissues [94].

Twenty-three of 1062 individuals from the East Sepik of Papua New Guinea were heterozygous for *FY*A* and a Duffy allele with the *FY*A* coding sequence (encoding Gly42), but with the GATA mutation characteristic of *FY*Null* (–67C), an allele frequency of 0.022 [95]. This allele (*FY*01N.01*) is probably silent in erythroid cells and red cells of these individuals had lower levels of Fy6 than those homozygous for normal *FY*A*. An erythroid-null allele with –67C and an *FY*A* sequence has also been reported in five Brazilians from the malaria-endemic region of the Amazon [96] and in two tribes of the Sudan [191].

Resistance of people with the Fy(a–b–) red cell phenotype to the malarial parasite *Plasmodium vivax* is described in Section 8.8.

8.4.2 Fy(a–b–) in other ethnic groups

Fy(a–b–) is very rare in ethnic groups not originating from sub-Saharan Africa [30,35]. None of 6000 white Australian donors was Fy(a–b–), as determined by testing red cells with the original anti-Fy3 [97], but a remarkably high incidence of 1% *FY*Null/Null* homozygotes was found in New York Caucasians by genotyping [34].

Most examples of non-African Fy(a–b–) have been found through the presence of strong anti-Fy3. The molecular background to the Fy(a–b–) phenotype has been determined in four non-Africans with anti-Fy3, all of whom had been transfused and/or pregnant. The Duffy gene of a white Australian woman [97] contains a 14 bp deletion resulting in a reading frameshift and introduction of a translation stop codon (281–295del, *FY*01N.02*) [37]. Homozygosity for nonsense mutations that introduced translation stop codons was found in three examples:

1 a white British woman with 408G>A (Trp136Stop) in *FY*A* (*FY*01N.03*) [98];

2 a Lebanese Jewish woman with 407G>A (Trp136Stop) in *FY*B* (*FY*02N.02*) [98];

3 a native Canadian (Cree) with 287G>A (Trp96Stop) in *FY*A* (*FY*01N.04*) [43,98].

Table 8.4 Reactions of anti-Fy3 (from different ethnic groups), anti-Fy5, and monoclonal anti-Fy6 with red cells of various phenotypes.

Race and phenotype	Anti-Fy3		Anti-Fy5	Anti-Fy6
	Black	Other		
All races				
Fy(a+b–)	+	+	+	+
Fy(a+b+)	+	+	+	+
Fy(a–b+)	+	+	+	+
Cord cells†	– or +	+	+	+
Papain-treated cells†	+	+	+	–
Black				
Fy(a–b–)	–	–	–	–
White				
Fy(a–b–)	–	–	+¶	nt
Fy(a–b+^{w}) (*FY*X/X*)	w	w	w	w
Rh_{null}†	+	+	–	+
D––†	+	+	w	+

†Not Fy(a–b–) cells.
¶White Australian [97,101].
nt, not tested; w, weakly positive.

An Fy(a–b–) Japanese woman without anti-Fy3, but who had never been transfused or pregnant, was homozygous for an *FY*A* allele with a deletion of 327C, which introduces a stop signal 12 codons downstream at codon 120 (*FY*01N.05*) [99]. Each of these mutations would be expected to result in no expression of Duffy antigen on red cells or in any other part of the body.

8.4.3 Fy3 and anti-Fy3

8.4.3.1 Fy3 antigen

Fy3 is present on all red cells apart from those of the Fy(a–b–) phenotype. Fy3 is a public antigen in people of European and Asian origin, polymorphic in many black populations, and a private antigen in some parts of Africa. In contrast to Fy^a and Fy^b, Fy3 is resistant to the treatment of red cells with proteases [43,59,97,100]. Red cells of some primates have Fy3, but not Fy^a or Fy^b.

8.4.3.2 Anti-Fy3

The original anti-Fy3 was found by Albrey *et al.* [97] in the serum of an Fy(a–b–) transfused white Australian woman during her third pregnancy. The antibody reacted equally strongly with Fy(a+b–), Fy(a+b+), and Fy(a–b+) cells, and could not be separated into anti-Fy^a and -Fy^b components. It did not react with Fy(a–b–) cells (Table 8.4). Four other examples of anti-Fy3 have been produced by people who are not of African origin [43,98,100], one of which appeared to show a preference for Fy(a+) cells [100].

Anti-Fy3 is rare in Fy(a–b–) black people, though several examples have been reported [58–61,102–104], including two demonstrated to be homozygous for the *FY*Null* erythroid-silent allele [104]. Most anti-Fy3 in black people are found in mixtures of antibodies to red cell antigens, which often include anti-Fy^a. In some black patients with anti-Fy3, anti-Fy^a had preceded development of anti-Fy3 but was no longer detected when the anti-Fy3 was present [62]. Anti-Fy^a is a far more common antibody in the serum of multiply transfused black patients than anti-Fy3. Antibody screening tests on sera from 566 transfused Fy(a–b–) black patients in France revealed no Duffy antibodies [105].

Fy(a–b–) black people have Fy:–3 red cells, but they have DARC in other parts of the body [92]. The *FY*Null* allele has the *FY*B* sequence, so it is likely that *FY*Null* homozygotes express Fy3 and Fy^b in non-erythroid tissues, explaining the rarity of anti-Fy3, the absence of anti-Fy^b, and the relatively common occurrence of anti-Fy^a in transfused Fy(a–b–) black people. Anti-Fy3 made in black people probably recognise subtle differences between DARC expressed in different tissues, possibly a glycosylation or conformational difference. Anti-Fy3 made by black people react either very weakly or not at all with cord cell samples [60,61,102], whereas anti-Fy3 from three non-black women all reacted equally strongly with red cells of adults and newborn infants [43,100]. Perhaps Duffy antigen on fetal red cells resembles that on adult endothelial cells.

Anti-Fy3 is potentially haemolytic and has been responsible for immediate and delayed HTRs [62,100, 103,106,107], including intravascular haemolysis in an acute reaction [106] and hyperhaemolyis in a patient with sickle cell disease [107]. Fy(a–b–) red cells should be selected for transfusion to patients with anti-Fy3. The third child of the white Australian and the eighth child of the Cree woman with anti-Fy3 showed signs of HDFN, but in neither case was any treatment beyond phototherapy required [97,43].

Several mouse monoclonal antibodies have been produced that resemble anti-Fy3 in reactivity and detect protease-resistant epitopes on DARC [87,108]. Protein analyses have shown that these antibodies detect either a non-linear conformational epitope involving charged amino acids in all three extracellular loops [109] or epitopes located on the third extracellular loop [108,110] (see Figure 8.1).

8.4.4 Fy5 and anti-Fy5

Fy5 closely resembles Fy3 [101,111]; it differs by its absence from Fy3-positive Rh_{null} cells (amorph and regulator type), weak expression on red cells of D–– phenotype, and presence on Fy(a–b–) cells from people of non-African origin. Like Fy3, Fy5 is a protease-resistant antigen. Fy5 is expressed equally strongly on red cells of adults and newborns (Table 8.4). DARC appears to be part of a protein complex that contains the Rh proteins [17], so Fy5 expression could be dependent on an interaction between those proteins.

Several examples of anti-Fy5 have been reported, all in multiply transfused black Fy(a–b–) patients, mostly with sickle cell disease [62,101,111–113]. All were present in a mixture of other red cell antibodies; in two anti-Fy^a was present [111,113] and in another two anti-Fy^a had preceded anti-Fy5 but was no longer detectable [62]. Anti-Fy5 could not be separated into other antibody components by adsorption and elution. Some Rh e-variant red cells have weak Fy5 antigen [114]. Red cells from *FY*X* homozygotes have depressed Fy5 (Section 8.3.4).

Anti-Fy5 has been incriminated in delayed HTRs [62,112,113]. One patient had two separate reactions, one caused by anti-Fy^a, the other by anti-Fy5 [113].

8.4.5 Monoclonal anti-Fy6

Some monoclonal antibodies produced by immunising mice with red cells have a specificity very similar to that of anti-Fy3 [4,5], but were named anti-Fy6 because, unlike anti-Fy3, the determinant they detect is destroyed by papain (Table 8.4), ficin, and chymotrypsin; Fy6 is resistant to trypsin. Peptide scanning and site-directed mutagenesis have demonstrated that anti-Fy6 detect a linear epitope on the N-terminal extracellular domain of DARC between amino acid residues 19 and 26 (see Figure 8.1) [108,115,116]. In its distribution on red cells of non-human primates, Fy6 differs from Fy^a, Fy^b, and Fy3, but shows close accord with susceptibility to *Plasmodium vivax* invasion (see Section 8.8). Monoclonal anti-Fy6 proved invaluable in the isolation of DARC [5,6].

A recombinant dromedary antibody fragment, known as a VHH or nanobody, had anti-Fy6 specificity [117]. VHHs are the smallest intact antigen binding fragment derivative from the heavy chain-only antibodies present in camelids. The VHH recognises native DARC on red cells, inhibits *Plasmodium vivax* invasion, and displaces interleukin-8 bound to DARC.

8.4.6 Fy4 and anti-Fy4

Behzad *et al.* [118] described an antibody, produced by an Fy(a+b+) sickle cell patient, which gave most of the reactions expected of an antibody to the product of the *FY*Null* allele, though weak reactions confused some of the results. The antibody was numbered anti-Fy4, but as no second example was been found and the original is no longer available, the number Fy4 is obsolete. Although it is now known that DARC is not present on Fy(a–b–) cells, Fy4 might have recognised a changed conformation of an associated membrane component that occurs in the absence of DARC.

Table 8.5 Prediction of Duffy phenotypes from separate tests of two SNPs in the *FY* gene.

SNP 125	SNP −67	Predicted phenotype
G	T	Fy(a+b–)
A	T	Fy(a–b+)
G+A	T	Fy(a+b+)
A	C	Fy(a–b–)
A	T+C	Fy(a–b+)
G+A	T+C	Fy(a+b–)
G	C	Fy(a–b–) Very rare
G+A	C	Fy(a–b–) Very rare
G	T+C	Fy(a+b–) Very rare
G+A	T+C	Fy(a–b+) Very rare

8.5 Duffy genotype determination

To obtain accurate Duffy phenotype predictions by molecular genotyping, it is essential that the SNPs at positions 125 (*FY*A/FY*B*) and −67 (*FY*Null/FY*AB*) are interrogated. If separate tests are employed for the two SNPs, the results can be interpreted as shown in Table 8.5 and will give a high level of accuracy, though a false result will occur if the rare allele with −67C and 125G is present. A method employing a pair of sequence-specific primers at each SNP that give four potential combinations for allele-specific amplification of the 700 bp sequence between the SNPs avoids this potential error [32,119]. Further tests of the SNP at position 265 can be added to detect the presence of *FY*X* [34,39,47,48,120]. If this is not used, an *FY*X* allele would be interpreted as an *FY*B* allele, which would have no adverse affect on transfusion.

Duffy genotyping is valuable for the determination of Duffy phenotype of fetuses of mothers with anti-Fy^a, in order to assist in assessing the risk of HDFN [74]. It will also avoid Fy^x red cells being mistyped as Fy(b–).

Sickle cell disease (SCD) patients are regularly transfused and often make multiple red cell antibodies, making subsequent transfusion difficult. Phenotype-matched blood is often requested for these patients and Duffy genotyping might assist in providing matched blood. Fy(a+b–) patients who are *FY*A/A* would be capable of making anti-Fy^b, but those who are *FY*A/Null* would not. In a study of Brazilian SCD patients, 28% were Fy(a+b–), but of these 96% were *FY*A/Null* and only 4% *FY*A/A* and capable of making anti-Fy^b [121].

8.6 Site density, development, and distribution of Duffy antigens

Fy(a+b–) and Fy(a–b+) red cells were estimated, by quantitative immunoferritin microscopy, to have 13 000–14 000 Fy^a or Fy^b sites; Fy(a+b+) cells have about half that number of Fy^a sites [122]. With two radioiodinated monoclonal anti-Fy6, estimates of 12 200 and 6000 sites per red cell were obtained [4,5].

Fy^a and Fy^b are fully developed at birth and have been detected on red cells from embryos as early as 6–7 weeks gestation [123,124]. The expression of Fy^a and Fy^b is as strong on red cells of very young fetuses as on those of adults, and remains unmodified throughout fetal life.

There is almost 50% higher level of expression of Fy6 on reticulocytes than on mature erythrocytes [125].

In addition to its presence on red cells, DARC, detected with anti-Fy6, is abundant on endothelial cells lining post-capillary venules throughout the body, except for liver [126,127], and on Purkinje neurons of the cerebellum [128]. Sequence analyses of cDNA indicate that the renal and erythroid isoforms of the Duffy polypeptide are identical and any small differences in MW of the glycoproteins are probably accounted for by altered glycosylation [10,126,127]. DARC was also detected, with a rabbit polyclonal antibody specific for the glycosylation of DARC, on some other vascular endothelial cells and on epithelial cells of renal collecting ducts and pulmonary alveoli [127]. The effect of the GATA mutation in the *FY*Null* allele is erythroid-specific, so DARC is present on non-erythroid tissues in black people with the Fy(a–b–) red cell phenotype [92] (Section 8.4.1). Duffy mRNA has been detected in lung, muscle, spleen, colon, heart, pancreas, kidney and brain [10,18,126]. This transcript is present in tissues from Fy(a–b–) black individuals [18].

Fy^a and Fy^b are not present on lymphocytes, monocytes, granulocytes, or platelets [129,130].

8.7 The Duffy glycoprotein is a chemokine receptor

The Duffy glycoprotein binds a variety of pro-inflammatory chemokines and is often known as the Duffy Antigen Receptor for Chemokines (DARC). Chemokines are chemotactic cytokines that are involved in many cellular processes, especially the recruitment, activation, and directional movement of leucocytes [15,131]. There are two main classes of chemokines, called CXC

and CC based on the position of two highly conserved cysteine residues at their N-termini, plus two minor classes, C and CXXXC. Most chemokine receptors belong to a very large family of integral cell-membrane glycoproteins, G protein-coupled receptors, which traverse the membrane seven times and have an extracellular N-terminal domain (Figure 8.1) [13]. Most chemokine receptors are specific for one or more chemokines of a single class, but DARC, a promiscuous receptor, binds with high affinity to 60% of inflammatory chemokines from both CX and CC classes, but not with homeostatic chemokines [14,132]. Unlike almost all other G protein-coupled receptors, DARC lacks the Asp-Arg-Tyr (DRY) motif in the second cytoplasmic loop and does not appear to be coupled to a guanosine triphosphate-binding protein (G-protein) [24]. Consequently, Duffy is a chemokine-binding protein with no signalling function and has been referred to as a 'silent receptor' or interceptor (internalising receptor) [133].

Fy(a–b–) red cells do not bind chemokines [47,134]. Fy(a–b+^{w}) (*FY*X/X*) bind chemokines in substantially reduced quantities compared with Fy(a–b+) cells [38,47].

The chemokine-binding pocket of DARC appears to involve charged residues in the first four extracellular domains, which are brought into close proximity by two disulphide bridges [109,135] (Figure 8.1). Anti-Fy^{a}, -Fy^{b}, and -Fy6 (including VHH), which bind to the first extracellular domain of DARC, and a monoclonal anti-Fy3, which binds the fourth extracellular domain, all compete with chemokines for binding [110,117,134–137]. Interleukin-8 (IL-8, CXCL8) bound to red cells treated with trypsin, sialidase, or *N*-glycanase, but not to cells treated with papain or α-chymotrypsin [138], which cleave the N-terminal extracellular domain of DARC (see Section 8.3.3). Glycosylation of the protein is not required for chemokine binding. The Asp42Gly (Fy^{a}/Fy^{b}) Duffy polymorphism is associated with about 20% of variation in serum concentration of the chemokine monocyte chemoattractant protein-1 (MCP-1, CCL2), with Asp42 (Fy^{b}) associated with the greater red cell-bound MCP-1, and also with serum concentrations of IL-8 and RANTES (CCL5) [139].

The function of DARC on red cells and on endothelial cells remains controversial. DARC on red cells has long been considered to function as a sink, binding excess chemokines to prevent inappropriate activation of neutrophils and disruption of chemokine gradients [140]. The sink theory, however, is supported by responses of healthy volunteers to infusions of endotoxin (lipopolysaccharide, LPS). Following LPS infusion, plasma chemokine levels were 2–3 times higher and red cell-associated chemokine levels 20–50 times higher in Duffy-positive Caucasians compared with Duffy-negative Africans [141]. However, lower levels of plasma chemokines in Fy(a–b–) persons and in Duffy knockout mice suggests that DARC on red cells could provide a reservoir of chemokines, maintaining plasma chemokine concentrations [142,143]. One mechanism for releasing chemokines from DARC is clotting [139].

A degree of neutropenia, often referred to as 'benign ethnic neutropenia', is prevalent in Africans and in people of African origin. It is closely associated with homozygosity for the erythroid-silent Duffy allele [144–146]. This provides further support for the proposal that red cell DARC is involved in the regulation of chemokine concentration and, as a consequence, neutrophil production and migration. Associations between Duffy phenotype and disease are described in Section 8.9.

DARC is present on the endothelial cells of post-capillary venules throughout the body, in both Duffy-positive people and Fy(a–b–) Africans (Section 8.6). The kidney isoform binds chemokines with the same affinity as the red cell isoform [126]. It has been assumed that DARC on endothelial cells functions as a chemokine reservoir or decoy receptor, dampening chemokine effects in the local circulation [133,147]. However, it now appears that a primary role of endothelial DARC is the transport of tissue-derived chemokines across the endothelial barrier to the luminal surface, where they are presented to receptors on leucocytes [148]. DARC internalises chemokines, but does not scavenge them. Instead, the chemokines remain active and support optimal leucocyte migration across the endothelial layers, a fundamental component of inflammation [148].

DARC in endothelial cells forms dimers with the chemokine receptor CCR5, inhibiting CCR5 signalling [7]. Therefore, DARC, which is non-signalling, could be involved in the regulation of chemokine signalling though heterodimerisation with other chemokine receptors.

Several people have the Fy(a–b–) red cell phenotype owing to homozygosity for inactivating mutations within the coding region of their Duffy genes (Section 8.4.2). These individuals would have no DARC in any of their tissues, yet all were apparently healthy.

8.8 Duffy antigens and malaria

Plasmodium vivax is responsible for between 70 and 390 million cases of malaria annually, mostly in South and

Southeast Asia. Although often considered to be a less severe form of malaria than that caused by *P. falciparum*, vivax malaria is far from benign and can often cause severe, life-threatening syndromes that are similar to those associated with falciparum malaria [149]. For several decades it had been known that most black people are resistant to *P. vivax* infection. When Miller *et al*. [150] showed that Fy(a–b–) red cells are refractory to invasion by the simian parasite *P. knowlesi*, which can invade human red cells *in vitro*, it was not long before the association between the Fy(a–b–) phenotype and resistance to *P. vivax* infection became apparent [151]. Of 11 black and six white volunteers exposed to *P. vivax*, all became infected except for the five Fy(a–b–) black subjects [151].

Fy(a–b–) human red cells are refractory to invasion by *P. knowlesi* and *P. vivax* merozoites, *in vitro*, whereas Fy(a+b+) cells are invaded [150,152,153]. This is not a purely racial characteristic as Fy(a–b–) red cells from two Cree Indians and a white Australian (Section 8.4.2) were not invaded by *P. knowlesi* [154]. Merozoites are able to attach to Fy(a–b–) cells, but they cannot form a junction and eventually become detached [150,154,155]. Invasion of Duffy-positive red cells by *P. knowlesi* and *P. vivax* can be blocked by the presence of monoclonal anti-Fy6; invasion of Fy(a+) cells can be partially blocked by anti-Fy^a [150,152]. The chemokines IL-8 and MGSA (CXCL1) blocked invasion of Duffy-positive cells [134]. Treatment of Duffy-positive red cells with chymotrypsin renders them resistant to invasion by *P. knowlesi* and *P. vivax*, whereas trypsin treatment has no effect [150,152]. This parallels the effects of these proteases on Fy^a, Fy^b, and Fy6 antigens, but not on Fy3 and Fy5, which are resistant to chymotrypsin cleavage. *P. vivax* prefers to invade reticulocytes, which have about 50% more Fy6 sites than mature erythrocytes [125].

Despite being Fy(b+) and Fy:3, red cells of the Old World rhesus monkey are Fy:–6 and are not invaded by *P. vivax*, but are invaded by *P. knowlesi*. New World capuchin monkey cells are Fy(a–b–) Fy:3,–6 and are not invaded by *P. knowlesi* or *P. vivax* merozoites. These data suggest that the Fy6 epitope is important for the invasion of *P. vivax* [154,156–158].

Proteins that bind Duffy-positive human red cells, but not Fy(a–b–) cells, have been isolated from the supernatants of cultured *P. knowlesi* and *P. vivax* at the time of merozoite release and reinvasion [159,160]. These Duffy-binding proteins (DBLs), Pkα-DBL and Pv-DBL for *P. knowlesi* and *P. vivax*, respectively, belong to the Duffy-binding-like erythrocyte-binding protein family (DBL-EPB), which includes *P. falciparum* EPBs [161]. Binding could be inhibited by anti-Fy6 and by the chemokines IL-8 and MGSA [157,159,160]. The purified parasite proteins bound specifically to purified DARC. The extracellular domains of each protein have been classified into six regions of amino acid homology [161]. COS-7 cells expressing the cysteine-rich region II (PvDBP-RII) formed rosettes with Duffy-positive, but not Fy(a–b–), red cells [162]. This rosetting could be blocked by a synthetic peptide representing amino acid residues 8–42 of the N-terminal domain of DARC [163]. Fine mapping restricts the binding site to between Gln19 and Trp26 on the N-terminal domain of DARC [164], the location of the Fy6 epitope, although sulphation of Tyr41 is also essential for association [165]. PvDBP-RII and PkαDBP-RII share extensive sequence homology with the *P. falciparum* glycophorin A-binding protein, EBA-175 [161] (Section 3.21.1). Analysis of the Duffy-binding ligands has led to the identification of the *var* genes that encode the variant endothelial cytoadherence proteins of *P. falciparum* (for review see [24]). Naturally occurring antibodies to PvDBP or, more likely, inhibitory antibodies produced by immunisation with recombinant PvDBP-RII, could prove valuable in vaccine production [166].

The Duffy gene is subject to opposing selection pressures: the need to maintain effective chemokine binding versus the benefits of reducing *P. vivax* invasion. It is likely, therefore, that the high incidence of the *FY*Null* allele in Africa results from the selective advantage it confers in providing resistance to vivax malaria. In parts of West Africa the frequency of *FY*Null* is almost 100%, yet *P. vivax* is not present in these areas [91]. It is probable that the parasite has been eliminated by the disruption of its life cycle resulting from a shortage of susceptible hosts. Heterozygosity for the silent allele *FY*Null* may have a selective advantage in areas where *P. vivax* is endemic: individuals heterozygous for *FY*Null* are less susceptible than *FY*Null* homozygotes to *P. vivax* infection [167] and their red cells bind substantially less PvDBP than those of individuals with two active Duffy alleles [168]. Furthermore, Fy(a+b–) (*FY*A/A*) red cells had up to 50% lower PvDBP binding than Fy(a–b+) (*FY*B/B*) cells and *FY*A/A* individuals had substantially reduced risk of clinical vivax malaria than individuals of *FY*B/B* genotype [169]. Consequently, *FY*A* appears to have a survival advantage over the ancestral allele, *FY*B*, which could explain the high incidence of *FY*A* in regions where vivax is endemic but *FY*Null* is rare.

Occasional cases of Fy(a–b–) individuals infected by *P. vivax* have been observed in the Brazilian Amazon and various parts of Africa [170–173] and a substantial number of Fy(a–b–) people in Madagascar were susceptible to *P. vivax* blood-stage infection and clinical vivax malaria [174]. *P. vivax* therefore, appears to be evolving alternative pathways for red cell penetration.

8.9 Other disease associations

In contrast to resistance to vivax malaria, susceptibility to a number of other diseases appears to be associated with the presence or absence of Duffy on red cells, probably as an effect of differences in chemokine regulation and their influence on leucocyte levels and inflammation. The association between the erythroid Duffy-null phenotype and benign ethnic neutropenia [144–146] was mentioned in Section 8.7.

HIV-1 attaches to red cells via DARC, effecting infection of target lymphocytes [175]. Individuals homozygous for *FY*Null* are reported to have a 40% increase in HIV-1 infection, but slower HIV disease progression once infected [175] (although the evidence is disputed [176]). In a cohort of South African women, the risk of acquiring HIV infection was about three-fold greater in those with Fy(a–b–) phenotype-associated low neutrophil counts, compared with other study participants [177]. Among leukopenic HIV-positive African Americans, however, there was a survival advantage of being *FY*Null/Null*, but this did not apply to *FY*Null/Null* patients with high white cell counts [178].

Red cell Duffy expression is strongly associated with severity of sickle cell disease (SCD) and especially with organ damage. Twice as many Duffy-negative patients had evidence of organ damage compared with Duffy-positive patients; Duffy-negative patients were nearly four times more likely to have proteinuria [144]. Duffy-positive SCD patients have higher plasma levels of chemokines IL-8 and RANTES than Duffy-negative patients [179]. Activation through clustering of integrin $\alpha 4\beta 1$, which may play an important role in vaso-occlusive crises, was induced by IL-8 and RANTES on Duffy-positive, but not Duffy-negative, sickle reticulocytes [180]. The presence of DARC, therefore, may determine the extent of inflammation in SCD and Anstee [181] has suggested that the enhanced propensity for alloimmunisation in SCD could be related to inflammation and that Fy(a–b–) phenotype may play a part in this process.

Fy(a–b–) African Americans have lower renal allograft survival in the presence of delayed graft function [182].

The release of chemokines from Duffy-positive red cells during clotting suggests localised increased chemokine levels in thrombotic states, which could modulate the inflammatory effects associated with haemostasis and thrombosis [139].

The surface expression of Duffy antigens and red cell chemokine scavenging function is reduced with red cell storage [183] and Duffy antigen has been identified on exocytic vesicles from stored red cells [184]. Although the overall loss of Duffy from banked red cells is modest, this might contribute to the 'storage lesion' associated with some adverse effects of transfusion. In mice, transfusion of red cells stored for 10 days increased neutrophil counts and chemokine concentrations in the air spaces of the lung and transfusion of red cells from Duffy knockout mice into endotoxaemic Duffy-positive mice increased airspace neutrophils, inflammatory cytokine concentrations, and lung microvascular permeability, compared with transfusion of Duffy-positive red cells [184]. Consequently, loss or modification of Duffy antigen could be a contributing factor in transfusion-associated lung injury in the critically ill.

A genetic study in the Caribbean and South America suggests that absence of red cell Duffy antigen accounts, at least in part, for higher prevalence and severity of asthma, associated with high total IgE concentration in people of African descent compared with those of European origin [185].

African American men have substantially higher levels of prostate cancer and associated mortality rates than Caucasian men. Some chemokines have angiogenic properties, and it has been proposed that DARC on red cells may reduce angiogenesis and consequently the progression of prostate cancer, by clearing angiogenic chemokines from the tumour [186]. A case-control study among Jamaican men of African origin, however, did not support any effect or red cell DARC expression on the risk or progression of prostate cancer [187].

DARC on vascular endothelium also interacts directly with a tetraspanin CD82 on cancer cells. CD82 is a suppresser of metastasis: interaction between Duffy and CD82 inhibits the spread of the cancer to remote sites and also appears to induce cancer cell senescence [188]. Duffy antigen expressed on breast cancer cells inhibits tumour growth and metastatic potential, through the clearance of angiogenic chemokines and inhibition of neovascularity

[189]. Similar effects were apparent with lung cancer cells [190].

References

1 Moore S, Woodrow CF, McClelland DBL. Isolation of membrane components associated with human red cell antigens Rh(D), (c), (E) and Fy^a. *Nature* 1982;295: 529–531.

2 Hadley TJ, David PH, McGinniss MH, Miller LH. Identification of an erythrocyte component carrying the Duffy blood group Fy^a antigen. *Science* 1984;223:597–599.

3 Tanner MJA, Anstee DJ, Mallinson G, *et al.* Effect of endoglycosidase F-Peptidyl *N*-glycosidase F preparations on the surface components of the human erythrocyte. *Carbohydrate Res* 1988;173:203–212.

4 Nichols ME, Rubinstein P, Barnwell J, Rodriguez de Cordoba S, Rosenfield RE. A new human Duffy blood group specificity defined by a murine monoclonal antibody. Imunogenetics and association with susceptability to *Plasmodium vivax*. *J Exp Med* 1987;166:776–785.

5 Riwom S, Janvier D, Navenot JM, *et al.* Production of a new murine monoclonal antibody with anti-Fy6 specificity and characterization of the immunopurified *N*-glycosylated Duffy-active molecule. *Vox Sang* 1994;66:61–67.

6 Chaudhuri A, Zbrzezna V, Johnson C, *et al.* Purification and characterization of an erythrocyte membrane protein complex carrying Duffy blood group antigenicity. Possible receptor for *Plasmodium vivax* and *Plasmodium knowlesi* malaria parasite. *J Biol Chem* 1989;264:13770–13774.

7 Chakera A, Seeber RM, John AE, Eidne KA, Greaves DR. The Duffy antigen/receptor for chemokines exists as an oligomeric form in living cells and functionally antagonizes CCR5 signaling through hetero-oligomerization. *Mol Pharmacol* 2008;73:1362–1370.

8 Wasniowska K, Eichenberger P, Kugele F, Hadley TJ. Purification of a 28 kD non-aggregating tryptic peptide of the Duffy blood group protein. *Biochem Biophys Res Comm* 1993;192:366–372.

9 Chaudhuri A, Polyakova J, Zbrzezna V, *et al.* Cloning of glycoprotein D cDNA, which encodes the major subunit of the Duffy blood group system and the receptor for the *Plasmodium vivax* malaria parasite. *Proc Natl Acad Sci USA* 1993;90:10793–10797.

10 Neote K, Mak JY, Kolakowski LF, Schall TJ. Functional and biochemical analysis of the cloned Duffy antigen: identity with the red blood cell chemokine receptor. *Blood* 1994; 84:44–52.

11 de Brevern AG, Wong H, Tournamille C, *et al.* A structural model of a seven-transmembrane helix receptor: The Duffy antigen/receptor for chemokine (DARC). *Biochem Biophys Acta* 2005;1724:288–306.

12 Smolarek D, Bertrand O, Czerwinski M, *et al.* Multiple interests in structural models of DARC transmembrane proteins. *Transfus Clin Biol* 2010;17:184–196.

13 Ji TH, Grossman M, Ji I. G protein-coupled receptors. I. Diversity of receptor-ligand interactions. *J Biol Chem* 1998;273:17299–17302.

14 Rot A, von Andrian. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. *Annu Rev Immunol* 2004;22:891–928.

15 Davenport RD. An introduction to chemokines and their roles in transfusion medicine. *Vox Sang* 2009;96:183–198.

16 Czerwinski M, Kern J, Grodecka M, *et al.* Mutational analysis of the *N*-glycosylation sites of Duffy antigen/receptor for chemokines. *Biochem Biophys Res Comm* 2007;356: 816–821.

17 Salomao M, Zhang X, Yang Y, *et al.* Protein 4.1R-dependent multiprotein complex: new insights into the structural organization of the red blood cell membrane. *Proc Natl Acad Sci USA* 2008;105:8026–8031.

18 Chaudhuri A, Polyakova J, Zbrzezna V, Pogo AO. The coding sequence of Duffy blood group gene in humans and simians: restriction fragment length polymorphism, antibody and malarial parasite specificities, and expression in nonerythroid tissues in Duffy-negative individuals. *Blood* 1995;85:615–621.

19 Iwamoto S, Omi T, Kajii E, Ikemoto S. Genomic organization of the glycoprotein D gene: Duffy blood group Fy^a/Fy^b alloantigen system is associated with a polymorphism at the 44-amino acid residue. *Blood* 1995;85:622–626.

20 Tournamille C, Colin Y, Cartron JP, Le Van Kim C. Disruption of a GATA motif in the *Duffy* gene promoter abolishes erythroid gene expression in Duffy-negative individuals. *Nature Genet* 1995;10:224–228.

21 Iwamoto S, Li J, Omi T, Ikemoto S, Kajii E. Identification of a novel exon and spliced form of Duffy mRNA that is the predominant transcript in both erythroid and postcapillary venule endothelium. *Blood* 1996;87:378–385.

22 Donahue RP, Bias WB, Renwick JH, McKusick VA. Probable assignment of the Duffy blood group locus to chromosome 1 in man. *Proc Natl Acad Sci USA* 1968;61:949–955.

23 Mathew S, Chaudhuri A, Murty VVVS, Pogo AO. Confirmation of Duffy blood group antigen locus (FY) at 1q22→q23 by fluorescence in situ hybridization. *Cytogenet Cell Genet* 1994;67:68.

24 Hadley TJ, Peiper SC. From malaria to chemokine receptor: the emerging physiologic role of the Duffy blood group antigen. *Blood* 1997;89:3077–3091.

25 Cutbush M, Mollison PL, Parkin DM. A new human blood group. *Nature* 1950;165:188–189.

26 Cutbush M, Mollison PL. The Duffy blood group system. *Heredity* 1950;4:383–389.

27 Ikin EW, Mourant AE, Pettenkofer HJ, Blumenthal G. Discovery of the expected hæmagglutinin, anti-Fy^b. *Nature* 1951;168:1077.

28 Chown B, Lewis M, Kaita H. The Duffy blood group system in Caucasians: evidence for a new allele. *Am J Hum Genet* 1965;17:384–389.

29 Lewis M, Kaita H, Chown B. The Duffy blood group system in Caucasians. A further population sample. *Vox Sang* 1972;23:523–527.

30 Mourant AE, Kopec AC, Domaniewska-Sobczak K. *Distribution of Human Blood Groups and Other Polymorphisms*, 2nd edn. London: Oxford University Press, 1976.

31 Shimizu Y, Hiroko A, Soemantri A, *et al.* Sero- and molecular typing of Duffy blood group in Southeast Asians and Oceanians. *Hum Biol* 2000;72:511–518.

32 Olsson ML, Hansson C, Avent ND, *et al.* A clinically applicable method for determining the three major alleles at the Duffy (*FY*) blood group locus using polymerase chain reaction with allele-specific primers. *Transfusion* 1998;38: 168–173.

33 Yan L, Fu Q, Jin L, Li L. Duffy blood group phenotypes and genotypes in Chinese. *Transfusion* 2001;41:970.

34 Hashmi G, Shariff T, Zhang Y, *et al.* Determination of 24 minor red cell antigens for more then 2000 blood donors by high-throughput DNA analysis. *Transfusion* 2007;47: 736–747.

35 Howes RE, Patil AP, Piel FB, *et al.* The global distribution of the Duffy blood group. *Nat Commun* 2011;2:266.

36 Tournamille C, Le Van Kim C, Gane P, Cartron J-P, Colin Y. Molecular basis and PCR-DNA typing of the Fy^a/fy^b blood group polymorphism. *Hum Genet* 1995;95:407–410.

37 Mallinson G, Soo KS, Schall TJ, Pisacka M, Anstee DJ. Mutations in the erythrocyte chemokine receptor (Duffy) gene: The molecular basis of the Fy^a/Fy^b antigens and identification of a deletion in the Duffy gene of an apparently healthy individual with the Fy(a–b–) phenotype. *Br J Haematol* 1995;90:823–829.

38 Yazdanbakhsh K, Rios M, Storry JR, *et al.* Molecular mechanisms that lead to reduced expression of Duffy antigens. *Transfusion* 2000;40:310–320.

39 Olsson ML, Smythe JS, Hansson C, *et al.* The Fy^x phenotype is associated with a missense mutation in the Fy^b allele predicting Arg89Cys in the Duffy glycoprotein. *Br J Haematol* 1998;103:1184–1191.

40 Schmid P, Ravenell KR, Sheldon SL, Flegel WA. *DARC* alleles and Duffy phenotypes in African Americans. *Transfusion* 2012;52:1260–1267.

41 Li J, Iwamoto S, Sugimoto N, Okuda H, Kajii E. Dinucleotide repeat in the 3′ flanking region provides a clue to the molecular evolution of the Duffy gene. *Hum Genet* 1997;99:573–577.

42 Cedergren B, Giles CM. An Fy^xFy^x individual found in Northern Sweden. *Vox Sang* 1973;24:264–266.

43 Buchanan DI, Sinclair M, Sanger R, Gavin J, Teesdale P. An Alberta Cree Indian with a rare Duffy antibody, anti-Fy3. *Vox Sang* 1976;30:114–121.

44 Habibi B, Fouillade MT, Levanra I, *et al.* Antigène Fy^x: etude quantitative chez les sujets Fy^bFy^x, Fy^aFy^x et Fy^xFy^x provenant de deux nouvelles familles. *Rev Franc Transfus Immuno-Hémat* 1977;20:427–438.

45 Parasol N, Reid M, Rios M, *et al.* A novel mutation in the coding sequence of the FY^*B allele of the Duffy chemokine receptor gene is associated with an altered erythrocyte phenotype. *Blood* 1998;92:2237–2243.

46 Habibi B, Perrier P, Salmon C. HD50 assay evaluation of the antigen Fy3 depression in Fy^x individuals. *J Immunogenet* 1980;7:191–193.

47 Tournamille C, Le Van Kim C, Gane P, *et al.* Arg89Cys substitution results in very low membrane expression of the Duffy antigen/receptor for chemokines in Fy^x individuals. *Blood* 1998;92:2147–2156. Erratum in *Blood* 2000;95:2753.

48 Gassner C, Kraus RL, Dovc T, *et al.* Fy^x is associated with two missense point mutations in its gene and can be detected by PCR-SSP. *Immunohematology* 2000;16: 61–67.

49 Tamasauskas D, Powell V, Saksela K, Yazdanbakhsh K. A homologous naturally occurring mutation in Duffy and CCR5 leading to reduced receptor expression. *Blood* 2001;97:3651–3654.

50 Castilho L, Rios M, Pellegrino Jr J, *et al.* A novel FY allele in Brazilians. *Vox Sang* 2004;87:190–195.

51 Moulds JM, Hayes S, Wells TD. DNA analysis of Duffy genes in American Blacks. *Transfusion* 1998;74:248–252.

52 Klein HG, Anstee DJ. *Mollison's Blood Transfusion in Clinical Medicine*, 11th edn. Oxford: Blackwell Publishing, 2005.

53 Schonewille H, van der Watering LMG, Loomans DSE, Brand A. Red blood cell alloantibodies after transfusion: factor influencing incidence and specificity. *Transfusion* 2006;46:250–256.

54 Takeshita A, Watanabe H, Fijihara H, *et al.* Collaborative study of irregular erythrocyte antibodies in Japan: results from the Japanese study group of allo-immunity and antigen diversity in Asian populations. *Transfus Apher Sci* 2010;43:3–8.

55 Issitt PD. Production of anti-Fy^a in Black Fy(a–b–) individuals. *Immunohematology* 1984;1:11–13.

56 Beattie KM. Letter. *Immunohematology* 1984;1:14.

57 Baldwin M, Shirey RS, Coyle K, Kickler TS, Ness PM. The incidence of anti-Fy^a and anti-Fy^b antibodies in Black and White patients. *Transfusion* 1986;26:546 [Abstract].

58 Sosler SD, Perkins JT, Fong K, Saporito C. The prevalence of immunization to Duffy antigens in a population of known racial distribution. *Transfusion* 1989;29:505–507.

59 Oberdorfer CE, Kahn B, Moore V, *et al.* A second example of anti-Fy3 in the Duffy blood group system. *Transfusion* 1974;14:608–611.

60 Molthan L, Crawford MN. Anti-Fy3. Second example in a Black. *Transfusion* 1978;18:386 [Abstract].

61 Kosinski KS, Molthan L, White L. Three examples of anti-Fy3 produced in Negroes. *Rev Franc Transfus Immuno-Hémat* 1984;27:619–624.

62 Vengelen-Tyler V. Anti-Fy^a preceding anti-Fy3 or -Fy5: a study of five cases. *Transfusion* 1985;25:482 [Abstract].

63 Rosenfield RE, Vogel P, Race RR. Un nouveau cas d'anti-Fy^a dans un sérum humain. *Rev Hémat* 1950;5:315–317.

64 Algora M, Barbolla L, Contreras M. Naturally occurring anti-D, anti-K, anti-Fy^a, and anti-Le^{ab}. *Vox Sang* 1991;61: 141.

65 Hardman JT, Beck ML. Hemagglutination in capillaries: correlation with blood group specificity and IgG subclass. *Transfusion* 1981;21:343–346.

66 Szymanski IO, Huff SR, Delsignore R. An autoanalyzer test to determine immunoglobulin class and IgG subclass of blood group antibodies. *Transfusion* 1982;22:90–95.

67 Chown B, Lewis M, Kaita H. Atypical Duffy inheritance in three Caucasian families: a possible relationship to Mongolism. *Am J Hum Genet* 1962;14:301–308, and *Am J Hum Genet* 1965;17:188.

68 Race RR, Sanger R, Lehane D. Quantitative aspects of the blood-group antigen Fy^a. *Ann Eugen* 1953;17:255–266.

69 Poole J, Daniels G. Blood group antibodies and their significance in transfusion medicine. *Transfus Med Rev* 2007;21:58–71.

70 Freiesleben E. Fatal hemolytic transfusion reaction due to anti-Fy^a ('Duffy'). *Acta Path Microbiol Scand* 1951;29: 283–286.

71 Badakere SS, Bhatia HM. A fatal transfusion reaction due to anti-Duffy (Fy^a). Case report. *Indian J Med Sci* 1970;24: 562–564.

72 Mollison PL, Cutbush M. Use of isotope-labelled red cells to demonstrate incompatibility *in vivo*. *Lancet* 1955;i: 1290–1295.

73 Gover PA, Morton JR. Transfusion reaction due to anti-Fy^a in donor blood. *Clin Lab Haematol* 1990;12:233–236.

74 Goodrick MJ, Hadley AG, Poole G. Haemolytic disease of the fetus and newborn due to anti-Fy^a and the potential clinical value of Duffy genotyping in pregnancies at risk. *Transfusion Med* 1997;7:301–304.

75 Branch DR, Scofield TL, Moulds JJ, Swanson JL. Unexpected suppression of anti-Fy^a and prevention of hemolytic disease of the fetus and newborn after administration of Rh immune globulin. *Transfusion* 2011;51:816–819.

76 Noizat-Pirenne F, Tournamille C, Bierling P, *et al*. Relative immunogenicity of Fy^a and K antigens in a Caucasian population, based on HLA class II restriction analysis. *Transfusion* 2006;46:1328–1333.

77 Picard C, Frassati C, Basire A, *et al*. Positive association of *DRB1*04* and *DRB1*15* alleles with Fy^a immunization in a Southern European population. *Transfusion* 2009;49: 2412–2417.

78 Lerut E, Van Damme B, Noizat-Pirenne F, *et al*. Duffy and Kidd blood group antigens: minor histocompatibility antigens involved in renal allograft rejection? *Transfusion* 2007;47:28–40.

79 Contreras M, Gordon H, Tidmarsh E. A proven case of maternal alloimmunization due to Duffy antigens in donor blood used for intrauterine transfusion. *Br J Haematol* 1983;53:355–356.

80 Michalewski B. Naturally occurring anti-Fy^b + C^w. *Vox Sang* 2001;80:235.

81 Badakere SS, Bhatia HM, Sharma RS, Bharucha Z. Anti-Fy^b (Duffy) as a cause of transfusion reaction. Case report. *Indian J Med Sci* 1970;24:565–567.

82 Kosanke J, Dickstein B, Chambers LA, Ng A, Wissel ME. Fatal delayed hemolytic transfusion reaction due to anti-Fy^b. *Transfusion* 2004;14(Suppl.):120A [Abstract].

83 Kim HH, Park TS, Oh SH, *et al.* Delayed hemolytic transfusion reaction due to anti-Fy^b caused by a primary immune response: a case study and a review of the literature. *Immunohematology* 2004;20:184–186.

84 Carreras Vescio LA, Fariña D, Rogido M, Sóla A. Hemolytic disease of the newborn caused by anti-Fy^b. *Transfusion* 1987;27:366.

85 van't Veer MB, van Leeuwen I, Haas FJLM, *et al.* Red-cell auto-antibodies mimicking anti-Fy^b specificity. *Vox Sang* 1984;47:88–91.

86 Harris T. Two cases of autoantibodies that demonstrate mimicking specificity in the Duffy blood group system. *Immunohematology* 1990;6:87–91.

87 Daniels G. Section 4: antibodies to other blood group antigens. Coordinator's report. *Transfus Clin Biol* 2002;9: 75–80.

88 Halverson GR, Chaudhuri A, Huang T, Yazdanbakhsh K, Reid ME. Immunization of transgenic mice for production of MoAbs directed at polymorphic blood group antigens. *Transfusion* 2001;41:1393–1396.

89 Sanger R, Race RR, Jack J. The Duffy blood groups of New York Negroes: the phenotype Fy(a–b–). *Br J Haematol* 1955;1:370–374.

90 Race RR, Sanger R. *Blood Groups in Man*, 6th edn. Oxford: Blackwell Scientific Publications, 1975.

91 Welch SG, McGregor IA, Williams K. The Duffy blood group and malaria prevalence in Gambian West Africans. *Trans R Soc Trop Med Hyg* 1977;71:295–296.

92 Peiper SC, Wang Z, Neote K, *et al.* The Duffy antigen/receptor for chemokines (DARC) is expressed in endothelial cells of Duffy-negative individuals who lack the erythrocyte receptor. *J Exp Med* 1995;181:1311–1317.

93 Iwamoto S, Li J, Sugimoto N, Okuda H, Kajii, E. Characterization of the Duffy gene promoter: evidence for tissue-specific abolishment of expression in Fy(a–b–) of black individuals. *Biochem Biophys Res Comm* 1996;222:852–859.

94 Chaudhuri A, Yuen G, Fang F, Storry J. Development of Duffy transgenic mouse: in vivo expression of human Duffy gene with –33T→C promoter mutation in non-erythroid tissues. *Br J Haematol* 2004;127:356–359.

95 Zimmerman PA, Woolley I, Masinde GL, *et al*. Emergence of *FY*A*^{null} in a *Plasmodium vivax*-endemic region of Papua New Guinea. *Proc Natl Acad Sci USA* 1999;96:13973–13977.
96 Langhi DM Jr, Bordin JO. Duffy blood groups and malaria. *Hematology* 2006;11:389–398.
97 Albrey JA, Vincent EER, Hutchinson J, *et al*. A new antibody, anti-Fy3, in the Duffy blood group system. *Vox Sang* 1971;20:29–35.
98 Rios M, Chaudhuri A, Mallinson G, *et al*. New genotypes in Fy(a–b–) individuals: nonsense mutations (Trp to stop) in the coding sequence of either *FY A* or *FY B*. *Br J Haematol* 2000;108:448–454.
99 Tsuneyama H, Uchikawa M, Shinozaki K, *et al.* A deletion in the Duffy gene of an apparently healthy individual with the Fy(a–b–) phenotype. *Transfusion* 2000;40(Suppl.):116S [Abstract].
100 Mannessier L, Habibi B, Salmon C. Un nouvel exemple anti-Fy3 comportant une réactivité pseudo-anti-Fy^a. *Rev Franc Transfus Immuno-Hémat* 1979;22:195–198.
101 Colledge KI, Pezzulich M, Marsh WL. Anti-Fy5, an antibody disclosing a probable association between the Rhesus and Duffy blood group genes. *Vox Sang* 1973;24:193–199.
102 Oakes J, Taylor D, Johnson C, Marsh WL. Fy3 antigenicity of blood of newborns. *Transfusion* 1978;18:127–128.
103 Jensen N, Crosson J, Grotte D, Anderson D. Severe hemolytic reaction due to anti-Fy3 following partial red cell exchange for sickle cell disease (SCD). *Transfusion* 1988;28(Suppl.):8S [Abstract].
104 Castilho L, Credidio DC, Ribeiro K, *et al.* Anti-Fy3 in sickle cell disease patients genotyped as *FY*B–33/FY*B–33*. *Transfusion* 2009;49(Suppl.):35A [Abstract].
105 Le Pennec PY, Rouger P, Klein MT, Robert N, Salmon C. Study of anti-Fy^a in five black Fy(a–b–) patients. *Vox Sang* 1987;52:246–249.
106 Olteneau H, Gerber D, Partridge K, Sarode R. Acute hemolytic transfusion reaction secondary to anti-Fy3. *Immunohematology* 2005;21:48–52.
107 Reyes MA, Illoh OC. Hyperhemolytic transfusion reaction attributable to anti-Fy3 in a patient with sickle cell disease. *Immunohematology* 208;24:45–51.
108 Wasniowska K, Lisowska E, Halverson GR, Chaudhuri A, Reid ME. The Fy^a, Fy6 and Fy3 epitopes of the Duffy blood group system recognized by new monoclonal antibodies: identification of a linear Fy3 epitope. *Br J Haematol* 2004;124:118–122.
109 Tournamille C, Filipe A, Wasniowska K, *et al*. Structure-function analysis of the extracellular domains of the Duffy antigen/receptor for chemokines: characterization of antibody and chemokine binding sites. *Br J Haematol* 2003;122:1014–1023.
110 Lu Z, Wang Z, Horuk R, *et al*. The promiscuous chemokine binding profile of the Duffy antigen/receptor for chemokines is primarily localized to sequences in the amino-terminal domain. *J Biol Chem* 1995;270:26239–26245.
111 DiNapoli J, Garcia A, Marsh WL, Dreizin D. A second example of anti-Fy5. *Vox Sang* 1976;30:308–311.
112 Chan-Shu SA. The second example of anti-Duffy^5. *Transfusion* 1980;20:358–360.
113 Bowen DT, Devenish A, Dalton J, Hewitt PE. Delayed haemolytic transfusion reaction due to simultaneous appearance of anti-Fy^a and anti-Fy5. *Vox Sang* 1988;55:35–36.
114 Meredith LC. Anti-Fy5 does not react with e variants. *Transfusion* 1985;25:482 [Abstract].
115 Wasniowska K, Blanchard D, Janvier D, *et al*. Identification of the Fy6 epitope recognized by two monoclonal antibodies in the N-terminal extracellular portion of the Duffy antigen receptor for chemokines. *Mol Immunol* 1996;33:917–923.
116 Wasniowska K, Petit-LeRoux Y, Tournamille C, *et al*. Structural characterization of the epitope recognized by the new anti-Fy6 monoclonal antibody NaM185-2C3. *Transfus Med* 2002;12:205–211.
117 Smolarek D, Hattab C, Hassanzadeh-Ghassabeh G, *et al.* A recombinant dromedary antibody fragment (VHH or nanobody) directed against human Duffy antigen receptor for chemokines. *Cell Mol Life Sci* 2010;67:3371–3387.
118 Behzad O, Lee CL, Gavin J, Marsh WL. A new anti-erythrocyte antibody in the Duffy system: anti-Fy4. *Vox Sang* 1973;24:337–342.
119 Mullighan CG, Marshall SE, Fanning GC, Briggs DC, Welsh KI. Rapid haplotyping of mutations in the Duffy gene using the polymerase chain reaction and sequence-specific primers. *Tissue Antigens* 1998;51:195–199.
120 Ansart-Pirenne H, Martin-Blanc S, Le Pennec P-Y, *et al.* *FY*X* real-time polymerase chain reaction with melting curve analysis associated with a complete one-step real-time *FY* genotyping. *Vox Sang* 2007;92:142–147.
121 Castilho L. The value of DNA analysis for antigens in the Duffy blood group system. *Transfusion* 2007;47(Suppl.):28S–31S.
122 Masouredis SP, Sudora E, Mahan L, Victoria EJ. Quantitative immunoferritin microscopy of Fy^a, Fy^b, Jk^a, U, and Di^b antigen site numbers on human red cells. *Blood* 1980;56:969–977.
123 Toivanen P, Hirvonen T. Fetal development of red cell antigens K, k, Lu^a, Lu^b, Fy^a, Fy^b, Vel and Xg^a. *Scand J Haematol* 1969;6:49–55.
124 Toivanen P, Hirvonen T. Antigens Duffy, Kell, Kidd, Lutheran and Xg^a on fetal red cells. *Vox Sang* 1973;24:372–376.
125 Woolley IJ, Hotmire KA, Sramkoski RM, Zimmerman PA, Kazura JW. Differential expression of the Duffy antigen receptor for chemokines according to RBC age and *FY* genotype. *Transfusion* 2000;40:949–953.

126 Hadley TJ, Lu Z, Wasniowska K, *et al*. Postcapillary venule endothelial cells in kidney express a multispecific chemokine receptor that is structurally and functionally identical to the erythroid isoform, which is the Duffy blood group antigen. *J Clin Invest* 1994;94:985–991.
127 Chaudhuri A, Nielsen S, Elkjaer M-L, *et al.* Detection of Duffy antigen in the plasma membranes and caveolae of vascular endothelial and epithelial cells of nonerythroid organs. *Blood* 1997;89:701–712.
128 Horuk R, Martin AW, Wang Z, *et al*. Expression of chemokine receptors by subsets of neurons in the central nervous system. *J Immunol* 1997;158:2882–2890.
129 Dunstan RA, Simpson MB, Rosse WF. Erythrocyte antigens on human platelets. Absence of Rh, Duffy, Kell, Kidd, and Lutheran antigens. *Transfusion* 1984;24:243–246.
130 Dunstan RA. Status of major red cell blood group antigens on neutrophils, lymphocytes and monocytes. *Br J Haematol* 1986;62:301–309.
131 Raman D, Sobolik-Delmaire T, Richmond A. Chemokines in health and disease. *Exp Cell Res* 2011;317: 575–589.
132 Gardner L, Patterson AM, Ashton BA, Stone MA, Middleton J. The human Duffy antigen binds selected inflammatory but not homeostatic chemokines. *Biochem Biophys Res Comm* 2004;321:306–312.
133 Pruenster M, Rot A. Throwing light on DARC. *Biochem Soc Trans* 2006;34:1005–1008.
134 Horuk R, Chitnis CE, Darbonne WC, *et al*. A receptor for the malarial parasite *Plasmodium vivax*: the erythrocyte chemokine receptor. *Science* 1993;261:1182–1184.
135 Tournamille C, Le Van Kim C, Gane P, *et al*. Close association of the first and fourth extracellular domains of the Duffy antigen/receptor for chemokines by a disulphide bond is required for ligand binding. *J Biol Chem* 1997;272:16274–16280.
136 Chaudhuri A, Zbrzezna V, Polyakova J, *et al.* Expression of the Duffy antigen in K562 cells. Evidence that it is the human erythrocyte chemokine receptor. *J Biol Chem* 1994;269:7835–7838.
137 Hausman E, Dzik W, Blanchard D. The red cell chemokine receptor is distinct from the Fy6 epitope. *Transfusion* 1996;36:4221–425.
138 Wasniowska K, Czerwinski M, Jachymek W, Lisowska E. Expression and binding properties of a soluble chimeric protein containing the N-terminal domain of the Duffy antigen. *Biochem Biophys Res Comm* 2000;273:705–711.
139 Schnabel RB, Baumert J, Barbalic M, *et al*. Duffy antigen receptor for chemokines (Darc) polymorphism regulates circulating concentrations of monocyte chemoattractant protein-1 and other inflammatory mediators. *Blood* 2010;115:5289–5299.
140 Darbonne WC, Rice GC, Mohler MA, *et al*. Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. *J Clin Invest* 1991;88:1362–1369.
141 Mayr FB, Spiel AO, Leitner JM, *et al*. Duffy antigen modifies the chemokine response in human endotoxemia. *Crit Care Med* 2008;36:159–165.
142 Jilma-Stohlawetz P, Homoncik M, Drucker C, *et al*. Fy phenotype and gender determine plasma levels of monocyte chemotactic protein. *Transfusion* 2001;41:378–381.
143 Fukuma N, Akimitsu N, Hamamoto H, *et al.* A role of the Duffy antigen for the maintenance of plasma chemokine concentrations. *Biochem Biophys Res Comm* 2003;303: 137–139.
144 Afenyi-Annan A, Kail M, Combs MR, *et al.* Lack of Duffy antigen expression is associated with organ damage in patients with sickle cell disease. *Transfusion* 2008;48: 917–924.
145 Grann VR, Ziv E, Joseph CK, *et al.* Duffy (Fy), DARC, and neutopenia among women from the United States, Europe and the Caribbean. *Br J Haematol* 2008;143:288–293.
146 Reich D, Nalls MA, Kao WHL, *et al*. Reduced neutrophil count in people of African descent is due to a regulatory variant in the Duffy antigen receptor for chemokines gene. *PLoS Gen* 2009;5(1).
147 Lee JS, Wurfel MM, Matute-Bello G, *et al*. The Duffy antigen modifies systemic and local tissue chemokine responses following lipopolysaccharide stimulation. *J Immunol* 2006;177:8086–8094.
148 Pruenster M, Mudde L, Bombosi P, *et al*. The Duffy antigen receptor for chemokines transports chemokines and supports their promigratory activity. *Nat Immunol* 2009;10: 101–108.
149 Baird JK. Resistance to therapies for infection by *Plasmodium vivax*. *Clin Microbiol Rev* 2009;22:508–534.
150 Miller LH, Mason SJ, Dvorak JA, McGinniss MH, Rothman IK. Erythrocyte receptors for (*Plasmodium knowlesi*) malaria: Duffy blood group determinants. *Science* 1975; 189:561–563.
151 Miller LH, Mason SJ, Clyde DF, McGinniss MH. The resistance factor to *Plasmodium vivax* in Blacks. The Duffy blood group genotype, *FyFy*. *New Engl J Med* 1976;295: 302–304.
152 Barnwell JW, Nichols ME, Rubinstein P. *In vitro* evaluation of the role of the Duffy blood group in erythrocyte invasion by *Plasmodium vivax*. *J Exp Med* 1989;169:1795–1802.
153 Miller LH, Haynes JD, McAuliffe FM, *et al.* Evidence for differences in erythrocyte surface receptors for the malarial parasites, *Plasmodium falciparum* and *Plasmodium knowlesi*. *J Exp Med* 1977;146:277–281.
154 Mason SJ, Miller LH, Shiroishi T, Dvorak JA, McGinniss MH. The Duffy blood group determinants: their role in the susceptibility of human and animal erythrocytes to *Plasmodium knowlesi* malaria. *Br J Haematol* 1977;36:327–335.
155 Miller LH, Aikawa M, Johnson JG, Shiroishi T. Interaction between cytochalasin B-treated malarial parasites and erythrocytes. Attachment and junction formation. *J Exp Med* 1979;149:172–184.

156 Hadley TJ, Miller LH, Haynes JD. Recognition of red cells by malaria parasites: the role of erythrocyte-binding proteins. *Transfus Med Rev* 1991;5:108–122.
157 Miller LH, McAuliffe FM, Mason SJ. Erythrocyte receptors for malaria merozoites. *Am J Trop Med Hyg* 1977;26(6): 204–208.
158 Palatnik M, Rowe AW. Duffy and Duffy-related human antigens in primates. *J Hum Evol* 1984;13:173–179.
159 Haynes JD, Dalton JP, Klotz FW, *et al*. Receptor-like specificity of a *Plasmodium knowlesi* malarial protein that binds to Duffy antigen ligands on erythrocytes. *J Exp Med* 1988;167:1873–1881.
160 Wertheimer SP, Barnwell JW. *Plasmodium vivax* interation with the human Duffy blood group glycoprotein: identification of a parasite receptor-like protein. *Exp Parasitol* 1989;69:340–350.
161 Adams JH, Kim Lee Sim B, Dolan SA, *et al.* A family of erythrocyte binding proteins of malaria parasites. *Proc Natl Acad Sci USA* 1992;89:7085–7089.
162 Chitnis CE, Miller LH. Identification of the erythrocyte binding domains of *Plasmodium vivax* and *Plasmodium knowlesi* proteins involved in erythrocyte invasion. *J Exp Med* 1994;180:497–506.
163 Chitnis CE, Chaudhuri A, Horuk R, Pogo AO, Miller LH. The domain of the Duffy blood group antigen for binding *Plasmodium vivax* and *P. knowlesi* malarial parasites to erythrocytes. *J Exp Med* 1996;184:1531–1536.
164 Tournamille C, Filipe A, Badaut C, *et al.* Fine mapping of the Duffy binding antigen site for the *Plasmodium vivax* Duffy-binding protein. *Mol Biochem Parasitol* 2005;144: 100–103.
165 Choe H, Moor MJ, Owens CM, *et al*. Sulphated tyrosines mediate association of chemokines and *Plasmodium vivax* Duffy binding protein with the Duff antigen/receptor for chemokines (DARC). *Mol Microbiol* 2005;55:1413–1422.
166 Arévalo-Herrera M, Chitnis C, Herrera S. Current status of *Plasmodium vivax* vaccine. *Hum Vaccines* 2010;6:1–9.
167 Albuquerque SRL, Cavalcante FdeO, Sanguino EC, *et al*. *FY* polymorphisms and vivax malaria in inhabitants of Amazonas State, Brazil. *Parasitol Res* 2010;106:1049–1053.
168 Michon P, Woolley I, Wood EM, *et al*. Duffy-null promoter heterozygosity reduces DARC expression and abrogates adhesion of the *P. vivax* ligand required for blood-stage infection. *FEBS Letts* 2001;495:111–114.
169 King CL, Adams JH, Xianli J, *et al*. Fy^a/Fy^b antigen polymorphism in human erythrocyte Duffy antigen affects susceptibility to *Plasmodium vivax* malaria. *Proc Natl Acad Sci USA* 2011;108:20113–20118.
170 Cavasini CE, de Mattos LC, Couto AAD'A, *et al*. *Plasmodium vivax* infection among Duffy antigen-negative individuals from the Brazilian Amazon region: an exception? *Trans R Soc Trop Med Hyg* 2007;101:1042–1044.
171 Ryan JR, Stoute JA, Amon J, *et al*. Evidence for transmission of *Plasmodium vivax* among a Duffy antigen negative population in Western Kenya. *Am J Med Hyg* 2006;75: 575–581.
172 Wurtz N, Lekweiry KM, Bogreau H, *et al*. Vivax malaria in Mauritania includes infection of a Duffy-negative individual. *Malaria J* 2011;10:e336.
173 Mendes C, Dias F, Figueiredo J, *et al*. Duffy negative antigen is no longer a barrier to *Plasmodium vivax* – molecular evidences from the African west coast (Angola and Equatorial Guinea). *PLoS Negl Trop Dis* 2011;5:e1192.
174 Ménard D, Barnadas C, Bouchier C, *et al*. *Plasmodium vivax* clinical malaria is commonly observed in Duffy-negative Malagasy people. *Proc Natl Acad Sci USA* 2010;107: 5967–5971.
175 He W, Neil S, Kulkarni H, *et al*. Duffy antigen receptor for chemokines mediates *trans*-infection of HIV-1 from red blood cells to target cells and affects HIV-AIDS susceptibility. *Cell Host Microbe* 2008;4:52–62.
176 Walley NM, Juig B, Dickson SP, *et al*. The Duffy antigen receptor for chemokines null promoter variant does not influence HIV-1 acquisition or disease progression. *Cell Host Microbe* 2009;5:408–410.
177 Ramsuran V, Kulkarni H, He W, *et al*. Duffy-null-associated low neutrophil counts influence HIV-1 susceptibility in high-risk South African Black women. *Clin Infect Dis* 2011;52:1248–1256.
178 Kulkarni H, Marconi VC, He W, *et al*. The Duffy-null state is associated with a survival advantage in leukopenic HIV-infected persons of African ancestry. *Blood* 2009;114: 2783–2792.
179 Nebor D, Durpès MC, Mougenel D, *et al*. Association between Duffy antigen receptor for chemokines expressions and levels of inflammation marker in sickle cell anemia patients. *Clin Immunol* 2010;136:116–122.
180 Durpès MC, Hardy-Dessources M-D, El Nemer W, *et al*. Activation state of $\alpha 4\beta 1$ integrin on sickle red blood cells is linked to the Duffy antigen receptor for chemokines (DARC) expression. *J Biol Chem* 2011;286: 3057–3064.
181 Anstee DJ. The relationship between blood groups and disease. *Blood* 2010;115:4635–4643.
182 Akalin E, Neylan JF. The influence of Duffy blood group on renal allograft outcome in African Americans. *Transplantation* 2003;75:1496–1500.
183 Mangalmurti NS, Xiong Z, Hulver M, *et al*. Loss of red cell scavenging promotes transfusion-related lung inflammation. *Blood* 2009;113:1158–1166.
184 Oreskovic RT, Dumaswala UJ, Greenwalt TJ. Expression of blood group antigens on red cell microvesicles. *Transfusion* 1992;32:848–849.
185 Vergara C, Tsai YJ, Grant AV, *et al*. Gene encoding Duffy antigen/receptor for chemokines is associated with asthma and IgE in three populations. *Am J Respir Crit Care Med* 2008;178:1017–1022.

186 Shen H, Schuster R, Stringer KF, Walthz SE, Lentsch AB. The Duffy antigen/receptor for chemokines (DARC) regulates prostate tumor growth. *FASEB J* 2006;20:59–64.

187 Elson JK, Beebe-Dimmer JL, Morgenstern H, *et al.* The Duffy antigen/receptor for chemokines (DARC) and prostate cancer risk among Jamaican men. *J Immigr Minor Health* 2011;13:36–41.

188 Bandyopadhyay S, Zhan R, Chaudhuri A, *et al*. Interaction of KAI1 on tumor cells with DARC on vascular endothelium leads to metastasis suppression. *Nature Med* 2006; 12:933–938.

189 Wang J, Ou Z-L, Hou Y-F, *et al*. Enhanced expression of Duffy antigen receptor for chemokines by breast cancer cells attenuates growth and metastasis potential. *Oncogene* 2006;25:7201–7211.

190 Addison CL, Belperio JA, Burdick MD, Strieter RM. Overexpression of the Duffy antigen receptor for chemokines (DARC) by NSCLC tumor cells results in increased tumor necrosis. *BMC Cancer* 2004;4:28.

191 Kempinska-Podhorodecka A, Knap O, Drozd A, *et al*. Analysis for genotyping Duffy blood group in inhabitants of Sudan, the fourth cataract of the Nile. *Malaria J* 2012;11:115.