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IMMUNOADSORPTION AND ITS APPLICATION FOR DESENSITIZING INCOMPATIBLE KIDNEY TRANSPLANT CANDIDATES WHO HAVE A POTENTIAL LIVING DONOR

Summary. Background. Plasmapheresis is widely used to remove potential deleterious antibodies from the blood. Because the volume of treated plasma is limited, plasmapheresis can be replaced by immunoadsorption (IA), a more tedious but sophisticated technique that enables treatment of larger volumes of plasma, i.e., > 4 L vs. 1.5–2 L. We have implemented in our department IA technique to replace plasmapheresis when we launched our ABO-incompatible (ABOi) and HLA-incompatible (HLAi) kidney-transplant programs with living kidney donors. In this setting, isoagglutinin titers (ABOi) or donor-specific alloantibodies (HLAi) have to be decreased drastically at pretransplant by apheresis and immunosuppression.

Methods. We designed a desensitization program based on IA, which was started in the first trimester of 2010 within the Acute Polyvalent Hemodialysis and Apheresis Unit (Toulouse University Hospital, France). We describe all the steps used to implement this IA technique. So far, we have performed > 225 IA sessions.

Results and Conclusions. The IA sessions were associated with a net body-weight gain of ~ 1 kg. Normally, IA is performed first and then hemodialysis on the same or following day; however, we were able to simultaneously perform IA with hemodialysis (tandem procedure). This tandem procedure has reduced costs. Implementation of IA has enabled the successful transplantation of 32 kidney patients.

Key words: ABO-incompatible kidney transplantation, desensitization, hemodialysis, HLA-incompatible kidney transplantation, immunoadsorption, living kidney transplantation.

Introduction

Immunoadsorption is a technique that might be used at the place of plasmapheresis because it allows treating within a session larger volumes of plasma. It is currently used for tissue connective diseases [1], as well as for resistant focal and segmental glomerulosclerosis [2]. Immunoadsorption can be used also to desensitize the kidney transplant candidates who are waiting for an incompatible kidney transplantation.

In France, ~ 36,000 patients have end-stage kidney disease treated by dialysis, chiefly hemodialysis (> 92 %) [3]. Of these, ~ 13,000 are on the national kidney-transplant waiting list; however, only ~ 3,000 patients per year receive a kidney transplant, and this figure is plateauing (Agence de la Médecine, 2010) because the yearly number of brain-dead donors is stable or even slightly decreasing. Meanwhile, the number of living related or unrelated kidney donors increased from < 10 to 12 % in 2012, now accounting for > 30 %

of the donors in our center (i.e., for ~ 180 kidney transplantations annually).

When implementing a living transplant-kidney program, to avoid denying a potential living donor, we also need to accept those with ABO-incompatibility. Moreover, many kidney-transplant candidates, such as those with a previous failed transplant and many women, are sensitized, i.e., they have anti-HLA alloantibodies (HLA incompatibility), which makes it difficult to find a suitable deceased and HLA-compatible donor. In this setting, it may be easier to find a potential suitable living-related donor. Of the ~ 500 patients with end-stage kidney disease and listed for a kidney transplant in our center, HLA-sensitized patients represent ~ 20 % of

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cases. Thus, in order to develop a living-kidney program, ABO-incompatible (ABOi) and/or HLA incompatible (HLAi) kidney pairs will be encountered. For HLAi, the only way to succeed is to implement a desensitization protocol. Several desensitization protocols have been published: they report good kidney-allograft outcomes, even though treatment costs are increased within the first year posttransplantation [4–6].

ABOi kidney transplantation was developed initially in Japan (in the 1980s) because the concept of a brain-dead donor was not recognized. It was achieved using preparatory plasmapheresis, intraoperative splenectomy, and maintenance immunosuppression based on calcineurin inhibitors, azathioprine or mycophenolate mofetil, and steroids. However, many postoperative infectious complications have occurred and many grafts have failed due to acute or chronic humoral rejection [7, 8]. In the early 2000s, many Japanese teams implemented ABO-incompatible transplantation by administering intravenous rituximab prior to transplantation instead of conducting a splenectomy. This enabled chemical elimination of B-lymphocytes, which are implicated in the humoral rejection that takes place after ABO-incompatible renal transplantation. Thereafter, ABOi kidney transplantation was disseminated worldwide with very good results in terms of graft survival.

Desensitization of Kidney-Transplant Candidates

In the setting of ABOi, pre-transplant desensitization currently relies upon: 1) removing antibodies, i.e., isoagglutinins, by means of several plasmapheresis ses-

sions, with the aim of lowering the titer of isoagglutinins to $< 1/10$; 2) preventing their subsequent synthesis by rituximab infusion; and 3) initiating conventional immunosuppression, i.e., tacrolimus, mycophenolic acid, and steroids, at 7–10 days pre-transplantation. The long-term posttransplant results for such patients are very good, particularly since pre-transplant splenectomy has been replaced by rituximab, i.e., results are as good as those observed in patients that have received an ABO-compatible living-kidney transplant [9, 10]. However, in candidates that have very high titers of isoagglutinins, pre-transplant plasmapheresis may be insufficient. This is why Tydén et al., in Sweden in the 2000s, replaced plasmapheresis with specific immunoadsorption (IA) sessions, using columns coated with either A or B blood-group antigens, which deplete isoagglutinins quickly and in a sustainable way [11].

In the setting of HLAi kidney transplantation, the recipient may have donor-specific alloantibodies (DSA) at pre-transplant. DSA can then act against HLA class I (A, B, or Cw) and/or HLA class II (DR, DQ, or DP) antigens. If DSAs are left, they quickly cause acute antibody-mediated rejection at posttransplant, despite immunosuppression [12]. Therefore, for pairs in whom the recipient has DSA(s) against the donor, we need to implement a desensitization protocol at pre-transplantation. This relies: 1) on removing DSAs by via plasmapheresis, 2) preventing their subsequent synthesis using rituximab infusion; and 3) starting conventional immunosuppression, i.e., tacrolimus, mycophenolic acid, and steroids at 7–10 days pre-transplantation [11]. Desensitization can also include intravenous IV-Ig infusions (for



Figure 1. The generators used to perform immunoadsorption. On the left of the image is the ADAorb® generator, and on the right hand side, the Com.Tec® generator

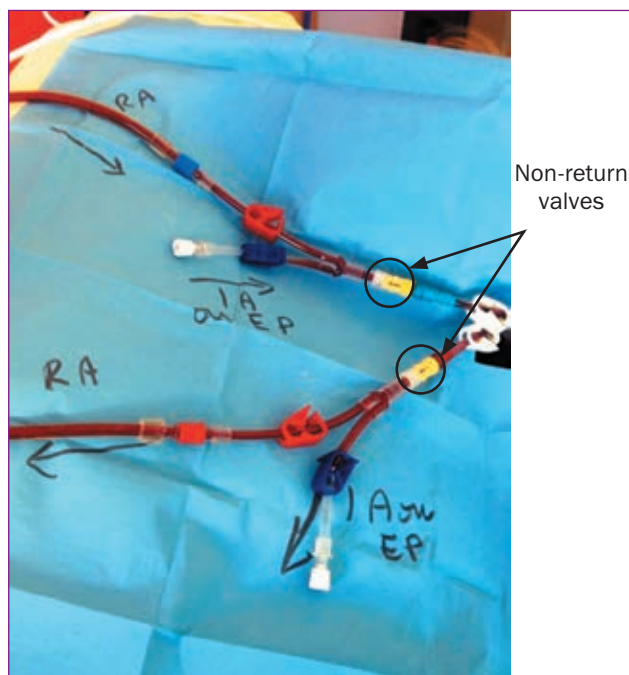


Figure 2. The Y-system, which allows concomitant hemodialysis plus immunoadsorption

its immunomodulatory properties); however, this treatment is costly and there is no conclusive evidence that it improves this protocol [5, 14].

Semi-Specific Immunoabsorption vs. Plasmapheresis

Immunoabsorption can replace plasmapheresis as a desensitization protocol for HLAi pairs. Because IA can treat greater volumes of plasma in one session (compared to plasmapheresis), it may be more efficient at reducing anti-HLA antibody titers. When addressing semi-specific IA, we use columns that are covered by *Staphylococcus* protein A and that can be reused up to 20 times provided they are carefully rinsed (Immunosorba & Globaffin, Fresenius Medical Care) [15], thus saving significant costs. The long-term post-transplant results for HLAi patients who undergo pre-treatment desensitization with IA are very good. In addition, this strategy is cost-effective when compared to matched kidney-transplant candidates who remain on a waiting list for a deceased kidney transplant [13, 16].

The kidney-transplant program at Toulouse University Hospital is one of the top three French kidney-transplant centers and performs the greatest number of living-related or unrelated kidney transplantations. Thus, the Nephrology/Transplantation Department decided to implement desensitization strategies in the setting of ABOi and/or HLAi kidney transplantation using pre-transplant IA instead of plasmapheresis.

Presentation of the Care Structure

The University Hospital of Toulouse (Rangueil, France) made the strategic choice to create one dialysis unit for acute polyvalent hemodialysis and apheresis (APHA), within the Department of Nephrology and Organ Transplantation (DNTO). Thus, concentrating expertise into a single location and creating high-quality collaboration between medical and paramedical personnel. The APHA team is composed of one attending physician, one senior nurse, twelve nurses, six nurse aides, and two biomedical assistants. In 2012, the APHA unit conducted 3,400 hemodialysis sessions, 520 plasmapheresis sessions, 130 IA sessions, 60 liver-dialysis sessions, and 580 continuous veno-venous hemodiafiltration sessions. The unit is open from 8:00 a.m. to 7:00 p.m., Monday through to Saturday, and a nurse is on call 24-h/7-days for emergencies.

The Immunoabsorption Technique

Below there are listed the prerequisites needed to implement IA in an apheresis unit, and our outcomes. Phase 1 took place in the first quarter of 2010. During this period, the medical team, led by Pr. L. Rostaing and Dr. A. Allal, implemented a desensitization program using IA to treat highly sensitized kidney-transplant candidates who could not receive a deceased renal allograft because they had high levels of anti-HLA antibodies (HLAi patients).

Immunoabsorption: Implications for Practice

Prerequisites

- Mastering hemodialysis basic procedures, mastering fistula and/or catheter procedures.
- Mastering plasmapheresis.

Implementation of immunoabsorption

- Training to use an ADAorb® monitor.
- Training on how to handle, save, and store IA columns.
- Use of non-specific usable columns, e.g., Immunosorba®, and specific non-reusable ABO columns.
- Set-up and manage hemodialysis and IA circuits simultaneously.

Outcomes

- Three goals:
 - increase patients' safety by developing a multi-skilled caregiver team;
 - time saving: only 6 h of treatment cf. 11 h with hemodialysis given after IA. Less stress and less tiring for patients during the desensitization sequence;
 - cost effectiveness: total procedure time of < 6 h, only one nurse, and reduction in total consumables.

Time saved means a nurse can look after two or three patients treated by apheresis or hemodialysis. Coupling IA and hemodialysis can be routinely performed by a single caregiver.

The care unit can treat patients 24 h/7 days a week: patients must be effectively desensitized whenever a transplant is available.

IA removes the antibodies of interest from the plasma, in this instance anti-HLA antibodies, by passing plasma through a column covered with *Staphylococcus* protein A (Immunosorba® system, Fresenius). Non-specific IA was initially performed in partnership with an experienced manufacturer (Fresenius Medical Care). In addition, a nurse from the company trained two nurses on our team. Training was carried out using two different monitors: Art Universal® and ADAorb®. The Art Universal separates the plasma by filtration: heparin and sodium citrate are used to prevent coagulation. The ADAorb® treats the plasma using two non-specific (protein A) columns. These columns can be reused up to 20 times after thorough rinsing with distilled water. Plasma flow within the columns is 40–50 mL/min for a blood flow of 70 mL/min. The procedure takes 4–5 h to treat the plasma plus ~ 1.5 h of total nursing time, making a total of 6.5 h. An IA session is associated with a weight gain of up to 1 kg.

The Four Phases in the Technique

During phase 1, i.e., implementing IA, the medical team maintains close surveillance for signs of blood contamination at the entry point of the column using a dipstick as an additional safety measure as well as the monitor's alarm. It is essential to prevent the columns becoming clotted, which would render them unusable.

The outcomes from phase 1 confirmed the usefulness of this technique. Hence, four highly sensitized patients on chronic hemodialysis, with high levels of anti-HLA alloantibodies and high mean fluorescence intensities were treated by IA (average of eight sessions per patient). There was a dramatic decrease in mean fluorescence intensity for some anti-HLA alloantibodies.

However, because these patients were waiting for a deceased donor-kidney transplant and were not prioritized on the national French transplant waiting list, they could not receive a kidney transplant within the 4 months following the IA sessions. Thus, at this point, we changed our strategy by offering IA only to highly sensitized kidney-transplant candidates who had a suitable potential living-kidney donor. We were then able to perform two kidney transplantations with living donors in two highly sensitized patients who had been desensitized by IA sessions with a negative complement-dependent cytotoxic crossmatch at transplantation, even though they still had DSAs at that time. At the last follow-up, these two patients have good renal function.

Phase 2 took place in the first trimester of 2011 with the goal of implementing IA in the setting of ABOi kidney transplantation (Table 1). In the context of ABO-incompatible renal grafts, IA can be either non-specific (cf. supra) or use specific columns that contain blood type A or B antigens on a sepharose matrix (GlycoSorb ABO[®]; Glycorex Transplantation AB, Lund, Sweden); the latter allows targeted elimination of isoagglutinins.

The following factors were needed: 1) a central line or arteriovenous fistula access; 2) plasma separation by centrifugation (no longer by filtration, i.e., we replaced the Art Universal monitor with the Com.Tec[®] monitor (Fresenius Kabi AG[®])) (see Figure 1); 3) adoption of a new circuit that allowed adaptation of specific IA columns on the Com.Tec[®] monitor, and iv) anticoagulation with citrate (citric-acid monohydrate) in the arterial line.

To respond to medical needs, the nursing team proposed the following recommendations: a 'Y'-connector on the return line to compensate, when appropriate, for blood-calcium depletion (citrate is a calcium chelator), and a three-way valve positioned at a point before plasma arrives at the column, dedicated to detecting blood contamination using a dipstick. Biological surveillance before and after the procedure included assessing calcium and magnesium levels, and anti-A or anti-B isoagglutinin levels. Samples were transported to the lab and blood-testing results were available within 30 min for calcium and magnesium, and in < 2 h for isoagglutinins.

Compared to phase 1, the nursing time in phase 2 was reduced to 2–3.5 hours for the IA procedure and to ≤ 15 min for setting-up the monitors and rinsing the columns.

The team used the Com.Tec[®] plasmapheresis monitor, which is mandatory for ABO-incompatible IA, in tandem with the ADA-sorb[®] plasma-treatment monitor (used during phase 1), which uses non-specific sepharose + protein A reusable columns (Immunosorba[®] Fresenius Medical Care). This combination made it pos-

Table 1. The four phases used to implement immunoadsorption in our apheresis unit

	First phase: non-specific IA	Second phase: specific/specific + non-specific IA		Third phase: coupling IA + HD	Fourth phase: generalization of coupling IA + HD
Generators/columns	Art Universal [®] ; ADA-sorb [®] /Immunosorba [®] system	Com.Tec [®] Glycosorb ABO [®]	Com.Tec [®] + ADA-sorb [®] /Immunosorba [®] + Glycosorb ABO [®]	Life 18TM, TherasorbTM/Therasorb [®] Ig flex + hemodialysis monitor	Any type of monitor: Com.Tec [®] ADA-sorb [®] -Life TM , Therasorb TM
Type of patients	Recurrent glomerulonephritis on the allograft (n = 4), or highly sensitized kidney transplant candidates (n = 4) Autoimmune peripheral neuropathy (n = 2)	ABO-incompatible kidney-transplant candidates	ABO-incompatible + HLA-incompatible kidney-transplant candidates	ABO-incompatible + HLA-incompatible patient	ABO incompatible/HLA incompatible/ABO + HLA incompatible patients
Number of transplant patients	Four: one with a living kidney Donor: no suitable cadaveric donors for the other three patients	8 patients with a living kidney donor	2 patients with a living kidney donor	1 patient	9 patients
Outcome	Excellent renal function	1 vascular rejection treated successfully; excellent renal function	Excellent renal function	Excellent renal function	3 acute rejections (1 cellular, 1 vascular, 1 humoral); or excellent renal function

sible to eliminate anti-HLA antibodies from sensitized patients as well as anti-A or anti-B isoagglutinins at the same time, and particularly benefited those patients with concomitant ABOi and HLAi living-kidney transplantation. This new procedure provided more security and comfort for the patients, and use of the non-specific columns cut costs. Thus far, seven patients have been successfully treated and grafted using this procedure.

Phase 3 was implemented concomitantly with phase 2. During phase 2, in October 2012, we encountered a difficult situation with a living-kidney transplant candidate on hemodialysis. This patient was highly sensitized with two DSAs that had high mean fluorescence intensities in the setting of ABO-incompatibility and elevated anti-A isoagglutinin titers ($> 1/128$), which were remarkably resistant to both non-specific and specific IA sessions (> 10), performed as described above. This resistance to IA led the team to implement a new strategy (phase 3) that coupled IA with hemodialysis. This patient was treated using a new LIFE18™ IA monitor (TheraSorb™). This monitor has two specialized functions: 1) plasma separation through centrifugation/filtration; and 2) it uses non-specific columns (TheraSorb® Ig Flex, Miltenyi Biotec GmbH: sepharose + sheep immunoglobulins directed against human anti-Ig). Because of its small-volume circuit (80 mL), IA and dialysis can take place simultaneously via the 'Y' assembly (Figure 2).

This configuration has multiple benefits for the patient: the procedure is quicker; it is better tolerated because hemodialysis corrects for electrolytic problems caused by citrate anticoagulation with IA (variations in calcium and/or magnesium levels, *de novo* alkalosis); and bodyweight increases can be avoided. In contrast, each IA session, when performed alone, leaves the patient with 0.5–1 L of hyperosmotic fluid. In addition, using our new method, less time is required by caregivers per patient and, thus, other patients can be treated simultaneously.

However, this procedure requires: 1) vigilance by the caretaker, i.e., proficiency in using the IA circuit assembly at the same time as the hemodialysis circuit; and 2) a small number of trained paramedics because of the complexity of the two new techniques.

Achieving a low isoagglutinin titer ($< 1/10$) in the patient described above at pre-transplantation was a long and difficult process because she required 20 non-specific immunoadsorptions, four specific IAs, and three plasmapheresis sessions at pre-transplant. Nonetheless, she successfully received a transplant and her current serum creatinine at 9 months posttransplant is 90 $\mu\text{mol/L}$.

Phase 4 was implemented in January 2013 (see Table 1). This consisted of systematically performing hemodialysis in tandem with an IA session; the latter could be either specific- or non-specific. Combined hemodialysis — IA has now become a common procedure: it achieves the best possible tolerance for the patient, regardless of the IA monitor used.

Conclusion

Today, thanks to excellent doctor/nurse/biomedical technician interactions, the Toulouse DNTO has performed 16 ABO-incompatible, 7 ABOi/HLAi, and 7 HLAi renal transplantations, which have resulted in very good outcomes with regards to kidney-allograft function.

Throughout the process of designing and implementing this new IA technique, the team has been creative, flexible, committed, and available (e.g., some posttransplantation IA sessions took place at night). In addition, it requires technical competence and knowhow.

The implementation of this new IA technique by the CHU Toulouse team did not exclusively involve the transplantation program. During this time, we also treated patients with other conditions, e.g., focal segmental glomerular sclerosis recurring after kidney transplantation, myasthenia gravis, and Guillain-Barré syndrome.

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ИММУНОАДСОРБЦИЯ И ЕЕ ПРИМЕНЕНИЕ ДЛЯ ДЕСЕНСИБИЛИЗАЦИИ НЕСОВМЕСТИМОГО ТРАНСПЛАНТАТА ПОЧКИ У КАНДИДАТОВ, КОТОРЫЕ ИМЕЮТ ПОТЕНЦИАЛЬНО ЖИВОГО ДОНОРА

Резюме. Введение. Плазмаферез широко используется для удаления потенциально вредных антител из крови. Поскольку объем очищаемой плазмы ограничен, плазмаферез может быть заменен иммуноадсорбцией (ИА), более трудоемким и сложным методом, обеспечивающим очищение больших объемов плазмы, т.е. > 4 л против 1,5–2 л. Мы внедрили в наш отдел технику ИА с целью заменить плазмаферез при АВО-несовместимой (АВОi) и HLA-несовместимой (HLAi) трансплантации почки от живых доноров. При этом титры изоагглютининов (АВОi) или донор-специфических аллогенных антител (HLAi) должны были резко снизиться в претрансплантационном периоде после афереза и иммуносупрессии.

Методы. Мы разработали программу десенсибилизации на основе ИА, которая была начата в первом триместре 2010 года в рамках работы отдела острого поливалентного гемодиализа и афереза (Университетская больница Тулузы, Франция). В этой статье представлены все шаги, используемые для реализации этого метода ИА. К настоящему времени мы выполнили > 225 процедур ИА.

Результаты и выводы. Процедуры ИА сопровождалась прибавкой массы тела ~ 1 кг. Как правило, сначала выполнялась ИА, а затем процедура гемодиализа в тот же или на следующий день, однако мы могли одновременно выполнять ИА и гемодиализ (тандем-процедура). Тандем-процедура сокращала расходы. Реализация ИА позволила провести успешную трансплантацию почек 32 пациентам.

Ключевые слова: АВО-несовместимая трансплантация почки, десенсибилизация, гемодиализ, трансплантация HLA-несовместимых почек, иммуноадсорбция, трансплантация почки живого донора.

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ІМУНОАДСОРБЦІЯ ТА ЇЇ ЗАСТОСУВАННЯ ДЛЯ ДЕСЕНСИБІЛІЗАЦІЇ НЕСУМІСНОГО ТРАНСПЛАНТАТА НИРКИ У КАНДИДАТІВ, ЯКІ МАЮТЬ ПОТЕНЦІЙНО ЖИВОГО ДОНОРА

Резюме. Вступ. Плазмаферез широко використовується для видалення потенційно шкідливих антитіл з крові. Оскільки обсяг плазми, що очищується, обмежений, плазмаферез може бути замінений імуноадсорбцією (ІА), більш трудомістким і складним методом, що забезпечує очищення великих обсягів плазми, тобто > 4 л проти 1,5–2 л. Ми впровадили в наш відділ техніку ІА з метою замінити плазмаферез при АВО-несумісній (АВО-i) і HLA-несумісній (HLA-i) трансплантації нирки від живих донорів. При цьому титри ізоагглютининів (АВОi) або донор-специфічних алогенних антител (HLAi) повинні були різко знизитися в претрансплантаційному періоді після аферезу та імуносупресії.

Методи. Ми розробили програму десенсибілізації на основі ІА, яка була розпочата в першому триместрі 2010 року в рамках роботи відділу гострого полівалентного гемодиализу та аферезу (Університетська лікарня Тулузи, Франція). У цій статті подані всі кроки, які використовуються для реалізації цього методу ІА. На цей час ми виконали > 225 процедур ІА.

Результати та висновки. Процедури ІА супроводжувалися збільшенням маси тіла ~ 1 кг. Як правило, спочатку виконувалася ІА, а потім процедура гемодиализу в той самий або наступний день, однак ми могли одночасно виконувати ІА і гемодиализ (тандем-процедура). Тандем-процедура скорочувала витрати. Реалізація ІА дозволила провести успішну трансплантацію нирок 32 пацієнтам.

Ключові слова: АВО-несумісна трансплантація нирки, десенсибілізація, гемодиализ, трансплантація HLA-несумісних нирок, імуноадсорбція, трансплантація нирки живого донора.