Bone Marrow Transplantation, (1997) 19, 491–495

Late onset haemolysis and red cell autoimmunisation after allogeneic bone marrow transplant

FE Chen, I Owen, D Savage, I Roberts, J Apperley, JM Goldman and M Laffan

Department of Haematology and LRF Adult Leukaemia Unit, Royal Postgraduate Medical School, London, UK

Summary:

We reviewed the medical records of 293 patients who underwent allogeneic bone marrow transplants at the Hammersmith Hospital between 1989 and 1994. There was clinical evidence of an autoimmune reaction against red cells in nine patients. Seven of these patients had significant haemolysis; the other two had red cell autoagglutination. Haemolysis was resistant to treatment in three cases. Six of the nine patients had monoclonal Ig bands identified within 1 year of transplant. The autoimmune reaction could be classified broadly into two types: an early onset type (n = 4) beginning 2 to 8 months post-transplant associated with a cold antibody, and a late onset type (n = 5) beginning 6 to 18 months post-transplant associated with warm antibodies. The predominant antibody in the two categories described may reflect the kinetics of immune reconstitution post-transplant, since serum IgM levels typically return to normal 2 to 6 months post-transplant, while IgG levels may not reach normal levels until 12–18 months post-transplant. We speculate that unbalanced reconstitution of B and T cell lymphopoiesis post-transplant may favour emergence of oligoclonal proliferation and that some of the resulting antibodies may have activity against red cells.

Keywords: bone marrow transplantation; haemolysis; autoimmune; antibody

Autoimmune haemolysis can present as a primary condition or as a phenomenon secondary to conditions such as lymphoproliferative or autoimmune diseases. A few cases of autoimmune haemolytic anaemia (AIHA) in patients following allogeneic bone marrow transplant have been described in the literature, usually associated with other cytopenias.[1–7] At the Hammersmith Hospital, nine documented cases of clinically significant autoimmune reaction against red blood cells occurring after allogeneic BMT have been identified between 1989 and 1994. These cases came to light because of abnormalities of blood count, blood film, biochemistry or cross-matching. During this period 293 patients had been allografted, giving an incidence of 3.1% for allogeneic BMT patients, which is much higher than would be expected for non-BMT patients. The patients reported here fall into two groups, an early onset group associated with cold autoantibodies and a late onset group associated with warm antibodies. In the early group the antibodies occurred between 2 to 8 months and in the late onset group between 6 and 18 months post-transplant. We discuss the relationship between these phenomena and the role of factors such as GVHD, relapse, CMV and immunosuppression and also the association with the regenerating immune system following BMT.

Correspondence: Dr M Laffan, Department of Haematology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0NN, UK
Received 30 July 1996; accepted 19 October 1996

Patients and methods

From the 293 patients who underwent allogeneic BMT at the Hammersmith Hospital, we identified nine patients with documented anti-red cell autoantibodies (red cell autoimmunisation, RCAI). These patients were identified because they had clinically evident haemolysis (falling Hb with elevated bilirubin and reticulocytosis) or blood film abnormality (eg agglutination). Patient and BMT details are shown in Table 1. Two donor–recipient pairs had minor ABO mismatch, one a major and one a bidirectional ABO mismatch. All were matched for Rh D type. Haemolytic episodes attributable to ABO and Rh incompatibility are not included in this report. One of the cases (No. 4) has been reported previously.[2]

Conditioning

The details of conditioning and anti-GVHD prophylaxis are given in Table 1.

Serology

Standard serological methods were employed.[8] The direct anti-globulin test (DAGT) was performed using polyspecific antihuman globulin. When the DAGT was found to be positive, further testing with specific anti-IgG, IgM, IgA and anti-C3d reagents was carried out. Eluates were prepared with the ether technique. Eluates were tested by the indirect antiglobulin test (IAGT) using monospecific reagents as for the DAGT. Serum was tested for the presence of agglutinating antibodies, lytic antibodies and by the IAGT. Samples positive in screening tests were tested for specificity in titration using panels of red cells with known antigens. Agglutination titres were measured at 20°C and 37°C. Appropriate controls were used in all cases.

Table 1 Clinical details of the nine patients

Case	*Diagnosis*	*BMT type*	*Sex*	*Age*	*Conditioning*	*GVHD prophylaxis*	*Onset of AIHA (months post-BMT)*	*Serology*
1	CML	MUD	M	36	Cyclo, TBI	CsA, MTX, Campath	12	Warm
2	CML	SIB	M	56	Cyclo, TBI	CsA, MTX	8	Warm
3	AA	SIB	F	21	Cyclo	CsA, MTX	18	Warm
4	AA	SIB	M	18	Cyclo	CsA, MTX	6	Warm
5	CML	MUD	F	34	Cyclo, TBI	CsA, MTX, Campath	7	Warm
6	MPD	SIB	M	44	Cyclo, busulphan	CsA, MTX	4	Cold
7	CML	MUD	M	4	Cyclo, TBI[a]	CsA, MTX, Campath	8	Cold
8	CML	SIB	M	41	Cyclo, TBI	CsA, MTX	2	Cold
9	CML	SIB	M	31	Cyclo, TBI	CsA, MTX	2	Cold

CML, chronic myeloid leukemia; AA, aplastic anemia; MPD, myeloproliferative disorder; MUD, matched unrelated donor; SIB, sibling donor; Cyclo, cyclophosphamide (60 mg/kg × 2 days for CML, 50 mg/kg × 4 days for AA and MPD); bulsulphan 4 mg/kg × 4 days, CsA; cyclosporin A; MTX, methotrexate.
[a]Marrow treated *ex vivo* with Campath 1M.

Results

Retrospective analysis of the 293 patients who underwent allogeneic BMT at the Hammersmith Hospital between 1989 and 1994 revealed nine cases with clinically evident autoimmune reaction against red blood cells giving an incidence of 3.1%. Eight of these had a positive DAGT and the last (case No. 8) had cold agglutination evident on the peripheral blood film with circulating cold reactive anti-red cell antibody but negative DAGT and eluate. The results of the serological investigations are shown in Table 2. The patients fall into two distinct groups on the basis of serological findings at the time at which haemolysis developed:

Warm type RCAI

This group comprised five patients (cases 1–5). The RCAI generally arose later than group 2, ranging from 6 to 18 months post-BMT, with haemolysis in all cases. Haemolysis was most severe in case No. 2 who required 91 units of red blood cells in a period of 7 months. The serological findings were typical of warm-type AIHA and the relative specificities where present were also typical of AIHA arising in non-transplant settings, being primarily within the Rhesus system.

Cold type RCAI

This group comprised the four patients (cases 6–9) in whom the RCAI tended to develop early, at 2, 2, 4, and 8 months post-transplant, respectively. The serology obtained in these cases was typical of cold-AIHA and showed some anti-Pr and anti-I specificity similar to that of cold-AIHA in non-transplant settings. Mycoplasma and EBV serology were negative in all four members of this group. Cases 7 and 8 had no evidence of clinically significant haemolysis despite titres against untreated cells of 1/64 and 1/256 respectively at 20°C. Case 6 required 25 units of red blood cells in a 10-week period.

In the warm reactive group, despite any apparent specificity, all cases demonstrated a clear non-specific or pan-reactive component. Testing against Rh-null cells was not performed. No ABO antibodies were detected and all patients were matched for Rh D type, excluding this antigen as a basis for the IHA. In the cold reactive group the anti-

Table 2 The results of serological investigation of the nine patients

Case	*DAT*		*Antibody specificity*		*Blood group*	
	IgG	*C3d*	*Serum*	*Eluate*	*Donor*	*Recipient*
1	+	+	NS + anti-e	Nonspecific	B+	A+
2	+	+	Nonspecific	Nonspecific	O+	O+
3	+	+	NS + anti-D	anti-D, E + NS	A+	A+
4	+	+	NS + anti-C, c, e	anti-D, e	O+	O+
5	+	+	Nonspecific	Nonspecific	O+	A+
6	–	+	anti-I	Negative	O+	A+
7	–	+	Nonspecific	ND	O+	O+
8	–	–	anti-Pr	Negative	A–	O–
9	–	+	anti-Pr	Negative	AB+	AB+

Cases 1–5 were warm type and detected at 37°C. Cases 6–9 were cold type and detected by saline methods at 20°C. Patients 2 and 5 also had an allo anti-E (ie donor is E neg). All Rh specificities were enhanced by papainised cells. Patient 6 was lytic at 20°C in saline; patient 7 lysed papainised cells at 20°C; patients 8 and 9 showed no lysis.
ND = not done; NS = nonspecific.

I and anti-Pr were extremely unlikely to be alloreactive and one showed no detectable specificity. Overall, the serological findings were typical of a true autoimmune reaction. This was not identified in any of the donors or recipients before bone marrow harvest who all underwent routine serological screening. The proportion of major and minor ABO mismatch in this group of patients was not significantly different from the allogeneic transplant group as a whole (0.444 *vs* 0.477; $P = 0.85$ by χ^2).

Relation of RCAI to clinical course

The clinical course of these patients is shown in Table 3. In the cold reactive group the detection of the autoantibody coincided with the onset of GVHD in three cases (Nos. 6, 8 and 9). Case 8 presented with severe skin and gut GVHD concurrently with the detection of agglutination which resolved with control of the GVHD. In case 6 haemolysis was preceded 1 month earlier by GVHD of the liver. In case 9 it coincided with an exacerbation of skin GVHD and transient cytogenetic relapse. In this case, haemolysis resolved with improvement of GVHD and return to cytogenetic remission after reduction of the cyclosporin dose. Cases 7 and 9 also suffered reactivation of CMV infection at the time of RCAI. In the warm reactive group there was no associated GVHD with onset of haemolysis. The coincidence in the cold group may therefore simply represent an overlap in the time course of these two complications. Two patients in this group (Nos 1 and 2) also developed CMV reactivation at the time of haemolysis making a total of four patients with this association.

Cytogenetic analysis revealed that three of the six cases of CML (Nos 1, 2 and 9) had a cytogenetic relapse prior to the haemolysis. In patient 1 this was further associated with the appearance of some recipient type red blood cells.

In patient 6 there was transient reversion of the ABO group to recipient type but the haemolysis persisted after it returned once more to donor type. Relapse does not, therefore, appear to be an important association with RCAI. All patients had immunoglobulin studies performed at several points following BMT. Six of the nine cases (Nos 1, 2, 6, 7, 8 and 9) had a monoclonal band visible by routine immunoelectrophoresis at some stage within a year of the transplant. However, only in cases 1 and 7 was it detectable at the time of RCAI. Immunofixation of the paraprotein in cases 7 and 8 revealed an IgG and an IgM respectively, whereas in both cases serology suggested that an IgM was likely to be responsible for the haemolysis. Three patients (5, 6 and 9) went on to develop chronic GVHD.

Treatment and outcome

Warm type RCAI: All five cases had clinically significant haemolysis requiring treatment. Cases 1 and 3 responded to prednisolone and immunoglobulin and prednisolone and splenectomy respectively, and have subsequently done well

Table 3 Clinical course of the patients

Case	*RCAI type*	*Clinical association*			*Treatment*	*Clinical outcome*	*Peak bilirubin (μmol/l)*
		CMV	*GVHD*	*Relapse*			
1	Warm	+	–	–	Prednisolone Immunoglobulin	Haemolysis resolved Alive and well	27
2	Warm	+	–	+	Prednisolone Immunoglobuin	Resistant haemolysis CML relapse Died from pneumonitis	623
3	Warm	–	–	+	Prednisolone Splenectomy	Haemolysis resolved after splenectomy Alive and well	42
4	Warm	–	–	–	Immunoglobulin Azathioprine Splenectomy Total lymphoid irradiation	Haemolysis eventually resolved but required further donor marrow infusion Alive with no haemolysis	30
5	Warm	–	–	+	Prednisolone Immunoglobulin Splenectomy Vincristine	Also developed ITP Died from multiple thromboembolism	427
6	Cold	–	+	–	Prednisolone Immunoglobulin	Resistant haemolysis Died with GVHD and sepsis	694
7	Cold	+	–	–	Nil	Relapsed with lymphoid blast crisis Subsequently reinduced	27
8	Cold	–	+	–	Nil	No overt haemolysis Resolved	20
9	Cold	+	+	+	Prednisolone	Haemolysis resolved but died from GVHD liver	70

Note that in patient 5 recipient cells were detected 2 months after haemolysis developed but no relapse of CML. In cases 1, 2 and 3, recipient cells were detected 12, 2 and 9 months respectively before haemolysis. In patient 9 there was transient cytogenetic relapse at the time of haemolysis both of which resolved after the cyclosporine dose was reduced. CMV+ indicates detection of CMV by DEAFF testing.

red cells and platelets. Other reports of isolated cytopenias[21–23] suggest that a common antigen is not involved. These autoimmune phenomena are therefore variable in their breadth of antigen specificity and likely to arise from the oligoclonal reconstitution of both B and T cells following BMT.

Hows *et al*[10] suggested that cyclosporin A may dysregulate T cells and allow the development of autoantibodies as seen experimentally in murine syngeneic BMT. However, the role of CsA cannot be determined from this study as all allogeneic BMT patients received CsA for GVHD prophylaxis. GVHD appears to be associated with cold AIHA. This could be a relevant association because IgM levels generally normalise more rapidly with acute GVHD. However, this is also true of IgG levels and in this report there is no association between GVHD and onset of warm AIHA. The association may therefore not be significant.

Treatment of the haemolysis in these cases was particularly difficult and overall prognosis appears to be poor. Four of the nine patients died; none died as a direct result of the haemolysis but from associated problems such as sepsis and GVHD. Two patients with a cold antibody recovered without treatment although one had subsequent relapse of leukaemia and two patients recovered from the haemolysis on medical treatment alone with steroids and intravenous immunoglobulins. Five patients remained resistant to medical treatment and three of these required splenectomy to which only one responded, and the one who developed trilineage cytopaenia responded only after total lymphoid irradiation. Thus, three of the resistant cases died. As noted above, this picture is similar to that in previous reports.

RCAI after BMT is thus a problem of clinical significance more frequently than hitherto indicated by the literature. Warm type autoantibodies associated with haemolysis are relatively difficult to treat, while marked degrees of cold agglutination may be clinically benign. The temporal pattern of RCAI and the spectrum of antibody specificities contribute further to the picture of immune regeneration following BMT.

References

1 Graze PR, Gale RP. Chronic graft-versus-host disease: a syndrome of disordered immunity. *Am J Med* 1979; **66**: 611–620.
2 Bashey A, Owen I, Lucas GF *et al*. Late onset immune pancytopenia following bone marrow transplantation. *Br J Haematol* 1991; **78**: 268–274.
3 Klumpp TR, Caligiuri MA, Rabinowe SN *et al*. Autoimmune pancytopenia following allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1990; **6**: 445–447.
4 Klumpp TR. Immunohematologic complications of bone marrow transplantation. *Bone Marrow Transplant* 1991; **8**: 159–170.
5 Klumpp T. Autoimmune pancytopenia following bone marrow transplantation. *Br J Haematol* 1992; **80**: 135–136 (letter).
6 Klumpp TR, Block CC, Caligiuri MA *et al*. Immune-mediated cytopenia following bone marrow transplantation. Case reports and review of the literature. *Medicine Baltimore* 1992; **71**: 73–83.
7 Tamura T, Kanamori H, Yamazaki E *et al*. Cold agglutinin disease following allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1994; **13**: 321–323.
8 Dacie JV, Lewis SM eds. *Practical Haematology*. 7th edn. Churchill Livingstone: London, 1991.
9 Sniecinski IJ, Oien L, Petz LD, Blume KG. Immunohaemohematologic consequences of major ABO-mismatched bone marrow transplantation in man. *Transplantation* 1988; **27**: 441–446.
10 Hows J, Beddow K, Gordon SE *et al*. Donor-derived red blood cell antibodies and immune hemolysis after allogeneic bone marrow transplantation. *Blood* 1986; **67**: 177–181.
11 Sparkes RS, Sparkes MC, Gale RP. Immunoglobulin synthesis following allogeneic bone marrow transplantation in man. *Transplantation* 1979; **27**: 441–446.
12 Witherspoon R, Schanfield MS, Storb R *et al*. Immunoglobulin production of donor origin after marrow transplantation for acute leukemia or aplastic anemia. *Transplantation* 1978; **26**: 407–408.
13 van Tol M, Gerritsen E, de Lange GG *et al*. The origin of IgG production and homogeneous IgG components after allogeneic bone marrow transplantation. *Blood* 1996; **87**: 818–826.
14 Verladi A, Speranza C, Terenzi A. Acquisition of Ig isotype diversity after bone marrow transplantation in adults. A recapitulation of normal B-cell ontogeny. *J Immunol* 1988; **141**: 815–820.
15 Storek J, Saxon A. Reconstitution of B cell immunity following bone marrow transplantation. *Bone Marrow Transplant* 1992; **9**: 395–408.
16 Kent EJ, Crawford J, Cohen HJ, Buckley RH. Development of multiple monoclonal serum immunoglobulins (multiclonal gammopathy) following both HLA-identical unfractionated and T cell-depleted haploidentical bone marrow transplantation in severe combined immunodeficiency. *J Clin Immunol* 1990; **10**: 106–114.
17 Hammarstrom L, Smith CIE. Frequent occurrence of monoclonal gammopathies with an imbalanced light chain ratio following bone marrow transplantation. *Transplantation* 1987; **43**: 447–449.
18 Drobyski WR, Potluri J, Sauer D, Gottschall JL. Autoimmune hemolytic anemia following T cell-depleted allogeneic bone marrow transplantation. *Bone Marrow Transplantation* 1996; **17**: 1093–1099.
19 Bierling P, Cordonnier C, Fromont P. Acquired autoimmune thrombocytopenia after allogeneic bone marrow transplantation. *Br J Haematol* 1985; **59**: 643–646.
20 Minchinton RM, Waters AH. Autoimmune thrombocytopenia and neutropenia after bone marrow transplantation. *Blood* 1985; **66**: 752 (letter).
21 Koeppler H, Goldman JM. Auto-immune neutropenia after allogeneic bone marrow transplantation unresponsive to conventional immunosuppression but resolving promptly after splenectomy. *Eur J Haematol* 1988; **41**: 182–185.
22 Anasetti C, Rybka W, Sullkivan KM *et al*. Graft-versus-host disease is associated with autoimmune-like thrombocytopenia. *Blood* 1989; **73**: 1054–1058.
23 Panzer S, Kiefel V, Bartram CR *et al*. Immune thrombocytopenia more than a year after allogeneic marrow transplantation due to antibodies against donor platelets with anti-PlA1 specificity: evidence for a host-derived immune reaction. *Br J Haematol* 1989; **71**: 259–264.

without further haemolysis. Case 4 has been reported previously.[2] His haemolysis progressed to pancytopaenia and subsequently resolved after a stormy course requiring prednisolone, azathioprine, splenectomy and total lymphoid irradiation. Cases 2 and 5 failed to respond to treatment and died from pneumonitis and multiple thromboembolism, respectively.

Cold type RCAI: The clinical courses of these patients are diverse. In two patients (7 and 8) with cold type antibodies there was no evidence of clinically significant haemolysis. However, both patients clearly had free antibody in the serum despite the negative DAGT in case 8. They were detected because of marked red cell agglutination. In the other two cases of cold type autoantibody, haemolysis was associated with fatal GVHD. The relationship of the antibody to BMT in these cases may be questioned but in no case was there any clinical or serological evidence of mycoplasma or EBV infection to account for it. In addition, the first three (6, 7 and 8) had detectable paraprotein within 2 months of detection of the antibody. In case 9, a paraprotein was detected 18 months later. Furthermore, three cases (6, 8 and 9) were associated with GVHD and the fourth (7) with reactivation of CMV. Overall, the clinical picture is of a cold autoantibody in association with defective regeneration of the immune system. Cases 7 and 8 resolved spontaneously, 9 after prednisolone but 6 was resistant to prednisolone and immunoglobulin.

Discussion

Immune haemolysis may arise at several points following BMT. The earliest type is the immediate reaction between donor red blood cells and recipient antibodies in the presence of major ABO/Rh mismatch.[9] A similar reaction may occur with minor mismatch when donor isohaemoagglutinins are infused. Immune haemolysis may also arise in the first 3 weeks following BMT due to antibodies from the engrafting donor lymphocytes reacting against residual recipient red blood cells (minor ABO/Rh mismatch). In this type, the antibodies usually disappear after 6 weeks post-transplant.[10]

The cases of RCAI described in this report fall into a distinct third group arising longer than 8 weeks post-BMT with features of a true autoimmune disorder. Despite the apparent specificity of some antibodies, there is in most cases a non-specific or pan-reactive component which is typical of AIHA. When present, the apparent specificities of the antibodies were against antigens shared by both donor and recipient. These features suggest that it is extremely unlikely that an alloimmune reaction was taking place, although it was not possible to demonstrate that the antibodies were of donor origin because allotype data were not available. It is reportedly unusual to detect any recipient antibody more than 4 months post-BMT,[11,12] although longer periods have been reported.[13]

These cases reported here fall into two distinct groups: an early onset group with autoantibodies of the cold reactive type occurring between 2 and 8 months, and a late onset group with autoantibodies of the warm reactive type occurring between 6 and 18 months after BMT.

The characteristics and the separate presentation of the two groups parallel the reconstitution of the donor immune system. Following BMT, donor B cells begin IgM production which reaches normal levels within 2 to 6 months followed by IgG at between 3 to 18 months.[14,15] This may provide a rational explanation for the separation of the two groups. Paraproteins were detected in six of the cases which suggests that post-transplant B cell lymphopoiesis may be initially oligo- or monoclonal.[16] The development of AIHA at a time when immunoglobulin levels are returning to normal may therefore reflect the relative overgrowth of autoreactive B cell clones by a purely stochastic process. Paraproteins are well known to arise after BMT but the incidence in this group (six of nine cases) is higher than the 18 in 42 cases studied by Hammarstrom and Smith.[17]

Although it is not known whether the antibody production is T cell-dependent in these cases, it is presumed that, like B cells, T cell reconstitution may be initially oligoclonal and that the network of T cell regulation of B cell function may be patchy, and with regard to some antigens, poorly represented. The absence of certain T cell clones with subsequent dysregulation of B cells may allow production of autoantibodies[3] which would thus explain the number of 'autoimmune' disorders following BMT such as other cytopenias, thyroid disease, acquired haemophilia and others. It may be argued that among these, only the haematological cytopenias are truly autoimmune.

Clinically significant RCAI following BMT has been reported only rarely in the past but from evidence presented here and in a recent report by Drobyski *et al* it may be more common than previously thought.[4,18] Moreover, the detected incidence of 3.1% is likely to be an underestimate as some cases produce mild or minimal haemolysis and might have escaped clinical attention. Drobyski *et al* found the frequency of autoimmune haemolysis in a group of patients following T cell depleted allogeneic BMT to be 3%. Interestingly, the onset was generally later in these patients (7–25 months post-BMT) and only one patient had a cold type antibody. As in the present report, the clinical outcome was determined largely by associated post-transplant problems resulting in death of four of their seven patients. Two of their patients responded to immunosuppressive therapy, which again is in keeping with the cases reported here.

Tamura *et al*[7] in 1994 reported a case of cold agglutinin disease arising 3 weeks following BMT which showed anti-Pr specificity as did two cases in this report. It arose in conjunction with CsA therapy and grade I GVHD. The earlier onset than in the cases reported here may have been hastened by a preceding episode of pericarditis, presumed to be viral in origin. Interestingly, the cold agglutinin was monoclonal which suggests a primary dysfunction of B cells rather than of T cell regulation.

Several other reports describe cases with multiple cytopenias following BMT.[4,5,19,20] These might be taken to suggest the presence of a common haemopoietic antigen against which the antibody reacts. The cases reported here do not support this hypothesis; moreover in adult Evans syndrome it is rare for the same antibody to bind to both