Reticuloendothelial Immune Serum (REIS)

III. The Effect of Strong Concentrations on the Growth of Walker Rat Sarcoma 319 in vitro

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(Received for publication July 10, 1945)

The importance of the reticuloendothelial system in relation to neoplasms has been recently brought into focus by a group of Soviet workers. According to Shimkin (18), blockading of the reticuloendothelial system has been used by Roskin (16) as a means of growing heteroplastic tumors. Bogomolets (3) has reported the effect of specific antireticular cytotoxic serum (ACS) on the genesis, survival, and metastasis of cancer. Anti-human spleen horse serum has been shown (9) to increase the carcinolytic capacity of the serum of cancer patients.

According to Bogomolets (4) "Whereas the macrophages penetrating the tumor tissue destroy the carcinogenetic cellular element, the fibroblasts and microphages form a strong line of demarcation around the cancerous foci." It is believed that this activity, along with the production of trephone-like substances by macrophages, assists in the resistance against cancerous proliferations. Bogomolets and Neuman found that at very low concentrations anticytotoxic serum was capable of reducing the number of positive inoculations of tumor grafts; the reverse was true with high concentrations. Bogomolets (4) states that, "... Dr. Neuman showed that stimulation of the connective tissue with anti-reticular cytotoxic serum in many cases leads to complete disappearance of large carcinomaous tumors in mice and decreases the number of metastases of the cancer to the lungs." Disappearance of pain, and of metastases in lymph nodes are given as evidence of the value of such sera in inoperable human cancer.

Research in our laboratories has corroborated the statement by Soviet workers that reticuloendothelial immune serum (REIS) prepared according to the method described by Marchuk (11) has both damaging properties and stimulating action. Thus homologous sera, when incorporated in the culture medium at high concentration, have been shown to restrict the outgrowth of splenic fragments and cause cell clumping (14), as well as total inhibition of heart fragments (15). Moreover, this effect was demonstrated in vivo by the experimental production of bartonellosis in latent carriers (1, 2). Preliminary experiments suggest that low concentrations of homologous sera stimulate the migration of cells from chick heart explants (13). These effects appear to be species specific, since they could not be produced by antisera made from antigen derived from a different species of animal.

MATERIALS AND METHODS

Walker rat sarcoma 319 was selected for a study of the effect of REIS on malignant tissue because its distinct cultural characters in vitro make it easy to distinguish it from other tumors and from normal tissues (10). Moreover, the metabolism of pure cultures of these cells has been carefully studied by Victor and Lewis (19) and by Gemmill, Gey, and Austrian (7). We are indebted to Dr. George O. Gey, of the Johns Hopkins Medical School, for his kindness in supplying us with a strain of Walker rat sarcoma 319.

All the results herein reported were conducted in hanging drop preparations. At least 8 slides were used for each experimental condition. The medium consisted of 50 per cent heparinized rooster plasma, 12.5 per cent extract from chick embryos incubated...
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Figs. 1-6
Figs. 7-12
6 to 10 days, 12.5 per cent rat serum, and 25 per cent REIS at various concentrations. Controls consisted of identical materials, but normal rabbit serum was substituted for the REIS.

The REIS used was rat anti-spleen serum with a complement-fixation titer of 1:1,600 and chick anti-identical materials, but normal rabbit serum was substituted for the REIS.

Tumor tissue was used either alone or in combination with fragments of spleen from newborn rats. Cultures were incubated at 37.5 °C for 3 to 5 days and fixed in 10 per cent formalin in saline. Harris hematoxylin and toluidin blue were used for staining.

RESULTS

In the first series of experiments, homologous (anti-rat) and heterologous (anti-chick) sera were incorporated in the media at high concentration. Clots containing 25 per cent of the undiluted serum; this is reported as a dilution of 1:4. Results were consistent in showing almost total inhibition in the migration of malignant cells from explants in the presence of anti-rat serum (Fig. 3). Moreover, small clusters of cells immediately adjacent to the explants were rounded and pycnotic (Fig. 4). In contrast to these results, explants in medium with anti-chick sera showed outgrowths (Figs. 5, 6) typical of control cultures containing normal rabbit serum (Figs.1, 2). These findings were obtained in 16 cultures representing each experimental condition, proving the species specificity of REIS.

Since antisera are prepared against an antigen consisting of splenic cells it was decided to conduct experiments with homologous REIS at various concentrations in conjunction with the spleen of newborn rats alone and in combination with fragments of tumor tissue. Outgrowth from Walker rat sarcoma was almost totally inhibited by REIS at 1:4, but results at 1:16 and 1:64 proved roughly comparable to controls. In contrast, migrating cells from splenic fragments were reduced in number and showed clumping (14) at 1:4, 1:16, 1:64, 1:128, and 1:256; but at 1:512 the results resembled those obtained in control cultures. Inhibition of the sarcoma cells at high concentration shows the overlapping specificity of REIS, while the injurious effects observed in spleen grown with considerably greater dilution of REIS demonstrates specificity of the antiserum when used against the type of cells originally employed as antigen.

Cultures in which both tumor and splenic cells were introduced into the clot (conjoint cultures) proved especially interesting. Controls containing no REIS, but in which 25 per cent normal rabbit serum was included, showed excellent growth and mixture of both species of cells (Figs. 11, 12). In media containing REIS as well as splenic fragments in addition to the sarcoma cells, inhibition was observed at all concentrations at which spleen alone was affected. That is, in the presence of spleen, sarcoma cells, which were not injured when cultivated in a medium with a 1:16 dilution of REIS, were found to be rounded and clumped in the presence of as little as 1:256 of the homologous REIS (Figs. 6, 7, 8, 9). Outgrowth of both the tumor and spleen fragments appeared normal with 1:512 REIS. It would seem, therefore, that in the presence of splenic elements, sarcoma cells were more susceptible in vitro to weaker concentrations of REIS than when cultivated alone.

DISCUSSION

In a recent paper Hungate and Snider (8) have reviewed and given additional experimental evidence for the capacity of living splenic cells to inhibit the growth of tumor tissues in chick eggs. Previous tumor immunization of hens and young pullets whose spleens were used against the tumors injected into eggs was not found to influence the results.

Workers in this field have tended to look upon the egg as a simple culture medium, and perhaps have not given sufficient consideration to immune responses of the living chick to splenic injections.

In an excellent contribution, Burke, Sullivan, Petersen, and Weed (5) have given experimental evidence...
for changes in organ antigenicity during ontogeny. Splenic cells from chickens 4 months to 1 year of age are certainly antigenic and unquestionably provide antibody formation in chicks by the 17th day. It appears, therefore, that such procedures may produce immune factors similar to those reported by Soviet workers and confirmed by us in studies on REIS.

In the preparation of antigen according to the method of Marchuk (11) spleen and bone marrow are ground in a mortar and centrifuged at 1,000 r.p.m. for 4 minutes. The resulting supernatant, which is injected into an antibody-forming host, contains intact cells. This fact is significant in the light of agreement between Murphy (12), Danchakoff (6), and Hungate and Snider (8) that cells are the agent essential for inhibition of tumor in eggs. But Stevenson (17), on the other hand, reported that tumor grafts and spleen fragments grew vigorously side by side in the egg.

Marchuk (11) wrote that frozen antigens produced immune sera of significantly lower titer than fresh ones. Refrigeration has also been found to lower the effectiveness of spleen in inhibiting tumors in eggs (8).

Sarcoma cells grown in vitro in intimate contact with splenic elements were not inhibited, but in the presence of REIS damaging effects were noted at concentrations not injurious to sarcoma cells cultivated in the absence of spleen fragments. It seems likely that in the inhibition of tumor grown in eggs the splenic cells that are added may exert their effect in association with an anti-spleen immune body progressively developed by the living chick. According to Danchakoff (6) it is essential to bring both tumor and splenic cells into intimate association to insure the inhibitory effect. Results obtained thus far in tissue culture offer no contradiction to this possibility, but argue for the presence of REIS to insure the effect.

CONCLUSIONS

Cells of Walker rat sarcoma 319 were inhibited in vitro by homologous (anti-rat) REIS, but not by heterologous (anti-chick) REIS at similar concentrations.

By the use of an anti-rat REIS with a complement-fixation titer of 1:1,600 sarcoma cells were inhibited at a concentration of 1:4 but not of 1:16. Splenic cells were clumped and limited in migration by concentrations up to 1:256, but not at 1:512. Outgrowth of tumor was reduced when splenic cells were included in the medium, but this effect was not found for controls containing no REIS.

It is suggested that the inhibitory action of spleen on tumor cells is exerted in the presence of relatively strong concentrations of REIS. These observations may explain the results reported for the inhibition of tumor in eggs by spleen; in such cases the injected spleen may cause the developing chick to produce the necessary REIS factor.

ACKNOWLEDGMENT

It is a pleasure to acknowledge indispensable technical assistance from Mrs. Madeline Lay in the preparation of tissue cultures.

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Reticuloendothelial Immune Serum (REIS). III. The Effect of Strong Concentrations on the Growth of Walker Rat Sarcoma 319 in vitro

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Cancer Res 1945;5:724-728.

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