12 Xg Blood Group System

12.1 Introduction

The human sex chromosomes began to arouse the interest of blood groupers with the discovery in 1962 of an X-linked blood group locus, *XG*. Mann *et al.* [1] found an antibody in the serum of a much transfused white man (Mr And) that detected an antigen with a frequency that, unlike all previous blood groups, differed in males and females. Family studies confirmed the suspicion that expression of the antigen, named Xg^a (XG1), was controlled by an X-linked gene. No antigen antithetical to Xg^a has been found, so the allele of *XG*A* is denoted *XG*0* here.

CD99, an antigen produced by a gene on both X and Y chromosomes, is closely related to the Xg blood group [2]. Because Xg^a and CD99 are encoded by closely linked homologous genes, CD99 has become XG2 of the Xg system. Xg^a and CD99 have been invaluable tools in the study of X- and Y-linkage, X–Y pairing and recombination, X inactivation, sex determination, X and Y aneuploidy, and various sex upsets, especially XX maleness.

12.2 Xg^a frequencies

The normal human chromosomal complement or karyotype is 46,XX in females and 46,XY in males. (Karyotypes are written with the total number of chromosomes first followed by the complement of sex chromosomes.) The *XG* gene resides on the X chromosome, but not on the Y chromosome. If a man has an *XG*A* allele on his X chromosome his red cells are Xg(a+) and if he has an *XG*0* allele they are Xg(a–). Homozygous *XG*A/A* and heterozygous *XG*A/0* women are Xg(a+); only homozygous *XG*0/0* women are Xg(a–). Consequently, more women than men express Xg^a on their red cells.

Results of testing 6784 unrelated Northern Europeans with anti-Xg^a were as follows: males, 66% Xg(a+) and 34% Xg(a–); females, 89% Xg(a+) and 11% Xg(a–) [3]. Phenotype, gene, and genotype frequencies are shown in Table 12.1. Results of other studies on a variety of ethnic groups are listed in Table 12.2. The incidence of *XG*A* differs remarkably little between most of the populations studied.

12.3 Xg^a inheritance

Analysis of the Xg groups of 2540 Northern European families with a total of 5824 children [3], together with many other families [20], proved that the *XG* locus is X-linked. With very few exceptions, Xg^a is inherited in a way expected of a character produced by an X-linked dominant gene. This expected pattern of inheritance is shown in Table 12.3.

Although the overwhelming majority of families with normal karyotypes fit the simple model presented in

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Table 12.1 Phenotype, gene, and genotype frequencies calculated from results of tests with anti-Xg^a on 6784 unrelated Northern Europeans [3].

		All	Males	Females
Phenotype	Xg(a+)	0.767	0.656	0.887
	Xg(a–)	0.233	0.344	0.113
Gene	*XG*A*	0.659		
	*XG*0*	0.341		
Genotype	*XG*A*		0.659	
	*XG*0*		0.341	
	*XG*A/A*			0.434
	*XG*A/0*			0.450
	*XG*0/0*			0.116

Table 12.3, there have been a few families that appear to break the rules of X-linked inheritance. According to these rules an Xg(a+) male must have received his *XG*A* gene from his mother, yet 16 Xg(a+) sons of Xg(a–) mothers are recorded [3,6,20–22]. Race and Sanger [21] suggested that a small portion of the father's X chromosome, including the *XG* locus, may have been translocated onto his Y chromosome, this Y chromosome being transmitted to his sons. A more probable explanation also involves X–Y recombination, with Xg^a expression in the sons being regulated by a gene (*XGR*) derived from the paternal Y chromosome (Section 12.9). In one of the families, one of two Xg(a+) men with an Xg(a–) mother has an Xg(a–) son [21]. Xg(a+) men must have Xg(a+) daughters, who inherit their father's single X chromosome. Cases of Xg(a–) daughters with Xg(a+) fathers have been considered less significant violations of X-inheritance because of the difficulties involved in ruling out non-paternity [3,20].

The first firm evidence that *XG* is located near the tip of the short arm of X was provided by an Xg(a–) woman who had failed to inherit her father's *XG*A* allele [23]: she had an X-Y translocation, 46,X,t(X;Y)(p22.3;q11), in which the distal segment of the short arm of one of her X-chromosomes was missing and replaced by part of the long arm of a Y-chromosome. The woman's son, who had inherited the abnormal chromosome and was nullisomic for Xpter–p22.3, was also Xg(a–). He suffered from a generalised ichthyosis as a result of zero steroid sulphatase activity, locating the steroid sulphatase (*STS*) gene, known to be closely linked to *XG*, on Xpter–p22.3.

Table 12.2 *XG* gene frequencies in various populations.

Population	No.	*XG*A*	*XG*0*	References
Northern European*	15716	0.66	0.34	[3–9]
Spaniards	636	0.59	0.41	[10]
Greeks	638	0.55	0.45	[11]
Sardinians	322	0.76	0.24	[12]
Israelis, non-Ashkenazi	201	0.68	0.32	[13]
Black New Yorkers and Jamaicans	219	0.55	0.45	[14]
Indians, Bombay	100	0.65	0.35	[15]
Thais	181	0.57	0.43	[16]
Japanese	529	0.68	0.32	[17]
Chinese	171	0.60	0.40	[5]
Chinese, Taiwan	178	0.53	0.47	[5]
Chinese, Hong Kong	1300	0.49	0.51	[18]
Taiwanese (Aboriginal)	164	0.38	0.62	[5]
Native Americans, Navajo	308	0.77	0.23	[5]
Australian Aborigines	352	0.79	0.21	[19]
New Guineans	263	0.85	0.15	[19]

*All the studies with people of Northern European extraction, which included people from North America, gave very similar gene frequencies.

12.4 Xg^a antigen

12.4.1 Biochemistry of Xg^a and the effects of enzyme treatments

Xg^a is destroyed by treatment of the red cells with the proteases bromelin, ficin, papain, pronase, trypsin, and chymotrypsin [24,25]. Xg^a is not destroyed by sialidase.

Immunoblotting with alloanti-Xg^a revealed that Xg^a resides on a red cell membrane component, probably a sialoglycoprotein, of apparent MW 22.5–28 kDa [25]. Sialidase treatment of red cells reduced the apparent MW of this structure by about 1.5 kDa. Whether the Xg glycoprotein is associated with CD99 in the red cell membrane is controversial [26,27] (see Section 12.6).

The sequence of *XG* (Section 12.8) predicts a 180 amino acid peptide with an extracellular N-terminal domain of about 142 residues, containing 16 potential *O*-glycosylation sites and no sites for *N*-glycosylation, a 20 residue transmembrane domain, and a 24 residue

Table 12.3 Phenotypes and genotypes of possible mating types and expected progeny.

Father		Mother		Sons		Daughters	
Xg(a+)	*XG*A*	Xg(a+)	*XG*A/A*	Xg(a+)	*XG*A*	Xg(a+)	*XG*A/A*
Xg(a+)	*XG*A*	Xg(a+)	*XG*A/0*	Xg(a+)	*XG*A*	Xg(a+)	*XG*A/A*
				Xg(a–)	*XG*0*	Xg(a+)	*XG*A/0*
Xg(a+)	*XG*A*	Xg(a–)	*XG*0/0*	Xg(a–)	*XG*0*	Xg(a+)	*XG*A/0*
Xg(a–)	*XG*0*	Xg(a+)	*XG*A/A*	Xg(a+)	*XG*A*	Xg(a+)	*XG*0/A*
Xg(a–)	*XG*0*	Xg(a+)	*XG*A/0*	Xg(a+)	*XG*A*	Xg(a+)	*XG*0/A*
				Xg(a–)	*XG*0*	Xg(a–)	*XG*0/0*
Xg(a–)	*XG*0*	Xg(a–)	*XG*0/0*	Xg(a–)	*XG*0*	Xg(a–)	*XG*0/0*

C-terminal cytoplasmic domain [28]. A putative 21 amino acid leader peptide is cleaved after membrane insertion.

12.4.2 Development and loss of Xg^a

Xg^a is developed at birth, but cord red cells may give weaker reactions than red cells of adults [8,29]. Xg^a appears to develop quite late in fetal life: of 54 samples from fetuses between 6 and 20 weeks' gestation, only 19 were Xg(a+), a significantly lower Xg^a frequency than that found in adults [30]. The youngest Xg(a+) fetus was 12 weeks old.

Expression of Xg^a declines exponentially with red cell age, with an *in vivo* half-life of 47 days [31]. Between 5 and 10% of red cells from an Xg(a+) male lack Xg^a antigen [32]. During *in vitro* erythropoiesis, Xg glycoprotein appears on the erythroblasts after glycophorin A and band 3, but before the Rh proteins [33].

12.4.3 Xg^a dosage and site density

Xg^a is expressed equally strongly on the red cells of hemizygous males as on those of homozygous females, but Xg^a expression on red cells of heterozygous females may be weaker. About 5–10% of Xg(a+) females, all heterozygotes, have very weak expression of Xg^a. Weak Xg^a in males is very rare.

The number of Xg^a binding sites per Xg(a+) red cell was estimated to be about 9000 in one study [32], but only 159 in another [34].

12.4.4 Xg^a on other cells

In 1974, Fellous *et al.* [35] utilised a microcomplement fixation test to detect Xg^a on cultured fibroblasts from Xg(a+) donors and on human–rodent somatic cell hybrids. Xg^a co-segregated with a number of known X-linked characters and the results also suggested that Xg^a on fibroblasts, like red cells, is not subject to X inactivation (see Section 12.7.1). Alas, Fellous and his colleagues were unable to repeat their feat of detecting Xg^a on cells other than red cells and neither, subsequently, could Hsu *et al.* [36].

XG transcripts were detected in fibroblasts [20], as well as in erythroid tissues and in heart, placenta, skeletal muscle, prostate, thyroid, spinal cord, and trachea [27]. Low levels of *XG* mRNA were also detected in some fetal tissues and in adult heart, lung, kidney, testis and some lymphoid cell lines [27,37].

12.5 Anti-Xg^a

Many examples of anti-Xg^a have been described [1,4,7,17,18,25,38–40]. Xg^a is apparently not very immunogenic yet, unlike other rare blood group antibodies, most sera containing anti-Xg^a are not 'contaminated' with other blood group antibodies. Twelve of the first 14 anti-Xg^a [21] and 11 of 13 anti-Xg^a found in Japanese volunteer donors [40] have been in men, somewhat surprising even considering the different frequencies of the Xg(a–) phenotype in men and women. In Hong Kong, four anti-Xg^a were identified in 325 serum samples referred for antibody investigation, and one anti-Xg^a was found in sera from 60 108 blood donors; all five antibodies were in men [18]. Anti-Xg^a often appear to be 'naturally occurring'. Although Xg^a antibodies occasionally

agglutinate red cells directly, they are generally IgG, react by the antiglobulin method, and are often capable of fixing complement.

Anti-Xg^a has never been held responsible for HDFN or for an HTR. One patient with anti-Xg^a received six units of Xg(a+) blood with no signs of a haemolytic reaction [38]. Repeated injections of small volumes of radiolabelled Xg(a+) red cells into a patient with anti-Xg^a survived normally [39]. Autoanti-Xg^a has been identified in a pregnant woman [41,42].

Murine monoclonal anti-Xg^a have been produced by immunising mice with human red cells [43] or with a peptide corresponding to a segment from the N-terminal of the Xg glycoprotein [37]. Two human monoclonal anti-Xg^a have also been described [44].

12.6 CD99, a quantitative polymorphism related to Xg

In 1981 Goodfellow and Tippett [2] started a new chapter in the Xg story when they observed that a monoclonal antibody to a determinant controlled by an X-borne gene (*MIC2*, now *CD99*), was defining a red cell polymorphism related to the Xg blood group. The monoclonal antibody, called 12E7, was produced as a result of immunising mice with human leukaemic T cells [45]. CD99, the antigen defined by the 12E7 antibody, is expressed on all human tissues tested [46], but on red cells, unlike other cells, the level of expression shows individual variation. By antiglobulin tests, radioimmunoassay, or flow cytometry, a quantitative polymorphism is observed and individuals can be subdivided into CD99 high expressors and CD99 low expressors [2,34].

Testing of red cells from over 300 Europeans demonstrated the following association between Xg and CD99 [2]:

all Xg(a+) individuals are CD99 high expressors;
all Xg(a–) females are CD99 low expressors;
about 68% of Xg(a–) males are CD99 high expressors and 32% low expressors.

Goodfellow and Tippett [2] postulated that a locus, *YG*, on the Y chromosome, controls the level of expression of the *CD99* gene. *YG* would have two alleles, *YG*A* (*Yg^a*) and *YG*0* (*Yg*). Individuals are CD99 high expressors if they have an *XG*A* or *YG*A* allele and CD99 low expressors if they have neither. Although family studies partially substantiated this theory [47], in light of information provided by some exceptional families the model was modified to involve a locus on X and Y chromosomes, which regulates both Xg^a and CD99 red cell expression [48] (described in Section 12.9). This putative regulator locus is called *XGR*.

CD99 became XG2 when two examples of alloanti-CD99 were found in healthy Japanese blood donors [49]. The antibodies gave the same pattern of reactions as monoclonal anti-CD99 with CD99 high- and low-expressor red cells.

Like Xg^a, CD99 is located on a sialoglycoprotein. CD99 is destroyed by the proteases papain, pronase, trypsin, and chymotrypsin [46,50,51]. It is generally sialidase-resistant [46,50], but one exceptional CD99-like antibody did not react with sialidase-treated cells [51]. Immunoblotting of red cells, lymphocytes, various human cell lines, and human–rodent hybrid cells containing a human X or Y chromosome showed that CD99 is associated with a glycoprotein of approximate MW 32 kDa [26,27,50–53]. Sialidase treatment of the cells resulted in a reduction in apparent MW of the CD99 structure [26,27,50,53]. Immunoblotting of immunoprecipitates confirmed that CD99 and Xg^a are located on separate structures [26,27]. Partially purified CD99 glycoprotein inhibited anti-CD99 and anti-Xg^a [54]. Petty and Tippett [26] found that alloanti-Xg^a co-precipitated Xg^a and CD99 glycoproteins, suggesting that these two homologous structures are associated in the membrane, but Fouchet *et al.* [27] were unable to repeat this result with a monoclonal antibody to the Xg glycoprotein.

CD99 is located on a protein of about 186 amino acids comprising an N-terminal signal peptide of 20 or 21 amino acids (cleaved after membrane insertion), about 100 extracellular residues, a hydrophobic transmembrane region, and a 36 amino acid cytoplasmic tail [54,55]. No difference in size or charge was detected between the X- and Y-encoded forms of the molecule [52].

There are an estimated 27 000 binding sites for anti-CD99 on lymphocytes, 4000 on platelets, and only 1000 on high-expressor and 100 on low-expressor red cells [50]. Reticulocytes from CD99 low expressors have lower levels of *CD99* transcripts than those from CD99 high expressors [34].

12.7 X-chromosome inactivation and the pseudoautosomal region

12.7.1 X-chromosome inactivation

Normal mammalian somatic cells have two X chromosomes in the female, but only one in the male. As most X-borne genes do not have a homologue on the Y

chromosome, the potential difference in dosage of X-linked genes between male and female cells is compensated by a process called X-chromosome inactivation [56,57]. In each somatic cell of an XX female only one of the X chromosomes is active, the other becoming permanently inactivated at an early stage in embryological development when a few million cells are formed. Whether the maternal or paternal X chromosome in any cell becomes inactivated is generally a matter of chance but, once inactivation has taken place, all descendants of that cell will have the same inactivated X chromosome. Female mammals are, therefore, mosaics of roughly equal numbers of cells with either the paternal or maternal X chromosome active. X inactivation is a *cis* phenomenon, which spreads along the chromosome from an X-inactivation centre, where the gene *XIST* produces a non-coding mRNA that appears to coat the inactive X.

Not all X-borne genes are subject to inactivation, with about 15% of the genes on the human X chromosome escaping inactivation [58]. The first locus known to deviate from the rule of X inactivation was *XG*. If *XG* were subject to inactivation, and assuming that Xg^a is a direct product of the *XG*A* gene and not manufactured outside the red cell, then heterozygous *XG*A/0* women would be expected to have a mixed population of Xg(a+) and Xg(a–) red cells. No such mosaicism occurs. Natural chimeras have proven that mixtures of Xg(a+) and Xg(a–) red cells can be produced by the same marrow [59,60] and post-bone marrow transplant conversion from Xg(a–) to Xg(a+) and *vice versa* provides further evidence that Xg^a production is restricted to the bone marrow [61].

CD99 also escapes inactivation [62]. Hybrid cell lines containing only an inactivated human X chromosome expressed CD99 and the antigen was expressed at increasing levels in hybrid cells with multiple inactive X chromosomes.

12.7.2 The pseudoautosomal region

During male meiosis it is important that the X and Y chromosomes segregate to separate spermatocytes. Consequently, like the autosomes, the X and Y chromosomes undergo pairing during the first meiotic division. This pairing, however, only involves the telomeric regions of the short and long arms of each chromosome. Within the pairing regions genes are shared and recombination occurs. The pairing regions are called pseudoautosomal regions (PAR): PAR1, with about 24 genes, is on the short arm and PAR2, with about five genes, is on the long arm [57,63].

PAR1 has a recombination rate about 20 times the average rate for the genome. Some genes within PAR1 and very close to the telomere undergo 50% recombination and cannot be distinguished from autosomal genes on family evidence. *CD99* is pseudoautosomal, but situated very close to the pseudoautosomal boundary and only recombines with X- and Y-linked genes on the other side of the boundary in about 2% of male meioses [64]. Pseudoautosomal genes are on both X and Y chromosomes, so no dosage compensation is required and they do not participate in X inactivation.

XG is not pseudoautosomal, but straddles to the pseudoautosomal boundary on the X chromosome [28]. It is likely that on very rare occasions *XG* is involved in recombination with the Y, providing an explanation for the rare families in which Xg(a–) mothers have Xg(a+) sons [3,6,20–22] (Section 12.3).

12.8 *CD99* and *XG* genes

Human–rodent somatic cell genetics has demonstrated that *CD99* is located on both X and Y chromosomes [65–67]. Hybrid cells express CD99 when either X or Y is the only human chromosome retained [66]. *CD99* cDNA was cloned by screening a cDNA expression library with a mixture of two monoclonal antibodies to CD99 epitopes [54,68]. A cDNA probe was used to show that *CD99* genes on the X and Y chromosomes are identical [68]. Somatic cell genetics [66] and *in situ* hybridisation [69] have mapped *CD99* to the tips of the short arms of the X and Y chromosomes, at Xp22.32 and Yp11.3.

Isolation of 95 kb of genomic DNA encompassing the entire *CD99* gene revealed a 52 kb gene orientated toward the centromere and with its 5′ end 95 kb from the pseudoautosomal boundary (*PAB1X*) [70] (Figure 12.1). The gene comprises 10 small exons: exon 1 encodes the leader peptide; exons 2–9 and 23 base pairs of exon 10 encode the CD99 protein (Table 12.4). No difference was detected between the organisation of the X- and Y-borne alleles.

Genomic sequences between the 3′ end of *CD99* and a CpG-rich region proximal to the pseudoautosomal boundary were used to isolate a 600 bp clone from a bone marrow cDNA library. This cDNA was, in turn, used to isolate an 820 bp transcript containing a 540 bp open reading frame capable of encoding a 180 amino acid polypeptide with a high level of homology with the CD99 protein, but not containing the sequence representing the CD99 epitope [28]. Mouse monoclonal and rabbit polyclonal antibodies raised to a 14 amino acid peptide

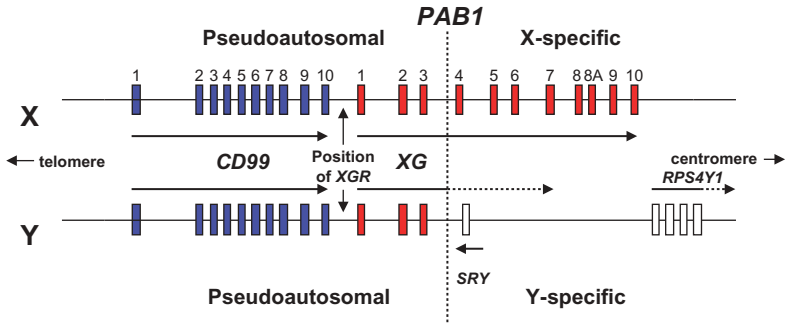

Figure 12.1 A region of about 250 kb the X and Y chromosomes spanning the pseudoautosomal boundary (*PAB1*), showing the organisation of *CD99* and *XG* [28,71]. Both genes contain 10 exons on X, but only 3 exons of *XG* are on Y. *CD99* and exons 1–3 of *XG* are pseudoautosomal; exons 4–10 of *XG* are X-specific. Exon 8A in *XG* was present in fibroblast RNA, but not erythroid RNA [20]. *SRY* and *RPS4Y1* are Y-specific genes. The position of *XGR*, a proposed regulator of *XG* and *CD99* expression on red cells [48], is shown between *CD99* and *XG*.

Table 12.4 Organisation of the *CD99* and *XG* genes [20,28,70].

	CD99			*XG*		
Exon	Exon size (bp)	Amino acids*	Intron size (kb)	Exon size (bp)	Amino acids*	Intron size (kb)
1	~244	1–23	23	246	1–21	12.5
2	33	23–34	3.2	42	21–35	3.3
3	48	34–50	2.4	24	35–43	8.3
4	45	50–65	0.6	63	43–64	7.0
5	69	65–88	1.0	63 (66)†	64–85	7.0
6	48	88–104	1.2	69	85–108	2.0
7	51	104–121	4.1	51	108–125	10
8A‡				45	(125–140)‡	
8	114	121–159	6.8	36	125–137 (140–152)	5.0
9	57	159–178	7.7	117	137–176 (152–191)	4.0
10	533	178–186		67 (244)	176–180 (191-195)	

*Amino acids encoded in CD99 by *CD99* and in the major erythroid form of Xg protein (fibroblast form in parentheses) by *XG*.
†In a minority of *XG* cDNA clones an additional 3 bp, inserting a serine at position 86, appeared between exons 5 and 6 [20].
‡Exon 8A is present in fibroblast RNA, but not in erythroid RNA [20].

synthesised to represent an N-terminal sequence encoded by this cDNA, behaved as anti-Xg^a in haemagglutination tests and were shown to bind the same membrane structure as anti-Xg^a by an antibody-specific immobilisation of antigens assay [37]. The antibodies gave an identical banding pattern to human anti-Xg^a on immunoblots of red cell membranes. The *CD99* homologue is, therefore, *XG*. Like *CD99*, erythroid *XG* transcripts consist of 10 exons, with exon 1 encoding the leader peptide and exons 2–10 the native protein (Table 12.4). An additional exon, exon 8A, is present in transcripts from fibroblasts [20,28].

XG spans the pseudoautosomal boundary, *PAB1X*. Exons 1–3 are situated within PAR1, whereas exons 4–10 are X-specific (Figure 12.1). Exons 1–3 of *XG* are also present on the Y chromosome. Transcription from the *XG* promoter on Y results in a low abundance of transcripts that contain exons 1–3 of *XG* plus sequences from two downstream Y-linked genes, the gene for the

testis-determining factor (*SRY*) and *RPS4Y1*. The *SRY* sequence is in antisense configuration and most of these transcripts do not maintain an open reading frame [71]. *XGPY*, an expressed pseudogene of *XG* with a frameshift mutation in exon 5, is on the long arm of Y at Yq11.21 [71].

XG has two modes of inheritance: the 5′ end is subject to a recombination rate 20 times that of the genome average and is also involved in X/Y recombination; the 3′ end is only subject to X/X recombination, with an average recombination rate.

There is substantial amino acid sequence homology between CD99 and the Xg protein, with corresponding blocks of regions rich in acidic amino acids, basic amino acids, proline residues, and glycine residues [20]. The Asp61-Gly-Glu-Asn sequence of CD99 recognised by 12E7 antibody (anti-CD99) is in a region rich in acidic amino acids not present in the Xg protein.

Transfection of mouse fibroblastic cells with *XG* and/or *CD99* cDNA molecules showed that Xg and CD99 proteins were expressed independently and at a similar level in single and double transfectants [27]. This suggests that the phenotypic association between Xg^a and CD99 polymorphisms is regulated primarily at the transcriptional level and not through association of the glycoproteins in the membrane.

12.8.1 Xg polymorphism

The molecular basis for the Xg polymorphism is not known. Monoclonal antibodies to at least two separate epitopes on the Xg glycoprotein, human anti-Xg^a, and rabbit antibodies raised to a 14 amino acid peptide corresponding to an N-terminal sequence of the Xg polypeptide, all reacted with Xg(a+), but not Xg(a–), red cells [37]. This suggests that the Xg(a–) phenotype results from absence of the Xg glycoprotein from the red cell. Reverse transcriptase PCR amplified *XG* fragments from Xg(a+) cord blood, but not Xg(a–) cord blood [20], indicating that substantially less RNA is produced by the *XG*0* allele of *XG*, than by the *XG*A* allele.

12.9 A model for explaining the association between the Xg and CD99 polymorphisms

When Goodfellow and Tippett [2] first recognised the unusual association between expression of Xg^a and CD99 on red cells, they proposed a hypothesis involving a gene (*YG*) on the Y chromosome. Both *XG* and *YG* regulate red cell expression of CD99 encoded by the structural gene *CD99*. However, families demonstrating recombination between *CD99* and *XG* and between *YG* and the X and Y chromosomes induced Goodfellow *et al.* [48] to modify their model.

In one family a normal 46,XX female had apparently received her father's *XG*A* on one of her X chromosomes and yet a *CD99* DNA probe showed that she had also received her father's Y chromosome PAR1 including his Y-borne *CD99* gene. Recombination must have separated *CD99* from the locus controlling expression of Xg^a.

Brothers receive the same Y chromosome from their father, yet seven of 172 Xg(a–) males did not have the same *YG* allele as their brother; that is, despite being Xg(a–), they differed in the level of their CD99 red cell expression [47]. In one family, part of which is shown in Figure 12.2, all the males had the same Y chromosome, but not all the Xg(a–) males were CD99 high expressors; one (II-1) was a low expressor [48]. A *CD99* RFLP showed that II-1 had inherited the same *CD99* gene from his father as that received on an X chromosome by his two sisters (II-2 and II-4) and that he had inherited a different *CD99* gene on a Y chromosome from his father from that received by his brother (II-3). Therefore, both *CD99* and

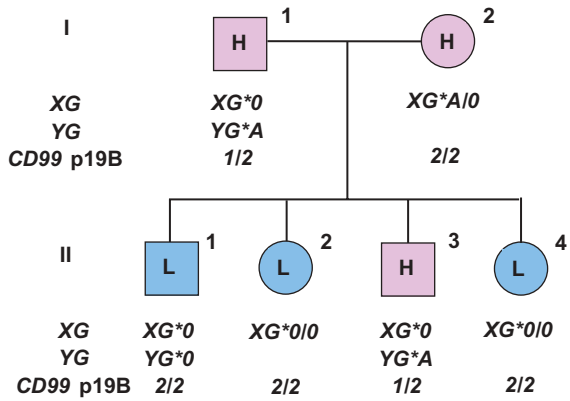

Figure 12.2 Part of family showing *XG* and *YG* genotypes and *CD99 Taq*I RFLP (p19B) genotypes, demonstrating recombination of *CD99* and the gene determining CD99 red cell expression (*YG*) with the remainder of the sex chromosomes [48]. The father (I-1) has passed an X chromosome with a *CD99 2* allele to his two daughters (II-2 and II-4) and a Y chromosome with *CD99 1* and *YG*A* (CD99 high-expressor) alleles to one of his sons (II-3), but a Y chromosome with *CD99 2* and *YG*0* (CD99 low-expressor) alleles to his other son (II-1). H, CD99 high; L, CD99 low.

YG have been involved in an X–Y exchange, are pseudoautosomal on the Y chromosome, and are distal to the male sex-determining gene *SRY*.

In order to explain these phenomena Goodfellow *et al.* [48] proposed the existence of a regulator locus, *XGR*, which controls *cis* expression of the structural loci *XG* and *CD99* on the X chromosome and of *CD99* on the Y chromosome. This gene is polymorphic. One allele (XGR^{high}) induces Xg^a antigen expression from the *XG* locus and high CD99 expression from the *CD99* locus; the other allele (XGR^{low}) prevents Xg^a expression and results in low level CD99 expression, probably by regulating transcription. All Xg(a+) individuals must have XGR^{high} on at least one X chromosome and so they have high expression of CD99. Xg(a–) females must have XGR^{low} on both X chromosomes and, therefore, must have low expression of CD99. Xg(a–) males must have XGR^{low} on their X, but can have either XGR^{high} or XGR^{low} on their Y, and so can have high or low expression of CD99 (Table 12.5). Although *XGR* is pseudoautosomal, it must be very close to the pseudoautosomal boundary (Figure 12.1) as recombination resulting in Xg^a inheritance contravening the rules of X-linkage is extremely rare (Section 12.3).

This model in which both Xg^a and CD99 expression on the red cells are controlled by the same gene (*XGR*) explains why the frequencies of Xg^a and CD99 high expression are so similar. It also provides an explanation for the families in which an Xg(a–) mother has an Xg(a+) son [3,10,20–22]. The son receives his mother's *XG* gene on his X chromosome, but his father's Y-derived XGR^{high} allele on the same chromosome as a result of X–Y recombination between *XGR* and *XG*. It should be emphasised that this theory is conjectural, but it does provide a useful model for explaining the data.

Table 12.5 Effects of *high*- and *low*-expression alleles of *XGR* on Xg^a and CD99 red cell phenotypes.

	XGR allele		Phenotype	
	X	X		
Females	*High*	*High*	Xg(a+)	CD99 high
	High	*Low*	Xg(a+)	CD99 high
	Low	*Low*	Xg(a–)	CD99 low
	X	Y		
Males	*High*	*High*	Xg(a+)	CD99 high
	High	*Low*	Xg(a+)	CD99 high
	Low	*High*	Xg(a–)	CD99 high
	Low	*Low*	Xg(a–)	CD99 low

12.10 XX males and sex chromosome aneuploidy

12.10.1 XX males (testicular disorder of sex development)

In order to maintain the chromosomal basis of sex determination, it is imperative that Y-borne genes outside the pseudoautosomal region are not normally involved in X–Y recombination, especially *SRY*, the gene controlling the testes determining factor. One occasion where this axiom appears to break down is in the rare case of XX males, sterile males with an apparently normal female karyotype. Approximately one in 20 000 men is 46,XX, yet is phenotypically and psychosexually male [72]. Xg and CD99 have played an important part in determining the aetiology of this phenomenon. In six families with informative Xg groups the XX male propositus had not received his father's *XG*A* allele, suggesting superficially that both X chromosomes were maternal in origin [21], probably caused by a small exchange of genetic material at the tips of the short arms of the X and Y chromosomes resulting in a paternally derived X chromosome that has lost its *XG* locus and gained the Y-derived testis determining genes [73]. This is consistent with the observed Xg distribution in XX males being much closer to that of XY males than to that of XX females [74,75]. A very informative family analysed for red cell expression of Xg^a and CD99 proved X–Y interchange as a cause of XX maleness [76]. Occasionally XX males express a paternally derived *XG*A* allele, suggesting that the X–Y recombination does not always involve the *XG* locus [21,72,77]. The X–Y interchange may involve an exchange of different amounts of genetic material, explaining how the recombinant chromosome could have both *XG* and *SRY* loci [78]. Although XX maleness can be explained by an exchange of genetic material between the X and Y chromosomes in about 80% of cases, other mechanisms, often involving autosomal genes, appear to be involved in the minority of examples of this form of sex reversal.

12.10.2 Sex chromosome aneuploidy

Aneuploidy is the term given to karyotypes in which the number of chromosomes is not a true multiple of the haploid number (23 in humans). Aneuploidies in which

one to five X chromosomes are involved can be viable, presumably because in somatic cells only one X chromosome is active (Section 12.7.1). One or more Y chromosomes may also be involved. The Xg blood group provided a great deal of information about the non-disjunctions that have caused these chromosomal upsets, in some cases pinpointing the meiotic division at which non-disjunction occurred [75,76]. More recent evidence on the origin of the X chromosomes in sex chromosome aneuploidy has been elicited from studies with DNA probes [79].

12.11 Functional aspects and association with disease

Although the natural ligands are not known, CD99 appears to function as a receptor and adhesion molecule. CD99 enhances α4β1 integrin-dependent T-cell adhesion to inflamed vascular endothelium under shear stress conditions [80] and modulates peripheral T-cell rosette formation and homotypic aggregation of thymocytes and B cells [81,82]. Yet CD99 can also induce apoptosis of thymocytes and mature T cells by a caspase-independent pathway [83,84]. A truncated isoform of CD99, which lacks most of the cytoplasmic domain as the result of alternative splicing of *CD99* transcripts, may function in an opposite way to full-length CD99 by inhibiting CD99-induced homotypic aggregation of B cells [82]. Expression of the CD99 long form in a T-cell line is sufficient to promote CD99-induced adhesion, whereas co-expression of both isoforms is required to trigger T-cell death [85].

Homophilic interaction between CD99 on endothelial cells and on monocytes and neutrophils is important in the migration of the leucocytes through endothelial vessel walls during inflammation [86,87]. CD99 may also play a role in migration of haemopoietic progenitors across the endothelium. CD99 is expressed on the majority of $CD34^+$ cells from bone marrow and mobilised peripheral blood, but only on 50% of $CD34^+$ cells from cord blood [88].

CD99 and HLA class I molecules co-localise both in the Golgi apparatus and at the cell surface, through a physical association via their transmembrane domains [89]. In B-cell and T-cell lines, decreased CD99 expression resulted in HLA class I retention in the Golgi [89,90]. In addition to HLA class I, both isoforms of CD99 are associated with HLA class II and the tetraspanin CD81 in T cells [91]. This tetraspanin web (see Section 23.2) may be involved in the regulation of T-cell proliferation. CD99 is expressed at around 8-fold higher levels on acute lymphoblastic leukaemia lymphoblasts than on normal T cells and could be used as a marker for minimal residual disease [92].

CD99 is expressed at the cell surface in most cases of Ewing sarcoma (EWS), a malignant, aggressive bone tumour, and is the best diagnostic marker for this disease [93]. CD99 contributes to oncogenesis by preventing neural differentiation through modulation of a mitogen-active protein kinase pathway [94]. Whereas 100% of EWS tumours express CD99, tumours in 24% of EWS patients also express Xg^a, which defines a subgroup of patients with worse prognosis than those not expressing Xg^a [95]. Forced expression of Xg^a in an EWS cell line enhanced cell migration and invasion *in vitro*, whereas knockdown had the opposite effect. Knocking down of CD99 expression in human EWS cell lines reduced their ability to form tumours and bone metastases when grafted into immunodeficient mice [94]; Xg^a expression increased the number of metastases [95].

CD99 is present in a variety of other tumours, including some carcinomas, leukaemias, and lymphomas. In osteocarcinoma, prostate cancer, and breast cancer cells the long isoform of CD99 (and in particular the presence of Ser168) inhibited metastasis, through increased motility, whereas the shorter variant appears to favour the phenomenon [96,97].

In contrast to CD99, almost nothing is known regarding the function of Xg^a, although structural homology between Xg^a and CD99 might suggest similar functions.

12.12 Xg^a and CD99 in animals

Gibbons (*Hylobates lar lar*) have Xg^a on their red cells: of 52 tested, 30% of males and 53% of females were Xg(a+), very suggestive of X-linkage. Other great apes (67 chimpanzees, two gorillas, 20 orangutans, and five gibbons of the species *Hylobates pileatus*) as well as various monkeys (including 60 baboons), and a few non-primates, were all Xg(a–). CD99 was not detected on the red cells or peripheral blood lymphocytes of 10 gibbons, regardless of their Xg phenotype. CD99 was detected on red cells and fibroblasts of chimpanzees and gorillas, but not of orangutans or any of the other mammals tested [21,98,99].

Genomic and cDNA analyses revealed *XG* and *CD99* in non-human primates. *XG* was also present in some other mammals and the position of *XG* at the pseudoautosomal boundary appears to be conserved in higher

primates [20]. The chromosomal organisation of the two homologous genes in tandem (Figure 12.1) probably arose from a duplication event occurring over 150 million years ago, before the mammalian radiation [20].

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