THE INFLUENCE OF ASCITIC FLUID ON THE ADHESION OF ASCITES TUMOR CELLS AND OF STRAIN L FIBROBLASTS TO GLASS

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Secretion by tumor cells of substances capable of killing surrounding cells and destroying tissue had been regarded by a number of authors as a factor which plays an important part in tumor growth [3]. Ascites tumors are convenient models for the study of metabolic products produced by cells and released into the surrounding medium. Consequently, some investigation in recent years has been devoted to the study of cytotoxic properties of the ascitic fluid; these properties may be due to the secretion by ascites cells of enzymes, especially proteolytic enzymes [1, 4, 5], as well as by the presence in the ascitic vluid of various toxic substances. Such a substance, polypeptide in nature, was recently described by Holmberg [2] who has shown that the acellular ascitic fluid induces an almost complete adhesion to glass and pseudopodal activity of strain L fibroblasts, and lyses them after a more prolonged contact.

The present work has been undertaken in order to confirm Holmberg's data and also to study the process of adhesion of ascitic cells to a solid substrate in a fluid,proteinaceous medium. The latter aim was considered important for the elucidation of mechanisms of the development of ascitic tumors.

EXPERIMENTAL

We have studied Ehrlich's mouse ascites cells and hepatoma 22 cells, as well as strain L fibroblasts. Ehrlich's ascites tumor was passaged through male mice strain C57BL, and ascitic hepatoma 22 through male mice strain C3HA.

Strain L fibroblasts were grown in monolayers on glass in medium 199 and bovine serum for 5-7 days. Ascitic cells were taken after 7-9 days of the growth of the tumor. In most cases the cells were suspended in medium 199 without a preliminary washing free of ascitic fluid; in some experiments the cells, strongly diluted with medium 199, were centrifuged at 150-200 rpm for 7 min, the supernatant fluid was removed and the cells in the sediment were resuspended in medium 199. The L fibroblasts were removed from glass by means of a rubber-tipped scraper and repeatedly mixed by pipetting in fresh medium 199 until a homogeneous suspension was obtained.

The concentration of cells in the suspension was determined by means of hemocytometer counts; the cells were diluted with medium 199 and the resulting suspension was introduced into the culture medium. The latter consisted of 70% medium 199 and 30% noninactivated bovine serum.

Acellular ascitic fluid was obtained after centrifugation of 10-12 day old (in some experiments 7-9 day old) ascitic tumors at 3500-4000 rpm for 30 min in a cold centrifuge. The resultant fluid was kept in a refrigerator at 4°C for several days until fibrin became separated. The supernatant fluid was used in the experiment after determinations were made of its protein content and of its NaCl content in some experiments. Because there was no difference in the activities of fresh ascitic fluid and of fluid kept at 4°C for as long as 14-16 days, it was possible to

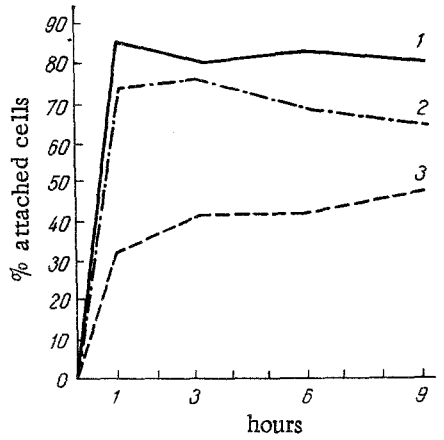

Fig. 1. Effect of Ehrlich's carcinoma ascitic fluid on the attachment of Ehrlich's ascites cells to glass 1) Serum; 2) Earle's fluid; 3) tumor ascitic fluid.

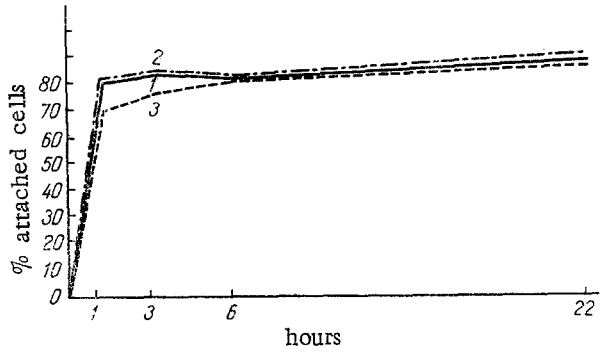

Fig. 2. Effect of Ehrlich's carcinoma ascitic fluid on the attachment of strain L fibroblasts to glass. Legend as in Fig. 1.

use the same fluid in several experiments. In order to obtain nontumor, acellular ascitic fluid, mice of strain C57BL were injected intraperitoneally with 0.5 ml of 20% NaCl solution. The animals were killed 40-60 min later, the abdominal cavity was opened and the peritoneal fluid was removed by means of a syringe (0.7-1.5 ml from each mouse); the peritoneal fluid was centrifuged in the same manner as the ascitic fluid.

The culture medium was placed in 3 ml amounts in Carrel flasks. In experimental cultures 1 ml of ascitic fluid was added, and in control cultures bovine serum in amounts containing equivalent concentrations of protein were added. In some experiments additional controls were used in which 1 ml of Earle's solution, a proteinless fluid, was added.

In experiments with nontumor ascitic fluid the latter was added in 1 ml amounts to the 3 ml of culture medium. In controls the culture medium was supplemented with: a) ascitic tumor fluid, b) bovine serum (both in amounts equivalent in protein content to 1 ml of nontumor ascitic fluid), c) ascitic tumor fluid in amounts equivalent in NaCl content to 1 ml of nontumor ascitic fluid. The experimental and the control media were supplemented with medium 199 to equal volume.

The final concentration of ascites cells was equal to 1 million per milliliter, that of L fibroblasts, 500,000 per milliliter. The flasks were incubated at 37°C. After 1, 3, and 6 h (in some experiments also after 9 and 22 h) the flasks were removed and revolved twice lightly in order to wash off the cells which had not adhered to the bottom. Then 0.3-0.4 ml of suspension was pipetted off for a count. After this the flasks were restoppered and replaced in the incubator. The number of cells which had not adhered to glass and the percentage of cells which adhered to glass were determined following hemocytometer counts. In some experiments cell suspensions, prior to counting, were mixed with 0.01% eosin solution in order to determine the percentage of cell injury. All glassware was treated in the usual manner. The data obtained were subjected to statistical analysis according to the method of Student-Fisher.

RESULTS

In controls in which bovine serum was added to the culture medium, as early as after 3 h of incubation, 70-80% of ascites cells and more than 80% of strain L fibroblasts became attached to glass. Subsequently, the percentage of attached cells remained at roughly the same level. The addition of Earle's solution did not result in statistically significant differences in the percentage of the attached ascites cells and L fibroblasts, as compared with serum controls.

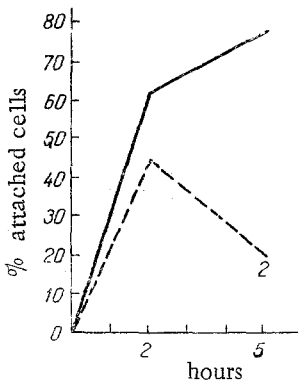

Fig. 3. Effect of Ehrlich's carcinoma ascitic fluid from fresh ascites (1) and from autolyzed ascites (2) on the attachment of strain L fibroblasts to glass.

The Effect of Ehrlich's Ascitic Fluid and of Hepatoma 22 on the Attachment of Ascites Tumor Cells and of Strain L Fibroblasts to glass

The addition of Ehrlich's ascitic fluid to the culture medium decreased sharply the attachment of Ehrlich's ascites cells to glass (Fig. 1).* After 1 h of incubation the percentage of attached ascites cells was 2.7 times lower than that in the serum control. Similarly, although somewhat less markedly, Ehrlich's ascitic fluid acted on the ascites cells of hepatoma 22.

A similar effect was produced by the ascitic fluid of hepatoma 22, whose addition to the culture medium lowered, after 1 h of incubation, the percentage of attached hepatoma 22 cells 1.8 times as compared with the control. This effect of ascitic fluids of Ehrlich's carcinoma and of hepatoma 22 on the attachment to glass of ascitic cells remained statistically significant throughout the entire period of incubation.

The addition of Ehrlich's ascitic fluid has no effect on the attachment of strain L cells to glass (Fig. 2), the difference between the control and experimental cultures was statistically insignificant for 22 h of incubation. A microscopical examination showed that L fibroblasts which attached to glass in the control as well as in the experimental cultures, were elongate and had outgrowths which permitted the cells to anastomose between themselves, producing in places complete monolayers. Ascitic fluid of hepatoma 22 also had no effect on the attachment to glass of strain L cells.

Eosin staining did not show a damaging effect of ascitic fluid on ascites cells or on strain L fibroblasts.

Effect of Ascitic Fluid from Autolyzed Ehrlich's Carcinoma on Strain L Fibroblasts

Because our results differed from these obtained by Holmberg [2] we decided to conduct some special experiments in order to determine the reason for the disagreement.

Ascites cells obtained from an animal with Ehrlich's carcinoma were divided into two parts. One part was immediately centrifuged in order to obtain tumor ascitic fluid; the other part was kept at 37°C for 20 h, after which it was also centrifuged in order to obtain an autolyzate. Both these fluids in 1 ml amounts were added to the cultures medium in experiments on the attachment of strain L fibroblasts to glass. Figure 3 shows a sharp inhibition of attachment to glass of strain L fibroblasts when the autolyzate was added. Microscopically, strain L cells on the surface of glass in the presence of the autolyzate in the medium were round, did not have outgrowths and were isolated from each other. This was identical to what was seen by Holmberg in his experiments in which he studied the effect of tumor ascitic fluid.

Effect of Nontumor Ascitic Fluid on the Attachment of Ehrlich's Carcinoma Ascites Cells to Glass

Compared to the serum control, the nontumor ascitic fluid, as well as the tumor fluid, produced a sharp inhibiting effect on the attachment of Ehrlich's ascites cells to glass. This effect remained statistcially significant during 3 h of incubation; subsequently, apparently as a result of the nature of the batch of the bovine serum used, there was a decrease in the percentage of attached cells in the control. There was no significant difference in the degree of the inhibiting effects of the tumor and the nontumor ascitic fluids; however, it must be noted that in these experiments, in order to equalize the protein content in the culture medium, the tumor ascitic fluid was diluted with medium 199 approximately twice, which corresponded to 1 ml of undiluted nontumor ascitic fluid.

Our results on the inhibiting action of the tumor ascitic fluid on the attachment to glass and on the pseudopodial activity of strain L fibroblasts, differed from those of Holmberg [2]. In our experiments such an effect was produced only by a strongly autolyzed ascites tumor. Holmberg used either fresh or weakly autolyzed tumor ascitic fluid. Apparently he used some specially sensitive substrain of L fibroblasts. Thus the accumulation of some cytotoxic products in the tumor ascitic fluid can hardly be regarded as a universal phenomenon.

*Each figure represents the results of several experiments.

On the other hand, we observed an inhibiting effect of the tumor ascitic fluid on the attachment to glass of ascites tumor cells. The same effect was produced by nontumor ascitic fluid, and apparently, as was seen in some preliminary experiments, by normal mouse serum. The reason for this interesting phenomenon is still not clear. It may be related to the qualitative differences in the proteins of bovine serum and of mouse plasma as well as to the nature of the surface of the ascites tumor cells.

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