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Isao Takahashi, *Okayama University*
Noritoshi Inagaki, *Okayama University*
Hiroshi Nakada, *Okayama University*
Eijiro Ohmoto, *Okayama University*
Makoto Takeuchi, *Okayama University*
Ken Osada, *Okayama University*
Toshiaki Matsuzaki, *Okayama University*
Shunichi Fukuda, *Okayama University*
Kozaburo Uchida, *Okayama University*
Fumikazu Kohi, *Okayama University*
Seiichi Yorimitsu, *Okayama University*
Ikuro Kimura, *Okayama University*
Koichi Kitajima, *Okayama University*
Hiroshi Sanada, *Okayama University Hospital*

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Abstract

Superoxide anion (O2-) production by neutrophils from 14 untreated patients with acute non-lymphocytic leukemia (ANLL) was significantly less than that of healthy controls (4.93 +/- 1.99 vx 6.20 +/- 1.53 nmol/min/10(6) neutrophils, p less than 0.05). In 10 patients with myelodysplastic syndrome (MDS), however, it was not significantly different from the control level although 6 of the 10 patients had low levels, when individual patients were compared with the lower limit of the control range. An inverse correlation between the O2- production of neutrophils and the percentage of leukemic cells in the marrow existed in ANLL (r = -0.55, p less than 0.01), but not in MDS. Three of 4 MDS patients who died of pneumonia prior to leukemic conversion showed a low level of O2- production. The impaired O2- production by neutrophils from some MDS patients, probably due to the faulty differentiation from leukemic clones, may be one of the causes of enhanced susceptibility to infection.

KEYWORDS: superoxide anion production, myelodysplastic syndrome, preleukemia

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Superoxide Anion Production by Neutrophils in Myelodysplastic Syndromes (Preleukemia)

Isao Takahashi*, Noritoshi Inagaki, Hiroshi Nakada, Eijiro Ohmoto, Makoto Takeuchi, Ken Osada, Toshiaki Matsuzaki, Shunichi Fukuda, Kozaburo Uchida, Fumikazu Kohi, Seiichi Yorimitsu, Ikuro Kimura, Koichi Kitajima[a] and Hiroshi Sanada[b]

Department of Internal Medicine, Okayama University Medical School, [a]School of Health Science, Okayama University, and [b]Central Laboratories, Okayama University Hospital, Okayama 700, Japan

Superoxide anion (O_2^-) production by neutrophils from 14 untreated patients with acute nonlymphocytic leukemia (ANLL) was significantly less than that of healthy controls (4.93 ± 1.99 vs 6.20 ± 1.53 nmol/min/10^6 neutrophils, $p < 0.05$). In 10 patients with myelodysplastic syndrome (MDS), however, it was not significantly different from the control level although 6 of the 10 patients had low levels, when individual patients were compared with the lower limit of the control range. An inverse correlation between the O_2^- production of neutrophils and the percentage of leukemic cells in the marrow existed in ANLL ($r = -0.55$, $p < 0.01$), but not in MDS. Three of 4 MDS patients who died of pneumonia prior to leukemic conversion showed a low level of O_2^- production. The impaired O_2^- production by neutrophils from some MDS patients, probably due to the faulty differentiation from leukemic clones, may be one of the causes of enhanced susceptibility to infection.

Key words : superoxide anion production, myelodysplastic syndrome, preleukemia

Myelodysplastic syndromes (MDS) are hematological disorders with the potential of progressing to acute leukemia (1). MDS presumably result from the transformation of pluripotent stem cells giving rise bi- or pancytopenia, morphological anomalies and functional defects of erythroid, myeloid and megakaryocytic cells (2). MDS are characterized clinically by refractory anemia and a slight to moderate increase of blasts in peripheral blood and bone marrow. MDS patients occasionally die of infection in spite of the absence of severe pancytopenia prior to overt leukemia.

Superoxide anion (O_2^-) production is related to the intracellular bactericidal activity by neutrophils, particularly in an oxygen dependent system. The measurement of O_2^- production of neutrophils has been widely applied to the clinical diagnosis of chronic granulomatous disease (CGD), in which neutrophils are defective in their intracellular killing activities in spite of having normal

*To whom correspondence should be addressed.

phagocytic capacity, resulting in recurrent bacterial infection (3, 4).

In this paper, O_2^- production by neutrophils in MDS was examined to evaluate one of the possible causes of enhanced susceptibility to infection, and also to gain some informations for concerning the prognosis of MDS patients.

Patients and Materials

Patients. Ten MDS and 23 acute nonlymphocytic leukemia (ANLL) patients were studied. Among the 23 ANLL patients, 14 were previously untreated and the remaining 9 had relapsed. Their ages ranged from 50 to 76 years (median: 63) among MDS and from 29 to 68 years (median: 48) among ANLL patients. MDS patients were diagnosed according to the FAB classification proposed in 1986 (5). One hundred and thirty-four healthy individuals, whose ages ranged from 16 to 78 years, were studied as controls.

Neutrophil preparations. Neutrophils were isolated from heparinized venous blood. One volume of 6% dextran in physiological saline was mixed with 5 volumes of blood and allowed to settle at room temperature. After sedimentation of the majority of the red blood cells, neutrophils were further isolated by the Ficoll-Hypaque gradient method, and the remaining red blood cells were hemolyzed in hypotonic solution. After two sedimentations (4℃, 10 min at 150 g) and washing with Krebs-Ringer phosphate (KRP) solution, a differential cell count was made. The neutrophils in KRP solution were refrigerated (0℃) until use. More than 95% of the cells were viable as assessed by the trypan blue dye exclusion method.

Measurement of O_2^- production. Release of O_2^- from neutrophils was measured by a method essentially similar to that described by Nakagawara (3). The reaction mixture consisted of 0.05 mM glucose, 65 μM ferricytochrome C and the cells (approximately 2.0×10^5 cells) in 2.0 ml of KRP solution. The mixture was preincubated at 37℃ for 5 min in a microcell. Concanavalin A and cytochalasin D were added simultaneously to the reaction mixture at the final concentrations of 100 μg/ml and 20 μg/ml, respectively. The reduction of cytochrome C was measured continuously by a double beam spectrophotometer UV-210 A (Shimadzu Ltd, Kyoto) at 550 nm. The rate of O_2^- production was expressed in terms of nmol cytochrome C reduced/min/10^6 neutrophils (band+segmented). Experiments were carried out in duplicates and the average was used for the rate of O_2^- production of neutrophils.

Results

Hematological and clinical findings of 10 MDS patients, including 6 with refractory

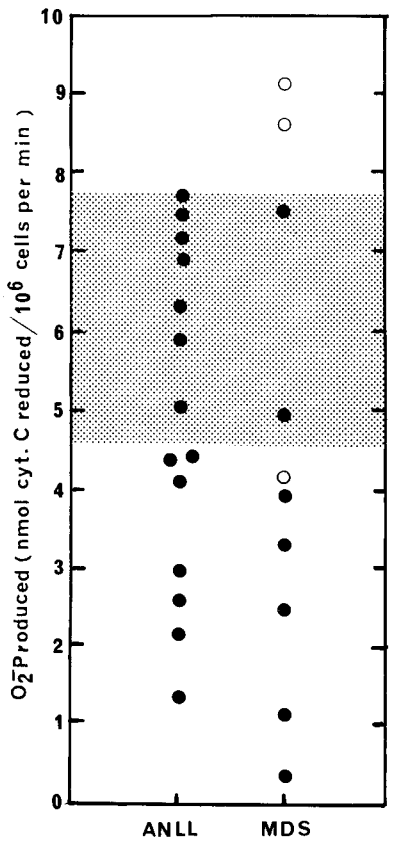

Fig. 1 The O_2^- production by neutrophils in myelodysplastic syndromes (MDS) and acute nonlymphocytic leukemia (ANLL). The shadowed portion indicates the control value range. The O_2^- production by neutrophils in ANLL is significantly lower than that in the control ($p < 0.05$). The MDS cases indicated by open circles terminated in overt leukemia. Number of cases examined were: MDS, 10; ANLL, 14.

anemia with excess of blasts (RAEB) and 4 with RAEB in transformation (RAEB-t), are shown in Table 1. Three of the 4 RAEB-t patients progressed to overt leukemia. Four patients died of pneumonia, and 3 died of heart failure. The O_2^- production by neutrophils of MDS and untreated ANLL patients is shown in Fig. 1. In ANLL patients, it was 4.93 ± 1.99 nmol/min/10^6 neutrophils, which was significantly lower than in healthy

Table 1 Hematological and clinical findings of patients with myelodysplastic syndrome

Case	Age	Sex	Diagnosis	Peripheral blood				Bone marrow		NAP score	Karyotype	Leukemic conversion	Alive/Dead (Cause of death)	O_2^- Production[a]
				RBC	Plt	WBC	Mybl	Erybl	Mybl					
1.	50	F	RAEB	223[b]	6.8[b]	2.0[c]	1.0[d]	2.8[d]	19.8[d]	4	46, XX, 9p−, 16q+	(−)	Dead (Pneumonia)	0.24
2.	76	M	RAEB	302	13.9	1.7	0	49.6	6.4	ND[e]	47, XY, +c	(−)	Alive	4.76
3.	66	M	RAEB	289	8.8	5.3	5.0	43.0	9.6	223	45, XY, −18/45, XY, −19 47, XY, +c	(−)	Dead (Heart failure)	7.44
4.	56	M	RAEB	211	18.0	2.2	0	17.2	14.2	172	46, XY	(−)	Alive	2.38
5.	64	F	RAEB	297	6.0	2.3	1.0	40.4	6.8	226	46, XX 46, XX, 1q+	(−)	Alive	1.07
6.	62	M	RAEB	252	3.6	3.0	0	35.4	6.4	94	46, XY	(−)	Dead (Heart failure)	3.88
7.	64	M	RAEB-t	346	2.0	1.8	0	22.6	26.0	21	45, X, −Y	(+)	Dead (Pneumonia)	4.17
8.	56	M	RAEB-t	263	29.5	2.9	0	34.0	25.8[f]	231	ND[e]	(+)	Dead (Heart failure)	8.33
9.	60	F	RAEB-t	256	9.1	1.6	0	14.6	9.4[f]	319	ND[e]	(+)	Dead (Pneumonia)	9.03
10.	68	M	RAEB-t	308	1.1	8.5	1.0	27.2	22.4	277	46, XY	(−)	Dead (Pneumonia)	3.18

a: nmol Cytochrome C reduced/10^6 neutrophils, *b*: $\times 10^4$ cells/μl, *c*: $\times 10^3$ cells/μl, *d*: %, *e*: not done, *f*: Auer body (+). Mybl: Type I+Type II, NAP: Neutrophil alkaline phosphatase, RAEB: Refractory anemia with excess of blasts, RAEB-t: RAEB in transformation.

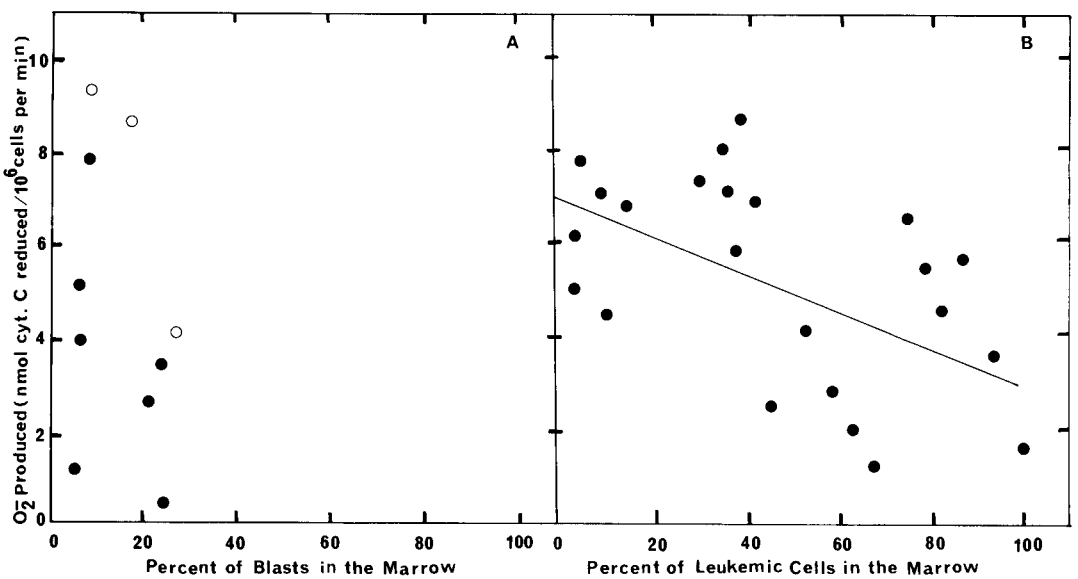

Fig. 2 Correlation between the O_2^- production by neutrophils and the percentage of leukemic cells or blasts in the marrow of myelodysplastic syndrome (MDS, Fig. 2-A) and acute nonlymphocytic leukemia (ANLL, Fig. 2-B). Cases of ANLL include 9 cases of relapse. A relation between O_2^- production and percentage of leukemic cells exists in ANLL ($r = -0.55$, $p < 0.01$), but not in MDS. The MDS cases indicated by open circles terminated in overt leukemia. Numbers of cases examined were: MDS, 10; ANLL, 23.

controls (6.20 ± 1.53 nmol/min/10^6 neutrophils). On the other hand, O_2^- production by neutrophils in MDS patients (4.45 ± 2.99 nmol/min/10^6 neutrophils) was not significantly different in comparison with that in healthy controls, although 6 of the 10 MDS patients had a low level of O_2^- production. The inverse correlation between the O_2^- production of neutrophils and the percentage of leukemic cells in the marrow existed in ANLL (Fig. 2, $r = -0.55$, $p < 0.01$). However, there was no correlation between the O_2^- production of neutrophils and blasts in MDS.

Among 9 MDS patients, 3 patients showed both a low neutrophil alkaline phosphatase (NAP) score and a low capacity for O_2^- production by neutrophils. However, there was no relation between the NAP score and the O_2^- production by neutrophils. Abnormal chromosome karyotypes, presented in Table 1, were recognized in 5 of 8 MDS patients. No specific chromosomal anomaly was found which correlated with the impairment of O_2^- production by neutrophils. Three patients with RAEB-t developed overt acute leukemia without showing a significant decrease in the O_2^- production by neutrophils. Three of the 4 patients who died of pneumonia showed a low level of O_2^- production by neutrophils.

Discussion

About 20% of MDS patients develop overt acute leukemia, and some patients die of infections in the absence of severe neutropenia before the leukemic conversion. MDS presumably result from the transformation of pluripotent stem cells, resulting in a functional defect of blood cells. Schreiner *et al.* (8) found the impairment of bactericidal activities in the preleukemic or early stage of acute leukemia. Schofield *et al.* (9) found a reduction in the activity of myeloperoxidase, and also of lactoferrin in MDS patients. Boogaerts *et al.* (10) found decreased adhesion, deficient chemotaxis, decreased myeloperoxidase content, slower chemiluminescence, decreased phagocytosis and impaired microbicidal capacity in MDS neutrophils, and they also found that an increasing number of blasts was associated with more severe granulocytic disability.

The production of active oxygen such as O_2^-, hydroxyl radical ($OH\cdot$), singlet oxygen ($_1O^2$) and hydrogen peroxide (H_2O_2), has been shown to be one of the metabolic events in phagocytosing neutrophils (11-13). Neutrophils have been found to exhibit microbicidal activities in the form of H_2O_2-myeloperoxidase(MPO)-halides (14). The release of O_2^- from phagocytosing neutrophils was first demonstrated by Babior *et al.* (15), and it was found to be low in patients with CGD, who suffer from severe and recurrent infection.

Previous studies from our laboratory have shown that: (a) O_2^- production by neutrophils is impaired not only in ANLL, but also in acute lymphocytic leukemia (ALL). In the state of complete remission, O_2^- production recovers, and in some cases exceeds the normal value. (b) Neutrophils in chronic myelocytic leukemia (CML) vary among patients with respect to O_2^- production. (c) Blasts from patients with acute leukemia release little O_2^- upon stimulation with concanavalin A and cytochalasin D (16). The O_2^- production by neutrophils in MDS, described herein, varied among patients. However, in 6 of the 10 patients it was lower than the lower limit of the control range. Although an inverse correlation between the O_2^- production by neutrophils and the percentage of leukemic cells in the marrow was present in ANLL, no correlation was found in MDS. Three of the 4 patients who died of pneumonia prior to overt leukemia showed a low level of O_2^- production. Therefore, the im-

paired O_2^- production by neutrophils seems to be one of the causes of the inability of MDS patients to prevent or combat infection. Why O_2^- production is impaired in MDS is not known. A human leukemic cell line, HL-60, was found to release O_2^- upon maturation *in vitro* (17). It was also found that a marker for immaturity, defined by the presence of the antigen recognized by the My 9 antibody persisted on the surface of mature neutrophils in a subgroup of MDS patients (18). On the basis of these findings, the decrease in O_2^- production by neutrophils in some MDS patients may be due to the faulty differentiation of neutrophils from leukemic clones.

Whether or not the measurement of O_2^- production by neutrophils is valuable for the early detection of the leukemic conversion in MDS is an interesting question. Out of 10 patients, 3 with RAEB-t who showed no significant impairment of O_2^- production by neutrophils developed overt acute leukemia. Two of them actually showed an increase in O_2^- production. On the basis of these findings, the measurement of O_2^- production by neutrophils in MDS seems to be valuable for determining the prognosis, particularly in terms of susceptibility to infection, but not for determining leukemic conversion.

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