Brief Definitive Report

A MACROPHAGE TUMOR CELL LINE AND PLASMINOGEN ACTIVATOR

A Potential Model System for Macrophage Regulation of Enzyme Production*

By J. A. HAMILTON, P. RALPH, and M. A. S. MOORE

From Memorial Sloan-Kettering Cancer Center, New York 10021

Downloaded from http://rupress.org/jem/article-pdf/148/3/811/1089467/811.pdf by guest on 15 June 2021

Certain inflammatory stimuli such as thioglycollate medium, asbestos, and endotoxin are able to elicit macrophages into the peritoneal cavity of mice which synthesize and secrete increasing amounts of the neutral protease, plasminogen activator (1, 2). The product of the interaction of this enzyme with the circulating proenzyme plasminogen is another neutral protease, plasmin, that is known to activate certain enzyme cascades relevant to the inflammatory response, viz., the complement (3), the clotting (4), and the kinin systems (5). Macrophages are an important cell type in chronic inflammatory lesions and it has previously been suggested that plasminogen-activator production by macrophages might play a role in the traffic of macrophages through the body (6). The inhibition of enzyme production by anti-inflammatory glucocorticoids and hence inhibition of cell migration might account for some of the anti-inflammatory properties of these drugs (6). In contrast, agents such as phorbol myristate acetate, concanavalin A, and lymphocyte-conditioned medium can cause macrophages to produce more enzyme (7, 8). Little is known about how macrophages modulate plasminogen activator levels in response to the various agents and a study of the regulation of enzyme production might be important for the understanding of macrophage physiology and function. In addition, information about the anti-inflammatory properties of glucocorticoids and about the mechanism of action of these drugs at a cellular level might be obtained by studying how they inhibit plasminogen-activator production by macrophages.

Recently, several mouse monocyte-macrophage tumor lines have been shown to exhibit certain properties similar to their nonneoplastic counterparts, viz., adherence properties, phagocytosis, lysozyme synthesis, production of colony-stimulating activity, receptors for Fc and complement, the ability to lyse tumor cell targets, and prostaglandin synthesis (9, 10). Thus, these cell lines offer the potential as models to study monocyte-macrophage physiology and function and, because large populations of homogeneous cells can be obtained, can provide sufficient material for the isolation of receptors, enzymes, etc. In addition, the problem of contaminating cell types in tissue culture experiments is avoided.

We show here that one such macrophage cell line, RAW264.10, synthesizes and secretes plasminogen activator spontaneously and that the synthesis of the enzyme can be inhibited by low concentrations of anti-inflammatory glucocorticoids but is stimulated by low concentrations of the tumor promoter, phorbol myristate acetate.

* Supported in part by grants PCM77-09114 and PCM75-19734 from the National Science Foundation.

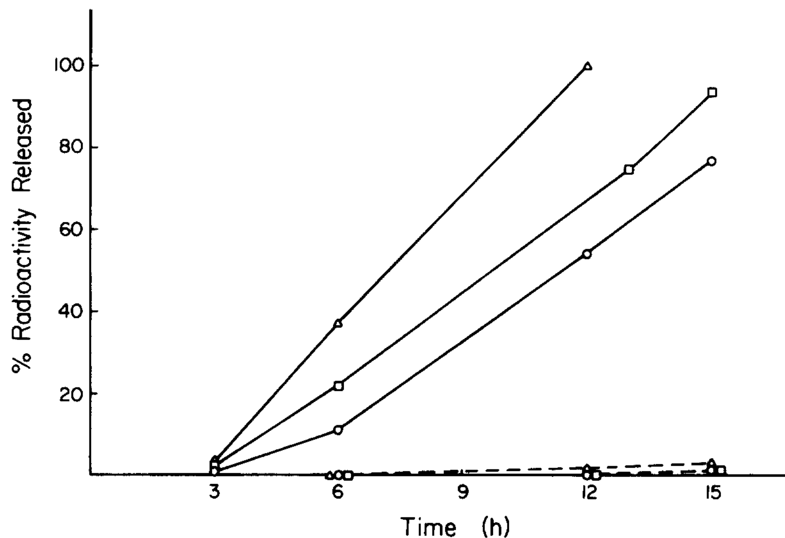

FIG. 1. Fibrinolysis by RAW264.10 cells. Varying numbers of washed cells were plated in duplicate cultures on ^{125}I-fibrin-coated Linbro wells (FB16-24TC) in Dulbecco's minimum essential medium (MEM) containing 5% acid-treated, heat-inactivated fetal bovine serum (ATHIFBS) (——) or 5% of plasminogen-depleted ATHIFBS (- - -). The total volume was 2 ml and 0.2-ml samples were removed as indicated (1). The following cell numbers were plated: 2×10^5 (○); 4×10^5 (□); and 8×10^5 (△). Each well contained 10 μg fibrin/cm^2, with a total radioactivity of 6×10^4 cpm.

Materials and Methods

Except when otherwise stated, all reagents and procedures were as previously described (1, 6). Phorbol myristate acetate and phorbol were obtained from Consolidated Midland Corporation, Brewster, N. Y.

RAW264.10 Cell Line. This is a clone derived from RAW264, a BALB/c tumor induced by Abelson leukemia virus whose macrophage nature has previously been described (9). All incubations were carried out at 37°C in a 5% CO_2-95% air atmosphere.

Results

Plasminogen-Dependent Fibrinolysis by RAW264.10. For monitoring the fibrinolytic activity of RAW264.10 cells, varying numbers of washed cells were plated directly onto ^{125}I-fibrin and the fibrinolytic activity measured by counting released radioactivity at various time points. As can be seen in Fig. 1, after an initial short lag period, there is an approximately linear fibrinolytic activity detected in these cells which is predominantly plasminogen dependent.

Inhibition of the Secretion of Plasminogen Activator by Anti-Inflammatory Glucocorticoids. Serum-free medium was collected from the cultures of RAW264.10 cells and assayed for plasminogen activator. As can be seen from Table I, these cells, as has been shown for activated mouse macrophages, secrete plasminogen activator. Anti-inflammatory glucocorticoids at low concentrations inhibit the synthesis and secretion of plasminogen activator from activated mouse macrophages (6). In Table I, the effect of 10^{-7} M dexamethasone on the extracellular and cell-associated enzyme levels in RAW264.10 cells is shown. As with peritoneal macrophages, this anti-inflammatory agent is able to decrease the enzyme levels without affecting the secretion of another enzyme, lysozyme (Table I).

Downloaded from http://rupress.org/jem/article-pdf/148/3/811/1089467/811.pdf by guest on 15 June 2021

TABLE I

Effect of Dexamethasone on the Secretion of Plasminogen Activator and Lysozyme

Drug in the medium	Plasmogen activator*		Lysozyme secreted
	Extracellular	Cell associated	
	U/mg cell protein‡		*μg/mg cell protein*
None	1,080	320	12.8
Dexamethasone (10^{-7} M)	277	50	11.7

RAW264.10 cells were washed in Dulbecco's MEM and placed at 4 × 10^5 cells in Dulbecco's MEM containing 0.05% lactalbumin hydrolysate (LH) in duplicate Linbro wells (Linbro Chemical Co., New Haven, Conn.). Dexamethasone was added at the start of the culture. After 24 h, the serum-free conditioned medium (CM) was collected, the cultures washed two times with phosphate-buffered saline (PBS), the cells lysed by the addition of Triton X-100 (Rohm & Haas Co., Philadelphia, Pa.) (0.2% vol/vol in water), and scraped from the surface of the dish with a plastic policeman. The conditioned media and the cells were then assayed for plasminogen activator and the conditioned media for lysozyme (1, 6).

* No plasminogen-independent fibrinolysis could be detected when plasminogen was omitted from the assay mixture.

‡ 1 U of plasminogen activator is defined as the amount that releases 10% of the initial radioactivity in 4 h (1).

TABLE II

Relative Effect of Glucocorticoids on Plasminogen-Activator Secretion

Drug	Control
10^{-7} M	*%*
PBS	100
Dexamethasone	24.7
Prednisolone	28.1
Fludrocortisone	31.5
Hydrocortisone	41.0
Corticosterone	47.8
Cortexolone	52.7
Desoxycorticosterone	60.7
Aldosterone	91.3
Progesterone	100
β-Estradiol	100
Testosterone	100

A similar experimental protocol to Fig. 2 and Table I was used. The results are expressed as percentages of the activity present in the control untreated cultures. The mean plasminogen-activator content of the control CM was 1,010 U/mg cell protein. The cell protein did not vary significantly between the control and experimental cultures

A dose response for dexamethasone inhibition on the extracellular enzyme levels is depicted in Fig. 2. The lysozyme secretion was not affected by these doses of dexamethasone (J. A. Hamilton, unpublished observations).

It has previously been shown that for the glucocorticoids, the anti-inflammatory drugs are the most effective at decreasing plasminogen-activator levels of mouse macrophages (6). The relative abilities of a series of glucocorticoids at 10^{-7} M to suppress extracellular plasminogen-activator levels from the RAW264.10 cells are listed in Table II. The relative inhibitory potencies are similar for these neoplastic cells and for the peritoneal mouse macrophages (6).

Stimulation of the Secretion of Plasminogen Activator by Phorbol Myristate Acetate (PMA). PMA, a tumor promoter in a mouse skin carcinogenesis model (11), has been shown to induce and stimulate plasminogen-activator production from mouse peritoneal macrophages (7). The parent alcohol, phorbol, is inactive. In Fig. 3, PMA is shown to increase plasminogen-activator secretion from RAW264.10 cells at low concentrations whereas phorbol itself is inactive. The cell-associated enzyme levels are increased at most two- to threefold (J. A. Hamilton, unpublished observations).

Downloaded from http://rupress.org/jem/article-pdf/148/3/811/1089467/811.pdf by guest on 15 June 2021

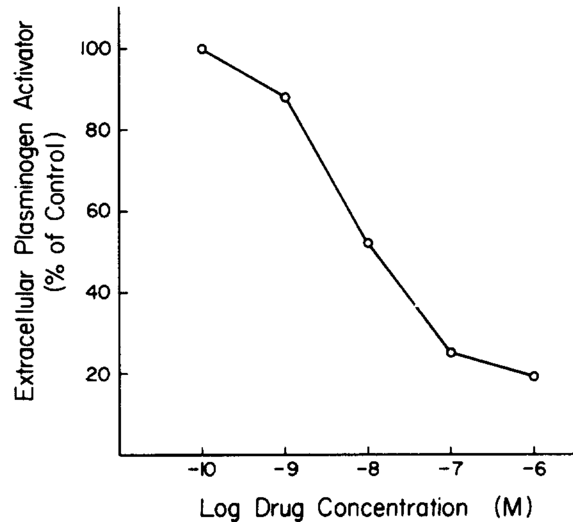

FIG. 2. Effect of dexamethasone on plasminogen-activator secretion from RAW264.10 cells. 4 × 10^5 cells were washed in Dulbecco's MEM and placed in Dulbecco's MEM + 0.05% LH in the presence of different concentrations of dexamethasone (duplicate cultures). After 24 h the CM was collected and assayed for plasminogen activator (6). The results are expressed as percentages of the activity present in the control untreated culture. Plasminogen-activator content of control CM was 890 U/mg cell protein. The cell protein content at the end of the cultures did not vary significantly between the control and experimental cultures.

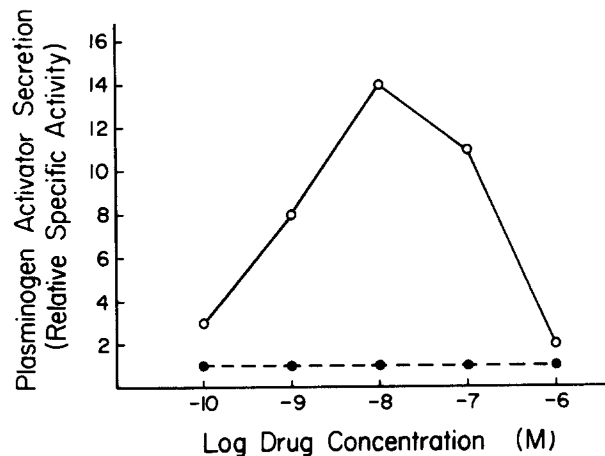

FIG. 3. Effect of PMA and phorbol on secretion of plasminogen activator. After washing in Dulbecco's MEM, 4 × 10^5 RAW264.10 cells were plated onto wells of Linbro trays in Dulbecco's MEM + 0.05% LH in the presence of PMA (○) or phorbol (●). At 24 h, the CM was collected and assayed for plasminogen activator. The amount of plasminogen activator secreted per milligram of cell protein in the control untreated cultures was defined as 1 (the actual mean value was 790 U/mg cell protein). No plasminogen-independent fibrinolytic activity was detected in the cultures. The cell protein content at the end of the cultures did not vary significantly between the control and experimental cultures.

Discussion

The above study indicates that a mouse macrophage cell line (RAW264.10) can spontaneously synthesize and secrete the neutral protease, plasminogen activator. In addition, this line produces less enzyme in the presence of low concentrations of glucocorticoids but more enzyme when treated with the tumor promoter, PMA.

Downloaded from http://rupress.org/jem/article-pdf/148/3/811/1089467/811.pdf by guest on 15 June 2021

Certain irritants, such as thioglycollate medium and asbestos fibers, are able to elicit macrophages into the peritoneal cavity of mice which synthesize much higher levels of plasminogen activator than the resident, unstimulated macrophages (1, 2). The amount of enzyme produced by the RAW264.10 line is comparable to that produced by the so-called activated macrophages and it is possible that this line is expressing a permanent state of activation. Further experiments are needed to understand the significance of the enzyme synthesis for RAW264.10 metabolism.

We have shown here that low doses of dexamethasone can inhibit production of plasminogen activator by the RAW264.10 line and the spectrum of the abilities of other glucocorticoids to inhibit enzyme production is similar to that found for thioglycollate-induced mouse peritoneal macrophages (6). Interestingly, the relative potencies of these drugs parallels their abilities to function as anti-inflammatory agents (12). Also, as with thioglycollate- and asbestos-induced macrophages, lysozyme secretion is not inhibited. These observations suggest that the cell line can modulate its enzyme production in response to glucocorticoids in a similar manner to mouse peritoneal macrophages. Thus the cell line may provide a useful model system for studying glucocorticoid-receptor interaction and possibly for receptor isolation from the macrophage. These cells may also enable the details for the mechanism of the glucocorticoid control of enzyme synthesis to be elucidated.

The cocarcinogenic effects of PMA and other promoting substances are known to be inseparable from a powerful inflammatory response that is associated with the presence of lymphocytes and macrophages in the affected areas of skin (13), and protease levels in the skin are shown to increase in conjunction with the inflammatory response. Furthermore, nanomolar concentrations of tumor-promoting phorbol esters (but not their inactive analogues) induce changes in cultured cells that resemble those seen on transformation with either chemical carcinogens or tumor viruses, and further enhance the expression of these transformation-specific phenotypic features in already transformed cells (14). They also reversibly inhibit terminal differentiation in a number of different cell types (14). We have shown here that the cell line RAW264.10 can increase plasminogen-activator production in response to low concentrations of PMA with a similar dose response to that found with mouse peritoneal macrophages and, as with the peritoneal macrophages, the parent alcohol is without effect (7). These observations suggest that the cell line possesses similar receptors or other cellular components to peritoneal macrophages which can respond to PMA and again points to the possible usefulness of this line in the study of macrophage physiology.

In summary, we suggest that the macrophage tumor cell line, RAW264.10, provides a cell type which should be useful in delineating how macrophages regulate the production of plasminogen activator. The cell line may be useful for the isolation of receptors for glucocorticoids and for studying molecular events associated with PMA-cell interactions. Studies on the relationship between plasminogen activator synthesis and the other functional properties of RAW264.10 (9, 10) are currently in progress.

Summary

The macrophage cell line, RAW264.10, synthesizes and secretes plasminogen activator. Production of this enzyme is inhibited by low concentrations of glucocorticoids and increased by phorbol myristate acetate. It is proposed that this line could be a suitable model for the regulation of enzyme synthesis by mouse peritoneal macrophages.

Downloaded from http://rupress.org/jem/article-pdf/148/3/811/1089467/811.pdf by guest on 15 June 2021

It is a pleasure to acknowledge the excellent technical assistance of Ms. K. Dugan and M. Minkoff.

Received for publication 4 May 1978.

References

1. Unkless, J., S. Gordon, and E. Reich. 1974. Secretion of plasminogen activator by stimulated macrophages. *J. Exp. Med.* **139:**834.
2. Hamilton, J., J.-D. Vassalli, and E. Reich. 1976. Macrophage plasminogen activator: induction by asbestos is blocked by anti-inflammatory steroids. *J. Exp. Med.* **144:**1689.
3. Ratnoff, O. D., and P. B. Naff. 1967. The conversion of C'1S to C'1 esterase by plasmin and trypsin. *J. Exp. Med.* **125:**337.
4. Cochrane, C. G., S. D. Revak, and K. D. Wuepper. 1973. Activation of Hageman factor in solid and fluid phases. A critical role of kallikrein. *J. Exp. Med.* **138:**1564.
5. Kaplan, A. P., and K. F. Austen. 1971. A prealbumin activator of prekallikrein. II. Derivation of activators of prekallikrein from active Hageman factor by digestion with plasmin. *J. Exp. Med.* **133:**696.
6. Vassalli, J.-D., J. Hamilton, and E. Reich. 1976. Macrophage plasminogen activator: Modulation of enzyme production by anti-inflammatory steroids, mitotic inhibitors, and cyclic nucleotides. *Cell.* **8:**271.
7. Vassalli, J.-D., J. Hamilton, and E. Reich. 1977. Macrophage plasminogen activator: induction by concanavalin A and phorbol myristate acetate. *Cell.* **11:**695.
8. Vassalli, J.-D., and E. Reich. 1977. Macrophage plasminogen activator: induction by products of activated lymphoid cells. *J. Exp. Med.* **145:**429.
9. Ralph, P., and I. Nakoinz. 1977. Antibody-dependent killing of erythrocyte and tumor targets by macrophage-related cell lines: enhancement by PPD and LPS. *J. Immunol.* **119:**950.
10. Kurland, J., and R. Bockman. 1978. Porstaglandin E production by human blood monocytes and mouse peritoneal macrophages. *J. Exp. Med.* **147:**952.
11. Boutwell, R. K. 1974. The function and mechanism of promotors of carcinogenesis. *CRC Crit. Rev. Toxicol.* **2:**419.
12. Nelson, D. H. 1962. Relative merits of the adrenocortical steroids. *Annu. Rev. Med.* **13:**241.
13. Troll, W., A. Klassen, and A. Janoff. 1970. Tumorigenesis in mouse skin: inhibition by synthetic inhibitors of proteases. *Science (Wash. D. C.).* **169:**1211.
14. Weinstein, I. B., and M. Wigler. 1977. Cell culture studies provide new information on tumour promoters. *Nature (Lond.).* **270:**659.

Downloaded from http://rupress.org/jem/article-pdf/148/3/811/1089467/811.pdf by guest on 15 June 2021