11 Yt Blood Group System

11.1 Introduction

Anti-Yt^a, an antibody detecting an antigen of high frequency, was first found through crossmatching in 1956 [1]. The antithetical antibody, anti-Yt^b, detects an antigen on red cells of about 8% of white people and was found eight years later [2]. Yt, also known as the Cartwright system, remains a two-allele system (Table 11.1); an inherited Yt(a–b–) phenotype has not been found.

Yt^a and Yt^b represent His353Asn in acetylcholinesterase (AChE) on red cells. *YT* (*ACHE*) is located on chromosome 7q22.

11.2 Yt antigens and red cell acetylcholinesterase

Yt^a and Yt^b are on the GPI-linked red cell glycoprotein, acetylcholinesterase (AChE). Immunoprecipitation with anti-Yt^a and -Yt^b isolated structures of apparent MW 72 kDa under reducing conditions and 160 kDa under non-reducing conditions; structures of identical electrophoretic mobility were obtained by immunoprecipitation with monoclonal antibodies to AChE [3]. The structures precipitated by anti-Yt^a and -Yt^b had AChE enzyme activity. Further proof that Yt antigens are on AChE was provided by a MAIEA assay with alloanti-Yt^a and -Yt^b, and with monoclonal anti-AChE [4].

Estimates of 3000–5000 sites per red cell were obtained in quantitative analyses with monoclonal IgG anti-AChE and estimates of 7000–10 000 sites were obtained with Fab fragments, suggesting that the enzyme exists in dimeric form in the red cell membrane [3].

The complement-sensitive population of red cells (PNHIII) from patients with paroxysmal nocturnal haemoglobinuria (PNH) are deficient in several glycoproteins, including AChE, that are anchored to the membrane by means of glycosylphosphatidylinositol (GPI) (see Chapter 19). Most anti-Yt^a were non-reactive with PNHIII cells, but reacted with the relatively normal, complement-insensitive population of cells (PNHI) from the same patient [5]. Furthermore, in two PNH patients, the PNHI cells were Yt(b+), yet their PNHIII cells failed to react with anti-Yt^b.

AChE plays an essential part in neurotransmission. Acetylcholine is a neurotransmitter that permits the transmission of an electrical signal when released from a nerve terminal at a neuromuscular junction. AChE rapidly hydrolyses acetylcholine to terminate the signal. AChE exists in different tissues in a variety of forms, a result of post-translational modification and alternative splicing [6,7]. The function of red cell membrane-bound AChE is not known.

Partial human *ACHE* cDNA clones were obtained by screening a cDNA library constructed from fetal muscle and adult brain RNA with an oligonucleotide complementary to the amino acid sequence of a peptide derived from *Torpedo* AChE [8]. The complete gene encoding human AChE was cloned by screening a human genomic cosmid library with an oligonucleotide probe prepared by PCR amplification of human DNA with primers corresponding to a conserved region of mouse AChE gene [7]. Three exons encode the signal peptide and N-terminal 535 amino acids; alternative splicing of the next exon results in structural divergence of the C-terminal domain so that a GPI-anchor may be attached in erythroid cells, but not in nervous tissue [7].

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ACHE was assigned to chromosome 7q22 by *in situ* hybridisation and by PCR-based somatic cell hybridisation techniques [9,10]. An analysis of 31 families informative for segregation of *YT* and *KEL* revealed loose linkage between these loci with maximum likelihood of a recombination fraction of 0.26 [11].

11.3 Yt^a and Yt^b

11.3.1 Frequency, inheritance, and molecular basis of the Yt antigens

Some population studies are shown in Table 11.2. Around 1 in 500 Caucasians are Yt(a–) [1,12,15]. The phenotype is less common in African Americans, with about 1 in 2000 Yt(a–) [15]. About 8–10% of Caucasians are Yt(b+) [12,14]. Genotype frequencies of *YT*A/A* 0.8966, *YT*A/B* 0.1006, and *YT*B/B* 0.0028 calculated from the results of tests with anti-Yt^a and -Yt^b on 659 white Canadians correlated closely enough with the observed figures to suggest that no third allele is present [14]. Tests with anti-Yt^a and -Yt^b on Israeli Arabs and Druse and on a variety of populations of Israeli Jews revealed a relatively high frequency of Yt^b [17]. None of 5000 Japanese was Yt(a–) and Yt^b has not been detected in relatively small samples of Japanese, Inuits, Thais, and Native Americans [18,19]. The results of two large series of family studies are consistent with simple Mendelian inheritance of *YT*A* and *YT*B* in a co-dominant fashion, and absence of a silent allele [12,14].

Two single nucleotide changes in *ACHE* are associated with Yt^a/Yt^b polymorphism. One, 1057C>A in exon 2, encodes His353Asn; the other, 1432C>T, is a silent mutation in exon 3 in the codon for Pro477 [20]. The His353Asn substitution has no effect on the catalytic activity of the enzyme [21].

Table 11.1 Antigens of the Yt blood group system.

No.	Name	Relative frequency	Molecular basis
YT1	Yt^a	High	1057C His353
YT2	Yt^b	Low	1057A Asn353

11.3.2 Effects of enzymes and reducing agents

Yt^a is not affected by trypsin, but is destroyed by α-chymotrypsin treatment of the red cells; papain and ficin may also destroy the antigen, but this appears to depend on the anti-Yt^a used. Yt^a is not sialidase-sensitive.

Yt^a and Yt^b are sensitive to disulphide bond reducing agents. Eight anti-Yt^a were non-reactive with red cells treated with 200 m*M* dithiothreitol (DTT) [22]. Nine of

Table 11.2 Population studies with anti-Yt^a and -Yt^b.

		Phenotype frequencies		Gene frequencies		
Population	No. tested	Yt(a+)	Yt(b+)	*YT*A*	*YT*B*	References
English	2568	0.998	nt	0.9559	0.0441	[1,12]
South Welsh	29 802	0.999	nt	0.9761	0.0239	[13]
European	1399	nt	0.081	0.9587	0.0413	[12]
White Canadians	659	1.000	0.106	0.9469	0.0531	[14]
Hispanic Americans	9933	0.999	nt	0.9638	0.0362	[15]
African Americans	10 622	0.999	nt	0.9783	0.0217	[15]
African Americans	714	nt	0.084	0.9571	0.0429	[16]
Israeli Jews	2549	0.986	0.213	0.8845	0.1154	[17]
Israeli Arabs	85	0.976	0.235	0.8706	0.1294	[17]
Israeli Druse	77	0.974	0.260	0.8571	0.1429	[17]
Japanese	5000	1.000	nt			
	70*		0	1.0000	0.0000	[18]

*These 70 Japanese donors are also included in the 5000 tested with anti-Yt^b.
nt, not tested.

15 anti-Yt^a did not react with cells treated with 6% 2-aminoethylisothiouronium bromide (AET) and the other six sera showed reduced reactivity [23]. Yt^b, determined by two anti-Yt^b, was destroyed by 200 m*M* DTT and 500 m*M* 2-mercaptoethanol [24].

11.3.3 Development and distribution of Yt^a and Yt^b

Yt antigens are present on red cells from cord blood samples. Yt^b appears to be fully developed at birth [25], but the strength of Yt^a on cord cells is weaker than that on red cells of adults [1,25,26]. Of 10 fetal red cell samples, taken at less than 32 weeks' gestation, eight did not react with anti-Yt^a and the other two reacted only very weakly [26].

AChE is present in nervous tissue and on erythroid cells [6], but little is known about the tissue distribution of Yt antigens. Yt^a was not detected by flow cytometry on lymphocytes, granulocytes, or monocytes [27].

11.4 Anti-Yt^a and -Yt^b

Despite the relatively low incidence of Yt(a–b+) phenotype, numerous examples of anti-Yt^a have been identified. Of 79 sera containing anti-Yt^a, 57 were monospecific and 22 contained a mixture of antibodies [28]. Yt^b appears to be a poor antigenic stimulus as anti-Yt^b is rare and generally found in antibody mixtures; only a few examples are reported [2,12,25,29–31]. Anti-Yt^a and -Yt^b have been stimulated by pregnancy or transfusion; neither has been 'naturally occurring'.

Yt antibodies are mostly IgG and require an antiglobulin test to agglutinate red cells. Of those anti-Yt^a sera that could be subclassed, most contained IgG1, some IgG1 plus IgG4, and a few IgG4 alone; none contained IgG3 [32–35]. Some anti-Yt^a bind complement [36], others do not [26,37].

Yt antibodies have not caused HDFN, despite several cases of women with anti-Yt^a having Yt(a+) children, and one case of a woman with anti-Yt^b having a Yt(b+) child [25]. Anti-Yt^a is alleged to have been responsible for a fatal delayed HTR in a patient with sickle cell disease [38] and has been implicated in an immediate HTR [39]. Many patients with anti-Yt^a, however, have received multiple transfusions of Yt(a+) red cells with no ill effects [28,33,35,40]. Of 18 patients with anti-Yt^a who received Yt(a+) red cells, only three showed evidence of decreased red cell survival [28]. Survival studies with radiolabelled antigen-positive red cells have given widely variable results with Yt antibodies [26,33,35–37,40–42]; in a few cases it was predicted that incompatible red cells would be removed rapidly from the circulation [36,36,37]. Cellular assays have also given variable results [26,28, 31,32,35,39,42,43]. Of 73 anti-Yt^a tested by a monocyte monolayer assay, 47 gave a positive result (>5% reactivity), 19 of those giving reactivity scores over 20%. Both anti-Yt^b tested gave scores >5%, and one of them was >20% [35]. One example of anti-Yt^a appeared benign before transfusion of incompatible blood, but subsequently *in vivo* and *in vitro* assays gave indications of haemolytic potential [43].

For transfusion purposes, each example of anti-Yt^a must be assessed independently. For patients with anti-Yt^a least incompatible blood should usually be selected for transfusion, but Yt(a–) red cells should be selected for patients with strong examples of the antibody.

An apparent alloanti-Yt^a in a Yt(a+) patient led to speculation of an inherited Yt^a variant [44]. The antibody did not react with the patient's own cells or with those of his Yt(a+) father, but did react with his mother's red cells. The antibody appeared 9 days after transfusion of 5 units of blood and disappeared after a few months.

11.5 Transient Yt(a–b–) phenotype, anti-Yt^{ab}, and red cell AChE deficiency

In view of the vital role of AChE in neurotransmission, it is not surprising that no inherited null phenotype resulting from deletion or inactivating mutation of *ACHE* has been found. A cardiac transplant candidate appeared to have the Yt(a–b–) phenotype, but some anti-Yt^a could be adsorbed and eluted from his cells [45]. His serum contained an antibody, anti-Yt^{ab}, that did not react with his own cells or with PNHIII cells, which lack GPI-linked proteins. Reduced 24-hour *in vivo* survivals of radiolabelled Yt(a–b+) and Yt(a+b–) red cells suggested that only autologous red cells would be suitable for transfusion. Red cells of the patient contained about 10% of normal AChE and about 15% of normal AChE enzyme activity was detected. Four months after the initial investigation, weak Yt^a activity was apparent by an antiglobulin test and red cell AChE content was 54–60% of normal.

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