Analysis of Specificity of Anti–Human Leukocyte Antigen Antibodies in Kidney Recipients in Reference to Clinical Outcome

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ABSTRACT

Background. Anti–human leukocyte antigens antibodies (HLA) are not always the main cause of graft injury but can be a marker of immune response to the graft. The aim of this study was to analyze anti-HLA specificities with the use of the most sensitive detection method (Luminex) in reference to clinical condition.

Methods. Sera of 65 kidney recipients (n = 443) were screened with the use of the mixed LABScreen kit, and, for 47 recipients, sera with maximal normalized background ratio (NBG) were subjected to specificity testing. NBG, numbers of specificities, donor-specific antibodies (DSA), and normalized mean fluorescence index (nMFI) of DSA and maximal anti-HLA were analyzed in reference to clinical (acute rejection [AR] diagnosis, immunosuppression), histopathological (C4d staining, chronic allograft nephropathy, AR type), and laboratory parameters (creatinine).

Results. We observed 1 to 51 specificities, class I DSA in 26.7%, class II in 10%, and estimated DQ-DSA in 63.3% of tested patients. Patients with AR and humoral AR had significantly higher NBG, number of anti-HLA class I, DQ and DQ-DSA types, and more frequently had anti-HLA and class II DSA-positive sera ($P < .052$). C4d staining was associated with higher anti-HLA class I ($P = .053$) and class I DSA ($P = .002$) type numbers, and maximal anti-HLA nMFI ($P = .036$) and was more frequent in AR ($P = .048$) and class II DSA positive patients ($P = .046$). Patients with chronic allograft nephropathy showed higher DQ-DSA-nMFI ($P = .036$). DQ-DSA-nMFI and maximal anti-HLA-nMFI correlated with creatinine increase (Spearman range [SR] = 0.64, SR = 0.41). Together with NBG, maximal class I and class II anti-HLA-nMFI correlated with the number of transplantation and maximal panel-reactive antibodies ratio (SR = 0.19–0.40).

Conclusions. Anti-HLA detection allows for humoral AR diagnosis but also for identification of patients with risk of any rejection. However, clear rules of anti-HLA interpretation and studies on their clinical impact are needed.

ANTI–HUMAN LEUKOCYTE ANTIGEN ANTIBODIES (HLA) are produced as a part of the response of the recipient's immune system against donor alloantigens. They are responsible for acute humoral rejection (AHR) and take part in chronic rejection [1,2]. This process is inhibited and modulated by immunosuppressive drugs, which are designed to block mainly cellular immune response, which is also important in the humoral immune response. Anti-HLA antibodies are used in laboratory diagnostic methods of rejection. Many different immune assays have been created over the years, including the complement-

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dependent cytotoxicity–lymphocytotoxic test, (CDC-LCT), enzyme-linked immunosorbent assay, flow cytometry, and, in the past decade, Luminex multiplexing xMAP technology, which is currently the most sensitive method [3,4]. They are only one of the elements required in rejection diagnostics besides clinical symptoms, serum creatinine level increase, and biopsy results. Complicated pathways of the human immune response make interpretation of laboratory assays that detect anti-HLA antibodies difficult [5,6].

The aim of this study was to analyze specificities of anti-HLA antibodies through the use of the most sensitive detection method available of multiplexing xMAP Luminex technology with reference to clinical condition, laboratory testing, and biopsy results.

METHODS

Sera of 65 kidney recipients, transplanted between June 2008 and April 2011 and monitored in the Clinic of Nephrology, Transplantology, and Internal Disease of Pomeranian Medical University were collected together with clinical data for up to 2 years after transplantation (at 2 weeks and at 1, 2, 3, 6, 9, 12, 15, and 18 months after transplantation). Each recipient was also diagnosed by use of 2 or more biopsies: protocol biopsies in 3, 12, or 24 months after transplantation and diagnostic biopsies for clinical conditions (rejection suspicion). The study project was approved by the institutional Bioethics Committee and is in accordance with the Helsinki Declaration.

Sera were stored at −40°C and tested at the Department of Microbiology and Immunology of the Pomeranian Medical University. Luminex multiplexing xMAP technology with LABScreen Mixed and single antigen reagents kits (One Lambda, Canoga Park, Calif, United States) were used in antibody detection according to the manufacturer's recommendation, and HLA Fusion (One Lambda) software was used for measurements analysis. Results of the Mixed LABScreen were reported as normalized background ratio (NBG) and as normalized mean fluorescence index (nMFI) for the single antigen assay. The cutoffs assigned by means of HLA Fusion were 1.5 for NBG and ×6 for nMFI.

Six to 11 sera of each recipient were screened with the Mixed LABScreen, and serum with the highest NBG value or significant NBG increase was selected for further NBG analysis and for specificity testing with the Single-Antigen LABScreen. The average and the minimal and the maximal values of NBG for class I and class II beads were used in the analysis of the Mixed LABScreen. In the single-antigen analysis, we used the number of anti-HLA and donor-specific antibody (DSA) types, the average of positive DSA nMFI, the total DSA nMFI (the sum of nMFI of all positive and negative DSA), and the maximal anti-HLA nMFI. Biopsy results were analyzed for the presence and the type (cellular or humoral) of acute rejection (AR), presence of positive C4d staining, and chronic allograft nephropathy/interstitial fibrosis/tubular atrophy (CAN/IF/TA) diagnosis. Basic laboratory indicators (creatinine level, also its increase from the previous testing and from the minimal level), immunosuppression levels (tacrolimus concentration and prednisone doses), immunological matching (the actual and the maximal panel reactive antibodies ratio [PRA], the number of transplantations and mismatches) as well as the clinical outcome (AR diagnosis based on the clinical status and biopsy result) were collected with each serum and correlated with sera testing results.

Results were analyzed with the use of Statistica 7.0 software. The Fisher's exact test, Kruskal-Wallis 1-way analysis of variance, and Mann-Whitney U tests were used in data comparisons, and the Spearman range (SR) test was used in correlation testing. Values of $P < .05$ were treated as statistically significant.

RESULTS

Sera (n = 443) of 65 kidney recipients (35% women), ages 19 to 71 years (mean, 44 years), were screened for the presence of anti-HLA antibodies. The medical histories of 23 patients showed a diagnosis of acute rejection (1 to 4 episodes): in 18 patients (27.7%) of cellular type (20% borderline type, 9.2% cellular, and 1.5% intestinal type) and in 7 patients (10.8%) of humoral etiology (7.7% C4d highly positive, 4.6% humoral). Twenty patients (30.8%) had positive results of C4d staining and 21 (32.3%) patients had CAN/IF/TA features in at least 1 of the biopsies.

Creatinine levels in tested sera were 0.74 to 6.7 mg/dL (mean level, 1.77 mg/dL). In 27 cases, the creatinine level increased by 0.01 to 2.48 mg/dL (on average, 0.14 mg/dL) from the previous testing, which is a 0.6% to 115% increase (on average, 19%; in 7 [11%] patients the increase was >20%) and by 0 to 5.31 mg/dL (on average, 0.57 mg/dL) from minimal level. Patients received the standard 3-drug immunosuppressive therapy composed of calcineurin inhibitor: tacrolimus (77% of patients) or cyclosporine (18.5%); anti-proliferative drug: mycophenolic acid (92%), azathioprine (3.1%), or everolimus (6.1%), as well as steroids in the mean dose of 12 mg (2.5–30 mg). Recipients had 1 to 6 mismatches (on average, 3.3). All of them had 0 to 4 (on average, 2.5) class I mismatches and 71% had 0 to 2 (on average, 0.83) class II mismatches ($P = .000$). The number of class II mismatches differed statistically between patients with and those without AR (mean number of mismatches, 1.04 vs 0.71, $P = .047$). Potential DQ mismatches were estimated in 90.8% of recipients, in all with AR and 85.7% without AR ($P = .082$). Actual and maximal PRA was 0% to 100% (on average, 6.9% and 9.88%), and only 2 patients (3.1%) were highly immunized (PRA >80%). For 56 patients it was the first transplantation, for 8 patients it was the second, and for 1 patient it was the third.

Mean values of the maximal, the minimal, and the average NBG of class I and class II anti-HLA in selected sera with maximal NBG for each patient in clinical groups are presented in Table 1. The values were significantly higher in patients with acute rejection in the medical history, especially of the humoral type, but not significantly higher for cellular rejection (except for class II minimal NBG). There were no significant differences between NBGs of sera with and without creatinine increase and C4d staining in biopsy (except minimal NBG), with and without CAN/IF/TA, or between sera of patients with different types of immunosuppressive drugs. Also, differences in NBGs of anti-HLA–positive and negative sera were not significant (except for class I maximal NBG), and, in DSA-positive sera, NBGs were significantly higher only for class I DSA. NBG values did not correlate significantly with creatinine levels, tacrolimus levels, or prednisone doses, but we