Blood transfusion enhances production of T-helper-2 cytokines and transforming growth factor β in humans

Uzi GAFTER, Yona KALECHMAN* and Benjamin SREDNI*
Department of Nephrology, Hasharon Hospital, Petah-Tiqva, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel and *Department of Life Sciences, Bar Ilan University, Ramat Gan, Israel

(Received 29 February/17 June 1996; accepted 25 June 1996)

1. Blood transfusion confers immune suppression with improved allograft survival. The aim of this study was to evaluate the effect of blood transfusion on the production of T-helper-2 cytokines and transforming growth factor β, which are associated with suppression of allograft rejection. An additional aim was to try to identify which blood cell type is mostly responsible for the blood transfusion effect. Production of interleukin-4, interleukin-10 and transforming growth factor β by peripheral blood mononuclear cells isolated from patients with end-stage renal disease was measured in vitro. These assays were performed before, and 4h, 4, 7 and 14 days after a single blood transfusion and the transfusion of one unit of leucocyte-free erythrocytes.

2. Blood transfusion stimulated a significant rise in the production of all three cytokines measured. Transfusion of erythrocytes had no effect on the production of interleukin-4 or interleukin-10.

3. It is suggested that blood transfusion enhances the production of interleukin-4, interleukin-10 and transforming growth factor β. These cytokines may inhibit production of T-helper 1 and pro-inflammatory cytokines, deactivate cytotoxic cells and thereby suppress allograft rejection. It is further suggested that the leucocyte is the transfused cell type which is mostly associated with induction of this immunosuppressive response.

INTRODUCTION

Blood transfusion improves renal and cardiac allograft survival in humans and animals [1-5]. Several mechanisms of immune suppression have been suggested to explain this beneficial effect of blood transfusion [5, 6-8]. A number of cytokines and cell surface molecules may participate in T-cell activation and can potentially be modified in an attempt to decrease rejection or induce tolerance [9]. Indeed, a single blood transfusion has been shown to suppress production of the T-helper (Th)-1 cytokines interleukin (IL)-2, interferon (IFN) and tumour necrosis factor by peripheral blood mono- nuclear cells (PBMCs) [7]. It has been suggested that IL-4 and IL-10 secreted by Th2 cells and IFN secreted by Th1 cells crossregulate and mutually inhibit their respective functions [10, 11]. It is therefore possible that blood transfusion may exert its immunosuppressive effect, in part, by stimulating Th2 cytokine production which in turn suppresses pro-inflammatory cytokines.

Unlike blood transfusion, transfusion of erythrocytes did not improve renal allograft survival [12]. We have shown potentiation of Th1 cytokine secretion by PBMCs after transfusion of erythrocytes [13], or by the addition of erythrocytes to the media in vitro [14]. This may suggest that the immunosuppressive effect of blood transfusion is independent of erythrocytes.

This study was undertaken to evaluate whether the effect of blood transfusion is initiated by enhanced production of suppressive cytokines and which blood cell type is mostly responsible for this effect.

The objectives of this study were first to determine the production of cytokines in vitro by PBMCs isolated from patients with end-stage renal disease before the blood transfusion and sequentially in the 2 weeks after it. The cytokines measured were Th2-derived cytokines IL-4 and IL-10 and transforming growth factor β (TGFβ) which is a potent suppressive cytokine [15]. The second objective was to examine the effect of transfusion of erythrocytes on IL-4 and IL-10 production following the same protocol.

METHODS

Protocol 1: effect of blood transfusion on cytokine secretion

Ten patients (six men and four women) with end-stage renal disease receiving regular haemodialysis treatment were studied. Their age range was 23 to 69 years (48 ± 5 years, mean ± SEM). The diseases leading to end-stage renal failure included: chronic glomerulonephritis (n = 3), interstitial nephropathy

Key words: blood transfusion, haemodialysis, T-helper 2 cytokines, interleukin-4, interleukin-10, transforming growth factor.

Abbreviations: IFN, interferon; IL, interleukin; PBMC, peripheral blood mononuclear cell; TGF, transforming growth factor; Th, T-helper.

Correspondence: Professor Uzi Gafter, Department of Nephrology, Rabin Medical Center, Hasharon Hospital, 7 Keren Kayemet St., Petah-Tiqva 49372, P.O.B. 121, Israel
Duration of chronic dialysis treatment ranged from 6 months to 8.5 years (3.2 ± 0.8 years). All patients received haemodialysis for 4 h thrice weekly with a cuprophane membrane dialyser. The patients were not transfused for at least 3 months before the study. They had not suffered from a recent infection and were not treated with immunosuppressive drugs. The patients were transfused with one unit of packed cells of 200–250 ml while on dialysis. Each patient was tested twice before being transfused and then 4 h, 4, 7 and 14 days post-transfusion. Blood for studies was withdrawn in the morning immediately before the haemodialysis, except for the blood taken 4 h after blood transfusion. PBMCs were isolated and production of IL-4, IL-10 and TGFβ was determined. The assays of all the samples of each patient were performed within the same assay run. The means of the two pretransfusion determinations were used as control values. All post-transfusion results were compared with the pretransfusion results.

Ethics committee approval and informed consent from each patient were obtained.

### Protocol 2: effect of erythrocyte transfusion on cytokine production

Six patients (five men and one woman) with end-stage renal disease receiving regular haemodialysis treatment were studied. Their ages ranged from 35 to 72 years (49 ± 6 years). The diseases leading to end-stage renal failure included: chronic glomerulonephritis (n = 3), obstructive uropathy (n = 1), diabetic nephropathy (n = 1) and nephrosclerosis (n = 1). Duration of chronic dialysis treatment ranged from 1 to 7 years (3.0 ± 1.0 years). All patients received haemodialysis for 4 h thrice weekly with a cuprophane membrane dialyser. They had not suffered from a recent infection and had not been treated with immunosuppressive drugs.

The patients received a transfusion of washed leucocyte-free erythrocytes. The erythrocytes were washed three times and transfused to the patients through a nylon filter (Miramed, Mirandola, Italy) to obtain a 97–98% leucocyte-free erythrocyte preparation. Blood for assays was obtained from the patient before transfusion and 4, 7 and 14 days post-transfusion. IL-4 and IL-10 production by PBMCs was determined.

### Reagents

Reagents used in this study were phytohaemagglutinin (Difco Lab., St Louis, MO, U.S.A.), lipopolysaccharide (from S. abortus-equi; Difco Lab., Detroit, MI, U.S.A.) and fetal calf serum (Maagar, Beit Haemek, Israel). Enriched RPMI-1640 (Gibco) was supplemented with 10% fetal calf serum, 2 mmol/l glutamine, 10 mmol/l non-essential amino acids, 3 mmol/l sodium pyruvate, 5 × 10^-5 mol/l 2-mercaptoethanol, 100 i.u./ml penicillin and 100 μg/ml streptomycin.

### Cell preparation

PBMCs were isolated after Ficoll–Hypaque gradient centrifugation. The isolated PBMCs were washed and suspended in enriched RPMI-1640 at a concentration of 10^6/ml.

### Production of cytokines

PBMCs (10^6/ml), were suspended in enriched RPMI-1640 medium with 10% fetal calf serum and 200 μg/ml phytohaemagglutinin. The cultures were incubated for 48 h. Supernatants were collected and assayed for either IL-4 or IL-10. For the production of TGFβ, PBMCs (5 × 10^6/ml) were suspended in enriched RPMI and incubated for 1 h at 37°C. Non-adherent cells were subsequently washed out of the tissue culture plate and the remaining adherent cells were cultured in enriched RPMI-1640 supplemented with 10 μg/ml lipopolysaccharide for 6 h.

### Immunoassays

The Biosource (CA, U.S.A.) human IL-4 and human IL-10 ELISA kits were used for quantitative measurements of IL-4 and IL-10 in the phytohaemagglutinin-stimulated PBMC supernatants. The R & D (MN, U.S.A.) TGF ELISA kit was used for quantitative measurements of TGFβ in lipopolysaccharide-stimulated PBMC supernatants.

### Statistical analysis

Data are presented as mean ± SEM. Student's t-test for paired data was used to compare post-transfusion results with pretransfusion results. A two-tailed P < 0.05 was considered significant.

### RESULTS

#### Clinical outcome

Nine patients were considered as possible candidates for renal transplantation. Their panel-reactive antibodies were 15.0 ± 8.5% before blood transfusion and did not change after it (13.1 ± 5.8%). Three women and four men were subsequently transplanted including all those who had a significant level of panel-reactive antibodies. Five patients received a cadaveric transplant and two patients received a living related transplant. Four transplanted patients are now 3.9 ± 0.9 years after transplantation with a serum creatinine of 1.8 ± 0.4 mg/dl. One patient returned to dialysis treatment 4 years after transplantation because of the recurrence of her original glomerular disease (membranous neph-
ropathy). One patient died 6 months after transplantation because of an acute myocardial infarction with a functioning graft, and another patient with diabetes mellitus died of sepsis shortly after a combined renal and pancreas transplantation.

**Effect of blood transfusion on cytokine secretion**

**IL-4.** The effect of blood transfusion on IL-4 production is illustrated in Fig. 1. As shown, all patients exhibited a rise in IL-4 production. A significant elevation in IL-4 production was observed until 4 days post-transfusion. From a pre-transfusion production of $39.4 \pm 5.8 \text{ pg/ml}$, it virtually doubled to $74.9 \pm 12 \text{ pg/ml} (P < 0.002)$ 4 h after blood transfusion. Four days after blood transfusion IL-4 levels reached $91.8 \pm 12 \text{ pg/ml} (P < 0.001)$. Thereafter, the production of IL-4 gradually decreased from the peak production to $48.9 \pm 6.9 \text{ pg/ml}$ 1 week post-transfusion ($P < 0.07$ compared with pre-blood transfusion level). By 2 weeks after blood transfusion, IL-4 production returned to the pre-blood transfusion level, $40.1 \pm 5.9 \text{ pg/ml}$.

**IL-10.** The effect of blood transfusion on IL-10 production is depicted in Fig. 2. IL-10 production by each patient rose, albeit in a different pattern from the rise in IL-4. Pre-blood transfusion, IL-10 production was $186.5 \pm 14.5 \text{ pg/ml}$, and 4 h post-blood transfusion it was similar at $185.7 \pm 14.2 \text{ pg/ml}$. Thereafter, it rose to $240.3 \pm 17.3 \text{ pg/ml} (P < 0.001)$ by day 4 after blood transfusion, to $275.8 \pm 22.4 \text{ pg/ml} (P < 0.001)$ 1 week after blood transfusion, and by 2 weeks after blood transfusion it reached $308.1 \pm 28.4 \text{ pg/ml} (P < 0.001)$.

**TGFβ.** The effect of blood transfusion on the production of TGFβ is illustrated in Fig. 3. The increase in TGFβ production by each patient was similar to the rise in IL-10 production. Before blood
transfusion, production of TGFβ was 602.2±23.7 pg/ml, and 4 h post-blood transfusion it was unchanged at 604.8±24.4 pg/ml. Four days after blood transfusion it rose slightly but significantly to 656.3±25.4 pg/ml (P<0.01). By 1 week it almost doubled to 1118.9±72.3 pg/ml (P<0.001), and at 2 weeks the high level of 907.4±33.9 pg/ml (P<0.001) was maintained.

Effect of erythrocytes on cytokine production

The effect of erythrocytes on the production of IL-4 and IL-10 is shown in Table 1. The production of both cytokines did not change after the transfusion throughout the 2 weeks of the study.

### DISCUSSION

A single blood transfusion given to haemodialysis patients led to the enhanced synthesis and secretion of IL-4, IL-10 and TGFβ by each one of the patients studied. IL-4 production increased early and lasted for 1 week. The production of IL-10 and TGFβ had risen by day four post-blood transfusion, and remained elevated during the 2 weeks of the study. It is noteworthy that haemodialysis patients studied under the same protocol expressed a substantial reduction in IL-2, IFN, tumour necrosis factor and colony stimulating factor production by PBMCs 2 weeks after a single blood transfusion [7]. It appears that blood transfusion may elicit a Th2-type production of cytokines by the PBMCs isolated from the recipient of the blood transfusion. IL-4 and IL-10 may in turn downregulate Th1-derived cytokine production [10, 11]. TGFβ is also capable of antagonizing Th1 cytokine production, albeit by a different mechanism, and suppressing other pro-inflammatory cytokines [15].

The Th2-like pattern response after blood transfusion may be related to prostaglandin E2 secretion. Prostaglandin E2 differentially modulates cytokine secretion profiles of human Th lymphocytes [16]. In the presence of prostaglandin E2, Th1-like responses are suppressed with the predominance of a Th2-like pattern [16]. It is of note that a single blood transfusion given to patients with end-stage renal disease led to an immediate rise in prostaglandin E2 secretion which peaked 4 days after blood transfusion [7]. It is suggested that blood transfusion enhances the production of IL-4, IL-10 and IFN. These cytokines may synergistically downregulate IL-2, IFN and other pro-inflammatory cytokines, deactivate cytotoxic and killer cells and thereby suppress allograft rejection.

Indeed, administration of recombinant IL-2 in mice in vivo prevented the beneficial effect of blood transfusion and allowed allograft rejection [17]. Furthermore, activation of Th2-like cells in murine recipients of heart allografts with donor-specific blood transfusion, anti-CD4+ monoclonal antibody pretreatment and cyclosporin A administration led to peripheral tolerance [18]. Th2-like cells isolated from these animals were transfused to other mice which received heart allografts. This adoptive transfer led to prolonged graft survival [18].

The pattern of cytokine production by PBMCs observed in the present study was also seen in non-rejecting allografts, i.e. increased expression of Th2 cytokines with suppressed expression of Th1 cytokines [19].

In contrast, rejecting allografts revealed enhanced expression of IL-2 and IFN with reduced expression of Th2 cytokines [19]. The expression of cytokines in endomyocardial biopsies from human cardiac transplant recipients was studied recently [20]. IL-2 was associated with severe, moderate and mild rejection, whereas IL-4 and IL-10 were found mostly in the mildly rejecting hearts [20].

The present study investigated the effect of a single blood transfusion on cytokine production shortly after the blood transfusion. It is impossible to correlate this effect with the long-term effect on renal transplantation, although it has been suggested that the beneficial effects of blood transfusion in humans last for many months. It is possible that blood transfusion induces proliferation of memory T-cells which may elicit an immune response similar to the pattern of the response observed shortly after the allogeneic stimulation.

Unlike the effect of blood transfusion, transfusion of erythrocytes had no effect on IL-4 and IL-10 production. In previous studies [13, 14] we have shown stimulation of PBMCs to produce IL-2, IFN, tumour necrosis factor and colony stimulating factor by both autologous and homologous erythrocytes. Lymphocyte activation was independent of allogeneic stimulation. Indeed, a co-stimulatory pathway via lymphocyte surface CD2 molecules could explain activation of lymphocytes by erythrocytes [14].

It is noteworthy that, unlike the poor kidney transplant survival after transfusion of erythrocytes in humans [12], class I antigens on purified erythrocytes can cause a donor-specific immune suppression with prolonged renal allograft survival in rats [21]. The difference in response between humans

Table 1. Effect of transfusion of erythrocytes on the production of IL-4 and IL-10 by PBMCs

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>No of patients</th>
<th>Pre-transfusion</th>
<th>Post-transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4 (pg/ml)</td>
<td>6</td>
<td>35.0 ± 0.9</td>
<td>35.6 ± 1.1</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>6</td>
<td>122.3 ± 8.8</td>
<td>120.3 ± 12.2</td>
</tr>
</tbody>
</table>

(All values are mean ± SD)
and rat models might be related to the dose of erythrocytes necessary to induce prolonged allograft survival, which could be species dependent. It may also be related to the level of class I antigen expression on the surface of the erythrocytes used before transplantation in the different species. In addition, there might be a difference in terms of strength of allograft rejection between humans and the various rat models used.

It seems that the immunosuppressive effects of blood transfusion via cytokine modulation may be attributed to the allogeneic stimulation provided by the leucocytes or, to a lesser degree, by platelets [22]. This suggestion was further tested by alloimmunization of women with spontaneous recurrent abortion by PBMCs from their husbands. Similarly to blood transfusion, this led to upregulation of Th2 cytokine production with suppression of Th1 cytokine production [23]. It is of note that infusion of trophoblast membranes that do not express human leucocyte antigens [24] produced similar clinical results to allogeneic stimulation [25].

The findings in the present study further elucidate the possible mechanisms whereby blood transfusion may exert immunomodulatory effects. Among the postulated beneficial effects of blood transfusion are improved graft survival, possible suppression of immuno-inflammatory illnesses and prevention of recurrent abortion [26]. Among the suspected negative effects are occurrence of malignancy, infections and reactivation of latent viruses and a possible rise in panel-reactive antibodies [24].

Blood transfusion has been shown to improve first-year and long-term renal graft survival in a recent retrospective study of about 30,000 transplanted patients [27]. However, most centres abandoned deliberate blood transfusion due to its side effects. Since blood transfusion effect results from the allogeneic stimulation, it is possible that in the future isolated human leucocyte antigens will be used, which will maintain the beneficial effect without the side effects of blood transfusion.

**Acknowledgment**

This study was partly sponsored by the Fetter Program for Clinical Research Collaboration.

**References**