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Donor white blood cell survival and cytokine profiles following red blood cell transfusion in Australian major trauma patients

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ABSTRACT

Background: The potential for the co-existence of genetically disparate cells (microchimerism) and associated cytokine profiles following red blood cell (RBC) transfusion in trauma patients has not been well characterized to date. This study investigated the incidence of surviving donor white blood cells (known as transfused-associated microchimerism (TAM)) and cytokine changes following blood transfusion in trauma patients.

Study design and methods: Trauma patients with an injury severity score (ISS) > 12 who had been transfused between 2012–2016 with at least 5 units of RBC units over a 4 h period were recruited. Trauma patients with ISS > 12 who did not require blood transfusion were recruited as controls. The incidence of TAM was determined using a panel of insertion/deletion (InDel) bi-allelic polymorphisms. Selected pro- and anti-inflammatory cytokine profiles were analyzed using cytometric bead array.

Results: The transfused cohort (n = 40) had median ISS of 28 [12–66], received a median of 11 RBC units [4–114] and had median hospital length of stay of 35 days [1–152]. Only 11 (27.5%) patients returned for follow-up blood sampling after discharge. Of these, one patient showed an InDel pattern indicating the presence of TAM. No patients in the control cohort (n = 49) showed TAM. Cytokines IL-10 and IL-6 were found to be elevated in the transfused trauma patients.

Conclusion: In this cohort, TAM was found to occur in one patient of the 11 who received a blood transfusion. Elevated IL-6 and IL-10 cytokines were detected in those patients who were transfused. However, the incidence of TAM could not be correlated with the elevated cytokine profiles for this cohort.

1. Introduction

Major trauma results in the activation of the immune system including the release of numerous pro- and anti- inflammatory responses mobilizing cellular and humoral elements (Brochner and Toft, 2009; Jackman, 2013; Svoboda et al., 1994). Compromised post-injury immune function often results in reduced T cell proliferation and increased suppressor T cell activity and cell-mediated lympholysis following allogeneic blood transfusion (Dunne et al., 2004; Dzik et al., 1996; Innerhofer et al., 1999). Some adverse patient outcomes associated with blood transfusion have been speculated to be mediated by the presence of viable donor white blood cells (leukocytes) present within the transfused red blood cell (RBC) units. (Murphy et al., 1986; Claas et al., 1981) To reduce the potential of leukocyte related adverse events following blood transfusion, donor white blood cell filtration (leukodepletion) was introduced for all Australian manufactured RBC units from October 2008. However, a leukodepleted RBC unit can still be within specification but contain up to one million donor leukocytes (Council of Europe guidelines) and clinical studies suggest that RBC transfusion may still be associated with undesirable transfusion-related

Abbreviations: RBC, red blood cell; ISS, injury severity score; SIRS, systemic inflammatory response syndrome; CI, confidence interval; InDel, insertion deletion polymorphisms; PBMC, peripheral blood mononuclear cell; PBS, phosphate buffered saline

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outcomes (Phelan et al., 2007; Titlestad et al., 2001).

One such consequence of transfusion is termed microchimerism, which is the co-existence of small numbers of genetically disparate cell populations within a single host (Bloch et al., 2013). Microchimerism can occur as a natural phenomenon between a fetus and mother but can also arise as a consequence of stem cell transplantation (Demetris et al., 1993; Ichinohe et al., 2004). Microchimerism is less frequently seen following blood transfusion, however it has been reported in 10–15% of major trauma patients who require blood transfusion. (Lee et al., 2005; Utter et al., 2004, 2008; Hirani et al., 2014). Usually transfused donor cells are recognized by the recipient's immune system and are removed within 90–100 days following transfusion (Luten et al., 2004). However, in cases of transfusion-associated microchimerism (TAM), donor leukocytes can endure for many years and the phenomenon may arise after transfusion of just a single unit of non-leukodepleted blood product (Lee et al., 1995, 1999). TAM can be detected using sensitive genetic analysis and these surviving donor leukocytes can account for up to 5% of the recipient's circulating hematopoietic cells. (Lee et al., 2005, 1999) The surviving donor leukocytes appear to be derived from a number of immunophenotypic lineages which suggests broad hematopoietic engraftment and proliferation within the recipient (Lee et al., 1999; Schechter et al., 1977).

It has been suggested that the incidence of TAM is more common in patients who receive large volumes of blood in one or more transfusion episodes, where there is a significant perturbation of the immune response at the time of transfusion, which can occur with trauma patients (Lee et al., 2005; Utter et al., 2004; Reed et al., 2007; Utter et al., 2006). A retrospective cohort of Australian major trauma patients reported that there was an incidence of TAM in 10% of patients (Hirani et al., 2014). This incidence of TAM was found despite the use of leukodepleted RBC units. These patients were transfused with a median of 9.9 RBC units and half of the patients with TAM had an associated spleen injury (Hirani et al., 2014).

The exact mechanism by which donor hematopoietic cells engraft in TAM is currently unknown. The presence of a spleen injury could affect the trauma patients' ability to remove abnormal cells and lymphocytes (Smith and Johnston, 1979). Cytokines are known to regulate the restoration of homeostasis following trauma, therefore, perhaps changes to cytokine profiles may cause disruption in how the donor cells are recognized and removed by the immune system (Svoboda et al., 1994). The response to trauma has been shown to modulate cytokines including IL-6, IL-10 and TNF-α which have been associated with poor patient outcomes, multiple organ failure or systemic inflammatory response syndrome. (Sapan et al., 2016, 2017; Keel et al., 1996; Lyons et al., 1997).

This study aimed to determine whether there was evidence of TAM in trauma patients who received a massive blood transfusion, and whether analysis of the cytokine profiles from these patients could provide evidence for modulation of immunological pathways, which may impact on the establishment of TAM.

2. Materials and methods

2.1. Selection of participants

The study was approved by the Western Sydney Local Health District Human Research Ethics Committee with governance granted at the participating New South Wales based hospital sites under the harmonization of multi-center ethical review (HoMER) process (National Health and Medical Research Council, Australia). The study was coordinated by the Australian Red Cross Blood Service (Blood Service). Patients were enrolled between the 1st of November 2012 and the 31st of December 2016. All study sites were located in the state of New South Wales, Australia and are as listed; Westmead Hospital, John Hunter Hospital, Royal North Shore Hospital and Concord General Repatriation Hospital.

2.1.1. Definition of major trauma

Major trauma included patients with the following criteria; multisystem blunt or penetrating trauma with unstable vital signs (systolic blood pressure < 90, heart rate > 120, respiratory rate < 10 or > 30, Glasgow Coma Scale < 14, revised trauma score < 11; penetrating injury of head, neck, torso or groin; total body surface area burns > 10% which are deep dermal and/or full thickness; amputation proximal to wrist or ankle; paralysis or other signs of spinal cord injury, flail chest, open or suspected depressed skull fracture, unstable pelvis or suspected pelvis fractures, two or more proximal long bone fractures, distended, rigid abdomen with signs of shock.

2.1.2. Massive transfusion definition

Massive transfusion was defined for this study as 10 RBC units or more transfused over 24 h or 5 RBC units or more transfused over 4 h.

2.1.3. Inclusion criteria

All major trauma patients over the age of 16 with an injury severity score (ISS) of > 12 were identified by clinical research staff upon admission into the hospital and were approached for consent for inclusion into the study as either transfusion recipients or as control participants (Baker et al., 1974).

Consent for inclusion into this study was conducted by the treating clinical staff by directly approaching the major trauma patient or by approaching the appointed next-of-kin where possible before the pre-transfusion blood sample was taken from the patient. If consent was provided by the patient's next-of-kin, retrospective consent for study participation was sought directly from the patient as soon as they were able. Any patient enrolled by their next-of-kin but wishing to withdraw had all samples collected and destroyed prior to analysis. Samples from patients were only analyzed when direct consent from the patient was obtained.

Patients that required blood transfusion had 2 x 6 mL sodium heparin blood tube samples (catalogue number 367876, BD Biosciences, San Jose CA) collected prior to the administration of any blood products. Further 2 x 6 mL sodium heparin blood tube samples were then taken; 5–7 days post-transfusion; 5–7 days after any further subsequent transfusion episodes required or every 30 days until the patient was discharged and an additional sample was taken upon discharge. Following hospital discharge, each transfusion recipient was approached by the study team with a follow-up letter every 6 months for a period of 36 months to provide a single 6 mL sodium heparin blood tube sample, which was collected by commercial pathology service providers. The original consent process included consent to be approached by the study team for the follow up samples, however another consent and participant information form was included with each letter as a reminder to participants of the study requirements.

Control participants were patients who did not receive any blood transfusion but had ISS > 12. Two 6 mL sodium heparin blood tube samples were taken upon hospital admission and a final 2 x 6 mL sodium heparin blood tube sample was taken upon discharge.

2.1.4. Exclusion criteria

Any female patients, including control participants, who were pregnant or had previously been pregnant were excluded to eliminate possible confounding results due to fetal-maternal microchimerism rather than as a result of blood transfusion. Minors under the age of 16; patients who did not receive a massive blood transfusion; patients not able to consent for themselves due to mental impairment; patients who had ISS < 12 and patients who have had any previous blood transfusion including hematological conditions or during transport to the hospital site were all excluded.

2.2. Medical and blood donation record analysis

Medical records were analyzed for each participant from the date of