ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION
-THE SURPRISING ABSENCE OF HYPERACUTE REJECTION-

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Abstract

　Owing to the shortage of deceased donors in Japan, since 1989, we have performed ABO-incompatible kidney transplantation(ABO-IKTx) to expand the indication for living donor kidney transplantation. During the past 2 decades, about 2,000 ABO-IKTxs were performed. Since 2001 the success rate for these kidney transplants has reached 96% for 1-year graft survival and 91% for 5-year graft survival, similar to outcomes of ABO-compatible kidney transplantation (ABO-CKTx). This dramatic improvement in results means that ABO-IKTx has become accepted as a therapeutic alternative for end-stage renal failure. Today ABO-IKTx accounts for 30% of all living donor kidney transplantations performed in Japan.

　In 1901 Karl Landsteiner discovered the presence of human ABO blood groups, and for many years ABO-IKTx was considered contraindicated, as the medical community assumed that this procedure would result in immediate/hyperacute ABO-related rejection followed by graft loss.

　When we actually attempted the procedure, however, we found that acute antibody-mediated rejection (AMR) developed, but not a single instance of ABO-related hyperacute rejection. This phenomenon can be demonstrated epidemiologically. Yet, that does not answer the fundamental question of "Why is hyperacute rejection absent?" This article addresses potential mechanisms of acute AMR across ABO mismatched barriers as well as summarizing graft/patient survival of ABO-IKTxs in Japan.

Hirosaki Med. J. **64**, Supplement：S28—S44, 2013

Key words: ABO-incompatiblity; kidney transplantation; hyperacute rejection; accommodation; antibody-mediated rejection

Introduction

　Time certainly flies. It has been more than 20 years now since we performed the first ABO-incompatible kidney transplantation in Japan, on January 19, 1989[1-7]. The very first ABO-IKTx was conceptualized and performed by Guy P.J. Alexandre in June of 1982[8-10]. However, transplant surgeons in Japan have done a great deal to popularize this surgery and to improve its outcome. As a result, Japan can boast of expertise second to none in the area of ABO-IKTx, a procedure which is relatively rare in the international medical community.

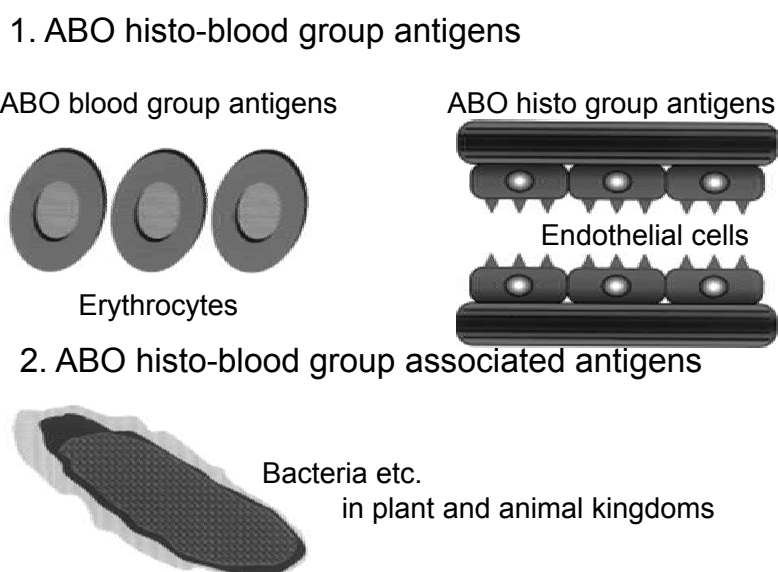　In 1901 Karl Landsteiner discovered the existence of human ABO blood groups[11], and for many years ABO-IKTx was considered to be contraindicated, as the medical community assumed that this procedure would result in immediate hyperacute rejection (HAR) followed by graft loss.

　However, when we actually attempted the procedure we found that acute AMR developed, but not a single instance of ABO-related HAR. This phenomenon can be demonstrated epidemiologically[13-15]. Yet, that does not answer the fundamental question of "Why is hyperacute rejection absent?" I was sure that my readers would not be satisfied until this mechanism was clearly elucidated, and neither would I. That is

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1. ABO histo-blood group antigens

ABO blood group antigens

ABO histo group antigens

Endothelial cells

Erythrocytes

2. ABO histo-blood group associated antigens

Bacteria etc.
in plant and animal kingdoms

Fig.1. ABO histo-blood group related antigens
ABO histo-blood group related antigens consist of the 2 types of antigens.

why this manuscript was written.

We have reported elsewhere two new ground-breaking findings in 2009 and 2010 regarding ABO- IKT[16, 17]. We are confident that these findings will overturn the conventional wisdom about ABO-incompatible transplantation, and will mark a fundamental change in therapeutic strategies.

I. Definitions and explanation of terms

To avoid misunderstanding by readers, I would like to define and explain the following important terms in advance.

1. Hyperacute rejection, and acute antibody-mediated rejection

HAR, as used in this manuscript refers to an acute AMR occurring within 24 hours of transplantation. Acute AMR is defined according to the Banff classification.

2. ABO histo-blood group related antigens

ABO histo-blood group related antigens consist of the following 2 types of antigens.

2.1. ABO histo-blood group antigens

These were formerly described as pure antigens. They are found on the surface of human erythrocytes, tissue cells, and vascular endothelial cells, and are antigens in the original sense of the word (Fig.1).

ABO histo-blood group antigens are further classified into two subtypes: ABO blood group antigens and ABO histo group antigens, according to the different proteins that are bound to the carbohydrate chain of each antigen.

2.2. ABO histo-blood group associated antigens

The process of evolutionary development has led to the presence of numerous ABO histo-blood group associated antigens within the plant and animal kingdoms. These carbohydrate antigens sometimes provoke antigen stimulation in humans, and can cause urticaria and tissue damage.Bacterial surfaces also harbor ABO histo-blood group associated antigens. From the perspective of the host defense mechanism, the healing process for complications such as sepsis in ordinary patients involves the production of neutralizing antibodies against the antigens of the causative bacteria. However, in ABO-
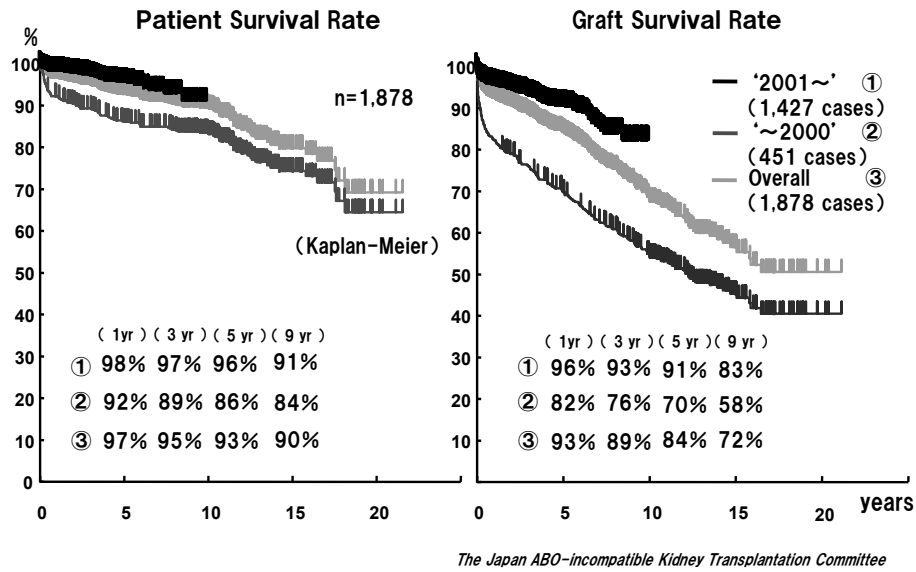
Fig.2. Results for ABO-incompatible kidney transplantation in Japan show a sharp improvement in findings for the 1,427 patients treated since 2001.Results are comparable to those obtained in ABO-compatible transplantation (ABO-incompatible Kidney Transplantation Committee 2010).

incompatible transplant patients the development of sepsis during the critical period can result in those neutralizing antibodies responding to ABO histo group antigens on the surface of the vascular endothelial cells in the transplant organ, causing acute AMR that damages the transplant organ[7, 15, 18].

II. Clinical outcome of ABO-incompatible kidney transplantation in Japan

1. Excellent long-term outcomes of ABO-incompatible living donor transplantation in Japan

In Japan, 1,878 ABO-IKTxs were performed between January 1, 1989 and December 31, 2010. Fig. 2 shows the patient and the graft survival rates for those procedures (Fig.2). The overall patient survival rate was 97% for the first year, 95% for the first three years, 93% for the first 5 years, and 90% for the first 9 years. The 1-year graft survival rate was 93%, 3-year 89%, 5-year 84%, and 9-year72%.

We subsequently analyzed survival outcomes for each of the earlier era (1989 – 2000) and recent era (2001 – 2010). The patient and graft survival rates for the 451 transplants performed during the earlier era were 92, 82% for the first year, 89, 76% for the first three years, 86,70% for the first 5 years, and 84, 58%, respectively. For the 1,427 procedures performed during the recent era, the patient and graft survival rates were 98, 96% for the first year, 97, 93% for the first three years, 96, 91% for the first 5 years and 91,83%, respectively, showing significant improvement (P < 0.01) in the recent era regarding both patient and graft survival.

Today ABO-IKTx accounts for 30% of all living kidney transplantations performed in Japan. This has made Japan the world-leading country in original research and clinical results for this procedure, which is not yet widely studied in most nations around the world[19].

Four main factors contributed to such rapid and sharp improvement as follows.

2. Changes over 20 years that potentially improve graft/patient survival rates.

Factor 1: The disproving of hyperacute rejection

The common wisdom has been that organ transplantation between incompatible blood groups would result immediately in HAR and graft loss. However, this concept turned out to be a completely unsupported assumption. We provided epidemiological proof that HAR was not caused by the ABO histo-blood group antigens[12-15].

The anti-A/anti-B antibodies that elicit acute AMR immediately after transplantation are clearly not natural antibodies. Instead, our results show them to be *de novo* antibodies that are produced after transplantation, as a result of stimulation and sensitization by the ABO histo group antigens present on the surface of the vascular endothelial cells in the graft and by ABO histo-blood group associated antigens (bacteria, etc.) [7, 15, 18]. The *de novo* antibodies are synthesized after the graft is introduced into the body of the recipient[17]. This fact has extremely important implications for therapeutic strategy in ABO-incompatible organ transplantation. If posttransplant antibody production can be suppressed, then acute AMR can be avoided; i.e., the most important treatment step for ensuring a successful graft outcome is pretransplant suppression of B cell function (desensitization therapy) [7, 15, 17, 18-30].

Factor 2: The mechanism of onset of acute antibody-mediated rejection, and types of rejection reaction

Factor 2, as I have described previously, involved clarification of the mechanism of onset of acute AMR as elicited by ABO histo-blood group related antigens. We found that the acute AMR induced by ABO histo-blood group related antigens (ABO histo-blood group antigens and ABO histo-blood group associated antigens) could be categorized into two different mechanisms: those resulting from antigen stimulation and those attributable to immunological response to such stimuli [7, 15, 18].

The acute AMR ceases to be elicited one to two weeks after transplantation. Thus, the condition termed "accommodation" will be established if the graft can survive for the first one to two weeks[7, 12-15, 18].

Factor 3: Multicenter cooperative research and statistical analysis

The third factor is associated with the establishment of the Japan ABO-incompatible Kidney Transplantation Committee to systematize the conducting of this type of kidney transplantation in multiple institutions across Japan in 1997. That organization conducts an annual statistical analysis of kidney transplantation procedures and outcomes in Japan, and sponsors two academic conferences each year to publicize and utilize those findings.

At the beginning of the earliest period, perioperative care was not yet fully developed. Our surgical experience was limited, the findings described above were still in the future, and we were struggling to establish effective therapeutic strategies. In the early period we classified all ABO-incompatible kidney transplant patients as immunologically high-risk cases, and we thought that splenectomy concurrently at transplantation was essential for inhibiting antibody production. However, the relevant morbidities including blood loss and acute pancreatitis led to fatal complications in a number of patients. There were also some cases in which overzealous antibody removal resulted in a loss of clotting factor. This produced bleeding tendencies and serum protein loss associated with conditions such as accelerated hypovolemia and ultimately resulting in acute renal failure.

As physicians had no knowledge of posttransplant induction and the establishing of accommodation, but instead assumed that acute AMR could occur at any time during the life of the graft, all ABO-incompatible kidney
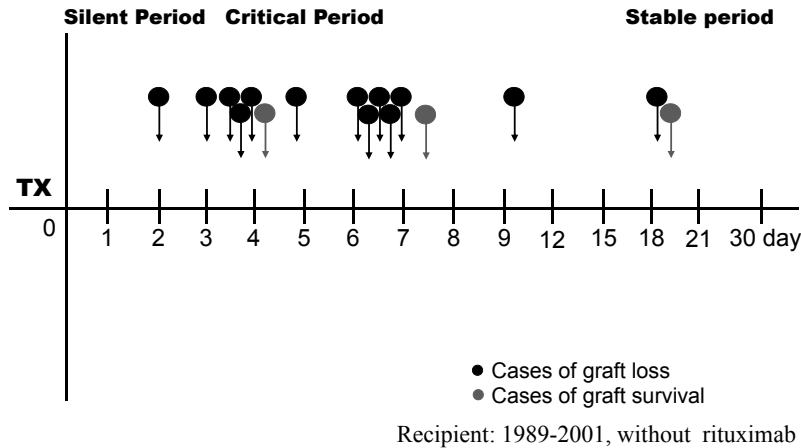
Fig.3. Onset of acute antibody-mediated rejection

transplant patients were considered high-risk, and immunosuppressive therapy was aggressively pursued. This increased the chance of opportunistic infection with organisms such as cytomegalovirus, and prolonged the pathological status of the patient.

Based on the statistical analysis of these data, we were able to make use of previous clinical experience and to increase both patient survival rate and graft survival rate[3-6].

Factor 4: Clinical applications of novel immunosuppressants

The fourth factor was the use of novel immunosuppressants, which we initiated from around the year 2000. That was when drugs that suppressed B cells involved in antibody production, specifically mycophenolate mofetil and rituximab, first became available. A monoclonal antibody against CD25 that suppresses activated T cells also became available for clinical use at about that time. From new findings based on the first and second factors described above, we realized that the most important point in our therapeutic strategy was the inhibition of antibody formation through pretransplant desensitization therapy. The clinical application of these new immunosuppressants contributed greatly to our improved therapeutic results[7, 15, 17, 18-30].

III. Mechanism of acceptance of ABO-incompatible kidney transplantation

1. The most common time of onset for acute antibody-mediated rejection

We provided epidemiological proof that HAR was not caused by the ABO histo-blood group antigens (Fig.3).

Fig.3 shows the time of onset of acute AMR as clearly documented in 494 patients who underwent ABO-IKTx in Japan between January 1989 and December 2001.

Only 13 patients lost their ABO-IKTxs by acute AMR and no HAR occurred. Notably, all of these patients received an early form of immunosuppressive therapy but none was treated with desensitization therapy, which is standard therapy today[7, 15, 17, 20-22]. Since they were considered immunological high-risk patients, they underwent intensive immunosuppressive therapy immediately pre- and post-transplants.

The time of onset of acute AMR shows a consistent trend. In the patients we studied, most of the cases of acute AMR occurred within one month, particularly during the first 2 to 7 days posttransplant. We have analyzed the cases of acute AMR developing within the first week posttransplant, and noted that rejection occurred

despite highly aggressive immunosuppressive therapy implemented in the immediate pre and postoperative period, and that graft loss was also encountered in almost every case. Subsequently the incidence of graft loss decreased. Beginning at approximately day 20 posttransplant, occurrences of AMR can be attributed primarily to factors such as inadequate immunosuppression and bacterial infection[7, 15].

The above data can be summarized followings.

1. HAR due to ABO histo-blood group related antigens does not arise within the first 2 days posttransplant. We refer to this period as the "silent period".

2. Acute AMR tends to occur within 2 to 7 days posttransplant. The incidence decreases after this period, and we have found no instances of acute AMR occurring more than 1 month posttransplant. We call this dangerous period the "critical period".

3. To look as the reverse side of the phenomenon described in item 2, accommodation was established 1 to 2 weeks posttransplant in many cases. Once accommodation has been established, there are no further instances of acute AMR, and this continues for the life of the graft. This period is termed the "stable period".

2. Why is ABO-related hyperacute rejection absent in ABO-incompatible kidney transplantation? -- Mechanism of onset for ABO-related acute antibody-mediated rejection

The statistical analysis performed in 1967 by Gleason, et al. on kidney transplant cases showed 1 year graft survival rate as low as 25% for ABO-IKTx. That report virtually shut the door to this type of transplantation[31]. I raised a question about this long-standing misconception and finally disproved the occurrence of HAR due to ABO incompatibility based on the above –mentioned epidemiological studies.

I defined the phenomenon of accommodation as the situation in which, although the vascular endothelial cells in the graft carry ABO histo-blood antigens on their surface, and the blood of the recipients contains antibodies to those antigens, no antigen-antibody reaction occurs, and there is no occurrence of acute AMR[5, 7, 12-15]. If I reflect on this definition, however, it contradicts itself. To explain this contradiction, I came up with the idea of key (antibody) and keyhole (antigen) mismatch.

We have reported elsewhere two new ground-breaking findings in 2009 and 2010 regarding ABO-IKTx[16, 17]. We are confident that these findings will overturn the conventional wisdom about ABO-incompatible transplantation, and will mark a fundamental change in therapeutic strategies.

The first finding involves ABO histo-blood group antigens, which are classed as carbohydrate antigens. Conventionally, emphasis has been placed on the saccharide chains only, and related phenomena have been interpreted accordingly. However, the saccharide chains in ABO histo-blood group antigens are present in the form of glycoproteins, and the binding proteins are termed "carrier proteins" or "anchor proteins." It has been reported that the carbohydrate chains of ABO histo-blood group antigens on erythrocyte membranes form glycoproteins by bonding to proteins such as band 3 or band 4.5[32, 33].

We performed proteomic analyses in order to determine which type of proteins would be bound to ABO histo-blood group antigens on the vascular endothelial cells (Fig.4). The results showed that those antigens, unlike the ones on erythrocyte membrane, primarily bind to proteins such as PECAM1 (platelet endothelial cell adhesion molecule 1), PLVAP (plasmalemmal vesicle associated protein), and vWf (von Wilebrand factor) (Fig. 5)[16].

The above data indicate that ABO histo-blood group antigens on vascular endothelial cells differ from those on the erythrocyte membrane,
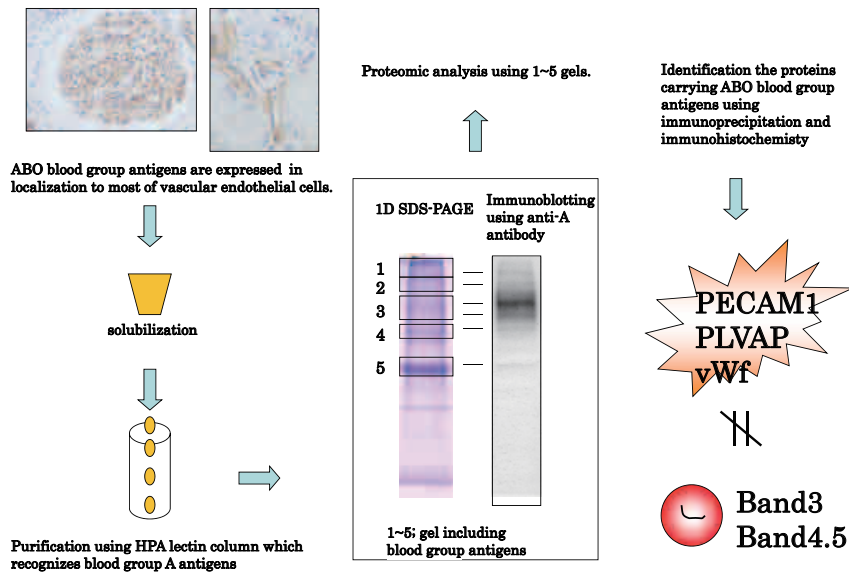
Fig.4. Proteomic analysis of proteins binding to human kidney ABO histo-blood group antigens

ABO histo group antigens are abundantly present on vascular endothelial cells in the kidneys, but the proteins binding to ABO histo group antigens on vascular endothelial cells had not been analyzed.We solubilized kidney tissue, collected blood group A carbohydrate chains using lectins that recognize those chains, used electrophoresis to extract specific bands, and then used a mass spectrometer to identify candidate proteins.
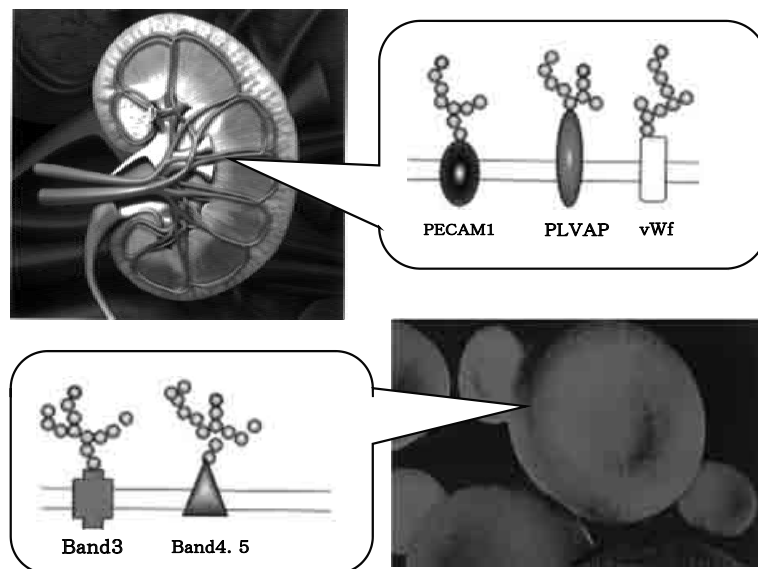

Fig.5. ABO histo-blood group antigens

They are found on the surface of human erythrocytes, tissue cells, and vascular endothelial cells, and are antigens in the original sense of the word.
ABO histo-blood group antigens are further classified into two subtypes: ABO blood group antigens and ABO histo group antigens, according to the different proteins that are bound to the carbohydrate chain of each antigen.

both in terms of structure and of antigenicity, depending on differences in the proteins to which they are bound. These antigens, which have been treated as the same type and categorized as ABO histo-blood group antigens, will now be categorized into two groups: "ABO blood group antigens" carried by the erythrocytes and "ABO histo group antigens" expressed in the vascular

endothelial cells.

The host immune system probably do not identify the saccharides and binding proteins separately, but instead recognize them as part of a whole, and then proceed to form antibodies that have a high affinity for that whole. It can also recognize the structural differences between ABO blood group antigens and ABO histo group antigens, and can initiate immunological responses accordingly. Such immunological responses will lead to anti-ABO blood group antibodies in the case of ABO blood group antigens, and anti-ABO histo group antibodies in the case of ABO histo group antigens. Thus, the serum natural anti-A/anti-B antibodies react well with erythrocyte membrane ABO blood group antigens but do not necessarily react with ABO histo group antigens on vascular endothelial cells having a different structure. This is the major factor contributing to the absence of HAR in ABO-IKTx .

If the "key" doesn't fit the "keyhole," the "door" won't open, and HAR cannot develop.

To further expand this theory, the antibody that is responsible for posttransplant ABO-related acute AMR must be an antibody that was newly synthesized after transplantation (*de novo* antibody, i.e. anti-ABO histo group antibody) after that has been sensitized by ABO histo group antigens on the vascular endothelial cells of the graft. If true, this theory explains the existence of approximately 2 days of "silent period" with no sign of HAR. This is the time required for sensitization, i.e. the time required for host immune system to recognize ABO histo group antigens and then to produce anti-ABO histo group antibodies.

In cases where pretransplant desensitization therapy has not been adequately implemented, memory B cells are sensitized by ABO blood group antigens and are class-switched to produce IgG antibodies. After being stimulated by ABO histo group antigens, the memory B cells produce anti-ABO histo group antibodies having a different affinity to these antigens. The anti-ABO histo group antibodies react in turn with ABO histo group antigens on the vascular endothelial cells to trigger a type I acute AMR[15]. Transplant procedures are followed by a two-day "silent period" free of acute AMR because it takes time for host immune system to recognize ABO histo group antigens on the vascular endothelial cells of the graft and to respond immunologically. That immunological response produces anti-ABO histo group antibodies to complete the antigen-antibody reaction, i.e., acute AMR[18].

3. Mechanism of acute antibody-mediated rejection in ABO-incompatible kidney transplantation: Which anti-A/anti-B antibodies are responsible, natural or *de novo*?

The second finding relates to the known fact that ABO-incompatibility associated acute AMR is caused by anti-A/anti-B antibodies. Conventionally it had been believed that this rejection response was caused by natural antibodies present in the recipient. If we consider the first theory, however, it becomes clear that the antibodies eliciting acute AMR could not be produced without transplantation of the donor organ. That is to say, the recipient host immune system must be sensitized to the ABO histo group antigens on the vascular endothelial cells, resulting in the new production of anti-A/anti-B *de novo* antibodies. Anti-ABO blood group antibodies and anti-ABO histo group antibodies are presumably very similar in structure. As of this writing, there are no assay systems available for demonstrating the differences between those two antibodies. I once considered the use of commercially-available umbilical cord veins (HUVEC), but realized that no ABO histo group antigens are present on the surface of the HUVEC. Then I investigated an indirect method to prove my theory.

Table 1. ABO-incompatible kidney transplant patients with severe acute AMR

Recipient	Age	Gender	Blood Type		Donor Age	HLA-A,B,DR Missmatch	Immuno-suppression		Antibody Titer		Acute AMR				Antibody Titer		PTCs C4d Deposition		
			Recipient	Donor					Preremoval	Pretransplant	Onset	Outcome			Onset	Post-graftectomy	0 hr	1 hr	AMR
1	38	M	O	B	Father 68	1	1st Era	IgM IgG	4 68	1 16	6 day	AMR III Graft loss	IgM IgG		32 64	32 128	(-)	(-)	(+)
2	33	M	O	B	Father 66	2	1st Era	IgM IgG	4 16	8 2	7 day	AMR III Graft loss	IgM IgG		256 64	512 512	(-)	(-)	(+)
3	68	M	B	A	Wife 61	3	2nd Era	IgM IgG	8 0	4 0	8 day	AMR III Graft loss	IgM IgG		16 0	256 0	(-)	(-)	(+)
4	54	M	B	A	Wife 45	5	2nd Era	IgM IgG	16 2	16 2	8 day	AMR II Graft surviving	IgM IgG		32 8		(-)	(-)	(+)

Abbrevidation   Antibody Titer : Anti-A, Anti-B Antibody Titer
AMR : Antibody-mediarted rejection
PTCs : Peritubular cpillaries

One day I was studying the development of acute AMR in four patients, and I happened to note the presence of Complement 4d (C4d) deposits in the peritubular capillaries (PTCs). In three of these four patients, the acute AMR was so severe that the graft was lost. However, perhaps coincidentally, after transplantation the postreperfusion one-hour biopsy findings were negative for C4d deposition in the PTCs in all four of these patients (Table 1). Since then, we have focused special attention on C4d deposition in PTCs in cases of ABO-CKTx and ABO-IKTx performed at Niigata University Medical and Dental Hospital between January 2000 and December 2008[17, 18].

C4d deposition in the PTCs is a comparatively specific and sensitive response associated with the immunological reaction to a kidney allograft. These deposits indicate that the antigens on the surface of the vascular endothelial cells are exposed to antibodies in recipient serum, and that an antigen-antibody reaction occurred[34-40]. Particularly in ABO-IKTx, if we see C4d deposits in the PTCs upon renal graft biopsy we can then infer that the ABO histo-blood group antigens on the vascular endothelial cells have been exposed to ABO histo-blood group antibodies. Therefore, by tracking the course of C4d deposition over time, we can infer whether these deposits are caused by natural antibodies or by *de novo* antibodies that were formed after transplantation. We thus began to monitor the time course of C4d deposits in the PTCs.

We performed consecutive 3 renal graft biopsies (0 hour, 1 hour, and protocol or episode biopsies) in 31 patients who underwent ABO-I TKx to examine the complement 4d (C4d) deposition in peritubular capillaries (PTCs) and to estimate when ABO blood type antigens on endothelial cells in PTCs reacted with these antibodies. As a control group, we also studied 37 ABO-CKTx patients during the same period.

In-depth reports on these forms of immunosuppressive therapy are available in the literature[7, 29, 30].

In ABO-I KTx group, C4d deposition was observed in 0%, 16.1%, and 70.9% of the patients at the first (0-hour), 1-hour, and the protocol/episode biopsy, respectively. In all 4 patients with acute AMR, both 0- and 1-h biopsy specimens were negative for C4d deposition, whereas those with the protocol/episode biopsy were positive. In ABO-C KTx group, all of 3 biopsy specimens were negative for C4d (Fig.6).

The first biopsy was the 0 hour biopsy, conducted prior to reperfusion of the kidney graft, so findings were negative, just as in the ABO-C KTx group. As the second renal biopsy was performed 1 hour after reperfusion, the C4d deposition that was detected at that time was attributable to natural antibodies. The C4d deposition detected at the third biopsy, after a certain posttransplant period, can be explained by three different mechanisms. Thus we have
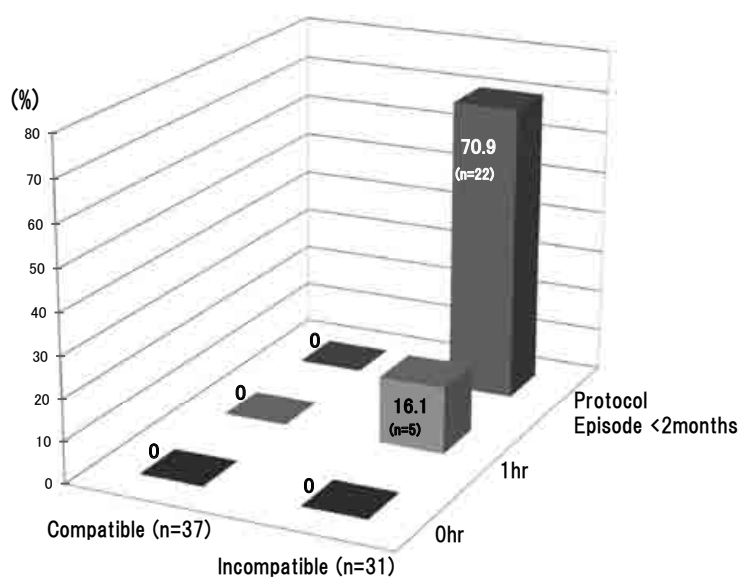
Fig.6. PTCs C4d Deposition in ABO-compatible and ABO-incompatible living donor ransplantation

（1）C4d deposition that is a continuation from the second period, （2）C4d deposition caused by *de novo* serum anti-A/anti-B antibodies, and （3）C4d deposition due to *de novo* serum anti-A/ anti-B antibodies in addition to the deposition caused by natural antibodies during the second period.

Even with repeated antibody removal pretransplant, it is impossible to remove all serum anti-A/ anti-B antibodies. There will naturally be some antibodies remaining in the blood. We assumed that those antibodies would be adsorbed on the transplant kidney, and that the positive rate would thus be nearly 100% at the second biopsy. However, the actual value of 16.1% was much lower than our expectations. In these 16.5% positive C4d recipients at the time of 1-hr biopsy（the second biopsy）, clinical acute rejection not occurred.

In considering possible causes of this outcome, we realized that we had been measuring serum anti-A/anti-B antibody titers, and that this actually measured the antibodies responding to ABO blood group antigens on the erythrocytes. Those antibodies were believed to respond in the same way as the ABO histo group antigens on the surface of the vascular endothelial cells[31]. Our clinical outcomes to date indicate that high anti-A/anti-B antibody titer in recipients pretransplant is not necessarily associated with the development of ABO-related acute AMR, and that the graft can survive in such patients. We noted this dissociation between pretransplant antibody titer and clinical results in many of our patients, but were unable to provide any specific explanation[7, 18, 41].

While the natural antibodies are highly reactive because of their strong affinity for the ABO blood group antigens on the erythrocyte surface, they may not respond so strongly to the ABO histo group antigens on the vascular endothelial cells, which have a different structure. These factors may contribute to the low incidence （16.1%）of positive C4d deposition at the second biopsy.

Acute AMR after ABO-IKTx is induced primarily by serum anti-A and anti-B antibodies produced *de novo* after transplantation, rather than by such antibodies remaining after pre-transplant antibody removal. Successful outcomes of ABO-I KTx require the prevention of acute AMR.

These findings are extremely important when applied to therapeutic strategies for ABO-incompatible organ transplantation. When antibody production can be suppressed, acute AMR rejection should also be suppressed. This means that pretransplant desensitization therapy to suppress B cell function would be the most effective treatment to ensure successful organ transplantation[7, 15, 18-31].

IV. Major Developments in ABO-incompatible Kidney Transplantation

Previous sections have focused primarily on explaining the development of acute antibody-mediated rejection in ABO-incompatible kidney transplantation. Because this emphasis on rejection might lead the reader to conclude erroneously that there is a high incidence of rejection associated with such transplantation procedures. This section will be devoted to a discussion of the usual clinical course in ABO-incompatible kidney transplantation.

This procedure can be divided broadly into three patterns of clinical course as shown in Fig.7. Currently, if desensitization therapy is implemented thoroughly and correctly before transplantation, over 90% of patients will experience the first pattern of clinical course. Accommodation will be established without development of acute antibody-mediated rejection, and the graft will survive. In a few percent of patients, acute antibody-mediated rejection will develop and the graft will be lost. The third pattern of clinical course occurs in those patients who develop complications, such as opportunistic infection in the early stage posttransplant, and also develop type II acute antibody-mediated rejection. In such cases, the rejection can be managed successfully with appropriate treatment. Let me briefly explain these three patterns of clinical course.

To make this explanation easier to follow, let's assume a combination in which the donor is type B and the recipient is type O（Fig.7）.

1. Cases where no reiection occurs and the graft survives

Almost all cases will follow the clinical course shown in the upper field in Fig.7①. There would be no problem at all if, after transplantation, the type B organ could change to a type O organ just like a chameleon changes the color of its skin. Unfortunately that doesn't happen. However, if desensitization therapy is implemented thoroughly and correctly before transplantation, B cell production will be inhibited and the formation of anti-ABO histo group antibodies will be suppressed. Regardless of how many times antibody removal is performed pretransplant, some natural antibodies will remain. However, up to a certain point such antibodies can be absorbed on the vascular endothelial cells without serious damage to the transplant kidney. In patients who have a high serum antibody titer or who experience a rebound of antibody titer following antibody removal, there is an apparent reduction in serum antibody titer following repeated antibody removal, but no reduction in host defense mechanism（antibody production capacity in the host）. In a situation such as this, it is important to repeat desensitization therapy once again.

If a satisfactory posttransplant clinical course can be maintained for one to two weeks, accommodation will be induced and established, and the graft will survive. There will be no subsequent development of acute antibody-mediated rejection due to ABO histo-blood group related antigens.

2. Cases in which type I acute antibody-mediated rejection develops
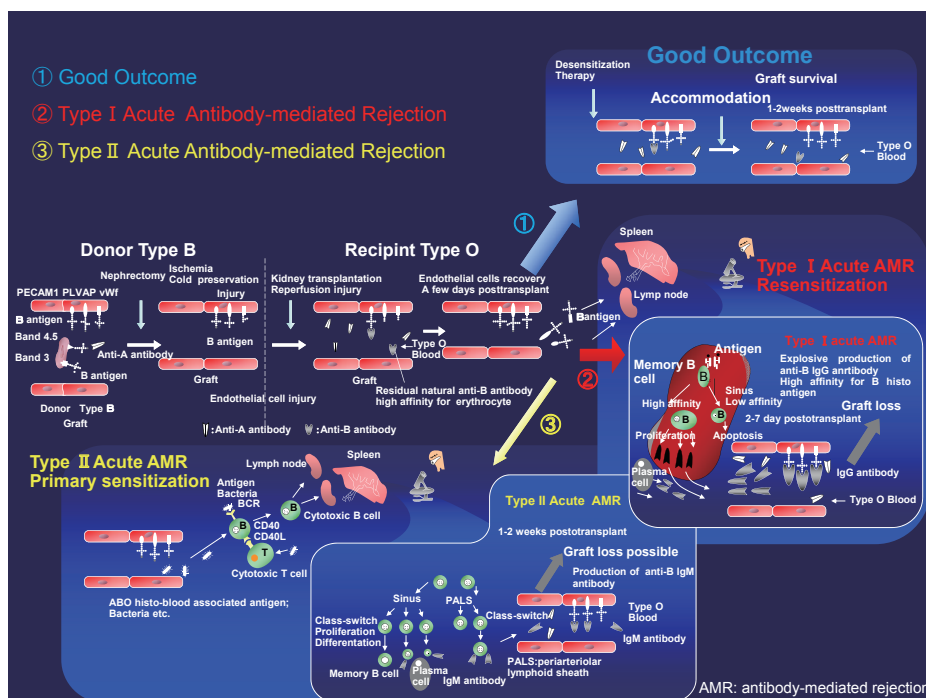
These are the cases of type I rejection

Fig.7. Major developments in ABO-incompatible kidney transplantation.

represented by the middle field in Fig7②. Patients at particularly high risk for this outcome are those in whom desensitization therapy has not been thoroughly and correctly implemented, and immunological high-risk patients prone to rebound following antibody removal.

If the recipient have been already sensitized by ABO blood group antigens and have undergone class switching to IgG. They are not suppressed, but instead remain "in hiding" in the lymph nodes and spleen, those cells will be stimulated by ABO histo group antigens on the vascular endothelial cells, and will promptly react by proliferating and differentiating, to give rise to an antibody response. This is a secondary response, so it can progress very rapidly. The proliferation of B cells, which have a strong affinity for ABO histo group antigens on the vascular endothelial cells, can result in explosive production of *de novo* anti-B antibodies (anti-ABO histo group antibodies), which results in the development of sudden and intense acute

antibody-mediated rejection, leading to the loss of kidney function.

Typically, type I acute antibody-mediated rejection is a clinically severe rejection response which begins with a transient high fever (38℃), precipitous reduction in platelet count, and swelling of the transplanted kidney. The fever is caused by factors such as thrombus formation and angiitis, with subsequent inflammation of the kidney, circulatory damage, and edema. Type I rejection responds very poorly to currently available treatment modalities, and graft function is almost always lost. Biochemical tests show elevated levels of LDH, an enzyme used as an indicator of renal problems, and slight elevation of transaminase may also be observed. Before long, antibody adsorption within the kidneys has reached the saturation point, and further antibody production will lead to a sudden and dramatic increase in serum anti-A/anti-B antibody titer. This occurs primarily as elevation of serum IgG antibody titer, frequently

accompanied by parallel elevation of IgM antibody titer.

3. Cases in which type II acute antibody-mediated rejection develops

The clinical course shown in the bottom field in Fig7③. represents cases in which type II acute antibody-mediated rejection has developed. After kidney transplantation but before accommodation is induced and established, if the recipient incurs initial sensitization by ABO histo-blood group associated antigens that are cross-reactive to donor ABO histo group antigens, then antibody production will be elicited as an immunological response, resulting in acute antibody-mediated rejection. As noted previously, the initial sensitization is a primary response, so its pathological progression is slower than type I rejection, and the clinical course is prolonged. This response generally develops late in the critical period. In situations such as this, serum IgM antibody titer is elevated, and IgG antibody titer generally remains unchanged.

This rejection is prone to develop in the early stage posttransplant as a result of opportunistic infection, particularly bacterial sepsis. If the recipient is exposed to a bacterial infection, naturally antibodies will be produced as part of the host defense mechanisms. However, this is truly a "two-edged sword" in the case of a posttransplant patient. The antibodies will act to neutralize the bacterial cells, but they will also produce a cross-reaction with the ABO histo group antigens of the vascular endothelial cells, giving rise to an acute antibody-mediated rejection.

At the microscopic level, the bacteria/ microorganisms act as antigens. When antigens react with B cell receptors, those antigens are engulfed, digested into peptides, and presented on the cell surface together with the histocompatible antigens. T cells activated by reacting with these same antigens then react with the B cell surface antigen peptides, so that there is contact between the T cells and the B cells. This kind of antigen-specific T cell-B cell contact is followed by a reaction between CD40L (ligand) on the activated T cells and CD40 on the B cells, inducing a signal mediated by CD40 in the B cells. This results in B cell activation in response to bacterial antigens. The activated B cells proliferate and differentiate to plasma cells which are thought to produce antibodies.

Because the condition progresses slowly in the type II rejection, the graft can be rescued with early diagnosis and appropriate treatment.

These are the three main patterns of clinical course that can be expected with ABO-incompatible kidney transplantation.

V. New strategies for immunosuppression in ABO-incompatible kidney transplantation

A simple summary of my therapeutic strategy for preventing acute AMR would be this: It is essential to implement immunosuppression during the critical period so that no immunological response will occur even in the event of antigen stimulation from ABO histo-blood group related antigens（ABO histo-blood group antigens and ABO histo-blood group associated antigens）, and it is also essential to prevent infections that could cause acute AMR.

Fig. 8 is indicated what I consider to be the "four major pillars" of immunosuppressive therapy for ABO-IKTx（Fig.8）. Although those "pillars" have not been changed since the beginning of ABO-IKTx, the importance of each measure has significantly changed. In the early days, pretransplant antibody removal and splenectomy were considered important[23-26,]
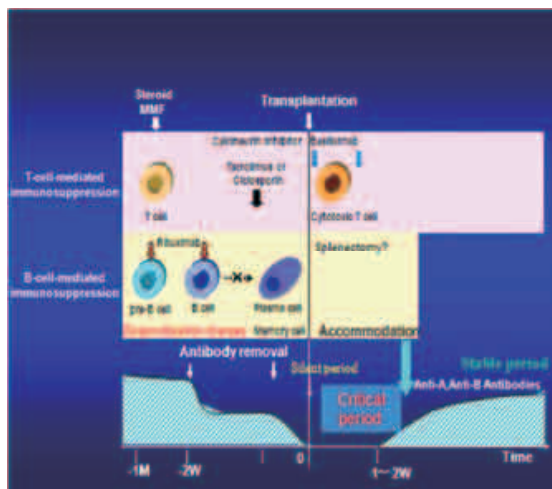
Fig.8. Strategy for immunosuppressive therapy in ABO-incompatible kidney transplantation (Pinpoint-targeted immunosuppression).
It is important to implement B cell mediated immunosuppression and T cell mediated immunosuppression at different times.

[42-48]. However, these measures lost their importance and desensitization therapy consisting mainly of pretransplant immunosuppressive pharmacotherapy for B cell suppression has become mainstream. This shift is a natural conclusion of elucidation of acute AMR mechanism as described in previous sections. This status is expected to remain unchanged[7, 18, 20, 21].

Summary

ABO-incompatible kidney transplantation is a radical, and effective treatment for end-stage renal disease.

Pretransplant desensitization therapy to suppress B cell function would be the most effective treatment to ensure successful ABO-incompatible kidney transplantation.

Acknowledgement

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List of abbreviations

ABO-CKTx	ABO-compatible kidney transplantation
ABO-IKTx	ABO-incompatible kidney transplantation
AMR	antibody-mediated rejection
C4d	Complement 4d
HAR	hyperacute rejection
PECAM1	platelet endothelial cell adhesion molecule
PLVAP	plasmalemmal vesicle associated protein
PTC	peritubular capillary
vWf	von Willebrand factor