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Enhancing Effect of Product of Activated Macrophages upon Resident Peritoneal Macrophages

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= Abstract =Macrophages having Fc and complement receptors can produce several factors which could activate the lymphocytes.

It was assumed that the activated macrophages might secrete certain factors which could activate the resident macrophages. Resident macrophages from rat peritoneal cavity were incubated with two kinds of supernatant obtained from the culture of thioglycollate-elicited macrophages with IgG-coated erythrocytes or complement-coated erythrocytes.

Supernatant from the culture of elicited macrophages and complement-coated erythrocytes enhanced the attachment with either IgG-or complement-coated erythrocytes, but supernatant induced by IgG-coated erythrocytes augmented the degree of attachment with IgG-coated erythrocytes only.

Any enhancing effect of both supernatants was not observed on the ingestion of IgG-or complement-coated erythrocytes.

Key Words: ***Activated macrophage, Fc receptor, Complement receptor***

INTRODUCTION

Macrophages elaborate a number of secretory products that may modulate the immunologic and inflammatory responses. The mononuclear phagocyte is essential for the development of cellular and humoral immunocompetence (Rosenthal 1980).

Macrophages produce a lymphocyte-activating factor, also known as interleukin 1, which stimulates T lymphocytes to produce a variety of lymphokines including a T-cell growth factor (Ruscetti and Gallo 1981). Macrophages secrete active proteases at neutral pH, including plasminogen activator, collagenase, and elastase. At inflammatory sites, macrophages will secrete acid hydrolase, capable of degrading collagen (Nathan *et al.* 1980).

On the other hand, a variety of soluble factors that influence macrophage's behavior have been described previously (North 1981). These products are elaborated by T lymphocytes and alter macrophage function as diverse as motility, cytotoxicity (Piessens *et al.* 1975), phagocytic activity (Rocklin *et al.* 1974), microbicidal capability (Nogueira and Cohn 1978), and the interactions of macrophages with other lymphocyte populations (Unanue 1976).

It was postulated that the activated macrophages might secrete certain factors which could also activate the unstimulated resident macrophages and enhance their opsonin-mediated attachment and phagocytosis (Fig. 1).

In order to clarify this assumption, resident macrophages in normal rat peritoneal cavity were incubated with two kinds of supernatant obtained from the culture of thioglycollate-elicited macrophages activated by binding to IgG-coated erythrocytes or complement-coated erythrocytes.

The degree of attachment (the percentage of rosette formation with attachment index) and the phagocytic activity (the percentage of ingestion with ingestion index) of the macrophages incubated with IgG-or complement-coated erythrocytes were measured.

MATERIALS AND METHODS

Macrophages: Rats (Wistar strain), weighing 180-200 g, were anesthetized with chloroform.

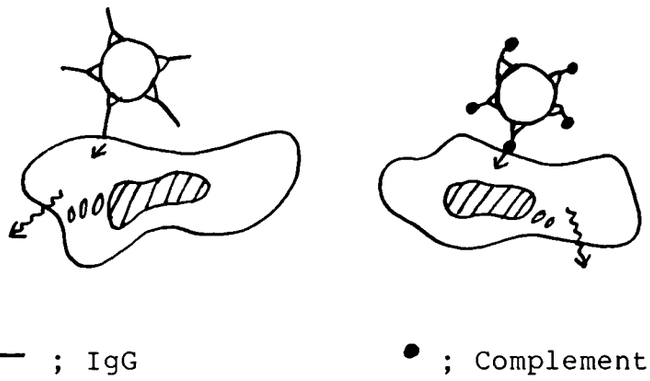

Fig. 1. Schematic representation of an assumption, which thioglycollate-elicited macrophages may secrete certain factors after their Fc or complement receptors bind to IgG- or complement-coated erythrocytes.

Peritoneal macrophages were harvested by peritoneal lavage with 60 ml of 4°C normal saline containing 50 U/ml of heparin (LipoHepin, Riker Lab.). The cells were washed twice in the medium and adjusted to a concentration of $5\text{-}10\times10^6$/ml in RPMI-1640 (GIBCO) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO) and containing a concentration of 50 μg/ml of gentamicin (GIBCO). 5 ml of the suspension was poured into 50 ml tissue culture flask (Falcon), and incubated for 6-8 hours in a 37°C, 5% CO_2 incubator. After washing the monolayer free of nonadherent cells, 4°C medium was added to the flask and allowed to stand at 4°C for 30 min. Macrophages were released from the flask by strong agitation with a pasteur pipette. Macrophage viability was tested for each set of experiments by 0.4% trypan blue exclusion method and macrophages were 91-94% viable.

Thioglycollate-elicited macrophages were harvested 3 days after intraperitoneal injection of 10 ml of fluid thioglycollate medium (Difco) and cultivated under conditions identical to those described for nonelicited macrophages (Conrad 1981).

IgG-coated eythrocytes: 1 ml of Rho(D)-positive, group O human erythrocyte (E) suspension (5×10^7/ml) was incubated with 1 ml of 1:250 diluted anti-Rho(D) antiserum [Rho(D) immunoglobuline, Cutter Lab.] for 1 hour at 37°C. The suspension was centrifuged for 5 min at 750 g, and pelleted human erythrocytes were suspended in the medium at a concentration of 4×10^7/ml (Hunt *et al.* 1981). This preparation is designated E(IgG).

IgM-coated erythrocytes: 1 ml of Rho(D)-positive, group A human erythrocytes(E) suspension ($5\text{-}10\times10^7$/ml), which had a regular agglutination titer of 1:32-1:64 against anti-A antiserum, was incubated with 1 ml of 1:64 diluted group B human pooled sera for 1 hour at room temperature (Greendyke *et al.* 1963). The suspension was washed and this preparation is designated E(IgM).

Complement-coated E(IgM): 0.1 ml of freshly thawed group AB human serum was mixed with 9.9 ml of a suspension of E(IgM) at a concentration of 5×10^7/ml. The mixture was incubated for 1 hour at 37°C and centrifuged for 5 min at 750 g. The pelleted E was adjusted to a concentration of 4×10^7 /ml (Reynolds *et al.* 1975). This preparation is designated E(IgM)C′.

Supernatants from the culture of activated macrophages: 5 ml of thioglycollate-elicited macrophages ($5\text{-}10\times10^6$/ml) was mixed with an equal volume of a suspension of E(IgG), or E(IgM)C′ at a concentration of 5×10^7/ml. The mixture was incubated for 4 or 24 hours at 37°C and centrifuged at 600 g for 30 min (Gery *et al.* 1981). The supernatants are designated S-G and S-C, respectively.

1 ml of resident peritoneal macrophages ($1\text{-}3\times10^7$/ml) was mixed with 4 mL of each supernatant and incubated for 24 hours at 37°C (Michl *et al.* 1979).

Rosetting and phagocytic assay: After 50 μl of E(IgG), E(IgM) or E(IgM)C′ at 4×10^7/ml put into each wells of tissue culture microplate (Nunclon), the indicator particles were then mixed with 50 μl of macrophages at 4×10^6/ml. The cells and indicator particles were centrifuged for 5 min at 50 g, incubated for additional 8 hours in a 5% CO_2, humidified incubator at 37°C and then gently resuspended and examined under light microscopy (Pommier *et al.* 1983).

The data from experiments examining the interaction of macrophages with the various erythrocyte preparations are presented as follows: percent of ingestion or attachment is the percentage of macrophages with erythrocytes ingested or attached. Ingestion or attachment index is (the percentage of macrophages which ingested or attached erythrocytes)×(average number of erythrocytes ingested or attached per macrophages) (Michl *et al.* 1979).

RESULTS

1. Attachment and ingestion of sensitized erythrocytes by resident macrophages

The attachment and ingestion indices obtained

when various preparations of human erythrocytes were incubated with resident rat peritoneal macrophages for 8 hours are shown in Fig. 2.

Neither group O nor group A erythrocytes were bound or ingested by these macrophages. E(IgM) and E(IgM)C′were attached but were not ingested. E(IgG) were ingested in significant numbers by resident macrophages. The unstimulated resident macrophages cultured without any activating factors showed higher degree of attachment with complement-coated erythrocytes, compared with that of IgG-coated erythrocytes.

2. Ingestion of E(IgG) and E(IgM)C′ by elicited macrophages

Elicited macrophages were more efficient in phagocytizing IgG-and complement-coated erythrocytes than nonactivated cells. As shown in Fig. 3, elicited macrophages ingested both E(IgG) and E(IgM)C′ more avidly than did resident cells.

Although only negligible ingestion occurred for complement-coated erythrocytes in case of the resident macrophages, thioglycollate elicited macrophages ingested E(IgM)C′ as much as resident macrophages ingested E(IgG).

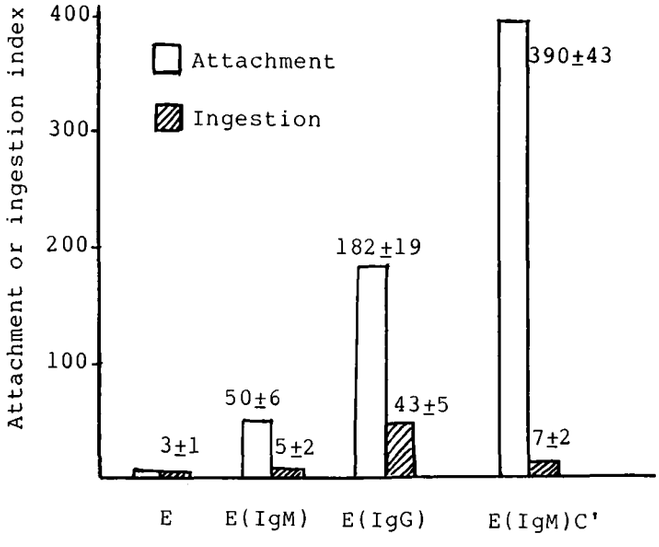

Fig. 2. Attachment and ingestion of sensitized human erythrocytes by resident macrophages.

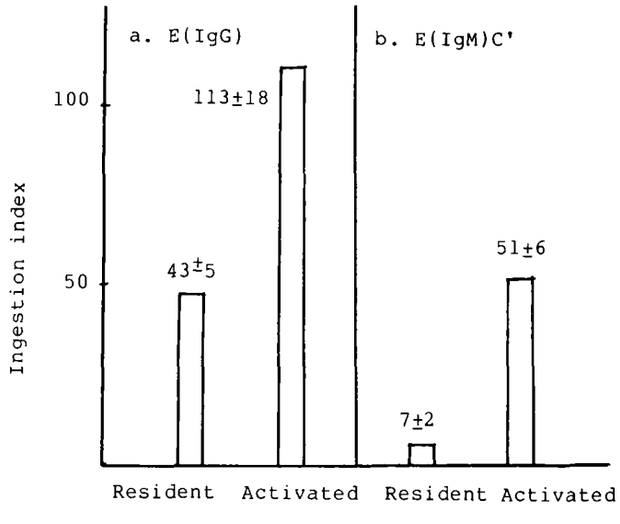

Fig. 3. Ingestion by activated and by resident macrophages of IgG-coated and complement-coated erythrocytes.

3. Effect of supernatants upon IgG-mediated attachment and ingestion

Incubation of resident macrophages with each supernatant (S-G, S-C) for 24 hours at 37°C generally increased binding of E(IgG) to the macrophage Fc receptors, as shown in Table 1.

In particular, supernatant of macrophage cultured with E(IgM)C′ for 24 hours remarkably increased the average number of E(IgG) bound to resident macrophages.

But most of supernatants did not have an effect upon the ingestion of E(IgG) by resident macrophages.

4. Effect to supernatants upon complement-mediated attachment and ingestion

Incubation of resident macrophages with the supernatant (S-C) of macrophage cultured with E(IgM)C′ for 4 hours at 37°C increased binding of E(IgM)C′ to the resident macrophages, as shown in Table 2.

Supernatants (S-G) of thioglycollate-elicited macrophages cultured with E(IgM)C′ did not have any considerable effect upon the attachment of E(IgM)C′ by resident macrophages.

Most of supernatants did not exert remarkable influence on the ingestion of E(IgM)C′ by resident macrophages.

Table 1. Attachment and ingestion of E(IgG) by resident macrophages incubated with supernatant

Supernatant	% Attachment	Attachment Index	% Ingestion	Ingestion Index
None	29±5	124±21	28±4	43±6
S-G(4)*	52±11	340±52	23±5	28±5
S-G(24)	50±9	340±44	32±5	61±7
S-C(4)**	62±9	384±42	24±3	25±5
S-C(24)	68±11	510±38	29±4	38±6

* S-G(4) or (24); supernatant of culture of macrophages activated by E(IgG) for 4 or 24 hours, **S-C(4) or (24); supernatant of macrophage cultured with E(IgM)C′ for 4 or 24 hours.

Table 2. Attachment and ingestion of E(IgM)C′ by resident macrophages incubated with supernatant

Supernatant	% Attachment	Attachment Index	% Ingestion	Ingestion Index
None	252±9	231±37	3±1	5±1
S-G(4)*	56±11	235±39	5±2	7±2
S±G(24)	57±11	343±38	6±1	13±2
S-C(4)	73±13	467±59	6±2	9±3
S-C(24)	85±15	569±58	9±2	16±4

* Abbreviation; See Table 1.

DISCUSSION

The degree of attachment of sensitized human erythrocytes by resident macrophages depends upon the kind of sensitizing material on erythrocytes and the presence of receptors on the plasma membrane of macrophages (Lasser 1983). Neither group O nor group A unsensitized erythrocytes were bound by resident macrophages, but E(IgM), E(IgG), and E(IgM)C′ were attached by macrophages in increasing order of attachment index.

Studies of Lay and Nussenzweig (1969) have demonstrated that murine macrophages have IgM receptors on their plasma membranes. Our studies showed some evidence of the presence of IgM receptors on macrophages, but this hypothesis is not generally accepted at the present time.

Thioglycollate-elicited macrophages are 1.5-3 times as efficient as nonactivated resident macrophages in ingesting E(IgG), and are 3-7 times as effective as those in ingesting E(IgM)C′ (Fig. 3).

Since we have not measured the time required for the ingestion of individual erythrocytes by elicited or resident macrophages, we cannot ascertain whether elicited cells merely have an increased total phagocytic activity or they exhibit an increased rate of ingestion as well.

Supernatants obtained from the culture of thioglycollate-elicited macrophages with E(IgG), or E(IgM)C′ were examined for the presence of any products from macrophages induced by the interactions between Fc receptor and E(IgG) or complement receptor and E(IgM)C′, respectively. A comparative study was performed between supernatants of 4-hour and 24-hour cultures(Gery *et al.* 1981). In general, it showed no significant difference between the supernatants.

Both supernatants of macrophage culture with E(IgG), or E(IgM)C′ could stimulate the macrophage Fc receptors and increase the number of variously sensitized erythrocytes bound to macrophages. Because we have not determined the number of Fc receptors per macrophage, we cannot recognize whether this increase of attachment ability results from the change of number or activity of macrophage receptors.

But most of supernatants could not influence the ingestion ability of E(IgG) by resident macrophages. So we can infer that the enhancement of ingestion ability of sensitized erythrocytes, induced by the stimulation of various supernatants is not always followed by rising of attached number. It has recently been proposed that phagocytosis results from a sequential interaction of ligands and receptors, but does not depend on the degree of attachment (Ehlenberger and Nussenzweig 1977).

In case of supernatants of macrophage cultured with E(IgM)C′ for 24 hours, binding of E(IgM)C′ to the resident macrophages was singificantly increased in number, but the attachment number of E(IgG) was not changed in comparison with the results of unincubated resident macrophages.

Supernatants of thioglycollate-elicited marcophages cultured with E(IgG), E(IgM)C′ did not exert any remarkable influence on the ingestion of E(IgM)C′ by resident macrophages, similar to the ingestion of E(IgG).

The data presented here demonstrate that products secreted by thioglycollate-elicited macrophages to be stimulated with E(IgG), E(IgM)C′ can affect the Fc receptor of resident macrophages and increase the attachment number of IgG-coated erythrocytes.

Our experiment also demonstrated that only the product of macrophage stimulated with E(IgM)C′ affects the complement receptor of resident macrophages, but other products secreted by macrophage to be stimulated with E(IgG) cannot influence the complement receptor of residents macrophages.

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= 국문초록 =

大食細胞의 活性化에 관한 實驗的 硏究

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金義鍾 · 金相仁

大食細胞의 活性化와 이에 수반되는 機能은 신체방어기전의 根幹을 이루는 현상이며, 細菌 또는 化學的 物質 등 많은 因子가 活性化에 관여하고 있다. 活性化된 大食細胞에서 由來되는 체액성 물질이 림프구를 活性化시킬 수 있음은 잘 알려진 사실이나 이러한 物質이 常在大食細胞에 어떠한 영향을 미치는가는 분명히 밝혀지지 않고 있다.

著者는 活性化된 大食細胞에서 由來된 物質이 常在大食細胞에 치는 영향을 追究하기 위하여 thioglycollate를 백서의 腹腔에 注入하여 얻은 大食細胞와 IgG結合赤血球 또는 補體結合赤血球를 함께 培養하여 배양상청액을 얻었으며, 이 上淸液을 첨가한 경우에 常在大食細胞와 IgG結合赤血球 및 補體結合赤血球사이에 일어나는 附着率과 貪食能을 대조군과 함께 비교측정하여 다음과 같은 結論을 얻었다.

常在大食細胞와 補體結合赤血球사이에 일어나는 附着率은 IgG結合赤血球와의 附着率에 비해 유의하게 높았으나, 補體結合赤血球보다 IgG結合赤血球를 더 많이 貪食하였다.

Thioglycolate로 자극된 大食細胞와 보체결합적혈구를 함께 배양하여 얻은 上淸液은 常在大食細胞와 IgG結合赤血球사이에 일어나는 부착율뿐만 아니라 보체결합적혈구와의 부착율도 증가시켰으나, thioglycollate로 자극된 大食細胞와 IgG결합적혈구를 함께 배양하여 얻은 上淸液은 常在大食細胞와 IgG결합적혈구사이에 일어나는 부착율만 증가시켰다. 두가지 상청액은 모두 大食細胞의 적혈구탐식능에는 영향을 미치지 않았다.