EFFECT ON LYMPHOCYTE FUNCTION OF TRANSFUSION-RELATED SERUM FACTORS
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Summary

IgG preparations from recipients of blood transfusion can block the Fcγ-receptor and inhibit phytohaemagglutinin-induced blastogenesis. Serum samples from subjects transfused with blood or blood products and from untransfused controls were fractionated over sucrose gradients. Transfused subjects showed Fcγ-receptor blocking activity and inhibition of phytohaemagglutinin-induced blastogenesis not only in the fraction containing IgG but also in higher molecular weight fractions. These higher molecular weight factors may be important in the modulation of T lymphocyte function occurring in transfused subjects.

Introduction

The enhancement of renal allograft survival by pre-transplant blood transfusion has been attributed to Fcγ-receptor (Fcγ-R) blocking antibodies [1]. We have previously shown that monomeric IgG preparations from recipients of blood or blood products can block lymphocyte Fcγ-Rs and inhibit phytohaemagglutinin (PHA)-induced T cell blastogenesis [2]. In this study we have set out to determine whether other serum factors, in addition to IgG, might affect lymphocyte function similarly.

Patients and methods

Patients  Serum samples were obtained (and stored at −20°C till use) from the following groups of subjects.

Group A  Six non-transfused healthy controls: three male, three female; mean age 37 years (range 21–52).

Group B  Six non-transfused uraemic subjects (serum creatinine >500μmol/L: three male, three female; mean age 38 years (range 21–59).

Group C  Six non-uraemic subjects, previously transfused with at least five
units of blood following gastrointestinal bleeding: two male, four female; mean age 40 years (19–65).

Group D Twenty-eight uraemic subjects receiving regular dialysis, previously transfused with at least five units of packed red cells: 16 male, 12 female; mean age 37 years (range 19–62).

Group E Six subjects with haemophilia A who were regular users of Factor VIII: all male; mean age 31 years (range 19–38).

**Methods** The samples were fractionated over discontinuous sucrose gradients [3] and the resulting fractions of graded molecular weight tested for their capacity to block human peripheral blood lymphocyte Fcy-receptors using an EA rosette inhibition assay [4] and to inhibit PHA-induced blastogenesis.

1. **Sucrose gradients** Five hundred microlitre aliquots of serum were fractionated by ultracentrifugation at 105,000G for 16 hours at 4°C over 4ml 20–40% discontinuous sucrose gradients using a model L2-65B Beckman ultracentrifuge. Five fractions (F1-5) each of 720μl were collected in turn from the bottom of the gradient, plus one larger fraction (F6) of 900μl. Each serum fraction was dialysed into phosphate buffered saline (PBS) at 4°C for 16 hours. The approximate molecular weight of serum components in each fraction was estimated by screening a small aliquot by radial immunodiffusion using four markers: IgM, IgG, C1q and transferrin. In all experiments, IgM was found to peak in F2, C1q in F3, IgG in F4 and transferrin in F5.

2. **Fcy-receptor blocking – the EA rosette inhibition assay** 1x10^6 monocyte depleted normal human peripheral blood lymphocytes were resuspended in 120μl serum fraction or in PBS alone as a control. Following incubation at 37°C for 30 minutes, cells were washed twice in Hepses buffered Earle's Medium (HEM), pH 7.3 (Gibco Laboratories, Glasgow), resuspended in 120μl HEM and mixed with an equal volume of chicken erythrocytes (E) which had been presensitized with rabbit IgG class antibody (A). Tubes were then centrifuged at 200G for five minutes prior to resuspension of the EA rosette pellet and fixation in 3% glutaraldehyde. Cells were resuspended in 0.75% trypan blue and inspected under sealed coverslips. Results were expressed as the percentage inhibition of EA rosette formation produced by IgG compared with the PBS control. Full details of this method have been previously described [4].

3. **Inhibition of T cell transformation** Normal human peripheral blood mononuclear cells (PBMC) were resuspended in RPMI 1640 culture medium supplemented with 10% heat-inactivated autologous serum and antibiotics at a final concentration of 4x10^6/ml. Fifty microlitres of PBMC (2x10^5 cells), 50μl serum fraction in PBS and 10μl PHA (100μg/ml) were incubated together in the wells of a flat bottomed microtitre plate. Following incubation at 37°C for 66 hours in a humidified atmosphere of 5% CO₂ in air the cells were pulsed with 14C-thymidine (0.02μCi/well) for six hours, harvested on to fibreglass filters using a multiple-channel automated cell harvester and washed in turn with distilled water, 5% trichloroacetic acid and methanol. The filters were dried at 37°C, placed in glass vials containing 5ml of scintillation fluid and the radioactivity counted in an automated β counter (Packard). Results were expressed
as the percentage inhibition of PHA response produced by the IgG as compared with control wells to which medium was added.

The results were analysed using a two-tailed Student’s ‘t’ test.

Results

Table I shows the mean (± standard error) percentage inhibition of EA rosette formation by serum fractions from test and control subjects. There was no significant difference in results between non-uraemic (Group A) and uraemic (Group B) subjects who had never been transfused. Both non-uraemic (Group C) and uraemic (Group D) subjects who had been transfused had significant Fcγ receptor blocking activity not only in fraction 4, the IgG peak, but in a wide range of molecular weight fractions. For example in fraction 1, the transfused subjects (Groups C and D) had significantly greater levels of inhibition of rosette formation than those in Groups A and B who were untransfused (p<0.01). Also in fraction 1, the recipients of Factor VIII (Group E) were significantly different from the untransfused subjects who comprised groups A and B (p<0.01).

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>Serum fraction</th>
<th>3</th>
<th>4</th>
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<tr>
<td>A Controls</td>
<td>21±4a</td>
<td>14±3</td>
<td>14±4</td>
<td>16±4</td>
<td>3±2</td>
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<td>B Uraemic non-transfused</td>
<td>18±5a</td>
<td>32±4</td>
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<td>21±5</td>
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<td>C Non-uraemic transfused</td>
<td>58±5b</td>
<td>62±5</td>
<td>55±5</td>
<td>33±6</td>
<td>30±8</td>
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<td>38±5b</td>
<td>37±2</td>
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<td>32±4</td>
<td>24±2</td>
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<tr>
<td>E Haemophiliacs</td>
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<td>38±3</td>
<td>43±9</td>
<td>33±9</td>
<td>16±3</td>
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a versus b, p<0.01

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<td>2±1e</td>
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a versus b, p<0.001; c versus d, NS; e versus f, p<0.05

Table II represents the mean (± standard error) inhibition of PHA induced blastogenesis by serum fractions from test and control subjects. Again looking at fraction 1, uraemic transfused subjects (Group D) showed highly significant
levels of inhibition of blastogenesis relative to the control subjects in Group A (p<0.001). The difference between recipients of Factor VIII (Group E) and control subjects (Group A) in fraction 1 was not statistically significant (0.05< p<0.1), although there was a significant difference between these two groups in fraction 2 (p<0.05).

Discussion

The production, following blood transfusion, of IgG class Fcγ-receptor blocking antibodies has been suggested as a mediator of the effect of transfusion in preventing renal allograft rejection [1]. The T lymphocyte has a central role in the rejection reaction and inhibition of PHA-induced blastogenesis provides an in vitro assessment of the capacity of such IgG preparations to affect T lymphocyte function. The present study shows that not only the IgG containing serum fraction (F4) but also high molecular weight fractions (e.g. F1) have the capacity both to block Fcγ-receptors and suppress PHA induced T lymphocyte transformation. Also we have found that transfusion of blood or blood products produces these high molecular weight Fcγ-receptor blocking factors as well as producing IgG. In addition to affecting T lymphocyte function in vitro, these high molecular weight factors may play an important role in vivo in preventing renal allograft rejection. We have shown that the production of these factors is not dependent on a uraemic environment as they are present in non-uraemic transfused patients as well. Also transfused cells are not essential in their production as the factors are found in haemophiliacs following infusions of Factor VIII, although the degree of inhibition of blastogenesis was greater in recipients of packed red cells. Finally, the composition of these high molecular weight factors is still uncertain. Immune complexes can block the Fcγ-receptors and initiate suppressor T cell function [5] and they could be responsible for the effects described in this study.

References

1  MacLeod AM, Power DA, Mason RJ et al. Lancet 1982; ii: 468
3  MacLean CA, Goudie BM, MacSween RNM et al. Immunology 1984; 53: 315
4  Sandilands GP, Reid FC, O’Keefe T et al. J Immunol Methods 1980; 36: 1