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LUMINOL-ENHANCED CHEMILUMINESCENCE OF PERIPHERAL BLOOD LEUKOCYTES AS AN EARLY INDICATOR OF GRAFT TAKE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOGENOUS LEUKAEMIA

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The luminol-enhanced chemiluminescence (CL) of peripheral blood leukocytes was studied daily in five patients with acute myelogenous leukaemia (AML) in first remission, who were undergoing allogeneic bone marrow transplantation (BMT). The CL was measured after stimulation of leukocytes with opsonized zymosan in highly diluted whole blood. All patients had an undetectable CL level on day +7, post BMT, simultaneously with severe pancytopenia caused by the pre-conditioning for BMT. Subsequently, CL started to rise, reaching the maximum level, twice that of healthy controls, on day +11. This preceded the rise of blood leukocytes above 1.0×10^9 l.$^{-1}$ and that of neutrophils above 0.5×10^9 l.$^{-1}$ by 3–14 days, but coincided with the appearance of large unstained cells (LUC; a parameter given by a Technicon H 6000 blood analyzer). One of the patients later had a transient decline of CL. This preceded the fall in white blood count and platelets by 7 days, suggesting marrow suppression. We conclude that in AML the measurement of leukocyte CL is a more sensitive test for prediction of graft take than the conventional blood counts.

Key words: **Chemiluminescence, Leukocytes, Bone marrow transplantation, Acute myelogenous leukaemia.**

INTRODUCTION

After successful bone marrow transplantation (BMT) the eradicated recipient haematopoiesis is replaced by the donor haematopoietic stem cells. To confirm the graft take, reticulocyte, leukocyte and platelet counts are monitored on a daily basis. However, it usually takes 2–4 weeks before the number of leukocytes and neutrophils exceeds 1.0×10^9 l.$^{-1}$ and 0.5×10^9 l.$^{-1}$, respectively. Recently, Martin *et al.*[1] reported that the appearance of large unstained cells (LUC; a parameter given by a Technicon Hemalog D 90 blood analyzer) in the peripheral blood after BMT could be a useful indicator of a successful graft take. To predict the outcome of BMT we have studied the zymosan-induced activation of peripheral blood leukocytes and correlated the findings to the white blood counts and the appearance of LUCs. The activation of leukocytes was measured by luminol-enhanced chemiluminescence on the day of transplantation and at least 25 successive days in five patients with acute myelogenous leukaemia (AML).

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PATIENTS AND METHODS

Patients

The patient material consisted of five patients with acute myelogenous leukaemia (AML) in first remission. All patients were given bone marrow transplants from an HLA-identical and MLC-negative sibling donor. Before BMT all patients were conditioned with cyclophosphamide 50 mg kg^{-1} day^{-1} for 4 days, followed by total body irradiation 2.5 Gy day^{-1} for 4 days. For graft vs host prophylaxis the patients received cyclosporin-A (cyA).

Reagents

Stock solution of 10 mM luminol (Sigma Chemical Co., St Louis, MO) was prepared in 0.2 M sodium borate buffer, pH 9.0. Zymosan (Sigma Chemical Co., St Louis, MO) was prepared by boiling 600 mg

in 30 ml of Hank's balanced salt solution (HBSS) at pH 7.4 for 20 min. Zymosan was opsonized by resuspending it in 60 ml of 65% pooled serum in HBSS and incubating at room temperature for 45 min. The opsonized zymosan was washed twice with HBSS and resuspended to a concentration of 20 mg ml^{-1} in HBSS.

Chemiluminescence (CL) measurement

Activation of leukocytes was measured without preliminary separation of leukocytes from whole blood. The measurements were performed every day except on Sundays for at least 25 days after BMT. Automated luminometer set-up allowing the simultaneous and continuous measurement of 25 samples (LKB Wallac 1251 Luminometer (Turku, Finland) connected to Olivetti M 20 microcomputer) was used as described earlier.[2,3] Cold suspensions of reagents and anticoagulated blood (EDTA) were pipetted into 4 ml polypropylene sample vials outside the luminometer. The activation of leukocytes commenced when the contents of the vials were warmed up to 37°C in the temperature controlled sample carousel of the instrument. CL emission was measured at 37°C for 60 min in a volume of 500 µl of HBSS buffer including 4×10^{-4} M luminol, 0.1% gelatin, 1 mg of opsonized zymosan and 50, 100 or 200 nl. of venous blood. The maximum CL emission in mV (obtained usually at 25 min after starting the reaction) was plotted against each blood sample volume. Linear regression was used to calculate the slope as an expression of the activation of leukocytes of the specimen. The CL emission was expressed as mV 1000^{-1} leukocytes and mV 1000^{-1} neutrophils. The sensitivity of Cl measurement is about 0.02 mV, allowing a reliable detection of approximately ten leukocytes in the sample vial.

Cell counting

The haematological parameters were measured by a Technicon H6000 automatic blood analyzer (Technicon Instruments Co., Tarrytown, NY).

RESULTS

After BMT all patients had a fall of CL to an undetectable level (Fig. 1, Table 1). This fall coincided with the development of severe pancytopenia caused by the preconditioning for BMT. After BMT platelet transfusions were given until the patients' own platelet production started. The platelet concentrates were irradiated (30Gy) to prevent the possible acute graft vs host reaction caused by contaminating lymphocytes.[4] For correction of anaemia the patients received filtered and irradiated red cell concentrates. These blood products had no measurable effect on CL. On day +11, Cl rose sharply in all patients to a two-fold level compared to healthy controls. This preceded the rise of blood leukocytes above 1.0×10^9 $l.^{-1}$ and of neutrophils above 0.5×10^9 $l.^{-1}$ by 3–14 days. Subsequently, CL levelled off to a normal range within 1–2 weeks. In all patients the rise of CL seemed to coincide with the appearance of large unstained cells (LUC, a parameter given by the Technicon H6000 blood cell analyzer) into the blood; the rise of LUCs was, however, marked (0.05×10^9 $1.^{-1}$) in only one patient (M.U.). The same patient showed later a sudden fall in CL, preceding a fall in peripheral blood leukocytes and platelets by 7 days (Fig. 2). This was probably due to cytomegalovirus activation, since simultaneously the patient started to excrete CMV in the urine. Because of severe thrombocytopenia he received high dose i.v. gammaglobulin (23 g/day) for 5 days (Sandoglobulin®), which corrected the thrombocytopenia and leukopenia. The effect of Sandoglobulin® on the possible CMV infection remained unclear.

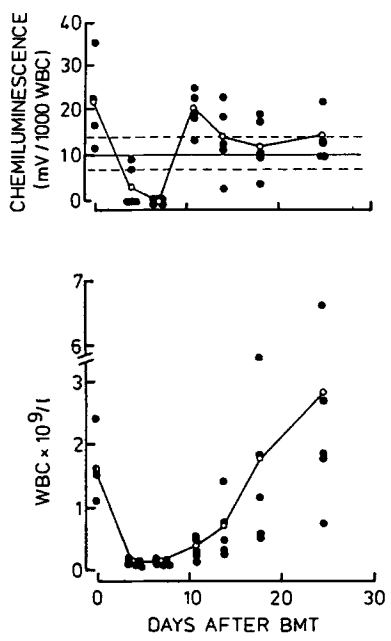

Fig. 1. The leukocyte chemiluminescence (CL) values ($mV/1000^{-1}$ WBC) and blood leukocyte values (WBC × 10^9 $l.^{-1}$) in five AML patients after BMT. The lines with open circles represent the mean values and dotted lines the ± 1 S.D. range for CL of healthy controls. The rise of CL preceded the rise of leukocytes above 1×10^9 $l.^{-1}$ by 3–14 days.

Table 1. Correlation of leukocyte chemiluminescence (CL) to the number of total leukocytes, neutrophils and large unstained cells (LUCs) in five AML patients tested daily, except on Sundays, after BMT

Patient		Days after BMT 0	+4	+7	+11	+14	+18	+25
S.L.	CL/1000 L	16	0	0	19	12	9.9	9.4
	CL/1000 N	18	0	0	36	NT	18	12
	WBC	1.60	0.08	0.15	0.18	0.50	0.60	0.74
	Neut	1.41	0.04	0.08	0.09	NT	0.33	0.56
	LUC	NT	0	0	0.01	NT	0.06	0
M.U.	CL/1000 L	22	6.6	0	25	2.8	3.5	9.8
	CL/1000 N	26	17	0	41	5.7	4.9	14
	WBC	2.43	0.21	0.14	0.55	0.79	4.70	6.70
	Neut	2.04	0.08	0.05	0.33	0.39	3.37	4.76
	LUC	0.01	0	0	0.05	0.06	0.08	0.06
M.K.	CL/1000 L	11	0	0	23	23	19	13
	CL/1000 N	11	0	0	30	26	25	24
	WBC	1.52	0.12	0.11	0.37	0.29	0.52	1.90
	Neut	1.47	0.07	0.06	0.28	0.26	0.40	1.01
	LUC	0	0	0	0.01	0	0.02	0.08
A.R.	CL/1000 L	35	8.9	0	13	11	10	13
	CL/1000 N	40	14	0	22	22	15	26
	WBC	1.10	0.22	0.22	0.38	0.71	1.16	1.89
	Neut	0.96	0.14	0.12	0.22	0.36	0.78	0.96
	LUC	0	0	0.00	0.01	0.02	0.01	0.04
J.P.	CL/1000 L	NT	0	0	21	18	17	21
	CL/1000 N	NT	0	0	35	32	29	35
	WBC	NT	0.18	0.17	0.50	1.42	1.85	2.74
	Neut	NT	0.12	0.09	0.30	0.81	1.07	1.65
	LUC	NT	0	0	0.02	0.04	0.03	0.06
Mean	± 1 S.D.							
	CL/1000 L	21 ± 10	3.1 ± 4.3	0	20 ± 4.6	13 ± 7.6	12 ± 6.2	13 ± 4.7
	CL/1000 N	24 ± 12	6.2 ± 8.6	0	33 ± 7.2	21 ± 11.3	18 ± 9.4	22 ± 9.4
	WBC	1.66 ± 0.56	0.16 ± 0.06	0.16 ± 0.04	0.40 ± 0.14	0.74 ± 0.43	1.77 ± 1.72	2.79 ± 2.30
	Neut	1.47 ± 0.44	0.09 ± 0.04	0.08 ± 0.03	0.24 ± 0.10	0.46 ± 0.24	1.19 ± 1.25	1.79 ± 1.71
	LUC	0	0	0	0.02 ± 0.02	0.03 ± 0.03	0.04 ± 0.03	0.05 ± 0.03

CL/1000 L = chemiluminescence as mV per 1000 leukocytes (opsonized zymosan); CL/1000 N = chemiluminescence as mV per 1000 neutrophils (opsonized zymosan); WBC = blood leukocyte count × 10^9 $l.^{-1}$; Neut = blood neutrophil count × 10^9 $l.^{-1}$; LUC = number of large unstained cells (given by a Technicon H6000 blood analyzer) × 10^9 $l.^{-1}$;
NT = not tested.
The WBC nadir varied between days +4 to +7 after BMT.

DISCUSSION

The activation of leukocytes by zymosan or other stimulators is accompanied by a burst in their oxidative metabolism. The principles of this activation and the resulting chemiluminescence are described elsewhere.[5,6,7,8]

We found the measurement of zymosan-induced activation of peripheral blood leukocytes to be a more sensitive test for prediction of marrow take than the conventional blood counts. This functional test was also highly reproducible in our five patients with acute myelogenous leukaemia (AML). The bone marrow recovery after allogeneic BMT was detected with CL 3–14 days prior to the rise of leukocyte or neutrophil counts above 1 × 10^9 $l.^{-1}$ and 0.5 × 10^9 $l.^{-1}$, respectively. CL showed a maximum response on day +11, which seemed to coincide with the appearance of LUCs in peripheral blood, detectable with the Technicon H6000 blood analyzer. This observation is in agreement with that of Martin *et al.*,[1] who proposed that such large cells might be early indicators of graft take after BMT. They found the LUCs to be mostly large mononuclear cells, predominantly belonging to the CD8-positive suppressor T-cell population. Our preliminary findings using isolated LUCs show that these CD8-positive cells are also HLADr-positive indicating that they could

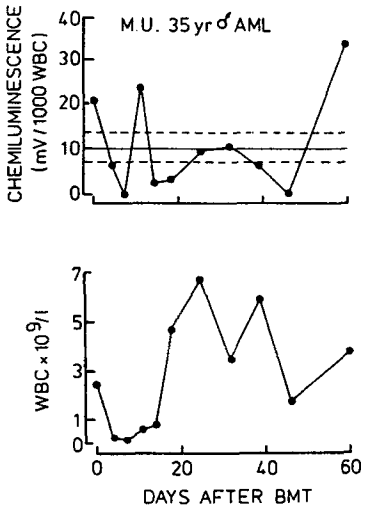

Fig. 2. A 35-year-old male (M.U.) with AML having a complicated course after BMT. On day +11 CL rose sharply about 7 days before the leukocyte count exceeded 1×10^9 l.$^{-1}$ Thereafter CL fluctuated until it suddenly fell, preceding the fall of leukocytes and platelets by 7 days. After treatment with high dose i.v. immunoglobulin, CL became measurable again followed by a rise in leukocytes and platelets. As to the symbols, see text to Fig. 1.

be activated suppressor T-cells. The proportion of these HLADr-positive cells seems to diminish during the course of recovery of bone marrow function.[9]

Our test modification is sensitive enough to detect approximately ten leukocytes per sample vial, provided that the blood is highly diluted in order to avoid inhibitory effect of red cells and plasma.[8] In our patients, transfusion of platelets or red cells had no effect on CL. Therefore we consider it improbable that the red cells or their ghosts would play any significant role in the CL assay as suggested by Peerless & Stiehm.[10] Consequently, we consider that the first few leukocytes emerging from the bone marrow transplant must be responsible for the strong rise in CL. The nature of this phenomenon remains to be studied. One possibility is that after the extensive changes in haematopoiesis linked to BMT, the first few cells — perhaps LUCs — are metabolically extremely active, and give rise to the vigorous CL responses. Another possibility could be that a subpopulation of neutrophils, capable of reacting with zymosan, is increased, leading to an impression that all neutrophils are abnormally active.

In conclusion, the measurement of leukocyte CL appears to be a promising new functional test for the early and sensitive prediction of graft take after BMT in patients with AML in remission. The test may also predict marrow suppression during recovery from BMT as seen in one of the patients (M.U.) The test is routinely done in all out patients undergoing allogeneic or autologous bone marrow transplantation. Whether this test gives any useful practical information in other patient groups than AML undergoing bone marrow transplantation is now under examination.

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