Natural and Antibody-Dependent Cellular Cytotoxicity of Polymorphonuclear Leukocytes

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Cytotoxic activity of polymorphonuclear leukocytes (PMN) in the peripheral blood of patients with various diseases was demonstrated to K562 cells (natural cytotoxicity, NC) and the antibody-coated P815 cells (antibody-dependent cellular cytotoxicity, ADCC), using a ^{51}Cr-release method. The NC values of normal PMN were lower than those of normal lymphocytes with mean values of 5.0% and 30%, respectively. The NC values of patients' PMN were also lower in malignancy, chronic hepatitis and connective tissue diseases. The ADCC values of normal PMN were moderately high with a mean value of 16.0%, which was almost a half of normal lymphocytes. Higher ADCC values of PMN were found in patients with chronic hepatitis, SLE and Behcet's disease, and in these cases the ADCC values of their lymphocytes were extremely low. The supernatants of PMN mix-cultured with unlabeled K562 or the antibody-coated P815 cells were fairly cytotoxic to both cells, though the similar supernatants of lymphocytes were cytotoxic only to K562 cells, but not to the antibody-coated P815 cells. — NC; ADCC; lymphocytes; PMN; SLE

Polymorphonuclear leukocytes (PMN) in the peripheral blood have been recently found to possess cytotoxic effect on the target cells either with direct contact (Hafemann and Lucas 1979) or in combination with antibody (Hafemann and Lucase 1979; Gale and Zighelboim 1975; Zighelboim et al. 1976; Clark and Klebanoff 1977). The mechanism of the antibody-mediated cytotoxicity of PMN has been conveniently explained by the presence of IgG-Fc receptor on the PMN (Zighelboim et al. 1976), and the cytotoxicity of PMN has been assumed to be mediated by enzymes and active oxygens from PMN (Goldstein et al. 1975; Cheson et al. 1977; Segal et al. 1978).

This paper reports the cytotoxicity of PMN of patients with various diseases tested to the ^{51}Cr-K562 cells and the antibody-coated ^{51}Cr-P815 cells in comparison with the cytotoxicity of lymphocytes.

In order to analyze the mechanism of the PMN-mediated cytotoxicity, the supernatants of PMN obtained in mix-culture with the unlabeled target cells were
assayed for cytotoxicity to the labeled target cells of K562 and antibody-coated P815.

**MATERIALS AND METHODS**

**Blood samples.** Blood samples were obtained from patients with bronchial asthma, malignancy, chronic hepatitis, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Behçet’s disease at the Hospital of Fukushima Medical College and from the staff members at the same Hospital as normal controls.

**Separation of polymorphonuclear leukocytes (PMN) and lymphocytes.** The heparinized peripheral blood was placed on the lymphoprep (Daichi Kagaku Co., Tokyo) with a specific gravity of 1.056 and centrifuged at 1,000 rpm for 5 min. The sediment and supernatant were separated. The sediment which contained mainly PMN was mixed with a 0.25% solution of NaCl in order to lyse the red blood cells contaminated. The purity of the obtained PMN was around 98%. The purified PMN was washed twice with the culture medium RPMI 1640 containing 10% fetal calf serum (FCS). The washed PMN was made in suspension in the culture medium at 2 X 10^6 cells/ml and used for the test. The supernatant contained mononuclear cells, from which lymphocytes were separated by removing the monocytes through adhesion to the plastic dish (Falcon, Tissue Culture Dish 3002) pre-coated with FCS. The purified lymphocytes were made in suspension in the culture medium at 2 X 10^6 cells/ml.

**Natural cytotoxicity (NC) by PMN and lymphocytes.** The established cell cultures K562 originated from a patient with erythroleukemia were kindly offered by Dr. K. Kumagai, Department of Microbiology, Tohoku University School of Dentistry, and used as target cells. A mixture of 0.1 ml of 1 x 10^7 K562 cells and 0.1 ml of 0.1 mCi of 51Cr was incubated at 37°C for 1 hr. The mixture was washed twice with the culture medium, and 1 x 10^7 51Cr-K562 cells were suspended in 1 ml of culture medium, radioactivity of which was usually 3 x 10^6 cpm or higher.

One tenth ml of 1 x 10^4 51Cr-K562 cells and 0.1 ml of 4 x 10^5 effector cells of PMN or lymphocytes were placed in a well of the microculture plate (Nuclon Delta SI, Denmark) and incubated at 37°C for 14 hr for PMN and 4 hr for lymphocytes. After incubation, 0.1 ml of supernatant of the culture medium was taken and its radioactivity was measured. Natural cytotoxicity of PMN and lymphocytes were measured by a percent of 51Cr released as follows:

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\text{% cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100
\]

The spontaneous release was the radioactivity of the culture medium containing 51Cr-K562 cells alone without effector cells.

**Antibody-dependent cellular cytotoxicity (ADCC).** The established culture cells P815 originated from the methylcholanthrene-induced mouse mastocytoma, which were offered by Dr. K. Kumagai, Department of Microbiology, Tohoku University School of Dentistry, were used as target cells. The cells were labeled with 51Cr as done for K562 cells.

One tenth ml each of 1 x 10^4 51Cr-P815 cells, 2 x 10^5 effector cells of PMN or lymphocytes and rabbit anti-P815 serum diluted at 1 to 10^4 were placed in a well of microculture plate. They were incubated at 37°C in an incubator containing 5% CO_2 for 14 hr for PMN and 4 hr for lymphocytes. After incubation, 0.1 ml of the culture supernatant was taken and its radioactivity was counted. The cytotoxicity was measured by a percent of 51Cr released as was done for K562 cells.

**Cytotoxicity of supernatant of the mix-cultures.** PMN or lymphocytes taken from normal subjects were cultured with the unlabeled target cells of K562 or antibody-coated P815 cells in a similar way to the NC and ADCC test.

One tenth ml of the supernatants taken from the mix-cultures of PMN and lymphocytes
Cytotoxicity of Polymorphonuclear Leukocytes was mixed with either 0.1 ml of \(1 \times 10^4\) \(^{51}\)Cr-K562 cells with 0.1 ml of culture medium or 0.1 ml of \(1 \times 10^4\) \(^{51}\)Cr-P815 cells with 0.1 ml of the diluted rabbit anti-P815 serum. Each mixture was incubated at 37°C for 4 hr and 0.1 ml of the supernatant was taken and its radioactivity was counted. The cytotoxicity of the mix-culture supernatant was measured by a percent of \(^{51}\)Cr released as in the tests with effector cells.

**RESULTS**

The cytotoxicity of PMN was tested using K562 cells and the antibody-coated P815 cells at three different incubation times; 4, 6 and 14 hr. In the test with 9 samples of PMN taken from healthy subjects, higher toxicity was obtained in 14 hr incubation in most of the samples as seen in Fig. 1.

Subsequently, 14 hr incubation time was used for the tests with PMN on both K562 and P815 cells, whereas 4 hr incubation time was used for the tests with lymphocytes.

The results of cytotoxicity test with PMN to K562 cells (natural cytotoxicity, NC) in 19 healthy subjects and 68 patients with various diseases are shown in Fig. 2. The mean NC value of PMN in 19 normal subjects was 5.0%. Among various diseases, PMN of 9 patients with bronchial asthma showed higher NC values with a mean of 10.8%. In other diseases, NC values were around normal or slightly lower with mean values of 1.2% in malignancy (17 samples), 3.8% in chronic hepatitis (14), 4.7% in rheumatoid arthritis (RA) (17), 1.5% in systemic lupus erythematosus (SLE) (4) and 1.8% in Behçet’s disease (7).

The cytotoxic values of PMN tested to the antibody-coated P815 cells (ADCC) widely ranged as seen in Fig. 3. The mean ADCC value of 25 normal subjects was 16.0%. Higher ADCC values were found in patients with chronic hepatitis, SLE and Behçet’s disease with mean values of 35.7%, 34.4% and 24.5% respectively, whereas lower values were found in patients with bronchial asthma, malignancy and RA with mean values of 2.6%, 15.0% and 5.0%, respectively.

![Fig. 1. Cytotoxicity of PMN on K562 cells (NC, left) and the antibody-coated P815 cells (ADCC, right).](image-url)
Fig. 2. Natural cytotoxicity of PMN on K562 cells.

Fig. 3. ADCC of PMN on the antibody-coated P815 cells.
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The NC values of PMN were compared with the NC of lymphocytes in testing both cells simultaneously to K562 cells in normal subjects and patients with malignancy, chronic hepatitis, RA, SLE and Behçet’s disease. In general, the NC values of PMN were fairly low as seen in Fig. 4, and a mean cytotoxic value of PMN in 10 normal subjects was approximately one sixth of that of lymphocytes. Out of 30 patients, 28 showed lower NC values of PMN than those of their corresponding lymphocytes. One patient with chronic hepatitis and one with SLE showed slightly higher NC values of PMN than those of their lymphocytes.

The ADCC values of PMN tested to the antibody-coated P815 cells were similarly compared with those of lymphocytes. As seen in Fig. 5, the mean cytotoxic value of normal PMN was approximately one half of that of lymphocytes.

In 9 normal subjects and 8 RA patients, cytotoxic values of all 17 PMN samples were lower than those of their lymphocytes. In 1 of 7 malignancy, 5 of 8 chronic hepatitis, all of 4 SLE and 2 of 6 Behçet’s patients, PMN showed higher ADCC values than those of their lymphocytes and interestingly enough in all these 12 patients, whereas ADCC values of their lymphocytes were lower than any of other samples in each group of the disease.

Subsequently, the cytotoxicity of the supernatants obtained from the mix-cultured cells was examined. Four samples each of supernatants obtained from
lymphocytes and PMN mix-cultured with K562 cells (Fig. 6) or the antibody-coated P815 cells (Fig. 6) were tested on $^{51}$Cr-K562 and the antibody-coated $^{51}$Cr-P815 cells.

As seen in Fig. 5, the supernatant of lymphocytes (Ly) obtained in a mix-culture with K562 cells was much cytotoxic to K562 cells than to the antibody-coated P815 cells, whereas supernatants of PMN mix-cultured with K562 cells showed a similar grade of cytotoxicity to both cells. Therefore, to the antibody-coated P815 cells, supernatants of PMN were more cytotoxic than those of L with mean values of 4.0% and 1.0%, respectively. Similar results of cytotoxicity were obtained by the supernatants taken from mix-culture with the antibody-coated P815 cells as seen in Fig. 5. The supernatants of lymphocytes were more cytotoxic to K562 cells than to the antibody-coated P815 cells, whereas the supernatants of PMN showed a similar cytotoxicity to both cells, though the cytotoxicity to K562 was slightly higher than to P815. Therefore, both mean values of cytotoxicity of PMN supernatants, 8.2% to K562 cells and 5.8% to P815 cells, were higher than those of lymphocytes, 7.0% and 1.5%, respectively.
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**Discussion**

A few studies have been reported on cytotoxic activity of PMN, though numerous reports have been accumulated on lymphocyte cytotoxicity (Schneider et al. 1975; Feldmann et al. 1976; Jouansen et al. 1976). The primary function of PMN is phagocytosis and subsequent release of enzymes, and active oxygen from PMN causes tissue damage in many diseases. Because of the release of such cytotoxic substances, PMN has been assumed to possess cytotoxicity to target cells in the NC and ADCC systems without phagocytosis.

When PMN was incubated with target cells longer enough for 14 hr than usual 4 hr for lymphocytes, cytotoxicity was demonstrated to some extent. The NC values of PMN were much lower than the ADCC values of PMN. The reason why 2 of 9 PMN samples of asthmatic patients showed high NC values is obscure. The pulmonary infection which is often accompanied in asthmatic patients may activate PMN to induce higher cytotoxicity. The ADCC of PMN has been reported by several investigators, and this function was assumed to depend on IgG-Fc receptors (Zighelboim et al. 1976) present on the surface of PMN similar to that of lymphocytes. The ADCC values of PMN were usually higher than NC values, and some of PMN showed much higher ADCC values than those of their lymphocytes.

In all of 11 cases of patients with various diseases in which PMN showed higher
ADCC values than those of their lymphocytes, the ADCC values of lymphocytes were the lowest in each group of disease. From this result, one could speculate that lower ADCC of lymphocytes might be compensated by higher ADCC of their PMN.

The lower ADCC of lymphocytes in SLE patients has been widely noticed and this phenomenon has been explained mostly by blocking of the IgG-Fc receptor on lymphocytes by the circulating immune complexes in SLE. In our preliminary experiments, heat-aggregated human IgG inhibited the ADCC of both lymphocytes and PMN equally well. If this is the case, the mechanism of ADCC of PMN should be explained differently from that of lymphocytes. The lysosomal enzymes and active oxygen are released from PMN, but not from lymphocytes, and such factors may participate in the cytotoxicity of PMN. Such assumption seemed to be plausible since the supernatants of PMN obtained in the mix-culture with unlabeled target cells showed significantly high cytotoxicity in the NC and ADCC tests. The results observed would suggest that soluble factors such as enzymes and/or active oxygen would play a significant role in cytotoxicity of PMN, whereas a coexistence of cytotoxic effector cells with the target cells might be necessary for cytotoxicity of lymphocytes. Further study will be necessary for characterization of the soluble factors responsible for PMN cytotoxicity.

References