Stimulation of Macrophage DNA Synthesis by Polyamionic Substances through Binding to the Macrophage Scavenger Receptor

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We previously demonstrated that ligands of macrophage scavenger receptors such as acetylated low density lipoprotein (LDL), oxidized LDL and advanced glycation-end products (AGE) of the Maillard reaction induce the growth of peritoneal exudate macrophages, and that the activity of AGE is inhibited by the presence of an antibody for granulocyte/macrophage colony-stimulating factor (GM-CSF). To evaluate the suggested role of the scavenger receptor in the induction of macrophage growth, we compared the effect of various polyamionic compounds which were reported to either have or not to have competent activity for the binding of acetylated LDL to scavenger receptors on macrophage DNA synthesis. Among the polyamions exhibiting such activity, polygalanine acid (poly G) and dextran sulfate strongly augmented macrophage DNA synthesis, although they did not increase macrophage cell number. On the other hand, polyamions which are not ligands for the scavenger receptors did not show a significant augmenting effect, suggesting that the binding of polyamions to the scavenger receptor is important but not, by itself, sufficient. The augmentation of DNA synthesis in macrophages cultured with dextran sulfate or poly G was inhibited by the co-presence of anti-GM-CSF antibody, suggesting that the reaction is mediated by GM-CSF. However, dextran sulfate did not augment the production of GM-CSF in macrophages. Therefore, GM-CSF spontaneously present in macrophages might be a prerequisite for the induction of DNA synthesis.

Key words: macrophage growth; scavenger receptor; polyamion; granulocyte/macrophage colony-stimulating factor (GM-CSF)

It has often been observed that macrophages in peripheral tissues proliferate locally, especially in some pathological states such as inflammation, tumors and atherosclerosis. This suggests that there are some systems that induce the local proliferation of peripheral macrophages. Two hemopoietic factors, namely, macrophage colony-stimulating factor (M-CSF) and granulocyte/macrophage colony-stimulating factor (GM-CSF) are reported to have growth-stimulating activity for peripheral macrophages. We have shown that macrophage growth is also induced by scavenging dead cells or cell debris, and that the active ingredients of the phagocytic materials are lipids such as triglycerides, cholesterol esters and negatively charged phospholipids such as phosphatidylethanolamine and cardiolipin. The activity of the phospholipids was very strong among these lipid species.

Several years ago, Kodama et al. identified type I and II scavenger receptors in macrophages which recognize chemically or oxidatively modified low density lipoprotein (LDL). It is now believed that the binding specificity of the scavenger receptors is very broad: they bind not only denatured proteins and acetylated or oxidized lipoproteins, but also various polyamionic molecules, e.g., dextran sulfate, several polynucleotides, and lipid complexes containing negatively charged phospholipids. Quite recently, using Chinese hamster ovary (CHO) cells overexpressed with the macrophage scavenger receptor type II, Araki et al. demonstrated that advanced glycation end products (AGEs) produced by the long-term incubation of proteins with sugar aldehydes such as glucose, are primarily endocytosed by macrophage type I and II scavenger receptors. From their broad specificity, macrophage scavenger receptors might play at least a limited role in the phagocytic recognition of dead cells or of the cellular component from damaged cells, although the mechanisms underlying the elimination of autologous dead cells by macrophages are not fully understood.

Our recent studies demonstrated that the growth of murine peritoneal macrophages is induced by ligands of macrophage scavenger receptors such as modified LDL and AGEs, suggesting that the receptors are closely related to the growth induction of this cell type as well as to phagocytic recognition. The growth-stimulating activity of AGEs was completely abrogated by the co-presence of a blocking antibody against GM-CSF. Moreover, since AGEs stimulated the expression of GM-CSF mRNA, macrophage growth is thought to be induced by ligands of scavenger receptors in the mediation of GM-CSF produced by macrophages themselves.

It is still unclear whether the growth stimulating signal(s) is delivered by the ligand binding to the scavenger receptor. An alternative possibility is that the growth induction is solely dependent on the molecular nature of the internalized ligands. This latter possibility is partly supported by the results that oxidized LDL was able to induce the growth of resident (not stimulated) peritoneal macrophages, whereas acetylated LDL had no substantial activity on the resident population, and that lysophosphatidylcholine is a key substance for the activity of oxidized LDL. To determine the exact growth-stimulating mechanism, chemically well-defined ligands with growth-stimulating activity would be desirable, since the structure of modified LDLs or AGEs are highly complex due to the presence of reactive oxygen intermediates and undefined chemical structures.

In this paper, we determined the capacity of various

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polyanions to induce macrophage DNA synthesis and compared these results with the competitive capacity of these ligands for the binding of acetylated LDL to scavenger receptors.\textsuperscript{16,17} We found that among polyanions that have binding capacity for the scavenger receptors, dextran sulfate and polyguanionic acid (poly G) have potent augmenting activity for macrophage DNA synthesis, whereas polyanions that are not ligands for the scavenger receptor do not show this activity.

To gain insight into the mechanism of the augmentation, we then examined the role of GM-CSF produced by macrophages in this phenomenon, when cultured with active polyanions, by using anti-GM-CSF monoclonal antibody. The augmenting effect on macrophage DNA synthesis was almost completely abolished by the presence of anti-GM-CSF monoclonal antibody. However, dextran sulfate, an active polyanion, did not stimulate macrophage GM-CSF production.

**MATERIALS AND METHODS**

**Reagents and Antibodies** The polyanions, dextran sulfate (5, 50 and 500 K), polyvinyl sulfate, fucoidin, hyaluronic acid, heparin, chondroitin sulfate B, colomic acid, poly-L-glumatic acid, heparin, and polynucleotides were all purchased from Sigma Chemical Co. (St. Louis, MO). Aphidicolin was also from Sigma. Oxidized LDL was prepared from human plasma LDL as described.\textsuperscript{21} L-Cell-conditioned medium was used as a source of crude M-CSF, and was collected from 3- to 4-d cultures of L-929 cells. Rat anti-GM-CSF mAb (IgG2a) against recombinant mouse GM-CSF was purchased from Genzyme Corp. (Cambridge, MA). Purified rat myeloma IgG2a was from Zymed Labs, Inc. (San Francisco, CA). Goat anti-coly-stimulating factor-I (CSF-1) antiserum, which was raised against purified CSF-1 (M-CSF), was a generous gift of Dr. E. Richard Stanley, Einstein College of Medicine, NY.

**Mice and Macrophages** Male C3H/He mice and C3H/HeJ mice were purchased from breeding colonies of the Shizuoka Experimental Animal Farm, Shizuoka, Japan. The former strain of mice was used in most experiments unless otherwise indicated. Peritoneal exudate cells were obtained 3—5 d after the i.p. injection of 30 mg of soluble starch into each mouse. These cells were suspended in RPMI 1640 medium (Nissui Seiyaku Co., Tokyo, Japan) supplemented with 5% heat-inactivated fetal calf serum (FCS, HyClone Lab, Inc., Logan, UT), penicillin (100 U/ml) and Kanamycin (60 μg/ml) (hereafter referred to as medium A). For [\textsuperscript{3}H]thymidine ([\textsuperscript{3}H]Tdr) incorporation assay, the peritoneal cells were incubated in 96-well microplates (Corning, Corning, NY) at 2 × 10\textsuperscript{4} cells/well. For cell counting, they were incubated in 24-well microplates (Nunc, OK-4000, Roskilde, Denmark) at 1 × 10\textsuperscript{5} cells/well. Cells were incubated for 90 min at 37°C in a CO\textsubscript{2} incubator to allow them to adhere to the culture plates. The medium was then removed, and the nonadherent cells were removed by three vigorous washings with pre-warmed phosphate buffered saline (PBS) solution. More than 95% of the adherent cells were judged to be macrophages both by Giemsa staining and carbon particle uptake. These macrophages were added with the sample to be tested and cultured at 37°C in a humidified atmosphere of 5% CO\textsubscript{2} in air with 0.2 ml of medium A without changing the medium.

**[\textsuperscript{3}H]Tdr Incorporation** The incorporation of [\textsuperscript{3}H]-Tdr into cultured macrophages from C3H/He mice was measured as described.\textsuperscript{22} Briefly, methyl-[\textsuperscript{3}H]thymidine (3008 TBq/mmol; New England Nuclear, Boston, MA) was added to each plate at 37 kBq/ml and incubated for 18 h. The medium was discarded, and the cells were dissolved in 100 μl of 0.5% sodium dodecyl sulfate (SDS), followed by the addition of 100 μl of cold 10% trichloroacetic acid (TCA). The TCA-insoluble material was collected on filters with Labo Mash LM-101 (Labo Science, Tokyo, Japan). The filters were dried, and their radioactivity was counted in a liquid scintillation spectrophotometer. All experiments were performed in triplicate. Results are presented as the mean ± S.D., and the Student's t-test was used to determine statistical significance.

**Estimation of Macrophage Cell Number in Culture** To determine the number of macrophages per well, the number of adherent cells within five standard-sized areas (0.25 mm\textsuperscript{2}) in each of three wells was counted.

**Measurement of Lipopolysaccharide (LPS)** The LPS content in polyanionic samples was measured by Toxicolor (Seikagaku Kogyo, Tokyo).

**Analysis of GM-CSF Production and GM-CSF mRNA Expression** The titer of GM-CSF produced by macrophages was measured by GM-CSF-specific ELISA (Endogen, Cambridge, MA). For mRNA analysis, RNA was extracted from macrophages which were cultured without or with test samples, using the guanidine hot phenol method. The CDNA was synthesized with Moloney murine leukemia virus reverse transcriptase (Perkin-Elmer Cetus, Norwalk, CT) and Taq DNA polymerase (Promega, Madison, WI). Amplification of reverse-transcribed total RNA was carried out by PCR in a buffer containing 50 mM KCl, 10 mM Tris—HCl (pH 9), 1.25 mM MgC\textsubscript{2+}, 0.125% glycerol, 0.1% Triton X-100, 0.5 μM Taq DNA polymerase (Promega) and 7.6 pmol of mouse GM-CSF primer (Clontech, Palo Alto, CA). The reaction consisted of 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min and extension at 74°C for 3 min. PCR was done for 35 cycles on the same samples with synthetic primers for β-actin as a control. PCR products were analyzed by electrophoresis on 1.5% agarose gel and visualized by ethidium bromide staining.

**RESULTS**

**Macrophage Growth-Stimulating Activity of Various Polyanions** We previously reported that the ligands of scavenger receptors, namely, acetylated LDL, oxidized LDL and AGE-proteins, induce murine peripheral macrophage growth in vitro.\textsuperscript{21,22} Brown and Goldstein originally described that the binding of acetylated LDL to its receptor was competitively inhibited by several polyanionic compounds. They include some polynucleotides, e.g. poly G, poly I and poly I: C, polysaccharides such as dextran sulfate, or an artificial compound, poly-
There are also many polyamions which are not competitive against acetylated LDL binding, including polycytidilic acid (poly C), polyadenilic acid (poly A), polyuridilic acid (poly U), heparin, colomic acid, and poly-d-glutamic acid (poly-d-glu). To determine the relevance of the scavenger receptor in the induction of macrophage growth, we examined the macrophage growth-stimulating activity of the effective or ineffective polyamions listed above.

We first observed that poly G and dextran sulfate (500 K), which are effective competitors of the scavenger receptor, markedly induced the augmentation of $[^3]$H]TdR incorporation into peritoneal exudate macrophages (Table 1). The extent of the augmentation was comparable with that obtained using M-CSF-containing L-cell supernatant and oxidized LDL. Poly I and poly I:C, however, did not show such augmentation, and the activity of polyvinyl sulfate was weak, although these are also ligands of the receptors. In spite of their polyamionic nature, ineffective competitors such as poly A, poly C, poly U, heparin, colomic acid and poly-d-glu did not show any augmenting effects. The kinetics curves of $[^3]$H]TdR incorporation into exudate macrophages cultured with dextran sulfate (500 K) and poly G are shown in Fig. 1. Both ligands exhibited marked enhancement from day 4 to 6. Each of the kinetics is similar to those obtained with modified LDLs as well as AGE-bovine serum albumin (BSA). In contrast, poly C did not show any enhancing effect at any time point examined, confirming its lack of growth-stimulating activity. The dose–response relationships were determined on day 6. As shown in Fig. 2, the enhancing effect of poly G was observed from 20 μg/ml whereas the effect of poly C was again very weak or nil at the concentrations investigated. Dextran sulfate (500 K) significantly enhanced $[^3]$H]TdR incorporation, from 0.8 μg/ml, and its maximum effect was observed at 4 and 20 μg/ml, while dextran (208 kDa) at up to 100 μg/ml had no enhancing activity (data not shown).

To determine the relationship between the molecular sizes of the ligands and the growth-stimulating activity, we compared the activity of dextran sulfates with different molecular weights. As shown in Fig. 2, the enhancing effect of dextran sulfate of smaller molecular sizes (5 and 50 K, on average) was much weaker than that of dextran sulfate of 500 K.

It was previously reported that the response of resident macrophages towards the growth-stimulating effect of M-CSF is weaker than that of stimulated macrophages. So, we next comparatively examined the effect of dextran

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### Table 1. Effects of Various Polyamions on $[^3]$H]TdR Incorporation into Starch-Induced Macrophages

<table>
<thead>
<tr>
<th>Sample</th>
<th>Binding competition with Ac-LDL</th>
<th>Relative increase in $[^3]$H]TdR incorporation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp. 1 (Polynucleotides)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly G</td>
<td>Yes</td>
<td>2.2 ± 0.3 **</td>
</tr>
<tr>
<td>Poly I</td>
<td>Yes</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Poly I:C</td>
<td>Yes</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Poly C</td>
<td>No</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>Poly A</td>
<td>No</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Poly U</td>
<td>No</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>L-Cell sup. (20%, v/v)</td>
<td></td>
<td>(5.5 ± 1.7)</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>(1.0 ± 0.1)</td>
</tr>
<tr>
<td>Exp. 2 (Polysaccharides)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextran sulfate (500 K)</td>
<td>Yes</td>
<td>4.6 ± 0.1 **</td>
</tr>
<tr>
<td>Heparin</td>
<td>No</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Colomic acid</td>
<td>No</td>
<td>1.8 ± 1.0</td>
</tr>
<tr>
<td>Oxidized LDL (100 μg/ml)</td>
<td></td>
<td>(3.6 ± 0.2)</td>
</tr>
<tr>
<td>L-Cell sup. (20% v/v)</td>
<td></td>
<td>(5.4 ± 2.9)</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>(1.0 ± 0.2)</td>
</tr>
<tr>
<td>Exp. 3 (Others)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyvinyl sulfate</td>
<td>Yes</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>Poly-d-glu</td>
<td>No</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>L-Cell sup. (20%, v/v)</td>
<td></td>
<td>(3.5 ± 0.9)</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>(1.0 ± 0.3)</td>
</tr>
</tbody>
</table>

a) Starch-induced macrophages were cultured with or without various polyamions. $[^3]$H]TdR incorporation was assayed on day 6 in each experiment. b) Capacity of each polyamion to inhibit the binding of Acetylated-LDL to macrophages was reported in refs. 16 and 17. c) Values represent mean ± S.D. of triplicate determinations. In experiments 1, 2 and 3, the values of $[^3]$H]TdR incorporation (dpm/well) into macrophages cultured without samples were 532 ± 77, 322 ± 72 and 1654 ± 516, respectively. d) p < 0.05 vs. without samples. e) p < 0.01 vs. without samples. f) p < 0.005 vs. without samples. g) p < 0.0001 vs. without samples.
polyanions had no effect at all on macrophage cell number, in spite of their augmenting activity for macrophage DNA synthesis. To study whether the augmented $[^{3}H]$TdR incorporation is due to DNA repair, the effect of aphidicolin, a specific inhibitor of DNA polymerase- $\alpha$ that is involved in DNA replication, was examined. Table 3 shows that as in the cases of L-cell supernatant or oxidized LDL, the augmenting effect of dextran sulfate (500 K) was completely abrogated by the presence of aphidicolin in its non-toxic concentration. Even when aphidicolin was added to the macrophages cultured with the polyanions 2 h before the addition of $[^{3}H]$TdR on day 6, the inhibitory effect was also observed (data not shown). These results suggest that the active polyanions enhance DNA replication in macrophages.

**Effect of Anti-CSF Antibodies on Dextran Sulfate-Induced Macrophage Growth**

We previously reported that the macrophage growth-stimulating effect of AGES was completely abrogated by the presence of anti-GM-CSF monoclonal antibody with GM-CSF-neutralizing activity. Moreover, we observed that the growth-stimulating activity of acetyl-LDL and oxidized LDL is also blocked by anti-GM-CSF antibody (un-
published observation). Here, we confirmed this phenomenon using dextran sulfate as a stimulator. As expected, the existence of anti-GM-CSF completely blocked dextran sulfate-induced macrophage DNA synthesis, whereas the control isotype-matched antibody did not (Fig. 4). In contrast, anti M-CSF (CSF-1) antiserum, which is able to inhibit the growth-stimulating activity of M-CSF-containing L-cell supernatant, did not significantly block the action of dextran sulfate. Similarly, not anti-M-CSF, but anti-GM-CSF antibody inhibited the effect of poly G (data not shown). In other experiments, we confirmed that anti-GM-CSF antibody did not inhibit the effect of 20% L-cell supernatant (data not shown). These results suggest that GM-CSF, but not M-CSF is an essential factor in the induction of macrophage growth by the ligands of scavenger receptors.

**Absence of Activity Stimulating Macrophage GM-CSF Production in Dextran Sulfate**

The next question we focused on was whether ligands for the scavenger receptors could indeed induce the production of GM-CSF from macrophages. Earlier we reported that AGE-BSA stimulates the expression of GM-CSF mRNA. It is possible, however, that this effect is due to reactive oxygen species presumably included in AGE-compounds, since the transcription factor NF-kB, which regulates the transcription of mRNA of several cytokines including GM-CSF, might be activated by the oxidative stress produced by AGEs. In this respect, dextran sulfate has some advantages since, in addition to its molecular simplicity, it does not contain reactive oxygen species. Moreover, in the preparation of dextran sulfate, the contamination of LPS which is known to be a potent macrophage-activating agent was negligible (less than 20 pg/ml LPS in 20 µg/ml dextran sulfate). As shown in Table 4, GM-CSF which was released spontaneously into the macrophage supernatant was approximately 40 pg/ml at 6 h but had disappeared by 24 h. Unexpectedly, dextran sulfate did not augment GM-CSF release at 6 or 24 h of culture, while LPS as a positive control induced about a four-fold increase in GM-CSF release at 6 h. This result was in agreement with our previous study that dextran sulfate did not stimulate macrophage growth and GM-CSF production in vitro. This result was also consistent with our previous study that dextran sulfate did not stimulate macrophage growth and GM-CSF production in vitro.

**Table 4. Quantification of GM-CSF Concentration in the Supernatants of Macrophages Cultured with Dextran Sulfate (500 K)***

<table>
<thead>
<tr>
<th>Culture period (h)</th>
<th>Concentration of GM-CSF (pg/ml)*&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>39 ± 19</td>
</tr>
<tr>
<td>24</td>
<td>7 ± 11</td>
</tr>
</tbody>
</table>

*<sup>a</sup>* Starch-induced macrophages were cultured without or with 20 µg/ml dextran sulfate (500 K) or 1 µg/ml LPS. *<sup>b</sup>* Concentration of GM-CSF in macrophage supernatants was measured by GM-CSF-specific ELISA.

![Figure 4](https://example.com/fig4.png)

**Fig. 4. Effects of Anti-CSF Antibodies on Macrophage Growth-Stimulating Activity of Dextran Sulfate (500 K)**

Starch-induced macrophages were cultured without or with 100 µg/ml recombinant mouse GM-CSF, 20 µg/ml dextran sulfate (500 K) or L-cell sup (20%, v/v). Anti-GM-CSF antibody (50 µg/ml), rat IgG2a (50 µg/ml) or anti-M-CSF antiserum (final 1000-fold dilution) were simultaneously added as indicated. [3H]Tdr incorporation was assayed on day 6. Bars represent S.D. The data in A, B and C were from separate experiments.

![Figure 5](https://example.com/fig5.png)

**Fig. 5. Lack of Augmentation in GM-CSF mRNA Expression by Dextran Sulfate**

Starch-induced macrophages were cultured with or without dextran sulfate (500 K) or LPS for the indicated periods. GM-CSF mRNA expression was analyzed by PCR normalized to β-actin as described in Materials and Methods. Lanes: 1, size marker; 2, without sample; 3, with 1 µg/ml LPS; 4, with 4 µg/ml dextran sulfate (500 K); 5, with 20 µg/ml dextran sulfate (500 K). The data in A and B were from different experiments.
sult was supported by analysis of the expression of GM-CSF mRNA. Dextran sulfate did not augment GM-CSF mRNA between 1 and 24 h (Fig. 5).

DISCUSSIONS

We previously reported that negatively charged phospholipids, acetylated LDL, oxidized LDL and AGEs, which are ligands of the macrophage scavenger receptors, were able to induce the growth of peripheral macrophages. In the present study, we found that dextran sulfate (500 K) and poly G, other ligands for these receptors, also have a stimulating activity for macrophage DNA synthesis which was not accompanied by an increase in macrophage cell number. Since the molecular nature of these active ligands is mutually different, the augmenting activity against exudate macrophages does not seem to depend on a specific structure or molecular nature of the ligands. Polyamines which are not ligands for scavenger receptors did not show any such activity. Nor could we find any augmenting activity in heat-aggregated human IgG or formaldehyde-modified BSA (unpublished observations), which have been thought to be endocytosed by macrophages through pathways distinct from acetylated LDL receptors. Therefore, it is probable that the internalization of these ligands by scavenger receptors generate a signal(s) leading to the stimulation of DNA synthesis. In addition to type I and II scavenger receptors, however, several receptors on macrophages were reported to bind modified proteins, modified LDLs or AGEs, although the binding capacity of polyamic substances in these receptors was not fully determined. It is necessary to identify which receptor(s) is responsible for the growth induction.

Although poly I: C and poly U compete with acetylated LDL binding to scavenger receptors, they did not induce macrophage growth. It is possible that the absence of DNA synthesis-inducing activity in poly I: C might result from its triggering activity of the release of interferons and tumor necrosis factor (TNF), which inhibit macrophage growth. We observed that pol I potentiated \( ^{3}H \)Tdr incorporation of macrophages derived from C3H/HeJ mice, which are known to be a low responder strain for LPS (data not shown), suggesting that contaminated LPS in poly I could inhibit the augmenting activity. Actually, Toxicor assay showed that the poly I preparation contained about a ten-fold higher amount of LPS than the other polyanines, poly G, poly A, poly C or poly U (when 50 \( \mu \)g/ml of poly I was added to the cultures, the final LPS concentration was estimated to be 50 ng/ml).

Unlike modified LDLs or AGE-proteins, poly G and dextran sulfate (500 K) did not increase macrophage cell number in spite of their augmenting activity for \( ^{3}H \)Tdr incorporation. However, it is unlikely that the \( ^{3}H \)Tdr incorporation is brought about by DNA repair because the activity of dextran sulfate was inhibited by aphidicolin, which is an inhibitor for the DNA replication enzyme, DNA polymerase-\( \alpha \). Although the reason why the division of macrophages is not induced by these polyanines is unknown, it is possible that the accumulation of these artificial compounds into macrophage cells becomes an obstacle for the progression of mitotic processes.

We previously reported that the macrophage growth-stimulating activity of negatively charged phospholipids was not inhibited by anti-M-CSF (CSF-1) antiserum, but the corresponding activity of AGE-BSA was effectively inhibited by anti-GM-CSF antibody. In this paper, we confirmed these points using dextran sulfate and poly G as growth inducers. The data clearly show that the activity of the polyanines was blocked by anti-GM-CSF antibody but was unaffected by anti-M-CSF antiserum. However, dextran sulfate did not augment GM-CSF production, as determined by both GM-CSF specific ELISA and GM-CSF mRNA expression. We observed that more than 100 ng/ml of recombinant mouse GM-CSF was required to induce the growth of starch-induced macrophages in our assay (data not shown). Therefore, the amount of GM-CSF released into the medium when macrophages were cultured with dextran sulfate (i.e., approximately, 40 pg/ml at 6 h) was too low to induce the growth. Considering these points, GM-CSF might be a prerequisite, but not, by itself, a sufficient factor to induce growth, and the very low release of GM-CSF or membrane-bound GM-CSF might support the augmentation of DNA synthesis induced by ligands of scavenger receptors. Several authors have reported that the presence of a subliminal concentration of GM-CSF is able to potentiate a hemopoietic effect or macrophage growth-inducing effect of M-CSF. A trace amount of GM-CSF might also support the activity of the ligands of scavenger receptors. In this respect, it is necessary to examine whether the active polyanines stimulate the expression of GM-CSF receptor on macrophages. Alternatively, it is also possible that DNA synthesis is induced by an unknown factor which reacts with the anti-GM-CSF monoclonal antibody. The significance of endogenous GM-CSF can be elucidated by using macrophages derived from GM-CSF-deficient mice. Further study along this line will be needed for a better understanding of the mechanism of macrophage growth induction as well as the physiological significance of GM-CSF.

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REFERENCES