32 HLA (Human Leucocyte-Associated) Class I Antigens on Red Cells

32.1 Bg antigens

Class I products of the genes of the major histocompatibility complex (MHC), *HLA-A*, *-B*, and *-C*, were initially detected on leucocytes but have since been shown to be present on virtually all nucleated cells. Their primary function is the presentation of foreign antigens to cytotoxic T cells. The very highly polymorphic MHC is located on chromosome 6p21. (For a more detailed description see any textbook on immunology.) Mature human red blood cells are not nucleated and do not generally have easily detectable HLA antigens. On occasion, however, certain HLA antigens are expressed strongly enough on red cells to be detected by conventional blood grouping techniques. Because red cells are unsuitable for HLA phenotyping, HLA is not considered a blood group system and HLA antibodies that react with red cells (often called Bg antibodies) are generally regarded by blood group serologists as unwelcome contaminants of antisera.

The discovery that HLA antigens can be expressed on red cells came when Morton and his colleagues [1,2] showed that an assortment of rather indeterminate and troublesome blood group antigens on red cells, called the Bg (Bennett–Goodspeed) antigens [3–5], were strongly related to the HLA antigens on white cells. Bg^a showed almost complete concordance with HLA-B7 [1], Bg^b correlated with HLA-B17, and Bg^c with HLA-A28 [2]. Bg(c+) red cells may also react with anti-HLA-A2, which is known to cross-react with HLA-28 [6,7], and many other cross-reactions occur. Other HLA antigens have been detected on red cells: HLA-A10 and -B8 are often quite strongly expressed [2,8] and HLA-A9, -B12, and -B15 may also be detected [9]. HLA expression on red cells is always weaker than on white cells [2,8]. Many individuals never express HLA on their red cells even though their lymphocytes carry an appropriate antigen. HLA expression on red cells varies substantially between individuals of the same HLA phenotype and within an individual over a period of time [8]. Red cells may be positive for a particular HLA antigen for months or years and then become negative for that antigen for a similar period. HLA antigen strength on red cells does not appear to be inherited in a conventional manner and an HLA antigen may be detected on the red cells of a person, but not on those of either parent [1,5,10,11]. Radioimmunoassay and flow cytometry revealed that red cells of about 50% of blood donors bound monoclonal antibodies directed at monomorphic determinants on HLA class I molecules [12]. Individuals with HLA-B7 on their lymphocytes always express measurable red cell HLA antigens [12,13]. Red cell-reactive antibodies were detected in all sera containing cytotoxic anti-HLA-B7 [14].

It has been estimated that the number of HLA sites on red cells is in the range of 40–550, compared with 100 000 on T lymphocytes [15]. This explains why it is very difficult to remove haemagglutinating HLA antibodies from sera by adsorbing with red cells even though they are readily removed by white cells [1,2,14,16].

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Red cells of people with HLA-B7 have significantly more HLA Class I molecules on their red cells than those without HLA-B7 [15]. Marked increase in red cell HLA expression is also associated with systemic lupus erythematosus (SLE) and, to a lesser extent, rheumatoid arthritis [17]. HLA-B7 patients with infectious mononucleosis have greatly increased expression of the HLA-B7 on their red cells, which sometimes takes years to return to normal [18]. Certain red cell HLA antigens are elevated in patients with leukaemia and a variety of other haematological diseases [19]. Strong expression of red cell HLA in healthy subjects is rare, but has been reported [10,20].

Class I MHC molecules are heterodimers consisting of a 45 kDa α-chain with three extracellular domains, α_1, α_2, and α_3, non-covalently associated with an 11 kDa polypeptide, β_2-microglobulin. HLA antigenic determinants reside on the α_1 and α_2 domains. β_2-microglobulin is not encoded by the MHC on chromosome 6, but by a gene on chromosome 15. Immunoblotting of red cells with monoclonal antibodies to class I α-chain and to β_2-microglobulin gave bands of apparent 45 kDa and 11 kDa respectively, suggesting that red cell HLA antigens are carried on structures similar or identical to those on nucleated cells [21].

Red cell-reactive HLA antibodies are inhibited by plasma from individuals with the corresponding antigens on their white cells [8]. This, together with the finding that red cell HLA activity is destroyed by treatment of the cells with chloroquine, led to a proposal that HLA antigens might be adsorbed onto the red cell surface from the plasma rather than being intrinsic red cell antigens [22]. There is a weight of evidence against this, however, and supporting the thesis that HLA antigens on red cells represent remnants of those antigens present in greater quantity on nucleated red cell precursors [23,24]. Treatment of red cells with chloroquine only removes the β_2-microglobulin; the α-chain remains intact and loss of antigenic expression probably results from configurational changes [25]. Some HLA activity returns if chloroquine-treated cells are incubated in purified β_2-microglobulin [25]. Furthermore, the number of HLA Class I molecules on red cells decreases with aging of those cells, the opposite of what would be expected if the antigens were acquired from the plasma [15]. The level of expression of HLA Class I and β_2-microglobulin are reduced during erythroid differentiation [26]. Red cell HLA antigens are not destroyed by treatment of the red cells with papain, ficin, pronase, trypsin, chymotrypsin, or with the sulphydryl reducing agents AET and DTT [8,25].

32.2 Clinical significance of Bg antibodies

Antibodies to HLA antigens on red cells have generally been considered clinically benign, but there is now substantial evidence that such antibodies have been responsible for both immediate and delayed HTRs [27–31]. In one patient with anti-HLA-A2, -A28, -B7, and -B7 cross-reactive group a delayed HTR was followed by an acute HTR after transfusion of two more HLA-incompatible units [28]. The patient was successfully transfused with one red cell unit from an HLA-compatible donor. There have been reports of increased destruction of radiolabelled red cells by HLA antibodies, although this appears to affect only a minority of red cells in the circulation [10,32,33]. Anti-HLA-B7 implicated in an acute HTR gave a negative result in a monocyte functional assay [29].

HLA antibodies have not been implicated in HDFN.

HLA antibodies reactive with red cells are often a nuisance in antibody investigations, but the difficulties can be reduced by stripping the HLA antigens from the red cells with chloroquine [22] or EDTA/glycine-HCl [34].

References

1 Morton JA, Pickles MM, Sutton L. The correlation of the Bg^a blood group with the HL-A7 leucocyte group: demonstration of antigenic sites on red cells and leucocytes. *Vox Sang* 1969;17:536–547.

2 Morton JA, Pickles MM, Sutton L, Skov F. Identification of further antigens on red cells and lymphocytes. Association of Bg^b with W17 (Te57) and Bg^c with W28 (Da15,Ba*). *Vox Sang* 1971;21:141–153.

3 Buchanan DI, Afaganis A. The Bennett–Goodspeed–Sturgeon or 'Donna' red cell antigen and antibody. *Vox Sang* 1963;8:213–218.

4 Chown B, Lewis M, Kaita H. The Bennett–Goodspeed antigen or antigens. *Vox Sang* 1963;8:281–288.

5 Seaman MJ, Benson R, Jones MN, Morton JA, Pickles MM. The reactions of the Bennett–Goodspeed group of antibodies tested with the AutoAnalyzer. *Br J Haematol* 1967;13:464–473.

6 Nordhagen R, Ørjasæter H. Association between HL-A and red cell antigens. An AutoAnalyzer study. *Vox Sang* 1974;26:97–106.

7 Nordhagen R. Association between HLA and red cell antigens. VIII. Haemagglutinins in another series of cytotoxic anti-HLA-A2 sera. *Vox Sang* 1978;35:375–377.

8 Nordhagen R. Association between HLA and red cell antigens. V. A further study of the nature and behaviour of the

HLA antigens on red blood cells and their corresponding haemagglutinins. *Vox Sang* 1978;35:49–57.
9 Nordhagen R. Association between HLA and red cell antigens. IV. Further studies of haemagglutinins in cytotoxic HLA antisera. *Vox Sang* 1977;32:82–89.
10 van der Hart M, Szaloky A, van den Berg-Loonen EM, Englefriet CP, van Loghem JJ. Présence d'antigènes HL-A sur les hématies d'un donneur normal. *Nouv Rev Franc Hémat* 1974;14:555–563.
11 Nordhagen R. Association between HLA and red cell antigens. VI. Family Studies. *Vox Sang* 1978;35:58–64.
12 Rivera R, Scornik JC. HLA antigens on red cells. Implications for achieving low HLA antigen content in blood transfusions. *Transfusion* 1986;26:375–381.
13 Salama A, Mueller-Eckhardt G, Strauss B-E, Mueller-Eckhardt C. HLA-B7 on human red blood cells. Improved detection by a radioactive anti-IgG test. *Tissue Antigens* 1982;19:183–188.
14 Nordhagen R. Association between HL-A and red cell antigens. III. Studies of haemagglutinins in cytotoxic anti-HL-A7 and anti-HL-A5 related sera. *Vox Sang* 1975;29:23–35.
15 Botto M, So AK-L, Giles CM, Mason PD, Walport MJ. HLA class I expression on erythrocytes and platelets from patients with systemic lupus erythematosus, rheumatoid arthritis and from normal subjects. *Br J Haematol* 1990;75:106–111.
16 Nordhagen R. Association between HL-A and red cell antigens. II. Absorption and titration analyses. *Vox Sang* 1974; 27:124–133.
17 Giles CM, Walport MJ, David J, Darke C. Expression of MHC Class 1 determinants on erythrocytes of SLE patients. *Clin Exp Immunol* 1987;69:368–374.
18 Morton JA, Pickles MM, Darley JH. Increase in strength of red cell Bg^a antigen following infectious mononucleosis. *Vox Sang* 1977;32:27–37.
19 Morton JA, Pickles MM, Turner JE, Cullen PR. Changes in red cell Bg antigens in haematological disease. *Immunol Commun* 1980;9:173–190.
20 Nordhagen R. HLA antigens on red blood cells. Two donors with extraordinarily strong reactivity. *Vox Sang* 1979;37: 209–215.
21 Giles CM, Botto M, King MJ. A study of HLA (Bg) on red cells and platelets by immunoblotting with monoclonal antibodies. *Transfusion* 1990;30:126–132.
22 Swanson JL, Sastamoinen R. Chloroquine stripping of HLA A,B antigens from red cells. *Transfusion* 1985;25:439–440.
23 Harris R, Zervas JD. Reticulocyte HL-A antigens. *Nature* 1969;221:1062–1063.
24 Brown G, Biberfeld P, Christensson B, Mason DY. The distribution of HLA on human lymphoid, bone marrow and peripheral blood cells. *Eur J Immunol* 1979;9:272–275.
25 Giles CM, Darke C, Rowe GP, Botto M. HLA Class 1 (Bg) antigens on red cells of SLE patients: a serological study with polyclonal and monoclonal antibodies. *Vox Sang* 1989; 56:254–261.
26 Daniels G, Green C. Expression of red cell surface antigens during erythropoiesis. *Vox Sang* 2000;78(Suppl. 1):149–153.
27 Nance ST. Do HLA antibodies cause hemolytic transfusion reactions or decreased RBC survival? *Transfusion* 2004;43: 687–690.
28 Benson K, Agosti SJ, Latoni-Benedetti GE, Leparc GF. Acute and delayed hemolytic transfusion reactions secondary to HLA alloimmunization. *Transfusion* 2003;43:753–757.
29 Takeuchi C, Ohto H, Miura S, *et al.* Delayed and acute hemolytic transfusion reactions resulting from red cell antibodies and red cell-reactive HLA antibodies. *Transfusion* 2005;45: 1925–1929.
30 Han K-S, Hyun J, Kim D-C, Lee S-H. A case of delayed hemolytic transfusion reaction caused by anti-Bg^a antibody. *Vox Sang* 2008;95(Suppl. 1):198–199 [Abstract].
31 Pisacka M, Bolckova HT, Matejkova E, Kralova M, Sklenarova M. Anti-HLA-B7 reactive in column agglutination and eluted after absorption from RBCs. *Vox Sang* 2012;103(Suppl. 1):201 [Abstract].
32 Nordhagen R, Aas M. Association between HLA and red cell antigens. VII. Survival studies of incompatible red blood cells in a patient with HLA-associated haemagglutinins. *Vox Sang* 1978;35:319–323.
33 Panzer S, Mueller-Eckhardt G, Salama A, *et al.* The clinical significance of HLA antigens on red cells. Survival studies in HLA-sensitized individuals. *Transfusion* 1984:24:486–489.
34 Champagne K, Spruell P, Chen J, Voll L, Schlanser G. EDTA/glycine-acid versus chloroquine diphosphate treatment for stripping Bg antigens from red blood cells. *Immunohematology* 1999;15:66–68.