Xenotransplantation 2006: 13: 166–170
Printed in Singapore. All rights reserved
doi: 10.1111/j.1399-3089.2006.00288.x

ABO histo-blood group antigen expression on the graft endothelium long term after ABO-compatible, non-identical heart transplantation

Koestner SC, Kappeler A, Schaffner T, Carrel TP, Mohacsi PJ. ABO histoblood group antigen expression on the graft endothelium long-term after ABO-compatible, non-identical heart transplantation. Xenotransplantation 2006; 13: 166–170. © Blackwell Munksgaard, 2006

Abstract: We recently reported a complete change in the endothelial ABO histo-blood group phenotype of a cardiac allograft long term after B to O mismatched transplantation. In the context of the current controversy on graft recolonization with recipient endothelial cells and its importance in the development of immunological unresponsiveness, we monitored the expression of endothelial ABH histo-blood group antigens of 10 ABO-compatible, non-identical cardiac allografts over an observation period of at least 30 months. ABH antigens as well as markers for endothelial cells, erythrocytes and thrombocytes were investigated retrospectively by immunohistochemistry using monoclonal antibodies on sections of formalin-fixed, paraffin-embedded biopsies and were evaluated semi-quantitatively by microscopy. In contrast to our earlier finding of the change in the endothelial ABO histo-blood group phenotype long term after ABO-mismatched transplantation, we could not confirm this change in 10 compatible but non-identical cases.

Simon C. Koestner,[1] Andreas Kappeler,[2] Thomas Schaffner,[2] Thierry P. Carrel[1] and Paul J. Mohacsi[1]

[1]Swiss Cardiovascular Center Bern and [2]Institute of Pathology, University Hospital (Inselspital), Bern, Switzerland

Key words: ABO blood-group system – endothelial cells – heart transplantation – immunohistochemistry

Abbreviations: Ab, antibody; AG, antigen; BIOT, biotinylated; DAB, 3,3-diaminobenzidine; D/R, donor/recipient; EC, endothelial cell; EDTA, ethylenediamine tetraacetic acid; EMB, endomyocardial biopsy; HTx heart transplantation; ISHLT, International Society for Heart and Lung Transplantation; mAb monoclonal antibody; RBC, red blood cell.

Address reprint requests to Paul Mohacsi, MD, FESC, FACC, Clinic and Policlinic of Cardiology, University Hospital (Inselspital), CH-3010 Bern, Switzerland (E-mail: paul.mohacsi@insel.ch)

Received 10 September 2005
Accepted 5 October 2005

Introduction

Endothelial cells (EC) play a major role in the immunological processes involved after allogeneic organ transplantation, being an interface between the graft and the immune system of the recipient. The ABO histo-blood group antigens (AG) on the surface of EC [1] cause hyperacute rejection in the presence of preformed antibodies (Ab) after incompatible transplantation. Medawar [2] suggested in 1965 that the replacement of the endothelium by cells of the recipient (chimerism) could help the graft to survive in the host. Thereafter, numerous investigations [3–6] were done to test this hypothesis and showed controversial results. Further investigations aimed at describing chimerism as an opportunity of organ regeneration after injury [7] and studied replacement of several cell types in the same organ (cardiomyocytes, smooth muscle cells and EC).

Using immunohistochemistry, we were recently able to demonstrate a progressive and complete change from B to H phenotype in the accommodated graft of a 19-yr-old asplenic patient, 2 yr after an accidentally mismatched B to O heart transplantation (HTx) [8]. The long observation necessary to see this antigenic shift remained unexplained.

These results convinced us to proceed with our investigations using a similar methodology to investigate cardiac allografts that were implanted into ABO-compatible, non-identical recipients and reached at least 30 months' graft survival.

Materials and methods

Among 19 patients with ABO-compatible but non-identical HTx of 108 heart-transplanted patients from the cardiac transplant program at the University Hospital Bern (Switzerland), 10 cases were selected retrospectively because of their long observation time (at least 30 months). Table 1 shows the clinical features of each selected patient, including age, sex and histo-blood group of both recipient and donor.

For each patient, routine endomyocardial biopsy (EMB) samples had been collected during the post-transplantation observation period. They were obtained from the right ventricle with a French 5.4 bioptome [9] according to standard follow-up protocols. All samples had been fixed in 4% neutral-buffered formaldehyde and processed to paraffin blocks according to standard protocols. Hematoxylin & eosin stainings of all biopsies were used to assess the rejection grade according to International Society for Heart and Lung Transplantation (ISHLT) criteria [10]. From each patient, one biopsy was taken in the early post-transplant period (1 to 2 weeks) and three samples were taken late in the observation period for the evaluation of the expression of ABH AG on EC using immunohistochemistry. To this end, serial sections of paraffin-embedded tissue were cut at 2 to 3 μm, dewaxed and stained with the following Ab: *mouse-anti-A* monoclonal antibody (mAb) (clone 81FR2.2; DakoCytomation, Glostrup, Denmark), diluted 1 : 100, *mouse-anti-B* mAb (clone 3E7; DakoCytomation), diluted 1 : 40, *mouse-anti-H* mAb (clone 92FR-A2; DakoCytomation), diluted 1 : 20 and *mouse-anti-CD31* (clone JC/70A; DakoCytomation), diluted 1 : 20, to demonstrate EC. To exclude a possible antigenic transfer from erythrocytes or thrombocytes to the EC, we also stained samples with *mouse-anti-glycophorin A* (clone JC159; DakoCytomation), diluted 1 : 200 and *mouse-anti-CD42b* (clone MM2/174; Novocastra Laboratories, Newcastle-upon-Tyne, UK) Ab. To restore formalin-fixed AG (except for glycophorin A), dewaxed slides were pretreated either by boiling in citrate buffer (A antigen in a pressure cooker at 121°C for 10 min, H antigen and CD31 in a microwave oven), by boiling in ehtylenediamine tetraacetic acid (EDTA) in a microwave oven (CD 42b) or by digestion with Pronase E (Sigma, St Louis, MO) (B antigen) before the application of the primary Ab. Two different secondary Abs were used: *rb-a-mo IgM/BIOT* (Dako Cytomation) for A, B and H antigen stainings and *gt-a-mo Ig/BIOT* (DakoCytomation) for CD31, glycophorin A and CD42b stainings. Sections were developed in 3,3-diaminobenzidine (DAB, Sigma)—H_2O_2, counterstained with hematoxylin and mounted. Since the H antigen is often difficult to visualize using standard immunohistochemistry, we used for this AG an amplification technique based on the formation of complexes of DAB with biotinyl tyramide. Tissues with known ABH type were included as positive controls.

The density of vessels positive for A, B or H antigen was established by counting the number of positive capillaries, venules and arterioles per defined surface area at a magnification of × 400, using a light microscope equipped with an eyepiece with a 10 × 10 counting grid. The results were expressed in percentages of capillaries positive for either A, B or H antigens compared with the total amount of capillaries (defined by the density of capillaries positive for CD31).

Results

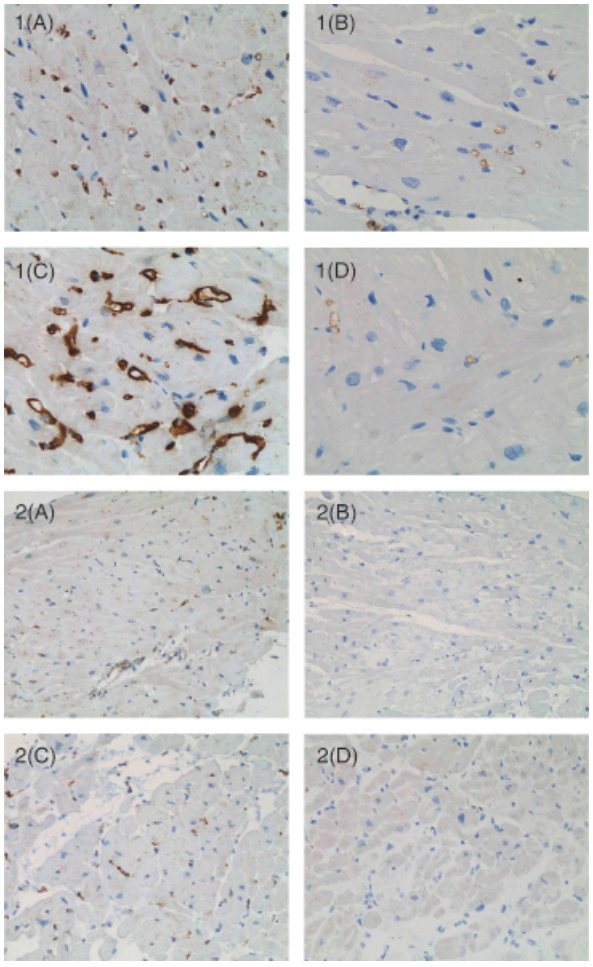
Five patients (#13, 40, 56, 66 and 73) showed no or very weak modification of their antigenic properties, keeping the AGs of the donor graft on all the

Table 1. Clinical features for each selected patient, including D/R blood group match, patient number (#), age, gender, total observation time (obs time) and percentage (%) of recipient antigen positive vessels

					% of vessels positive for recipient antigen[a]			
BG match	Patient #	Age at HTx	Gender	Obs time (months)	Early[b]	Late (months)		
0 to A	2	61	M	101	3.4	12.8 (96)	13.0 (98)	6.9 (101)
	17	55	M	102	5.2	4.8 (79)	4.5 (88)	1.5 (102)
	24	49	M	78	0.5	2.8 (65)	2.6 (73)	2.0 (78)
	37	47	M	65	0.0	1.5 (56)	3.9 (60)	1.8 (65)
	47	31	M	56	2.4	16.4 (40)	3.3 (50)	2.6 (56)
0 to B	56	48	M	31	0.0	0.0 (24)	0.0 (30)	0.0 (31)
	66	33	M	43	0.0	0.0 (35)	0.0 (41)	0.0 (43)
	73	62	F	37	0.0	0.0 (24)	0.0 (31)	0.0 (37)
A to AB	13	20	M	97	0.1	0.3 (85)	0.0 (93)	0.2 (97)
B to AB	40	58	M	65	0.0	0.0 (53)	0.0 (59)	0.0 (65)

[a]Compared with $CD31^+$ vessels (= 100%).
[b]Early = first month (< 2 weeks post-Htx).
HTx, heart transplantation.

biopsies we analyzed (see Fig. 1 and Table 1). All O to B transplants as well as the B or A to AB transplants belong to this group. The maximal post-transplant follow-up for these patients was 97 months. The five other patients with O to A compatible mismatch (#2, 17, 24, 37 and 47) showed weak to moderate presence of the AG of the recipient on a maximal post-transplant follow-up of 102 months. Figure 1 shows two biopsies of a patient 2 weeks and 97.5 months after O to A transplantation with moderate appearance of the recipient's AG several months after transplantation (Fig. 1(A–D)) and two biopsies of an other patient 1 week and 43 months after O to B transplantation without any appearance of the recipient's antigen (Fig. 2(A–D)).

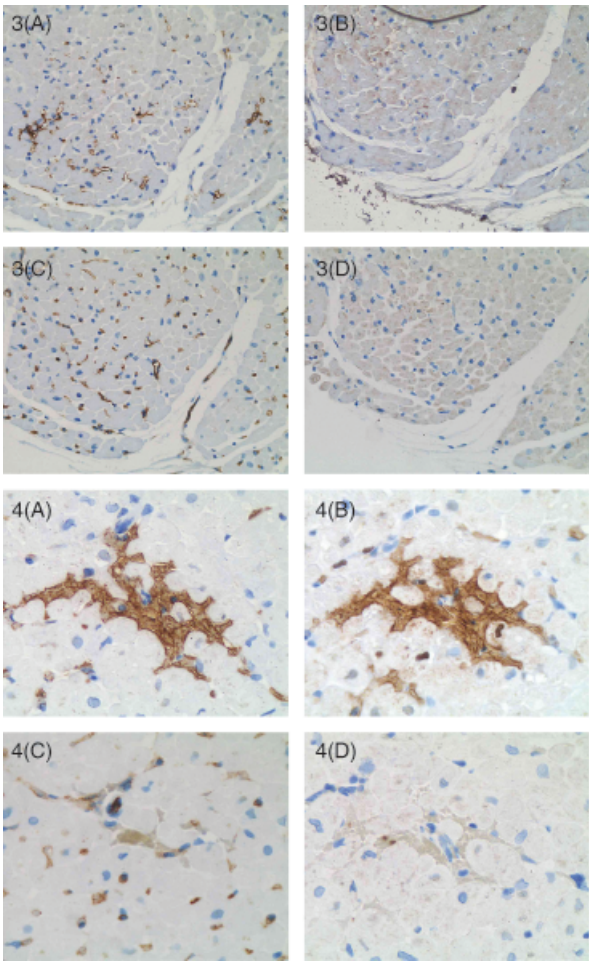


Figures 1 and 2. Expression of ABH antigens in two ABO-compatible non-identical transplanted patients (left side = anti-H; right side = anti-A (1B and 1D) and anti-B (2B and 2D)). Patient #2 (O to A): 1A and 1B = 2 weeks post-Htx; 1C and 1D = 97.5 months post-HTx. Patient #66 (O to B): 2A and 2B = 1 week; 2C and 2D = 43 months post-HTx. Despite the appearance of some vessels positive for the recipient's antigen early after O to A transplantation (1B), the graft of patient #2 remains strongly of blood group O (1C and 1D). The graft of patient #66 never showed any antigen of the recipient (2B and 2D).

The control stainings with glycophorin A (platelets) and CD42b (erythrocytes) allowed us to attribute the presence of ABH antigens only to EC (see Figs 3(A)–4(D)).

Discussion

We monitored the expression of endothelial ABH antigens of 10 ABO-compatible but non-identical cardiac allografts that reached a follow-up observational time of at least 30 months post-HTx. In contrast to our earlier finding of a complete change in the endothelial ABO histo-blood group phenotype long term after B to O mismatched transplantation [8], we could not confirm this change in 10 compatible but non-identical cases.



Figures 3 and 4. Figures 3(A) and 4(A) depict recipients antigens (anti-A) (anti-B not shown, since this match showed no occurrence of recipient antigens). 3B and 4B = anti-glycophorin A (red blood cell (RBC) antigen). 3C and 4C = anti-CD 31 endothelial cells (EC). 3D and 4D = anti-CD42b (thrombocytes). The positive-looking area in 4b represents anti-glycophorin A positive cells (= RBCs) of blood group A (positive in 4A = anti-A) from the recipient and not the endothelium of the graft (anti-$CD31^{-}$).

A change in the endothelial phenotype after transplantation of solid organs has been presumed to play a potential role in the acceptance of the allograft [2]. Several studies published on this topic used different rationales and technical approaches, and showed controversial results [3–6].

EC serve an important role in augmenting immune responses through enhanced expression of major histocompatibility complex class II Ag. Immune-mediated vascular injury associated with rejection requires re-endothelialization to restore vascular integrity. The origin of the reparative EC can be determined when ABO antigens expressed on these cells differ in the donor and recipient. Therefore, O'Connell et al. [6] stained serial EMB for ABO antigens in 34 (13%) compatible, non-identical cardiac allograft recipients of 268 cardiac transplant procedures. In 10 of these cases the allograft EC expressed the characteristics of the recipient (five partial and five complete) within 7.5 months after transplantation.

Because of the poor outcome of ABO-incompatible transplanted cardiac allografts, these types of investigation, especially in the long-term follow-up, are very rare. In infants transplanted in the first months of life and whose grafts further accommodated, perhaps as a result of delayed development of antigraft antibodies, no chimerism was observed [11].

Our present information suggests that, even though the methods used are similar and even though the observation time is quite long, no complete change in the graft antigenicity occurs in compatible non-identical ABO match. Limitations of our current results are as follows:

- Everybody working in the area of ABH tissue immunohistochemistry is aware of the tricky situation with respect to reliability and reproducibility of the results. In fact, before starting our investigations, we validated our immunohistochemical technique during a period of almost an entire year. The aim was to improve the signal strength by using an amplification method called PolyMICA® [8]. This amplification technique was especially important for the visibility of the H antigen. Our current work did not allow us to reuse the PolyMICA® technique, as this product is no longer available on the market. Therefore, we adjusted our amplification methodology, this time with biotinyl tyramide.
- The patchy pattern of the positive area illustrated by immunohistochemistry may raise further discussions about the reliability of our data. Therefore, we counted all positive vessels obtained on the entire surface of all biopsy specimens. Some investigators may argue that the patchy area may represent a location of immune-mediated vascular injury associated with rejection requiring re-endothelialization. These kinds of pathophysiological thoughts might be well taken. Why such a kind of patchy pattern occurs without any obvious correlation to cellular rejection and without any time dependency on the post-Htx period and the respective levels of Ab (data published elsewhere [12]), however, remains unanswered.

The above-mentioned problems led us to use different controls, namely CD31 (as an EC marker), CD42b (as a platelet marker) and glycophorin A (as an RBC marker).

Taken together, in ABO-compatible grafts, published data do not allow us to understand the rules for endothelial chimerism after transplantation, because these studies used different methods and were done in different clinical settings. The current work in our laboratory investigates whether and under which preferential conditions the possible change of ABO phenotpye may occur (e.g., after vascular injury resulting from ABO-incompatible organ transplantation).

Acknowledgments

This work was supported by the Katharina Huber-Steiner Foundation, Bern, Switzerland.

References

1. Nydegger U, Mohacsi P, Koestner S et al. ABO histo-blood group system-incompatible allografting. Int Immunopharmacol 2005; 5: 147.
2. Medawar P. Transplantation of tissues and organs: introduction. Br Med Bull 1965; 21: 97.
3. Lagaaij E, Cramer-Knijnenburg G, Van Kemenade F et al. Endothelial cell chimerism after renal transplantation and vascular rejection. Lancet 2001; 357: 33.
4. Thiele J, Varus E, Wickenhauser C et al. Regeneration of heart muscle tissue: quantification of chimeric cardiomyocytes and endothelial cells following transplantation. Histol Histopathol 2004; 19: 201.
5. Sedmak D, Sharma H, Czajka C, Ferguson R. Recipient endothelialization of renal allografts. Transplantation 1988; 46: 907.
6. O'Connell J, Renlund D, Bristow M, Hammond E. Detection of allograft endothelial cells of recipient origin following ABO-compatible, nonidentical cardiac transplantation. Transplantation 1991; 51: 438.
7. Quaini F, Urbanek K, Beltrami AP et al. Chimerism of the transplanted heart. N Engl J Med 2002; 346: 5.
8. Koestner S, Kappeler A, Schaffner T et al. Histo-blood group change of the graft from B to O after mismatched heart transplantation. Lancet 2004; 363: 1523.

9. Caves P, Schulz W, Dong EJ, Stinson E, Shumway N. New instrument for transvenous cardiac biopsy. Am J Cardiol 1974; 33: 264.
10. Billingham M, Cary N, Hammond M et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection. J Heart Transplant 1990; 9: 587.
11. West LJ, Pollock-Barziv SM, Lee KJ et al. Graft accommodation in infant recipients of ABO-incompatible heart transplants: donor ABH antigen expression in graft biopsies. J Heart Lung Transplant 2001; 20: 222.
12. Mohacsi P, Rieben R, Sigurdsson G et al. Successful management of a B-type cardiac allograft into an O-type man with 3½-year clinical follow-up. Transplantation 2001; 72: 1328.