SUPPLEMENTAL DATA*

Desensitization therapy.

Patients and methods

Luminex SAFB assay.

Detection of anti-HLA abs directed against HLA Class I and Class II antigens by Luminex assay (LSA, One Lambda, Canoga Park, CA) was performed in routine in the Immunology laboratory of Saint-Louis hospital. Patients were screened for anti-HLA antibodies by Luminex assay, and Luminex-detected abs directed against HLA Class I and Class II antigens were also identified by SAFB Luminex assay (One Lambda, Canoga Park, CA). Identification of anti-HLA-A, -B, -Cw, -DR, -DQ, and -DP antibodies by Luminex used the references LS1A04 and LS2A01 from One Lambda for classes I and II respectively. Antibodies were detected by the use of goat anti-human IgG coupled with phycoerythrin. The fluorescence of each bead was detected by use of a reader (LABscan; Luminex, Austin, TX) and recorded as mean fluorescence intensity (MFI). The antibody positivity threshold was set at a normalized MFI of 500 according to the baseline formula of the Fusion software (One Lambda). The first time-point of anti-HLA antibodies identification before LTx was taken just before the date of listing of patients. In case of a wait time more than 6 months or in case of immunologic event as blood transfusion, blood sampling for anti-HLA antibody detection was repeated. The last result of the anti-HLA antibodies was taken into account for our desensitization protocol. During follow-up, a cleared DSA was arbitrary defined as MFI <1000. This cut-off was chosen because it corresponded to the threshold of MFI used to start our protocol of desensitization."

HLA typing and cross-matching

HLA typing of recipients was performed by molecular biology (Innolipa HLA typing kit; Innogenetics, Gent, Belgium). For all LTx donors, HLA-A/B/DR/DQ tissue typing involved the microlymphocytotoxicity technique with tissue-typing trays (One Lambda Inc.), and was controlled by molecular biology (1). Within 24 hours of transplantation, LTx recipients underwent auto- and allo-anti-human globulin complement-dependent cytotoxicity (AHG-CDC) T-cell and CDC B-cell crossmatching with and without dithiotreitol reduction, using current (day of transplantation) and historic sera (1) .

Immunosuppression

Immunosuppression after LTx was based on an induction therapy with rabbit antithymocyte globulin (Thymoglobuline; Genzyme, Lyon, France; 1.5mg/kg per day for 3 days, except for: (1) cytomegalovirus (CMV)-infection–negative recipients with CMV infection–positive donors, (2) candidates colonized with *B. cepacia or M. abcessus; (*3) recipient age > 65 years,(4) preoperative long-term administration of corticosteroids $\geq$ 10-15 mg/day, and (5) high probability of postoperative ECMO, who received basiliximab (Simulect; Novartis, Basel, Switzerland) for induction therapy. Some LTx candidates, considered as at particularly increased risk of postoperative infections (among those with conditions that precluded ATG [conditions (1),(2),(3),(4),(5)], had no induction. All lung-transplant recipients received maintenance immunosuppressive therapy with tacrolimus, mycophenolate mofetil (2g/day) and prednisolone (500-mg intravenous [i.v.] methylprednisolone before surgery and before reperfusion of the graft; 1mg/kg/day on the following days and

thereafter replaced by oral prednisone, which was progressively tapered to 0.1 mg/kg/day after 12 weeks). Tacrolimus monitoring involved the predose concentration (C0), with target predose concentration levels of 8 to 12 ng/mL. AR episodes $\geq$ A1 grade were treated with iv methylprednisolone (15 mg/kg/day) for 3 days iv course, then an oral taper of prednisolone. Steroid-resistant cellular AR was treated with rabbit antithymocyte globulin therapy (2.5 mg/kg/day for 5 days) in case of failure of a 3-day course of i.v. methylprednisolone (15 mg/kg/day). Proven and probable AMR (2) were treated with plasmapheresis (5 sessions) rituximab (375 mg/m2), and IVIg (2 g/kg). Our institutional protocol for treatment of AMR during the study period (3) has progressively evolved, but probable AMR diagnosed within the 6 first months in desensitized patients with persistent DSAs at 6 months had additional treatment at the date of probable AMR which consisted of new administration of rituximab (375 mg/m2), followed by monthly prolonged IVIg (n=6 patients).

Desensitization protocol

The decision to accept a graft was made according to the use of virtual crossmatch (VCXM) that was performed according to pf-DSA in historical serum sampled at listing. First, our pre-defined policy of allocation of lung graft was to exclude all pf-DSA>5000 MFI, considered as not permitted, and to allow allocation of the proposed graft with corresponding pf-DSA between 500 and 5000 of MFI in immunized candidates. Patients with pf-DSA MFI between 3000 and 5000 were assessed for the probability to obtain a graft allocation at the time of listing, and only those with an unfavorable benefit-risk balance to obtain a graft allocation without a high risk of death while on waiting list were allowed to be listed with anticipated allocation of a

proposed graft with corresponding pf-DSA between 3000 and 5000. The MFI threshold of 5000 was chosen because it has been previously reported that an MFI>5000 correlates with a positive flow cytometry crossmatch (4).

Hence, in case of allocation, a prophylactic perioperative desensitization protocol was initiated in all LTx candidates with historical pf-DSA with MFI >500 and <5000. It included (i) perioperative plasmapheresis sessions (One performed immediately before LTx, then 5 sessions over 5-10 following days; 2/3 fresh frozen plasma fibrinogen substitution if serum fibrinogen <2 g/L) associated with (ii) rituximab (375 mg/m2) at day-1 post-PE and (iii) IVIg infusion (2g/kg) at day-3 post-PE (iv) mycophenolate mofetil (3g/day, whereas 2g/day was administered to all other patient). Importantly, the desensitization treatment was readjusted day-1 after LTx according to the MFI results at the time of LTx (day 0 serum). Patients with preformed DSA with MFI>500 and <5000 on historical serum but with an immunodominant DSA (iDSA) that remained at MFI<1000 at day-0 stopped the desentization protocol at day-1 (low DSA group), while they had already received a pre-LTx plasmapheresis session before available results of SAFB Luminex assay. Hence, we finally used MFI.1000 for pf-DSA at day-0 as a standard cutoff for administration of desensitization therapy. In case of LTx with pf-DSA>1000 at day-0, the complete desensitization treatment was performed. DSA screening was performed after the 5 plasmapheresis, then every month for 3 months, every 3 months until year 1 and then yearly. Retrospective true CXM was performed within the 24H of LTx by complement-dependent cytotoxicity (CDC and AHG-CDC) on T and B donor lymphocytes in historical and current sera. During the study period, 39 patients with preformed SAFB-detected abs were consecutively transplanted, and compared with 216 consecutive transplanted patients without any preformed anti-

HLA SAFB-detected abs. Some desensitized patients with high pfDSA had iDSA MFI>5000 at day 0, due to the fact that graft allocation was based on result of historical pf-DSA, with possible increase at day-0.

Plasmapheresis sessions were performed in dedicated beds located in the ICU department, first just before LTx surgery for all patients considered immunized at the arrival to our hospital for performing LTx (n=105 patients). No intraoperative session was performed during surgery. Just after surgery at day 1, results of day 0-DSA were obtained, allowing to continue and start full desensitization protocol (in the n=39 patients with high DSA day 0) or to not perform the desensitization for patients with DSA with day 0 MFI< 1000 (n=66 patients with low DSA at day 0). Any post-LTx plasmapheresis sessions were performed in the ICU under the control of the apheresis team, with a 24/7h availability.

Our study was exclusively focused on the impact of preformed DSAs and the effect of a desensitization protocol applied in case of pre-existing DSAs with MFI>1000, and *de novo* DSAs were not analyzed.

Monitoring protocols of patients.

During follow-up of patients, surveillance of cellular acute rejection (AR) and antibody-mediated rejection episodes was performed with systematic transbronchial biopsies (TBBx) (month 1, 2, 3, 6, 9, 12 post-LTx) and in case of clinical, physiological or radiographic changes. During fibroptic endoscopy, bronchoalveolar lavage fluid (BALF) was systematically tested to determine the presence of bacteria,

viruses and fungi(5). Multiplex polymerase chain reaction (PCR) assay was used to detect respiratory viruses in BALF. All TBBx specimens were systematically assessed for AR, and an AR episode was diagnosed histologically and graded according to the International Society for Heart and Lung Transplantation (ISHLT) criteria(6). An AR score was defined by the number of biopsy-proven cellular AR episodes, graded according to the International Society for Heart and Lung Transplantation (ISHLT) criteria(6), during the first 12 months after LTx (7). Diagnosis of antibody-mediated rejection (AMR) was retrospectively made according to criteria of ISHLT(2). CLAD were classified into two clinical phenotypes: bronchiolitis obliterans syndrome (BOS)(8, 9), defined by the classical ISHLT definition, restrictive allograft syndrome (RAS)(10), or mixed phenotype.

PGD was diagnosed and graded according to ISHLT criteria (11).

Outcomes

Patients with CLAD were classified into two clinical phenotypes: bronchiolitis obliterans syndrome (BOS) defined by the classical ISHLT definition (12) or restrictive allograft syndrome (RAS) (13). RAS was defined as a decline in total lung capacity (TLC) of at least10% from baseline: however, in case not enough TLC measurements were available, a forced expiratory volume in 1 s/forced vital capacity (FEV1/FVC) ratio that remained normal or increased above normal with an FVC decline of at least 20% from baseline was also considered restrictive, as used previously (27) whereas a FEV1/FVC index of less than 0.7 was considered obstructive. Severe RAS was defined as those requiring oxygen therapy. All patients were followed at least every 3 months, and for those with pulmonary complications,

additional fiberoptic bronchoscopy for BALF and TBBx sampling was performed to determine clinical, physiological or radiographic changes.

Immunosuppression

All lung-transplant recipients received maintenance immunosuppressive therapy with tacrolimus), mycophenolate mofetil (2 g/day for all patients, except those with perioperative desensitization treated by 3g/day of mycophenolate mofetil) and prednisolone (500-mg intravenous [i.v.] methylprednisolone before surgery and before reperfusion of the graft; 1 mg/kg/day on the following days and thereafter replaced by oral prednisone, which was progressively tapered to 0.1 mg/kg/day after 12 weeks). Rabbit antithymocyte globulin induction therapy was given during the postoperative period (2.5 mg/kg/day for 3-5 days), except for cytomegalovirus (CMV)-infection–negative recipients with CMV infection– positive donors. Tacrolimus monitoring involved the predose concentration (C0), with target predose concentration levels of 8 to 12 ng/mL. AR episodes $\geq$ A1 grade (5) were treated with iv methylprednisolone (15 mg/kg/day) for 3 days iv course, then an oral taper of prednisolone. Steroid-resistant cellular AR was treated with rabbit antithymocyte globulin therapy (2.5 mg/kg/day for 5 days) in case of failure of a 3-day course of i.v. methylprednisolone (15 mg/kg/day).

Some desensitized patients with high pfDSA had iDSA MFI>5000 at day 0, due to the fact that graft allocation was based on result of historical pf-DSA, with possible increase at day-0.

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