Leukocyte transfusions for the prophylaxis and treatment of infections associated with granulocytopenia

J.E. CURTIS, MD, FRCP[C]; R. HASSELBACK, MD, FRCP[C]; D.E. BERGSAGEL, MD, D PHIL, FRCP[C]

The role of leukocyte transfusions in the prevention and treatment of infections in adults with granulocytopenia was investigated. Leukocytes were obtained from healthy volunteers by continuous-flow centrifugation. Histocompatibility antigen (HLA)-matched leukocytes were used to assess the prophylactic value of leukocyte transfusions.

Seven patients with acute myelogenous leukemia received HLA-matched leukocytes during the period of maximal granulocytopenia associated with initial remission induction therapy; 20 concurrently treated patients who did not receive leukocyte transfusions were the control group. The patients receiving HLA-matched leukocytes had significantly fewer ($P = 0.043$) infectious episodes (not bacteriologically proven) during the study period, and remission occurred in 5 of the 7, compared with 10 of the 20 controls.

In addition, 52 series of two or more ABO-compatible transfusions were given to 50 patients with proven infection or elevated temperature presumed due to infection and a granulocyte count of less than 0.5×10^9/L. Response, indicated by a decrease in temperature, occurred in 23 patients.

Leukocyte transfusions thus have an important adjuvant role in the management of patients with severe granulocytopenia.

On a étudié le rôle de la transfusion de leucocytes dans la prévention et le traitement des infections des adultes atteints de granulocytopénie. Les leucocytes ont été obtenus de volontaires sains par centrifugation à débit continu. Des leucocytes compatibles pour le système des antigènes d'histocompatibilité (HLA) ont été utilisés pour établir la valeur prophylactique des transfusions de leucocytes.

Sept patients souffrant de leucémie myéloïde ont reçu des leucocytes compatibles pour le système HLA pendant la période de granulocytopénie maximale associée au traitement d'induction de rémission initiale; 20 patients traités concurremment et qui n'ont pas reçu de transfusion de leucocytes ont été employés comme groupe témoin. Les patients qui ont reçu des leucocytes compatibles pour le système HLA ont eu significativement moins ($P = 0.043$) d'épisodes infectieux (sans confirmation bactériologique) pendant la durée de l'étude, et une rémission a été obtenue chez 5 sujets sur 7, comparativement à 10 témoins sur 20.

De plus, 52 séries d'au moins deux transfusions compatibles pour le système ABO ont été administrées à 50 patients souffrant d'une infection démontrée ou d'une température élevée présumément due à une infection et ayant un décompte des granulocytes de moins de 0.5×10^9/L. Une réponse, indiquée par une détervescence, est survenue chez 23 patients.

Les transfusions de leucocytes ont donc un rôle d'appoint important dans le traitement des patients présentant une granulocytopénie profonde.

From the department of medicine, University of Toronto, and the Ontario Cancer Institute, Toronto

Presented in part at the 45th annual meeting of the Royal College of Physicians and Surgeons of Canada, Quebec City, Jan. 22, 1977

Reprint requests to: Dr. J.E. Curtis, Department of medicine, Princess Margaret Hospital, 500 Sherbourne St., Toronto, Ont. M4X 1K9

Infection is the major cause of death of patients with acute leukemia,[1,2] and granulocytopenia is the most important predisposing factor to infection in these patients.[3] Chemotherapy during remission induction temporarily increases the granulocytopenia.[3,4] Since granulocytes are required to kill even antibiotic-inhibited bacteria, antibiotic therapy during granulocytopenia is less effective.[5,6] Transfusions of peripheral blood leukocytes provide a means of reducing the degree and duration of granulocytopenia.[7-9] Leukocyte transfusions given to granulocytopenic patients with infection frequently result in decreased temperature and clinical improvement.[10-14]

This report describes (a) a prospective study demonstrating the effectiveness of transfusions of histocompatibility antigen (HLA)-matched leukocytes in the prevention and control of infection during remission induction therapy in adults with acute leukemia, and (b) our experience at Princess Margaret Hospital, Toronto, in treating infections in granulocytopenic patients with leukocytes from healthy volunteers.

Methods

Leukocyte procurement

Peripheral blood leukocytes were collected by a continuous-flow centrifugation technique[15] with anticoagulant citrate dextrose solution, National Institutes of Health formula A.

Donors were healthy family members, friends or unrelated volunteers between 18 and 66 years of age. Donor–recipient pairs were of the same ABO blood type. HLA typing[16] of the donor and recipient was done on occasion.

Procedures in leukocyte transfusion

The leukocytes, suspended in citrated autologous plasma, were transfused within 1 hour after completion of the leukapheresis. Hemoglobin concentration, leukocyte count and differential,

and platelet count were determined before, at 1 hour and at 24 hours after each leukocyte transfusion.

Assessment of leukocyte function

The function of the buffy-coat cells was assessed by three parameters. Aliquots of 20 leukocyte collections were examined by the trypan-blue dye exclusion test,[17] and functional activity was assessed from the concentration of colony-forming units in culture (CFU-C)[18] and the production of colony-stimulating activity (CSA).[19] CFU-C concentration provides an estimate of the concentration of granulocyte progenitor cells and depends on cell proliferation. The production of CSA depends on intact metabolic cellular processes.

The number of CFU-C per transfusion was calculated by multiplying the concentration of CFU-C in the buffy-coat cell collection by the number of leukocytes transfused. The CSA of a transfusion was the product of the number of leukocytes transfused and the number of irradiated buffy-coat cells necessary for 50% reconstitution of CFU-C formation by nonadherent normal marrow cells.

Prospective study of prophylactic leukocyte transfusions

The role of leukocyte transfusions in the prevention of infection was assessed in 27 adult patients receiving similar remission induction therapy, antibiotic therapy and erythrocyte and platelet transfusions; their clinical details are summarized in Table I. Remission was induced with either a 4-day course of cyclophosphamide, arabinosyl cytosine and vincristine (CAV),[4] or adriamycin and a 7-day continuous infusion of arabinosyl cytosine (ADR–ARA-C).[20]

Seven patients with acute myelogenous leukemia (AML) receiving initial remission induction therapy were given a series of leukocyte transfusions from HLA-compatible siblings; except where indicated in Table I, donor–recipient pairs were HLA-identical. The transfusions were given between the second and third courses of CAV or between the first and second courses of ADR–ARA-C (Fig. 1) — times of maximum granulocytopenia and peak incidence of infection.

The 20 control patients were being

Table I—Clinical details* of patients in prospective study of leukocyte transfusions

No.	Age (yr), sex	Remission induction chemotherapy (no. of courses); response	No. of leukocyte transfusions	No. of days of temperature > 38°C	Initial cell counts (x 10^9/L)		Clinical status during study period
					Leukocytes	Platelets	
Recipients of HLA-matched leukocyte transfusions							
1	47, F	CAV (2); CR	5	*0*	*4.9*	140	Afebrile until day 13; perianal infection (*Escherichia coli*); CAV therapy delayed
2	67, M	CAV (4); CR	*4*	1	1.8	12	Became afebrile; no proven infection
3	*47*, M	CAV (6); F	5	0	8.4	*110*	Afebrile; no infection
4	53, F	CAV (6); CR	5	0	1.3	78	Afebrile; no infection
5	42, F	CAV (7); CR	3	2	1.8	160	Pneumonia (*Klebsiella*); improved during transfusion
6	58, M	ADR–ARA-C (1); CR	4	0	9.4	150	Afebrile; no infection
7	41, M	ADR–ARA-C (2); F	4	2	142.0	40	Afebrile; no infection
Patients not receiving HLA-matched leukocyte transfusions							
1	49, M	CAV (4); CR	—	4	1.8	36	Oropharyngeal infection and bronchopneumonia with right pleural effusion (*Klebsiella*)
2	29, F	CAV (7); CR	—	0	3.2	34	Afebrile and asymptomatic
3	*54*, F	CAV (4); CR	—	8	22.0	16	Pneumonia and right pleural effusion (*Pseudomonas aeruginosa*)
4	60, M	CAV (3); CR	—	0	1.8	160	No fever or infection
5	61, F	CAV (8); F	—	0	104.0	78	Pharyngitis (*Staphylococcus aureus*); responded in 48 hours to antibiotics
6	56, M	CAV (4); F	—	6	1.6	40	Bronchopneumonia; clinical and radiographic diagnosis; cultures negative
7	58, F	CAV (3); F	—	3	46.0	42	Multiple painful inflamed skin lesions; cultures negative
8	56, M	CAV (5); F	—	*2*	128.0	100	No clinical evidence of infection
9	50, M	CAV (2); F†	—	1	200.0	*60*	Afebrile to day 15; bronchopneumonia and septicemia (*E. coli*)
10	28, F	CAV (6); F	—	10	23.0	< 10	Febrile; bronchopneumonia; cultures negative
11	19, M	CAV (6); F	—	0	4.9	31	Afebrile; no infection
12	16, F	CAV (4); CR	—	6	4.8	240	Pneumonia (*Klebsiella*)
13	28, M	CAV (6); CR	—	4	*12.8*	< 10	Pleural effusion: tuberculosis?
14	60, F	ADR–ARA-C (1); CR	—	1	8.2	430	Pneumonia (*Klebsiella*)
15	47, M	ADR–ARA-C (1); CR	—	15	0.9	31	Bilateral bronchopneumonia (*S. aureus*)
16	16, F	ADR–ARA-C (1); CR	—	2	12.8	210	Dental abscess; cultures negative
17	23, M	ADR–ARA-C (2); F	—	1	98.6	77	Presented with dental sepsis; became afebrile
18	77, F	ADR–ARA-C (1); F	—	0	158.0	200	Cutaneous necrotic ulcers (*Pseudomonas*)
19	74, M	ADR–ARA-C (1); PR	—	4	14.9	150	Pneumonia and septicemia (*P. aeruginosa*)
20	51, F	ADR–ARA-C (2); F	—	6	2.4	40	Perianal abscess (*Bacteroides* and *Streptococcus faecalis*)

*Median values italicized. Abbreviations: HLA = histocompatibility antigen; CAV = cyclophosphamide, arabinosyl cytosine (ARA-C) and vincristine; CR = complete remission; F = failed; ADR = adriamycin; PR = partial remission.
†Early death; donor and recipient differed by one haplotype.

treated concurrently. They did not have an HLA-compatible sibling and did not receive leukocyte transfusions during this part of remission induction therapy unless evidence of life-threatening infection developed. ABO-compatible leukocyte transfusions were made available for these patients. Those in whom infection developed after the study period often received ABO-compatible leukocyte transfusions as described below.

Leukocyte transfusions for established infection

Adult patients with various malignant diseases who had (a) an infection or an unexplained fever not responsive to at least 48 hours of appropriate antibiotic therapy and (b) potentially reversible granulocytopenia with a granulocyte count of less than 0.5 x 10^9/L were candidates for a series of transfusions of leukocytes from ABO-compatible donors. Transfusions were given daily for as long as the clinical condition necessitated it. Data for patients who received fewer than two leukocyte transfusions were excluded from analysis. A total of 52 consecutive series of transfusions of ABO-matched leukocytes were given to 50 patients.

Definition of response to leukocyte transfusions

A response or improvement was defined as a persistent decrease of the mean daily temperature to normal, or by more than 2°C when associated with clinical improvement, within 24 hours and for more than 2 days. Transient clinical improvement alone was not considered a response to leukocyte transfusions.

Results

Characteristics of leukocyte collections

Data from 50 consecutive leukocyte collections from healthy donors are summarized in Table II.

Leukocytes from 20 consecutive procedures were examined by the trypan-blue dye exclusion test; 98 to 100% of the cells were found to exclude the dye. CFU-C formation, tested on 136 occasions, was generally greater than that observed for unseparated peripheral blood leukocytes, indicating no impairment of proliferative capacity (Fig. 2a). Intact metabolic function was indicated by the CSA production by irradiated leukocytes obtained from the leukapheresis cell collection (Fig. 2b).

Table II—Data from 50 consecutive leukocyte collections from healthy donors

Datum	Mean ± standard deviation
Volume of blood processed (L)	8.1 ± 1.1
No. of cells collected per procedure (x 10^9)	
Leukocytes	3.0 ± 2.5
Lymphocytes	2.4 ± 1.8
Granulocytes	0.7 ± 0.8
Monocytes	0.08 ± 0.1
Volume of blood loss (mL)*	35.2 ± 19.2
Volume of cell collection (mL)	93.2 ± 35.7

*Hematocrit of cell collection (%) x volume of cell collection (mL) ÷ hematocrit of donor (%).

Prospective study of prophylactic leukocyte transfusions (Tables I and III)

The two groups of patients were comparable with respect to the prognostic factors of age and initial leukocyte and platelet counts. The mean absolute granulocyte and platelet counts at the beginning of the study period and the nadirs of these counts were similar for the two groups. Remission occurred in 5 of the 7 patients who received leukocyte transfusions during remission induction therapy, and in 10 of the 20 patients who did not receive prophylactic leukocyte transfusions. The number of episodes of infection (those clinically compatible with infection but not bacteriologically proven) was significantly less ($P = 0.043$) in the patients receiving prophylactic leukocyte transfusions. There was no significant difference ($P > 0.10$) in the number of bacteriologically proven infections in the two groups. The number of days of elevated temperature (mean daily temperature greater than 38°C) was significantly less ($0.05 > P > 0.025$) in the patients who received transfusions than in the control group.

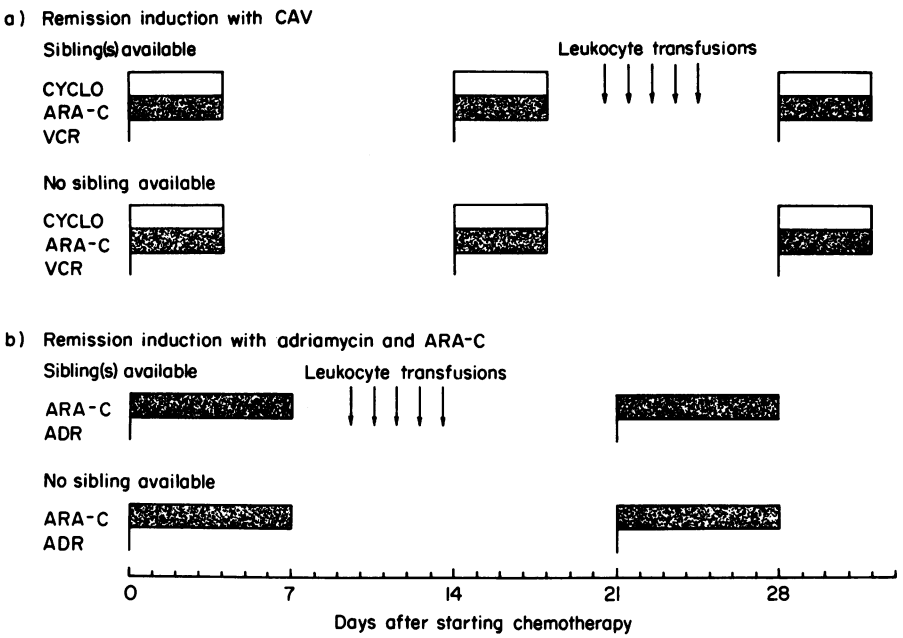

FIG. 1—Treatment plan for patients in prospective trial of leukocyte transfusions from histocompatibility antigen (HLA)-matched sibling donors. CAV = cyclophosphamide (CYCLO), arabinosyl cytosine (ARA-C) and vincristine (VCR); ADR = adriamycin.

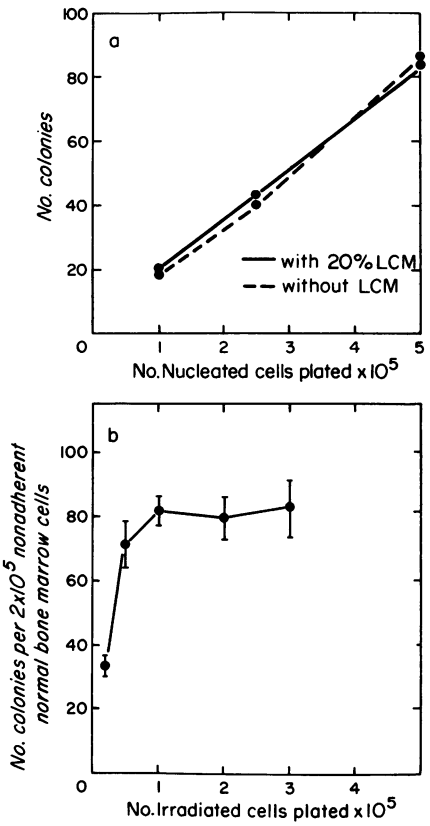

FIG. 2—Function of leukocytes collected by leukapheresis: a, production of colony-forming units by leukocytes cultured for 14 days in methylcellulose with or without leukocyte-conditioned medium (LCM); b, production of colony-stimulating activity by irradiated cells from buffy-coat collection incubated for 14 days in methylcellulose with 2 x 10^5 nonadherent normal bone marrow cells and 20% LCM (138 colonies).

Table III—Clinical results of propective study of prophylactic leukocyte transfusions

Datum	Group: Transfusion recipients (n = 7)	Group: Controls (no transfusions) (n = 20)	Significance of difference*
Age (yr)			
Median	47	54	NS
Range	41 — 67	16 — 77	
Initial leukocyte count (x 10^9/L)			
Median	4.9	12.8	NS
Range	1.3 — 142.0	0.9 — 200.0	
No. of remissions, complete and partial	5	10	NS
Total no. of episodes of infection	2	15	P = 0.043†
No. of bacteriologically proven infections	1	10	P > 0.10†
No. of days of temperature >38°C			
Median	0	2	0.05 > P > 0.025‡
Range	0 — 2	0 — 15	

*NS = not significant.
†By Fisher's exact test.
‡By rank-sum test (one-sided).

Leukocyte transfusions for established infection

After 23 of the 52 series of ABO-matched leukocyte transfusions given because of infection the patient's condition improved (Table IV). Infection was proven microbiologically in 12 of the 23 episodes, as well as in 17 of the 29 episodes not associated with response to leukocyte transfusion. Of the 36 patients with AML 19 were given leukocyte transfusions during the period of initial remission induction therapy; complete remission[21] occurred in 8 of the 14 who responded to the transfusions but in only 1 of the 5 who did not respond.

The number of leukocyte transfusions, the frequency of transfusions and the numbers of leukocytes, lymphocytes and granulocytes were not related to response to the leukocyte transfusions.

An increase in the absolute granulocyte count in a leukocyte transfusion recipient of at least 0.4 x 10^9/L for 3 days or more that tended to progress was considered significant. Such an increase was observed in 15 of the 23 series of transfusions associated with clinical improvement ($P > 0.005$) (Table V). In eight patients improvement occurred without a sustained increase in absolute granulocyte count.

Discussion

Our study has demonstrated the effectiveness of HLA-matched leukocyte transfusions in the prevention and control of severe infections in granulocytopenic patients with AML. Significant reductions in the number of infectious episodes and days with elevated temperature were found in the group receiving transfusions.

HLA-matched leukocytes were chosen for transfusion because these types of cells have the highest percent recovery in the recipient and therefore may be assumed to be the most effective type for transfusion.[22] The study design enabled us to compare the clinical course of patients who received leukocyte transfusions with that of similarly treated patients not receiving transfusions.[23] Leukocyte transfusions were not withheld from any patients whom it was thought they might benefit.

Higby and colleagues[13] randomly assigned patients with clinically evident infection and granulocyte counts of less than 0.5 x 10^9/L to receive antibiotics alone or antibiotics plus leukocyte transfusions for 4 days. Of the 17 patients in the group receiving transfusions 15 became afebrile and survived for 20 days. Of the 19 patients in the control group only 5 survived for 20 days from the onset of the study period. However, preliminary results of two other randomized prospective trials have indicated the usefulness of leukocyte transfusions in the management of infection in neutropenic patients.[24,25]

The present study has also shown that a series of transfusions of ABO-compatible leukocytes was often beneficial in neutropenic patients with severe infections. The role of these transfusions is difficult to assess because of variabilities of treatment schedules, stage of disease and degree of myelosuppression. In our study we controlled these variables: patients were studied only during the period of initial remission induction therapy and they received similar chemotherapy.

The mechanism by which leukocyte transfusions may be effective in controlling infection in granulocytopenic patients is not clear. We obtained good results with relatively small numbers of cells, predominantly lymphocytes.[11-13,20] This suggests that the number of granulocytes transfused may not be important. Indeed, the clinical condition of eight patients improved in the absence of a sustained increase in the absolute granulocyte count. The buffy-coat collections obtained by centrifugation leukapheresis are rich in cells capable of CFU-C and CSA production. Thus, the important cells for transfusion may be granulocyte progenitor cells or cells capable of producing factors essential for the maturation of granulocyte precursors.[26]

We thank Dr. J.E. Till for the statistical analyses; Mrs. Judy Falk, tissue typing

Table IV—Clinical response to leukocyte transfusions for established infection

Diagnosis	Clinical response to transfusions; no. of episodes of infection: Improvement	No improvement
Acute myelogenous leukemia	18 (14*)	18 (5*)
Acute lymphoblastic leukemia	2	7
"Hairy-cell" leukemia	0	1
Chronic myelogenous leukemia, blast crisis	1	2
Malignant lymphoma	1	0
Solid tumour	1	1
Total	23	29

*The patients received leukocyte transfusions during the period of initial remission induction therapy.

Table V—Clinical response to leukocyte transfusions and increase in absolute granulocyte count

Clinical response	Significant increase* in absolute granulocyte count: Present	Absent
Improvement, n = 23	15	8
No improvement, n = 29	1	28

*An increase of at least 0.4 x 10^9/L for 3 days or more that tended to progress; $P < 0.005$ (by Fisher's exact test for association of granulocyte increase and improvement).

laboratory, Toronto Western Hospital, for the tissue typing; and Dr. G.M. Kouroupis, Wellesley Hospital, Toronto, for the tests for hepatitis A antigen.

This work was supported by a demonstration model grant, Ontario Ministry of Health, and by grants from the Ontario Cancer Treatment and Research Foundation, Toronto.

References

1. HERSH EM, BODEY GP, NIES BA, et al: Causes of death in acute leukemia. *JAMA* 193: 99, 1965
2. CHANG HY, RODRIGUEZ V, NARBONI G, et al: Causes of death in adults with acute leukemia. *Medicine (Baltimore)* 55: 259, 1976
3. BODEY GP, BUCKLEY M, SATHE YS, et al: Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. *Ann Intern Med* 64: 328, 1966
4. ABU-ZAHRA H, CLARYSSE A, COWAN DH, et al: Treatment of acute myeloblastic leukemia in adults: remission induction with a combination of cyclophosphamide, cytarabine and vincristine. *Can Med Assoc J* 107: 1073, 1972
5. SHOHET SB: Morphologic evidence for the in vivo activity of transfused chronic myelogenous leukemia cells in a case of massive staphylococcal septicemia. *Blood* 32: 111, 1968
6. BODEY GP, MIDDLEMAN E, UMSAWADI T, et al: Infections in cancer patients. Results with gentamicin sulphate therapy. *Cancer* 29: 1697, 1972
7. MCCREDIE KB, FREIREICH EJ, HESTER JP, et al: Leukocyte transfusion therapy for patients with host-defense failure. *Transplant Proc* 5: 1285, 1973
8. BOGGS DR: Transfusion of neutrophils as prevention of treatment of infection in patients with neutropenia. *N Engl J Med* 290: 1055, 1974
9. HIGBY DJ, HENDERSON ES: Granulocyte transfusion therapy. *Annu Rev Med* 26: 289, 1975
10. GRAW RG JR, HERZIG G, PERCY S, et al: Normal granulocyte transfusion therapy: treatment of septicemia due to gram-negative bacteria. *N Engl J Med* 287: 367, 1972
11. LOWENTHAL RM, GROSSMAN L, GOLDMAN JM, et al: Granulocyte transfusions in treatment of infections in patients with acute leukaemia and aplastic anaemia. *Lancet* 1: 353, 1975
12. SCHIFFER CA, BUCHHOLZ DH, AISNER J, et al: Clinical experience with transfusions of granulocytes obtained by continuous flow filtration leukapheresis. *Am J Med* 58: 373, 1975
13. HIGBY DJ, YATES JW, HENDERSON ES, et al: Filtration leukapheresis for granulocyte transfusion therapy. *N Engl J Med* 292: 761, 1975
14. FORTUNY IE, BLOOMFIELD CD, HADLOCK DC, et al: Granulocyte transfusion: a controlled study in patients with acute nonlymphocytic leukemia. *Transfusion* 15: 548, 1975
15. FREIREICH EJ, JUDSON G, LEVIN RH: Separation and collection of leukocytes. *Cancer Res* 25: 1516, 1965
16. KISSMEYER-NELSON F, THORSBY E: Human transplantation antigens. *Transplant Rev* 4: 11, 1970
17. PHILLIPS HJ: Dye exclusion tests for cell viability, in *Tissue Culture Methods and Applications*, KRUSE PR JR, PATTERSON MK JR, New York, Acad Pr, 1973, pp 406-8
18. RUBIN SH, COWAN DH: Assay of granulocytic progenitor cells in human peripheral blood. *Exp Hematol* 1: 127, 1973
19. MESSNER HA, TILL JE, MCCULLOCH EA: Interacting cell populations affecting granulopoietic colony formation by normal and leukemic human marrow cells. *Blood* 42: 701, 1973
20. MCCREDIE KB, BODEY GP, GUTTERMAN JV, et al: Sequential adriamycin-Ara-C (A-OAP) for remission induction (RI) of adult acute leukemia. *Proc Am Assoc Cancer Res* 15: 62, 1974
21. HASSELBACK R, CURTIS J, SOOTS M, et al: The influence of morphology on prognosis in acute leukemia. *Can Med Assoc J* 96: 1610, 1967
22. MCGRAW RG JR, GOLDSTEIN IM, EYRE HJ, et al: Histocompatibility testing for leucocyte transfusion. *Lancet* 2: 77, 1970
23. GEHAN EA, FREIREICH EJ: Non-randomized controls in cancer clinical trials. *N Engl J Med* 290: 198, 1974
24. VOGLER WR, WINTON EF: The efficacy of granulocyte transfusions (GT) in neutropenic patients (abstr). *Proc Am Soc Clin Oncol* 17: 252, 1976
25. HERZIG R, HERZIG G, BULL M, et al: Efficacy of granulocyte transfusion therapy for gram-negative sepsis: a prospectively randomized controlled study (abstr). Ibid, p 253
26. MCCULLOCH EA, MARK TW, PRICE GB, et al: Organization and communication in populations of normal and leukemic hemopoietic cells. *Biochim Biophys Acta* 355: 260, 1974