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ABNORMAL NEUTROPHIL CHEMOTAXIS IN BONE MARROW TRANSPLANT PATIENTS CORRELATES WITH IMPAIRED 31D8 MONOCLONAL ANTIBODY BINDING

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ABSTRACT.

Background. **31D8 monoclonal antibody (mAb) has been shown to bind heterogeneously to human neutrophils, identifying subsets of cells which differ in their functional response to chemotactic stimuli. In this study we used 31D8 mAb to determine whether differences in neutrophil subpopulations might explain the long-lasting decreased chemotaxis observed in bone marrow transplant recipients.**

Methods. **Thirty patients with self-sustaining hematopoiesis 1 to 5 years after bone marrow transplantation (BMT) (15 allogeneic and 15 autologous) performed for acute lymphocytic leukemia (ALL, 10 patients) or acute myelogenous leukemia in complete remission (8 patients), Hodgkin's lymphoma (2 patients), chronic myeloid leukemia (8 patients) and severe aplastic anemia (2 patients) were included in the study. Neutrophil chemotaxis was evaluated using a modified Boyden chamber assay and 31D8 binding was determined by indirect immunofluorescence and cytofluorimetric analysis.**

Results. **Neutrophil chemotaxis was significantly impaired in the BMT group with respect to controls. The chemotactic defect strikingly correlated with autologous BMT and, in particular, with ALL as the pre-existing disease. No differences between patients and controls were observed in the percentage of 31D8 *bright* and *dull* neutrophils. However, when mean fluorescence intensity (MFI) was analyzed as a relative measure of 31D8 antigen expression on the overall neutrophil population, a significant decrease was observed in neutrophils from BMT patients with respect to controls. As for chemotaxis, the impairment of 31D8 binding was more evident in autologous BMT and strikingly correlated with ALL as the pre-existing disease regardless of age, sex and time since BMT. Moreover, a significant positive correlation between impaired chemotaxis and decreased 31D8 binding was found in our patients.**

Conclusions. **These findings suggest that the decreased neutrophil chemotaxis observed in some BMT patients may be due in part to circulating 31D8 *dull* neutrophils, although the causes for the decreased 31D8 binding and for the quite pronounced neutrophil defect in ALL patients remain unknown.**

Key words: neutrophils, bone marrow transplantation, 31D8 mAb, chemotaxis

Systemic bacterial and fungal infections, together with graft vs host disease (GVHD), have been increasingly recognized as significant causes of morbidity and mortality in bone marrow transplant (BMT) patients.[1-3] While neutropenia and immunosuppressive therapy may be responsible for the high incidence of infectious complications in the early postgrafting period, a neutrophil functional defect has been suggested as a possi-

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ble component-cause of immunodeficiency in successful BMT recipients.[3-5] A pioneer study by Clark et al.[4] described abnormal chemotaxis in allogeneic BMT patients that was associated with an increased risk of infections; moreover, both GVHD and treatment with anti-thymocyte globulin (ATG) were implicated in the neutrophil defect. Subsequent studies confirmed the presence of abnormal neutrophil function in patients receiving both allogeneic and autologous BMT,[5-10] but the cause of the neutrophil defect and its clinical significance remained uncertain. We have recently shown long-term abnormal neutrophil chemotaxis in patients receiving allogeneic and, above all, autologous BMT;[11] none of these patients had been treated with ATG and no signs of GVHD were present at the time of the study, suggesting that other mechanisms were implicated in the abnormality.

The functional heterogeneity of neutrophils has been recognized for many years,[12] and this was recently offered as a possible explanation for certain acquired chemotactic defects.[13] Over the last few years a number of monoclonal antibodies (mAb) that bind to neutrophils in a heterogeneous fashion have been described.[13] Among these is a mouse IgG1 mAb (31D8) which heterogeneously binds to mature neutrophils, identifying subsets of cells which differ in their functional response to chemotactic stimuli: a major subpopulation of 31D8 *bright* cells (approximately 80% of normal neutrophils) which respond optimally to chemoattractants, and a minor subpopulation of *dull* cells which respond poorly to chemoattractants.[14]

To verify the hypothesis that an alteration in neutrophil subsets may justify decreased chemotaxis in BMT patients, the present study examined neutrophil 31D8 binding and chemotaxis in patients who had successfully undergone autologous or allogeneic BMT.

Table 1. Patient population.

Patient	*Age/Sex*	*Diagnosis**	*Months from BMT (type of BMT)*	*WBCx10⁹/L (% neutrophils)*
1	43/F	ALL	27 (auto)	6.8 (60)
2	41/F	CML	44 (allo)	3.9 (48)
3	36/F	CML	69 (allo)	7.6 (33)
4	32/M	SAA	23 (allo)	8.7 (48)
5	41/M	AML	51 (auto)	7.6 (44)
6	44/F	ALL	12 (auto)	3.5 (56)
7	18/F	ALL	61 (auto)	6.8 (52)
8	26/F	CML	54 (allo)	7.8 (41)
9	35/M	SAA	14 (allo)	3.8 (57)
10	19/F	ALL	12 (auto)	4.1 (58)
11	35/F	ALL	72 (auto)	7.4 (58)
12	22/M	AML	12 (auto)	5.4 (70)
13	35/F	AML	25 (allo)	3.8 (47)
14	41/M	HL	12 (auto)	4.5 (60)
15	48/M	AML	46 (auto)	6.7 (39)
16	24/F	ALL	46 (allo)	2.8 (65)
17	17/F	ALL	12 (allo)	5.5 (68)
18	46/F	HL	12 (auto)	6.6 (47)
19	55/F	AML	60 (auto)	7.9 (56)
20	33/M	AML	51 (auto)	7.3 (61)
21	30/F	CML	57 (allo)	11.9 (53)
22	24/F	CML	65 (allo)	4.2 (64)
23	53/M	AML	23 (allo)	7.6 (55)
24	32/M	AML	32 (auto)	7.3 (50)
25	30/F	CML	46 (allo)	8.0 (41)
26	35/M	ALL	50 (allo)	6.0 (52)
27	41/F	CML	89 (allo)	5.2 (49)
28	36/F	CML	69 (allo)	10.0 (66)
29	44/F	ALL	12 (auto)	8.8 (49)
30	18/F	ALL	61 (auto)	7.3 (55)

*ALL, acute lymphocytic leukemia; CML, chronic myelogenous leukemia; SAA, severe aplastic anemia; AML, acute myelogenous leukemia; HL, Hodgkin's lymphoma.

Materials and Methods

Patients and patient management

Thirty patients with self-sustaining hematopoiesis 1 to 5 years after BMT were selected for this study (Table 1): 10 males and 20 females whose ages ranged from 17 to 55 years (mean 34). Eight patients with chronic myelogenous leukemia (CML), 3 with acute lymphocytic leukemia (ALL), 2 with acute myelogenous leukemia (AML) and 2 with severe aplastic anemia (SAA) received allogeneic non T-depleted BMT; seven patients with ALL, 6 with AML and 2 with Hodgkin's lymphoma (HL) received unpurged autologous BMT during remission.

The conditioning regimen for the leukemias included ARA-C (3 g/sqm/12 h×4) and/or etoposide (30 mg/kg/day ×2), cyclophosphamide (60 mg/kg/day ×2) and total body irradiation (TBI) (3.33 Gy/day ×3; dose rate 5.55

cGy/min). In CML patients, additional spleen irradiation (10 Gy) was performed. Lymphoma patients received BCNU (200 mg/sqm/day ×2) or etoposide (30 mg/kg/day ×2) plus TBI or CBV (cyclophosphamide, 1.5 g/sqm/day ×4; BCNU, 200 mg/sqm/day ×4; etoposide, 250 mg/sqm/day×4). The patients with SAA were conditioned with cyclophosphamide 50 mg/kg/day×3 and thoraco-abdominal irradiation (6 Gy). Cyclosporin A (CyA) was given in combination with methotrexate according to the Seattle schedule to prevent GVHD.[15] None of the patients was receiving drug therapy and no signs of GVHD or infections were present at the time of the investigation. Thirty healthy volunteers, matched for sex and age, were selected as controls.

Cell separation procedure

Peripheral blood neutrophils were purified by centrifugation of peripheral blood on a Lymphoprep density gradient (Nyegaard, Oslo, Norway) followed by dextran sedimentation and lysis of erythrocytes.[16] Samples of cell suspensions in RPMI 1640 (Flow Laboratories) were spun onto slides with a cytocentrifuge and stained with May-Grünwald-Giemsa for evaluation of gross morphology and the level of neutrophil maturity by light microscopy. In all cases nuclear morphology demonstrated that more than 90-95% of cells were mature neutrophils; band form neutrophils were the only immature cells occasionally observed in some BMT patients (5%). The viability of purified neutrophils was assessed by means of trypan blue exclusion; more than 95% of the cells were viable.

Chemotaxis

Neutrophil chemotaxis was evaluated using a modified Boyden chamber assay,[17] with blind-well chambers and 3 μm micropore filters (Millipore); 200 μL of the cell suspension, containing 2.5×10^6 PMN/mL in RPMI 1640+0.4% bovine serum albumin (BSA; Sigma) were layered on top of the filter, and the lower compartment was filled with 200 μL of the chemotactic factor (see below). Following incubation at 37°C for 90 min in a humidified atmosphere with 5% CO_2, the filters were fixed with ethanol and stained with hematoxylin-eosin. The chemotactic response was then determined by evaluating the number of cells per *high power field* (hpf) that had migrated through the entire thickness of the filter; triplicate chambers were used in each experiment and 5 fields were examined in each filter. In all cases the person scoring the assay had no knowledge of the experimental groupings.

The chemoattractants utilized were zymosan-activated serum (1 mg/mL for 30 min at 37°C; ZAS) at a 10% (vol/vol) final dilution in RPMI 1640, and the synthetic peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP; Sigma) at a 10^{-8}M final concentration.

31D8 labeling and analysis by flow cytometry

The labeling of neutrophils with 31D8 mAb was performed as previously described.[14] Briefly, the 5×10^5 neutrophils contained in each reaction tube were mixed with 25 μL of a 1/40 dilution of 31D8 mAb stock (ICN Biomedicals, Irvine, Scotland). Following 30-min incubation at 4°C, the samples were washed twice in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). The cells were then washed before adding 100 μL of FITC-conjugated $F(ab')_2$ goat anti-mouse IgG. After incubation for 30 min at 4°C, the fluorescein-labeled cells were washed twice and fixed in buffer containing 1% paraformaldehyde (pH 7.2). The samples were kept in the dark at 4°C until they were analyzed with a flow cytometer. Flow cytometric analysis was performed using an Ortho Cytoron flow cytometer (Ortho Diagnostic System, Westwood, MA, USA). The forward and right angle light scattering properties of the cells were used to identify neutrophils from contaminating mononuclear cells. The mean fluorescence intensity (MFI) of 10,000 neutrophils was determined and displayed on a one-parameter histogram consisting of 256 channels. A relative measure of 31D8 antigen (Ag) expression was obtained by establishing the MFI[18] converted from log to linear scale after subtraction of the background control fluorescence of cells incubated without specific mAb or with mouse IgG1 isotype control mAb.

The percentage of *bright* and *dull* cells was quantitated according to Seligmann et al.,[14]

assuming that the 31D8 *bright* population generated a symmetric curve and that all other cells were part of the *dull* population. With the flow cytometer the area of the entire curve was determined, and the area of the symmetric curve about the major peak (*bright* curve) was subtracted from this valve to determine the size of the *dull* area. This approach made it possible to quantitate changes in the relative numbers of 31D8-stained cells regardless of any differences in labeling intensity.

Statistical analysis

Standard error was used throughout as an estimate of variance and means were compared with Student's t-test. Correlations between chemotaxis and 31D8 Ag expression were determined using Pearson's correlation coefficient. P values < 0.05 were accepted as significant.

Results

31D8 binding to neutrophils

The 31D8 mAb bound to the neutrophils of the controls and BMT patients in a heterogeneous manner, with the majority of cells strongly binding 31D8 (*bright* cells). However, no significant difference in the percentage of *bright* neutrophils was observed between BMT patients (87.8±5%) and controls (89.9±3.9%). The approach used to define *bright* and *dull* cells quantitated changes in the relative numbers of 31D8-stained cells regardless of their labeling intensity. When MFI was analyzed as a relative measure of 31D8 Ag expression on the overall neutrophil population, a significant decrease was observed in the neutrophils from BMT patients with respect to those from controls, but when autologous and allogeneic BMT patients were considered separately, the decrease was significant only in the patients receiving autologous BMT (Table 2). The possible correlations between 31D8 binding and pre-existing disease in BMT patients are shown in Table 2. Although the unbalanced distribution of pre-existing disease is a limitation of this analysis, it is clear from the table that decreased 31D8 Ag expression correlated strikingly with ALL. Since the majority of ALL patients (7/10) underwent autologous BMT, it was of interest to define whether pre-existing ALL, autologous BMT, or both, correlated with the neutrophil phenotypic abnormality. MFI values for 31D8 obtained in ALL patients receiving autologous BMT (38.9±2.2; n=7) were significantly lower than in controls (56.4±2.4; $p<0.001$) and in all non-ALL patients receiving autologous BMT (53.5±4.4; n=8, $p<0.02$). Although it is true that 9/10 of our ALL patients were females and a sex-related difference in 31D8 Ag expression has been previously reported,[14] the MFI values of neutrophils

Table 2. 31D8 mAb binding and chemotaxis of neutrophils from BMT patients and controls.

Subjects (n)	*31D8 (MFI)*		*Chemotaxis (cells/hpf)*			
			ZAS		*FMLP*	
		p[d]		p[d]		p[d]
Controls (30)	56.4 ± 2.4[a]		39.1 ± 2.5[b]		41.9 ± 2.8[b]	
BMT patients (30)	48.0 ± 2.0	<0.01	28.1 ± 3.2	<0.01	29.5 ± 2.5	<0.005
autologous BMT (15)	46.6 ± 3.1	<0.025	25.7 ± 4.9	<0.02	28.0 ± 3.3	<0.005
allogeneic BMT (15)	49.4 ± 2.7	NS	30.6 ± 4.2	NS	30.9 ± 3.9	<0.05
ALL (10)[c]	40.1 ± 2.4	<0.001	17.9 ± 5.0	<0.001	22.2 ± 3.0	<0.001
CML (8)	49.6 ± 3.6	NS	29.9 ± 5.7	NS	26.8 ± 3.2	<0.005
AML (8)	54.8 ± 4.7	NS	36.0 ± 6.9	NS	33.4 ± 5.1	NS
SAA (2)	45.9 ± 2.9	NS	25.5 ± 8.8	NS	58.1 ± 13.3	NS
HL (2)	56.5 ± 0.9	NS	43.1 ± 6.6	NS	32.2 ± 1.2	NS

[a]Mean ± s.e.m. for mean fluorescence intensity (MFI) corrected for non-specific staining. [b]Mean ± s.e.m. for number of migrated cells (x hpf) toward zymosan-activated serum (ZAS) or FMLP. [c]Pre-existing diseases in BMT patients: ALL, acute lymphocytic leukemia; CML, chronic myelocytic leukemia; AML, acute myelocytic leukemia; SAA, severe aplastic anemia; HL, Hodgkin's lymphoma. [d]Student's t-test for statistical analysis (versus controls).

from ALL females (38.9±2.4) were significantly lower than those observed in controls ($p<0.001$) and in all of the other females in our BMT group (49.9±3.7; n=11, $p<0.02$). Finally, no correlation was observed between 31D8 Ag expression and patient age or the time interval after marrow infusion (data not shown).

Neutrophil chemotaxis and correlations with 31D8 mAb binding

Neutrophil chemotactic responsiveness was significantly impaired in the BMT group as a whole with respect to controls (Table 2). It is worth noting that, like 31D8 Ag expression, the chemotactic defect strikingly correlated with autologous BMT and, in particular, with ALL as the pre-existing disease (Table 2), thus suggesting a possible relationship between the chemotactic behavior and 31D8 Ag expression. Using Pearson's correlation coefficient, a significant positive correlation was observed between chemotaxis and 31D8 Ag level on neutrophils from BMT patients (Figure 1a and 1b).

Four patients, 2 with impaired and 2 with normal chemotaxis and 31D8 Ag expression, were tested twice in each of the assays; their functional and phenotypic behavior was maintained over time, suggesting stable individual differences in both parameters.

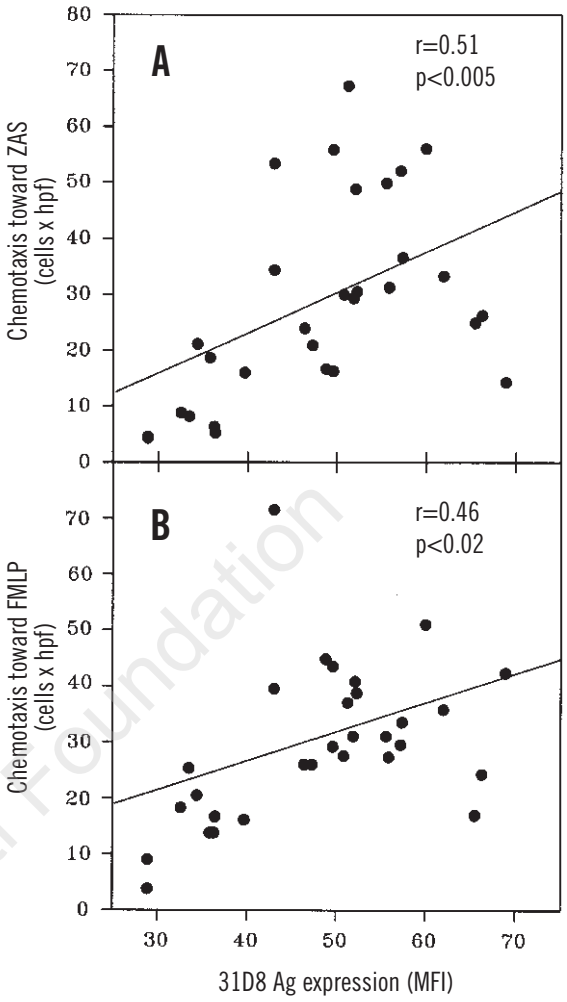

Figure 1. Analysis of correlations between 31D8 Ag expression and chemotaxis toward ZAS (A) of FMLP (B) in neutrophils from BMT patients.

Discussion

In the present study, we confirm defective neutrophil chemotaxis in some patients 1 to 5 years after successful BMT. The functional defect was more evident in the group receiving autologous BMT and strikingly correlated with ALL as the pre-existing disease; in fact, among those receiving autologous BMT, it was found only in ALL patients.

Since the causes previously suggested for the neutrophil defect in BMT patients (i.e. GVHD and ATG)[4,5] were not implicated in our subjects, we evaluated 31D8 mAb reactivity in an attempt to define whether neutrophil heterogeneity may be a possible explanation for the functional abnormality. This mAb binds to neutrophils in a heterogeneous manner, identifying subsets of cells with different functional responses to chemotactic stimuli.[14] Previous studies of 31D8 labelling patterns suggested a correlation between the increased number of circulating 31D8 *dull* neutrophils and the decreased chemotaxis observed in neonates[19] and trauma patients.[20] When the analysis was performed in our BMT patients, no differences between patients and controls were observed in the percentage of *bright* and *dull* neutrophils. However, the method used to define 31D8 *bright* and *dull* neutrophils does not consider the relative level of 31D8 Ag expression on labeled cells (MFI). When this parameter was evaluated, a significant decrease in MFI was observed in the neutrophils from BMT patients. As for chemotaxis,

the impairment of 31D8 Ag expression was more evident in autologous BMT patients and strikingly correlated with ALL as the pre-existing disease regardless of age, sex and time since BMT. Moreover, a significant positive correlation between impaired chemotaxis and decreased 31D8 Ag levels was found in our patients, and this agrees with previous reports showing that 31D8 *dull* cells are less responsive to chemoattractants.

The cause for decreased 31D8 Ag expression in the neutrophils from some BMT patients is not clear, and the same is true for the quite pronounced neutrophil defect in ALL patients receiving autologous BMT. An increase in the number of eosinophils (which weakly bind 31D8) or differences in cell size can be excluded as causes of the 31D8 dull labeling pattern. The previously observed correlation between morphologic immaturity and 31D8 dull labeling[19,20] also seems unlikely as a possible explanation for our results. In fact: first, in our BMT patients only few band form neutrophils were observed by light microscopy; second, 31D8 Ag expression is not a late maturational event but appears at the myelocyte stage in the bone marrow;[14] third, previous data by Gallin et al.[21] suggested that the 31D8 labeling pattern may reflect true neutrophil subpopulations that originate from distinct stem cell pools. Furthermore, even though we did not examine the activation state of neutrophils in this work, it has already been demonstrated that 31D8 mAb binding is not affected by cell activation.[14,19,22] Moreover, previous results[11] obtained in a different cohort of BMT patients did not suggest differences in neutrophil activation between patients and controls, at least in terms of chemiluminescence production and adhesion molecule expression.

The most relevant impairment of neutrophil chemotaxis and 31D8 Ag expression was observed in ALL patients receiving autologous BMT and suggests that some specific drug used in the induction, consolidation and/or maintenance phases, rather than BMT itself, may contribute to the neutrophil defects. Of interest, long-term damage of myeloid progenitor cells as a result of chemotherapy-induced long-term damage to the stem cells and/or the regulatory microenvironment has been described in children treated for ALL.[23]

While if greater knowledge about the function and structure of the molecule defined by the 31D8 mAb is required to define the significance of our results, the correlation that we observed between impaired chemotaxis and decreased 31D8 Ag levels suggests another possible explanation for the long-lasting functional defect in some BMT patients.

References

1. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. Transplantation 1974; 18:295-304.
2. Clif RA, Buckner CD, Fefer A, et al. Infectious complications of marrow transplantations. Transplant Proc 1974; 6:389-93.
3. Winston DJ, Gale RP, Meyer DV, Young LS. Infectious complications of human bone marrow transplantation. Medicine 1979; 58:1-32.
4. Clark RA, Johnson FL, Klebanoff SJ, Thomas ED. Defective neutrophil chemotaxis in bone marrow transplant patients. J Clin Invest 1976; 58:22-31.
5. Zimmerli W, Zarth A, Gratwohl A, Speck B. Neutrophil function and pyogenic infections in bone marrow transplant recipients. Blood 1991; 77:393-9.
6. Territo MC, Gale RP, Cline MI. The UCLA Bone Marrow Transplantation Team: neutrophil function in bone marrow transplant recipients. Br J Haematol 1977; 35:245-50.
7. Sosa R, Weiden PL, Storb R, Syrotuck J, Thomas ED. Granulocyte function in human allogeneic marrow graft recipients. Exp Hematol 1990; 8:1183-9.
8. Gadish M, Kletter Y, Flidel O, Nagler A, Slavin S, Fabian I. Effects of recombinant human granulocyte and granulocyte-macrophage colony-stimulating factors on neutrophil function following autologous bone marrow transplantation. Leuk Res 1991; 15:1175-82.
9. Van den Broek PJ, van der Meer JWM, Leijh PCJ, Zwaan F, van den Barselaar M, van Furth R. Functions of granulocytes after allogeneic bone marrow transplantation. Blut 1981; 42:253-7.
10. Locatelli F, Giorgiani G, Pession A, Bozzola M. Late effects in children after bone marrow transplantation: a review. Haematologica 1993; 78:319-28.
11. Capsoni F, Minonzio F, Ongari AM, et al. Abnormal neutrophil chemotaxis after successful bone marrow transplantation. Leuk Lymphoma 1991; 4:335-41.
12. Gallin JI. Human neutrophil heterogeneity exists, but is it meaningful? Blood 1984; 63:977-83.
13. Gallin JI. Disorders of phagocytic cells. In: Gallin JI, Goldstein IM, Snyderman R, eds. Inflammation: basic principles and clinical correlates. New York: Raven Press, 1992:859-74.
14. Seligmann B, Malech HL, Melnick DA, Gallin J. An antibody binding to human neutrophils demonstrates antigenic heterogeneity detected early in myeloid maturation which correlates with functional heterogeneity of mature neutrophils. J Immunol 1985; 135:2647-53.

15. Storb R, Deeg HJ, Whitehead J, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation. N Engl J Med 1986; 14:729-35.
16. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest 1968; 21(suppl. 97):77-89.
17. Boyden S. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. J Exp Med 1962; 115:453-66.
18. Poncelet P, Carayon P. Cytofluorometric quantification of cell-surface antigens by indirect immunofluorescence using monoclonal antibodies. J Immunol Methods 1985; 85:65-74.
19. Krause PJ, Malech HL, Kristie J, et al. Polymorphonuclear leukocyte heterogeneity in neonates and adults. Blood 1986; 68:200-4.
20. Krause PJ, Maderazo EG, Bannon P, Kosciol K, Malech HL. Neutrophil heterogeneity in patients with blunt trauma. J Lab Clin Med 1988; 112:208-12.
21. Gallin JI, Jacobson RJ, Seligman BE, et al. A neutrophil membrane marker reveals two groups of chronic myelogenous leukemia and its absence may be a marker of disease progression. Blood 1986; 68:343-6.
22. Brown CC, Malech HL, Gallin JI. Intravenous endotoxin recruits a distinct subset of human neutrophils, defined by monoclonal antibody 31D8, from bone marrow to the peripheral circulation. Cell Immunol 1989; 123:294-306.
23. Bhavnani M, Morris Jones PH, Testa NG. Children in long-term remission after treatment for acute lymphoblastic leukaemia show persisting haemopoietic injury in clonal and long-term cultures. Br J Haematol 1989; 71:37-41.