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Use of leucocyte-poor blood components and HLA-matched-platelet donors to prevent HLA alloimmunization

M. F. MURPHY, P. METCALFE, H. THOMAS,* J. EVE,† J. ORD,†
T. A. LISTER* AND A. H. WATERS *Department of Haematology and*
**ICRF Department of Medical Oncology, St Bartholomew's Hospital, London, and*
†*Regional Blood Transfusion Centre, Brentwood, Essex*

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SUMMARY. Recent studies have shown that the incidence of alloimmunization due to repeated platelet transfusions from random donors may be reduced by the use of leucocyte-poor blood components. These results were confirmed by this study, where 16% of patients with acute leukaemia undergoing initial chemotherapy and receiving leucocyte-poor blood components developed lymphocytotoxic antibodies, compared with 48% of patients in a control group receiving standard (non-leucocyte-depleted) blood components. In a third group, who received leucocyte-poor blood components and HLA-matched platelets, none of the patients developed lymphocytotoxic antibodies. There was a low incidence of platelet-specific antibodies (8%) but no difference between the three groups. Improved methods of removing leucocytes from blood components appear to offer the best approach for minimizing HLA alloimmunization, as the provision of HLA-matched platelet donors for prophylactic platelet support of all patients is not feasible.

One of the major problems associated with repeated platelet transfusions from random donors is poor survival of the transfused platelets because of HLA alloimmunization (Stefanini *et al*, 1952; Aster *et al*, 1964; Shulman, 1966; Grumet & Yankee, 1970; Howard & Perkins, 1978), which occurs in at least 50% of multitransfused patients (Schiffer *et al*, 1976; Howard & Perkins, 1978; Eernisse & Brand, 1981; Pegels *et al*, 1982). Refractoriness due to platelet specific antibodies is less of a problem (Waters *et al*, 1981; Pegels *et al*, 1982).

There is increasing evidence that HLA alloimmunization by platelet transfusions is induced by contaminating lymphocytes. Welsh *et al* (1977) showed that pure platelet preparations were non-immunogenic in rats. They suggested that this was due to the absence

Correspondence: Dr M. F. Murphy, Department of Haematology, St Bartholomew's Hospital, London EC1A 7BE.

of class-II antigens on platelets, as primary immunization against HLA antigens is thought to be a two-signal process requiring both class-I and class-II antigen differences (Bach *et al*, 1976). Further studies by the same group showed that it was necessary for the class-II antigen difference to be presented by viable lymphocytes (Batchelor *et al*, 1978). Claas *et al* (1981) confirmed these results in mice, and human transfusion studies by Eernisse & Brand (1981) have shown that the incidence of HLA alloimmunization is reduced by the use of leucocyte-poor blood components. However, even using leucocyte-poor blood components about 20% of patients still become alloimmunized (Eernisse & Brand, 1981; Schiffer *et al*, 1983; Robinson, 1984), probably because of residual contaminating leucocytes. The aim of the present study was to assess the effect on alloimmunization of using HLA-matched platelet donors in addition to leucocyte-poor blood components.

PATIENTS STUDIED

Eighty-six patients with newly diagnosed acute leukaemia entered the study. Those found to have HLA or platelet-specific antibodies at presentation, and those subsequently receiving granulocyte transfusions or dying within 2 weeks of commencing chemotherapy, were excluded. The remaining 61 patients received one of three transfusion programmes (shown below), depending on the availability of leucocyte-poor blood components and HLA-matched platelet donors for each patient:

'Control' group. Plasma-reduced red cell transfusions. Single platelet concentrates from random donors.

'Leucocyte-poor' group. Filtered red cell transfusions. Leucocyte-poor single platelet concentrates from random donors.

'HLA-matched' group. Filtered red cell transfusions. Leucocyte-poor platelet concentrates from platelet donors matched for at least three out of the four HLA-A and HLA-B loci.

Patients received red cell transfusions for anaemia (Hb < 10 g/dl), and prophylactic platelet transfusions when the platelet count was less than 20×10^9/l. In the few patients where prophylaxis failed to prevent bleeding, additional platelet transfusions were given.

METHODS

Red cell transfusions. Leucocyte-poor red cell transfusions were prepared using the Imugard IG500 filter (McGregor *et al*, 1983). The maximum permitted white cell count was 8×10^6 per unit of filtered blood.

Platelet transfusions. Platelet concentrates were obtained by plateletpheresis of single donors using either the Haemonetics Model 30 or IBM 2997 cell separators (mean platelet yield $3{\cdot}55 \times 10^{11}$, mean WBC contamination $5{\cdot}38 \times 10^9$). Leucocyte-poor platelet concentrates were prepared either by extra centrifugation (150 *g* for 10 min using an MSE Mistral 6 L Centrifuge) of the plateletpheresis concentrates (mean platelet yield $2{\cdot}55 \times 10^{11}$, mean WBC $0{\cdot}22 \times 10^9$) or by using the 'dual-stage' technique with the IBM 2997 cell separator (mean platelet yield $2{\cdot}09 \times 10^{11}$, mean WBC $0{\cdot}09 \times 10^9$).

HLA-typed donor panel. HLA-matched platelet donors were selected from an HLA-typed donor panel at the Regional Blood Transfusion Centre, Brentwood. This panel has approximately 2300 donors. Platelet donors were matched for at least three out of four of the HLA-A and -B loci of the patient.

Assessment of platelet recovery. The recipient's platelet counts were measured before platelet transfusions and at 1 h and 20 h post-transfusion. The percentage of transfused platelets circulating at these times was calculated by the formula:

$$\text{Recovery} = \frac{\text{Platelet increment} \times \text{Blood volume}}{\text{Total platelets transfused}} \times 100\%$$

Blood volume was calculated as body surface area (m^2) × 2·5.

Patients were said to be refractory to platelet transfusions as a result of alloimmunization if there was no measurable recovery 20 h after transfusion in the absence of non-immune factors that might have caused increased platelet consumption, such as bleeding, fever (> 38°C), septicaemia or splenomegaly.

Statistics. Statistical comparisons were made using the Fisher exact test.

Antibody screening. Serum samples from each patient were tested at presentation and at regular intervals during the period of chemotherapy, using the following methods: *Lymphocytotoxicity test (LCT)* (Mittal, 1978). Serum samples were tested for HLA antibodies against a comprehensive HLA-typed lymphocyte panel from 20 selected donors. *Platelet immunofluorescence test (PIFT)* (Borne *et al.* 1978). Serum samples were tested for platelet-specific antibodies against platelets from two Group O donors. Serum samples from patients with HLA antibodies were tested against chloroquine-treated platelets to determine whether platelet-specific antibodies were also present (Nordhagen & Flaathen, 1983).

RESULTS

The three groups of patients were similar in age, previous transfusions, and in the case of women, previous pregnancies (Table I). The average number of red cell and platelet transfusions received by each group of patients during remission induction therapy was similar, except that the 'HLA-matched' group received fewer platelet transfusions (Table I).

Table I. Clinical details of patients studied

Patient group	No. of patients	Age (years) (mean and range)	Sex (M:F)	Previously transfused	Previously pregnant	Units of blood per patient (mean)	Platelet concentrates per patient (mean)
Control	31	51 (21–78)	15:16	7	14	26	16
Leucocyte-poor	19	47 (17–71)	9:10	2	7	33	19
HLA-matched	11	49 (27–66)	7:4	2	4	23	9

Table II. Incidence of alloimmunization

Patient group	No. of patients	HLA antibodies	HLA + platelet-specific antibodies	Platelet-specific antibodies only	Patients refractory to platelet transfusions because of HLA immunization
(a) All patients					
Control	31	15 (48%)	3	0	7/31 (23%)
Leucocyte-poor	19	3 (16%)	0	2	1/19 (5%)
HLA-matched	11	0 (0%)	0	0	0
(b) Patients not previously transfused or pregnant					
Control	14	7 (50%)	1	0	2/14 (14%)
Leucocyte-poor	11	1 (9%)	0	2	0
HLA-matched	5	0 (0%)	0	0	0

Table IIa shows the incidence of alloimmunization in all patients studied. Fifteen out of 31 patients (48%) in the 'control' group developed HLA antibodies, between 1 and 7 weeks (mean 3·7 weeks) of beginning treatment. HLA antibodies were found in three out of 19 patients (16%) in the 'leucocyte-poor' group, but in none of 11 patients in the 'HLA-matched' group. The incidence of HLA alloimmunization in the 'control group' was significantly different from that in the 'leucocyte-poor' group ($p=0·02$) and the 'HLA-matched' group ($p=0·01$). The incidence of alloimmunization was lower in the HLA-matched group compared with the leucocyte-poor group, but this was not statistically significant ($p=0·28$). Platelet-specific antibodies were found in three of the patients in the 'control' group, two of the patients in the 'leucocyte-poor' group, and in none of the patients in the 'HLA-matched' group.

In the 'control' group, seven patients (23%) became refractory to platelet transfusions from random donors because of HLA alloimmunization, compared with one patient (5%) in the 'leucocyte-poor' group, and none in the 'HLA-matched' group. All patients who were refractory to platelet transfusions from random donors because of HLA alloimmunization had multispecific HLA antibodies. All five patients with platelet-specific antibodies were refractory to platelet transfusions, but multispecific HLA antibodies were also present in three of these patients. The specificities of the platelet-specific antibodies were not determined.

The results were similar in the patients who had not been previously transfused or pregnant (Table IIb).

Death from bleeding occurred in two out of the 61 patients in the study; both were in the 'control group' and were refractory to platelet transfusions from random donors because of HLA alloimmunization.

DISCUSSION

It has been suggested by Gmur *et al* (1983) that it is possible to minimize alloimmunization by using single donor rather than multiple donor platelet concentrates. The present study was

unable to reproduce the low incidence of HLA alloimmunization (15%) found by Gmur *et al* (1983) in a group of multitransfused acute leukaemia patients receiving single random donor platelet concentrates: the incidence of HLA alloimmunization in our 'control' group, who also received single random donor platelet concentrates, was 48%, and seven out of the 15 patients with HLA antibodies were refractory to platelet transfusions from random donors. However, the benefit of leucocyte depletion of blood components in reducing HLA alloimmunization was confirmed, with only 16% of patients developing lymphocytotoxic antibodies, similar to the results of previous studies using leucocyte-poor blood components (Eernisse & Brand, 1981; Schiffer *et al*, 1983; Robinson, 1984). In an attempt to reduce the incidence of HLA alloimmunization even further, the present study combined the use of HLA-matched donors with leucocyte-poor blood components, and no patients became alloimmunized.

The suggestion that the use of HLA-matched platelet donors from the beginning of treatment might select out the production of platelet-specific antibodies (Schiffer & Slichter, 1982) was not supported by this study; there was a marked reduction in the incidence of HLA antibodies in the 'leucocyte-poor' and 'HLA-matched' groups, but no increase in the incidence of platelet-specific antibodies in these two groups.

It is difficult to provide HLA-matched platelet donors for patients with acute leukaemia from the beginning of treatment. They often require urgent platelet transfusions before the results of HLA typing are available, and sometimes accurate HLA typing is difficult because of lymphopenia and the problem of HLA typing blast cells. In addition, it would strain the limited resources of the available HLA typed donor panels to provide sufficient numbers of HLA-matched donors for all patients. Furthermore, the routine use of HLA-matched platelet donors would require facilities for plateletpheresis which are not available in all centres. On the other hand, the provision of leucocyte-poor red cell and platelet concentrates is a relatively simple alternative and improvements in methods of providing blood components with even fewer leucocytes than was possible with the techniques used in this study would seem to offer the best approach for minimizing alloimmunization. In this respect, a clinical trial is warranted using the virtually leucocyte-free platelet concentrates prepared by the filtration procedure described by Sirchia *et al* (1983).

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