23 Raph Blood Group System

23.1 Introduction

The Raph system contains just one antigen, MER2 (RAPH1), located on the tetraspanin CD151. The true MER2– phenotype, associated with the presence of anti-MER2, is very rare and results from mutations in *CD151*, but there is a quantitative red cell polymorphism in which red cells of about 8% of Caucasians are serologically MER2–.

23.2 CD151 and the tetraspanin superfamily

The four-transmembrane domain superfamily (TM4SF) of integral proteins (tetraspanins) comprises 33 members in humans (for reviews see [1,2,3,4]). Tetraspanins are widely distributed on animal cells and several are known to be expressed on human haemopoietic cells including CD9, CD37, CD53, CD63, CD81, CD82, CD151, and CD231, though only CD151 and CD82 have been detected on mature red cells [5,6]. In addition to the characteristic four membrane-spanning domains, tetraspanins have short, cytosolic N- and C-termini and one small (EC1) and one large (EC2) extracellular loop. EC2 comprises 78–150 amino acids and contains four conserved cysteine residues, two of which are in a CCG motif. EC2 of most tetraspanins also contains a PxxCC motif, one or two disulphide bonds, and at least one *N*-glycan (Figure 23.1). Tetraspanins in the cell membrane aggregate with each other and with a variety of other transmembrane proteins, especially integrins, to form clusters known as tetraspanin-enriched microdomains. Integrins are heterodimeric, transmembrane receptors, consisting of non-covalently linked α and β subunits (see Section 16.8). They are two-way signalling molecules that dynamically link the extracellular matrix (ECM) with the cytoskeleton [4].

CD151 (TSPAN24) was originally identified as a platelet and endothelial cell marker [8]. Isolation of CD151 cDNA from megakaryoblastic and T-cell leukaemia cell lines revealed that CD151 is a 253 amino acid protein with a single *N*-glycosylation site and six cysteine residues in EC2 [9,10].

CD151 was located on chromosome 11p15.5 by *in situ* hybridisation and radiation-hybrid mapping [11,12]. It comprises 8 exons: exon 2 encodes the N-terminal domain; exon 3, the first transmembrane domain (TM1), EC1, TM2, and the cytoplasmic loop; exon 4, TM3; exons 5 and 6, EC2; exon 7, TM4; and exon 8, the C-terminal domain [12] (Figure 23.1).

23.3 CD151 is the Raph glycoprotein

The gene encoding MER2 was assigned to chromosome 11p15 by somatic cell hybridisation in 1987 [13]. In 2004 Karamatic Crew *et al.* [5] noticed that the genes for MER2 and CD151 were on the same region of chromosome 11. They confirmed that CD151 expresses the MER2 antigen by showing that (i) a monoclonal anti-CD151 reacted with red cells and recognised the MER2 polymorphism, (ii) incubation of MER2+ red cells with human alloanti-MER2 blocked binding of murine anti-CD151 and murine anti-MER2, and (iii) MER2– individuals who

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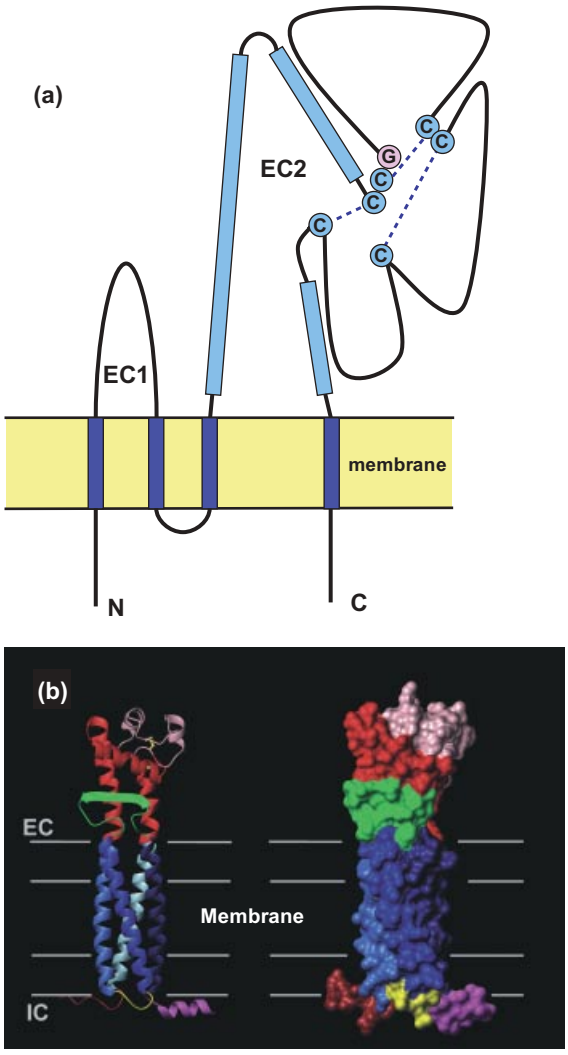

Figure 23.1 (a) Diagram of CD151, a typical tetraspanin, showing the four membrane-spanning domains, the cytosolic N- and –termini, the small extracellular loop (EC1) and large extracellular loop (EC2), comprising three α-helices (blue boxes), six Cys residues (C), one of which is part of the Cys-Cys-Gly (CCG) motif, and three disulphide bonds (dotted lines). (b) Ribbon and space-fill models of a tetraspanin, showing EC1 in green, the three α-helices of EC2 in red, and the remainder of EC2 in brown (reproduced from Seigneuret [7], with permission from Elsevier). EC, extracellular; IC, intracellular.

had made anti-MER2 were homozygous for mutations in *CD151*.

23.4 MER2 (RAPH1) antigen and anti-MER2

23.4.1 Effects of enzymes and reducing agents

MER2 is resistant to papain and sialidase treatment of red cells, but is destroyed by trypsin, chymotrypsin, pronase, and the disulphide bond reducing agent AET.

23.4.2 MER2– phenotype and alloanti-MER2

True MER2– phenotype is very rare and has only been found in individuals with alloanti-MER2. Human anti-MER2 was found in three individuals living in Israel, but originating from a Jewish community in India [14]. Two were siblings and the third was unrelated. All three were homozygous for a single nucleotide insertion (G383) in exon 5 of *CD151*, causing a frameshift and premature stop signal at codon 140 (*RAPH*–01N.01*) [5]. This would result in a translated protein lacking most of EC2 and TM4. It is unlikely that this truncated protein would reach the plasma membrane or that the severely truncated EC2 region would fold to a functional protein domain, and so these individuals could be considered to have a Raph-null phenotype. All had renal failure requiring dialysis and regular blood transfusion (Section 23.5). In two cases the antibodies were detected before commencement of dialysis and before the patients had been transfused [14]. All three antibodies react by an antiglobulin test with anti-IgG.

Alloanti-MER2 has been identified in four women, all of whom had been pregnant and are homozygous for *CD151* exon 6 mutations encoding amino acid substitutions in EC2: 511C>T, Arg171Cys (*RAPH*–01.01*) in women of Pakistani and Turkish origin [15]; 533G>A, Arg178His (*RAPH*–01.02*) in a Turkish woman [5]; 494G>A, Arg165Gln in a Caucasian woman [16].

23.4.3 The MER2 quantitative polymorphism and the effect of *In(Lu)*

The strength of MER2 varies considerably between red cell samples [13]. Of 1016 English blood donors, 8% were MER2– by an antiglobulin test. Analysis of 103 Northern European families with a total of 294 children confirmed that this polymorphism is inherited. The serological

MER2– phenotype, with no production of anti-MER2 and no detected mutations in *CD151*, probably represents the low end of a spectrum of red cell expression, with a site density below the threshold for detection by indirect agglutination techniques. Expression of MER2 on erythroid cells decreases throughout *ex vivo* erythropoiesis and MER2 is detectable on erythroid progenitors of apparent MER2– individuals [5,17]. In addition, MER2 is abundant on platelets of individuals with both MER2+ and MER2– red cell phenotypes, though there may be some degree of correlation between strength of antigen expression on red cells and platelets [5]. The reason for the individual variation in the expression of MER2 on red cells and platelets is unknown.

Titration scores with anti-MER2 and red cells of members of a large Sardinian family demonstrated that the dominant inhibitor gene *In(Lu)* (representing mutations in *EKLF*) exerted a slight depressing effect on MER2 expression (see Section 6.8.2.5). Scores for nine family members with *In(Lu)* (Lu_{mod}) varied from 0–15 with a mean of 6, whereas scores for 12 members without *In(Lu)* [Lu(a–b+)] varied from 12–21 with a mean of 16.

23.4.4 Clinical significance of anti-MER2

The two Israeli siblings with anti-MER2 and hereditary nephritis have received numerous transfusions of cross-match incompatible blood over a number of years with apparently no ill effects or indications of reduced red cell survival [14]. An 81-year-old woman with anti-MER2 showed indications of an HTR following transfusion of a third unit of red cells and results of a monocytes monolayer assay suggested that the antibody had the potential to be clinically significant [15]. Least incompatible red cells should be selected for transfusion to patients with anti-MER2.

23.5 Disease associations and functional aspects

The functions of tetraspanins are multifarious. Together with their partner molecules in the tetraspanin-enriched microdomains, they are involved in cell adhesion, signalling, and intracellular trafficking. They appear to function as molecular facilitators and transmembrane linkers, by recruiting signalling enzymes and tethering them to integrins, and by engaging other transmembrane proteins in specific lateral associations to form a network referred to as the tetraspanin web. Expression of certain tetraspanins and their partners is deregulated in some human malignancies [4,18]. Enhanced CD151 expression correlates with increased risk of metastasis or reduced survival in colon, lung, and prostate cancers. Metastasis is reduced in *Cd151* knockout mice, demonstrating that CD151 expression on host cells, as well as on tumour cells, plays a role in tumour progression [19]. CD151 is present on nearly all epithelial, endothelial, and fibroblastic cells, and associates tightly in cell membranes with the laminin-binding integrins α3β1, α6β1, α6β4, and α7β1. For reviews see [1–4].

MER2 demonstrates well how the study of an obscure blood group of little clinical importance in blood transfusion can provide important information on the functions of proteins in a variety of tissues. All three Israeli patients with CD151 deficiency had end-stage renal failure and the siblings also had severe blistering of the shins, neurosensory deafness, bilateral lachrymal duct stenosis, nail dystrophy, and β-thalassaemia minor [5,20]. CD151 binds integrin α3β1 on glomerular podocytes, enhancing adhesion strength between the podocytes and laminin of the glomerular basement membrane, important in the maintenance of a functional filtration barrier [21]. CD151 is also involved in dermal–epidermal adherence in the skin. Deafness in the CD151-deficient patients suggests that CD151 plays a similar role in the inner ear.

Severe anaemia observed in the CD151-deficient patients can be attributed, in part at least, to the occurrence of β-thalassaemia minor, but these patients require much higher doses of recombinant erythropoietin to maintain their haemoglobin levels between 80 and 100 g/L than other patients with β-thalassaemia trait undergoing chronic haemodialysis [22]. This suggests that their marrow response to erythropoietin is impaired, which may result from defects in the membrane assembly of integrins in erythroid progenitors that impact on signalling pathways also utilised by the erythropoietin receptor [5].

Modelling of CD151 EC2 suggested that both Arg178His and Arg171Cys, present in other MER2– patients with anti-MER2, are consistent with a protein that has lost the MER2 epitope, but retained integrin binding functionality. This explains why these patients showed none of the symptoms seen in the CD151-deficient Israelis [5,15].

CD151-deficiency in some strains of mice leads to similar kidney defects to those in CD151-deficient humans, but to no skin lesions or hearing impairment [21,23,24].

23.6 Tetraspanin CD82 is also present on red cells

The only other tetraspanin to be detected on mature red cells is CD82 (TSPAN27) and, like CD151, CD82 displays a quantitative polymorphism. About 86% of 115 blood donors had CD82-negative red cells, as determined by an antiglobulin agglutination test, yet CD82 was detected on erythroid progenitor cells of an individual with CD82-negative red cells [6].

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