Supplemental data*

Patients and methods

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. The transplant allocation system was identical for the 3 centers and followed the rules of the French national agency for organ procurement (Agence de la Biomédecine).
Within 24 hours of transplantion, these patients 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 peak sera.

Monitoting protocols of patients.

During follow-up of patients, surveillance of cellular acute rejection (AR) episodes was performed with systematic transbronchial biopsies (TBBx) (hospital Foch and Marie-Lannelongue, and Bichat hospital during the 2009-2012 period) 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 (1). 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 (2).

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 (2), during the first 12 months after LTx (3).

Outcomes

Patients with CLAD were classified into two clinical phenotypes: bronchiolitis obliterans syndrome (BOS) defined by the classical ISHLT definition (4) or restrictive allograft syndrome (RAS) (5). 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 cyclosporine (n=49; Bichat Hospital) or tacrolimus (Bichat n=14; Foch and Marie-Lannelongue Hospital), mycophenolate mofetil (2 g/day) and prednisolone

(500-mg intravenous [i.v.] methylprednisolone before surgery and before reperfusion of the graft; 0.5 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). In Foch and Marie-Lannelongue hospitals, rabbit antithymocyte globulin induction therapy was given during the postoperative period (1.5 mg/kg/day for 3 days), except for cytomegalovirus (CMV)-infection–negative recipients with CMV infection– positive donors. Cyclosporine monitoring involved the 2-hr post-dose concentration (C2) with target C2 levels of 1200 and 800 kg/L at 1 week and 3 months, respectively. Tacrolimus monitoring involved the predose concentration (C0), with target predose concentration levels of 8 to 12 ng/mL. AR episodes $\geq$ A1 grade (1) 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).

Historical 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, which centralized all sera analyses of the 3 transplant centers. 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). For all patients included in this study, all sera screened by SAFB Luminex assay within 6-12 months post-transplantation were reviewed, with a mean number of DSA screenings within the 6 to 12 months after transplantation of 2.6 $\pm$ 1.9 SAFB assays/patient.

EDTA-Luminex assay

For all included patients with DSAs detected within 6 to 12 after Tx, the selected historical serum sample was additionally retested for identification of circulating DSA antibodies with a SAFB Luminex assays modified by using EDTA pre-treatment of serum, in order to more accurately determine anti-HLA antibody strength through the MFI criterion. All assays were performed as recommended by the manufacturers, in the same laboratory (Immunology Laboratory, Saint-Louis Hospital, Paris). EDTA was used to circumvent the complement interference phenomenon, in order to more accurately determine the anti-HLA antibody strength through the MFI criterion. For this purpose, the assay was modified as follows: serum was supplemented with 1/20 volume pH 8.0 solution of EDTA at 160 mM then incubated for 10 min at room temperature before addition to the SAFB. Sera were also pre-treated with Adsorb-Out (One Lambda) to remove the putative non-specific background. For each patient, we recorded the presence or absence and number of DSA, and for each DSA, its strength defined as the mean MFI without and with EDTA of the beads bearing the donor antigen.

The antibody positivity threshold was set at a normalized MFI of 500 according to the baseline formula of the Fusion software (One Lambda). For EDTA-treated samples, the interpretation was as follows: for antigens present as only one allele in the SAFB kit, the reactivity against the antigen was assigned to this bead. For antigens represented by more than one allele, two situations could occur: 1) the reactivity was assigned to the antigen if all the alleles were positive and mean MFI was calculated from all beads of this same antigen, or 2) the reactivity was assigned to the donor allele if known or to the most probable one from expected linkage disequilibrium, if not all alleles were positive. For DQ, we also considered the alpha chain and composite epitopes created by the juxtaposition of both chains, because the SAFB kit combinations for DQ allow for separating anti-DQbeta from anti-DQalpha antibodies, especially when not all DQbeta beads for the same antigen are positive. DSAs identified by reinterpreted SAFB Luminex assay with EDTA were named reinterpreted EDTA-DSA (r-DSA) results.

C1q assay

For all patients, each historical selected serum with presence of DSA or r-DSA (detected by SAFB Luminex assay or re-interpreted EDTA-Luminex assay, respectively) was then rested with Luminex SAFB C1q assay. The assay involved the C1q-kit (One Lambda) used as instructed. Briefly, serum was first pre-treated with Adsorb-Out, then heat inactivated for 30 min at 56°C, then centrifuged, and 5 microliters were added to the SAFB in the presence of 5 microliter of the recombinant C1q diluted 1/5 in the dedicated buffer, both reagents being provided in the kit. After 20-min incubation on a rotating platform at room temperature, 5

microliters of the phycoerythrin-labelled anti-C1q conjugate were added, and incubated for 20 minutes. After centrifugation, the bead pellet was resuspended in PBS, centrifuged again, resuspended in 80 microliters of PBS, and read of a Luminex 200® (Luminex Corporation, Austin, TX) fluoroanalyzer. The MFI positivity threshold was set at 300 after removal of the signal obtained for the patient's serum on the negative control bead.

ADDITIONNAL RESULTS

Freedom from CLAD and graft survival according to detection of r-DSA and complement-binding properties.

Classification of patients

Patients were classified according to the presence **of r-DSA and their complement-binding properties into 3 groups:** 1) Patients without r-DSA antibodies (n=114), 2) Patients with non–complement-binding r-DSAs (n=40), and 3) Patients with complement-binding DSAs (n=13) (*See Figure S1*, supplementary figures).

FREEDOM FROM CLAD according to r-DSA and complement-binding properties.

Freedom from CLAD was lower in patients with r-DSAs (n=53) than without r-DSA (n=114): 1 and 3 year-freedom frequencies were 65.6% (95% CI 53.9–79.8) and

41.4% (29.8–57.6) versus 87.6% (81.7–97.9) and 72.5% (64.6–81.4) (P<0.00001 by log-rank test) (*Figure S2-A*). When patients with r-DSAs after transplantation were subsequently categorized according to complement-binding capacity, patients with C1q-binding r-DSAs had the poorest 1-year and 3-year freedom from CLAD (34.2% [15.5–75.2] and 17.1% [4.9-60.1], as compared with patients with non–C1q-binding r-DSAs (75.0% [62.7–89.7] and 48.6% [35.0–67.4]), and patients without r-DSAs (87.4% [81.5–93.8] and 72.3% [64.3–81.3]) (p<0.0001) (*Figure S2-B*).

GRAFT SURVIVAL according to r-DSA and complement-binding properties.

Graft survival frequency was lower in patients with r-DSAs compared to those without r-DSAs: 1 and 3 year graft survival frequencies were 84.9% (95% CI: 75.8–95.1) and 63.5% (51.7–78.1) versus 95.7% (92.0–99.5) and 85.0% (78.7–91.8) (p=0.0005 by log-rank test) (Figure S3-A). When patients with r-DSAs after transplantation were subsequently categorized according to complement-binding capacity, patients with C1q-binding r-DSAs had the poorest 1-year and 3-year graft survival (76.9% [57.1–100] and 38.5% [19.3–76.5]), as compared with those with non–C1q-binding r-DSAs (87.5% [77.8 -98.4] and 71.6% [58.6–87.5]), and without r-DSAs (95.6% [91.9–99.4] and 84.9% [78.5–91.8]) (p=<0.0001), (Figure S3-B).

In univariate analysis, the presence of donor-specific anti-HLA antibodies and C1q-binding donor-specific anti-HLA antibodies according to classification by r-DSAs was associated with CLAD onset and graft survival (Table 2). On multivariable Cox regression analysis, we observed that: (i) C1-binding r-DSA detection was an independent predictor of both CLAD onset (hazard ratio, 8.35; 95% CI, 3.44 to 20.28; p<0.0001) and graft loss (hazard ratio, 6.05; 95% CI, 2.50 to 14.69;

p<0.0001), and that (ii) detection of non-complement binding r-DSA was an independent predictor of CLAD (hazard ratio, 1.89; 95% confidence interval [CI],1.04-3.41, p=0.03) (Table 2).

Value of DSA MFI detected by standard SAFB, rEDTA-SAFB and C1q-assay.

Mean (SEM) MFI value of reinterpreted-SAFB-detected DSAs with corresponding C1q-binding was higher than those without corresponding C1q-binding (6295 ± 1235, n=13, versus 1975 ± 355,2, n=34 <0.0001) (Figure S-5 in online Supplemental data).

References

1. Brugiere O, Thabut G, Mal H, Marceau A, Dauriat G, Marrash-Chahla R, Castier Y, Leseche G, Colombat M, Fournier M. Exhaled NO may predict the decline in lung function in bronchiolitis obliterans syndrome. *Eur Respir J* 2005; 25: 813-819.
2. Stewart S, Fishbein MC, Snell GI, Berry GJ, Boehler A, Burke MM, Glanville A, Gould FK, Magro C, Marboe CC, McNeil KD, Reed EF, Reinsmoen NL, Scott JP, Studer SM, Tazelaar HD, Wallwork JL, Westall G, Zamora MR, Zeevi A, Yousem SA. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. *J Heart Lung Transplant* 2007; 26: 1229-1242.
3. Swerdlow DR, Trotter A, Girlanda R, Matsumoto C, Fenelly E. Computed tomography (CT) colonography with CT arteriography and venography for the workup of intestinal transplant candidates. *Clin Transplant* 2013; 27: 126-131.
4. Cooper JD, Gaissert HA, Patterson GA, Pohl MS, Yusen RD, Trulock EP. [Lung volume reduction surgery in advanced emphysema--results of the Washington University, St. Louis]. *Wien Med Wochenschr* 1996; 146: 592-598.
5. Verleden S, Vingerhoets G, Santens P. Heterogeneity of cognitive dysfunction in Parkinson's disease: a cohort study. *Eur Neurol* 2007; 58: 34-40.