Vox Sanguinis (2002) 82, 207–210

ORIGINAL PAPER

Acute haemolytic transfusion reactions due to weak antibodies that *in vitro* did not seem to be clinically significant

B. Hoppe,[1] L. Pastucha,[2] A. Seltsam,[2] A. Greinacher[3] & A. Salama[1]

[1]*Institute of Transfusion Medicine, Charité Campus Virchow-Klinikum, Humboldt-Universität Berlin, Germany*
[2]*Department of Transfusion Medicine, Medizinische Hochschule Hannover, Germany*
[3]*Institute for Immunology and Transfusion Medicine, Ernst-Moritz-Arndt-Universität, Greifswald, Germany*

Vox Sanguinis

Background and Objectives In the present article, we report on two patients with acute haemolytic transfusion reactions (AHTRs), and whom we were unable to transfuse, owing to alloantibodies that *in vitro* did not seem to be clinically significant.

Materials and Methods The patients were a 67-year-old male and a 64-year-old female, both of whom developed antibodies to red blood cells (RBCs) after repeat blood transfusions. Serological analyses were carried out using standard techniques.

Results Both patients developed an AHTR of the intravascular type following blood transfusions. Serological re-examination revealed weakly reactive alloantibodies with anti-JMH specificity in one patient, and with unclear specificity in the second. Rechallenging the patients with 15–30 ml of packed RBCs caused AHTRs, and blood transfusion became impossible in both cases.

Conclusion Weak alloantibodies that *in vitro* do not seem to be clinically significant may cause severe AHTRs.

Received: 7 December 2002, revised 8 February 2002, accepted 12 February 2002

Key words: acute haemolytic transfusion reactions, JMH.

Introduction

Alloantibodies to high-frequency antigens (Ab-HFA) react with red blood cells (RBCs) of almost all individuals. However, the clinical significance of these antibodies is variable and depends on the antigen involved. While some antibodies such as anti-Vel and anti-Tj^a are known to have the potential for causing acute haemolytic transfusion reactions (AHTRs), many other antibodies are benign or thought to be only clinically significant in a subset of patients. The so-called high-titre, low-avidity antibodies (HTLAs) usually produce only weak reactions in the indirect antiglobulin test (IAT) and, in almost all cases, do not cause haemolytic transfusion reactions [1]. In this article, we report on two patients who developed an AHTR owing to the presence of alloantibodies exhibiting serological characteristics of the class formerly called HTLA.

Correspondence: Prof. Dr A. Salama, Institute of Transfusion Medicine, Charité Campus Virchow-Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany
E-mail: abdulgabar.salama@charite.de

Patients

Patient 1

A 67-year-old man was admitted for elective aorto-coronary bypass surgery. The patient received two packed RBC units without any complications during surgery. The serological crossmatches and the autocontrol were negative. One month later two RBC units were given during an emergency operation. The compatibility tests performed during the latter blood transfusion showed only weak reactions in the IAT. Immediately after this transfusion, however, the patient developed macrohaemoglobinuria, and his haemoglobin decreased from 11·1 g/dl to 7·7 g/dl without any sign of significant blood loss. Free plasma haemoglobin increased to 430 mg/l, lactate dehydrogenase (LDH) to 1199 U/l, and total serum bilirubin rose to 122 μM. The patient required further blood transfusions, but the administration of a test dose of 30 ml of packed RBCs resulted in an AHTR (lumbar and sternal pain, dyspnoea and haemoglobinuria). Family donors were not available and no compatible RBCs could be found. The patient had to be discharged from the hospital without a blood transfusion.

Patient 2

A 64-year-old woman suffering from a severe and therapy refractory osteomyelosclerosis developed alloantibodies (anti-Fy^a, -S, -Jk^a, and -E) following multiple blood transfusions. Two weeks after the last uncomplicated RBC transfusion, severe haemolysis (including lumbar pain and macrohaemoglobinuria) developed during transfusion of two Fy(a-), S-, Jk(a-), and E- RBC units that were negative in compatibility testing performed before transfusion. Serological re-examination with a blood sample drawn after transfusion revealed positive reactions to all RBCs tested in the IAT and a slightly positive autocontrol. Application of 30 ml of another Fy(a-), S-, Jk(a-), and E- RBC unit again resulted in an AHTR. Family donors were not available and no compatible RBCs could be found. The patient died 3 weeks later as a result of decompensated anaemia.

Methods

The direct antiglobulin test (DAT), the IAT and the cross-matches were performed by conventional tube testing or gel card test, as previously described, using the Micro-Typing System (DiaMed, Cressier sur Morat, Switzerland) and commercially available antiglobulin reagents: anti-C3d (Dako, Hamburg, Germany); and anti-IgG, anti-IgM and anti-IgA (Biotest, Dreieich, Germany) [2]. Antibodies were identified using a large panel of rare test cells negative for high-frequency antigens (see the Results). A panel of serum samples containing antibodies to such antigens was used to type the RBCs of the patient for high-frequency antigens. RBC-bound antibodies were eluted using the RBC acid-elution system, R-E-S (Immucor, Rödermark, Germany). IgG subclasses of antibodies were estimated using IgG subclass-specific antiglobulin reagents (anti-human subclass set; CLB, Amsterdam, the Netherlands). All reagents were used according to the manufacturer's instructions.

A modified monocyte monolayer assay (MMA) was used to test the *in vitro* RBC destruction potential of the antibodies [3,4]. In brief, monocyte monolayers were prepared by incubating mononuclear cells on slides at 37 °C for 1 h. Non-adherent lymphocytes were removed. RBCs were incubated with serum in the presence or absence of complement (fresh AB serum) at 37 °C for 1 h. Human AB serum was used as a negative control, and anti-D (Partobulin™; Immuno, Heidelberg, Germany) was used as positive control. Sensitized and washed RBCs were added to the monolayer and incubated for 1 h at 37 °C. The slides were then rinsed to remove non-adherent RBCs and the cells were stained with Wright/Giemsa. The slides were examined by light microscopy, and the frequency of monocytes with phagocytosed red cells was estimated.

Results

Serological findings

Patient 1

Subsequent compatibility testing with blood samples drawn after transfusion revealed weak agglutination in the IAT and a slightly positive autocontrol. The DAT was positive for IgG_1 and C3d. The patient's serum was weakly reactive (score 1+ to 2+) with all routine RBC panels tested. This reactivity disappeared in RBCs treated with ficin or papain, but not in those treated with neuraminidase, trypsin, α-chymotrypsin or pronase. Patient's serum in the presence of fresh AB serum did not lead *in vitro* to lysis of test RBCs. The patient's blood group was A1 ccD.Ee. Extensive RBC antigen and serum tests were performed (Tables 1 and 2). Interestingly, the patient's serum was not reactive with five of six JMH-test cells, and

Table 1 Results of red blood cell (RBC) antigen testing

Patient	RBC antigen
No. 1	A_1, D+, C-, c+, E+, e+, Rh:17[a],29[a], M+, N-, S+, s+, U+[a], En (a+)[a], K-, k+, Kp(a-b+), Js(a-b+), Ku+[a], Fy(a+b+), Fy:3[a], Jk(a+b+), Jk:3[a], Ge:2[a],3[a],4[a], Le(a+b-), P+, P_1+, Er(a+)[a], AnWj+, Di(a-b+)[a], Wr(a-b+)[a], Lu(a-b+), Lu:3,8[a], LW(a+), LW(ab+)[a], Ch(a+), Co(a+b-), Co:3[a], Sc:1,-2[a], In(b+)[a], Ok(a+)[a], Cr(a+), Tc(a+), Dr(a+)[a], IFC+[a], Yt(a+)[a], Do(b+), Gy(a+), Hy+, Jo(a+)[a], Vel+, Lan+, Jr(a+), At(a+)[a], EMM+[a], JMH-
No. 2	0, D-, C-, c+, E-, e+, Rh:17[a], hr(b+)[a], hr(s+)[a], M+, N+, S-, s+, U+ [a], En(a+)[a], K-, k+, Kp(b+), Js(b+), Fy(a-b+), Fy:3[a], Jk(a-b+), Jk:3[a], Ge:2,3,4[a], Le(a-b-), P+, P_1+, Er(a+)[a], AnWj+, Di(a-b+)[a], Wr(a-b+)[a], Lu(a-b+), Lu:8[a], LW(a+), Ch(a+), Rg(a+), Co(a+), Sc:1[a], Ok(a+)[a], Cr(a+)[a], Tc(a+)[a], Dr(a+)[a], Yt(a+)[a], Do(a+b+)[a], Gy(a+)[a], Vel+, Lan+, Jr(a+), At(a+)[a], EMM+ [a], JMH+, Kn(a+), McC(a+), Sl(a+)[a], Yk(a+)[a], Cs(a+), MER2+[a], Xg(a+)[a], Au(a+)[a], MAM+[a], PELL+[a]

[a]Antigens tested at the International Blood Group Reference Laboratory, Bristol, UK.

Table 2 *Results of serum/absorbate testing*

Patient	Test RBCs	
	Non-reactive	Reactive
No. 1	JMH- (*n* = 5), Tc(a-b-c-) (*n* = 1), Gy(a-) (*n* = 1), Hy- (*n* = 2), Jo(a-) (*n* = 1), Co(a-b-) (*n* = 1), i adult (*n* = 1)	JMH- (*n* = 1), Do(b-), Gy(a-) (*n* = 1), Hy- (*n* = 2), Jo(a-) (*n* = 1), Rh_{null}[a], Lu(a-,b-)[a], Lu:-3, Lu:-6[a], Lu:-8, Lu:-12[a], Lu:-13[a], Kp(b-), Js(b-), Jk(a-b-), Jk:-3, Ge:-2,-3,-4, Ge:-4, U-, En(a-), Kn(a-), McC(a-), Yk(a-)[a], Ch(a-)[a], Rg(a-), Jr(a-), Yt(a-), Lan-, Vel-, Co(a-), Di(b-), Dr(a-), PELL-[a], Tou-[a], i adult (*n* = 1), Er(a-), H-, Tj(a-), P-
No. 2	None	Vel-, Kn(a-), McC(a-), Kp(b-), Co(a-), Rg(a-), Js(b-), Cs(a-), Lu(b-), Yt(a-), AnWj-, Sd-.

[a]Antibody specificities were tested at the American Red Cross Blood Services, Los Angeles, CA, USA. Patients' serum (no. 1) and absorbate (no. 2) were assessed in an indirect antiglobulin test (IAT) with test red blood cells (RBCs) lacking the antigens mentioned.

his blood showed minor and major compatibility with one JMH-blood sample containing anti-JMH, so we consider anti-JMH to be present in the patient's serum. The patient's antibody titre was 1 to 8 before and after AHTR. Absorption and elution studies were positive, but the antibody could not be absorbed completely from the serum. No signs of additional allo- or autoantibodies were found. A modified MAA showed no increase in RBC phagocytosis after sensitization of different RBCs with the patient's serum as compared to negative controls.

Patient 2

This patient developed an AHTR immediately after receiving two matched RBC units. The DAT was weakly positive for anti-C3d, and the patient's serum drawn after transfusion reacted with all Fy(a-), S-, Jk(a-) and E- RBCs tested (score 2+ to 4+). The titre before and after application of 30 ml of another Fy(a-), S-, Jk(a-) and E- RBC unit was 1 to 64. The patient's serum did not lead *in vitro* to lysis of test RBCs in the presence of fresh AB serum. The eluate prepared from RBCs obtained after the transfusion was negative. Absorption of the serum with RBCs that were positive and negative for Fy^a, S, Jk^a and E, resulted in the elimination of antibodies to Fy^a, Jk^a, S and E, but not the new Ab-HFA. Moreover, the Ab-HFA could not be recovered in eluates. The patient's blood group was O ccddee. The results of antigen testing for this patient are shown in Table 1. The IAT performed using serum following threefold absorption with Fy(a+), S+, Jk(a+), E- RBCs was positive (score 2+ to 3+) for all E- RBCs tested (Table 2). The specificity of this antibody could not be defined. The antibody reactivity was resistant to papain, bromelain, trypsin, α-chymotrypsin and AET. Ultimately, a modified MAA showed no increase in RBC phagocytosis after sensitization of different RBCs with the patient's serum compared to negative controls.

Discussion

Both patients described above developed AHTRs owing to the presence of weak alloantibodies with a relatively high IAT titre, no increase in antibody concentration after the transfusion of incompatible RBCs, and incomplete absorption and elution of the antibodies. Additionally, they were directed against very-high-frequency antigens. The serum of patient no. 1 reacted with only one of the six JMH- RBCs tested. Thus, this antibody seems to be directed against a JMH variant. Indeed, a clinically significant anti-JMH has previously been described in one patient who developed a mild haemolytic transfusion reaction [5,6]. Unfortunately, in patient no. 2 we were unable to identify the specificity of the antibody. Whatever the specificity of the antibody of the latter case, the development of an AHTR could not be expected in these patients based either on their serological behaviour (including tests for *in vitro* haemolysis) or by the results obtained from the MMA of the antibodies used to induce *in vitro* phagocytosis of sensitized RBCs. It remains unresolved as to why the antibodies did not lead to cell phagocytosis *in vitro*. However, monocyte-based functional assays do not invariably reflect the clinical significance of alloantibodies [7,8]. Thus, the *in vivo* compatibility testing using small volumes of RBCs was the only way to accurately assess the clinical significance of the antibodies described here. Both patients repeatedly developed AHTRs associated with haemoglobinuria, suggesting that the haemolysis was predominantly intravascular. Such reactions are usually observed in situations where ABO-incompatible RBCs are transfused and are less commonly the result of other complement-activating antibodies, e.g. potent anti-Vel. The majority of immune antibodies against blood group systems such as Rh, Kidd, Duffy and Kell cause predominantly extravascular haemolysis that usually occurs within a few days after blood transfusion [9]. However, these antibodies

may also infrequently cause intravascular immune haemolysis [9–13]. The reason why the antibodies of the patients described above caused rapid *in vivo* RBC haemolysis or phagocytosis, but not *in vitro* destruction, is unknown. In this context, it must be noted that AHTRs can also occur without detectable antibodies in isolated cases [14–16]. Thus, it remains unclear whether the AHTRs described here were solely related to the antibodies or whether they could, at least in part, be caused by other, yet unidentified, factors.

Acknowledgements

We would like to thank Mrs Joyce Pool from the International Blood Group Reference Laboratory and Mr George Garratty from the American Red Cross Blood Services, Los Angeles, California, for their help in characterizing the antibodies to high-frequency antigens.

References

1 Rolih SD: High-titer, low-avidity (HTLA) antibodies and antigens: a review. *Transfus Med Rev* 1989; 3:128–139
2 Salama A, Berghofer H, Mueller Eckhardt C: Detection of cell–drug (hapten)–antibody complexes by the gel test. *Transfusion* 1992; 32:554–556
3 Nance SJ, Arndt P, Garratty G: Predicting the clinical significance of red cell alloantibodies using a monocyte monolayer assay. *Transfusion* 1987; 27:449–452
4 Neppert J, Mueller Eckhardt C: Improved monocyte cytotoxicity test. *Immunobiology* 1983; 165:415–420
5 Mudad R, Rao N, Issitt PD, Roy RB, Combs MR, Telen MJ: JMH variants: serologic, clinical, and biochemical analyses in two cases. *Transfusion* 1995; 35:925–930
6 Issitt PD, Anstee DJ: *Applied Blood Group Serology*, edn 4. Durham, North Carolina, USA, Montgomery Scientific Publications, 1998
7 Douglas R, Rowthorne NV, Schneider JV: Some quantitative aspects of the human monocyte erythrophagocytosis and rosette assays. *Transfusion* 1985; 25:535–539
8 Zupanska B, Thompson E, Brojer E, Merry AH: Phagocytosis of erythrocytes sensitized with known amounts of IgG1 and IgG3 anti-Rh antibodies. *Vox Sang* 1987; 53:96–101
9 Mollison PL, Engelfriet CP, Contreras M: *Blood Transfusion in Clinical Medicine*, edn 10. Oxford, United Kingdom: Blackwell Science Ltd, 1997
10 Patten E, Reddi CR, Riglin H, Edwards J: Delayed hemolytic transfusion reaction caused by a primary immune response. *Transfusion* 1982; 22:248–250
11 Pineda AA, Taswell HF, Brzica SM: Delayed hemolytic transfusion reaction. An immunologic hazard of blood transfusion. *Transfusion* 1978; 18:1–7
12 Salama A, Mueller-Eckhardt C: Delayed hemolytic transfusion reactions. Evidence for complement activation involving allogenic and autologous red cells. *Transfusion* 1984; 24:188–193
13 Molthan L, Matulewicz TJ, Bansal-Carver B, Benz EJ: An immediate hemolytic transfusion reaction due to anti-C and a delayed hemolytic transfusion reaction due to anti-Ce+e: hemoglobinemia, hemoglobinuria and transient impaired renal function. *Vox Sang* 1984; 47:348–353
14 Fudenberg H, Allen FH: Transfusion reactions in the absence of demonstrable incompatibility. *N Engl J Med* 1957; 256:1180–1184
15 Stewart JW, Mollison PL. Rapid destruction of apparently compatible red cells. *Br Med J* 1959; 1:1274–1275
16 van der Hart M, Engelfriet CP, Prins HK, van Loghem JJ: A haemolytic transfusion reaction without demonstrable antibodies *in vitro*. *Vox Sang* 1963; 8:363–370