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BLOOD TRANSFUSION INDUCED CHANGES IN CELL-MEDIATED LYMPHOLYSIS: TO IMMUNIZE OR NOT TO IMMUNIZE

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We have recently observed that the HLA-DR match between recipients and transfusion donors influences the beneficial effect of blood transfusions on allograft survival. To examine the immunologic effects of one-HLA-DR-Ag-matched and completely DR-mismatched transfusions, transfusion-induced changes in cell-mediated lympholysis (CML) were investigated. Blood donor directed CTL activity was measured in vitro before and after blood transfusion in 56 candidates for organ transplantation who received planned HLA-typed blood. We report that blood donor-directed CTL activity increased substantially after a single transfusion mismatched with the recipient for two HLA-DR Ag (p < 0.0001). A transfusion matched for one HLA-DR Ag did not enhance CTL activity. No correlation was found between CTL reactivity and sharing of HLA class I Ag. The present study supports our previous observation that matching for at least one HLA class II Ag (HLA-DR) between transfusion recipient and blood donor is required if immunization by blood transfusion is to be avoided. These data show that the presence or absence of "autologous" HLA-DR Ag on the leucocytes of the transfusion donor plays a decisive role whether immunization or immune suppression will ensue.

Although the use of blood transfusions is very common in clinical medicine, it is not known to which extent blood transfusions influence the immune responsiveness of the transfusion recipient. It is known that blood transfusions can immunize the transfusion recipient against the Ag of the blood donor (1, 2). It is, however, not clear to which extent blood products can suppress the recipient's immune response. Evidence is growing that blood transfusions not only improve the prognosis of heart and kidney transplantation (3-5), but also influence the outcome of autoimmune (6) and malignant (7) diseases.

The mechanisms underlying the transfusion-induced suppression are poorly understood. Several studies have tried to elucidate "the transfusion effect" by investigating transfusion-induced changes in cell-mediated immune responses. These studies have shown divergent results; blood transfusions resulted in an increase (8-12), a decrease (10, 13) or no change in CTL reactivity (8, 9, 11, 13).

We have recently reported that immunization or suppression by blood transfusion is influenced by the HLA-DR match between blood donor and transfusion recipient (5). A beneficial effect on allograft survival was observed only when donor and recipient were matched for at least one HLA-DR Ag. A transfusion mismatched for both HLA-DR Ag led to increased HLA-antibody formation and accelerated graft rejection (5).

The present study was undertaken to evaluate the effect of one-HLA-DR-Ag-matched and completely HLA-DR-mismatched transfusions on in vitro cell-mediated immune responses. Peripheral blood lymphocytes were measured for donor-specific CTL activity before and after blood transfusion. Our results show that transfusions that do not share an HLA-DR Ag with the recipient activate donor-specific CTL activity before and after blood transfusion. We report that blood donor-directed CTL activity was measured in vitro before and after blood transfusion. One sample from the donor was taken at the time of blood donation. Mononuclear leucocytes were isolated from the blood donor, the kidney donor, as well as against K562 in a 4-h 51Cr release assay using three E:T ratios (E:T: 50:1; 25:1; 12.5:1). All

MATERIALS AND METHODS

Patient group. Candidates for kidney or heart transplantation who had not previously received blood, each received leukocyte-poor blood from a randomly chosen donor. The transfusion donor was HLA typed after the transfusion. The patients received no immunosuppression during the transfusion protocol. ABO blood groups, sex, age, and HLA-A,B mismatches with the transfusion donor were evenly distributed over the patients groups who received one-HLA-DR-Ag-matched or completely DR-mismatched transfusions.

Blood transfusion. The transfusion consisted of 1 U of fresh (<24 h) leukocyte-poor RBC (containing 3 ± 3.5 x 10^8 leukocytes). Blood samples. Heparinized blood samples were obtained from the kidney transplant patients before, and 14 and 21 to 28 days after transfusion. One sample from the donor was taken at the time of blood donation. Mononuclear leucocytes were isolated by Ficoll-isopaque separation, washed with HBSS and stored in liquid nitrogen (14).

Cell-mediated lympholysis. Cell-mediated lympholysis was measured in the standard cell-mediated lympholysis test (14). PBL of the transfusion recipient were sensitized in vitro against irradiated cells from the blood donor, the kidney donor, as well as against HLA-A,B,C and DR incompatible control cells. The percentage lysis was determined on PHA blasts of the transfusion donor, the kidney donor, the unrelated control and the celline K562 in a 4-h 51Cr release assay using three E:T ratios (E:T: 50:1; 25:1; 12.5:1). All
assays were performed in duplicate. Responses before and after transfusion were tested in the same experiment. The percentage lysis was calculated using the following formula:

\[
\text{experimental mean cpm} - \text{spontaneous release mean cpm} \\
\times \frac{\text{maximum release mean cpm} - \text{spontaneous release mean cpm}}{100}
\]

\text{Statistics.} \text{ Differences between the groups were tested by analysis of variance.}

\text{HLA typing.} \text{ Typing for the HLA antigens A, B, and C (class I Ag) was performed with the standard NIH lymphocytotoxicity test. Typing for HLA-DR (class II Ag) was performed with two-color fluorescence with a set of highly selected class II allo-antisera. Blood donor and transfusion recipient were considered to be matched for one HLA-DR Ag when one of the following HLA-DR Ag was shared: DR1, DRw15(2), DRw16(2), DR3, DRw11(5), DRw12(5), DRw13 Dw1, DRw13 Dw19, DRw14 Dw6, DR7, DRw9, DRw8, DRw10.}

\text{Transplantation.} A total of 20 of the recipients who were given blood has been transplanted with a renal allograft. After transplantation the patients received immune suppression with azathioprine and prednisone. Graft survival was calculated according to the actuarial life table method. Differences between the groups were tested with a two-tailed \( \chi^2 \) test derived from a log-rank analysis.

\text{RESULTS}

HLA-A, B, DR matches between blood donors and transfusion recipients. CML reactivity of the individual patients and outcome of transplantation are given in Table I.

CML reactivity increased substantially when blood donor and transfusion recipient were mismatched for both HLA-DR antigens (Fig. 1). The mean lysis increased from 26.6 + 2.4% before transfusion to 50.7 ± 3.9% percent at 2 wk after transfusion (increase 24.1 ± 3.7%; \( p < 0.0001 \)). It decreased again to 42.1 ± 4.3% at 21 to 28 days after transfusion.

CTL reactivity did not increase significantly when donor and recipient shared one HLA-DR Ag. The mean lysis increased from 23.7 ± 4.2% before transfusion to 26.2 ± 4.2% at 2 wk after transfusion (mean increase in lysis 2.5 ± 2.8%; \( p = \text{NS} \)). Consequently, CTL reactivity after transfusion was significantly different between the two groups (\( p < 0.0001 \) at 2 wk after transfusion, \( p = 0.03 \) after 4 wk).

No correlation was found between CTL reactivity and sharing of HLA-A or -B antigens (Table I). The number of HLA class I mismatches was similar in the two groups (2.62 and 2.87 in the one-HLA-DR-Ag matched and completely DR-mismatched groups, respectively).

The mean percentage lysis before and after transfusion against different target cells, SEM and the number of patients tested are given in Table II. The increase in the mean percentage lysis is illustrated in Figure 2. CTL reactivity increased after a completely HLA-DR-mismatched transfusion both against cells of the transfusion donor (\( p < 0.001 \)) and against splenocytes of the kidney donor (\( p = 0.026 \)) (Fig. 2). Reactivity against HLA-A,B,C incompatible control cells or against K562 did not increase significantly. No significant changes in reactivity against neither blood donor, unrelated control cells,

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{No. of matches} & \textbf{CML on day} & \textbf{Increase/ decrease in percent lysis} & \textbf{F/S} & \textbf{No. of matches} & \textbf{CML on day} & \textbf{Increase/ decrease in percent lysis} & \textbf{F/S} \\
\hline
\textbf{A} & \textbf{B} & \textbf{DR} & \textbf{0} & \textbf{14} & & & \\
\hline
1 & 0 & 0 & 39 & 29 & -7 & S & 1 & 0 & 1 & 51 & 29 & -22 \\
0 & 0 & 0 & 23 & 18 & -5 & S & 1 & 1 & 1 & 65 & 43 & -20 \\
0 & 0 & 0 & 31 & 26 & -5 & S & 0 & 0 & 1 & 19 & 10 & -9 \\
0 & 0 & 0 & 40 & 36 & -4 & S & 2 & 2 & 1 & 25 & 16 & -9 \\
0 & 0 & 0 & 29 & 24 & -4 & S & 1 & 1 & 1 & 6 & 7 & -1 \\
1 & 0 & 1 & 9 & 10 & 1 & S & 1 & 1 & 1 & 25 & 26 & 1 \\
0 & 0 & 0 & 62 & 46 & 2 & S & 2 & 0 & 1 & 9 & 11 & 3 \\
1 & 0 & 0 & 28 & 32 & 4 & S & 0 & 0 & 1 & 3 & 7 & 4 \\
1 & 0 & 0 & 22 & 32 & 10 & S & 1 & 1 & 1 & 11 & 16 & 5 \\
1 & 0 & 1 & 3 & 6 & 1 & S & 1 & 0 & 1 & 36 & 42 & 6 \\
0 & 0 & 0 & 37 & 27 & 15 & S & 1 & 0 & 1 & 46 & 52 & 6 \\
0 & 0 & 0 & 39 & 55 & 16 & S & 0 & 0 & 1 & 0 & 7 & 7 \\
0 & 0 & 0 & 19 & 35 & 16 & S & 0 & 1 & 1 & 12 & 19 & 7 \\
0 & 0 & 0 & 57 & 46 & 17 & S & 0 & 0 & 1 & 36 & 43 & 7 \\
0 & 0 & 0 & 44 & 62 & 18 & S & 1 & 1 & 1 & 58 & 59 & 8 \\
1 & 1 & 0 & 28 & 46 & 18 & S & 0 & 1 & 1 & 18 & 29 & 11 \\
0 & 0 & 0 & 1 & 27 & 19 & S & 1 & 0 & 1 & 25 & 60 & 34 \\
1 & 0 & 0 & 25 & 46 & 21 & S & 0 & 0 & 1 & 26 & 60 & 34 \\
1 & 1 & 0 & 6 & 28 & 22 & S & 1 & 0 & 1 & 6 & 28 & 26 \\
0 & 0 & 0 & 5 & 27 & 22 & S & 1 & 0 & 1 & 46 & 68 & 22 \\
0 & 0 & 0 & 26 & 55 & 29 & S & 0 & 0 & 0 & 20 & 57 & 37 \\
1 & 1 & 0 & 49 & 86 & 37 & S & 0 & 0 & 0 & 38 & 86 & 37 \\
0 & 0 & 0 & 9 & 81 & 40 & S & 1 & 1 & 0 & 9 & 50 & 41 \\
0 & 0 & 0 & 13 & 56 & 43 & S & 1 & 0 & 1 & 26 & 70 & 44 \\
0 & 0 & 0 & 24 & 79 & 55 & S & 1 & 0 & 0 & 15 & 71 & 56 \\
1 & 1 & 0 & 30 & 89 & 59 & S & 1 & 0 & 0 & 31 & 39 & 62 \\
1 & 0 & 0 & 3 & 85 & 82 & S & 0 & 0 & 0 & 3 & 85 & 82 \\
\hline
\end{tabular}
\caption{Number of HLA-A, -B, and -DR matches between transfusion recipient and blood donor, outcome of blood donor directed CML, and outcome of transplantation.}
\end{table}

* F. Failure; S. success.
BLOOD TRANSFUSION AND CELL-MEDIATED CYTOTOXICITY

Figure 1. Cell-mediated lympholysis in recipients of one-HLA-DR-Ag-matched and completely DR-mismatched transfusions. The percentage lysis (mean ± SE) before, 14, and 21 to 28 days after transfusion. Recipients and transfusion donors matched for one HLA-DR Ag. Recipients and transfusion donors mismatched for both HLA-DR Ag. P = p values of the difference between the one-HLA-DR-Ag-matched and completely DR-mismatched groups.

K562, nor against the kidney donor were observed after one-HLA-DR Ag-matched transfusions.

Four patients received a selected transfusion that was HLA-class l compatible with the recipient. These transfusions were selected such that they were mismatched for one HLA-DR Ag and either or not shared one HLA-DR Ag with the recipient (Table III). The results so obtained are in agreement with the results with the randomly chosen blood donors. CTL reactivity did not increase significantly in the patients who received a transfusion that shared one DR Ag with the recipient (increase in lysis: patient 1: 8%; patient 2: 1%; patient 3: -3%). CTL reactivity increased substantially in the two patients who received a transfusion that did not share an HLA-DR Ag with the recipient (increase in lysis: patient 3: 54%; patient 4: 59%).

Graft survival. Twenty patients who were given blood have been transplanted with a renal allograft. None of the eight patients who were given blood and shared one HLA-DR Ag with their donors rejected their grafts (Table I). Consequently graft survival was 100% after 5 yr follow-up (Fig. 3). Four of the 12 patients who received a completely DR mismatched transfusion and were transplanted rejected their graft (5-yr graft survival: 66.7%, Fig. 3, p is not significant).

DISCUSSION

The present study shows that the presence or absence of "autologous" HLA class II Ag (HLA-DR) on the leukocytes of the transfusion donor plays a decisive role whether in vitro activation of cytotoxic T cell responses will ensue. Transfusions mismatched with the recipient for two DR Ag led to a significant increase in donor-directed cytotoxicity. Transfusions, equally mismatched for HLA class I Ag, but matched with the recipient for a single HLA-DR Ag did not enhance CTL reactivity. Sharing of HLA class I Ag without sharing class II Ag did not prevent activation of CTL responses.

The increased response after a completely DR-mismatched transfusion appears to be specific for the Ag of the transfusion donor. The response against control cells that are A,B, DR incompatible with the transfusion donor or against K562 did not increase. The response to the kidney donor also increased after a completely HLA-DR-mismatched transfusion. This may be due to shared Ag between transfusion donor and kidney donor.

The present study is in agreement with our previous studies in which we showed that completely DR-mismatched transfusions activated humoral immunity. The sharing of an HLA-DR Ag between blood donors and transfusion recipients reduced the risk of leucocytotoxic antibody formation (5). Graft survival was improved only when transfusion donor and recipient were matched for one HLA-DR Ag (5).

The absence of increased CTL reactivity is unlikely to be due to differences in the number of other Ag on the

Table II

| Cell-mediated lympholysis (CML) against different target cells, SE, number of patients tested, and p values comparing patients who received blood sharing or not HLA-DR Ag with recipients |
|---|---|---|---|---|---|---|---|---|---|
| No DR Matched | One DR Matched | | | | | | | |
| | | | | | | | | |
| Day | Percent lysis | SE | n | Percent lysis | SE | n | p* | |
| 0 | 26.6 | 2.4 | 37 | 23.7 | 4.2 | 19 | | |
| 14 | 50.7 | 3.9 | 37 | 26.2 | 4.2 | 19 | <0.0001 | |
| 21 | 42.1 | 4.3 | 19 | 25.6 | 6.2 | 10 | 0.03 | |
| CML against spleenocytes of kidney donor | | | | | | | | |
| 0 | 21.7 | 5.3 | 11 | 25.4 | 4.7 | 8 | | |
| 14 | 34.6 | 6.5 | 11 | 25.0 | 5.5 | 8 | 0.039 | |
| CML against unrelated control | | | | | | | | |
| 0 | 33.0 | 2.6 | 36 | 25.0 | 4.5 | 19 | | |
| 14 | 35.4 | 3.3 | 36 | 29.1 | 4.9 | 18 | NS | |
| 21 | 34.8 | 4.7 | 19 | 32.8 | 7.4 | 10 | NS | |
| CML against cell line K562 | | | | | | | | |
| 0 | 26.8 | 4.5 | 15 | 24.1 | 4.9 | 9 | | |
| 14 | 24.3 | 4.4 | 15 | 17.7 | 2.5 | 9 | NS | |

* p value of the difference between one-HLA-DR matched and completely mismatched transfusion.
lymphocytes of the transfusion donor. The number of HLA class I mismatches is similar in the two groups (2.62 and 2.87 in the HLA-DR Ag-matched and completely DR-mismatched groups, respectively). Class I Ag sharing, without sharing class II Ag was not found to influence CTL reactivity. Blood donors and recipient who are matched for one HLA-DR Ag, are mismatched for several other class II Ag (the second DR Ag, HLA-DQ and HLA-DP Ag). This is sufficient to activate cellular immune responses in vitro.

It is, therefore, not clear how the presence of a single autologous HLA-DR Ag can negate the immunizing effect of several mismatched HLA class I and class II Ag. It is possible that activation or induction of T cell nonresponsiveness are dependent on the way in which Ag are presented. If donor and recipient are mismatched for both HLA-DR Ag host APC will probably take up, process and present donor Ag to host T cells in context of host HLA Ag. This will lead to activation of donor-directed immune responses. If donor and recipient are matched for HLA class II Ag, possibly donor APC, which share MHC class II Ag with the recipient can present Ag to host T cells. Presentation of peptides by HLA class II" donor leucocytes that lack assessor cell function, such as B cells or activated T cells, may result in T cell unresponsiveness. Presentation of Ag by cells that lack accessory function has been shown to induce T cell unresponsiveness in stead of activation of T cells (15–17). This has been suggested to be an extrathymic mechanism for maintaining self tolerance (15).

It should be noticed that there was no deletion of donordirected CTL after an one-HLA-DR-Ag-matched transfusion; the responses after transfusion remained at background levels. This is in agreement with observations made in animal models. Specific cytotoxic T cells are found in the nonrejected kidney of blood transfused rats (18). However, we can not exclude that deletion of donor-directed CTL requires a longer time interval.

It has recently been shown that one mismatched haplotype between blood donor and transfusion recipient is required to induce a beneficial transfusion effect (19). This is in agreement with the results in our studies. The patients who shared one HLA-DR Ag with their donors were mismatched for the second HLA-DR Ag.

The clinical consequences for transplant recipients are self-evident. The present data show that completely HLA-DR-mismatched transfusions activate donor-directed cytotoxic T cell responses. We have previously shown that such transfusions did not significantly improve graft survival and led to increased HLA-antibody formation. It seems, therefore, that such transfusions are contraindicated in candidates for organ transplantation. The use of a single one-HLA-DR-Ag-matched transfusion may be considered to improve graft survival rates.
As yet, it is not clear to which extent or to which antigens the immune response is suppressed by a one-HLA-DR-antigen-matched blood transfusion. The implication of our studies may not be restricted to candidates for organ transplantation. Further studies may reveal the effects of matched and mismatched blood products on cancer recurrence, autoimmune diseases, or on responses to infectious microorganisms. The observation that one-HLA-DR-antigen-matched and completely DR-mismatched transfusions have different immunologic effects may have important implications for the clinical use of blood products.

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