31 Sid Antigen

31.1 Introduction

Numerous antibodies of identical specificity were studied in several laboratories for at least 10 years before Macvie *et al.* [1] and Renton *et al.* [2] simultaneously reported the specificity in 1967 and named it anti-Sd^a. About 91% of white people have Sd(a+) red cells and so Sid qualifies for inclusion in the 901 series of high frequency antigens and has the number 901011. As Sid differs so significantly, both in frequency and characteristics, from the other 901 series antigens, it has been given a separate chapter. Although the gene encoding the β1,4-*N*-acetylgalactosaminyltransferase responsible for Sd^a expression has been isolated, Sid has not been made a blood group system because the genetic basis of the Sid polymorphism has not been elucidated.

31.2 Sd^a and Cad

31.2.1 Variation in strength of Sd^a: the Sd(a++) or Cad phenotype

The strength of expression of Sd^a on Sd(a+) cells varies from barely recognisable to extremely strong. A 'mixed field' reaction, with small agglutinates among a sea of unagglutinated cells, is a characteristic of reactions with anti-Sd^a. If the unagglutinated cells are separated and retested with anti-Sd^a, a 'mixed field' picture is again apparent [2]. In haemagglutination tests with a strong anti-Sd^a, about 1% of individuals have strong agglutination with big clumps of cells, about 80% have moderate sized agglutinates consisting of 10–20 cells, about 10% have only occasional tiny agglutinates, and about 9% have no agglutination [1]. Weak examples of anti-Sd^a react with only a few Sd(a+) samples and may be mistaken for antibodies to a low frequency antigen.

In 1968, Cazal *et al.* [3] described a new private antigen, Cad, in a Mauritian family. Cells of the Cad+ members were polyagglutinable and, despite being group O or B, were agglutinated by *Dolichos biflorus* seed extract, a lectin that generally only agglutinates A_1 cells (Chapter 2). After demonstrating that red cells with very strong Sd^a expression are also agglutinated by *Dolichos* lectin and that Cad cells are very strongly Sd(a+), Sanger *et al.* [4] concluded that Cad represents the high end of a continuous curve of Sd^a antigen strength. There is considerable variation in agglutination strength with *Dolichos* and with human anti-Sd^a of these 'super-Sid' or Sd(a++) cells between individuals and within families [5–11]. As Cad probably represents the expression of an extra strong Sd^a gene, the term Sd(a++) will be used here to describe all phenotypes in which the red cells have sufficiently strong Sd^a to be agglutinated by *Dolichos* lectin. The term Cad will be restricted to describe the rare phenotype of members of the Cad family, still probably the strongest Sd(a++) cells encountered.

Sd(a++) cells of the Cad family are polyagglutinable [3]; that is, they are agglutinated by most human sera. Although other Sd(a++) cells are generally polyagglutinable, this is usually less obvious and selected sera and sensitive methods are required to detect the agglutination [9,10]. Sd(a++) polyagglutination differs from all other forms of polyagglutination (Chapter 33).

Weak anti-Sd^a appears to be present in the sera of most people and responsible for the polyagglutination

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Table 31.1 Frequencies of Sd(a++) in various populations as detected by different preparations of *Dolichos biflorus* lectin.

Population	No. tested	No. positive	Frequency	References
French, Montpellier	250 000	0		[3]
French (four centres)	78 526	56	0.0007	[10]
Canadian, Winnipeg	1425	2	0.0014	[8]
Canadian, Toronto	2191	1	0.0005	[5]
Japanese	51 420	15	0.0003	[7]
Hong Kong Chinese	36 037	110	0.0031	[18]
Thai	14 261	37	0.0026	[12,19]

of Sd(a++) cells. Polyagglutinable Sd(a++) cells are not agglutinated by sera from people with Sd(a++) cells and the stronger the Sd^a antigen on Sd(a++) cells, the greater the number of 'normal' sera that would agglutinate them [12].

31.2.2 Sd^a in body fluids and other tissues

Sd^a can be detected, by haemagglutination inhibition, in the saliva of individuals with Sd(a+) red cells, with greater quantities present in neonatal than adult saliva [1,13]. Sd^a can also be detected in human serum and milk, but by far the greatest abundance is found in meconium and urine [13]. Inhibition of anti-Sd^a with urine is considered the most reliable method of determining Sd^a phenotype.

Sd^a has been found, in variable quantities, in the urine of 12 species of mammals, but not in birds [13]. Guinea pig urine is an extremely concentrated source of Sd^a and a useful tool in blood group antibody identification.

Of the human tissues, kidney is a very rich source of Sd^a [13], with activity localised to the distal convoluted tubules and collecting ducts [14]. Sd^a is also present in colon and stomach, but was not detected in small intestine, muscle, liver, spleen, or brain [15,16].

31.2.3 Frequency and inheritance

The frequency of Sd^a, as determined by testing red cells, is about 90%. Three surveys gave the following results: 290 English blood donors, 91.4% Sd(a+) [1]; 131 English donors, 91.0% Sd(a+) [2]; 1307 Italian donors, 89.3% Sd(a+) [17]. A higher incidence of Sd(a+) is obtained when urine is tested by haemagglutination inhibition. Sd^a was detected in the urine of 96.1% of English blood donors [13] and 93.4% of north Italians [17]. The gene and genotype frequencies in English donors determined by urine inhibition are: Sd^a 0.8030; *Sd* 0.1970; Sd^a/Sd^a 0.6448; Sd^a/Sd 0.3164; *Sd/Sd* 0.0388.

Sd(a++) is very rare in Europeans, but may be less infrequent in the Far East. Table 31.1 shows some frequency determinations on group O and B donors tested with *Dolichos* lectin. These frequencies are somewhat arbitrary as they are partly dependent on the potency of the lectin preparation used.

Tests with anti-Sd^a on 55 families with a total of 168 children, demonstrated that Sd^a is inherited as a dominant character [1]. Sd(a++), as detected by *Dolichos* lectin, is also inherited in a dominant manner [3–8,10–12,20]. Family studies have shown that Sd^a is not part of the ABO, MNS, P1PK, Rh, Lutheran, Kell, Duffy, Kidd, Xg, Dombrock, or XK systems [1,5,8,10]. One Canadian family provided a suggestion of linkage between the genes for Sd(a++) and Wr^a (*SLC4A1* or *DI*), with six non-recombinants and no recombinants, providing a lod score of 1.806 at a recombination fraction (θ) of zero [8]. Both *SLC4A1* and *B4GALNT2*, the Sd^a gene (Section 31.4.3) are on the long arm of chromosome 17.

31.2.4 Sd^a in babies and pregnant women

Red cells of fetuses and newborns are Sd(a–) and become Sd(a+) about 10 weeks post-natally [1,2,15]. Red cells of the son of an Sd(a++) father were non-reactive with *Dolichos* lectin at birth, but were agglutinated by the lectin 6 months later [10]. Saliva, urine, and meconium from neonates generally contain abundant Sd^a substance [13].

An increased frequency of Sd(a–) red cell phenotype is found among pregnant women [1,15,21]. In different studies, 36% and 75% were Sd(a–) at term, and 25% six weeks post-partum had Sd(a–) red cells [15,21]. Sd^a is present in the urine of most Sd(a–) pregnant women [13].

31.3 Sid antibodies and lectins

31.3.1 Anti-Sd^a

Anti-Sd^a is disclosed in about 1% of donor sera when Sd(a+) cells are used for detection [1,2]. The incidence of anti-Sd^a is increased when Sd(a++) cells are used [21], and the exceptionally strong Sd(a++) Cad cells are agglutinated by most sera. Sd^a antibodies are usually IgM and active at 20°C [1,2,15], although IgG anti-Sd^a has been identified [21,22].

Anti-Sd^a are not generally considered a transfusion hazard [1,15,21,23], though transfusion of Sd(a+) red cells to patients with anti-Sd^a may evoke a significant increase in IgG antibody titre [21]. Most survival studies with ^{51}Cr-labelled cells in patients with anti-Sd^a have shown insignificant reduction in red cell survival, even when Sd(a++) cells were used [24,25]. A couple of HTRs, allegedly caused by transfusion of Sd(a++) cells to a patient with IgM anti-Sd^a, are reported [24,25].

31.3.2 Lectins and other non-human sources of anti-Sd^a

A single lectin in *Dolichos biflorus* extracts specific for terminal GalNAc agglutinates A_1, Tn-positive, and Sd(a++) red cells [26]. These reactions can be inhibited by GalNAc [4]. Sd(a++) cells express more determinants than A_1 or Tn cells, so an apparently specific anti-Sd(a++) can be produced by dilution of the lectin or by partial adsorption with A_1 or Tn cells; further adsorptions, or adsorption with Sd(a++) cells, removes all activity.

The seed lectins *Salvia horminum*, *S. farinacea*, and *Leonurus cardiaca* [27–29], and the snail lectins *Helix pomatia* and *H. aspersa* [6,30–33] agglutinate Sd(a++) cells and these reactions are inhibited by GalNAc. Sd(a++) and Tn specificity in the *Salvia* lectins can be separated by adsorption [27]. The anti-A_1 lectins *Phaseolus lunatus* and *P. limensis* do not agglutinate even the strongest of Sd(a++) cells [30–32].

Chicken serum contains a 'naturally-occurring' antibody reactive with Sd(a++) cells [34]. Separable anti-Tn and an antibody that agglutinates Sd(a++) cells were identified in the serum of the snake *Python sebae* [35].

31.4 Biochemistry

31.4.1 Sd^a in urine

In 1970, Morton and Terry [36] partially separated Sd^a-active material from human urine by ethanol precipitation. Ten years later, Soh *et al.* [37] showed that Sd^a activity is associated with Tamm–Horsfall protein, now usually called uromodulin, the most abundant glycoprotein in urine [38]. Uromodulin is secreted by the kidney and protects against urinary tract infection (UTI). It is composed of 70% protein and 30% carbohydrate, with eight potential *N*-glycosylation sites. Uromodulin in urine is a key anti-adherence factor for *Escherichia coli*, the principal cause of UTI. Mutations in *UMOD*, the gene encoding the uromodulin protein, lead to rare autosomal dominant diseases, referred to as uromodulin-associated kidney disease, and uromodulin is also a risk factor for chronic kidney disease [38].

Sd(a+) uromodulin contains between 1–2% GalNAc, whereas that from Sd(a–) individuals contains virtually none [37,39]. Sd^a-active uromodulin can be precipitated by *Dolichos* and *Helix pomatia* lectins and by human anti-Sd^a [39,40]. A pentasaccharide isolated from Sd^a-active uromodulin is shown in Table 31.2b [41,42]. This pentasaccharide strongly inhibits the reactivity of anti-Sd^a with Sd(a+) cells and of *Dolichos* lectin with Sd(a++) cells. The pentasaccharide is not present on uromodulin from Sd(a–) urine, whereas a tetrasaccharide lacking the GalNAc in β-linkage with Gal (Table 31.2a), is present on both Sd^a-active and -inactive glycoproteins [43]. Tetrasaccharides prepared by the cleavage of GalNAc or sialic acid residues from the Sd^a-active pentasaccharides lack Sd^a activity [44]. A mucin with high GalNAc content isolated from urine with *H. pomatia* lectin inhibited anti-Sd^a as effectively as uromodulin [45], so at least two substances in human urine carry the Sd^a determinant.

31.4.2 Sd^a on red cells

Most biochemical analysis of Sd^a on red cells has been carried out on the Sd(a++) cells of members of the Cad family, which have exceptionally high levels of Sd^a activity. SDS PAGE revealed that glycophorin A (GPA) and glycophorin B (GPB) from Cad red cells have an increase in apparent MW of 3 and 2 kDa, respectively, compared with normal red cells [46]. This results from the addition of a β-linked GalNAc residue to most of the disialotetrasaccharides characteristic of these glycoproteins [47] (Table 31.2c and d, and Chapter 3). The resulting pentasaccharide (Table 31.2d) inhibited *Dolichos* lectin and anti-Sd^a, and the altered GPA molecules bound to the lectin in affinity columns [46–48]. Red cells from the original Cad propositus had approximately 12 pentasaccharides per GPA molecule, cells from two other Sd(a++) individuals had 2–3 pentasaccharides per GPA molecule, and Sd(a+) and Sd(a–) cells had no pentasaccharides

Table 31.2 Some structures associated with different Sd^a phenotypes (for abbreviations see Table 2.4).

Tetrasaccharide and pentasaccharide isolated from uromodulin

(a) Sd(a–) and Sd(a+)

Galβ1→4GlcNAcβ1→3Gal
3
↑
NeuAcα2

(b) Sd(a+)

GalNAc β1→4Galβ1→4GlcNAcβ1→3Gal
3
↑
NeuAcα2

Tetrasaccharide and pentasaccharide from glycophorin A

(c) Sd(a–), Sd(a+), and Sd(a++)

Galβ1→3GalNAc—Ser/Thr
3 6
↑ ↑
NeuAcα2 NeuAcα2

(d) Sd(a++)

GalNAcβ1→4Galβ1→3GalNAc—Ser/Thr
3 6
↑ ↑
NeuAcα2 NeuAcα2

Sialosylparagloboside and Sd^a ganglioside

(e) Sd(a–), Sd(a+), and Sd(a++)

Galβ1→4GlcNAcβ1→3Galβ1→4Glc—Cer
3
↑
NeuAcα2

(f) Sd(a+), and Sd(a++)

GalNAcβ1→4Galβ1→4GlcNAcβ1→3Galβ1→4Glc—Cer
3
↑
NeuAcα2

[49]. Increased apparent MW of decay-accelerating factor (CD55) in Cad cells was also assumed to result from the presence of additional GalNAc residues [50].

Thin layer chromatography of Cad red cell membranes demonstrated an unusual profile characterised by reduction in the content of sialosylparagloboside (Table 31.2e), the major ganglioside in normal cells, and the presence of a new ganglioside of lower mobility [51]. The unusual ganglioside, which binds *H. pomatia* lectin and inhibits anti-Sd^a, represents sialosylparagloboside with an additional terminal β-GalNAc residue (Table 31.2f) [51,52]. The structure of the terminal pentasaccharide of this ganglioside is identical to that of Sd^a-active uromodulin shown in Table 31.2b [52]. Small quantities of this ganglioside were detected in membranes from Sd(a+), but not Sd(a–) red cells, and might represent the major Sd^a-active structure in Sd(a+) red cells [51].

31.4.3 The Sd^a glycosyltransferase and the gene that encodes it

The product of the *Sd^a* gene is a β1,4-*N*-acetylgalactosaminyltransferase that catalyses the transfer of GalNAc in β-linkage from a nucleotide carrier to an appropriate tetrasaccharide acceptor substrate molecule. Such enzymes have been detected in human urine from Sd(a+) but not Sd(a–) individuals [43,53], in human kidney [54], in human large intestine [55], and in guinea pig kidney [56,57]. Sd(a–) uromodulin was the best acceptor for the human urine enzyme, but the disialotetrasaccharides of GPA were also suitable acceptors [53]. Preparations from human kidney catalysed the transfer of GalNAc to sialosylparagloboside, but not to native GPA, although tryptic peptides of GPA were good acceptor substrates [51,54].

In 2003, two groups exploited the sequence of a mouse β1,4-*N*-acetylgalactosaminyltransferase gene to isolate human Sd^a cDNA from an expressed sequence tag database and from colon carcinoma (Caco2) cells [58,59]. The Sd^a gene, *B4GALNT2*, is on chromosome 17q23.1 and consists of at least 12 exons encoding two proteins of 506 and 566 amino acids, identical in their sequence with the exception of their cytoplasmic tail. Through alternative splicing of one of two exons 1 most of either a very long (exon 1_L) or very short (exon 1_S) cytoplasmic N-terminal domain is encoded; exon 2 encodes the membrane-spanning domain and the stem region; exons 3–11 the putative catalytic site. Transient transfection of Cos-7 cells with the catalytic domain of the enzyme produced a soluble form of the protein that catalysed the transfer of GalNAc to α2,3-sialylated acceptor substrates to form the GalNAcβ1-4(Neu5Acα2-3)Galβ1-R trisaccharide common to Sd^a antigens [58]. The short form of the enzyme is associated with a higher level of activity [60]. Northern blot analysis indicated that five transcripts were highly expressed in colon and to a lesser extent in kidney, stomach, ileum, and rectum [58].

31.5 Sd^a and gastrointestinal cancer

Sd^a antigen is expressed abundantly in normal gastrointestinal mucosae [14,61], but neither Sd^a antigen nor the Sd^a-transferase is detected in gastrointestinal cancer cells [14,55]. Transfection of colonic or gastric cell lines with the Sd^a-transferase cDNA resulted in increased cell surface Sd^a expression with concomitant loss of the E-selectin ligands sialyl-Le^x (sLe^x) and sialyl-Le^a (sLe^a) (Section 2.3.4), and significant decreased adhesion to activated human umbilical vein endothelial cells [62]. Kawamura *et al.* [62] propose that the Sd^a-transferase competes with fucosyltransferases, blocking expression of sLe^x and sLe^a biosynthesis and eliminating metastasis. From experiments on colorectal cancer specimens, Malagolini *et al.* [60] found that Sd^a and sLe^x are expressed on different proteins and consider that the effect described by Kawamura *et al.* [62] is an artefact of the experimental model and not applicable to colon cancer tissues.

31.6 Malaria

Sd(a++) cells of the Cad family are relatively resistant to invasion by the malarial parasite *Plasmodium falciparum* [63] (see Section 3.21.1). Sialic acid on glycophorin molecules is required by *P. falciparum* merozoites for invasion of the red cells and this sialic acid might not be available on the *O*-glycans of Cad cells because of chemical interaction with the acetyl group of the extra GalNAc residues [63].

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