Monocyte chemiluminescence and macrophage precursors in the aged.

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Abstract

Age-related alterations in the host defense system have been vigorously investigated because of increased susceptibility to infection and neoplasms in the aged. Although monocyte-macrophages form a major part of the cellular defense against microorganisms, the majority of investigations has been limited to neutrophils and lymphocytes. The present study, designed to determine the influence of age on mononuclear phagocytes, revealed no significant decrease in the absolute number of blood monocytes, but did reveal a tendency for the chemiluminescence of blood monocytes to decrease (p less than 0.10) and a significant decrease in the numbers of macrophage precursors (p less than 0.05) in the aged (over 70 year old), in comparison with controls (under 40 years old). On the basis of these findings, functional alterations of monocyte-macrophages seem to participate in the increased susceptibility to infection in the aged.

KEYWORDS: monocyte chemiluminescence, macrophage precursor, monocyte function in the aged, susceptibility to infection in the aged

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MONOCYTE CHEMILUMINESCENCE AND MACROPHAGE PRECURSORS IN THE AGED

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Abstract. Age-related alterations in the host defense system have been vigorously investigated because of increased susceptibility to infection and neoplasms in the aged. Although monocyte-macrophages form a major part of the cellular defense against microorganisms, the majority of investigations has been limited to neutrophils and lymphocytes. The present study, designed to determine the influence of age on mononuclear phagocytes, revealed no significant decrease in the absolute number of blood monocytes, but did reveal a tendency for the chemiluminescence of blood monocytes to decrease (p<0.10) and a significant decrease in the numbers of macrophage precursors (p<0.05) in the aged (over 70 years old), in comparison with controls (under 40 years old). On the basis of these findings, functional alterations of monocyte-macrophages seem to participate in the increased susceptibility to infection in the aged.

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Age-related alterations in the host defense system have received much attention because of greater susceptibility to neoplasms and infection in the aged. Recent studies have found an impairment of T-lymphocyte function in the aged (1). Impaired B-lymphocyte function and altered humoral immune response with advanced age have also been demonstrated (2). Peripheral phagocytic cells, such as neutrophils and monocytes, are functionally activated at the early phase of inflammatory response; therefore, their age-related alteration might be in part responsible for the increased susceptibility to infection. Some neutrophil functions, such as random mobility and oxidative metabolism, have been found to be impaired in the aged (3, 4); however, monocyte-macrophages of the aged have received little consideration.

Active oxygen produced in phagocytosing cells in the forms of superoxide anion (O$_2^-$), singlet oxygen (O$_2^*$), hydroxyl radical (OH·) and hydrogen peroxide (H$_2$O$_2$) is known to have microbicidal activity and to act independently or in the form of H$_2$O$_2$-halides (5). The chemiluminescence of phagocytes is thought to result from the relaxation of electrically excited molecules produced in association with oxidative events (6).
In the present study, the chemiluminescence of monocytes and numbers of macrophage precursors were investigated in order to evaluate possible causes of increased susceptibility to infection in the aged.

MATERIALS AND METHODS

Study population. Eighty-two volunteers were enlisted among office workers, medical doctors, nurses, and old people living in a home for the aged. Individuals with infection, diabetes mellitus, hepatic dysfunction, renal diseases and collagen diseases were excluded. Except for hypertension and arteriosclerotic diseases, volunteers had few health problems during the study. The age distribution was 24 years old to 90 years old, and the male to female ratio was 26 : 56.

Direct monocyte counting. Using alpha-naphthyl butyrate esterase (alpha-NBE) staining (7), the absolute number of monocytes was determined. Briefly, peripheral blood was diluted 1 : 10 with the staining solution, which consisted of alpha-NBE (substrate) and hexazotized pararosaniline (coupling agent), in a standard leukocyte pipette. After shaking gently for 5 min at room temperature, peripheral monocytes stained red-brownish were counted on a hemocytometer.

Measurement of chemiluminescence. Peripheral blood mononuclear cells were isolated from 10 ml of heparinized venous blood by the Ficoll-Hypaque gradient method. The mononuclear cell fraction was washed twice and suspended in Medium 199. Luminol (10⁻² μmol) was added to 0.08 ml of the suspension containing 10⁵ monocytes (pH 7.2, 37.0 °C), adjusted by using alpha-NBE staining, and spontaneous chemiluminescence (Base Line : BL) was measured for 5 min by means of a photon counter (BM 2000, LUMAC). After 5 min, 200 μg of concanavalin A was added to the sample, and the maximum chemiluminescence (Peak Level : PL) and time to PL (Peak Time : PT) were measured. The value of chemiluminescence was expressed by the relative light unit/10⁵ monocytes. All experiments were carried out in duplicate.

Macrophage precursor test. This test was done by a modification of Krikorian’s method (8). Briefly, 2 × 10⁴ monocytes/ml, adjusted by alpha-NBE staining, were suspended in RPMI 1640 containing 50 % human AB serum, and 100 μl of the suspension was cultured in a microtiter plate for 7 days. After 7 days, the culture medium was discarded, and the remaining monolayer of cells was rinsed 3 times in saline to remove nonadherent cells. The remaining cells were stained with 1 : 2000 crystal violet in 0.1 ml citric acid for 30 min. After vigorous agitation, cells were counted on a hemocytometer and the macrophage precursor index (MPI) was calculated as follows:

\[
\text{MPI} = \frac{\text{Mean number of macrophages per well}}{\text{Mean number of monocytes per well}} \times 100
\]

RESULTS AND DISCUSSION

The recent increase in the population of the aged has raised much attention with respect to the clinical management of various diseases of the aged. Some investigations of the host defense system in the aged have been prompted by the clinical observations that old people can not effectively handle infection and suffer from neoplasms. Although monocyte-macrophages form part for the cellular defense against microorganisms, the majority of investigations has been limited to neutrophils and lymphocytes.

The present study, designed to determine the influence of age on mononuclear
phagocytes, revealed no significant decrease in the absolute numbers of peripheral monocytes among the elderly (269 ± 112/cmm in the aged vs 269 ± 96/cmm in the controls, shown in Fig. 1). On the other hand, the PL of monocyte chemiluminescence, shown in Fig. 2, tended to be lower in the aged than in the controls (3,767 ± 2,301 RLU in the aged vs 5,209 ± 2,076 RLU in the controls, p < 0.10). Phagocytosis by neutrophils initiates a marked increase in oxidative metabolism. This increase includes oxygen consumption associated with the formation of active oxygen as well as increased glucose oxidation via the hexose monophosphate shunt (9-11). Killing of engulfed microorganisms can be brought about by two different

![Fig. 1. Blood monocyte count. a: individuals under 40 years of age. b: individual over 70 years of age. c: Mean ± SD RLU: relative light unit](image1.png)

![Fig. 2. Monocyte chemiluminescence. a: individuals under 40 years of age. b: individuals over 40 years of age. c: Mean ± SD RLU: relative light unit, Sec: Second](image2.png)
mechanisms: an oxygen-dependent one involving MPO and the formation of $\text{H}_2\text{O}_2$, $^1\text{O}_2$, $\text{O}_3$ and $\text{OH}^-$, and an oxygen-independent one involving graule proteins such as lactoferrin and lysozyme. Human blood monocytes are similar to neutrophils in many aspects of their phagocytic behavior. In patients with chronic granulomatous disease, who suffer from severe and recurrent bacterial infections, oxidative metabolism impairment is found in both neutrophils and monocytes (12). It seems to us that the tendency for monocyte chemiluminescence to decrease in the aged might to be one possible cause of easy susceptibility to infection. A previous study in our laboratory revealed that certain immunomodulators accelerated the chemiluminescence production of neutrophils in man (13). Therefore, immunomodulators are expected to act in the prevention and management of infection in the aged.

As monocytes mature into macrophages, phagocytic activity, protein synthetic capacity, surface receptors for IgG and certain complement compartments develop. Major functions of macrophages are the collection of garbage of the body and disposing of damaged cells and cell debris, the defense against intracellular pathogenic bacteria and fungi, and the interaction with lymphocytes in the immune response (14). The MPI in the aged, presented in Fig. 3, was significantly decreased as compared to the younger controls ($2.51 \pm 1.82\%$, in the aged vs $4.41 \pm 4.16$ in the control, $p < 0.05$). The lower MPI in the aged is thought to indicate the retardation or the impairment of the maturation of monocytes into macrophages, resulting in the impairment of the initiation of cellular immunity to microorganisms.

On the basis of these findings, functional alterations in the monocyte-macrophage system seem to participate in increased susceptibility to infection in the aged. Further studies are now being conducted to elucidate the relationship of monocyte chemiluminescence and MPI to other monocyte-macrophage functions in the aged.

![Graph](http://escholarship.lib.okayama-u.ac.jp/amo/vol39/iss6/4)

Fig. 3. Monocyte precursor index. a: individuals under 40 years of age. b: individuals over 70 years of age. c: Mean ± SD
REFERENCES


