Anti–Human Leukocyte Antigen Antibodies Are Present in Blood of Blood Donors: Is Therapy With Blood Preparations Safe for Graft Recipients?

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ABSTRACT

Background. Blood products infusions are often administrated to graft recipients. Post-transfusion reactions of anti–human leukocyte antigen antibodies (anti-HLA) are responsible for transfusion-related acute lung injury, but cases of graft rejection after blood product infusions were recently also proven.

Methods. The aim of this study was to assess, with the use of the very sensitive Luminex technology and traditional lymphocytotoxic test, the prevalence and cytotoxic activity of anti-HLA in blood donors with different medical histories to evaluate a potential risk of post-transfusion immune complications. Data were analyzed according to different normalized background cutoffs (1.5, 2.2; and the high cutoffs−10.8 for I class and 6.9 for II class anti-HLA).

Results. We observed that anti-HLA may be present in 36% of donors, and even in up to 73.6% of risk groups. Significant risk factors included female sex (23.9% to 64.2% for different cutoffs) and pregnancy history (30% to 72.5%), regardless of the cutoff used in analysis, whereas sera from female donors showed lower cytotoxicity (panel reactive antibodies). Anti-HLA were also detected in men (3.7% to 37%), in donors after a transfusion (0% to 62.5%), and even with no known risk factors (3.8% to 26.9%).

Conclusions. Luminex technology is a sensitive tool in anti-HLA detection, but consensus in measurement interpretation for blood donors is needed. Selection of blood products on the basis of medical history can be a useful alternative for routine testing of blood donors. The clinical significance of treatment of graft recipients with blood products requires further study; until then, more attention should be paid to possible complications.

THE HUMAN LEUKOCYTE ANTIGENS (HLA) system constitutes a set of glycoproteins present on every nucleated cell of the body and blood platelet that is unique to each individual. It is an important element of human immune response but also a major barrier for transplantation. Foreign alloantigens induce an immune response including the production of anti-HLA antibodies. An alloimmunization occurs as a result of transplantation but also after blood transfusions and pregnancies [1]. Anti-HLA antibodies were also found in the course of some infections as the result of cross-reactivity; however, this relationship is not clearly understood [2,3]. The presence of these antibodies in allograft recipients or in transfused blood may lead to immunological complications; the most

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common is transfusion-related acute lung injury (TRAIL) [4,5]. However, 2 cases of laboratory-proven acute rejection resulting from transfusions of blood products containing anti-HLA antibodies were recently published [6,7].

The aim of this study was to evaluate, with the use of the newest and the most sensitive method—Luminex xMap technology—the prevalence of anti-HLA antibodies in blood of healthy blood donors to assess a potential risk of immune complications resulting from an infusion of blood products.

METHODS

Serum samples (100 mL) were collected during the period from January 11, 2011, to January 26, 2011, from healthy blood donors in the West Pomeranian Blood Donation Center in Szczecin. They were qualified according to the standard procedure, after a medical examination and basic laboratory tests. From the total of 1080 blood donations we chose all derived from donors with positive medical history. We obtained 53 samples from donors after pregnancies or transfusions. There were no donations after transplantation since transplantation history excludes from blood donation. On January 24 to 26, 2011, samples were also collected from donors without a positive history (as a control group) who were asked about infections in the past 3 years. The study project was approved by our institutional Bioethics Committee and is in accordance with the Helsinki Declaration. Individual consent of each donor was also obtained.

Samples were divided into 4 groups according to the medical history of donors: the pregnancy subgroup, transfusions, pregnancies and transfusions, and the control group, including a healthy subgroup and an infections subgroup (Table 1).

Samples were stored frozen (−40°C) and tested for the presence of anti-HLA antibodies at the Department of Microbiology and Immunology, Pomeranian Medical University in Szczecin. Luminex multiplexing xMAP technology and a LABScreen Mixed reagents kit (One Lambda, Canoga Park, Calif, United States) were used for antibody detection and HLA Fusion software (One Lambda) was used for analysis. Results are reported as fluorescence values normalized according to the manufacturer's recommendation as normalized background (NBG) ratio, which is proportional to the amount of anti-HLA antibodies in the serum sample. NBG ratio cutoff of 1.5 was used for positives in HLA Fusion analysis. Additionally, measurements were classified with a cutoff of 2.2 recommended by the manufacturer, as well as with the high cutoffs of 10.8 for HLA class I and 6.9 for class II as proposed for blood donors by Triulzi et al [8] (mean +3 standard deviation of the natural log-transformed distribution of NBG values in the 1138 non-transfused male blood donors). Maximal positive NBGs of sera were also used in data analysis.

Sera were also tested for cytotoxicity of antibodies with the use of the conventional lymphocytotoxic test (complement-dependent cytotoxicity [CDC]) with determination of panel-reactive antibodies (PRA, the percentage of positive reactions of sera with a panel of 30 lymphocyte samples). The CDC test was performed according to the standard procedure: 2 hours of serum and lymphocyte incubation, 15 minutes of incubation with complement, and 5 minutes of eosin staining and fixation with formalin. Samples with 30% more destroyed cells than in the negative control reaction were qualified as positive.

Statistical Analysis

Results were analyzed with the use of Statistica 6.0 software. The Fisher's exact test, Kruskal-Wallis 1-way analysis of variance (KW), and Mann-Whitney U tests were used in data comparisons and the Spearman range test was used in correlation testing. Values of $P < .05$ were treated as statistically significant.

RESULTS

We tested the sera of 94 blood donors (67 women and 27 men), ages 19 to 61 years (average age, 33.2 years). Positive medical history was obtained from 53 donors, ages 21 to 61 years (mean age, 38.51 years), including 45 women and 8 men. In this group, 40 women reported a history of pregnancies, 8 donors reported transfusions, and 5 women reported both risk factors. The control group contained 41 donors ages 19 to 52 years (mean age, 26.34 years); 19 men and 22 women. Fifteen control subjects reported an infection (11 of the respiratory tract, 4 of the urinary tract) during the last 3 years: in 13 cases in the last year and single cases 2 and 3 years ago (Table 1).

The presence of anti-HLA antibodies according to the 1.5 cutoff was detected in 56.4% (53 of 94) tested donors. The percentage of positive sera was significantly higher in the risk group than in the control group (73.6% vs 34.1%, Fisher's exact test; $P = .0001$). We estimated that the percentage of anti-HLA–positive sera in the population of blood donors could be 36% (in the group of 1080 donations, 39 of 53 risk group donors and 34.1% of the tested 41 control donors of 1027 were positive). Percentages of anti-HLA–positive sera statistically differed between the study subgroups (KW, $P = .001$). Among donors with a positive history, the percentage of sera containing anti-HLA antibodies was the highest in the group with both risk factors (100%) and the lowest was in the transfusion group (62.5%), but differences were not statistically significant (KW, $P = .32$). In the control group, the percentage of positive sera was almost 2 times higher in the subgroup with an infection in the medical history (46.7% vs 26.9%), but the difference was not significant (Fisher's exact test, $P = .17$) (Table 1).

Tested donors produced class I anti-HLA antibodies more often than did class II: 50% versus 24.5% (Fisher's exact test, $P = .0002$); 18.1% of donors produced antibodies against both groups. The highest percentage of anti-HLA class I antibodies was found in the group with both risk factors (80%), the lowest was found in the control group (34.4%). The differences are statistically significant (KW, $P = .0473$). Similarly, significant differences were observed for class II anti-HLA positive sera: 60% of the pregnancy/transplantation group and 7.3% for the control group (KW, $P = .001$); for both class I and class II anti-HLA antibodies positive sera: 40% in the pregnancy/transplantation group and 7.3% of control group (borderline significance, KW, $P = .062$) (Table 1).

Antibody levels characterized as average of maximal NBG ratios were higher in the risk group than in the control group and in the risk group were the highest in the pregnancies group, but differences in NBG among the 4 subgroups were not statistically significant (KW, $P = .4493$ for