ONCOLOGY

Soluble Factor Produced by Nonactivated Syrian Hamster Peritoneal Macrophages: Cytostatic Activity Towards Normal, Transformed, and Tumor Cells

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It is generally accepted that macrophages (Mph) are an important component in host antitumor defence. The capacity of activated and in some cases nonactivated Mph to depress the proliferation of dividing tumor cells seems to be of special interest. According to some authorities [4, 7, 10], *in vivo* selected malignant cell variants can differ from parent cells in susceptibility to cytostasis induced by activated Mph.

We have recently shown a high susceptibility of spontaneously *in vitro* transformed Syrian hamster embryo cells (strain STHE) to the cytostatic activity (CSA) of nonactivated resident peritoneal Mph during effector and target cell contact. The susceptibility of *in vivo* selected highly tumorigenic and metastatic variants of this strain was significantly reduced, as well as that of highly tumorigenic hamster emabryo cells *in vitro* transformed by Rous sarcoma virus (RSV) [1].

It has previously been shown [2] that one of the mechanisms of nonactivated macrophagal cytostatic activity may be the release of soluble cytostatic factor(s) (CSF). These results were demonstrated with the use of low-malignant highly susceptible STHE cells. Thus, the aim of the present research was to compare the CSF activity towards normal, transformed, and tumor cells.

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MATERIALS AND METHODS

Some characteristics of the Syrian hanster monolayer cell cultures used in this study are presented in Table 1.

Syrian hamster resident peritoneal macrophages were obtained after their adhesion in 24-well plates and intensive washing to remove nonadherent cells, as described previously [2]. Macrophages were incubated in 1.4 ml RPMI 1640 culture medium with 5mM HEPES buffer without serum. Macrophage supernatant containing cytostatic factor(s) were centrifuged at 1000 rpm and embedded (0.2 ml/well in a 96-well plate with target cells (TC) (2×10^4/well) seeded 2 h before. Immediately, 0.02 ml of 10% bovine serum was added to the wells. After 20 h of incubation, 3H-TdR was added (0.05 ml/well, 0.25 μCi or 5 Ci/mM) to the target cells for 4 h. Target cells were then harvested on fiberglass filter with the use of a cell harvester (LKB, Sweden), and 3H-TdR incorporation was estimated with a liquid scintillator β-counter. Cytostatic activity was determined as a percentage by the formula: CSA=1-[experiment(cpm)/control(cpm)]×100, where "control" is 3H-TdR incorporation in target cells grown in fresh medium and "experiment" is 3H-TdR incorporation in target cells grown in Mph supernatant.

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TABLE 1. Characteristics of Malignant Properties of Target Cells

Cells	Type of transformation	TG (log TrD_{50})	EMA	SMA
HE (normal)	–	–	–	–
STHE	spontaneous *in vitro*	1.7–2.4	$>2\times10^6$	–
STHE–ML–6	spontaneous + *in vivo* selection	1.5–1.7	5×10^4	±
STHE–ML–8	« »	1.2–1.4	5×10^3	+
STHE–75/18	« »	0.7–1.2	5×10^4	+
STHE–83/20	« »	« »	5×10^4	+
HET–SR	RSV* (*in vitro*)	0.5–0.9	10^4	–
HET–SR–1	RSV (*in vitro*)	0.8–1.4	10^4	+

Note. TG – tumorigenic activity in log TrD_{50} (transplanted tumor cell dose leading to tumor growth in 50% of inoculated animals. EMA – experimental metastatic activity (minimal dose of i.v. inoculated cells inducing growth of 10 and more metastases in the lungs); SMA – spontaneous metastatic activity – (+) (the appearance of 20 and more spontaneous metastases in the lungs and other organs after s.c. transplantation for 2 months); (±) – late appearance of spontaneous netastases (after 3 months); * – Rous sarcoma virus (Schmidt–Ruppin strain).

The results were statistically processed using Student's *t* test.

RESULTS

The susceptibility to the cytostatic action of soluble CSF of nonactivated Mph of three types of embryo cells, that is, of normal (HE), *in vitro* spontaneously transformed (STHE), and RSV transformed (HET-SR) was compared. The most demonstrative experiment is represented with due to consideration for the CSF secretion time course by diagrams showing CSF susceptibility of three cell types at macrophage doses 5×10^5 (Fig. 1, *a*) and 5×10^4 Mph/well (Fig. 1, *b*). It is evident that normal and spontaneously transformed cells virtually did not differ in susceptibility to CSF secreted by the higher dose of Mph, whereas some differences were seen when we used a lower dose of secreting cells: HE cells were a little more susceptible to CSF as compared to STHE cells. These differences were repeatedly observed in experiments with the use of the same Mph dose (1.7×10^5) and the same incubation period (18-24 h) for CSF secretion (Table 2, $p\leqslant0.05$).

CSF susceptibility of HET-SR cells which have never been selected *in vivo* (as well as STHE cells) but possess a significantly higher level of tumorigenicity (TG) and experimental metastasizing as a result of RSV transformation is presented in the figure. These data demonstrate that HET-SR cells are much more resistant to CSF produced by both Mph doses used in the experiment as compared with STHE cells. Only weak cytostasis (up to 20%) was observed when higher Mph doses were used, whereas HE and STHE cell proliferation was inhibited by more than 60%.

In subsequent experiments the susceptibility to CSF of cells differing in level of malignancy, including the HET-SR strain and another highly malignant and spontaneously metastasizing *in vitro* RSV-transformed strain (HET-SR-1), was studied (Table 3). The susceptibility of the HET-SR-1 strain to CSF also proved to be significantly lower as compared with spontaneuosly transformed STHE cells (35.9 vs. 57.9%).

Hence, as shown in Table 2, both RSV-transformants were seen to be significantly less sensitive to CSF Mph as compared to spontaneously transformed cells. There are approximately twofold ($p<0.02$-0.001) differences in the cytostasis levels of the compared strains, whereas the differences in susceptibility to CSF between HET-SR and HET-SR-1 are statistically insignificant.

In general the results suggest that of the examined cells, HE and STHE cells (i.e., normal and low-malignant) were significantly more susceptible to CSF than cell strains characterized by high tumorigenicity and metastatic activity; these latter strains differed in their capacity for spontaneous metastases as well, and therefore it can be assumed that resistance to CSF is not a sign determining metastasizing capacity.

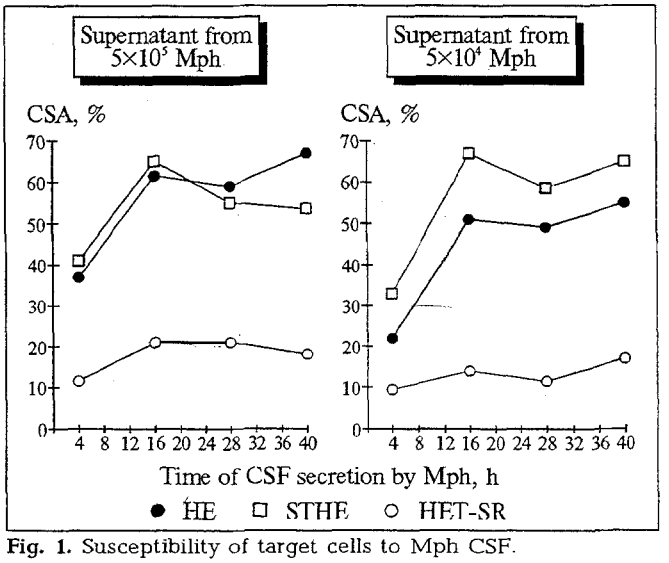

Fig. 1. Susceptibility of target cells to Mph CSF.

TABLE 2. Susceptibility of Normal and Spontaneously *in Vitro* Transformed Cells to Mph–Secreted Soluble Factor CSA

Target cells	№ of experiment								Mean ($M\pm m$)
	1	2	3	4	5	6	7	8	
HE	23	38	39	69	41	56	55	68	48.6±5.7
STHE	21	31	20	31	10	38	31	58	30.0±5.1

Note. For CSF secretion 1.7×10^5 Mph/well of 24–well plate were incubated for 18–24 h.

TABLE 3. Susceptibility of Transformed and Tumor Cells to Mph–Secreted Soluble Factor CSA

Target cells	CSA, % ($M\pm m$)	Number of experiments	Significance of differences, (p)*
STHE	57.9±3.0	16	–
STHE–ML–8	45.6±1.8	8	>0.05
STHE–ML–6	22.6±6.7	6	<0.001
STHE–75/18	23.3±5.2	10	<0.001
STHE–83/20	18.0±4.8	6	<0.001
HET–SR	22.9±4.8	13	<0.001
HET–SR–1	35.9±4.5	7	<0.02

Note. 1.7×10^6 Mph/well of 24–well plate were used for CSF secretion; * – significance of differences between investigated strain and STHE strain in their sensitivity to CSF.

As was previously shown, tumor cells obtained by *in vivo* selection of STHE cells were usually characterized by a higher level of tumorigenisity and metastasizing activity as compared with the original parent STHE cells never selected *in vivo* [5]. Moreover, other scientists have shown that *in vivo* selected variants can differ in resistance to activated Mph CSA [7, 10], thus suggesting that Mph participate in tumor selection *in vivo*.

In light of this, in the next series of experiments we compared low-malignant parent STHE cells and highly-malignant *in vivo* selected variants of this strain for susceptibility to CSA of soluble CSF secreted by nonactivated Mph. Table 3 presents the data indicating the inhibition of 3H-TdR incorporation in the cells examined. As shown, STHE cells were highly susceptible to CSF and 3 out of 4 of its *in vivo* selected variants (STHE-ML-6, STHE-75/18, and STHE-83/20) proved less susceptible to soluble CSF CSA. Approximately twofold differences were observed in the levels of cytostasis of the compared parent strain and *in vivo* selected variants under the effect of CSF obtained by incubation of 1.7×10^6 Mph/well. The acquisition of a higher resistance to Mph CSF by these cells correlated with their higher *in vivo* survival rate as compared with the parent STHE cells. The only exception was highly malignant STHE-ML-8 cells which virtually did not differ from the parent cells in susceptibility to CSF ($p<0.05$). The susceptibility of malignant STHE-ML-8 cells to CSF may be connected with some unknown individual properties of these cells essential for *in vitro* cytostasis but negligible for *in vivo* survival or with the acquisition of alternative properties compensating for their high susceptibility to Mph CSF. It is known that omacrophages can serve as a sourse of a number f soluble factors regulating normal and tumor cell growth.Most of these factors are secreted by Mph activated by different agents. According to published data [3, 6, 9], antitumor CSF secreted by activated Mph, such as IL-1, TNF, and TGF-B, depress tumor cell growth but stimulate the growth of normal fibroblasts. Sugimura *et al.* [8] discovered a soluble factor secreted by macrophage cell line U937 which possesses growth inhibitory activity with respect to a number of tumor cells and proliferating thymocytes. For comparison of CSF discovered by us with known Mph-secreted factors, the properties of CSF and the conditions of its secretion are being investigated.

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