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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(Received 2 June 1987; revised 2 November 1987; accepted 7 November 1987)

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 $1.0 \times 10^9 \text{L}^{-1}$ and that of neutrophils above $0.5 \times 10^9 \text{L}^{-1}$, respectively, 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 $1.0 \times 10^9 \text{L}^{-1}$ and $0.5 \times 10^9 \text{L}^{-1}$, 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).

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 $50 \text{mg kg}^{-1} \text{day}^{-1}$ for 4 days, followed by total body irradiation $2.5 \text{Gy day}^{-1}$ 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\textquotesingle 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$^\circ$C in the temperature controlled sample carousel of the instrument. CL emission was measured at 37$^\circ$C for 60 min in a volume of 500 $\mu$l of HBSS buffer including $4 \times 10^{-4}$ M luminol, 0.1\% gelatin, 1 mg of opsonized zymosan and 50, 100 or 200 $\mu$l 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 CI 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, CI rose sharply in all patients to a two-fold level compared to healthy controls. This preceded the rise of blood leukocytes above $1 \times 10^9 l^{-1}$ and of neutrophils above $0.5 \times 10^9 l^{-1}$ 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 ($0.05 \times 10^9 l^{-1}$) 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\textsuperscript{®}), which corrected the thrombocytopenia and leukopenia. The effect of Sandoglobulin\textsuperscript{®} on the possible CMV infection remained unclear.