INCREASED SECRETION OF PLASMINOGEN ACTIVATOR BY HUMAN MACROPHAGES AFTER EXPOSURE TO LEUKOCYTE INTERFERON

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Received 13 April 1981; revised version received 18 May 1981

1. Introduction

Interferons are known to affect mononuclear phagocyte functions both in vitro and in vivo. Enhanced spreading on growth substratum [1,2] as well as increased phagocytic [3] and tumoricidal [1,4] activities have been described in cultured macrophages after exposure to interferons [IFN). Increased phagocytic activity of macrophages is likewise evident in vivo in animals treated with interferon [5,6]. Human monocytes exposed in vitro to interferon have also been reported to show enhanced phagocytic activity [7] as well as increased capacity to kill tumour [8] and virus-infected cells [9]. However, in [10] both leukocyte and fibroblast interferon reversibly inhibited morphological differentiation of human monocytes to macrophages in culture; this phenomenon might have had a role in IFN-induced immunosuppression [10].

We have also found that interferons can inhibit monocyte–macrophage transition in culture as judged by morphology, but we show here that this does not necessarily result in an inhibition of the development of cellular functions such as secretion of plasminogen activator. Moreover, low concentrations of interferon α were found to effectively enhance the secretion of this specific protease by human blood monocyte-derived macrophages.

2. Materials and methods

2.1. *Monocyte cultures*

Mononuclear leukocytes were isolated by standard techniques [11] from the buffy coat fraction of human blood provided by the Finnish Red Cross Blood Transfusion Centre (Helsinki).

Monocytes were purified by a double-adherence method (O. S., T. H., A. V., submitted) and cultured in a 1:1 mixture of medium 199 and RPMI 1640 (obtained in powder form from Flow Labs., Glasgow) supplemented with 10% newborn calf serum [12,13]. More than 95% of the adherent cells in these cultures showed the monocyte marker nonspecific esterase as assayed according to [14].

2.2. *Interferons and interferon antisera*

All interferon preparations and antisera were kindly put at our disposal by Dr K. Cantell, Helsinki. Stock solutions of partially purified human leukocyte interferon [15], containing 6×10^6 IU/mg protein, were made in Hank's buffered salt solution, usually at 50 000 IU/ml, Millipore®-filtered and stored in small aliquots at −20°C. Mock-interferon preparations were adjusted to the same protein concentrations. Recombinant DNA-coded *Escherichia coli* interferon (Hu-IFN-α_1 (*Eco*) [16] a gift from Dr C. Weissman, Zürich) had spec. act. 4000 IU/ml of protein. Antisera to leukocyte interferon used in these experiments had been produced in rabbit or sheep [17] and had anti-α-interferon titres of 1:75 000 and 1:450 000, respectively.

2.3. *Caseinolysis-in-gel assay for plasminogen activator*

The method used is detailed in [18], and allows measuring of plasminogen activator activity in serum-containing as well as in serum-free medium samples. Plasminogen-dependence of the observed caseinolysis was controlled for each sample.

2.4. *Protein determination*

The Lowry method [19] was applied using bovine serum albumin as standard.

3. Results

We have found that secretion of plasminogen activator, a specific serine protease [20], by human monocyte–macrophages shows a temporal concurrence with the morphological transition (O. S., T. H., A. V., submitted). Supernatants of fresh monocyte cultures were devoid of this enzyme activity while those of differentiated cell cultures showed increasing levels of activity measurable by the sodium dodecyl sulfate (SDS)-enhanced caseinolysis-in-gel method (fig.1). In contrast, cell-associated plasminogen activator activity was already readily demonstrable in fresh monocyte cultures without SDS treatment, as in [21–23]. A detailed description of the kinetics of plasminogen activator release from monocyte–macrophages is described elsewhere (O. S., T. H., A. V., submitted).

Secretion of plasminogen activator by monocyte–macrophages was greatly enhanced when 24 h monocyte cultures were exposed to low concentrations of human leukocyte interferon. Higher doses of interferon caused less stimulation of the enzyme secretion (fig.1). Mock-interferon preparations had no effects on plasminogen activator activities (fig.1) while two different antisera against human leukocyte interferon inhibited the stimulation of plasminogen activator secretion (not shown). When the cultures were exposed to interferon at a stage when definite morphological

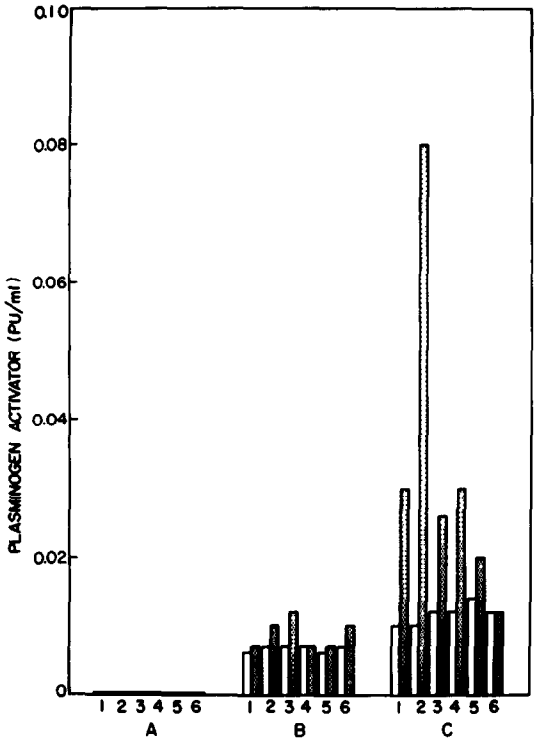

Fig.1. Effect of interferon on plasminogen activator secretion by human monocyte–macrophage cultures. Culture media of 24 h monocytes were supplemented with various concentrations of interferon (dotted columns 1–6), mock-interferon (white columns 3–6) or left without additions (white 1–2). Interferon concentrations (IU/ml) were: 1.7 (1), 5 (2), 17 (3), 50 (4), 167 (5) and 500 (6). Mock-treated cultures received respective amounts of mock-protein. Supernatants were harvested every second day (A, 0–2 days; B, 2–4 days; C, 4–6 days), cleared from detached cells by a brief centrifugation and assayed for plasminogen activator activity. Medium replacements were carried out without readding interferon; PU, Ploug units [24].

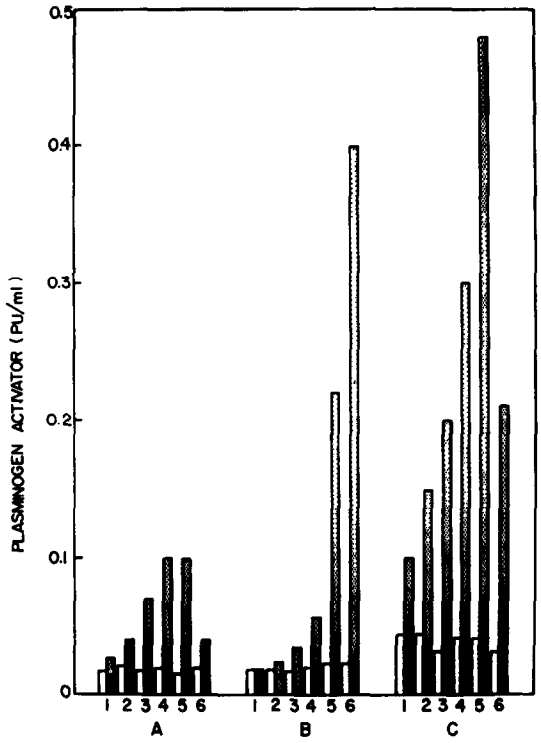

Fig.2. Effect of interferon on plasminogen activator secretion by differentiated macrophages. Cultures were prepared as for fig.1, but interferon was added only after 4 days' incubation when most of the cells already showed morphological signs of differentiation. For symbols see legend for fig.1. The interferon-induced increase in plasminogen activator activity in the medium was maximally ~20-fold, but when calculated per protein in the cell layer, the increase was even greater, maximally 30–40-fold. The final cellular protein masses in the experiment were 100 (1), 105 (2), 100 (3), 80 (4), 65 (5) and 42 μg/culture (6) in the interferon-treated cultures, and between 100 and 120 μg/culture in all control and mock-treated cultures.

Table 1
Effect of recombinant DNA-coded interferon on plasminogen activator production by human macrophages

Culture		Plasminogen activator activity in culture medium (PU/ml)	
		4 days	6 days
Control		0.01	0.08
+ IFN-α_1(*Eco*)	5 IU/ml	0.01	0.10
+ IFN-α_1(*Eco*)	17 IU/ml	0.01	1.10
+ IFN-α_1(*Eco*)	50 IU/ml	0.03	1.50
+ IFN-α(*Le*)	5 IU/ml	0.01	0.20
+ IFN-α(*Le*)	17 IU/ml	0.02	1.00
+ IFN-α(*Le*)	50 IU/ml	0.04	1.70

Recombinant DNA interferon α_1 produced in *E. coli* (IFN-α_1-(*Eco*)) and leukocyte interferon (IFN-α(*Le*)) were added to one-day monocyte cultures at indicated doses and aliquots of culture media were assayed for plasminogen activator activity after 4 and 6 days' incubation

differentiation was already visible a dose-dependent enhancement of the secretion of plasminogen activator was seen. Even the highest concentration of interferon used (500 IU/ml) was still stimulatory in this type of experiments (fig.2). This enhancement was not due to a general increase in the rate of protein synthesis since interferon inhibited the accumulation of cellular protein mass (fig.2).

As reported in [10] interferon doses >15–50 IU/ml, when applied on fresh monocytes, reversibly inhibited the morphological differentiation with most of the cells remaining roundish. This inhibition of differentiation was prevented if the interferon preparations were preincubated with antisera to leukocyte interferon (O. S., T. H., A. V., submitted).

Both the enhancement of plasminogen activator secretion and the inhibition of morphological differentiation were readily reproducible but the degree of both phenomena varied between different batches of monocyte cultures. Inhibition of differentiation was always more or less directly proportional to the applied interferon dose while the maximal enhancement of plasminogen activator secretion was obtained within a dose range of 10–180 IU/ml and ranged from 10–40-fold.

The 'PIF'-grade interferon preparations [15] used in the above experiments are only ~1% pure (K. Cantell, personal communication). The antibodies raised against them, in spite of producing a high titre anti-interferon activity, probably also react against a variety of other leukocyte-derived components in the preparations. Thus it is necessary to repeat these experiments with highly purified interferon or with interferon preparations free of leukocyte-derived contaminants. We have so far tested one of the various recombinant DNA-coded leukocyte interferons produced by *E. coli* [16]. The effects of this preparation on cellular morphology in monocyte–macrophage transition (not shown) as well as on plasminogen activator secretion were similar to those of the PIF-grade leukocyte interferon (table 1).

4. Discussion

Our results indicate that human interferon at low concentrations can specifically enhance the secretion of plasminogen activator, a specific serine protease, by human monocyte-derived macrophages in culture. This is the first time that a specific proteolytic enzyme is shown to be involved in the chain of biochemical events following the action of interferon on animal cells. That the enhanced enzyme activity indeed was due to the action of interferon on the cells and not caused by a contaminating leukocyte-derived substance in the partially purified interferon preparations was suggested by the lack of respective activity in adequately prepared mock-interferon, and was further documented by showing a similar dose-dependent effect by a recombinant DNA-coded interferon preparation which most probably has only interferon in common with the PIF-interferon. It will be of interest to see if the increased secretion of plasminogen activator can be correlated to increased motility or to alterations in the host-defense functions of these cells.

Macrophages generally have a greater phagocytic activity than monocytes and thus our observation that interferons at moderate concentrations appear to inhibit monocyte–macrophage transition might be considered controversial with the earlier reports that interferons enhance phagocytosis both in vitro and in vivo [3,5,6]. However, it is important to note that concentrations of IFN up to 500 IU/ml, which inhibited morphological differentiation, did not prevent the concomitant induction of plasminogen activator secretion but rather potentiated it. This observation indicates that development of biochemical characteristics of monocyte–macrophages in culture can be

dissociated from morphological differentiation and reinforces the requirement of exact parameter definitions in describing mononuclear phagocytes at different stages of maturation and activation.

Acknowledgements

We thank Dr Kari Cantell for interferon and anti-interferon preparations and Ms Helena Kainulainen and Ms Anja Virtanen for technical assistance.

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