Leukocyte-depleted blood: a comparison of available preparations

G. ROCK,* PH D, MD
A. BAXTER, ART
E. GRAY, RT

Febrile nonhemolytic transfusion reactions due to leukoagglutinins are frequently seen in patients who have been given multiple blood transfusions. To prevent or reduce the severity of these reactions, leukocyte-poor blood (that containing fewer than 0.3×10^9 leukocytes per unit) is frequently requested by clinicians. Four methods commonly used in Canada to produce leukocyte-poor blood were examined for their relative effectiveness and appropriate use. The mean total leukocyte count per unit was reduced to 0.22×10^9 in buffy-coat-poor red blood cell preparations produced by centrifugation with the blood bag inverted, to 0.19×10^9 by perfusion through an Imugard filter, to 0.21×10^9 by the use of an IBM 2991 automated cell washer and to 0.13×10^9 with the use of frozen blood. The proportion of red cells recovered varied from 62% with the inverted-spin method to 85% with the use of frozen blood. Comparison of these data and the percentage of leukocytes removed, the shelf life of the product, the cost of supplies and the preparation time indicated that the use of sophisticated machinery, such as the IBM cell washer, or of glycerolization plus washing of frozen cells is not warranted for most patients. Instead, patients who have febrile nonhemolytic transfusion reactions should initially be treated with a leukocyte-poor red cell preparation produced by the inverted-spin method; only if such reactions recur should the blood bank be requested to provide filtered, washed or frozen red cells.

Des réactions transfusionnelles non hémolytiques fébriles causées par des leucoagglutinines sont fréquemment observées chez des patients qui ont reçu plusieurs transfusions sanguines. Afin de prévenir ces réactions ou d'en réduire la gravité, les médecins réclament fréquemment du sang pauvre en leucocytes (contenant moins de $0,3 \times 10^9$ leucocytes par unité). Quatre méthodes couramment utilisées au Canada pour produire du sang pauvre en leucocytes ont été comparées quant à leur efficacité et leur bonne utilisation. La numération leucocytaire moyenne par unité a été réduite à $0,22 \times 10^9$ dans des préparations de globules rouges pauvres en couche leucocytaire produites par centrifugation du sac de sang inversé, à $0,19 \times 10^9$ par perfusion sur un filtre Imugard, à $0,21 \times 10^9$ par l'utilisation d'un appareil automatisé pour laver les globules (modèle IBM 2991) et à $0,13 \times 10^9$ par l'utilisation de sang congelé. La proportion de globules rouges récupérés a varié de 62% avec la centrifugation du sac inversé à 85% avec l'emploi de sang congelé. La comparaison de ces résultats et du pourcentage de leucocytes enlevés, de la durée d'utilisation du produit, du coût des approvisionnements et du temps de préparation a indiqué que l'emploi d'équipements sophistiqués, comme l'appareil IBM pour laver les globules, ou de la glycérolisation suivie du lavage des globules congelés ne se justifie pas pour la plupart des malades. Les malades souffrant de réactions transfusionnelles non hémolytiques fébriles devraient plutôt recevoir initialement une préparation de globules rouges pauvre en leucocytes produite par centrifugation du sac inversé; ce n'est qu'après récidive que l'on devrait réclamer de la banque de sang des globules rouges filtrés, lavés ou congelés.

Febrile nonhemolytic transfusion reactions frequently follow infusion of blood products containing leukocytes,[1-3] at rates of 0.2% with red blood cell concentrates and 0.5% with whole blood.[4] In previously sensitized patients a dose of between 0.25 and 0.5×10^9 leukocytes can produce these reactions.[1] Therefore, efforts have been directed towards production of leukocyte-poor blood products for transfusion in these patients.

Methods currently available to produce leukocyte-poor blood include, among the more common, centrifugation to remove the buffy coat, filtration, washing with saline and freezing the blood. However, the preparations produced by these methods vary considerably in efficacy, shelf life, cost and time required for their production. Meryman and colleagues[2] compared 13 procedures for preparing leukocyte-poor blood and found that a single centrifugation of packed cells diluted with saline followed by removal of the buffy coat was second only to freezing blood in efficacy of reducing the leukocyte content. Menitove and associates[3] later reported that the use of leukocyte-poor red cell preparations obtained by inverting the blood bag during centrifugation (the inverted-spin method) usually prevented the recurrence of febrile nonhemolytic transfusion reactions due to leukoagglutinins. Various filtration techniques have also been used to reduce leukocyte content: Wenz and colleagues[5] used a microaggregate filter to obtain granulocyte-poor red cell preparations, and Kikugawa and Minoshima[6] used the Imugard filter, which incorporates cotton wool and a nylon fabric with pores 25 μL in diameter, to remove 95% of the leukocytes.

In our centre we collect and process approximately 65 000 units of blood per year. The 4075 units of leukocyte-poor blood that we provided to clinicians last year represented approximately 7% of our total collection. While national statistics on the total number of units of leukocyte-poor blood issued are not available, we know that 4.5% of the units of blood issued by the Calgary Centre of the Canadian Red Cross are leukocyte-poor preparations, produced by the inverted-spin

From the blood transfusion service, Ottawa Centre, Canadian Red Cross, and * the department of medicine, University of Ottawa

Reprint requests to: Dr. G. Rock, Medical director, Blood transfusion service, Ottawa Centre, Canadian Red Cross, 85 Plymouth St., Ottawa, Ont. K1S 3E2

method (T. Bowen: personal communication, 1984). Many hospitals also process their own leukocyte-poor blood. Because of existing constraints on financial expenditures and technologists' time, we decided to re-evaluate both the demand for leukocyte-poor blood and the methods that produce optimal products.

Our previous studies had shown that clinicians requesting frozen blood usually wanted leukocyte-poor blood, and we found that washing red cell concentrates with an automated cell washer (model 2991, IBM Instruments, Inc., Danbury, Connecticut) provided a product that was cheaper than frozen blood but equally effective.[7] Using this technique we reduced the number of units of frozen blood that we processed from a peak of 1000 in 1979 to 103 in 1983. This represented a substantial cost saving in view of the much greater cost of processing and storing frozen blood. While the IBM cell washer is extremely effective in reducing the leukocyte content of blood,[2,7] it lowers the content well below the levels associated with most transfusion reactions,[1] and we wanted to determine whether a less expensive alternative procedure would suffice.

Methods

Blood collection and processing

Blood was collected from volunteers considered healthy according to the criteria used by the Canadian Red Cross blood transfusion service. The units of blood either were kept as whole blood for subsequent production of leukocyte-poor red cell preparations by the inverted-spin method or were made into red cell concentrates by centrifugation at 7000 × *g* for 5 minutes and removal of plasma to give us a packed cell volume of 0.70 ± 0.05 L/L. The leukocyte-poor red cell preparations made from whole blood collected into a multiple pack[8] were produced in a closed system; since the unit was not opened during processing, this product had a normal shelf life. The red cell concentrates were processed by one of three open systems: obtaining filtered red cells by perfusing red cell concentrate through an Imugard (IG 500) filter (Terumo Corporation, Ste-Thérèse, PQ) at 150 mm Hg of pressure, following the manufacturer's instructions; washing fresh red cell concentrate with saline in the IBM 2991 cell washer;[7] and deglycerolizing and washing frozen blood[9] in the IBM 2991 cell washer.[10]

Sample testing

Packed cell volume, blood volume and leukocyte counts were determined before and after processing in aliquots of each unit of whole blood or red cell concentrate used in the study. Leukocyte counts were performed manually because glycerolization produces leukocyte fragments and debris in frozen blood, and this results in artificially high values when an electronic particle counter is used: previous experience in our laboratory has shown that electronic cell counting gives values up to 10% higher than manual counting. Differential counts could not be made with frozen blood, again because of cellular rupture due to glycerolization. For the various products the efficacy of leukocyte removal and red cell recovery was compared.

Results

As shown in Table I, each of the four methods reduced the leukocyte count below 0.25×10^9 per unit, with freezing and filtration producing the lowest counts and highest percentage removal. Differential leukocyte counts showed that 99% of lymphocytes were removed by each of the three methods studied. Attempts to use an Imugard filter to produce more than one unit of red cell concentrate were not successful: the proportion of leukocytes removed decreased with each successive unit filtered, with average values of 85% for the first unit, 52% for the second and 40% for the third.

The proportion of red cells recovered was similar (about 82%) with the three open-system methods but much lower (62%) with the closed inverted-spin method.

The shelf life of the products prepared by the three open-system methods was considerably shorter (24 hours) than that of the product prepared in a closed system (35 days). In addition, since the latter was

Table I—Comparison of different methods of preparing leukocyte-poor blood

Product	Mean ± standard deviation or absolute value per unit							
	Weight (g)	Packed cell volume (L/L)	Leukocyte count per bag ($\times 10^9$)	% of leukocytes removed	% of red cells recovered	Shelf life	Cost of supplies ($)	Preparation time (min)
Leukocyte-poor red cell preparation (n = 23)	160 ± 21.35	0.68 ± 0.09	0.22 ± 0.17	91 ± 5.45	62 ± 7.69	35 d	–	30
Filtered cells* (n = 8)	261 ± 13.12	0.63 ± 0.08	0.19 ± 0.15	93 ± 6.08	82 ± 8.61	24 h	15	30
Washed cells† (n = 21)	226 ± 33.12	0.74 ± 0.03	0.21 ± 0.18	90 ± 7.46	80 ± 8.23	24 h	24‡	30
Frozen blood (n = 11)	213 ± 39.12	0.75 ± 0.06	0.13 ± 0.15	96 ± 2.47	85 ± 7.30	24 h	60	90

*Imugard filter was used to process each unit.
†Saline and an IBM 2991 cell washer were used.
‡Cost of processing two units in one bag, $35.

processed in its original collection pack it was much cheaper to make than the other three products.

It took 30 minutes to process units by the inverted-spin method, automated cell washing or filtration, whereas it took 90 minutes to thaw and process frozen blood.

Discussion

The data from this study suggest that the inverted-spin method of preparing leukocyte-poor blood, aside from being faster and cheaper than the other three methods tested, yields a product that is sufficiently depleted in leukocytes to satisfy most requirements for leukocyte-poor blood. Furthermore, this preparation has a shelf life of 35 days and thus can be provided by the Canadian Red Cross blood transfusion service at any time.

Alternatively, when leukocyte-poor blood is required immediately, hospitals can use Imugard filters. Unfortunately, the filter is not intended for, nor does it easily adapt to, bedside use since it requires priming with 250 mL of saline before the blood is perfused and since the perfusate line is open to allow saline to be removed before transfusion begins. Also, processing more than one unit of blood with an Imugard filter greatly reduces the method's efficacy: only 40% of the leukocytes will be removed after a filter is used for a third unit. Using a new filter for each unit thus greatly increases the method's cost.

The literature suggests that a reduction of the leukocyte content to levels below 0.3×10^9 per unit will protect most patients against febrile nonhemolytic transfusion reactions; thus, the dedication of time and effort toward production of a preparation with even fewer leukocytes is not warranted in most cases.[1,2]

In a study of 99 658 transfused units Menitove and associates[3] found that 0.5% caused febrile nonhemolytic transfusion reactions. Only 15% of the patients experienced another such reaction and required transfusion of leukocyte-poor blood thereafter. Their experience showed that leukocyte-poor blood prepared by the inverted-spin method was acceptable, causing only one additional febrile nonhemolytic transfusion reaction. Thus, efforts at producing a purer red cell preparation should be reserved for a few selected patients.

We recommend that clinicians who require leukocyte-poor blood first request the product prepared by the inverted-spin method, which will be suitable in most cases. If febrile nonhemolytic transfusion reactions persist, leukocyte-poor blood prepared by one of the more efficient but more expensive and time-consuming methods should then be requested.

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References

1. PERKINS HA, PAYNE R, FERGUSON J, WOOD M: Nonhemolytic febrile transfusion reactions. Quantitative effects of blood components with emphasis on isoantigenic incompatibility of leukocytes. *Vox Sang* 1966; 11: 578–600
2. MERYMAN HT, BROSS J, LEBOVITZ R: The preparation of leukocyte-poor red blood cells: a comparative study. *Transfusion* 1980; 20: 285–292
3. MENITOVE JE, MCELLIGOTT MC, ASTER RH: Febrile transfusion reaction: What blood component should be given next? *Vox Sang* 1982; 42: 318–321
4. MILNER LV, BUTCHER K: Transfusion reactions reported after transfusions of red blood cells and of whole blood. *Transfusion* 1978; 18: 493–495
5. WENZ B, GURTLINGER KF, O'TOOLE AM, DUGAN EP: Preparation of granulocyte-poor red blood cells by microaggregate filtration: a simplified method to minimize febrile transfusion reactions. *Vox Sang* 1980; 39: 282–287
6. KIKUGAWA K, MINOSHIMA K: Filter columns for preparation of leukocyte-poor blood for transfusion. *Vox Sang* 1978; 34: 281–290
7. GRAY E, BAXTER A, LABERGE A, ROCK G: Preparation of leukocyte-poor blood: a comparison of IBM 2991 washing and Huggins freeze-thawing. *Vox Sang* 1981; 40: 323–328
8. Canadian Red Cross Society, blood transfusion service, blood components working group, BARR R (chmn): *Blood Components Manual*, 2nd ed, Can Red Cross Soc, Toronto, 1983: 12
9. HUGGINS CE: Practical preservation of blood by freezing. In *Red Cell Freezing: a Technical Workshop*, Am Assoc Blood Banks, Washington, 1973: 31
10. VALERI CR: *Blood Banking and the Use of Frozen Blood Products*, CRC Pr, Cleveland, Ohio, 1976: 205–206

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June

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Canadian Ophthalmological Society annual meeting
Quebec Hilton Hotel, Quebec
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June 25–28, 1984

75th Annual Conference of Canadian Public Health Association
Calgary Convention Centre, Calgary
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