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 plateausing (Agence de la Medicine, 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 suitable deceased and HLA-compatible 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 the donors.
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 sessions, 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 [4–6].

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

**Semi-Specific Immunoadsorption vs. Plasmapheresis**

Immunoadsorption 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 polynvalent 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 Immunoadsorption 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).

**Immuoadsorption: Implications for Practice**

**Prerequisites**

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

**Implementation of immunoadsorption**

— Training to use an ADAsorb® 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 ADAsorb®. The Art Universal separates the plasma by filtration: heparin and sodium citrate are used to prevent coagulation. The ADAsorb® 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 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 ADAsorb® 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>
<thead>
<tr>
<th>Table 1: The four phases used to implement immunoadsorption in our apheresis unit</th>
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<tbody>
<tr>
<td><strong>Generators/columns</strong></td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Art Universal®; ADAsorb®/Immunosorba® system</td>
</tr>
<tr>
<td><strong>Type of patients</strong></td>
</tr>
<tr>
<td>Autoimmune peripheral neuropathy (n = 2)</td>
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<tr>
<td><strong>Number of transplant patients</strong></td>
</tr>
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<td>Donor: no suitable cadaveric donors for the other three patients</td>
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<td><strong>Outcome</strong></td>
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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 electrolyte 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 hypervolemic 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 μmol/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.

Acknowledgements

We would like to thank all the APHA team for its enthusiasm and commitment, which has allowed the implementation of this new IA procedure in our department. We also thank Fresenius Medical (France) for their long-term help in setting-up this program.

References

ІМУНОАДСОРБЦІЯ ТА ЇЇ ЗАСТОСУВАННЯ ДЛЯ ДЕСЕНСИБІЛІЗАЦІЇ НЕСУМІСНОГО ТРАНСПЛАНТАЦІЙНОГО ПОЧКИ У КАНДИДАТОВ, ЯКІ МАЮТЬ ПОТЕНЦІЙНО ЖИВОГО ДОНОРА

Резюме. Вступ. Плазмафізер широко використовується для видалення потенційно шкідливих антитіл з крові. Оскільки обсяг плазми, що очищується, обмежений, плазмафізер може бути замінений імуноадсорбцією (ІА), більш трудомістким і складним методом, що забезпечує очищення великих обсягів плазми. Ми розробили програму десенсибілізації на основі ИА, яка була впроваджена в першому тримострумі 2010 року в рамках роботи відділу острого полівалентного гемодіалізу (Університетська лікарня Тулуза, Франція). У цій статті подані всі кроки, які використовуються для реалізації цього методу ИА. На цей час ми виконали > 225 процедур ИА.

Методи. Ми розробили програму десенсибілізації на основі ИА, яка була впроваджена в першому тримострумі 2010 року в рамках роботи відділу острого полівалентного гемодіалізу (Університетська лікарня Тулуза, Франція). У цій статті подані всі кроки, які використовуються для реалізації цього методу ИА. На цей час ми виконали > 225 процедур ИА.

Результати та висновки. Реалізація ІА дозволила провести успішну трансплантацію почки 32 пацієнтам.

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