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NATURAL KILLER (NK) ACTIVITY IN PATIENTS WITH CHRONIC GRANULOMATOUS DISEASE*)

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ABSTRACT

Natural killer (NK) activity against K 562 target cells was explored in 4 patients with chronic granulomatous disease (CGD). Although the NK activity of these patients was normal, the activity did not change after removal of monocytes from the effector cell population, contrary to normal adult controls.

The possibility of a defect in the regulatory function of monocytes on NK activity in CGD patients is discussed.

INTRODUCTION

Chronic granulomatous disease (CGD) is an inherited disease of the phagocytes characterized by reduced ability to kill ingested bacteria. Its clinical manifestations are recurrent and intractable bacterial infections and widespread granulomatous lesions of the skin, lungs and lymph nodes[1-4]. Although monocytes in CGD are known to have abnormal bactericidal activity, it is not known whether other functions of monocytes, especially their regulatory function in lymphocyte-mediated immune responses, are abnormal or not. The functions of T-and B-lymphocytes are normal in CGD[4,5].

In this study, the natural killer (NK) activity of peripheral blood mononuclear cells against an erythroleukemic cell line K 562[6-8] was explored in patients with CGD. The results suggest that some defect in monocyte-NK cell interaction may be present in patients with CGD.

MATERIALS AND METHODS

Patients

The diagnosis of CGD was established by clinical and laboratory findings: decreased nitroblue tetrazolium (NBT) reduction[9,10] and defective intracellular killing of catalase-positive bacteria by phagocytes[2]. The laboratory and clinical findings in 4 patients are shown in Table. The clinical courses of these patients have been described in detail in previous reports[5,11]. The NK activity of these patients was assayed at a time when obvious infections were not present.

Informed consent was obtained from patients and/or guardians.

Cytotoxicity assay

Techniques for the isolation of the effector cells, radiolabeling of the target cells (K 562), and cytotoxicity assay are described in the previous report[12]. Briefly, the effector cells were isolated from heparinized peripheral blood by

*) 田中義人，坂野　堯，臼井朋包：慢性肉芽腫症患者のナチュラル・キラー (NK) 活性について

Table. Clinical and Laboratory Findings in 4 patients with CGD

Case	1	2	3	4
Name	C.I.	Y.M.	N.I.	S.K.
Age (year)	10	8	15	6
Sex	F	M	M	M
Onset by 1 year of age	−	+	+	+
Lymphadenitis	+	+	+	+
Hepatosplenomegaly	+	+	+	+
Pneumonitis	+	+	+	+
Liver Abscess	−	−	+	+
Granulocytosis	−	+	+	+
Anemia	−	+	+	+
Elevated ESR	+	+	+	+
Hypergammaglobulinemia	+	+	+	+
Decreased NBT reduction	+	+	+	+

ESR=Erythrocyte sedimentation rate; NBT=Nitroblue tetrazolium.

centrifugation with Ficoll-diatrizoate sodium gradient. The monocytes were removed by adhesion to Falcon plastic dishes for 2 hours. Approximately 2×10^6 target cells were labeled with 100 μCi of ^{51}Cr (Na_2 $^{51}CrO_4$, 1 mCi/ml, specific activity 250–450 μCi/mg Cr, New England Nuclear, Corp, Boston, Mass.) for 50 minutes at 37°C in a humidified CO_2 incubator. The labeled K 562 cells were dispensed into wells of Nunc U-bottomed microtiter plates, each well receiving 0.1 m*l* containing 10^4 cells. Equal volumes of various dilutions of effector cells were added to triplicate wells, yielding ratios of effector to target cells (E/T ratio) of 6.25, 12.5, 25, 50, and 100. The plates were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 3 hours. Assays were terminated by centrifuging plates at room temperature, and 0.1 m*l* of supernatant was collected for counting in a well type gamma counter (Shimazu Model RAW-600, Shimazu, Japan).

The NK activity (per cent specific lysis) was calculated from the following formula:

$$\text{per cent specific lysis} = \frac{\text{CPM (experimental)} - \text{CPM (spontaneous)}}{\text{CPM (maximum)} - \text{CPM (spontaneous)}} \times 100$$

where experimental release was determined with effector cells present, and spontaneous release was determined from incubation of target cells in medium only (always less than 20% of the maximum release). The maximum release was determined by adding 0.1 m*l* of 0.5% Triton X-100 to the target.

RESULTS AND DISCUSSION

The NK activity of peripheral blood mononuclear cells from patients with CGD has not been previously reported. As shown in Fig. 1, the NK activity was normal in 4 patients with CGD. However, when the NK activity of monocyte-depleted and not-depleted effector cells from these patients was compared, there was no change in NK activity after monocyte depletion. The per cent increment of NK activity after monocyte depletion was 105.9±6.9 % in patients with CGD; this was much smaller than that of normal adult controls (131.3± 31.1%) (Figs. 2 and 3).

We previously reported that the NK activity in infants and children was as high as that of adult controls[12], and this finding was used as the control value in our laboratory. The monocyte-NK cell interaction seems to be complex, and at the present time, the precise relationship between NK cells and monocytes is not completely understood[12]. However, in normal healthy adult controls, as shown in Fig. 3., monocyte depletion of the effector cell population resulted in augmentation of the NK activity in most cases. Furthermore, the effect of monocyte depletion on NK activity varied from donor to donor, i. e., not only various

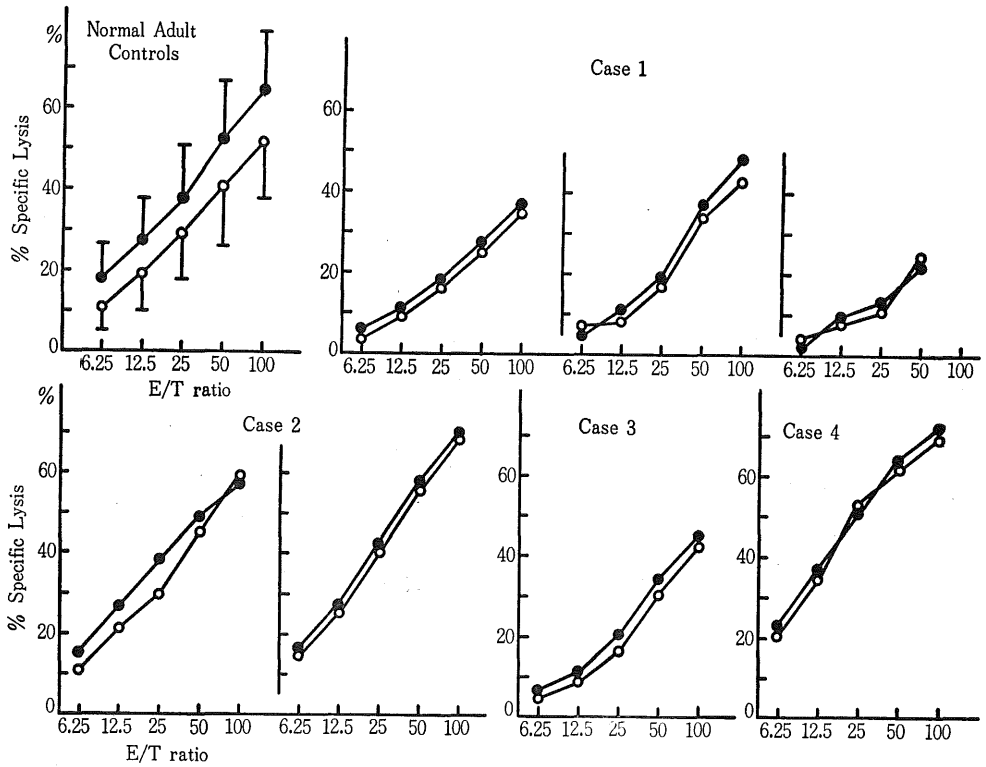

Fig. 1. The peripheral blood mononuclear cells from 26 healthy adult controls and 4 patients with CGD were isolated by centrifugation with Ficoll-diatrizoate sodium gradient. These cells consisted of 80–85% lymphocytes, 15–20% monocytes and less than 2% granulocytes. After adhesion to a plastic surface, harvested non-adherent cells consisted of more than 96% lymphocytes. The monocyte-depleted (●) and not-depleted (○) effector cells reacted with ^{51}Cr-labeled K 562 cells for 3 hours at E/T ratios of 6.25 : 1, 12.5 : 1, 25 : 1, 50 : 1, and 100 : 1. Case 1 was studied on 3 separate occasions, case 2 on 2 separate occasions, and cases 3 and 4 were studied once. NK activity of 26 healthy adult controls is depicted as mean±S. D.

degrees of augmentation of NK activity, but also a significant decrease was observed in some cases. This suggests that monocytes and NK cells might interact dynamically in accordance with differences in the *in vivo* situation. On the contrary, in patients with CGD, monocytes seemed to play no role in the regulation of NK activity, they acted like innert by-standers. The results of this experiment suggest that the regulatory function of monocytes on NK activity is defective in patients with CGD. To clarify this point further experiments are needed, and the study of monocyte-NK cell interaction is proceeding in our laboratory.

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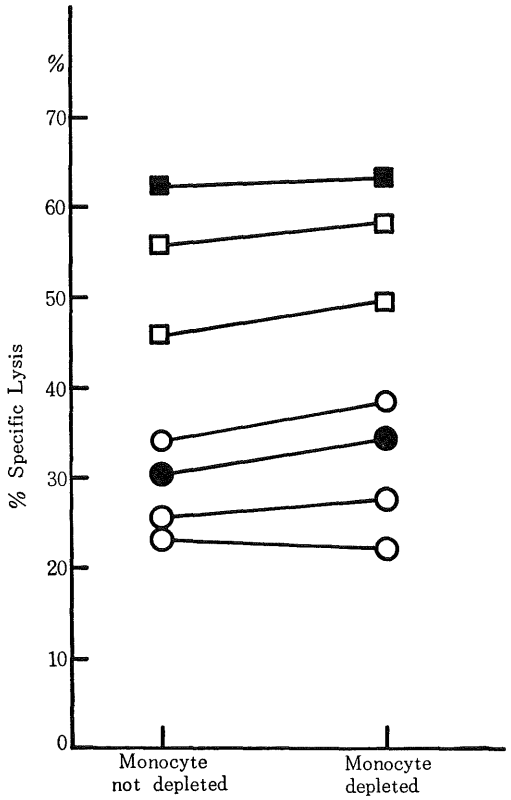

Fig. 2. Effect of monocyte depletion in patients with CGD
Effector cells were prepared from 4 patients with CGD and monocyte-depleted and not-depleted cells were simultaneously caused to react with ^{51}Cr-labeled K 562 cells at the E/T ratio of 50 : 1 for 3 hours. Case 1 (○) was studied on 3 separate occasions, case 2 (□) on 2 separate occasions, and case 3 (●) and case 4 (■) were studied once. The per cent increment of NK activity after monocyte depletion was 105.9±6.9% (mean±S. D.) (n=7).

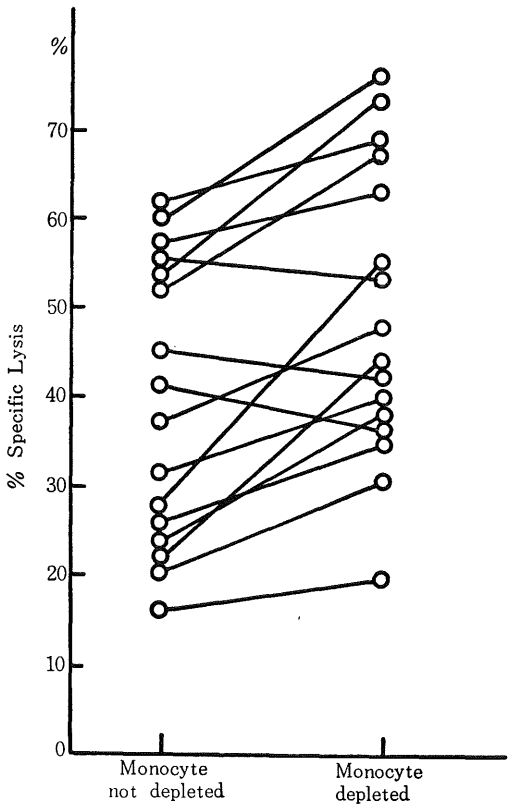

Fig. 3. Effect of monocyte depletion in healthy adult controls
Effector cells were prepared from 16 healthy adult donors, and monocyte-depleted and not-depleted cells were simultaneously caused to react with ^{51}Cr-labeled K 562 cells at the E/T ratio of 50 : 1 for 3 hours. The per cent increment of NK activity after monocyte depletion was 131.3±31.1 % (mean±S. D.) (n=16).

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