Impaired Leukocyte Mobility and Production of Monocyte-Derived Granulotactic Factor in Pediatric Malignant Disease during Chemotherapy

TATSUHITO TONO-OKA, MASAYUKI NAKAYAMA, MASATO OHKAWA, TAKEO TAKEDA and SHUZO MATSUMOTO

Department of Pediatrics, Hokkaido University School of Medicine, Sapporo 060


Random mobility and chemotactic responses of granulocytes and monocytes, and production of monocyte-derived chemotactic factor for granulocytes (CFG) in the 17 patients with childhood malignant disease were estimated using an agarose plate method. Mononuclear cells (MNC) of the majority of the patients contained a significantly decreased number of monocytes with normal migratory capacity, whereas random mobility of granulocytes of all the patients was within normal range. Chemotactic responses of granulocytes and monocytes to zymosan activated serum (ZAS) were decreased in 11 to 17 occasions. All these impairments seemed to be induced by chemotherapeutic agents, because there were no differences in the degree of the impairment between group in remission with maintenance chemotherapy and that during induction chemotherapy.

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Infection is a major cause of mortality and morbidity in patients with leukemia or solid tumors. It may be due to decreased number and/or impaired function of leukocytes, which are induced by the disease or chemotherapy. There are numerous papers concerning cellular immunological state in malignant patient (Dupuy et al. 1971; Wybran and Fudenberg 1973; Gross et al. 1975; Leikin et al. 1978), however, only a limited amount of work concerning the functions of phagocytic cells, especially leukocyte mobility and chemotaxis, are available (Boetcher and Leonard 1974; Hausman et al. 1975; Pickering et al. 1975). It is because functions of phagocytic cells are not completely understood and few simple and quantitative in vitro methods are available.

We reported, previously, the usefulness of the agarose plate method (Tono-oaka et al. 1978, 1979b) that was introduced by Nelson and his co-workers (1975), to estimate various kinds of granulocyte mobility. Furthermore, using agarose plate we have developed a method which measures the degree of production of monocyte-derived chemotactic factor for granulocytes (CFG) by human mononuclear cells...

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cells (MNC) (Tono-oka et al. 1980). Using these methods we evaluated the ability of granulocytes and monocytes to migrate spontaneously and to respond to chemotactic factor, and the ability of MNC to produce CFG in pediatric malignant patients during chemotherapy.

**PATIENTS AND METHODS**

*Patient selection*

Heparinized blood (20 units of heparin per ml of blood) was obtained on 19 occasions from 17 children with malignant disease 2 to 14 years of age (Table 1) at various times during the course of hospitalization, or during routine follow-up visits. Their malignancies included acute lymphocytic leukemia (ALL), acute myelocytic leukemia (AML), neuroblastoma (NB) and rhabdomyosarcoma (RMS). Patients with acute leukemia consisted of group during induction therapy, group in remission while receiving consolidation therapy, and group in remission while receiving maintenance therapy. All the leukemic patients except one patient who was in end stage had leukemic cells in bone marrow, but not in peripheral blood. All the patients with NB had stage IV degree of involvement and had received previous surgical treatment and radiotherapy, but at the time of the study they were receiving 1000 mg to 1800 mg/M² of cyclophosphamide (CPM) because of

<table>
<thead>
<tr>
<th>Clinical diagnosis*</th>
<th>Age (years)</th>
<th>Stage†</th>
<th>Chemotherapy‡</th>
<th>Infection</th>
<th>Result</th>
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* ALL, acute lymphatic leukemia; AML, acute myelocytic leukemia; NB, neuroblastoma; RMS, rhabdomyosarcoma.
† CR, complete remission; PR, partial remission
‡ 6-MP, 6-mercaptopurine; MTX, methotrexate; P, prednisolone; VCR, vincristine; DN, duanomycin; DCVP, DM+ara-C (cytosine arabinoside)+VCR +P; CPM, cyclophosphamide.
residual tumors. A patient with RMS was in remission and receiving VCR and CPM as the maintenance therapy.

Cell preparations

MNC and granulocytes were purified by methods previously described (Tono-oka et al. 1978). These cells were suspended to $1 \times 10^8$ cells/ml in PBS. MNC contained about 85% of nonphagocyting cells and 15% of phagocyting cells. The granulocyte preparation contained more than 99% of granulocytes.

Preparation of agarose plate

Agarose plates were made as previously described (Tono-oka et al. 1978). When leukocytes mobility was measured, 3 ml of agarose solution were added to each 35 X 10 mm Falcon plastic dish, and when CFG production was measured 4 ml were added. In the present study no serum was added to medium of TC 199 (Gibco). We confirmed that random mobility and chemotaxis of granulocytes can be observed in an agarose plate containing no serum. 3 mm x 3 mm wells were made as shown in Fig. 1.

Assay of leukocytes mobility and production of CFG

Granulocyte mobility, namely random mobility and chemotaxis, was measured as previously described (Tono-oka et al. 1978). Zymosan activated serum (ZAS) and Escherichia coli-derived chemotactic factor (bacterial chemotactic factor; BCF) were used as chemotactic factor. In the case of the measurement of monocytes random mobility and chemotaxis, 10 µl of mononuclear cell suspension was placed instead of granulocyte suspension into the inner well, and migration distance was measured after a 20-hr culture. ZAS alone was used as the chemotactic factor, because BCF had no effect. The modes of measurement of migration distance and calculation of chemotactic index (C.I.) were the same as those previously described (Tono-oka et al. 1978).

Measurement of CFG production by MNC was performed by the measurement of the chemotactic response of indicator cells, namely normal granulocytes. Each of the outer wells received a 10 µl of MNC suspension containing $1 \times 10^4$ cells and 5 µl of lipopolysaccharide obtained from Escherichia coli (LPS, Difco) at a concentration of 10 µg/ml. Then each of the inner wells received 10 µl of a normal granulocytes suspension containing $1 \times 10^4$ cells. Then dishes were incubated at 37°C in a humidified atmosphere containing 5% CO₂ in air. After a 3-hr incubation, the distance of migration of granulocytes from the margin of the inner well toward the outer well where CFG was produced by MNC was measured with an ocular micrometer.

Chemotactic index (C.I.) of indicator granulocytes which expresses the degree of CFG production by MNC was calculated as follows; linear distance moved from the margin of the well toward the outer well (chemotaxis)/linear distance moved from the margin of the

![Fig. 1. Agarose plate method.](image-url)
well toward the inside (random mobility or spontaneous migration).

CFG productions by normal MNC stimulated by various kinds of stimulants were shown in Fig. 2. MNC stimulated by LPS or anti-β₂ microglobulin goat serum produced a significant amount of CFG.

All the experiments were carried out in duplicate or triplicate for each material, and the mean values were determined.

![Graph showing CFG production by mononuclear cells stimulated by various kinds of stimulants.](image)

**Fig. 2.** CFG production by mononuclear cells stimulated by various kinds of stimulants.

### Results

**Random mobility and chemotaxis of granulocytes**

All the granulocytes obtained on 18 occasions from 16 patients showed normal random mobility, and there was no difference in mobility between groups in remission and in induction therapy as shown in Fig. 3. Then chemotaxis was estimated using the two kinds of chemotactic factor, and granulocytes of malignant patients showed different chemotactic responses against two kinds of factor, namely ZAS and BCF. Chemotactic responses of all the granulocytes to BCF were within normal range; however, approximately 50% of patients had decreased chemotactic responses to ZAS (Fig. 4). There seemed to be no difference in chemotactic response between groups in remission and in induction therapy.

**Random mobility and chemotaxis of monocytes**

In the present study, random mobility of monocytes depends upon the number of normal migrating monocytes in MNC, because the number of monocytes was not standardized. As shown in Fig. 5, many patients had decreased monocytes random mobility, namely, malignant patients during chemotherapy had decreased numbers of normal migrating monocytes. Then chemotaxis was estimated using ZAS, and approximately half of the patients had decreased chemotactic responses (Fig. 6). In the monocytes mobility, there was also no difference between groups in remission and in induction therapy.
Leukocyte Mobility and CFG Production in Malignancies

Fig. 3. Random mobility of granulocytes from pediatric patients with malignancies.

![Graph showing migration distance for normal adults, acute leukemia, and solid tumor patients.]

ZAS, E. coli-derived factor

Fig. 4. Chemotaxis of granulocytes from pediatric patients with malignancies. ZAS, zymosan activated serum.

![Graph showing chemotactic index for normal adults, acute leukemia, and solid tumor patients.]
Fig. 5. Random mobility of monocytes from pediatric malignancies. 
(p<0.02 between groups of leukemic patients and normal adults)

Fig. 6. Chemotaxis of monocytes from pediatric malignancies to zymosan activated 
serum. (p<0.05 between groups of leukemic patients and normal adults)
Leukocyte Mobility and CFG Production in Malignancies

CFG production by MNC

CFG productions in response to LPS in 11 of 17 occasions or 10 of 15 patients fell below the mean minus one standard deviation of normal adults. Patients in remission also tended to show decreased CFG production (Fig. 7).

The degrees of the impairment in the various kinds of leukocytes function observed in the present study were not likely to be due to age factor, because younger patients did not always have impaired functions.

DISCUSSION

In the patients with acute leukemia, generally, susceptibility to bacterial infections seems to be due to the decreased number of circulating granulocytes, but some workers demonstrated impaired functions of granulocytes and monocytes in these patients. Pickering et al. (1975) reported that resting hexose monophosphate shunt (HMPS) activity of all patients with childhood malignancies was significantly greater, and ability to stimulate HMPS activity was significantly less than that noted in healthy control patients. Moreover, unstimulated NBT dye reduction of leukocytes obtained from patients was also significantly less than that observed in healthy control children. Recently he and his co-workers (1978) studied effect of chemotherapeutic agents on metabolic and bactericidal activity of polymorphonuclear leukocytes, and demonstrated that resting and stimulated HMPS activity of normal granulocytes and the killing of E. coli by normal granulocytes were...
significantly decreased by the various kinds of anti-leukemic agents. These two reports suggest that anti-neoplastic agents impair granulocytes functions. However, there are some opposite findings concerning anti-neoplastic agents or immunosuppressive agents (Rosner et al. 1970; Strauss et al. 1970; Skell et al. 1971). These differences may be due to the modes of estimation. Not only granulocytes functions, but also monocytes functions are thought to be impaired in patients with malignant disease. Many studies demonstrated that monocyte functions, especially chemotactic function, are impaired in patients with solid tumors, and the functions tend to correlate with the stage of disease (Boeteker and Leonard 1974; Hausman et al. 1975; Snyderman et al. 1976; Leb and Merritt 1978).

In the present study, we demonstrated that random mobility of granulocytes is normal, but that of monocytes is impaired in the patients with childhood malignant disease. This impaired random mobility of monocytes means the decreased number of monocytes with normal migratory capacity in the peripheral blood. We did not standardize the number of monocytes placed into a well, because it may be doubtful whether all populations of monocytes can be completely identified by different kinds of methods, namely morphological examination, examination of phagocyting ability, peroxidase staining or esterase staining. This problem seems to be important especially in the case of malignancies during chemotherapy in which monocytes functions may be impaired. The fact that patients in remission as well as those in induction therapy had decreased monocytes random mobility suggests that anti-neoplastic agents impair monocytes and decrease the number of normal migrating monocytes. No differences in the effects could be detected among the drugs given in these patients. The impairment may be dependent upon the duration of chemotherapy, rather than the kind of drug. All the patients in this study were given chemotherapeutic agents during 2 months to 2 years.

In both granulocytes and monocytes, chemotactic responses tended to be decreased. It is noteworthy that many patients showed decreased chemotactic responses of granulocytes to ZAS, but showed normal chemotactic responses to BCF. We obtained the analogous results using cord blood granulocytes (Tono-oka et al. 1979b). There are some papers demonstrating that localized leukocytes mobilization in vivo was impaired in leukemic patients (Holland et al. 1971; Senn and Jungi 1975). This fact may be explainable by not only the decreased number of circulating granulocytes, but also the impaired function of chemotaxis.

Snyderman and Pike (1976) demonstrated that the impairment of monocyte or macrophage chemotaxis is due to the presence of tumor cells, and that neoplasms produce an inhibitor of macrophage chemotaxis. Maderazo et al. (1978) also reported that a majority of patients with cancer showed a chemotactic defect of granulocytes and monocytes that is serum associated and due to the presence of an inhibitor. However, these explanations are not applicable directly to our results, because the patients in remission had impaired chemotaxis of granulocytes and monocytes. We speculate that anti-neoplastic agents impaired both granulopoiesis
and monopoiesis and decrease the number of normal phagocytizing cells with migratory capacity.

These are few studies regarding CFG, although many reports regarding LDCF for monocytes are available. It is reported that lymphokine productions in malignant diseases are impaired. Leikin et al. (1978) studied production of both mitogenic lymphokines and monokines in pediatric malignant disease, and demonstrated that leukemia patients in remission on chemotherapy as well as patients with extensive tumors receiving treatment showed depression of production of these mediators. Our results in the present study are analogous to their results, namely CFG productions in many malignant children are suppressed during anti-neoplastic chemotherapy regardless of whether maintenance or induction therapy.

From the present study it was suggested that susceptibility to bacterial infections in the patients receiving anti-neoplastic chemotherapy is partly due to suppressed mobility and/or chemotaxis of phagocytic cells and CFG production by MNC, which are induced by chemotherapeutic agents.

References


