
Defective Function of Peripheral Blood Monocytes from Patients with Non-Hodgkin's Lymphoma

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ABSTRACT

The aim of the study was to analyze the function of peripheral blood monocytes from patients with Non-Hodgkin's lymphoma before and after incubation with zymosan and indomethacin. Peripheral blood samples were collected from 28 patients with malignant lymphoma (13 males and 15 females with age range 20-65) years. Their clinical record and pathologic material were reviewed. The control group consisted of 17 normal subjects, (9 men and 8 women) of age range of 20-45 years. The following investigations were carried out in all patients: Bactericidal activity against *Escherichia coli*. Level of superoxide anion and Chemiluminescence's Technique for analysis of oxygen metabolite Results. The mean bactericidal indices of *E. coli* by peripheral blood monocytes without indomethacin were 56.75 (SD \pm 10.5) in control group at 60 minutes and it was 36.88% (SD \pm 11.3) in Non-Hodgkin's lymphoma patients. The level of INT was greater in healthy control than NHL-patients. The improvement after addition of zymosan was significant in all groups. The peak generation of chemiluminescence in Non-Hodgkin lymphoma patients was 11256×10^{-3} CPM at 20 minutes and in healthy controls 16575×10^{-3} CPM at 5 minutes and after incubation with zymosan and indomethacin were 13843×10^{-3} CPM at 5 minutes in NHL patients and 16312×10^{-3} CPM in healthy controls. The time of appearance of CL peak improved in Non-Hodgkin's lymphoma patients ($p < 0.01$) but there is no difference in the time of CL peak of the healthy controls.

Key Words: Non-Hodgkin's lymphoma, Function, Monocyte, Bactericidal activity, Chemiluminescence.

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INTRODUCTION

Monocytes are human macrophages of great importance to human health. They are essential accessory cells in most immune responses and act directly to kill a variety of microbial pathogen.

The major functions of the monocyte-macrophage system in resistance to and killing of bacteria and tumor cells and this play an important role in the degradation of dead cells and extracellular debris and also in the tissue repair by promoting proliferation of fibroblast and development of vasculature^[1]. The role of monocyte-macrophage in cellular immunity is of great importance to body defense^[2]. Of particular interest is the finding of a number of Immunological process that alter the morphology, metabolism and function of monocytes: This involves the activation of an enzyme (s) in the membrane, NAD (P) H oxidase, capable of the one-electron reduction of molecular oxygen into the superoxide anion (O_2^-) radical and is termed the respiratory burst^[3].

Singlet oxygen (1O_2) is also produced during spontaneous dismutation of O_2^- and is responsible for the emitted light or chemiluminescence (CL) detectable after activation of the respiratory burst. Such highly reactive oxygen products have all been implicated as microbicidal agent within the phagocytes, either by themselves or in concert with lysosomal enzyme^[4]. Immunological deficiency in lymphoma is one of the most characteristic features of a group of diseases related to the direct involvement of the immune system in the malignant process and indirectly to the extent that the tumor burdens is immunosuppressive. In the present study, we tested monocytes from a number of Non-Hodgkin's Lymphoma patients to evaluate peripheral blood monocytes before and after stimulation with zymosan and indomethacin.

MATERIALS and METHODS

Peripheral blood samples were collected from 28 patients with malignant lymphoma (13 males and 15 females with age range 20-65 years. Their clinical record and pathologic material were reviewed. The control group consisted of 17 normal subjects, 9 men and 8 women) of age range of 20-45 years.

The following investigations were carried out in all patients:

Isolation of Blood Monocytes

Heparinized venous blood was collected in graduated glass tubes. (Heparinized blood was diluted 1:1 with 0.15 M NaCl and divided into aliquots of 25 mL which were slowly layered over a mixture containing ficoll-visotrast (SERVA, Heidelberg, visotrast 370), in falcon centrifuge tubes.

After centrifugation at 1500/minutes at 37°C the mononuclear cells layer appearing between the plasma and the sediment cells was washed twice with Eagle-MEM (37°C). Separated cells added to 14 mL MEM (minimal essential media) and 1.4 mL AB-serum incubated for 90 minutes at 37°C with slowly swinging. After incubation they were washed twice with cold MEM.

Leukocytes and differential counts were performed on both cell suspensions. Papenheim and nonspecific esterase staining determined the percentage of monocytes in the separated mononuclear suspensions and it was found to be approximately 10-15% contamination of mononuclear cell preparation by lymphocytes. The monocyte number was adjusted to 1×10^6 /mL in MEM mixture. The cell vitality was tested by using Acridinorange.

Isolation of *E. coli*

A strain of *E. coli* (NCTC 10418) was prepared. The bacteria were cultured for 24 hours at 37°C. They were then collected and washed in 0.15 M NaCl by centrifugation adjusted to 1×10^7 /mL in Eagle - MEM. The actual number of bacteria was determined by usual plating procedures and the number of colonies was recorded after 24 hours of incubation at 37°C.

Bactericidal Function Method

With modification of the method of Alexander et al^[5], two mL of the monocyte mixture were incubated with 1 mL (1×10^7) of bacteria suspended in MEM with an approximate bacteria: Monocyte ratio of 5: 1 and to this suspension 0.5 mL of NHPS was added as well as 1.5 mL of MEM solution. The control tubes containing 0.2 mL bac-

teria (1×10^7), 0.2 mL NHPS and 1.6 mL MEM. Both control specimens and the bacteria-monocyte suspensions were rotated at 37°C end over end at a rate of 10 rotations per minute to promote contact between bacteria and monocytes. Samples of 0.5 mL of the incubated monocyte bacteria suspension removed at 60 minutes (group A (control group), group B without indomethacin and group C with 1 µg/mL indomethacin) then cultured on the blood agar plate. The plates were counted 24 hours after incubation at 37°C and the results were expressed as the index of monocyte bactericidal activity.

Index of bactericidal activity:

1. No. colonies after monocyte lysis at time t X 100

No. colonies at 0 hour

Superoxide Anion Assay

Superoxide anion was measured by the photometric measurement of the product of INT^[6]. INT (2{4-Iodophenyl}-5 phenyl tetrazolium chloride. INT was measured spontaneous and after stimulation with opsonized zymosan after reduction of tetrazolium salts eg NBT to formazan. The modified method used for this micro assay involved 75 µL INT instead of NBT (P-nitroblue tetrazolium chloride)^[7,8]. The test was accompanied on microtiter plates with 50 µL cell suspension (1×10^6 monocytes) per well. The formazan was extracted from the pellet with 150 µL dimethylsulphoxide. After extraction 50 µL NaOH were added to the supernatants. The absorbance of supernatants was measured in a photometer at 620 nm.

Chemiluminescence Technique

Chemiluminescence where emitted light was measured by appropriate detection^[9]. Samples of 1 mL cell suspension (10^6 cell in Hanks solution stimulated with zymosan (200 µL) or zymosan and indomethacin (1 µg/mL indomethacin of MERCK SHARP & DOHME-USA) in clinilumat LB 9502 (Fa Bethold-Germany) at 37°C after 30 minutes incubation. 100 mL of luminol (10^4 M PBs) were added as sensitizer to each sample. The count per minute (CPM) was observed in the 5, 10, 15 and 20 minutes.

Statistical Analysis: Data were processed by microcomputer which was also used for making statistical tables. Correlation between immunologic characteristics were investigated by means of analysis of variance and the Chi-Square test.

RESULTS

Monocyte-Bactericidal Function From Patients with Non-Hodgkin's Lymphoma

The mean bactericidal indices of *E. coli* by peripheral blood monocytes without indomethacin were 56.75 (SD 10.5) in control group at 60 minutes and it was 36.88% (SD 11.3) in Non-Hodgkin's lymphoma patients.

It was observed that bactericidal activity of the patients with Non-Hodgkin's Lymphomas was significantly lower than that in the healthy control. The bactericidal activity after incubation the blood monocytes with 1 µg/mL indomethacin show increased bactericidal indices in NHL to 56.6 (SD ± 8.7) and in healthy control to 66.6 (SD ± 12.6) The P value was 0.01 when the patients with Non-Hodgkin's lymphomas were compared with the control group.

INT Assay

INT from patients with Non-Hodgkin's lymphoma: The production of reduced formazan was measured. The level of INT was greater in healthy control than NHL-patients. The improvement after addition of zymosan was significant in all groups.

The P Value after stimulation with zymosan was < 0.001 in healthy and < 0.0001 in Non-Hodgkin's lymphoma.

Chemiluminescence Assay

Table 2 and 3 show that the maximum peak of CL response by peripheral monocytes from Non-Hodgkin's lymphoma.

The peak generation of chemiluminescence by 1×10^6 monocytes in the presence of luminol (10^4 M in PBS) in Non-Hodgkin lymphoma patients was 11256×10^{-3} CPM at 20 minutes and in healthy controls 16575×10^{-3} CPM at 5 minutes.

The generation of chemiluminescence by $1 \times$

Table 1. Level of INT

Patients	Spontaneous INT	Stimulation with zymosan
NHL	0.038 (SD 0.05)	0.326 (SD 0.188)
Healthy control	0.100 (SD 0.061)	0.300 (SD 0.135)

10^6 monocytes in presence of luminol (10^4 M in PBS) incubated with zymosan and indomethacin were (13843×10^{-3} CPM) at 5 minutes in NHL patients and in healthy controls were 16312×10^{-3} CPM.

The time of appearance of CL peak improved in Non-Hodgkin's lymphoma patients ($p < 0.01$) but there is no difference in the time of CL peak of the healthy controls. The CL peak in Non-Hodgkin's lymphoma remained higher than that of healthy controls at the time between 10 to 20 minutes that means the indomethacin inhibits the cyclooxygenase activity and increase the activity of monocytes in Non-Hodgkin's lymphoma patients more than the healthy controls.

DISCUSSION

Blood monocytes from Non-Hodgkin's lymphoma patients in late stages are impaired in their ability to kill *E. coli*. Non-Hodgkin's lymphomas in early stage show that results are equivalent to the low responding control group, but still at the lower limit of normal immune function and its bactericidal activity of monocytes is normal.

Blood monocytes of Non-Hodgkin's lymphoma in disseminated stages, show a decreased ability to lyse *E. coli* due to mature lacking hydrolyze activity, Ig changes, deficiency of C3 and uncontrolled of PGE₂^[10]. The uncontrolled released of PGE₂ by adherent cells is considered to be one of the factors that suppress monocytes and T-cells function in malignant lymphoma in advanced stages.

The bactericidal activity of monocytes in patients with Non-Hodgkin's lymphoma was lower than in healthy^[11]. After exposure of monocytes to indomethacin, the functional activity of monocytes from patients and healthy was modified^[12].

The measurement of O₂⁻ by the INT method,

which depends on the reduction of INT were decreased in Non-Hodgkin's lymphoma and there were restore of INT after incubation with zymosan as improvement of respiratory burst by initiation of the secretion as a direct result of interaction of the particles with cell surface restore and as a result of phagocytosis itself.

The monocytes release in O₂⁻ to phagocyte stimuli and this O₂⁻ dismutation can account for all of the H₂O₂ found released under the same conditions^[13]. The study have shows a correlation between O₂⁻ production by phagocytes and microbial killing of microorganisms by monocytes as well as destroying the O₂⁻ produced by these phagocytes. However O₂⁻ itself is not particularly toxic and may contribute a little directly to the microbicidal activity of phagocytes^[14,15].

The addition of zymosan acted as opsonization active substance which led to the increase of complement component production and changed of cell membrane and activated the phospholipase A₂ and the NAD (P) H-oxidase; and there were improvement of the respiratory burst of monocytes of Non-Hodgkin's lymphoma patients^[16].

The results demonstrate that decreased lytic activity of monocytes and dysfunction in release of O₂⁻ as a source of H₂O₂ and also in its ability to react with H₂O₂ to generate ¹O₂^[17].

In Non-Hodgkin's lymphoma patients, the CL peak (a peak of H₂O₂ species) was delayed with significant values but after incubation with indomethacin, there was a restore of lytic activity and oxidative metabolism. These data suggest that the delay in appearance of the CL peak seem to be compensated after addition of indomethacin. The production of oxygen radicals by macrophages is inhibited by PGE₂ which suggests that PGE₂ is a factor that regulates the production of toxic oxygen metabolites by monocytes which ha-

Table 2. CPM zymosan stimulated monocytes

	Without stimulation	5 minutes	10 minutes	15 minutes	20 minutes
NHL	8050 (SD 750)	9500 (SD 780)	10550 (SD 820)	10900 (SD 810)	11256 (SD 835)
Control	12400 (SD 1300)	16575 (SD 1650)	13600 (SD 1250)	12500 (SD 1100)	11800 (SD 1100)

Table 3. CPM Zymosan and indomethacin stimulated monocytes

	Without stimulation	5 minutes	10 minutes	15 minutes	20 minutes
NHL	8060 (SD 650)	13843 (SD 1100)	13600 (SD 1050)	13000 (SD 1150)	12200 (SD 950)
Control	12500 (SD 1150)	16312 (SD 1575)	13400 (SD 1280)	12800 (SD 1260)	12000 (SD 1100)

ve a very important role in the killing of microorganisms and the tumor cells^[15]. Monocytes are stimulated to release H₂O₂ by the zymosan or indomethacin. This model was set up to ask whether monocytes secretion of H₂O₂ could be sufficient to kill the microorganism and the tumor cells.

Bacterial killing, the paramount functional features of the monocytes are drastically affected by PGE. The production of intraphagolysosomal toxic oxygen metabolites by monocytes has a very important role in the killing of microorganism^[15]. Therefore, the excess in the production of prostaglandin by monocyte in-patients with malignant lymphomas could explain the impaired lytic function of monocytes.

A partial restoration of monocyte functions after invitro incubated with PGE2 inhibitors, this study demonstrates decreased lytic activity and dysfunction in the generation of toxic oxygen metabolites of monocytes from Non-Hodgkin's lymphoma patients in disseminated stages, and this is associated with excessive production of PGE2 which corrected in vitro by addition of indomethacin^[18,19]. Further more, inhibitors of prostaglandin synthesis could correct the impaired kinetics of oxidative metabolism of monocytes^[20].

Finally the study has shown correlation between the monocytes dysfunction in Non-Hodgkin's

lymphoma patients and impaired level of chemiluminescence, INT level and bactericidal activity. These functions corrected by addition of indomethacin as a PGE2 inhibitor, and/or zymosan, which is responsible for the release of lysosomal acid hydrolases during the phagocytosis.

Abbreviation

NCTC: Natural collection of type cultures, NHPS: Normal Pool Human Serum,

MEM: Minimal essential media,

INT: (2{4-Iodophenyl}-5 phenyl tetrazolium chloride.

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