25 I and i Antigens, and Cold Agglutination

25.1 Introduction

The subject of cold agglutination, as defined by Roelcke [1], involves, 'the occurrence and reaction of autoantibodies, reacting optimally in the cold (0°C) with red blood cells'. These autoantibodies are termed cold agglutinins. Low titre cold agglutinins are present in the sera of all adults. The most prevalent of these autoantibodies is a heterogeneous assembly of specificities called anti-I; antibodies that react with the red cells of almost all adults, but do not react, or at best react only weakly, with the red cells of neonates. Anti-I are generally weak, but potent examples may be found as autoantibodies in patients with cold agglutinin disease (CAD) or following *Mycoplasma pneumoniae* infection. In a rare inherited phenotype called adult i the red cells express very little I antigen. Alloanti-I is generally present in sera of i adults.

The i antigen has a reciprocal relationship with I. It is expressed only very weakly on the cells of most adults, but strongly on fetal, neonatal, and adult i red cells. Anti-i cold agglutinins may be haemolytic and are often present in sera of patients with infectious mononucleosis.

I and i determinants are carbohydrate structures carried on glycolipids and glycoproteins. They are internal structures of ABH-active oligosaccharides. The i-active structure is a linear chain of repeating *N*-acetyllactosamine units and is the precursor of the branched I-active structures. In normal development, i antigen is converted to I antigen by the branching of linear oligosaccharide chains. This conversion is catalysed by a β1,6-*N*-acetylglucosaminyltransferase, the product of *GCNT2*. Adult i results from *GCNT2* mutations.

I and i are not the product of alleles. Before *GCNT2* was recognised as being responsible for I biosynthesis, I and i comprised the Ii collection, but subsequently I (I1) formed the I blood group system and i (207002) remained in the Ii collection.

Although most cold agglutinins are Ii antibodies, many other specificities are known that detect determinants on carbohydrate structures of membrane glycoproteins and glycolipids (Section 25.9).

25.2 I (I1) and i antigens

Wiener *et al.* [2] were the first to give the name I to an antigen of variable strength detected by an autoantibody of very high titre. Reports of more anti-I soon followed and most reacted only very weakly with cord red cell samples [3,4]. I antigen strength varies from person to person and titration scores follow a normal distribution curve [5]. Treatment of red cells with proteases or with sialidase generally enhances expression of I and i antigens. The number of I antigen sites per red cell has been estimated at between 32 000 and 500 000 [6–8].

When Marsh and Jenkins [9,10] found two cold agglutinating antibodies that reacted strongly with cord cells

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and adult i cells, but only very weakly with normal adult cells, the opposite of anti-I, they named these antibodies anti-i. Some potent anti-i react quite strongly with adult cells, but their titre may be 1000 times higher with cord cells. Adult i cells generally have higher expression of i than cord cells. The number of i antigen sites has been estimated to be between 20 000 and 65 000 on cord cells and between 30 000 and 70 000 on adult i cells [8].

Expression of i is depressed on red cells of adults with an *In(Lu)* gene, a dominant inhibitor of various red cell antigens resulting from *EKLF* mutations (Section 6.8).

25.3 Biochemistry and molecular genetics

25.3.1 Structure of I and i

Ii antigens are carbohydrates and present on the interior structures of the complex oligosaccharides that carry ABH and Lewis antigens (Sections 2.2 and 2.3). Like the ABH-active structures, Ii determinants on red cell membranes are detected on three major classes of macromolecules:

1 *N*-linked oligosaccharides of glycoproteins;
2 simple glycolipids; and
3 complex glycolipids (polyglycosylceramides).

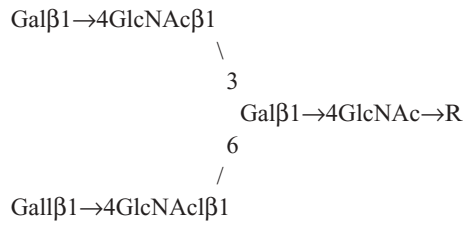
Ii determinants are either accessible or present as partially or totally masked antigens. They are not only detected on red cells, but on many other cell types and in various body secretions (Section 25.5). Recommended reviews on Ii biochemistry are found in [1,11–14] and some important papers on I and i biochemistry are [15–23].

I and i antigens are based entirely on Type 2, Galβ1→4GlcNAc (*N*-acetyllactosamine), chains. They may be concealed in ABH-active oligosaccharides and revealed by the stepwise removal of terminal monosaccharides by chemical degradation [15,16]. Anti-i detect linear structures, prevalent on fetal cells. The basic i structure is an unbranched polylactosamine comprising at least two *N*-acetyllactosamine units:

$$\text{Gal}\beta1 \rightarrow 4\text{GlcNAc}\beta1 \rightarrow 3\text{Gal}\beta1 \rightarrow 4\text{GlcNAc} \rightarrow \text{R}.$$

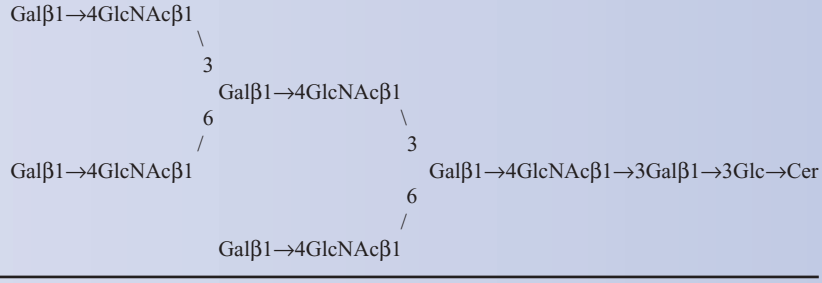
Paragloboside, which has a single lactosamine unit, is not i active; hexasaccharides of three lactosamine units are generally better inhibitors of anti-i than the tetrasaccharide shown above [20,23].

I antigen activity is associated with the branched structure



There are relatively few of these branched structures present on the glycolipids and glycoproteins of fetal cells because most branching occurs after birth [17]. Highly branched polyglycosylceramide molecules, not present in the membranes of fetal or neonatal cells, have a high level of I activity. Ii-active oligosaccharide chains on glycoproteins are generally more complex and linked through GlcNAc to asparagine. Examples of i- and I-active glycolipids are shown in Table 25.1.

Table 25.1 Examples of i- and I-active glycolipids.

i	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→Cer
I	

Much of the chemistry of Ii antigens has been worked out with the aid of numerous monoclonal autoantibodies that differ in their fine specificities. Although no two anti-I appear to be identical they can be subdivided into three general categories [21]:

1 those recognising the Galβ1→4GlcNAcβ1→6 branch (typified by the antibodies Ma. and Woj.);

2 those recognising the Galβ1→4GlcNAcβ1→3 sequence with branching (Step.);

3 those requiring both branches for activity (Phi.).

The basic i and I structures are usually further glycosylated. Ii antibodies vary in their ability to combine with determinants that have additional glycosylation. Terminal Gal residues may be fucosylated by H-transferase to produce an H-active structure, which, in turn, may act as an acceptor substrate for A- and B-transferases to catalyse the addition of GalNAc and Gal respectively (Section 2.3). In red cells of the rare O_h (Bombay) phenotype, in which terminal fucosylation of Ii-active oligosaccharides does not occur because of the absence of H-fucosyltransferase, I antigen expression is enhanced (Section 2.12.6). Destruction of H antigen by treatment of red cells with α1,2-fucosidase from *Aspergillus niger* elevates I expression [24]. Alternatively, the terminal Gal of Ii-active oligosaccharides may be sialylated. This prevents fucosylation and subsequent conversion to A- and B-active structures. These sialylated structures have moderate I or i activity, which is enhanced by sialidase treatment [11,22,25].

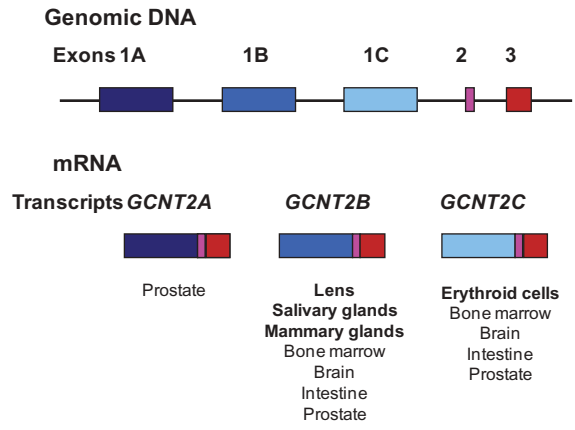


Figure 25.1 Organisation of *GCNT2*, showing the three exons 1 and exons 2 and 3. Below are the three transcripts and some examples of cells and tissues where they are expressed.

25.3.2 Biosynthesis of I

The biosynthesis of i requires the sequential action of β1,3-*N*-acetylglucosaminyltransferase and β1,4-galactosyltransferase. The i antigen is transformed into an I-active structure by the I branching enzyme, a β1,6-*N*-acetylglucosaminyltransferase [11,22,25].

Chinese hamster ovary (CHO) cells usually express i, but no I. Bierhuizen *et al.* [26] used a gene transfer procedure (similar to that described in Section 2.3.1.1 for isolation of an α1,2-fucosyltransferase gene) in order to clone cDNA encoding a β1,6-*N*-acetylglucosaminyltransferase from a cDNA expression library derived from human teratocarcinoma cells, which express large quantities of I-active branched structures. Following primary and secondary transfection, CHO cells expressing I antigen were isolated by panning with anti-I (Ma). The cloned cDNA contained an open reading frame and hydropathy analysis predicted a type II transmembrane protein with topology characteristic of a glycosyltransferase (Section 2.3). I activity of CHO cells transfected with the cloned cDNA appeared to result from branching of i-active *N*-acetyllactosamine chains at GlcNAcβ1→6Gal linkages, suggesting that the cloned gene, *GCNT2*, encodes the I-branching enzyme, a β1,6-*N*-acetylglucosaminyltranferase (IGnT). This was confirmed by the identification of mutations in *GCNT2* responsible for the adult i phenotype [27] (Section 25.4.3).

The coding sequence of *GCNT2* is divided over three exons, but there are three alternative versions of exon1 – exons 1A, 1B, and 1C – encoding 402, 400, and 402 amino acids, respectively [28,29] (Figure 25.1). Transcripts, active in different tissues, contain one of the three first exons plus exons 2 and 3. The transcript active in erythroid precursors contains exons 1C, 2, and 3 (*GCNT2C*). Expression of *GCNT2C* is almost restricted to bone marrow [28,29]. During *ex vivo* erythropoiesis there was a dramatic increase in expression of the *GCNT2C* transcript, with concomitant decrease in *GCNT2B* transcript [29]. The conversion of i to I during early infancy suggests activation of *GCNT2C* during that period. *GCNT2C* is regulated by the transcription factor CCAAT/enhancer binding protein α (C/EPBα) [30]. Dephosphorylation of Ser21 of C/EPBα stimulates transcription of *GCNT2C* and, ultimately, poly-*N*-acetyllactosamine branching [31].

Mapping of 39 expressed sequence tags (ESTs), one of which was identical to *GCNT2*, located *GCNT2* on chromosome 6p24-p23 [32].

Table 25.2 Frequency of adult i phenotype in various populations, determined by testing red cells with anti-I.

Population	No. tested	Adult i		References
		No.	Frequency (%)	
England, London	17 000	0		[3]
France	10 090	1	0.01%	[33]
USA, New York	22 000	5†	0.02%	[2]
African Americans, Detroit	8552	1	0.01%	[34]
Japan	1017	0		[35]
Taiwan	562	0		[36]

†Four black, one white.

25.4 Adult i and other rare phenotypes

25.4.1 Serology, inheritance, and frequency of adult i

Red cells of the rare adult i phenotype are rich in i antigen and but have very low levels of I. Anti-I is usually present. Black people with adult i (i_2) usually have less i and more I than white people (i_1) [3,4,9,10]. Anti-I from i_1 individuals can be adsorbed by i_2, but not i_1, cells.

Numerous family studies have shown that adult i is inherited as a recessive character. In some families with adult i members, red cells of obligate heterozygotes have reduced I and enhanced i compared with cells from unrelated controls [10].

Table 25.2 shows results of several population studies carried out by screening with anti-I. Molecular testing of 51 white donors revealed one heterozygote for an allele responsible for adult i (505A) [28] (see Section 25.4.3), which predicts an incidence of adult i phenotype of about 1 in 10 000.

Only two i adults, one white and one black, were found by screening 2.5 million serum samples from American donors for the presence of anti-I [37]. In another study, eight i adults were found as a result of testing sera of 22 700 pregnant women (0.035%) for anti-I, but only four were found among 135 100 non-pregnant patients (0.003%) [38]. These figures suggest that I depression may be a transient phenotype in pregnancy.

25.4.2 Association between adult i and congenital cataracts

In 1972, Yamaguchi *et al.* [39] reported four adult i propositi with a total of four i and seven I siblings. All eight adult i individuals had congenital cataracts (inherited lens opacity); all seven I individuals had normal vision. In Japan, adult i phenotype is almost invariably accompanied by cataracts [40,41]. Of 31 Japanese adult i donors and patients, 29 suffered impaired vision due to cataracts, and in the 11 families studied there was no recombination between Ii phenotype and cataracts [41]. Two of 92 Chinese in Taiwan with congenital cataracts had the adult i phenotype [36]. In people of European origin, adult i phenotype is not generally accompanied by congenital cataract [42], but the association has been reported in two Caucasian families [43,37], in four Arab and one Persian Jewish families from Israel [44,45], and in two Pakistani families [46].

25.4.3 Molecular basis of adult i and its association with congenital cataracts

Adult i results from homozygosity (or compound heterozygosity) for mutations in *GCNT2*, that include missense mutations, nonsense mutations, and a deletion of most of the coding region of the gene (Table 25.3). All prevent poly-*N*-acetyllactosamine branching in red cells and, therefore, conversion of i to I. Heterozygosity for a pair of *cis* mutations in *GCNT2* (1054G>A, Gly352Arg; 1184C>Tm Ala395Val) was associated with weak I and no i red cell expression [49], a phenotype previously reported as I_{int} [10].

Poly-*N*-acetyllactosamine branching appears to be important in maintaining transparency of the lens. Whereas only *GCNT2C* is expressed in erythroid cells, only *GCNT2B* is expressed in lens epithelial cells [28]. Mutations in exon 1C only inactivate the product of *GCNT2C* and cause adult i red cell phenotype, but leave

Table 25.3 Some *GCNT2* mutations responsible for adult i phenotype.

Mutation†	ISBT *GCNT2**	Exon	Amino acid change†	Cataracts	Ethnicity	References
243T>A	*01W.01*	1C	Asn81Lys	No	East Asian	[47]
505G>A	*01W.02*	1C	Ala169Thr	No	Caucasian	[28]
651delA	*N.07*	1C	Val244stop	No	East Asian	[48]
683G>A	*01W.03*	1C	Arg228Gln	No	Caucasian	[28]
816G>C, 1006G>A	*N.04*	2, 3	Glu272Asp, Gly336Arg	Yes	East Asian	[29]
935G>A		2	Gly312Asp	Yes	Persian Jewish	[45]
978G>A	*N.05*	2	Trp328stop	Yes	Arab	[44]
1043G>A	*N.01*	3	Gly348Glu	Yes	East Asian	[27,29]
1148G>A	*N.02*	3	Arg383His	Yes	East Asian	[27,29]
del exon 1B,1C,2,3	*N.06*	1B,1C,2,3	No protein	Yes	East Asian, Pakistani	[27,28,46]

†Nucleotides and amino acids counted for *GCNT2B* and its product [26]; *GCNT2C* has six more nucleotides and two more amino acids [28,29].

GCNT2B and, therefore, the lens unaffected. Mutations in exon 2 or 3, however, affect all three *GCNT2* transcripts, resulting in adult i and congenital cataracts [28,29].

The equivalent gene in mice is organised in a similar fashion to human *GCNT2*, but mice deficient in the common exon did not develop cataracts earlier than wild-type mice [50].

25.4.4 Other rare phenotypes

Red cells of seven of 5864 healthy blood donors in Mumbai reacted very weakly with anti-I, but did not react with anti-i [51]. The I antigen on these red cells was either weaker or of about the same strength as that on cord cells. Analysis of two large three-generation families showed the rare phenotype to be inherited, although the mode of inheritance appeared to be complex, depending partly on ABO group as all individuals with the unusual phenotype were group A_1 or A_1B [51,52]. Similar phenotypes with depressed expression of both I and i have been found outside India in A_1, B and O individuals [53,54].

25.5 Distribution of Ii antigens

Clausen and Hakomori [13] refer to I and i as histo-blood group antigens because, like ABH, they are not restricted to red blood cells, but are found on the surface of most human cells and on soluble glycoproteins in body fluids.

25.5.1 Body fluids

I antigen can be detected by haemagglutination-inhibition in milk with most anti-I [55–57] and in saliva with rare examples of anti-I [56,58,59]. A high-titred autoanti-I was inhibited to a varying extent by all 181 salivas tested, including saliva from neonates, with no correlation between degree of inhibition and the presence of ABH or Lewis substances [59]. With anti-I Sti., however, the concentration of I substance in saliva was a function of ABH secretor status, non-secretors having much greater quantities of salivary I substance than secretors [60,61]. This result is not surprising as non-secretor salivas lack the H-transferase responsible for fucosylation of the I-active structures. I antigen is generally difficult to detect in plasma by haemagglutination-inhibition [58,59], but one anti-I serum was inhibited by all 39 plasmas tested [62]. Unlike saliva, there is no relationship between I concentration in plasma and ABH secretor status [60–62].The average level of I antigen in plasma from neonates was 25% of that in plasma from adults [62]. Milk from four I women contained I substance and anti-I (or anti-HI) [57].

Saliva, milk, and plasma from i adults contained normal quantities of soluble I antigen, and milk from one i adult inhibited her own anti-I [55,59,61]. It is likely that these studies were carried out with body fluids from i adults of the European type, without cataracts and with mutations in exon 1C of *GCNT2*. The transcripts active in salivary and mammary glands are predominantly *GCNT2B* (Figure 25.1), explaining the presence of

secreted I in these i adults. It would be expected that body fluids from i adults with cataracts, and with mutations affecting exons 2 or 3, would be deficient in I.

Plasma from an individual with I– i– red cells had a reduced quantity of plasma I antigen [61].

There appears to be some i antigen present in saliva and milk, which can be detected by a minority of anti-i [55,58,63]. Haemagglutination by anti-i is inhibited by serum or plasma from most adults and from cord blood samples [58,62–64]. An i-active glycoprotein, with no detectable I, A, B, or H activity, was isolated from serum on an anti-i affinity column [64].

I and i antigens have been detected in amniotic fluid, urine, and ovarian cyst fluid [58,65,66].

25.5.2 Other blood cells

I and i are present on lymphocytes from cord and adult blood [67,68]. Anti-I and -i are potent cold lymphocytotoxins, effective at killing B and T lymphocytes [67,69,70]. Monoclonal anti-i were specific for subsets of B lymphocytes and for most pre-B cells in adult bone marrow [71]. Anti-I and -i are also cytotoxic for peripheral blood monocytes and macrophages, and about 25% of granulocytes; they are equally effective at killing granulocytes obtained from either maternal or cord blood samples [72,73]. Platelets tested with anti-I by flow cytometry produced a broad distribution curve, with the majority of platelets having a low density of I antigens compared with A and B antigens [74].

25.5.3 Other tissues

The gastrointestinal mucosae and the mucins they secrete have been studied in detail for Ii antigens, especially with anti-I (Ma), which detects Galβ1→4GlcNAcβ1→6. This I (Ma) structure is detected in gastrointestinal glycoproteins from ABH non-secretors, but not those from secretors, where it is concealed by the ABH immunodominant monosaccharides [75,76]. The expression of the three *GCNT2* transcripts in various cells and tissues are shown in Figure 25.1 and in references [28,29].

The i antigen is a characteristic of dividing cells present on a variety of cell types including lymphoblasts, fibroblasts, erythroblasts, and thymocytes [77].

25.5.4 Other animal species

Wiener *et al.* [78] tested red cells from over 160 different animal species and found that the distribution of Ii antigens cuts across taxonomic lines. Red cells of most adult primates, including chimpanzees and various monkeys, resemble human neonatal and adult i cells by lacking I and expressing i [78–80]. In tests on red cells of various non-primate species (cat, dog, guinea pig), there was no evidence of a developmental change of i to I [80].

25.6 Ontogenesis and oncogenesis

Ii antigens represent developmental antigens on red cells and in many other tissues. In red cells, and probably most other tissues, conversion begins around the time of birth. Fetal and neonatal red cells have very little I antigen expression and very few branched chains. The increase in I strength and concomitant reduction in i antigen expression reaches adult levels at the age of between 6 and 18 months [10,81] and coincides with the branching process of oligosaccharide chains [17]. High expression of i antigen is also a characteristic of immature and less differentiated adult cells [13]. Conversion from i to I is part of a continual process of erythroid differentiation; circulating 'young' red cells have higher i expression than 'old' cells [82]. The expression of the *GCNT2C* transcript increases dramatically during differentiation of erythroid progenitors from $CD34^+$ cells [29]. The i antigen is detected in the germinating layer of squamous epithelium, such as that from the intestine, whereas branching of the oligosaccharides occurs in the more differentiated cell layers and i is no longer present [83].

I and i often demonstrate altered expression on neoplastic cells and may be considered onco-developmental antigens (reviews in [12,84,85]). *GCNT2* expression, regulated by transforming growth factor (TGF) β, is closely related to metastasis in breast cancer. Enzyme activity enhances cell detachment, migration, and invasion [86].

25.7 I and i antibodies

25.7.1 Anti-I

25.7.1.1 Autoanti-I

The original anti-I was an autoantibody that caused AIHA [2] as were most subsequent examples of high titred anti-I agglutinins [5,87,88]. Potent cold reactive antibodies responsible for cold agglutinin disease (CAD) are usually of I specificity. CAD is reviewed in [1,68,89,90]. These antibodies are generally monoclonal, accounting for the heterogeneity of their specificity. They are usually IgMκ, but IgMλ and IgG autoanti-I occur [1].

They directly agglutinate I-positive red cells at 4°C with varying thermal amplitude, but are generally inactive above 30°C. One autoanti-I active at 30°C caused an acute HTR in a small child when two units of blood were transfused immediately after their removal from the refrigerator [91].

Transient, polyclonal, or oligoclonal autoanti-I may arise from infection, most typically by *Mycoplasma pneumoniae* [1,89]. At least 50% of patients with pneumonia induced by *M. pneumoniae* produce high-titred cold agglutinins during the three weeks after the onset of respiratory symptoms. It is likely that the pathogen modifies a sialylated I-active receptor making it immunogenic and that antibody is then produced to the modified structure [92–94]. Sialylated I determinants are recognised by anti-Sia-lb2 (anti-Gd) and anti-Sia-b1 (anti-Fl) cold agglutinins (Section 25.9) and these antibodies occur together with anti-I in the majority of cases of *M. pneumoniae*-induced cold agglutinin production [95,96]. Most people have weak, cold-reactive autoanti-I in their serum [97]. Analysis by adsorption and elution of 22 sera containing cold agglutinins that could not be clearly defined as anti-I or -i revealed that all contained a separable mixture of both antibodies [98].

25.7.1.2 Alloanti-I

Anti-I of fairly high titre is usually present in the sera of i adults. Although adult i red cells are not totally devoid of I, the anti-I can be referred to as alloanti-I as it does not react with autologous cells. These antibodies are almost invariably IgM and usually only active at low temperatures.

Rare examples may be haemolytic and have a thermal range up to 37°C [55] and some anti-I with a thermal range below 37°C can cause shortened survival of transfused I+ red cells [99]. Less than 1% of radiolabelled I+ red cells survived 15 minutes after injection into an i adult with anti-I, a clear indication that his antibody had the potential to provoke a dangerous transfusion reaction [100]. One anti-I became potentially clinically significant after transfusion of 6 units of I+ blood [101]. Judd [102] has described in detail how he would manage cold agglutinins in a transfusion setting.

25.7.1.3 Anti-I lectin

Lectin prepared from the gonads of *Aplysia depilans*, a marine mollusc (sea slug), behaved serologically as anti-I [103]. All other lectins resembling anti-I have specificities dependent on the presence of ABH or P antigens [103] (Section 25.7.6).

25.7.2 Anti-i

Alloanti-i has not been recognised. The first three examples of autoanti-i were from patients with reticulosis, one of whom died with AIHA [3,4]; a fourth example was in a patient with myeloid leukaemia [88]. Anti-i is a rare alternative to anti-I in CAD [89]. Anti-i are heterogeneous in specificity [8,16,77,104].

Anti-i is often found in the serum of patients with infectious mononucleosis and occasionally causes haemolysis. Estimates of the proportion of infectious mononucleosis patients with anti-i vary between 8 and 90%, but only very few develop haemolytic complications [79,105–107]. These antibodies may be IgM or, in some cases, may be IgG anti-i combined with IgM anti-IgG [79,106–111]. One of the IgM anti-i behaved like a Donath-Landsteiner antibody (haemolysis in the presence of complement following incubation of red cells in serum at 4°C and subsequent warming to 37°C) [110], as did an IgG anti-i detected in a patient with chronic paroxysmal cold haemoglobinuria [112].

The presence of anti-i is associated with immunodeficiency. Autoanti-i activity was detected in 50% of patients with Wiskott–Aldrich syndrome, a rare X-linked recessive immunodeficiency [71], and in 64% of patients with HIV/AIDS [113]. Infection with Epstein-Barr virus, the pathogen associated with infectious mononucleosis, is endemic in HIV/AIDS.

Maternal IgG autoanti-i can cross the placenta and has resulted in positive DATs with cord cells and mild neonatal jaundice [114,115]. Acute intravascular haemolysis in a patient with anti-i followed infusion of two units of blood deemed compatible by an immediate spin cross-match technique [116].

Monoclonal anti-i have been produced by heterohybridomas of mouse myeloma and human lymphoid cells [71,83].

25.7.3 Structure of I and i antibodies

A rat monoclonal antibody, 9G4, recognises a cross-reacting idiotypic determinant present on virtually all pathogenic anti-I and -i cold agglutinins and specifically inhibits haemagglutination by these antibodies [117,118]; it does not generally react with cold agglutinins of other specificities (anti-Pr, etc.) [119]. The epitope recognised by 9G4 is on an IgM heavy chain variable region derived from a single, highly conserved common gene segment V4-34 (previously called V_H4-21) [71,120–122]. Although all non-pathogenic monoclonal, naturally occurring anti-i also appear to be V4-34 encoded antibodies [123], this is not the case for non-pathogenic anti-I where V_H3

genes are often involved [124]. Site-directed mutagenesis analysis demonstrated that both Trp7 and an Ala23-Val-Tyr motif of framework region 1 (FR1) of V4-34 encoded immunoglobulin are required for I binding [125]. Based on the three-dimensional structure of an anti-I cold agglutinin [126], those amino acids essential for I antigen binding appear to form an extensive hydrophobic patch in FR1 [125].

A majority of IgM Rh antibodies also have the V4-34 encoded heavy chain segment. In addition to their Rh specificity, these antibodies have cold agglutinin activity directed against I/i antigens, although patient-derived cold agglutinins never detect Rh antigens [127,128]. The same amino acid residues in FR1 were found to be essential for both anti-D and anti-i reactivity of V4-34-encoded IgM anti-D [129].

25.7.4 Anti-I^T

Anti-I^T defines an antigen expressed strongly on cord cells, weakly on most adult cells, and very weakly on adult i cells [130]. I^T is expressed very weakly on red cells of Melanesians with South-East Asian ovalocytosis [131,132] (Section 10.9). Cold agglutinins were detected in 76% of Melanesians from Papua New Guinea; of the six analysed in detail, one was anti-I and five were anti-I^T [130]. Anti-I^T was found in 84% of Yanomama Indians of Venezuela [133]. Outside of those populations, rare examples of anti-I^T are often IgG and found in patients with Hodgkin's lymphoma or other lymphoproliferative diseases [134–136]. These antibodies may be active at 37°C and responsible for AIHA [134,136]. Six examples of IgG autoanti-I^T were not apparently clinically significant as judged by response to incompatible transfusion and *in vitro* survival studies [137,138]. IgM anti-I^T can also cause AIHA [139,140].

25.7.5 Anti-j

Cold agglutinins in two patients, which agglutinated adult and cord red cells, behaved as anti-Ii and were named anti-j [141]. These antibodies reacted with protease- and sialidase-treated red cells, but not with cells treated with endo-β-galactosidase, which cleaves Type 2 oligosaccharide chains. The antibodies were inhibited by linear (i) and branched (I) Type 2 structures. The two anti-j were unusual cold agglutinins as they were IgMλ molecules; they resembled most pathogenic anti-I and -i by expressing the 9G4 idiotype, a characteristic of antibodies encoded by a gene utilising a V4-34 sequence.

25.7.6 Ii antibodies and the H, ABO, and P groups

Considering the heterogeneity of Ii antibodies and the close biochemical association between Ii and the H, A, and B antigens, it is of no surprise that some antibodies appear to show a preference for I determinants with attached H, A, or B immunodominant monosaccharides. The most abundant of these antibodies is anti-HI, which does not react, or at least reacts only very weakly, with I-positive cells of the rare H-deficient phenotypes (described in Section 2.14.8). Some anti-I resemble anti-HI by giving stronger reactions with O or A_2 cells than with A_1 red cells [3,88,142]. Anti-Hi has also been reported [143]. Some anti-I react more strongly with A, B, or AB cells than with O cells. These antibodies have been called anti-AI (or -A_1I) [4,142,144,145], -BI [144,146–148], and -(A+B)I [149]). Anti-HILe^b (or -ILe^{b}H) agglutinated only O or A_2, I-positive, Le(a–b+) cells [150,151].

P1 antibodies that do not agglutinate P1+ cord or P1+ adult i red cells are called anti-IP1 [152]. An antibody reacting only weakly with cord and adult i cells, and not at all with p cells, was named anti-IP [153]. Anti-I^TP was responsible for fatal AIHA [154].

25.8 I and i antigens and disease

Red cells of patients with dyserythropoietic conditions often have elevated expression of i [73,155–158]. These conditions include thalassaemia, sickle cell disease, congenital dyserythropoietic anaemia II, Diamond–Blackfan syndrome, myeloblastic erythropoiesis, sideroblastic erythropoiesis, refractory anaemia, paroxysmal nocturnal haemoglobinuria, and acute leukaemias. Red cells with increased i antigen also appear in the circulation of people subjected to repeated phlebotomy [159]. I expression is not generally decreased in these conditions [156]. When red cell production is inadequate to meet demands, the proliferative stress on the erythroid precursors results in shortened maturation time before the red cell precursors appear in the peripheral blood. This could account, at least in part, for enhanced i expression.

The i antigen is detected on lymphocytes from adults and cord samples [67]. In chronic lymphocytic leukaemia (CLL) there is a reduction in lymphocyte i expression, as detected by some anti-i [160]. In acute lymphoid leukaemia, blast cells have as much i antigen as normal lymphocytes, whereas in acute myeloid leukaemia, blast cells have much less i antigen [161]. Lymphoblasts can be

distinguished from myeloblasts by their i expression in undifferentiated acute leukaemia and in chronic myeloid leukaemia in blast-cell crisis [162].

The late activation of *GCNT2* may have evolved as a mechanism to prevent ABO HDFN [31]. Despite the common occurrence of maternal-fetal ABO incompatibility, and the prevalence of IgG ABO antibodies, particularly in group O individuals, severe HDFN caused by ABO antibodies is rare (Section 2.14.3). This results, in part, from the linear structure of A- and B-active oligosaccharides on fetal and neonatal red cells, which prevent monogamous bivalency (the binding of both Fab arms) of IgG, and weakens the interaction between antibody and antigen.

25.9 Other cold agglutinins

In addition to anti-I and -i, cold agglutinins of numerous other specificities have been defined, mostly by Roelcke and his colleagues (for reviews see [1,163]). These antibodies and some characteristics of the determinants they define are listed in Table 25.4. All comply with the definition of cold agglutinins: autoantibodies to red cell antigens that react optimally in the cold (0°C) [1]. Apart from anti-j, they are usually IgMκ monoclonal antibodies.

The most abundant cold agglutinins, after anti-I and -i, are the Pr antibodies. These detect protease-labile determinants on the *O*-linked sialotetrasaccharides and sialotrisaccharides found predominantly on glycophorins A and B. These glycoproteins also carry blood group M, N, S, and s antigens and so Pr is described in Section 3.6.4. Pr antibodies are heterogeneous and have been subdivided. Anti-Pr_1, -Pr_2, and -Pr_3 are distinguished from each other by the effects of certain chemical modifications of their determinants [1]. Anti-Sa, like anti-Pr_2, detects an antigen present on glycophorin A and also on some gangliosides [179,180]. All of 15 anti-Pr contained Vκ light chains, with Vκ IV subgroup predominating [181]. A few anti-Pr and -Sa are IgAκ [165,182]. Pr antibodies may be associated with rubella infection [183] and Pr autoantibodies may cause severe AIHA (Section 3.6.4).

Anti-Sia-lb (anti-sialo-linear-branched, formerly anti-Gd) represents a heterogeneous collection of antibodies detecting protease-resistant, sialidase-sensitive antigens

Table 25.4 Antigens defined by cold agglutinins, showing reactions of the antibodies with adult (Ad), cord (Cd), adult i (i ad), papain- (Pap) and sialidase-treated (Sial) red cells.

Antigen	Ad	Cd	i ad	Pap	Sial	Comments	References
I	+	w	w	+	+	Branched; glycoproteins and glycolipids	See text
i	w	+	+	+	+	Linear; glycoproteins and glycolipids	See text
I^T	w	+	w	+	+		See text
j	+	+	+	+	+	Linear and branched; glycoproteins and glycolipids	[141]
$Pr_{1–3}$	+	+	+	0	0	*O*-glycans of glycophorins	*
Pr_a	+	+	+	0	+		*
Sa	+	+	+	w	0	*O*-glycans of glycophorins and gangliosides	[164,165] *
Sia-lb1 (Gd_1)	+	+	+	+	0	Sialylated linear and branched; glycolipids	[166,167]
Sia-lb2 (Gd_2)	+	+	+	+	0	Sialylated linear and branched; glycolipids	[166,167]
Sia-b1 (Fl)	+	w	w	+	0	Sialylated branched; glycolipids	[168,169]
Sia-l1 (Vo)	w	+	+	+	w	Sialylated linear; glycolipids	[169,170]
Li	w	+	+	+	0	Sialylated linear; glycolipids	[169,171]
Lud	+	w	+	w	0		[172]
Me	+	+		+	+	Enhanced by human milk	[173]
Om	+	+		+	+	Not enhanced by human milk	[174]
Ju	+	+		w	w		[175]
IgM^{WOO}	0	0	0	0	+	Type 1 chain	[176]
Rx	+	w	+	+	+	pH optimum 6.5; previously Sd^x	[177,178]

*See Section 3.5.4.

+, strong agglutination; w, relatively weak agglutination compared with +; 0, no agglutination.

located on gangliosides and created by the α2,3-sialylation of I- and i-active structures (branched and linear) on glycolipids [1,166,169]. Anti-Sia-lb1 require only a terminal sialic acid residue for activity (NeuNAcα2,3–); anti-Sia-lb2 also require subterminal Gal (NeuAcα2→3Galβ1–) [167]. Sia-lb antibodies also bind sialyl-Le^a (sLe^a) and sialyl-Le^x (sLe^x) structures (see Table 2.3) expressed on nucleated cells and in soluble cancer-related mucins [184].

Sia-b1 (Fl) is located on glycolipids with sialylated branched structures, whereas Sia-l1 (Vo) and Li are probably α2,3-sialylated linear structures on glycolipids [94,168–171,184].

Very little is known about the biochemistry of the Lud, Om, Me, and Ju antigens. Anti-Lud recognises α2,3-sialylated Type 1 chain sequences [1,185]. Activity of anti-Me (which is not related to anti-M^e of the MNS system), is enhanced by preheated human milk, but not by individual milk sugars [173]. Anti-Om activity is slightly reduced by human milk [174], which distinguishes it from anti-Me. The cold agglutinin IgM^{WOO}, which agglutinates sialidase-treated cells, but not untreated cells, recognises the Type 1 chain Galβ1→3GlcNAcβ1→3Galβ1→4Glc/GlcNAc [176]. Anti-Rx was originally named anti-Sd^x because it appeared to be inhibited by Sd(a+) but not Sd(a–) urine [177,186]. This was shown to be a non-specific effect, probably resulting from the extreme pH dependency of the antibody [178].

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