Pathol Immunopathol Res 1989;8:287–299

0257–7761/89/0086–0287$2.75/0

Anti-Class II Antibody Production Prolongs Renal Allograft Survival

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Introduction

It is widely accepted that conditioning of recipients by blood transfusions exerts a favorable influence on the survival of renal allotransplants [1–7]. In the past, some investigators suggested that the beneficial effect of transfusions was only the result of selection, and many patients were thought to be rendered nontransplantable as a consequence of sensitization by transfusions [8–12]. A low rate of rejection in some studies does not support a selecting out process as the mechanism for improved allograft survival and argues for a modification of the immunologic responses [13]. The effects of blood transfusions are found to improve the outcome, especially in the best HLA-matched groups [5]. In contrast, others described a highly significant allograft survival in the transfused DR-mismatched cadaveric kidney allograft recipients [14]. Many authors have suggested that HLA-DR antigen matching alone improves allograft survival [15–20]. However, this finding is not universal [21]. The timing of transfusions as well as the number of units of blood needed for optimum graft survival are still unresolved. HLA-A and -B antigen matching has shown a disappointing correlation with graft survival [7].

The immunologic stimulus of pregnancy, blood transfusion, tissue allograft and possibly some viruses and bacteria may induce lymphocytotoxic antibodies. Transfusions alone have a minimal effect on alloimmmunization in men and in multiparous women [14, 22]. Some indicate that transfusions may induce both a specific and a nonspecific suppression of the cell-mediated immune response, while others disagree [23–25]. Alloimmunization does not appear to decrease graft survival in those patients with a negative lymphocyte crossmatch by sensitive techniques who receive grafts [14, 26–29].

We hypothesize that pretransplant alloimmunization with nondonor-specific blood transfusions will sometimes stimulate class II (B cell) antibodies which act as enhancing factors extending the survival of cadaveric kidney allografts. This study was designed to evaluate the lymphocytotoxic antibody response by observing for a difference in donor T and B cell panel reactivity in a pre- and post-transfusion serum sample from uremic patients on hemodialysis awaiting

their first cadaveric kidney allotransplants. The data were analyzed to determine whether or not an association exists between HLA class I and II lymphocytotoxic antibodies and longevity of renal allograft survival.

Methods

Transplant Recipients

Frozen sera were stored at −70 °C from 40 primary recipients of cadaveric renal allotransplants collected from May 1981 through September 1987 prior to the transplant. Samples collected before and after transfusions of whole blood or packed red cells from each patient were randomly chosen. Transfusions were administered before the patient was placed on the transplant list with intervals of 1 week between transfusions. The last unit was usually given after adding the patient to the list. HLA-class I (T cell) and class II (B cell) antibodies were detected using an Amos modified complement-dependent microlymphocytoxicity assay [30, 31].

Serological Techniques

A randomly selected donor panel of 40 peripheral blood lymphocyte samples separated by Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) were reacted with patient serum to screen for HLA-class I (T cell) antilymphocytotoxic antibodies. The serum and cells were incubated at room temperature for 30 min, rabbit complement was added and incubation continued for 1 h. Trypan blue was added to distinguish live from dead cells. The reaction was graded as 8, 90–100% dead; 6, 50–90% dead; 4, 20–50% dead; 2, 10–20% dead and 1, 0–10%. The percentage of donor panel cells reacting was expressed as percent reactive antibody (PRA). When greater than 10% of the panel cells reacted with a grade of 4 or better, the panel was considered to be significant. When a particular cell specific for a given antigen was lysed by antibody-rich serum greater than 50% of the time, that antibody specificity was considered to be present. Multiple serum samples frozen after blood transfusions were completed prior to transplantation were analyzed and the mean PRA was calculated as the post-transfusion HLA-class I (T cell) PRA.

A randomly selected donor panel of 29 B-cell enriched lymphocytes, fractionated by adsorption to nylon-wool columns with subsequent elution, was used to identify HLA-class II lymphocytotoxic antibodies (B-cell antibodies) [32]. The serum and cells were incubated at 37 °C for 1 h, rabbit complement was added and incubation continued for 1.5 h at room temperature. The complement-mediated lysis was graded as above utilizing the same criteria for significance of reactivity. The percentage of donor cell panel reacting was expressed as class II (B cell) PRA.

Patients' sera identified to contain HLA-class II (B cell) antibodies were absorbed with pooled blood bank platelets from at least 10 donors. After absorbing twice for 30 min at room temperature, the sera were reanalyzed for class I (T cell) and class II (B cell) antibodies to determine the effectiveness of the platelet absorption and antibody specificity, respectively. For reevaluation of T cell PRA, a warm (37 °C) incubation was carried out as with the B cell PRA.

Control HLA-class I (T cell) and HLA-class II (B cell) PRA were assayed using the sera of 27 normal nontransfused volunteers of similar age and sex. Positive and negative controls were included on each microtiter plate for both HLA-class I (T cell) and HLA-class II (B cell) microlymphocytotoxicity assays.

Crossmatch Technique

A modification of the technique described by Amos et al. [31] with anti-IgG to increase the sensitivity was performed on potential donor-recipient pairs prior to transplantation.

Statistical Analysis

Survival distributions of differences in the PRA before and after blood transfusions (transfusion-related cytotoxic antibodies) as well as post-transfusion PRA levels were analyzed. The relationship of survival (greater than or less than 1 year) was evaluated when the time lag between initial dialysis, transplantation workup or the last transfusion was compared to the time of the subsequent transplant. Other factors which influence kidney allograft survival such as the number of blood transfusions, immunosuppressive therapy, HLA matches, age, sex, parity, prior dialysis and the production of monospecific or multispecific HLA antibodies were also considered. These variables were examined to determine whether an association existed between the level of transfusion-induced cytotoxic antibody production and the presen-

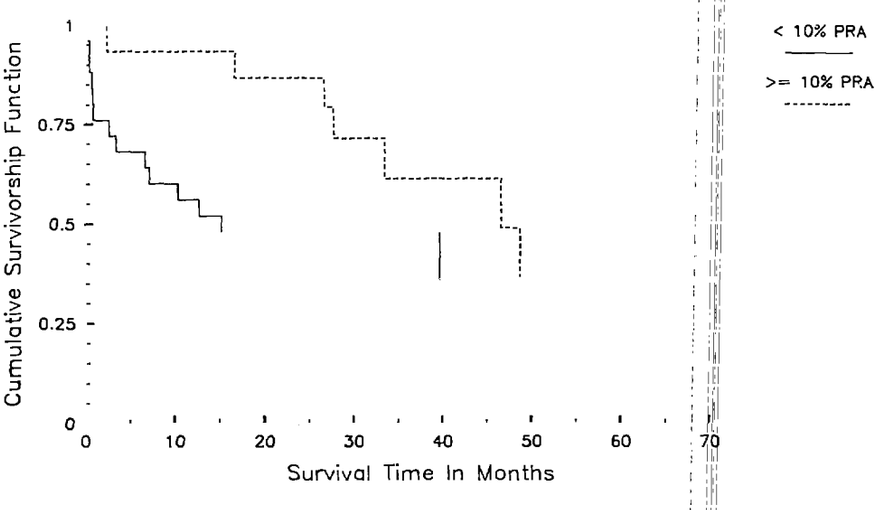

Fig. 1. The effect of differences between pre- and post-transfusion B cell PRA values, expressed as transfusion-related HLA class II B cell antibodies, on renal allograft survival. Transplant survival in patients with greater than or equal to 10% PRA is compared to those with less than 10% PRA. The cumulative survivorship function is displayed as 100, 75, 50 and 25% allograft survival.

sitization caused by pregnancy, specific HLA antibody synthesis, sex, waiting time on the transplant list, the number of packed red cells or whole blood transfusions and previous positive anti-IgG crossmatches. Statistical analyses employed both the χ^2 test of independence, medial test and Gehan's generalized Wilcoxon test.

Results

Significant differences in survival of cadaveric renal allografts were demonstrated when transfusion-related increases in B cell PRA values were determined and analyzed. Recipients with B cell PRA differences of less than 10% between pretransfusion and post-transfusion serum samples manifested a median graft survival of 14 months whereas those with values of greater than 10% attained a median graft survival of 46 months ($p = 0.0291$; fig. 1). The difference between pre- and post-transfusion T cell PRA classified by the same criteria was not significant ($p = 0.5788$). When only the post-transfusion class II (B cell) PRA was considered, there was a highly suggestive correlation with the extension of survival ($p = 0.0653$; fig. 2). The post-transfusion T cell PRA was not associated with lengthening the survival of the allograft ($p = 0.1611$).

Packed red cells or whole blood sensitized less than half the recipients measured by the production of monospecific and/or multispecific antibodies to HLA-A and -B and/or HLA-DR antigens. The patient population under investigation was sensitized to a

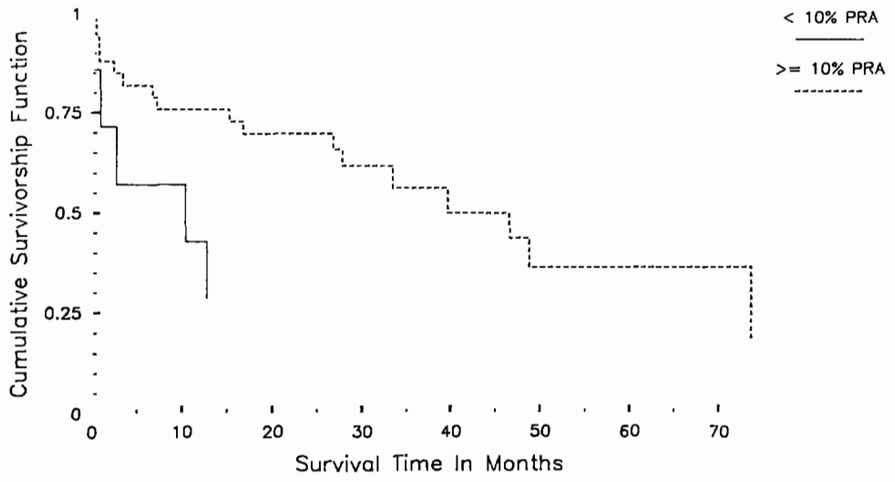

Fig. 2. The effect of post-transfusion HLA class II B cell antibodies, expressed as greater than or equal to 10% PRA or less than 10% PRA, on renal allograft survival. The cumulative survivorship function is expressed as 100, 75, 50 and 25% allograft survival.

slightly greater extent by class II HLA-DR antigens (17 of 40 patients or 43%), compared to the class I HLA-A, -B antigens (12 of 40 patients or 33%). The production of monospecific or multispecific HLA-DR antibody was shown to prolong the survival of renal allografts by a median of 31 months ($p = 0.0090$; fig. 3). No prolongation in allograft survival could be demonstrated with HLA-A or -B antibody ($p = 0.3443$).

Prominent among the many factors that influence renal allograft survival is HLA-A, -B and -DR histocompatibility between donor and recipient. When one to four HLA-A, -B matches were combined and compared to those recipients with no matches, a significant difference in allograft survival was observed between the two groups. The median survival for those with no matches was 11 months compared to the group where 60% of the recipients continued to maintain their allografts by the end of follow-up, ($p = 0.0255$; fig. 4). Comparison of HLA-DR matches between no match and one to two HLA-DR matches did not significantly extend allograft survival ($p = 0.1852$). Circulating antibodies specific for allograft antigens failed to influence allograft survival ($p = 0.3704$ for HLA-DR antibodies and $p = 0.7736$ for HLA-A, -B antibodies.

Neither sex, parity nor age affected allograft survival in the group studied. The number of whole blood or packed red blood cell transfusions, whether 3–5 or greater, produced no detectable difference ($p = 0.2506$). Likewise, the choice of prednisone or cyclosporin immunosuppressive therapy led to no difference in allograft survival ($p = 0.8347$).

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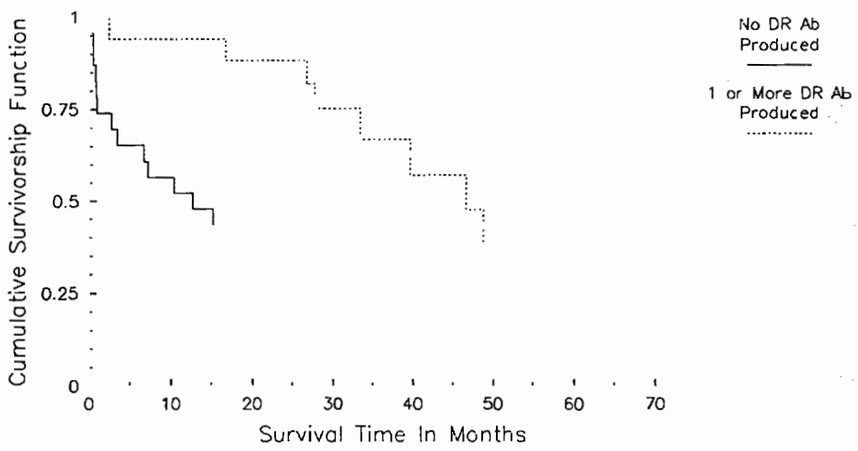

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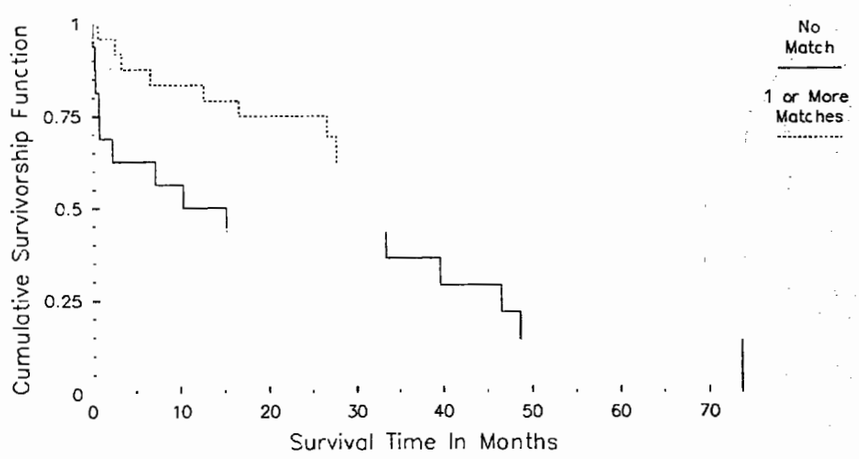

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Fig. 3. The effect of monospecific or multispecific HLA-DR antibody (Ab) on transplant survival. The cumulative survivorship function is represented as 100, 75, 50 and 25% allograft survival.

Fig. 4. The effect of HLA-A and -B antigen matching, expressed as no match and as 1 or more matches on transplant survival. The cumulative survivorship function is displayed as 100, 75, 50 and 25% allograft survival.

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A historical positive crossmatch that subsequently became negative produced no deleterious effect on allograft survival (p = 0.7723). The majority of the study recipients (25 of 40 or 63%) were diagnosed with underlying hypertension which progressed to end stage renal disease. These patients were divided equally between the two groups with an allograft survival of less than 1 year, GpA (8 of 12 or 67%) or greater than 1 year, GpB (17 of 27 or 61%). Groups A and B contained an equal distribution of other primary diagnoses that included diabetes (10%), chronic glomerulonephritis (13%), polycystic kidney disease (5%), nephrosclerosis (5%) and progressive glomerulonephritis (3%). There was no significant effect of hemodialysis on graft survival (GpA = mean 627 ± 124, med. 436; GpB = mean 812 ± 176, med. 529 days; p = 0.4956), transfusion timing before transplantation (GpA = mean 185 ± 50, med. 141; GpB = mean 160 ± 24, med. 113; p = 0.4956), and waiting time on the transplant list (GpA = mean 211 ± 36, med. 190; GpB = mean 236 ± 29, med. 208; p = 0.5809). The presence of B cell and T cell cytotoxic antibodies following transfusion therapy, with a negative crossmatch, did not preclude proceeding with allotransplantation, with a success rate of 85% in spite of increased cytotoxic antibody activity, ≥ 50% T cell PRA (n = 5, mean 289 ± 85 days waiting), < 50% T cell PRA (n = 35, mean 216 ± 24 days waiting) and ≥ 50% B cell PRA (n = 11, 192 ± 33 days waiting), < 50% B cell PRA (n = 29, mean 253 ± 28 days waiting). When ≥ 50% B cell and T cell cytotoxic antibody activity was compared to the number of days on hemodialysis, a T cell PRA of greater than 50% was associated with the longest number of days on dialysis [≥ 50% T cell PRA (n = 5, mean 1,193 ± 727) and < 50% T cell PRA (n = 35, mean 625 ± 85)]. No difference appeared in ≥ 50% B cell PRA (n = 5, mean 289 ± 85%) and < 50% B cell PRA (n = 35, mean 216 ± 24%) when compared to the time on hemodialysis.

To further define the significance of the B cell and T cell PRA difference between pre- and post-transfusion samples, the influence on these quantitative parameters by sex, parity, antibody production and number of whole blood or packed red blood cell transfusions was analyzed. Sex influenced the production of transfusion-related B cell and T cell cytotoxic antibodies quantitated as PRA (males; n = 21, mean 4 ± 3%; females, n = 19, mean 18 ± 0.03%, p = 0.0018 and males, med. 3 ± 3%; females, med. 10 ± 0.1, p = 0.0286, respectively) (fig. 5). When males and nulliparous females were grouped together and compared to parous females, an increase in the transfusion-related B cell PRA was associated with parous females (n = 25, mean 6 ± 3% and n = 14, mean 20% ± 4, p = 0.0059, respectively) (fig. 6). This association was not observed with transfusion-related T cell PRA (p = 0.2074). There was,

Fig. 5. The influence of sex on transfusion-related B and T cell cytotoxic antibodies expressed as the difference in the pre- and post-transfusion PRA level is represented by boxes. The median values (50th percentile) are displayed as vertical lines within the boxes, while the 25th and 75th percentiles form the outer edges of each box.

Fig. 6. The influence of sex and parity on transfusion-related B and T cell cytotoxic antibodies represented as the difference in the pre- and post-transfusion PRA level is depicted as boxes. The median values (50th percentile) are displayed as vertical lines within the boxes, while the 25th and 75th percentiles form the outer edges of each box.

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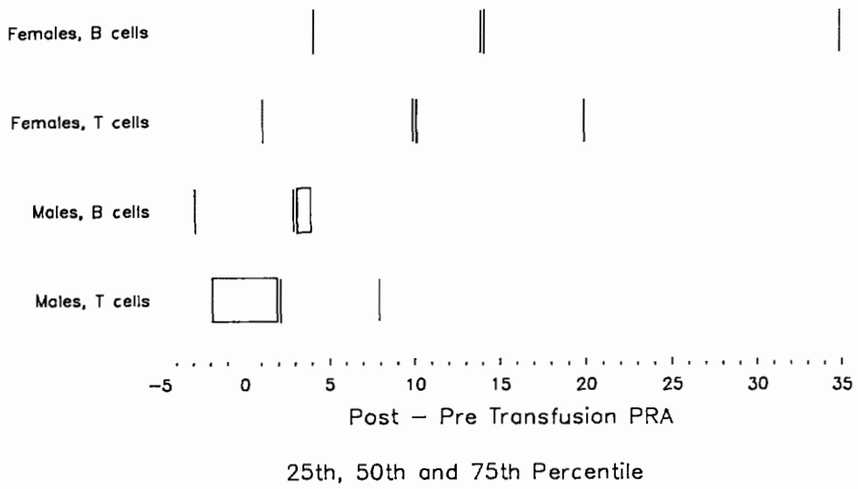

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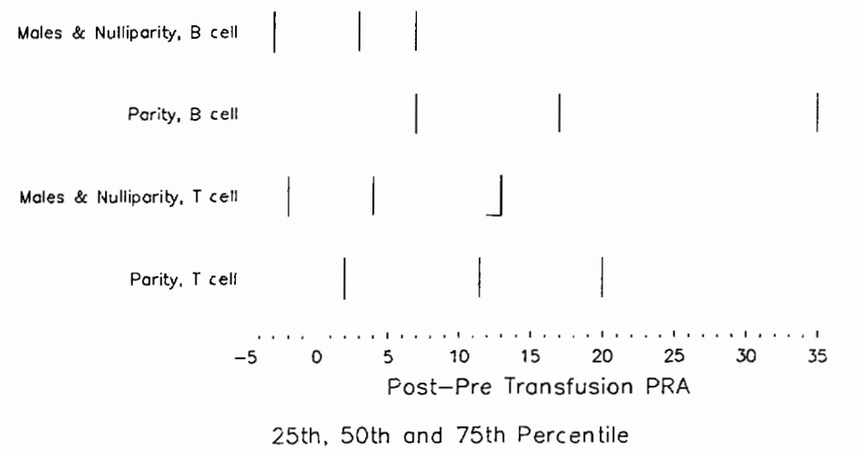

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however, no association of either transfusion-related B cell or T cell PRA with the number of pregnancies, p = 0.8738 and 0.2969, respectively. The production of monospecific or multispecific HLA-A, -B antibodies was not correlated with an increase in the transfusion-related T cell PRA (p = 0.3173). In contrast, the production of monospecific or multispecific HLA-DR antibody was significantly related to an increase in transfusion-related B cell PRA, p = 0.0001. To account for sex differences in the transfusion-related PRA, the preceding observations were categorized and analyzed by sex. Females and males alike demonstrated a significant production of transfusion-related monospecific or multispecific HLA-DR antibodies, p = 0.0162 and 0.00984, respectively. In contrast, neither females nor males produced significant monospecific or multispecific HLA-A, -B antibodies associated with increases in transfusion-related T cell PRA levels, p = 0.2579 and 0.6015, respectively.

Baseline B cell PRA levels for females and males entering the study were mean 29 ± 6 and mean 13 ± 2%, respectively. The mean PRA levels for females and males after three or more transfusions of whole blood or packed red blood cells were 47 ± 6 and 17 ± 4%, respectively. Females in this investigation demonstrated a higher baseline B cell PRA and attained a higher B cell PRA level after transfusion than did their male counterparts. The control group of 27 volunteers (14 females, 13 males) manifested a mean B cell PRA of 9 ± 1%. When the group was divided by sex, there was no difference in the mean B cell PRA levels (females = 9 ± 2%, males = 8 ± 2%). Parity in the control group was relatively low with only 4 of 14 females known to be parous. This could explain the diminished B cell PRA levels in this group of females.

The majority of males (18 of 21 or 85%) failed to respond to multiple blood transfusions with the production of increased B cell cytotoxic antibodies. More than half of the male nonresponders (11 of 18 or 61%) were included in GpB, the successful allograft group. Three males (3 of 21 or 14%) with elevated transfusion-related B cell cytotoxic antibodies produced monospecific or multispecific HLA-DR antibody. One male with an increase in B cell PRA after transfusion of slightly less than 10% developed a specific HLA-DR antibody. All males who responded with an increased B cell PRA, attributable to monospecific or multispecific HLA-DR antibodies were determined to have a successful allograft. Females who produced monospecific or multispecific HLA-DR antibody (13 of 14 or 93%) in response to blood transfusions successfully accepted cadaveric renal allografts. Only 1 of 14 (7%) of the female responders experienced allograft survival of less than 1 year. By contrast, only 1 parous nonresponder had a successful allograft, whereas 4 of 5 (80%) female nonresponders developed allograft failure in less than 1 year.

In conclusion, the number of blood transfusions, whether more or less than 5, showed no effect on the increased production of transfusion-related B cell or T cell cytotoxic antibodies detected by PRA, p = 0.7296 and 0.5302, respectively.

Discussion

Successful transplantation of renal allograft recipients with historical positive crossmatches but negative pretransplant sensitive

anti-IgG crossmatches [26–28] has provoked interest in the conditioning effect of blood transfusion therapy and its mechanism of action. Terasaki [34] has suggested that the beneficial effect of transfusions on allograft survival can be attained only in conjunction with immunosuppressive therapy. Anderson et al. [13] concluded that donor-specific transfusions with or without Imuran® improved allograft survival with the former developing minimal sensitization. Abouna et al. [2] postulated that immunization by the intravenous route with whole-blood transfusions in mongrel dogs probably modified the antigen in some manner to produce a blocking effect on cell-mediated immunity or a stimulation of suppressor T cells. Opelz et al. [3] presented evidence that mixed lymphocyte blocking antibodies appeared after transfusion and improved the fate of transplants. Kaltzmann et al. [23] concluded that suppression of the mixed lymphocyte reaction after blood therapy was attributable to suppressor cells, indicating that nonspecific immunosuppression could be generated. In contrast, Jeffery et al. [25] found no correlation between the number of transfusions and magnitude of the mixed lymphocyte response. The phenomenon of prolonged renal allograft surivival following blood transfusion was postulated by Van Es et al. [4] to be immunologically nonspecific since RhLA product sharing between blood and kidney donors in rhesus monkeys was less than could be expected for immunologic enhancement. Keown and Descamps [6] suggested a nonspecific RBC-mediated modulation of the immune response reflecting the RBC-filled macrophage's impaired antigen processing and phagocytic function. Fehrman et al. [24] concluded that transfusions led to a 'selection effect' where fewer patients with antibodies received grafts. Trends that Horimi et al. [29] observed were most compatible with the concept that leukocytes produce the transfusion effect since leukocyte-rich whole blood was more effective than leukocyte-deficient frozen blood in prolonging allograft survival.

Our results indicate that 3, 4, 5 or more transfusions of packed red blood cells or whole blood extended allograft survival through both monospecific or multispecific B cell cytotoxic antibodies. A boost in B cell antibody activity following blood transfusions prolonged graft survival, whereas the associated T cell cytotoxic antibody activity had no effect. Opelz et al. [22] in a large study of 737 hemodialysis patients receiving up to 20 transfusions found no elevation in the T and B cell cytotoxic antibody reactivity which they expected. After five transfusions Fehrman et al. [24] detected only B cell cytotoxic antibodies. Whereas Horimi et al. [29] showed that cytotoxic antibodies were not necessarily injurious to renal allotransplants, there was a slightly diminished graft survival rate in recipients manifesting warm reactive anti-T lymphocyte antibodies. In the rhesus monkey, Van Es et al. [4] argued against B cell alloantibodies extending allograft survival, as no correlation was observed between positive B cell crossmatches and prolonged graft survival.

HLA-A, -B antigen matching in several studies revealed no correlation with graft survival [7, 11, 16, 18, 19, 21]. In contrast, others suggested improvement in graft survival with HLA-A, -B antigen matching [5, 15]. The present investigation detected a significant facilitation of allograft survival when at least one HLA-A, -B antigen was matched. Many authors proposed that HLA-DR antigen matching alone improved graft

survival [15–20]. This finding, however, was not universal [21]. Similar to the report by Opelz and Terasaki [21], we did not observe an extension of graft survival through HLA-DR matching in this particular group of randomly selected recipients.

Females were reported by Opelz et al. [3] to have twice the incidence of cytotoxic antibodies as males yet the same survival rates. By contrast, a Norwegian study showed that the beneficial effect of blood transfusions was more pronounced in males [10]. In the present investigation, sex influenced the production of transfusion-related B cell and T cell cytotoxic antibodies with females producing higher levels than males. The incidence of both cytotoxic antibodies in females was 4 times the incidence in males. All females producing both B cell and T cell cytotoxic antibodies were shown to have graft survival rates of greater than 2 years with the exception of 1 at greater than 1 year. Males demonstrating B cell cytotoxic antibodies were also protected against graft loss. Although the majority of males failed to respond to multiple blood transfusions, greater than half experienced successful transplantation. Therefore, other factors not yet revealed contributed to allograft survival in males. Females responding to multiple transfusions with the appearanace of monospecific or multispecific HLA-DR antibodies are almost assured of a successful allograft.

Baseline B cell cytotoxic antibodies were highest in females and rose to higher levels compared to males after blood transfusions. In the control group divided equally among males and females, there appeared to be no sex difference in the production of B cell cytotoxic antibodies. Since the number of parous females in the control group was equal to the number of nulliparous females in the study group, this could explain why no sex differences were observed in the control group.

Many authors have noted a propensity in parous females to elaborate cytotoxic antibodies following blood transfusion [14, 22]. Parity is believed by some to improve allograft survival [26] while others disagree [9]. Our results did not confirm increased allograft survival with parity, although parity correlated well with increased transfusion-related B cell cytotoxic antibody production. In contrast to Opelz et al. [22], we were unable to correlate a rise in transfusion-related B or T cell cytotoxic antibodies with the number of pregnancies. The increased reactivity to blood transfusions in females may be related to an anamnestic response where the initial sensitization to lymphocytes may be through the transplacental exchange of blood during parturition. Another explanation could be the propensity of females to produce higher levels of immunoglobulins compared to males, which is linked to the effect of estrogen on the immune system [33].

Also of significance was the association of HLA-DR monospecific or multispecific antibodies with the increased production of transfusion-related B cell cytotoxic antibodies. In contrast, there was no association of monospecific or multispecific HLA-A, -B antibodies with an increase in transfusion-related T cell cytotoxic antibodies. The occurrence of B cell cytotoxic antibodies did not differ from that of T cell cytotoxic antibodies although monospecific or multispecific HLA-DR antibodies occurred more frequently after transfusions than did HLA-A, -B antibodies. In addition, there was no sex difference in the correlation of HLA-DR an-

tibodies with increased transfusion-related B cell cytotoxic antibody levels.

Barnes [8] projected that allograft losses caused by sensitization would equal or exceed the gains anticipated from a universal transfusion policy. Opelz et al. [3] concluded that the higher risk of graft failure with cytotoxins was attributed to mismatching of HLA antigens with the production of antigraft antibodies. The danger of patients developing high levels of cytotoxins was suggested to be the most urgent caveat of blood transfusions. With the identification of specific cytotoxins prior to surgery and the selection of a suitable mismatch, graft failures could be avoided [3]. The risk of rendering a recipient nontransplantable with blood transfusions was revealed to be minimal [22]. Only T cell sensitization was considered to result in hyperacute rejection [22, 29]. Good graft survival could be obtained in many hypersensitized recipients of blood transfusions primarily with the initial renal allograft [26]. In those who were alloimmunized, a sensitive negative crossmatch abolished any decrease in graft survival [26, 27]. In our study, the production of specific HLA-A, -B antibody and transfusion-related T cell cytotoxic antibodies was not observed to decrease significantly the survival of the kidney allograft, whereas production of antibodies specific for HLA-DR antigens and transfusion-related B cell cytotoxic antibodies actually prolonged graft survival. When circulating monospecific or multispecific HLA-DR or HLA-A, -B antibodies matched or cross-reacted with the kidney allograft, no significant decrease in graft survival occurred. The same was true when a positive crossmatch was obtained prior to the negative crossmatch.

Salvatierra et al. [26] observed that the occurrence of preformed cytotoxic antibodies at greater than 50% PRA were associated with longer periods on dialysis which reflected difficulty in finding compatible cadaver kidneys. Hormini et al. [29] also proposed that transfused patients must wait longer than nontransfused patients for a transplant. In contrast, a Norwegian study detected no differences in the waiting time for a transplant or the length of time on dialysis in the transfused compared to nontransfused group [10]. Likewise, we observed no effect when waiting times and length on dialysis were compared to allograft survival. Neither B cell nor T cell reactivity of greater than 50% PRA after transfusion therapy was associated with a longer waiting period. Recipients demonstrating T cell cytotoxic antibodies of greater than 50% PRA were, however, found to be associated with longer periods on hemodialysis. Sensitization to T cells may have occurred during hemodialysis treatment over an extended period rather than through a series of blood transfusions administered just prior to transplantation.

Summary

It is widely accepted that transfusions are beneficial to the outcome of renal allotransplantation. Whereas some investigators suggested that transfusions may induce both specific and nonspecific suppression of the cell-mediated immune response, others disagree. To lend clarity to this discrepancy, we collected 40 serum samples before and after blood transfusion therapy of first-time cadaveric renal allograft recipients and evaluated each for T cell and B cell cytotoxic antibodies using an Amos modified complement-dependent microlymphocytotoxicity assay. When greater than 10% of the panel cells reacted with a grade 4 or better, the panel was considered significant, and when a lymphocyte specificity was lysed by antibody-rich serum greater than 50% of the

time, the antibody was considered specific. Control T and B cell PRA assays employed sera from 27 normal nontransfused volunteers of similar age and sex. Survival distributions of differences in the PRA before and after blood transfusions and posttransfusion PRA levels were compared using the Gehan generalized Wilcoxon test. Other factors which influence allograft survival such as HLA-A, -B and -DR matches, number of blood transfusions, immunosuppressive therapy, age, sex, parity, previous positive crossmatch, circulating cytotoxic antibodies matching the graft, prior dialysis, lenght of time on the waiting list, lapse of time between transfusion and transplantation and the underlying primary diagnosis were also considered using the Gehan generalized Wilcoxon test or the χ^2 approximation.

Transfusion-related B cell cytotoxic antibodies, HLA-DR monospecific or multispecific antibodies and HLA-A, -B matching extended graft survival in a significant manner. Sex influenced the production of B and T cell transfusion-related cytotoxic antibodies with females producing greater quantities of antibodies than males. Parity and the production of monospecific or multispecific antibody were associated with an increase in transfusion-related B cell cytotoxic antibody. A difference in sex was not linked to the production of monospecific or multispecific HLA-DR antibodies. The majority of males failed to respond to multiple blood transfusions with the production of B cell cytotoxic antibodies although more than half were successfully grafted. All females and males who responded with the production of B cell cytotoxic antibodies monospecific or multispecific, with the exception of 1 female, demonstrated an allograft survival of greater than 1 year.

In conclusion, differences between pre- and posttransfusion B cell PRAs and monospecific or multispecific HLA-DR antibodies identified in patient sera following transfusions were good predictors of renal allograft survival in both males and females. Other prognosticators of good allograft outcome for males failing to produce B cell cytotoxic antibodies will be elucidated through further research.

Acknowledgement

The exceptional secretarial skill of Ms. Berlinda Harris in preparing the manuscript for publication is gratefully acknowledged.

References

1 Festenstien, H.; Sachs, J.A.; Paris, A.M.I.; Pegrum, G.D.; Moorhead, J.F.: Influence of HLA matching and blood transfusion on outcome of 502 London transplant group renal-graft recipients. Lancet *i:* 158–161 (1976).
2 Abouna, G.M.; Barabas, A.Z.; Pazderka, V.; Boyd, N.; Betters, J.M.; Kinniburgh, D.W.; Loo, V.S.; Schlaut, J.; Kavithavongs, T.; Dossetor, J.B.: Effect of pretreatment with multiple blood transfusions and with skin grafts on the survival of renal allografts in mongrel dogs. Transplant. Proc. *9:* 265–268 (1977).
3 Opelz, G.; Singar, D.P.S.; Michey, M.R.; Terasaki, P.I.: Effect of blood transfusions on subsequent kidney transplants. Transplant. Proc. *5:* 253–259 (1973).
4 Van Es, A.A.; Marguet, R.L.; Van Rood, J.J.; Kalff, M.W.; Balner, H.: Blood-transfusions induce prolonged kidney allograft survival in Rhesus monkeys. Lancet *i:* 506–509 (1977).
5 Mohanakumar, T.: Ellis, T.M.; Dayal, H.; DuVall, C.; Mendez-Picon, G.; Lee, H.M.: Potentiating effect of HLA matching and blood transfusion on renal allograft survival. Transplantation *32:* 244–247 (1981).
6 Keown, P.A.; Decamps, B.: Improved renal allograft survival after blood transfusion: a nonspecific, erythrocyte-mediated immunoregulatory process? Lancet *i:* 20–22 (1979).
7 Reinitz, E.R.; Rhodes, C.; Ellis, T.; Goldman, M.H.; Bacquero, A.; Mendez-Picon, G.; Lee, H.M.; Carter, W.H.; Mohanakumar, T.: HLA-A, -B and renal transplantation. Transplanat. Proc. *16:* 1415–1418 (1984).
8 Barnes, B.A.: Implications of a universal transfusion policy in renal tranplant recipients. Transplantation *23:* 451–452 (1977).
9 Editorial: Blood transfusions and renal transplantation. Lancet *ii:* 193–194 (1978).
10 Solheim, B.G.: The role of pretransplant and renal transplantation. Transplant. Proc. *11:* 138–144 (1979).
11 Williams, K.A.; Ting, A.; Cullen, P.R.; Morris, P.J.: Transfusions: their influence on human renal graft survival. Transplant. Proc. *11:* 175–178 (1979).
12 Glass, N.R.: Blood transfusions are not for everyone yet. Transplantation Proc. *29:* 264–265 (1980).

13 Anderson, C.B.; Tyler, J.D.; Rodney, G.E.; Etheredge, E.E.; Anderson, C.K.; Flye, M.W.; Jendrisak, M.D.; Sicard, G.A.: Preoperative immunomodulation of renal allograft recipients by concomitant immunosuppression and donor-sepcific transfusions. Transplant. Proc. *19:* 1494–1497 (1987).
14 Moore, S.B.; Serioff, S.; Pierides, A.M.; Watts, S.K.; Ruud, C.M.: Transfusion-induced alloimmunization in patients awaiting renal allografts. Vox Sang. *47:* 354–361 (1984).
15 Albrechtsen, D.; Brattie, A.; Kiss, E.; Solheim, B.-G.; Thoresen, A.B.; Winlher, N.; Thorsby, E.: Significance of HLA matching in renal transplantation. Transplantation *28:* 280–284 (1979).
16 Moen, T.; Albrechtsen, D.; Flatmark, A.; Jakobsen, A.; Jervell, J.; Halvoroen, S.; Solheim, B.G.; Thorsby, E.: Importance of HLA-DR matching in cadaveric renal transplantation. New Engl. J. Med. *303:* 850–854 (1980).
17 Ting, A.; Morris, P.J.: Powerful effect of HLA-DR matching on surivival of cadaveric renal allografts. Lancet *ii:* 282–285 (1980).
18 Persijn, G.; Van Leeuwen, A.; Hoogboom, J.; Gabb, B.W.; Nagtegaal, A.; Van Rood, J.J.: Matching for HLA antigens of A, B and DR loci in renal transplantation by Eurotransplant. Lancet *ii:* 1278–1301 (1978).
19 Ayoub, G.; Terasaki, P.I.: HLA-DR matching in multi-center, single-typing laboratory data. Transplantation *33:* 515–517 (1982).
20 Martins-da-Silva, B.; Vassalli, P.; Harder, F.; Largiader, F.; Montandon, A.: Influence of HLA-DR matching in cadaver kidney transplantation in Swisstransplant. Transplant. Proc. *11:* 760–762 (1979).
21 Opelz, G.; Terasaki, P.I.: International histocompatibility study in renal transplantation. Transplantation *33:* 87–95 (1982).
22 Opelz, G.; Graver, B.; Mickey, M.R.; Terasaki, P.I.: Lymphocytotoxic antibody response to transfusions in potential kidney recipients. Transplantation *23:* 177–183 (1981).
23 Klatzmann, D.; Gluckman, J.C.; Foucault, C.; Bensussan, A.; Assobga, U.; Duborst, A.: Suppression of lymphocyte reactivity by blood transfusions in uremic patients. Transplantation *35:* 332–338 (1983).
24 Fehrman, I.; Ringden, O.; Moller, E.: Blood transfusions as pretreatment for kidney transplantation: immunization rate and effect on cellular immune response in vitro. Transplantation *35:* 339–343 (1983).
25 Jeffrey, J.R.; Cheung, K.; Masniuk, J.; Taylor, D.: Mixed lymphocyte culture responses. Transplantation *38:* 42–45 (1984).
26 Salvatierra, O., Jr.; Perkins, H.A.; Amend, W., Jr.; Feduska, N.J.; Duca, R.M.; Potter, D.E.; Cochrum, K.C.: The influence of presensitization on graft survival rate. Surgery *81:* 146–151 (1977).
27 Ferguson, R.M.; Simmons, R.L.; Noreen, H.; Yunis, E.J.; najarian, J.S.: Host presensitization and renal allograft success at a single institution: first transplants. Surgery *81:* 139–145 (1977).
28 Feduska, N.J.; Vincenti, F.; Amend, W.J., Jr.; Duca, R.; Cochrum. K.; Salvatierra, O., Jr.: Do blood transfusions enhance the possibility of a compatible transplant? Transplantation *27:* 35–38 (1979).
29 Horimi, T.; Terasaki, P.I.; Chia, D.; Sasaki, N.: Factors influencing the paradoxical effect of transfusions on kidney transplants. Transplantation *35:* 320–323 (1983).
30 MacQueen, J.M.; Tardif, G.: Tissue typing reference manual; 2nd ed., SEOPF, sec. C-13, pp. 1–7, Richmond 1987.
31 Amos, D.B.; Baskir, H.; Boyle, W.; MacQueen, M.; Tulikainen, A.: A simple microcytotoxicity test. Transplantation *7:* 220–222 (1969).
32 Fotino, M.; Mannon, A.K.; Nylon wool separation of T and B lymphocytes. J. Immun. *112:* 420–423 (1974).
33 Grossman, C.J.: Interactions between the gonadal steroids and the immune system. Science *227:* 257–261 (1985).
34 Terasaki, P.I.: The beneficial transfusion effect on kidney graft survival attributed to clonal deletion. Transplantation *37:* 119–125 (1984).

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