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Rapid Effect of Interferon-γ on Human Monocyte Chemiluminescence

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The effect of lymphokines and interferon-γ on human monocyte chemiluminescence was studied by measuring the phorbol myristate acetate-induced luminol-dependent chemiluminescence. When human peripheral blood monocytes were incubated with lymphokines (culture supernatants of concanavalin A-stimulated human leukocytes), their ability to generate chemiluminescence increased rapidly, reaching a maximal level at about 4 h, and then decreased on further incubation. Similar results were obtained in experiments using a commercially available human interferon-γ in place of crude lymphokines. The increasing effect of interferon-γ on monocyte chemiluminescence was dose-dependent in the range of 0.1—10^2 U/ml. These results show that the monocyte chemiluminescence may be clinically useful as a simple and rapid method for the determination of human interferon-γ.

Keywords——interferon-γ; lymphokine; monocyte; chemiluminescence; luminol; phorbol myristate acetate

Introduction

Lymphokines, which are soluble factors derived from T lymphocytes and well-known chemical mediators of cell-mediated immunity, have aroused increasing interest in recent years. In particular, macrophage activating factor (MAF) which renders macrophages cytotoxic to tumor cells *in vitro* is thought to play an important role in tumor immunity. So, in clinical practice, it is important to know the immunological activity of the patients, such as the ability of lymphocytes to produce lymphokines and the responsiveness of macrophages to lymphokines.

At present, the tumoricidal activity is usually used as a marker of lymphokine-mediated macrophage activation. However, the method is complicated and time-consuming. So, it is desirable to establish a simple and rapid method for the determination of lymphokines in clinical practice.

Recently, the chemiluminescence method has begun to be used for the monitoring of lymphokine-mediated macrophage activation.[1] Previously, we found a rapid effect of lymphokines on murine macrophages by use of the chemiluminescence method.[2] That is, the ability to generate chemiluminescence of macrophages increased markedly after only 4 h of treatment with lymphokines. We have devised a new type of apparatus suitable for the measurement of macrophage chemiluminescence, and developed a simple and rapid method for the determination of murine lymphokines.[3] Subsequently, we examined the effect of lymphokines on human monocyte chemiluminescence in order to apply this phenomenon clinically. In addition, the effect of interferon-γ (IFN-γ) was also examined, since it has been identified as one of the MAFs in murine[4] and human[5] systems by use of recombinant IFN-γ.

In this paper, we wish to show that measurement of the monocyte chemiluminescence is clinically useful as a simple and rapid method for the determination of IFN-γ.

Materials and Methods

Media and Culture Conditions——Unless otherwise stated, cells were cultured in RPMI 1640 medium (Nissui Seiyaku, Tokyo, Japan) supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin and 10% heat-inactivated newborn calf serum (GIBCO, Grand Island, N. Y.). All cultures were performed at 37 °C in a humidified atmosphere containing 5% CO_2.

Preparation of Lymphokines——Human mononuclear cells (MNC) were separated from peripheral blood of healthy adults by centrifugation at $400 \times g$ for 30 min on a lymphocyte separation medium, Lymphosepar I (Ficoll-Conray solution, $d = 1.077$; Immuno Biological Laboratories, Fujioka, Japan), and washed three times with 0.01 M phosphate-buffered saline (pH 7.2). The MNC were suspended in medium at a cell density of 2.5×10^6 cells/ml, and then cultured with 5μg/ml of concanavalin A (Con A; Sigma Chemical, St. Louis, M. O.) for 48 h. Then the culture was centrifuged and the supernatant was retained as the source of lymphokines. As a control, a supernatant obtained from MNC cultured without Con A and then supplemented with Con A was used.

IFN-γ——Human IFN-γ, purified from culture supernatants of Con A-stimulated human leucocytes by the use of monoclonal anti-human IFN-γ antibody, was purchased from Japan Chemical Research Co., Ltd., Kobe, Japan. The IFN-γ (purity, $> 10^7$ U/mg) was first dissolved in distilled water at a concentration of 1×10^4 U/ml, and then diluted to the desired concentrations with medium.

Preparation of Monocytes and Lymphokine Treatment——The MNC obtained from the peripheral blood as above were plated on plastic dishes (Lux 5221, 35 × 10 mm) at a cell density of 1×10^6 cells/dish. After a 1—2 h incubation, nonadherent cells were removed by washing twice, and then the dish-adherent cells, regarded as monocytes, were incubated with 2 ml of media containing lymphokines or IFN-γ for various times. The purity of monocytes was greater than 90% as determined by nonspecific esterase staining.

Measurement of Monocyte Chemiluminescence——The measurement of monocyte chemiluminescence was performed as described previously using an apparatus suitable for the dish-adherent cells.[3] Briefly, a dish with adherent monocytes was washed twice with Hanks' balanced salt solution without phenol red (HBSS) and then placed in the reaction chamber warmed at 37 °C. The chemiluminescence was measured with a Lumicounter 1000 (Niti-On Medical and Physical Instruments Mfg. Co., Japan) equipped with an integrator-timer, immediately after the addition of 1 ml of the chemiluminescent reagent solution which consisted of 100 μg/ml of luminol (Wako Pure Chemical Ltd., Japan) and 500 ng/ml of phorbol-12-myristate-acetate (PMA; Sigma) in HBSS. The amount of chemiluminescence was expressed as the integrated counts for 5 min, and the time course of chemiluminescence was similtaneously recorded. The chemiluminescence index was calculated as follows:

$$\text{chemiluminescence index} = \frac{\text{chemiluminescence of lymphokine (or IFN-}\gamma\text{)-treated monocytes}}{\text{chemiluminescence of control monocytes}}$$

Statistical Analysis——The statistical significance of differences was determined by applying Student's t-test.

Results

Figure 1 shows the pattern of PMA-induced chemiluminescence of monocytes incubated with medium, control supernatants and lymphokine supernatants for 4 h. In all cases, after a time lag about 20 s, the chemiluminescence increased rapidly, showing a maximal level at about 1 min, and then decreased. Although there is little difference in the time course of the appearance of chemiluminescence among the three, the intensity of chemiluminescence of lymphokine-treated monocytes increased markedly in comparison with the other two. In another experiment, the increasing effect of lymphokine supernatants on monocyte chemiluminescence could be seen even at the dilution of 3% (data not shown). Although the chemiluminescence pattern of monocytes varied a little with the incubation time, measuring the chemiluminescence for only the initial 5 min was sufficient for our purposes.

Figure 2 shows the time course of the effect of lymphokines on monocyte chemiluminescence. When human monocytes were cultured *in vitro*, their ability to generate chemiluminescence decreased as the incubation time proceeded. However, the chemiluminescence generated from the lymphokine-treated monocytes increased rapidly, reaching a maximal level (about 1.5 times the control) at about 4 h, and then decreased to the control level. When the incubation time was further prolonged, in some cases, the chemiluminescence of the

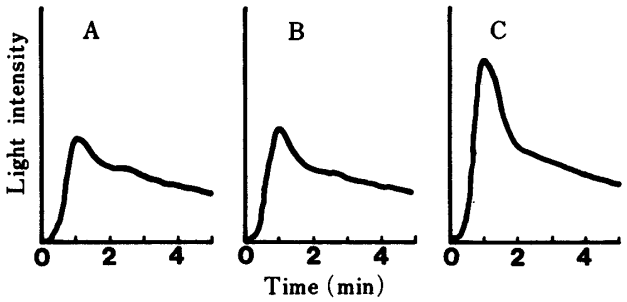

Fig. 1. Time Course of PMA-Induced Monocyte Chemiluminescence

Monocytes, which were incubated for 4 h with medium alone (A), or media containing 30% control supernatants (B) or 30% lymphokine supernatants (C), were exposed to 100 μg/ml of luminol and 500 ng/ml of PMA, and the chemiluminescence generated from the monocytes was recorded for 5 min.

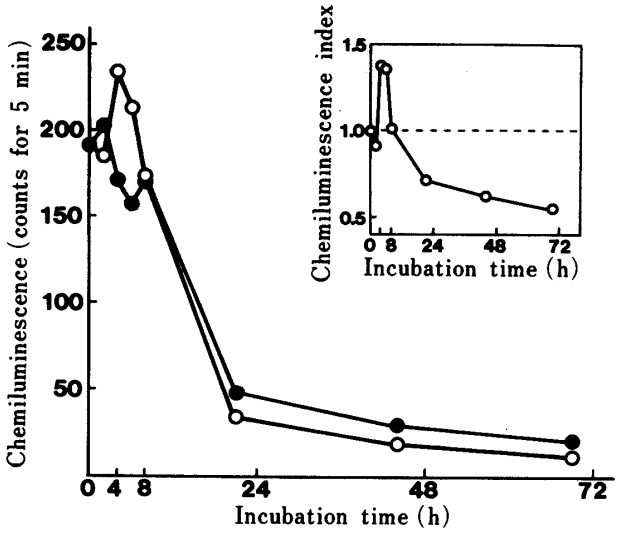

Fig. 2. Time Course of the Effect of Lymphokines on Monocyte Chemiluminescence

Monocytes were incubated with media containing 30% lymphokine supernatants (○) or 30% control supernatants (●) for various times, and then the chemiluminescence was measured. The ratio of the chemiluminescence of lymphokine-treated monocytes to that of the control (chemiluminescence index) is shown in the inset.

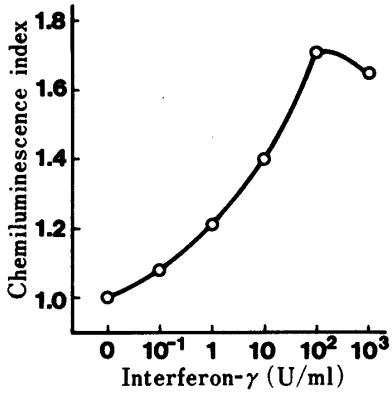

Fig. 3. Effect of Concentration of IFN-γ on Monocyte Chemiluminescence

Monocytes were incubated for 3—5 h with media containing various concentrations of IFN-γ, and then the chemiluminescence was measured. Each point was calculated by dividing the mean of duplicate cultures by the mean of the control ($n=5$). The differences between the chemiluminescence of IFN-γ (more than 10 U/ml)-treated monocytes and control monocytes were statistically significant ($p<0.05$).

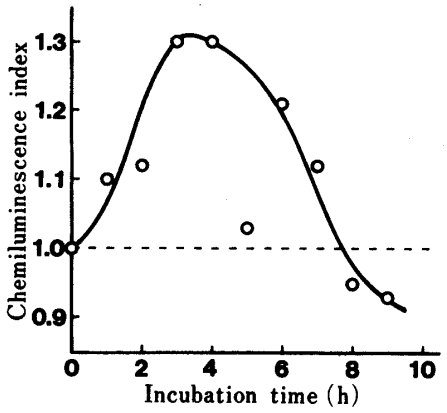

Fig. 4. Time Course of the Effect of IFN-γ on Monocyte Chemiluminescence

Monocytes were incubated with or without 100 U/ml of IFN-γ for various times, and then the chemiluminescence was measured. The results were expressed as Chemiluminescence Index at that time.

lymphokine-treated monocytes fell below that of the control. Such a rapid increase in chemiluminescence of lymphokine-treated monocytes was reproducibly observed with statistical significance ($p<0.05$) in repeated experiments.

To ascertain what kind of lymphokines induces a rapid chemiluminescent response in human monocytes, we examined the effect of IFN-γ on monocyte chemiluminescence, since IFN-γ is well-known as one of the lymphokines which activate monocytes and is commercially available.

As shown in Fig. 3, the chemiluminescence generated from the monocytes, which were incubated for 3—4 h with various concentrations of IFN-γ, increased dose-dependently in the concentration range of 0.1—10^2 U/ml.

Next, the effect of incubation time on monocyte chemiluminescence was examined at the concentration of 100 U/ml IFN-γ. The time course of the effect of IFN-γ on monocyte chemiluminescence is quite similar to that of crude lymphokines. As shown in Fig. 4, the chemiluminescence of IFN-γ-treated monocytes increased with incubation, reaching a maximal level after 3—4 h, and then decreased on further incubation. These results show that IFN-γ is at least one of the lymphokines which induce a rapid chemiluminescent response in human monocytes.

Discussion

In this paper, we showed that the effect of lymphokines on human monocytes could be determined simply and rapidly by use of the chemiluminescence method, as in the murine system. However, the effect of lymphokines on monocyte chemiluminescence was short-lived, that is, it became maximum at about 4 h, and then disappeared on further incubation. Seim[6] has already reported concerning the effect of lymphokines on monocyte chemiluminescence, but he did not observe the marked increase in chemiluminescence of lymphokine-treated monocytes. Since he measured monocyte chemiluminescence only after a 24 h incubation with lymphokines, such prolonged incubation may be the reason for his failure to observe the effect of lymphokines on monocyte chemiluminescence.

On the other hand, Matsumoto and Imanishi[7] have recently reported that recombinant human IFN-γ increased the ability of human monocytes to generate chemiluminescence. Interestingly, they measured the monocyte chemiluminescence after a 20 h incubation with IFN-γ. It is unclear why they could detect the increasing effect of IFN-γ on monocyte chemiluminescence at 20 h while we could not, although there are some differences in experimental conditions.

In the case of the murine peritoneal exudate macrophages, we previously observed that the chemiluminescence of the lymphokine-treated macrophages increased biphasically during incubation for 3 d.[8] In the present study, however, the chemiluminescence of lymphokine-treated monocytes did not increase on further incubation. In some cases, it decreased below that of the control after 2—3 d. At present, we can not explain this.

In the early stage of incubation, however, the time course of the effect of lymphokines on human monocyte chemiluminescence was quite similar to that observed in the murine macrophage system. In the murine system, the time course of the early effect of lymphokines on macrophage chemiluminescence was well correlated with that of the priming step to induce cytotoxic macrophages.[8] However, it is unknown at present whether the increase in chemiluminescence of lymphokine-treated monocytes is also correlated with their cytotoxic activity. Further investigations will be required.

Quite recently, we observed in the murine system that recombinant murine IFN-γ increased the chemiluminescence of murine peritoneal exudate macrophages similarly to that of crude lymphokines. It is very interesting that IFN-γ could induce a rapid chemiluminescent response in both monocytes and macrophages, although they are different in maturation.

In this investigation, we showed that the ability of human monocytes to generate chemiluminescence increased markedly after only 4 h of incubation with lymphokines or IFN-γ, and that the increasing effect of IFN-γ on monocyte chemiluminescence was dose-dependent in the range of 0.1—10^2 U/ml. These results show that measurement of the monocyte chemiluminescence may be clinically useful as a simple and rapid method for the determination of IFN-γ. We are expecting that this chemiluminescence method will be applied

clinically to monitor the immunological activity of the patients, such as the ability of lymphocytes to produce IFN-γ and the responsiveness of monocytes to IFN-γ.

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