EDITORIAL

ABO, alemtuzumab and allogeneic transplantation

This article has been corrected since Advance Online Publication and a corrigendum is also printed in this issue.

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ABO antigens are widely distributed carbohydrate histo-blood group antigens on red cells, platelets, vascular endothelium and epithelial tissues. Although ABO incompatibility is a major barrier to solid organ transplantation, 30%–50% of all allogeneic hematopoietic stem cell transplants (allo-HSCTs) are ABO mismatched.¹ ABO incompatibility has no impact on engraftment of stem cells or myeloid cells, which lack A/B antigens; however, ABO mismatching has been associated with delayed red cell engraftment, pure RBC aplasia, hemolysis, increased transfusion requirements, venous occlusive disease, severe GvHD and decreased survival.^{1–9}

Early studies have suggested that ABO incompatibility may be a particular risk factor in reduced-intensity conditioning peripheral blood HSCT due to the higher number of T- and B cells in those products.^{1,2} Most large studies examining ABO and HSCT outcomes have consisted predominantly of marrow allo-HSCT (Table 1).^{1,7–9} In this issue, Brierley *et al.*² report their 18 years of experience, using alemtuzumab-based reduced-intensity conditioning, including 504 (92%) peripheral blood HSCT. As expected, ABO mismatching had no impact on myeloid or platelet engraftment. Nor did the authors find evidence of clinically significant delays in red cell engraftment, using RBC transfusion during the first 100 days as a surrogate indicator. RBC utilization was essentially equivalent in matched and ABO-mismatched transplants, although there was a trend toward increased RBC transfusion in ABO major-incompatible HSCTs (Supplementary Table 2; $P=0.06$),² especially in patients requiring >35 units, and in patients with GvHD. This differs from marrow transplants, which have reported a 10- to 20-day delay in erythroid engraftment in ABO major-incompatible allo-HSCT.^{5–9} Unfortunately, the authors did not measure either the reticulocyte count, isoagglutinin titer or the appearance of donor red cells—more valid metrics of red cell engraftment. They also did not report the incidence of pure RBC aplasia, which is reported to occur in up to 24% of ABO major-incompatible HSCTs.^{1,4}

There is ongoing debate regarding the impact of ABO mismatching on GvHD and transplant outcomes (Table 1).¹ Consistent with many other European studies,^{1,5,7} Brierley *et al.*² found no significant difference in relapse-free or overall survival among ABO-mismatched HSCT. The authors also did not find any significant increase in stage II–IV acute GvHD in ABO-mismatched HSCT. Interestingly, the authors did find a novel association between ABO major incompatibility and the development of extensive chronic GvHD ($P=0.0036$), which has not been reported by others.^{1,6,7}

A major difference between prior studies and the results reported by Brierley *et al.*² is the routine use of alemtuzumab in the conditioning regimen (Table 1). Alemtuzumab is a humanized murine monoclonal against CD52, a relatively small glycosphosphatidylinositol-linked glycoprotein and a ligand for SIGLEC10. CD52 is widely distributed on both B and T cells, natural killer (NK) cells, NKT cells and dendritic cells, but is absent on hematopoietic stem cells.¹⁰ Alemtuzumab administration rapidly depletes B and T cells for several months due to a long circulating

half-life, whereas NK cells are relatively spared with recovery within 3–4 weeks.¹¹ Alemtuzumab-based regimens have been shown to be highly effective in reducing acute GvHD and graft failure, even in haploidentical transplants.¹⁰ In early studies, the major drawback of alemtuzumab-based regimens was the potential for autoimmune cytopenias and a high risk of CMV and other infections due to delayed B- and T-cell recovery.^{10,12} Subsequent protocols have reduced alemtuzumab dosage without compromising engraftment, GvL effect or GvHD prophylaxis.^{2,10,11} In the study by Brierley *et al.*,² the incidence of autoimmune cytopenias was 7.5%, which is comparable to other reports (2%–15%).¹² One major oversight was the absence of data regarding CMV and other infections. Over 60% of study patients were at potential risk for CMV infection. In addition, patients received different doses of alemtuzumab over the 18-year study period, including many patients who had received high-dose alemtuzumab.

Alemtuzumab-based reduced-intensity conditioning regimens may be particularly effective in ABO-mismatched transplants due to their effects on B cells and invariant NKT cells (iNKT), which strongly express CD52.^{13,14} iNKT cells are a small but critical T-cell population involved in the B-cell response to glycolipid antigens via CD1d, a major histocompatibility complex-like molecule.¹⁴ iNKT cells promote B-cell proliferation, B memory responsiveness, plasma cell generation and antibody production.^{14,15} Their relative absence in common variable immunodeficiency may contribute to both B-cell defects and hypogammaglobulinemia. Recent studies suggest that iNKT can stimulate B-cell proliferation and antibody production through cell–cell contact, probably due to the expression of stimulatory glycolipid self-antigens on B cells.^{14,15}

Recently, iNKT cells were identified as necessary for the development of allo-anti-A formation in a murine mouse model.¹⁶ Tazawa *et al.*¹⁶ were able to induce high-titer anti-A in wild-type mice after repeated injections with group A human red cells, whereas little or no anti-A was observed in nude mice, which are deficient in iNKT cells. Similar results were observed in CD1d-knockout mice and in wild-type mice pretreated with an anti-CD1d monoclonal antibody. Finally, monoclonal anti-CD1d successfully prevented anti-A formation in a group O, humanized NOD/SCID/yc^{null} mouse model. These results suggest that depletion or inhibition of iNKT cells could be highly effective in ameliorating the potential adverse effects due to ABO incompatibility. NKT cells are highly susceptible to alemtuzumab even at low doses, with depletion of NKT cells for up to 3 months.^{11,13}

In ABO major-mismatched transplants, loss of recipient ABO antibodies typically precedes the appearance of donor-type circulating red cells: a persistence of isoagglutinins can be associated with delayed red cell recovery and pure RBC aplasia.^{4,5} Several studies have observed accelerated isoagglutinin loss and red cell recovery among MUD transplants, and in patients with grade II–IV GvHD, suggesting a possible graft-versus-plasma cell effect.^{5,6,9} A small study of alemtuzumab in allo-HSCT showed a low incidence of pure RBC aplasia (2%) with appearance of donor red cells and loss of isoagglutinins within 2–3 months—consistent with clearance of preformed antibodies and suppression of endogenous antibody production.⁹

Table 1. Comparison of ABO-mismatched transplants among selected published studies

Study	Brierley et al. ²	Resnik et al. ³	Ozkurt et al. ⁴	Blin et al. ⁵	Kimura et al. ⁶	Seebach et al. ⁷	Stussi et al. ⁸	Mielcarek et al. ⁹
No. of patients	594	221	147	809	5,549	3103	562	1676
Country	England	Israel	Turkey	France	Japan	CIBMTR	Switzerland	USA
No. of institutions	Three	One	One	One	Multiple	Multiple	Two	One
HPC source (%)								
Bone marrow	8.2	9	—	54.3	100	100	93	100
Blood (PBSC)	91.7	91 ^a	100	33.7 ^b	—	—	7	—
Transplant type (%)								
MUD	61	22.6	8.2	65	100	—	9	55
MRD	39	70	91.8	35	—	100	91	45
Conditioning (%)								
Myeloablative	0	0	64	90	88	100	100	100
RIC	100	100	36	18.8	12	—	—	—
Alemtuzumab	100	—	—	—	—	—	—	—
ATG/OKT-3	0	100	64.6	1.3 ^c	8.3	—	9.6	0
ABO identical (%)	53	67.5	54.4	48.8	50.8	32.1	64.2	57.3
ABO-mismatch (%)	47	42.5	45.6	51.2	49.2	67.9	35.8	42.7
Major	21.5	25.3 ^d	17	41.6	24.9	13.9	17.4	18.7
Minor	19.6	17.2	20.4	9.5 ^e	21.7	14.5	15.3	17.8
Bidirectional	5.9	— ^d	8.2	—	2.6	3.7	3	6.1
HLA identical (%)	89.6	92	98	—	61.9	100	96	100 (?)
GVHD prophylaxis (%)								
Cyclosporin	96.2	100	100	97	48.2	100	100	100
Methotrexate	11.1	—	100	77.1	—	100	59.8	100
Mycophenolate	0.3	—	—	19.6	—	—	—	—
Tacrolimus	—	—	—	—	47.2	—	—	—
Clinical outcomes (MM versus matched)								
Delayed RBC engraftment	N	N	Y (major)	Y	Y	Y (major)	Y (major)	Y
Pure RBC aplasia (%)	—	1.2	24	5	—	—	20	—
Acute GvHD (II–IV)	Equivalent	Equivalent	Equivalent	↑ MM –PBSC (83% versus 63%)	↑ (Minor)	Equivalent	Equivalent	Equivalent
Graft failure	—	1.1%	3.0%	—	Equivalent	—	—	—
Overall survival	Equivalent	Equivalent	↑ (Minor)	Equivalent	↓ (Major, minor)	Equivalent	↓ (Bidirectional)	Equivalent
NRM	Equivalent	↑ (Minor)	↑ (Minor)	—	↑ (Major, minor)	Equivalent	—	—

Abbreviations: —, no data or discussion; HPC, human progenitor cell; MM, ABO-mismatched; MRD, matched-related donor; MUD, matched unrelated donor; NRM, non-relapse mortality; RIC, reduced-intensity conditioning. ^aEight patients received both bone marrow and PBSC. ^bTwelve percent of patients received cord cells (not shown). ^cSubanalysis of outcomes in 154 MUD patients, including 102 patients that received ATG. ^dPatients with bidirectional mismatched transplants included with ABO major incompatible transplants. ^ePatients with ABO minor- and bidirectional-mismatched transplants analyzed as a single group.

In contrast, ATG appears to have little impact on either isoagglutinin titers or NKT cells. Blin *et al.*⁵ and Ozkurt *et al.*⁴ found no difference in isoagglutinin levels or red cell engraftment in patients who received ATG. Furthermore, Mielcarek *et al.*⁹ reported a rapid rebound and prolonged persistence of isoagglutinins in patients receiving pre-transplant plasma exchange (ABO titers > 1:16), despite routine use of ATG and methotrexate. ATG does contain antibodies reactive with NKT cells, including iNKT cells, although activity appears to be weak based on antibody clearance.¹⁷ In mice and humans, ATG leads to only mild decreases in iNKT cells (9%) compared with other lymphocyte populations, leading to a 200-fold relative increase in circulating iNKT cells.¹⁸ As a consequence, there is increasing interest in the use of alemtuzumab over ATG in ABO-mismatched solid organ transplantation, with encouraging results. The results reported by Brierley *et al.*² suggest that alemtuzumab may also be superior to ATG in ABO-mismatched allo-HSCT, especially using peripheral blood stem cells. Transplant regimens using ATG in

ABO-mismatched peripheral blood allo-HSCT showed an increase in non-transplant-related mortality and decreased overall survival, despite a significantly higher percentage of matched-related donor transplants (70%–91% versus 39%, Table 1) and myeloablative conditioning.^{3,4}

ABO antibodies may also be cleared by adsorption via ABO-active substances present in the recipient or in transfused blood products. The latter could account for the apparent association between GvHD, isoagglutinin levels and red cell engraftment, as GvHD is often accompanied by increased blood utilization.^{2,4} ABO-soluble substances could also theoretically protect against severe GvHD by preventing binding of donor-derived antibody to ABO antigens on endothelium and epithelium. Finally, soluble ABO may be necessary for long-term tolerance in minor-incompatible and bidirectional transplants. In mice, circulating A-antigen/antibody complexes lead to a specific deletion of B splenocytes secreting anti-A.¹⁹ Indirect evidence for a similar process in humans can be observed in a humanized NOD/SCID/yc^{null} mouse model.²⁰

A neglected area of study is the role of blood group genetics on transplant outcomes, which may account for some of the differences between published studies. Cellular and soluble ABO expression is determined by *ABO*, *Secretor* (*FUT2*) and *Lewis* (*FUT3*) genes, with several alleles showing enzymatic, ethnic and geographic variation. In particular, Caucasians have a high incidence of A₂ (20%), a weak A subtype characterized by little or no A-antigen in the plasma, platelets, endothelium or epithelial tissues. As a consequence, A₂ individuals are considered 'group O' compatible in solid organ transplantation. In the American and European studies, it is likely to be that a significant proportion of A–O mismatched HSCT included A₂–O transplants, thereby diluting the potential impact of ABO incompatibility. In contrast, A₂ is extremely rare in Japan and this could explain the higher morbidity among both ABO major- and minor-mismatched HSCT reported by Kimura *et al.*⁶

Soluble ABO is also dependent on *Secretor*/*FUT2* and is influenced by *Lewis*/*FUT3*. The majority (70%–80%) of Americans and Europeans are 'secretors' with ABO-active glycolipids and proteins in the plasma and on mucosal epithelium. In Japan and other Asian populations, however, there is a high prevalence of weak *FUT2* and *FUT3* alleles. These individuals have diminished or absent soluble ABO (and Le^b) antigens in the plasma, especially among heterozygous individuals, although ABO expression is retained on gut mucosa (and hepatic endothelium). This might also contribute to the reported increase in severe hepatic and gastrointestinal GvHD among Japanese ABO-mismatched HSCT recipients.⁷ Similar to HLA, allelic variation among *ABO* and *FUT2* could subtly influence allo-HSCT and could be the subject of future studies.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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