PART XXV

TRANSPLANTATION POSTERS I

Chairmen: C Ponticelli
          A Vercellone
TRANSFUSION-INDUCED ENHANCEMENT OF PROSTAGLANDIN AND THROMBOXANE RELEASE IN PROSPECTIVE KIDNEY GRAFT RECIPIENTS

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Summary

Pre-transplant blood transfusions (BT) improve the survival of kidney grafts. Apart from specific immunoregulation by T suppressor cells or anti-idiotypic antibodies, the role of non-specific immunoregulatory factors, such as prostaglandins, is being discussed as a possible mechanism for this effect. We studied the in vitro prostanoid release from peripheral mononuclear cells following three deliberate blood transfusions. Twenty-five previously non-transfused dialysis patients were studied. Spontaneous and LPS-induced prostaglandin E (PGE) and thromboxane B₂ (TXB₂) were determined in cell-free culture supernatants by fluid phase RIA. Transfused patients exhibited a more rapid onset and steeper increase of prostanoid production. After 24 hours incubation, the spontaneous and LPS-induced PGE release of pre- and post-BT cells was significantly different (pre-BT: 2.1 and 5.1 ng/ml; post-3-BT: 5.0 and 7.9 ng/ml; p<0.01). Pre-BT cells released considerably lower amounts of TXB₂ than post-BT cells (spontaneous release: 39 vs 88 ng/ml; LPS-induced release: 62 ng/ml vs 129 ng/ml; p<0.05). After correction for monocytes as defined by monoclonal antibodies, post-BT cells again showed increased prostanoid release as compared to pre-BT cells. Therefore, the enhanced PGE and TXB₂ release of post-BT cells is not caused by an increase merely in the number of monocytes. Rather, BT appear to induce an enhanced release of prostanoids by activation of monocytes. We also found a correlation between the number of BT and the amount of prostanoid release.

Introduction

The mechanisms whereby blood transfusions (BT) induce kidney graft protection are unknown. In addition to specific immunoregulation by T suppressor cells or anti-idiotypic antibodies, the influence of non-specific factors is being considered. Prostaglandins (PG) have attracted particular attention because...
they have been shown to inhibit cell-mediated immunity as well as antibody responses [1,2]. PGE appears to act as a negative feedback signal in immune responses that require macrophage/monocyte-lymphocyte interactions [3]. It was shown in mice [4] and rats [5] that the administration of PGE delayed graft rejection. When indomethacin, an inhibitor of endogenous PG synthesis, was administered, grafts were more rapidly rejected [4]. However, paradoxically, PGE serum levels were found to be elevated during graft rejection [6,7]. Macrophages from mixed lymphocyte cultures produce strikingly increased amounts of PG when the cultures are set up with cells from sensitised allograft recipients and the respective donors [8]. The intravenous injection of sheep erythrocytes stimulates PG production in mice [9]. We studied the influence of planned BT in dialysis patients on PG release from peripheral mononuclear cells in vitro.

**Patients and methods**

Twenty-five patients (9 females, 16 males, aged between 7 and 38 years) on chronic haemodialysis were the subjects of this study; the underlying renal diseases leading to end-stage renal insufficiency were glomerulonephritis (16 patients), pyelonephritis (4), polycystic kidneys (2), nephrotic syndrome (2), and unspecified renal disease (1). None of the patients received drugs interfering with PG release and none had been transfused previously. The patients received three BT at two week intervals. Nineteen patients were transfused with whole blood, and six patients received packed red cells. Blood samples were obtained prior to transfusion, 14 days after each BT, and three months after the last BT. Mononuclear cells were prepared by Ficoll-Hypaque gradient centrifugation, deep-frozen, and stored in liquid nitrogen until use.

Mononuclear cells (4 x 10⁶) were cultured in bicarbonate-buffered medium (RPMI 1640, supplemented with L-glutamine, penicillin (100 IU/ml), streptomycin (100µg/ml), and 10% heat-inactivated fetal calf serum. To stimulate monocytes, 20µl/ml LPS (lipopolysaccharide from *E coli* 0127, Gibco, Glasgow, UK) were added to the cultures. In kinetic studies, the spontaneous and LPS-induced release of PGE and TXB₂ in cell-free culture supernatants were determined by fluid phase radioimmunoassay (RIA). The antibody raised against PGE₁ cross-reacted with PGE₂; values are therefore given as PGE without subclass specification. The anti-TXB₂ antibody cross-reacted less than 0.5 per cent with other arachidonic derivatives. Both antibodies have been described in detail previously [10]; they were kindly provided by Professor D Gemsa, Medizinische Hochschule, Hannover, and Dr M Seitz, Medical Policlinic, Heidelberg, FRG. PG determinations were performed according to the method described by Jaffée et al [11]. Prostanoid concentrations were calculated using a logit-log computer program.

Monocyte numbers contained in the mononuclear cell preparations were defined by indirect immunofluorescence using two different anti-monocyte monoclonal antibodies (OKM₁, Ortho Diagnostics, Raritan, USA; CM₁ provided by Dr P Terasaki, Los Angeles, USA). The percentage of stained cells was measured using a Spectrum III flowcytometer (Ortho).
Prostanoid release from patient cells before BT was compared to that after one to three BT using the Wilcoxon and Wilcox test for multiple comparisons of dependent samples.

**Results**

Spontaneous and LPS-induced PGE release in patients before and after three blood transfusions as well as in healthy controls is shown in Figure 1. The kinetics revealed that peripheral mononuclear cells of transfused dialysis patients exhibited a more rapid onset and a steeper increase of prostanoid production. By six hours of incubation the spontaneous and LPS-induced PGE release in transfused patients was significantly increased as compared to pre-transfusion values. After 24 hours or 48 hours the differences became highly significant (pre-BT: 2.1 and 5.1ng/ml; post-3-BT: 5.0 and 7.9ng/ml; p<0.01).

Similar findings were obtained when TXB₂ release from peripheral mononuclear cells was examined. The kinetic analysis revealed at six hours, a steeper increase of TXB₂ production in post-transfusion cells than in pre-transfusion or in control cells. After 24 hours incubation, spontaneous and LPS-induced release of TXB₂ in transfused patients was significantly enhanced as compared to pre-transfusion values (spontaneous TXB₂ release: 88ng/ml post-3-BT vs 39ng/ml pre-BT; LPS-induced TXB₂ release: 129ng/ml post-3-BT vs 62ng/ml pre-BT; p<0.05).

We attempted to clarify whether enhanced prostanoid release of post-transfusion cells was merely caused by an increase in the number of monocytes or by
monocyte activation. PGE and TXB2 values were corrected for 1x10^6 monocytes according to the monocyte numbers defined in the mononuclear cell preparations. Table I gives relative monocyte numbers and corrected PGE and TXB2 values pre-transfusion and after one, two and three blood transfusions. In controls 23.7 per cent of the mononuclear cells were characterised as monocytes. In non-transfused patients comparable monocyte numbers were observed (23.9%). Following a first BT monocytes rose to 26.4 per cent and reached 27.5 per cent after three BT; they returned to pre-transfusion values within three months after the last BT. Spontaneous PGE release rose significantly from 10.2ng/ml pre-transfusion to 18.7ng/ml after three BT (p<0.01). The LPS-induced PGE release from 21.1ng/ml to 28.9ng/ml (p<0.05). Similarly, post-BT monocytes released spontaneously and after stimulation with LPS higher amounts of TXB2 than pre-BT monocytes (326.8ng/ml vs 158.9ng/ml, p<0.01; 476.2ng/ml vs 243.6ng/ml, p<0.05). In addition, there was a strong correlation between the number of BT and the amount of prostanoid release.

TABLE I. In vitro PGE and TXB2 release of peripheral mononuclear cells, related to the percentage of monocytes, in dialysis patients before and after blood transfusions (BT) and in controls

<table>
<thead>
<tr>
<th></th>
<th>PGE-release‡</th>
<th>TXB2-release‡</th>
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<tbody>
<tr>
<td></td>
<td>% monocytes</td>
<td>Spontaneous</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n=22)</td>
<td>23.7±1.1</td>
<td>12.6±2.0</td>
</tr>
<tr>
<td>Patients (n=25)</td>
<td></td>
<td></td>
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<tr>
<td>Pre 1 BT</td>
<td>23.9±1.3</td>
<td>10.2±2.5</td>
</tr>
<tr>
<td>Post 1 BT</td>
<td>26.4±1.1</td>
<td>12.1±2.7</td>
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<tr>
<td>Post 2 BT</td>
<td>26.7±1.2</td>
<td>14.4±2.6</td>
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<tr>
<td>Post 3 BT</td>
<td>27.5±1.0*</td>
<td>18.7±2.5**</td>
</tr>
<tr>
<td>3 months</td>
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<tr>
<td>post 3 BT</td>
<td>21.0±0.5</td>
<td>10.5±0.9</td>
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Values are given as x ± SEM; ‡ng/ml/1x10^6 monocytes after 24 hours incubation
*p<0.05; **p<0.01; post-BT vs pre-BT values

Discussion

The present study demonstrates that peripheral mononuclear cells of transfused dialysis patients release higher amounts of prostanoids than cells of non-transfused patients. After BT, the spontaneous as well as the LPS-induced release of PGE and TXB2 was significantly enhanced. This effect was 'dose' dependent; whereas pre-transfusion prostanoid release was normal (as compared to controls), it increased with the number of BT. There is little doubt that the increased prostanoid release occurred from monocytes as the mononuclear cell preparations were free of platelets. Though activated granulocytes, in particular neutrophils, and to a much lesser degree eosinophils are capable of releasing PG, their production is much lower than that of monocytes/macrophages and does not persist
for periods longer than six to eight hours in vitro [3]. During this early cell incubation period, the amount of prostanoid release was relatively small in our studies.

In previous experiments we observed increased monocyte numbers in dialysis patients following BT and this was confirmed in the current study. Although at first sight the increased monocyte numbers might be held responsible for the increase in prostanoids, our data argue against this simple explanation. After correction for monocyte numbers, post-transfusion cells still showed enhanced prostanoid release.

Whether our in vitro findings are relevant to the transfusion-transplant effect in vivo remains speculative. PG of the E and F types are nearly completely inactivated during a single lung passage; the half-life of these substances when released into the circulation is less than one minute [3]. It is therefore unlikely that BT induce a persisting high level of PG activity that mediates systemic immunosuppression. A more likely possibility is that PG may regulate the immune response within the graft. Recipient lymphocytes sensitised by previous BT might produce high amounts of lymphokines in the grafted kidney and thereby activate macrophages resulting in enhanced PG production. Lymphokine-mediated activation of macrophage functions, including PG release, has been described in animal models [8]. Further studies of BT-induced PG release in vivo, may prove relevant in explaining the beneficial effect of BT on kidney graft survival.

References

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