Note

Multinucleation of Macrophages with Dextran Sulfate *in Vitro*

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When macrophages obtained from rat bone marrow were incubated with dextran sulfate at 37°C, giant cell formation took place. Large multinucleated cells contained more than 200 nuclei. Multinucleation occurred at 0.5–100 µg/ml of dextran sulfate.

Macrophages play an important role in the immune

system, and their function is regulated by lymphokines produced by sensitized lymphocytes. In *in vitro* experiments, it was shown that 1,25-dihydroxyvitamin D₃,¹⁾ recombinant γ-interferon,²⁾ and interleukin 4³⁾ induced formation of multinucleated giant cells of macrophages. In addition, 1,25-dihydroxyvitamin D₃ induced fusion of human promyelocytic leukemia cells (HL60).⁴⁾ Quite recently, we showed that lignin derivatives also induced formation of multinucleated giant cells of macrophages.⁵⁾ These results led us to examine whether or not other compounds such as dextran sulfate and lipopolysaccharides induce multinucleation of macrophages.

Rat bone marrow macrophages were prepared by the method of Saotome *et al.*⁶⁾ The bone marrow cells (3×10^6 /ml) obtained from the femurs of JAR-2 rats (150–200 g) were cultured in 12-well plastic culture plates (Corning, N.Y., U.S.A.). After 48 hr of culture, the medium was removed and the attached cells (macrophages) were washed three times with culture medium. After these procedures, the purity of macrophages was more than 95%.⁶⁾ Macrophages were then cultured with and without dextran sulfate (average molecular weight, ~8,000) (Sigma Chemical Company, St Louis, Mo., U.S.A.) at 37°C. The cultures were observed under a binocular dissecting microscope and a phase contrast microscope every day.

Most of the macrophages were single cells after 5 days of culture without dextran sulfate (Fig. 1A). In contrast, after macrophages were cultured with dextran sulfate at 10 µg/ml, several giant cells appeared at day 3 as shown

Fig. 1. Micrographs of Macrophages.

The cells were cultured without (A) and with dextran sulfate (B) at 10 µg/ml for 3 days at 37°C, after removing floating cells, and then fixed with methanol and stained with 10% Giemsa solution. The magnification was $\times 100$.

Table I. DOSE RESPONSE EFFECT OF DEXTRAN SULFATE ON THE MULTINUCLEATION OF MACROPHAGES

Dextran sulfate ($\mu\text{g/ml}$)	Number of multinucleated cells per dish*
0	12
0.5	27
1.0	40
5.0	344
10	394
50	315
100	126

Cells washed three times with new culture medium were cultured for 3 days in the presence and absence of dextran sulfate at various concentrations.

* Multinucleated cells were formed at a part of culture dish where cell population density was high.

in Fig. 1B. The central giant cell contained more than 20 nuclei arranged in a ring.

An established mouse macrophage cell line (TtT/M-87)⁷⁾ did not form giant cells with dextran sulfate, nor was giant cell formation observed with other cell lines derived from rat liver, monkey kidney, rat hepatomas, human urinary bladder carcinomas, or mouse sarcoma. Lipopolysaccharides (10–100 $\mu\text{g/ml}$) did not induce giant cell formation of macrophages.

Macrophages were incubated with dextran sulfate at various concentrations (0.5–100 $\mu\text{g/ml}$). As shown in Table I, multinucleation took place in a dose dependent manner with dextran sulfate; a significant effect was observed at 0.5 $\mu\text{g/ml}$ and the giant cell formation was very active at 5–10 $\mu\text{g/ml}$ of dextran sulfate. However, at 100 $\mu\text{g/ml}$ giant cell formation decreased slightly. The largest multinucleated cell contained more than 200 nuclei.

Multinucleation hardly took place during the first 24 hr of incubation of macrophages with dextran sulfate at 37°C, but increased markedly during the next 24 hr and reached an almost plateau level at 72 hr. A similar course of giant cell formation was observed with lignin derivatives.⁵⁾ In the case of 1,25-dihydroxyvitamin D₃, it was reported that multinucleation of mouse macrophages occurred with a time lag and needed both RNA synthesis and protein synthesis.⁸⁾ In contrast, cell fusion by polyethylene glycol⁹⁾ or electrical pulses¹⁰⁾ takes place without a time lag, since the fusion is due to fast changes in membrane structure. Therefore, polyethylene glycol or electrical pulses initiate cell fusion at random. The multinucleation by dextran sulfate took place with a time lag. Therefore it might need

certain metabolic changes in macrophage cells.

The multinucleation was due to cell fusion.⁵⁾ In this study, colchicine at 0.3 μM completely inhibited multinucleation of macrophages. This indicates that microtubules are important in multinucleation of macrophages.

1,25-Dihydroxyvitamin D₃, γ -interferon, and interleukin 4 induced multinucleation of macrophages *in vitro*.^{1–3)} It is known that these compounds have certain biological activities not only *in vitro* but also *in vivo*. To our knowledge, on the other hand, dextran sulfate was thought to be biologically inactive. In a recent *in vitro* study, however, it was shown that dextran sulfate inhibited the growth of human immunodeficiency virus (HIV), which induces the acquired immune deficiency syndrome (AIDS).¹¹⁾ Our study clearly shows that dextran sulfate induced multinucleation of macrophages *in vitro*. In addition, our recent study showed that lignin derivatives (carboxylated or sulfonated) induced multinucleation of macrophages *in vitro*.⁵⁾ These results suggest that polyanions generally induce multinucleation of macrophages. Further studies on multinucleation of macrophages by dextran sulfate may contribute to a better understanding of the effects of dextran sulfate on macrophages in the immune system.

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