TIME COURSE OF CYTOSTATIC FACTOR PRODUCTION BY NONACTIVATED RESIDENT SYRIAN HAMSTER PERITONEAL MACROPHAGES

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Peritoneal macrophages (Mph) can exert a cytotoxic action on tumor cells, which takes the form of inhibition of cell proliferation (cytostasis) or cell lysis. Cell lysis requires activation of Mph, whereas nonactivated monocytes and macrophages are capable of exerting a cytostatic action (CSA) [5, 8]. According to data in the literature, for nonactivated Mph exert their CSA, direct contact between Mph and target cells is essential [4, 6], but according to our own data [2], when the possible mechanisms of CSA by nonactivated peritoneal Mph were investigated by the use of highly sensitive cells of the STHE strain were used, a soluble cytostatic factor (CSTF) was found to be secreted into the medium.

The aim of this investigation was to study the time course and conditions of secretion of CSTF into the culture medium by resident Mph, using cells of the STHE strain as highly sensitive target cells.

EXPERIMENTAL METHOD

Among the conditions potentially affecting secretion of CSTF, various numbers of Mph, their spreading, and the role of serum in the culture medium were tested. It was also interesting to compare these data with those on CSTF secretion by Mph of tumor-bearing animals, for we know that such Mph may be either activated, and possess cytolytic activity, or suppressed [10-12].

As target cells we used cells of the STHE strain (spontaneously transformed hamster epithelium), with a low level of malignancy. Peritoneal resident Mph were obtained from intact animals or animals with tumors after subcutaneous inoculation of cells of the STHE or STHE-SR strains (highly malignant embryonic cells transformed by Rous sarcoma virus). Peritoneal exudate cells were transferred in 24-well plates ("Falcon") in doses differing by a factor of 10, from $7 \cdot 10^5$ to $7 \cdot 10^3$ cells/well in 1.4 ml of medium. Nonadherent cells were washed off 2 h after transfer; most of the cells which remained were Mph (more than 93%, when stained by the Giemsa method). To judge by the number of cells washed off, the number of macrophages which remained was about half the number initially transferred. The Mph were incubated in Eagle' medium + lactalbumin (1:1) with 10% bovine serum or in medium RPMI-1640 with or without 10% bovine serum (+ HEPES). At various times after the nonadherent cells were washed off, the supernatants (Su) of the test Nph were transferred to test tubes and centrifuged at 1000 rpm for 10 min, after which they were added in a volume of 0.2 ml to target cells, transferred for 2 h in a dose of $2 \cdot 10^4$ to a well of a 96-well plate, having first removed the medium. Target cells treated in this way were incubated in the presence of Su of Mph for 24 h. As the control for treatment of the target cells, the cultural medium was used. To prevent the Mph from spreading, plates treated with a solution of poly HEMA [poly(2-hydroxyethyl methacrylate), $2 \cdot 10^{-5}$, 10^{-4}, and $5 \cdot 10^{-4}$ M, "Aldrich"], were used in some experiments. Cytostatic activity (CSA) of Su was determined as incorporation of ^{3}H-thymidine (1 μCi, 5 Ci/mmole) by target cells during the last 4 h, as described previously [1]. The results were subjected to statistical analysis by Student's t test.

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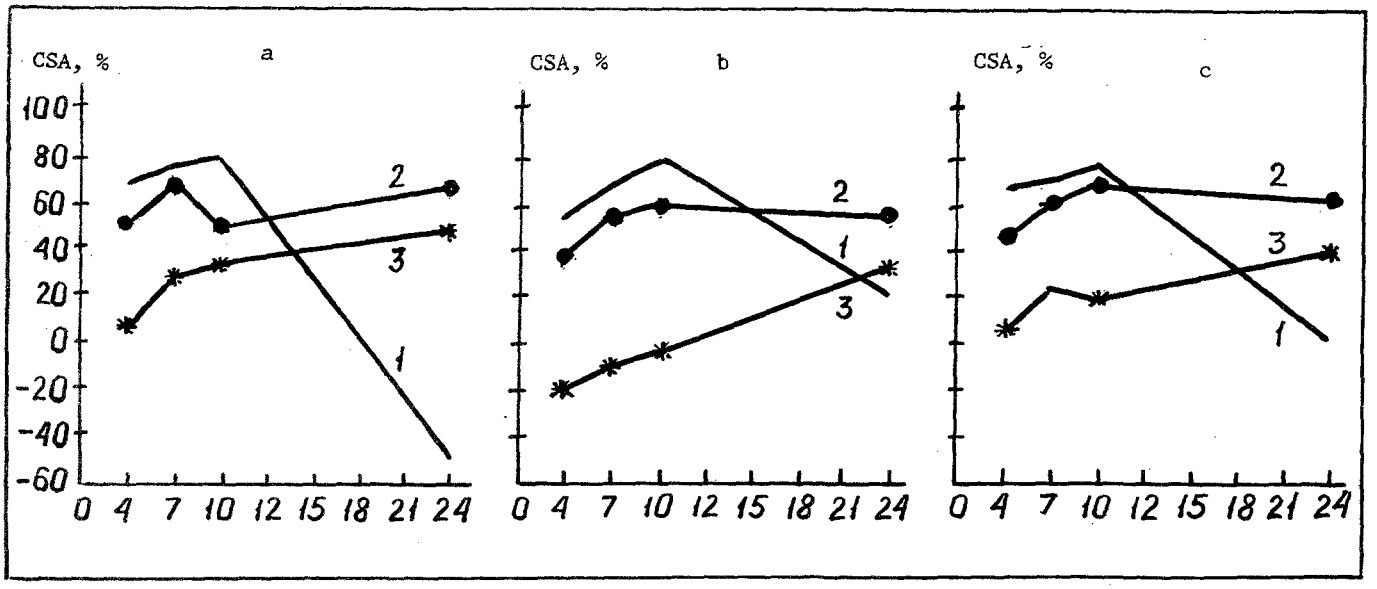

Fig. 1. Time course of production of cytostatic factor (CSTF) by macrophages (Mph) from intact animals and animals with tumors. Test supernatant (Su) taken from Mph of intact animals (a), from Mph of tumor-bearing animals after subcutaneous inoculation of SHTE (b) and SHTE-SR (c) cells. Abscissa, duration of incubation of Mph secreting CSTF (in h); ordinate, CSA, inhibition of incorporation of ^{3}H-thymidine into SHTE cells after incubation for 24 h in Su of Mph (in %). Values below zero correspond to potentiation of target cell proliferation. 1) Su from $3.5 \cdot 10^5$ Mph; 2) Su from $3.5 \cdot 10^4$ Mph; 3) Su from $3.5 \cdot 10^3$ Mph.

EXPERIMENTAL RESULTS

To analyze conditions potentially affecting secretion of CSTF by Mph, experiments were carried out to determine CSTF production in serum-free medium and to compare it with production in medium with 10% bovine serum, which may perhaps potentiate the action of Mph The results showed that CSTF production by Mph in serum-free medium is the same as in medium with serum, a fact which could prove useful during its subsequent identification (data not given).

Another condition potentially affecting the CSA of Mph is the ability of Mph to spread out on a plate [3]. In our experiments, to reduce this spreading the Mph were incubated on an ordinary polystyrene plate or on a plate treated with three concentrations of poly HEMA ($2 \cdot 10^{-5}$, 10^{-4}, and $5 \cdot 10^{-4}$ M). The state of Mph on the plate treated with poly HEMA indicated significant inhibition of their spreading (from slightly adherent to completely nonadherent Mph). If Su from Mph located on an untreated plastic surface or one treated with poly HEMA was transferred to target cells, it was found that CSA of Su of Mph was independent of their degree of spreading (data not given). Next, the time course of production of the CSA of Mph of intact animals, secreted into the medium, as a function of dose of Mph and of the duration of their incubation on the plastic, was investigated in six experiments. Although CSA of Su in these experiments varied, a general tendency of its course and its dependence on dose of Mph could be detected in all the experiments. For instance, with a dose of $3.5 \cdot 10^5$ Mph significant accumulation of CSTF in Su was found after only 4 h of incubation, with a maximum of CSA toward 9-11 h, and to a decrease in this activity, or sometimes even an increase in target cell proliferation toward 24 h of incubation of Mph. Mph in a smaller dose ($3.5 \cdot 10^4$) secreted CSTF at a lower level throughout the period of observation (4-24 h). On incubation of Mph in a dose of $3.5 \cdot 10^3$, CSTF accumulation could be detected only toward 7 h, and throughout the period of observation the CSA of these Su gradually increased, although it did not reach the level of CSTF secretion by higher doses of Mph.

The reduction of CSA of Su of Mph taken in the maximal dose, mentioned above, and their growth-stimulating action suggest that Mph secrete several factors into the medium, some inhibiting, some stimulating proliferation of target cells. These factors may perhaps be secreted simultaneously into the medium and may compete to

manifest their activity with gradual predominance of activity of the factor potentiating target cell proliferation. However, it is also possible that accumulation of CSTF of Mph up to a certain high level may suppress its secretion and initiate the secretion of another factor, stimulating target cell proliferation. The possibility of a bimodal action of the same factor on target cells likewise cannot be ruled out, when, if its concentration in Su increases, it begins to have an action stimulating proliferation instead of a CSA.

Secretion of CSTF by Mph has been described by many investigators, but most research has been done with effectors activated in vivo and/or in vitro [5, 7, 9]. A similar time course of secretion of CSTF by trehalose-dimycolate-induced Mph with additional activation in vitro by LPS was demonstrated in [9]. CSA of Su from resident and thioglycollate-induced Mph was not found by these workers. Differences in the results obtained by these workers and ourselves, when studying CSA of Su of resident Mph can be explained by differences in activity of the Mph used and also by differences in sensitivity of the target cells in the two groups of studies compared.

It was interesting to compare CSA of Su of nonactivated Mph from intact animals with CSA of Su of Mph from tumor-bearing animals, inoculated subcutaneously with cells of two tumor strains differing in carcinogenicity – STHE and STHE-SR. According to some evidence [12] intraperitoneal injection of tumor cells also led to potentiation of the cytolytic activity of peritoneal Mph.

The writers previously [2] studied CSA of Mph of intact animals and tumor-bearing animals. Mph from the latter, unlike those from intact animals, possessed cytolytic action, but no difference could be found between them for CSA. Nevertheless, differences might be expected in the CSA of resident Mph of intact animals and of tumor-bearing animals when the time course of CSF secretion was studied. To investigate this problem two series of experiments were carried out to determine the dynamics of secretion of CSTF by Mph of animals with the two different strains of tumors (STHE and STHE-SR; Fig. 1b, c). Comparison with Fig. 1a reveals no significant differences in the time course of CSTF accumulation by Mph of intact animals and animals with tumors.

On the whole the facts described above are evidence of the ability of Mph of normal Syrian hamsters (and also of tumor-bearing animals) to secrete a factor with a marked degree of CSA. Production of CSTF by nonactivated Mph may perhaps be an essential mechanism for monitoring proliferation of transformed cells in vivo.

REFERENCES

1. N. A. Lavnikova and L. G. Burdelya, Byull. Éksp. Biol. Med., No. 3, 333 (1989).
2. N. A. Lavnikova and L. G. Burdelya, Byull. Éksp. Biol. Med., No. 6, 647 (1991).
3. G. Berton and S. Gordon, Immunology, **49**, 693 (1983).
4. S. Gabilly and R. Gallily, Immunology, **42**, 149 (1981).
5. S. K. Chapes, D. Duffin, and A. O. Paulsen, J. Immunol., **140**, No. 2, 589 (1990).
6. S. Hashimoto, M. Nazaoka, T. Yokokura, et al., Scand. J. Immunol., **27**, No. 3, 261 (1988).
7. M. B. Hevin, B. Friguet, and R. M. Fauve, Int. J. Cancer, **46**, No. 3, 533 (1990).
8. O. Kildehl-Andersen and S. Seim, Acta Path. Microbiol. Immunol. Scand., Sect. C, **92**, 181 (1984).
9. M. Lepoivre, H. Boudbid, G. Lemaire, et al., Cell. Immunol., **115**, No. 2, 273 (1988).
10. H. Mashiba, K. Matsunaga, K. Yagawa, et al., Gann, **72**, No. 6, 848 (1981).
11. Y. Nakata, J. Yamashita, T. Kishi, et al., Cancer Immunol. Immunother., **20**, 43 (1985).
12. M. K. Ribinson and E. F. Wheelock, Cell. Immunol., **73**, 230 (1982).