Effect of autologous salvaged blood on postoperative natural killer cell precursor frequency

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Summary

Background Immunosuppression after major surgery increases the risk of infections. Natural killer cells play a pivotal part in defence against infection. We aimed to investigate the immunomodulatory effects of different types of postoperative blood transfusion by use of a new assay for measuring the frequency of peripheral blood natural killer precursor cells (NKpf assay).

Methods We measured the natural killer cell precursor (NKp) frequency before and 5 days after surgery in 120 patients undergoing joint replacement surgery. The patients were assigned to one of five groups according to the type of transfusion received: non-transfused (n=32), allogeneic non-leukodepleted blood (eight), allogeneic leukodepleted blood (30), autologous predeposited blood (ten), and autologous salvaged blood collected within the first 24 h after surgery (40). We also measured interferon γ and interleukin 10 concentrations before and after surgery.

Findings The mean postoperative NKp frequency for all patients was lower than the preoperative values, except in patients receiving autologous salvaged blood, which was higher than all other groups ($p<0·0001$). Postoperative NKp frequencies for patients receiving allogeneic or autologous predeposited blood responded similarly ($p=0·99$), but these patients had lower NKp frequencies than did the non-transfused group ($p<0·0001$). Postoperative interferon γ concentrations were higher in the autologous salvaged blood group ($p<0·0001$) than in other groups, which did not differ from each other. Interleukin 10 concentrations were similar across all groups ($p=0·49$).

Interpretation Immunosuppression associated with surgery and blood loss was reflected in a reduced frequency of NKp and decreased interferon γ. This immunosuppression was reversed by transfusion of autologous salvaged blood, suggesting that this fluid contained immunostimulants.

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Introduction

Major surgery and allogeneic transfusion suppress the body's immune defences against pathogenic micro-organisms, and the effect is additive when the two interventions occur together. Systemic immuno-suppression has been shown by increased incidence of infections and in vitro by lymphocyte culture studies.[1,2] The amount of immunosuppression varies according to diagnosis, patient characteristics, and surgical technique. Thus, cholecystectomy and resection of colonic carcinoma done by laparoscopy lead to less immunosuppression than do similar operations done by laparotomy.[3,4] Major surgery is followed by suppression of proinflammatory cytokines and, after a delay, increased production of anti-inflammatory cytokines.[5,6] These trends are sometimes affected by the action of prostaglandin E_2 released by macrophages. Anaesthetics and opioids are also associated with a transient anti-inflammatory condition.[7]

Immunosuppression induced by allogeneic transfusions is well documented and was initially recognised for its beneficial effects in organ transplantation.[8] A similar process might account for suppression of recurrent autoimmunity after allogeneic transfusions, as exemplified in Crohn's disease.[9] In people, allogeneic transfusion is associated with increased T-cell mediated cytolysis, more regulatory T cells, and interleukin 10 synthesis, and fewer natural killer cells, delayed hypersensitivity, and resistance to infection. In orthopaedic surgery some of these sequelae can be avoided by giving predeposited autologous blood or blood salvaged during surgery. For similar reasons we and others have advocated use of blood salvaged postoperatively.[10–14]

The main objective of this study was to measure NKp frequency because their progeny are thought to have an important role in first-line defence against micro-organisms. NKp cells are present in very low numbers in peripheral blood, but undergo rapid clonal expansion in response to interleukin 2 and interleukin 15.[15]

Methods

Study design

This prospective non-randomised observational study complied with the Declaration of Helsinki regarding investigations into human beings, and was fully approved by the local (Southmead Hospital) research ethics committee. We obtained signed informed consent from all volunteering patients.

We enrolled 120 patients undergoing primary elective total knee or hip replacement surgery at the Avon Orthopaedic Centre, Southmead Hospital, Bristol, UK, into the study. Criteria for exclusion included pre-existing infection, previous blood transfusion, malignant disease, autoimmune disorders, and diabetes. No patients undergoing revision operations were included. We used a tourniquet routinely to control blood flow at the operation site in knee replacement operations. Blood samples for

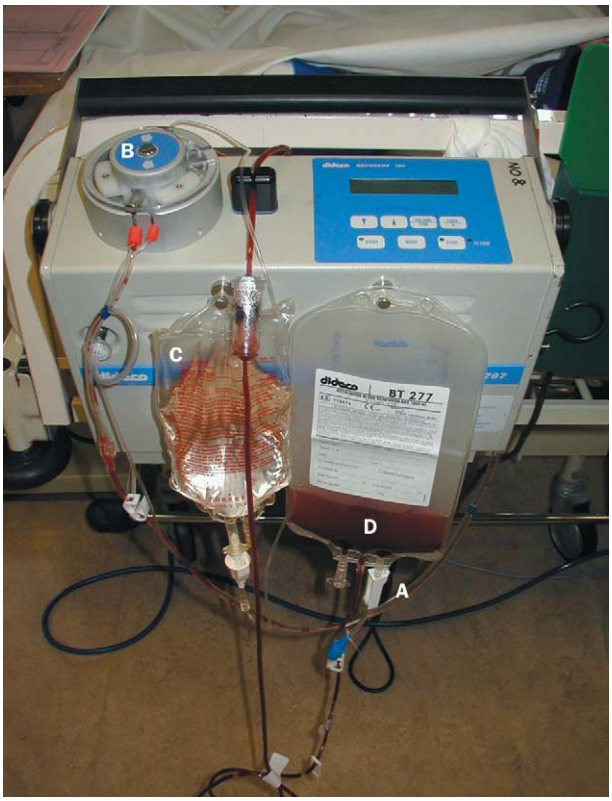

Figure 1: **Recovery device for postoperative autologous salvaged blood from knee replacement operation site**
A tube (A) is inserted into the joint space during the operation, where it removes wound fluid under negative pressure exerted by the rotary pump (B). After exiting the wound site the tube is connected to a smaller diameter tube that delivers acid citrate dextrose to the fluid from the reservoir (C). The anticoagulated fluid is then pumped via the rotary pump (B) to the collection bag (D), which is subsequently reinfused into the patient.

haematological and immunological analyses were obtained on admission and 5 days after surgery. We assigned patients to one of five groups according to the type of transfusion procedure: group 1, no blood; group 2, allogeneic non-leukodepleted blood; group 3, allogeneic leucodepleted blood; group 4, autologous pre-deposited blood; and group 5, autologous salvaged blood. Transfusions were given 1–4 days after surgery in groups 2, 3, and 4 and within 1–2 days in group 5. The absolute trigger for transfusion was haemoglobin of lower than 80 g/L, but transfusions might have been given to some patients with higher haemoglobin concentrations for various clinical reasons. General and spinal anaesthesia were used in all cases.

Transfusion groups and procedures

No blood transfusions were given to the 32 patients in group 1. For group 2 (eight patients), we obtained allogeneic non-leucodepleted blood according to the National Blood Service published guidelines.[17] From each donor, 450 mL blood was transferred into 65 mL citrose phosphate dextrose adenine. We then removed plasma, leaving a suspension of 70–90% packed cells. All blood units were stored at 4°C for up to 35 days. Reinfusion was done over 2–4 h.

We obtained allogeneic leucodepleted blood for the 30 patients in group 3 as described above for normal blood donors. Within 48 h of collection, we filtered leucocytes through a third generation filter, which depleted leucocytes to fewer than 5×10^6 per unit of red cells with at least 99% frequency, in accordance with the current National Blood Service standard for leucodepletion.[17] Leucocyte-depleted blood units were monitored in accordance with guidelines and then stored at 4°C for up to 35 days.[18]

Autologous pre-deposited blood (group 4) was donated by ten patients who were in good health, aged between 17 and 70 years, weighed more than 50 kg, had good venous access, and had a haemoglobin concentration of at least 110 g/L at referral. We invited well-motivated patients who were able to travel to the transfusion centre to participate in this group. We obtained two to three units of predeposited blood once a week; the final collection was a minimum of 3 days before the operation. Blood was collected and stored in the same way as allogeneic non-leucodepleted blood.

From the 40 people in group 5, we obtained autologous salvaged blood postoperatively from a catheter inserted into the wound site. A rotary pump applied negative pressure through the catheter. Below the wound exit point the catheter was joined by a smaller diameter tube that delivered acid citrate dextrose as anticoagulant and preservative to the aspirated wound fluid giving a final ratio of 1 unit of acid citrate dextrose to 12 units of blood. This anticoagulated fluid was delivered to a collection bag and transfused into the patient (figure 1). Most wound drainage fluid was collected within the first 24 h after surgery. If the volume of retrieved fluid reached a minimum of 100 mL by 6 h or a maximum of 500 mL within 6 h it was re-infused through a 40-μm filter similar to that used in a standard blood-giving set. No attempt was made to separate red blood cells before reinfusion. We repeated this protocol every 6 h or until a maximum of 1500 mL had been reinfused. None of the patients included in this group received allogeneic or predeposited blood transfusions.

Assays

We obtained 20–30 mL venous blood in sterile tubes containing preservative-free sodium heparin (final concentration 25 IU/mL) before and 5 days after the operation. Peripheral blood mononuclear cells were separated by density gradient centrifugation and cryopreserved until tested. We used the HLA-negative

	Group 1 (n=32)	Group 2 (n=8)	Group 3 (n=30)	Group 4 (n=10)	Group 5 (n=40)	p
Sex						
Female	17 (53%)	6 (75%)	19 (63%)	3 (30%)	20 (50%)	0·30
Male	15 (47%)	2 (25%)	11 (37%)	7 (70%)	20 (50%)	
Operation						
Knee replacement	22 (69%)	5 (63%)	13 (43%)	0	40 (100%)	
Hip replacement	10 (31%)	3 (37%)	17 (57%)	10 (100%)	0	
Age (median [IQR], years)	69 (62–78)	74 (64–79)	71·5 (66–78)	58 (45–65)	71·5 (65–78)	0·005
Hospital stay (median [IQR], days)	8 (7–10)	8 (7–12)	9 (7–14)	7 (7–7)	8·5 (7–14)	0·03
Blood transfused (mean [SD], mL)*	0	1013 (318)	1305 (583)	900 (0)	596 (226)	

*Whole blood equivalents (less anticoagulant).
Table 1: **Patients' characteristics**

	Group 1 (n=32)	Group 2 (n=8)	Group 3 (n=30)	Group 4 (n=10)	Group 5 (n=40)
Haematological data					
Packed-cell volume [%][Aw] (mean, SD)					
Preoperative	0·41 (0·04)	0·37 (0·05)	0·38 (0·05)	0·36 (0·04)	0·40 (0·04)
Postoperative	0·32 (0·04)	0·33 (0·05)	0·31 (0·05)	0·30 (0·02)	0·33 (0·04)
Postoperative least squares mean* (SE)	0·32 (0·01)	0·34 (0·01)	0·32 (0·01)	0·30 (0·01)	0·34 (0·01)
White blood count (mean [SD], $\times10^{-9}$/L)					
Preoperative	6·90 (1·61)	7·95 (2·08)	7·93 (1·95)	7·20 (1·84)	6·95 (1·65)
Postoperative	8·89 (2·54)	7·99 (2·23)	9·59 (2·77)	7·94 (1·20)	9·60 (2·63)
White blood count (mean $\log_e$, [SD], $\times10^{-9}$/L)					
Preoperative	1·91 (0·23)	2·04 (0·26)	2·04 (0·25)	1·95 (0·23)	1·91 (0·24)
Postoperative	2·15 (0·28)	2·04 (0·27)	2·22 (0·27)	2·06 (0·15)	2·22 (0·30)
Postoperative least squares mean* (SE)	2·17 (0·04)	2·03 (0·08)	2·22 (0·04)	2·01 (0·08)	2·27 (0·05)
Lymphocyte count (mean [SD], $\times10^{-9}$/L)					
Preoperative	1·66 (0·53)	1·50 (0·47)	1·94 (0·54)	1·63 (0·51)	1·84 (0·64)
Postoperative	1·38 (0·45)	1·18 (0·27)	1·59 (0·57)	1·36 (0·43)	1·42 (0·52)
Postoperative least squares mean* (SE)	1·47 (0·06)	1·40 (0·11)	1·47 (0·06)	1·29 (0·11)	1·38 (0·06)
Immunological data					
NKp frequency (mean [SD] per 10^{-6} PBMC)					
Preoperative	1512 (382)	1013 (487)	1404 (592)	1447 (539)	1163 (473)
Postoperative	1094 (415)	567 (186)	714 (377)	764 (361)	1757 (477)
NKp frequency (mean $\log_e$ [SD], per 10^6 PBMC$^{1/2}$)†					
Preoperative	38·54 (5·25)	31·10 (7·20)	36·65 (7·94)	37·29 (7·92)	33·20 (7·89)
Postoperative	32·43 (6·66)	23·53 (3·88)	25·81 (7·02)	27·96 (6·35)	41·51 (5·94)
Postoperative least squares mean* (SE)	31·42 (0·94)	26·49 (1·85)	25·28 (0·94)	25·05 (1·85)	43·62 (1·06)
Interferon γ (median [IQR], μg/L)					
Preoperative	4·55 (9·42)	8·95 (5·60)	5·37 (6·10)	6·05 (9·60)	5·10 (7·49)
Postoperative	3·58 (7·60)	3·86 (4·55)	3·48 (5·47)	2·30 (4·25)	6·56 (7·28)
Interferon γ (mean [SD] μg/L)$^{1/2}$†					
Preoperative	2·56 (1·97)	2·90 (0·79)	2·60 (1·35)	2·49 (0·99)	2·46 (1·30)
Postoperative	2·18 (1·77)	2·06 (0·96)	2·07 (1·04)	1·90 (1·20)	2·62 (1·39)
Postoperative least squares mean* (SE)	2·05 (0·15)	1·76 (0·29)	2·05 (0·15)	1·85 (0·29)	2·92 (0·16)
Interleukin 10 (median [IQR], ng/L)					
Preoperative	19·0 (29·0)	42·0 (61·5)	31·0 (60·0)	14·0 (29·0)	28·5 (86·0)
Postoperative	39·5 (77·5)	23·0 (44·0)	25·0 (46·0)	28·5 (31·0)	40·5 (63·5)
Interleukin 10 (mean $\log_e$ [SD], ng/L)†					
Preoperative	2·63 (1·70)	3·05 (1·73)	3·17 (1·59)	3·15 (1·05)	2·83 (1·87)
Postoperatie	3·29 (1·58)	2·93 (1·11)	2·92 (1·55)	3·00 (1·20)	3·56 (1·60)
Postoperative least squares mean* (SE)	3·29 (0·30)	2·99 (0·56)	2·81 (0·29)	2·61 (0·57)	3·57 (0·32)

NKp=precursor natural killer cells. PBMC=peripheral blood mononuclear cells. *Adjusted for age, sex, type of operation, and preoperative value with outliers omitted. †Data transformed for analysis.

Table 2: **Haernatological and immunological data**

natural-killer-cell-sensitive human erythro-leukaemic cell line, K562 (ECACC, UK) as a target for functional NK-cells. The NKp frequency was estimated by a new assay on the basis of the limiting dilutions analysis as applied in vitro. Clonal progeny of NKp were sustained in culture by adding recombinant interleukin 2 and interleukin 15. Results were expressed as frequency of NKp per 10^6 peripheral blood mononuclear cells. Details of this assay have been published previously.[15]

For the cytokine synthesis assays, we cultured peripheral blood mononuclear cells with cytokines interleukin 2 and interleukin 15 as in the NKp assay, but at a single cell concentration of 4×10^6/mL. After 5 days, culture supernatants were obtained and assayed by ELISA. Immunosorbent 96-well plates (Nunc) coated with interferon γ or interleukin 10 (Pharmingen) capture antibodies were blocked with phosphate-buffered saline containing 1% bovine serum albumin (Sigma) at 37°C. We washed plates with phosphate-buffered saline containing 0·1% Tween and 50 μL dilutions of recombinant interferon γ or interleukin 10 (Pharmingen) added to derive a standard curve. Supernatants from cultures were added in duplicate (50 μL per well) to the plates and incubated for 2 h at room temperature. They were then washed and biotinylated detection antibodies (Pharmingen) were added. Plates were incubated for 1 h, and then washed, and 50 μL streptavidin peroxidase (Sigma) was added. After incubation for 30 min, plates were washed and 100 μL of 3,3,5,5-tetramethyl benzidine, hydrogen peroxide, and phosphate citrate solution was added. The colour reaction was stopped by addition of 50 μL of 2 mol/L sulphuric acid and plates were read with a scanner (Dynatech) at 450 nm (reference 590 nm). The average concentration of each duplicate assay was derived with a standard curve and expressed as μg/L for interferon γ and ng/L for interleukin 10.

Statistical analysis

Demographic data and preoperative haematological and immunological measurements were compared by analysis of variance or the Kruskal-Wallis test (continuous variables) or Fisher's exact test (binary variables). Where differences were indicated, paired comparison tests were done and a Bonferroni correction applied to maintain an overall 5% significance level. We used analysis of covariance with adjustment for age, sex, type of operation, and preoperative haematological values to compare the five groups. Interactions between the patient group and preoperative haematological or immunological measurements were investigated and retained if significant at the 5% level. Model assumptions were assessed graphically and the square root and natural logarithmic transformations were used when the assumptions of constant variance or normally distributed data were untenable. The transformation that best satisfied the assumptions was chosen in each case. Hence, we analysed the following values: $\log_e$ white blood-cell count; $\log_e$ interleukin 10 (ng/L); square root of NKp frequency per 10^6 peripheral blood mononuclear cells, and square root of interferon γ (mg/L). For all other data, non-transformed values were analysed. For

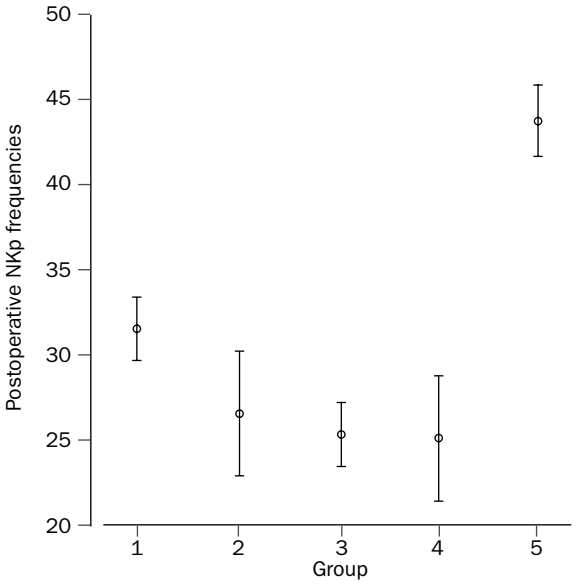

Figure 2: **Natural killer cell precursor frequencies expressed as square root of NKp counts**
Least squares postoperative mean values, adjusted for age, sex, type of operation, and preoperative value, are plotted with 95% CIs by patient group.

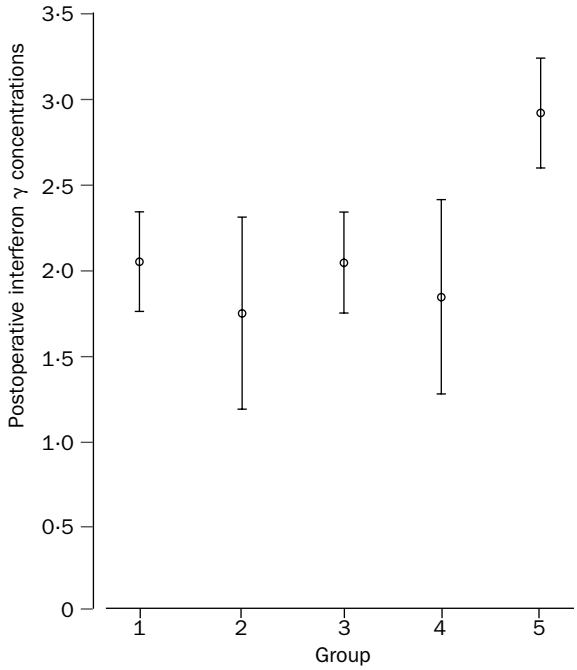

Figure 3: **Interferon γ concentration expressed as square root of interferon γ values**
Least squares postoperative mean values, adjusted for age, sex, type of operation, and preoperative value, are plotted with 95% CIs by patient group.

interleukin 10, we used Tobit analysis since readings of less than 1 ng/L were not detectable. In the analysis of postoperative white blood-cell count three outliers, one from group 3 and two from group 5 were omitted. In the analysis of postoperative lymphocyte counts, we omitted three outliers, one from group 3 and two from group 5. In the analysis of postoperative frequency of NKp, one outlier from group 5 was omitted. In the analysis of postoperative interferon γ concentrations, three outliers one each from groups 1, 3, and 5 were omitted.

Role of the funding source

The sponsor did not influence this study or its publication in any way.

Results

Between 1999 and 2001 a total of 120 patients were enrolled in the study. Assignment of patients to receive allogeneic non-leucodepleted blood (group 2) was stopped in June, 2000, because of a new national policy requiring leucodepletion of all transfusions. Autologous predeposit transfusion (group 4) was offered only to patients undergoing hip replacement surgery who were young and fit enough to get to the blood centre. Autologous salvaged blood transfusion was offered only to patients undergoing knee replacement surgery (group 5).

In groups 2 and 3 the volume of allogeneic blood given ranged from 515 to 3605 mL with most (78%) receiving 1030–1545 mL. Patients assigned to receive autologous predeposit blood (group 4) received about 1030 mL (two donations) each. The mean volume of autologous salvaged blood reinfused in group 5 was 650 mL per patient. These volumes are expressed as whole blood equivalents (less anticoagulant) in table 1.

The packed-cell volume (haematocrit) on admission varied significantly between the groups ($p<0·0005$). The mean values were higher for patients in groups 1 and 5 than in the other groups (table 2). After adjustment for age, sex, operative procedure, and packed-cell volume on admission, we recorded no differences in postoperative packed-cell volume between the five groups ($p=0·12$).

White blood-cell count on admission did not differ by much between the groups ($p=0·09$). The mean postoperative $\log_e$ white blood count differed between the five groups ($p=0·01$). The mean values were lower for patients in groups 2 and 4 compared with the other groups, although after Bonferroni correction for multiple comparisons the differences between individual groups were not significant at the 5% level.

Lymphocyte counts on admission did not differ by much between groups, with group means between 1·50 and $1·94\times10^{-9}$/L ($p=0·14$). The lymphocyte counts were also similar after surgery ($p=0·43$).

The NKp frequency on admission differed significantly between the five groups ($p=0·01$), with mean NKp frequencies of between 1013 and 1512 per 10^6 peripheral blood mononuclear cells (table 2). After adjustment for age, sex, type of operation and frequency of NKp on admission, the postoperative frequency of NKp varied significantly between the patient groups ($p<0·0001$). The mean postoperative frequency of NKp was significantly higher for patients in group 5 than for all other groups ($p<0·0001$). With one exception, all patients in group 5 had a postoperative NKp frequency that was higher than that before surgery. The mean postoperative NKp frequency for the untreated group 1, although lower than for group 5, was significantly higher than for groups 2, 3, and 4 ($p=0·0001$ for overall comparison of group 1 against 2, 3, and 4), where the results were similar ($p>0·99$) (table 2 and figure 2).

Admission concentrations of interferon γ were similar in all groups ($p=0·68$). The postoperative concentrations followed a similar pattern to the frequency of NKp insofar as the mean response was significantly higher for patients in group 5 ($p=0·0001$) than in the other groups where the results were similar ($p>0·99$) (table 2 and figure 3).

Concentrations of interleukin 10 on admission were much the same across the five groups ($p=0·59$) and did not differ significantly between the groups after surgery ($p=0·49$).

Discussion

This study compared the effects of major joint replacement surgery and transfusion on the immune system. Although this type of surgery is now commonplace and safe, on the rare occasions that it is complicated by infection, the results can be catastrophic. Our main findings were that postoperative NKp frequencies were substantially suppressed after joint replacement surgery. The effect was further exacerbated in groups that received transfusions of allogeneic non-leucodepleted, allogeneic leucodepleted, and autologous predeposit blood. However, autologous salvaged blood containing wound drainage fluid was invariably associated with an increased frequency of NKp at 5 days after surgery compared with the preoperative frequency. Furthermore, synthesis of interferon γ reflecting these changes suggests not only that the innate immune system, but also the adaptive immune system, is activated.

We did a prospective non-randomised observational study because of restrictions imposed by criteria for assigning patients to different postoperative transfusion regimens. Both hip and knee replacement surgery are associated with similar blood loss and duration of procedure. The non-randomised design is a clear limitation of the study. We have attempted to keep the confounding to a minimum through use of analysis of covariance. However, all such adjustments are imperfect. We could only partly control for type of operation, since this is completely confounded with type of transfusion in groups 4 and 5. Nonetheless, the magnitude of the NKp frequency and interferon γ effects in group 5 suggest that it is unlikely to be an artefact of study design. A randomised controlled trial is needed to provide more robust, high quality evidence of these effects.

Limiting-dilutions analysis is an accurate and reproducible method for measuring the frequency of NKp. The frequency of NKp cells identified by this functional assay is close to the threshold for accurate detection by routine flow-cytometric methods (0·01–0·5%). Moreover, the exact phenotype of NKp is unknown, so quantitation by flow cytometry is not feasible. In previous studies[15] we showed that the NKp assay accurately measured precursors of cytolytic natural-killer cells at frequencies below 0·25% of peripheral blood mononuclear cells. In this study NKp frequencies as low as 0·01% were accurately measured. Despite their low frequency, NKp driven by interleukin 2 and interleukin 15 differentiate rapidly into clones of mature cytolytic natural-killer cells with the capacity to kill target cells by the perforin-granzyme pathway. The cell-cycle time has been estimated at 8–12 h under some conditions of antigen stimulation.[19] Assuming that the same time applies to these cytokine-driven cells, each individual NKp cell could produce between 800 and 27 000 mature progeny within 5 days.

Mature natural-killer cells can be defined by the immunophenotype CD3–CD56+CD16+. This subset constitutes about 14% of peripheral blood mononuclear cells in healthy adults. Natural-killer cells thus defined are derived from marrow and are thymus independent originating from CD34+ multipotent stem cells. The immunophenotype of natural killer cells measured in our NKp assay is unknown, but their capacity to kill target cells implies a role in immunity. In innate immunity natural-killer cells have been directly implicated in antiviral defence and indirectly in antibacterial defence.[20] They interact with macrophages in control of sepsis and seem to regulate dendritic cells in evolving adaptive immunity.[21,22] Patients with congenital immunodeficiency disease with reduced, non-functional, or absent natural-killer cells have a high frequency of infections and reduced immunosurveillance against malignant disease.[20] Our observation of a substantial decrease in the frequency of NKp 5 days after surgery was only a snap-shot of one aspect of immune status, and further studies are indicated.

The aim of the cytokine studies was to gain insight into changes in other parts of the immune system. Our studies suggested that the surgery was associated with a reduction in postoperative synthesis of interferon γ without any detectable changes in synthesis of interleukin 10 on day 5. Postoperative synthesis of interferon γ was restored by autologous salvaged blood transfusion but no change was detectable in interleukin 10 synthesis. This observation might have reflected function in several cell types involved in innate and adaptive immunity to infection. While mature natural-killer cells synthesise interferon γ, tumour necrosis factor α, interleukin 3, interleukin 8, granulocyte macrophage colony stimulating factor (GM-CSF), and interleukin 10 under special conditions, activated T cells and macrophages produce interleukin 10 and interferon γ.[20,23,24] Interferon γ has an important role in activating dendritic cells and T cells to differentiate along the proinflammatory pathway responsible for adaptive immune responses to infection. Interleukin 10 inhibits production of interferon γ by macrophages, but enhances interferon γ production by mature natural-killer cells. An added complication is that synthesis of interleukin 10 and interferon γ varies between individuals in accordance with allelic differences in the gene promoter region. Hence, further studies using specific subsets of peripheral blood mononuclear cells are needed to identify contributions from these sources.

In the non-transfused patients (group 1), the inhibitory effects of surgical trauma on NKp frequency and interferon γ synthesis occurred independently of transfusion. The additional inhibitory effect on the frequency of NKp associated with allogeneic transfusion in groups 2 and 3, and especially with the autologous predeposited blood group 4, raises the question as to whether the inhibition is an attribute of blood transfusion per se, or an attribute of blood loss,[25] or both. Since many clinical factors affect the decision to transfuse a patient this issue cannot be resolved without a properly controlled trial.

The surprising finding that autologous salvaged blood transfused intravenously reversed the decrease in the frequency of NKp and synthesis of interferon γ associated with surgery was contrary to expectations. It suggested that agents originating in the operation site targeted key stages in the migration and development of NKp cells either in bone marrow, liver, thymus, or blood. The agents could act as immunostimulants or competitors of naturally occurring immunosuppressants, or both. That these agents originate from within the wound site is supported by the observation that autologous predeposited blood transfusion (group 4) did not give the same effect.

Substances known to be released into autologous salvaged blood from the knee joint site include interleukin 1β, interleukin 6, interleukin 8, interleukin 10, complement degradation products (C′3a, C′5b, SC′5b-9), constituents of methyl methacrylate cement such as gentamycin, microparticles of polyethylene, and some metals (cobalt, cromium, molybdenum, nickel).[26–30]

The clinical relevance of these observations can be inferred from a study by Newman and colleagues,[12] who suggested that transfusion of autologous salvaged

blood was accompanied by a significant decrease in postoperative infections. Removal of leucocytes or fluid from autologous salvaged blood might remove this benefit. Nonetheless, some constituents of autologous salvaged blood are thought to be harmful, such as fat particles.[31] The risk associated with these effects needs to be weighed against the risk of harmful effects of allogeneic transfusions that include transfer of infectious agents such as pathogenic prions and viruses, immunosuppression and immunological diseases such as transfusion-induced graft versus host disease, and transfusion-associated lung infiltration.[14]

In summary, we showed that autologous salvaged blood activated systemic immunity after joint replacement surgery as shown by increased frequencies of NKp and concentration of interferon γ, thereby reversing the immunosuppression associated with surgical trauma and blood loss. The acknowledged benefits of autologous salvaged blood for reducing the risks associated with allogeneic transfusions might be further enhanced by these findings.

Contributors

A Gharehbaghian developed the NKpf assay and undertook the bulk of the practical work as part of a PhD project (supervised by B A Bradley) successfully submitted to the University of Bristol in 2002. K M G Haque and B A Bradley made the original observations that led to the design of this study. C Truman provided essential assistance for all practical laboratory work on a day-to-day basis. R Evans was the research nurse responsible for the identification and tracking of study patients. R Morse instructed and supervised A Gharehbaghian with the cytokine release assays. J Newman and G Bannister are orthopaedic surgeons whose patients were studied and who provided intellectual input at the planning and interpretation stages of this study. C Rogers was responsible for analysing the data. B A Bradley was responsible for directing and financing this study and with assistance from A Gharehbaghian, and C Rogers interpreted the findings and prepared this manuscript.

Conflict of interest statement

None declared.

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