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REVIEW ARTICLE

ABO-incompatible renal transplantation: From saline flushes to antigen-specific immunoadsorption-Tools to overcome the barrier

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On April 23, 1951, a 30-year-old woman received the first intentional ABOi (ABO incompatible) renal transplantation in Boston. At that time, it was commonly believed that intensely rinsing the graft to remove blood would be sufficient to overcome any immunological problems associated with blood type incompatibility. However, when the abovementioned patient and another ABOi transplant recipient died within a month, Humes and colleagues arrived at the same conclusion: "We do not feel that renal transplantation in the presence of blood incompatibility is wise." In the decades that followed, we learned that the oligosaccharide surface antigens representing the ABO-blood group antigens are expressed not only on erythrocytes but also on cells from various tissues, including the vascular endothelium. The growing gap between organ demand and availability has sparked efforts to overcome the ABO barrier. After its disappointing results in the early 1970s, Japan became the leader of this endeavor in the 1980s. All protocols are based on 2 strategies: removal of preformed antibodies with extracorporeal techniques and inhibition of ongoing antibody production. Successful ABOi renal transplantation became possible with the advent of splenectomy, new immunosuppressive drugs (e.g., rituximab, a monoclonal antibody against CD20), and extracorporeal methods such as antigen-specific immunoadsorption. This review summarizes the underlying pathophysiology of ABOi transplantation and the different protocols available. Further, we briefly touch potential short- and long-term problems, particularly the incidence of infectious complications and malignancies, that can arise with high-intensity immunosuppressive therapy.

Key Words Graft survival, Graft loss, Rejection, Preconditioning

INTRODUCTION

1. Mind the gap

The optimal treatment for most patients with chronic kidney disease stage 5/5D is successful renal transplantation, which, compared to dialysis, results in significantly lower mortality and morbidity rates. Despite intensive efforts promoting postmortem organ donation and the change in legislation that regulates organ donation in some countries, there is an ever-increasing organ shortage worldwide. Thus, this treatment is becoming increasingly difficult to implement. As a result, nearly 10% of patients in the European Community die while awaiting a suitable graft. Because of the shortage of donor organs, the number of transplantations involving living organ donors who are related to the patient is increasing steadily. However, about 20% of eligible living kidney donors are blood-group incompatible. Since blood group antigens are considered the most antigenic in the transplantation process, there is a high risk of hyperacute transplant rejection mediated by preformed blood groups antibodies.

2. The ABO system

The ABO system was introduced by the Austrian scientist Karl Landsteiner in 1900 [1]. The blood group designation is dependent on the presence or absence of A and B antigens on the surface of the red blood cells. ABO blood group antigens are oligosaccharide surface antigens expressed on erythrocytes, tissue cells, and in saliva and other body fluids. All blood group antigens contain the H-antigen as a common precursor. Expression of the A- or B-allele-encoded glycosyltransferase catalyzes the addition of specific carbohydrate determinants to the H-antigen. The H-antigen is the only ABO structure present in blood type O; expression of the O allele induces no functional glycosyltransferase [2]. The

ABO subgroups are determined by the density of A-, B-, or O (H)-antigen expression on the surface of red blood cells. A_1 is the most frequent A subtype in Europe; it has a surface expression of 1,000,000 antigens per red blood cell. A_1 has the highest expression rate and is distinguished from A_2, A_3, A_x, A_{end}, A_m and A_{e1} subtypes, which are significantly lower in antigenic surface expression [3, 4]. Similarly, the antigen density determines the B subtypes as well. The B subtype with the highest surface expression is type B, which has 700,000 antigens expressed on the surface of each red blood cell. B_3, B_x, B_m and B_{e1} express lower amounts of antigen on the surface [5]. Using the same system, AB can be subclassified into 9 different subtypes. Interestingly, serum antibodies are specific for other blood types. Blood type O contains antibodies against A and B; blood type A contains antibodies against B and vice versa; and blood type AB expresses both antigens but no antibodies. Interestingly, these antibodies are not present in newborns, but develop during the first year of life, presumably in response to food and environmental antigens [6]. The presence of preformed antibodies and the expression of blood group antigens on blood vessel endothelia are the basis for the initial paradigm that organ transplantation across blood group barriers is impossible.

3. Preformed antibodies and hyperacute rejection

Without preconditioning, transplantation across the ABO barrier will lead to hyperacute rejection within minutes (Fig. 1). The recipient's preformed "natural antibodies" react with the ABO carbohydrate antigens expressed on the vascular endothelial cells in the graft. Antibody binding leads to fixation and activation of complement, which induces endothelial cell activation and damage, and finally results in the formation of microthrombi and microhemorrhages (Fig. 1). The origin and function of "natural antibodies" remain obscure; they are produced without prior antigen exposure by a subset of B-cells expressing CD5 (CD5-negative B-cells are responsible for antigen-induced antibody production) [7]. In addition to the preformed antibody response, maximized immune activation occurs against the graft; the blood group antigens are considered the most antigenic in the transplantation process [8]. Therefore, patients require preconditioning to remove natural antibodies and to buffer the initial immune response until "graft-accommodation" has occurred (see below).

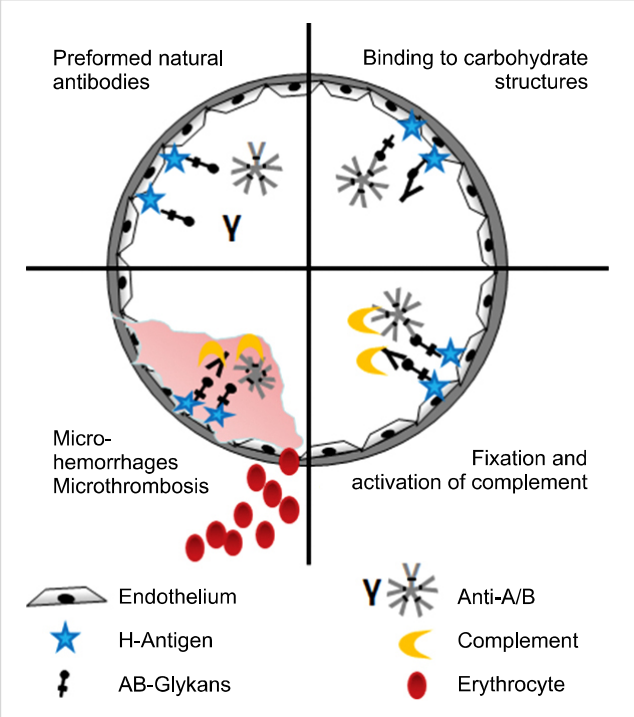

Fig. 1. Hyperacute rejection mediated by ABO antibodies. Preformed natural antibodies bind to blood group carbohydrate structures on endothelial cells. This leads to complement fixation, microthrombosis and microhemorrhages.

4. Role of the von Willebrand factor

vWF (von Willebrand factor) is a large plasma glycoprotein synthesized by endothelial cells and megakaryocytes, and it has a key role in the initial phase of hemostasis [9]. vWF is continuously secreted into the plasma and is stored in endothelial Weibel-Palade bodies or in the alpha-granules of platelets. Interestingly, vWF mediates platelet adhesion to the site of damage, and one of its unique features is to facilitate the covalent linking of ABO antigens to the sugar chain [10]. This sort of glycosylation is also mediated by glycosyltransferase activity in the endothelial cells. The exact biological function of ABO antigens on vWF and the influence of vWF on hemostasis or thrombotic complications is still unclear. However, it is known that persons with blood type O have a significantly lower vWF concentration. Interestingly, studies in patients who had received an ABO-mismatched bone marrow transplantation have revealed that the majority of glycosylated vWF is synthesized in the renal endothelium. vWF molecules derived from the renal endothelium expressed blood group antigens, whereas vWF molecules derived from platelets did not. Therefore, vWF glycosylation plays a role in increased presentation of donor-ABO antigens in the recipient as well, and it may be associated with the development of immunological tolerance in recipient plasma [11].

TREATMENT PROTOCOLS FOR ABO-INCOMPATIBLE TRANSPLANTATION-FROM NORMAL SALINE FLUSH TO ANTIGEN-SPECIFIC IMMUNOADSORPTION

1. The pioneers of ABO-incompatible renal transplantation

On April 23, 1951, a 30-year-old woman received the first intentional ABOi (ABO incompatible) renal transplantation in Boston. Hume and colleagues [12] wrote in their seminal paper: "The donor's blood type was O, Rh +, while that of the recipient was A, Rh +, thus presenting compatibility in the major grouping but not in the minor." Based on the pathophysiological knowledge available at that

time, the pioneers of renal transplantation believed that the graft would be made acceptable by simply perfusing the graft intensely with normal saline. However, the graft infracted early in the process and never gained function. They sensed that the problem may have been related to blood type incompatibility in the graft, and thus, for the second intentional ABOi renal transplantation (performed on May 7, 1952), the graft was "perfused with 500 mL of Ringer's solution to which 25 g of albumin and 200,000 units of penicillin had been added." Both recipients died 25 and 19 days after the surgery. The authors arrived at the following conclusion: "We do not feel that renal transplantation in the presence of blood incompatibility is wise" [12]. In the years that followed, several reports documented accidental ABOi transplantations that led to rapid graft loss within the first year after transplantation [13, 14]. A report on the first successful series of 26 patients was published by Alexandre and coworkers [15]; the treatment protocol included a simultaneously performed splenectomy. Later, this protocol was adopted by Japan, the country with the most experience in this area, and 851 ABOi transplantations were conducted in 82 centers between 1989 and 2005 [16].

All 3 of the major protocols (from Japan, USA, and Europe) are based on 2 strategies: removal of preformed antibodies by extracorporeal techniques and inhibition of antibody production.

2. Removal of preformed antibodies

The circulating antibodies produced against ABO antigens that are not present in the recipient will cause antibody-mediated rejection of the graft, and hence, these antibodies have to be removed prior to the transplantation. The antibodies can be removed by either specific or non-specific extracorporeal treatment methods.

Bier and colleagues have conducted important preclinical studies since the early 1970s; these studies had paved the road to the clinical application of therapeutic plasma exchange (TPE) [17]. They demonstrated the ability of TPE to selectively reduce the circulating antibody and to significantly delay the rejection of porcine kidney xenografts in dogs. A decade later, Bensinger et al. showed that TPE (or immunoadsorption) could be used to remove ABO antibodies, thus allowing successful ABOi bone marrow transplantation [18]. This technique also was used to rescue the graft of a patient who had mistakenly received an ABOi kidney [19]. Interestingly, TPE not only reversed the acute rejection in this patient but also reduced the damage to such a degree that long-term (20 months) graft function was normal. These publications were critical to the decision of Alexandre and coworkers [15] to employ TPE as an essential tool for removing antibodies prior to initiating an ABOi transplantation; it is still the method of choice in Japan and the USA [20-22]. A variation on the theme of TPE is DFPP (double-filtration plasmapheresis). In DFPP, plasma is separated using a plasma separator, in which plasma passes through a plasma component separator (small pores). Large molecular weight proteins are discarded, and the small molecular weight substances, including albumin, are cycled back to the patient. Compared to standard TPE, this approach reduces the volume of replacement fluid required, i.e., albumin [23].

A more specific method for removing isoagglutinins is IA (immunoadsorption). In the early 1980s, a solid-phase immunoadsorbent column with blood group A trisaccharide had been used to demonstrate the specific removal of anti-A antibody from human plasma *in vitro*; moreover, dogs immunized *in vivo* displayed no toxicity [24, 25]. The Glycosorb ABO column, a single-use column that efficiently reduces donor-specific anti-A and anti-B IgM and IgG by 81% and 56%, respectively, at the first treatment [26], is currently used in all published European protocols [27-29]. Some authors believe that antigen-unspecific immunoadsorption by the Globaffin or Ig-Therasorb device is equivalent in efficacy to antigen-specific immunoadsorption, despite the absence of comparative studies [30].

3. The Japan protocol

Because of the decreasing number of deceased organ donors, Japan had started a program on ABOi transplantation in 1989. In this program, the natural antibodies are preoperatively removed by DPFF, and the kidney transplantation is combined with a splenectomy in addition to immunosuppressive therapy with CNIs, anti-metabolites, and steroids. This protocol resulted in graft survival that was comparable to the survival outcomes following ABO-compatible transplantation [16]. One of the major disadvantages of this protocol is the high rate of infection and postoperative complications that are associated with splenectomy, such as postsplenectomy septic syndrome, atelectasis, pancreatitis/fistula, pulmonary embolism, and bleeding at the operative site [31]. Therefore, instead of performing a splenectomy, many institutions now use anti-CD20 antibody (rituximab), which markedly reduces the incidence of acute antibody-mediated rejection [21].

4. The Johns Hopkins protocol

The Johns Hopkins (USA) protocol is based on rituximab and TPE. Depending on the pretransplant antibody titer, 2-15 TPEs are performed preoperatively [32] and is followed by low-dose CMV hyperimmunoglobulin and rituximab (formerly splenectomy). The patient and graft survival rates in ABOi transplantation are comparable to national statistics for compatible live donor transplants [33].

5. The Stockholm protocol

Tyden and coworkers developed a novel protocol in 2003 [28]. Preoperative B-cell ablation therapy is performed using anti-CD20 antibodies (375 mg/m^2), and the TPE component is replaced by a more specific approach for removing the preformed natural antibodies by using specific anti-A or anti-B directed IA. In addition, the recipient receives a combination of immunosuppressants with mycophenolate, tacrolimus, and steroids for 10 days before the planned transplantation.

6. The Hannover protocol

In Hannover, the Tyden-Protocol is used with minor modifications. The patients receive an anti-CD20 treatment 4 weeks before the planned transplantation, and they begin immunosuppressive therapy with tacrolimus (trough level, 8 ng/mL) combined with mycophenolate (2×0.5 g/d) and steroids (0.3 mg/kg). One week before the planned transplantation, daily IA is conducted using Glycosorb columns selected to fit the anti-erythrocyte antibody constellation until the isoagglutinin titer is at or below 1 : 8. The day before transplantation, the patients receive 30 g immunoglobulins i.v. (intravenously), and 500 mg of a steroid is administered i.v. during transplantation. The mycophenolate dosage is increased to 2×1 g/d. The tacrolimus dosage is adapted to reach trough levels-12 ng/mL for up to 4 weeks and 10 ng/mL for up to 3 months, with further reduction as usual and according to the clinical situation. Steroids are tapered as is typical after kidney transplantation. Recently, routine IA after transplantation was switched to an on demand approach. IA is continued throughout the first 2 weeks, if the titer is higher than 1 : 8 during the first week and higher than 1 : 16 during the second week. Regular additional application of anti-interleukin-2 antibody on days 1 and 4 after transplantation were discontinued since a higher rate of infection was observed for that combination. Higher rejection rates were not experienced after the anti-interleukin-2 antibody was removed from the treatment regimen.

ACCOMMODATION

The most critical phase after ABOi transplantation is the early postoperative phase. The risk for developing an acute rejection related to blood group antigens is low after the initial phase despite the fact that blood group-specific antibodies are produced continuously by the recipient. This type of tolerance is referred to as "accommodation," and its underlying mechanisms are poorly understood. One explanation might be the presence of a defective glycosyltransferase, an enzyme that facilitates blood group antigen synthesis in the donor organ. The ischemia and reperfusion injury of the donor organ leads to inactivation of this enzyme; therefore, the donor endothelium expresses fewer blood group antigens, which leads to reduced immunogenicity [34].

The activity of the glycosyltransferase is lost and the blood group antigens lose their immunogenicity within 2 weeks after transplantation and after the initial functional recovery, but the antigens are still present. The second paradox involves positive C4d complement in the peritubular capillaries, which is one of the hallmarks of antibody-mediated rejection according to the Banff Classification; however, this occurrence in ABOi transplantation is not associated with a higher rejection rate. A recent study on protocol biopsies that compared ABO-compatible and ABOi renal transplants revealed C4d positivity in 94% of the ABOi cases (N=89 biopsies of 45 patients), with other signs of rejection occurring in less than 50% of those cases; however, 11% C4d positivity was detected in the compatible cases (N=250), which were all associated with graft rejection [35].

The success in ABOi renal transplantation has even encouraged physicians to perform intentional ABOi liver transplantation [36, 37]. Using a pre-, peri-, and postoperative antibody depletion protocol, which included TPE, i.v. Ig administration, rituximab therapy, and IA, we also performed the first successful ABOi lung transplantation at the Medical School, Hannover [38].

1. Potential side effects

The high intensity of immunosuppression increases the risk for both infections and neoplasms. Interestingly, the use of Glycorex antigen-specific immunoadsorption is associated with the removal of antibodies against *Pneumococcus* and *Haemophilus* polysaccharide antigens, but anti-tetanus and anti-diphtheria protein antibodies are not affected [39]. Nevertheless, after more than 3 years, outcome data indicate that ABOi kidney transplantation does not differ from ABO-compatible transplants [29, 40]. More recent data from Hannover indicate that intense immunosuppression might lead to a significant increase in viral infections [27].

In summary, ABOi living-donor kidney transplantation represents an accepted therapeutic procedure for long-term graft survival, which is comparable to that in ABO-compatible living kidney donations. Thus far, the risk of infection and neoplasm linked to immunological preconditioning with modern immunosuppressive drugs and specific or non-specific elimination of preformed antibodies seem to fall within acceptable limits; however, long-term data are necessary to confirm this. More sophisticated monitoring of immune function may be helpful for developing tailor-made immunosuppression regimens, particularly for this patient population.

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