Evidence in Vivo and in Vitro of a Role for the Pituitary in the Growth of Malignant Lymphomas in Nb Rats

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ABSTRACT

The growth of a transplantable malignant lymphoma in Nb rats, the Nb 2 node, was accelerated in rats bearing an estrogen pellet and, in particular, in rats bearing transplanted tumors of the anterior pituitary gland. "Lymphoma cell growth-promoting activity" in the sera from the rats was detected and assayed using cultures of Nb 2 node cells. The "activity" of the sera paralleled the degree to which lymphoma growth was accelerated in the animals. Serum from normal rats at a concentration of 1% was moderately active in stimulating culture growth; serum from estrogenized rats was at least 10 times more active; serum from rats bearing pituitary tumors was extremely active and stimulated growth even at a concentration of 0.001% (1:10^5 dilution). In contrast, serum from hypophysectomized rats was devoid of activity, even if the animals were estrogenized. The evidence indicates (a) that the growth of the lymphoma in Nb rats was stimulated by factor(s) in the peripheral blood, the levels of which were subject to control by the pituitary, and (b) that estrogen stimulated lymphoma growth indirectly, through the pituitary gland.

INTRODUCTION

It is well established that hormones, including estrogens and androgens, can influence the growth of lymphomas and leukemias in rodents (for a review, see Ref. 6). For example, in mice, the incidence of lymphomas has usually been found to be higher in females than in males and to increase in animals treated with estrogens (1, 5). In the Nb rat, malignant lymphomas are not uncommon and have been found in approximately 0.1% of rats over the past 7 years. They may develop spontaneously or after prolonged treatment with estrogens (9, 10). Transplants of 13 Nb rat lymphomas have shown varying degrees of dependency on estrogen for growth in the host (7, 8). Relatively little, however, is known of the role of estrogen in the development and growth of the lymphomas.

One of the transplantable Nb rat lymphomas, the Nb 2 node, which grows approximately twice as rapidly in estrogenized rats as in untreated rats, has recently been established in continuous suspension culture (3). We have used the cultured cells to study the mechanism(s) by which estrogen stimulates the growth of the lymphoma in the animal. The results described here show that the blood of the rats contains "lymphoma cell growth-promoting activity," the levels of which are elevated when the animals are estrogenized. In contrast, serum from hypophysectomized animals has been found to lack growth-promoting activity even if the animals are estrogenized. It appears that the acceleration of the growth of the lymphoma in the intact animal by estrogen is an indirect effect which is mediated by the pituitary, as suggested earlier by less direct observations (8).

MATERIALS AND METHODS

Rats and Tumors. Nb rats (150 to 250 g) were obtained from the laboratory colony at the University of British Columbia. The origin of this strain of rat has already been described (9).

The Nb 2 node lymphoma used in this study arose in a male Nb rat 10 months after implantation of an estrogen pellet. The animal showed generalized diffuse enlargement of lymph nodes and the thymus (Fig. 1). The lymphoma has been maintained for over 7 years by serial transplantation in Nb rats using methods already described (9). The initial 14 transplant generations grew slowly and only in estrogen-conditioned rats; thereafter, the transplants grew more readily and would also grow, although more slowly, in nonestrogenized males. Sublines were obtained in which the transplant remained localized, which allowed satisfactory monitoring of the growth of the tumor; terminal, however, tumors had a tendency to spread, contributing to the death of the animal. Microscopically, the lymphoma appeared as a typical, moderately well-differentiated reticulum cell lymphoma (Fig. 2). In most experiments, the lymphoma was transplanted as small pieces (about 1-mm cubes) with a trocar into the s.c. tissues at the back of the neck of lightly etherized animals; estrone pellets, when used, were implanted s.c. into the flank at the same time (9). The time taken for transplants to become palpable (about 0.5 cm diameter) was noted, and the size of the tumor was thereafter measured daily with calipers and expressed as the sum of its length and width in cm. A 10-g tumor measured approximately 9 cm (e.g., 5 + 4 cm). In some experiments, the lymphoma was transplanted into the flanks of rats which were already bearing growing autonomous pituitary tumor transplants in the neck region. The transplantable pituitary tumors used in these experiments originated in the anterior pituitary of Nb rats after prolonged exposure to estrogen (9). Most of these tumors caused excessive growth of the rats or the adrenal glands, indicative of effects on the host of somatotropic or adrenocorticotropic hormones, respectively.

Hypophysectomized and normal male Sprague-Dawley rats (weighing about 150 g) were obtained from Simonsen Laboratories, Inc., Gilroy, Calif.

Estrone Pellets. Compressed pellets of estrogen (2 mm in diameter, 10 mg) were prepared and implanted as previously described (9); they contained 90% estrone and 10% cholesterol or 20% estrone and 80% cholesterol.

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Assay of Lymphoma Cell Growth-promoting Activity of Rat Serum. Rat serum was obtained by exsanguinating rats by heart puncture under light ether anesthesia and centrifuging the clotted blood for 15 min at 400 x g; samples were stored at -20°. The lymphoma cell growth-promoting activity of serum samples was assayed with "stationary" cultures of Nb 2 node lymphoma cells. The establishment and maintenance of suspension cultures of these cells are described in an accompanying paper (3); the appearance of actively growing cultured cells is shown in Fig. 3. For the assay, the cells were transferred from Fischer's medium supplemented with fetal calf serum (in which they were propagated) to medium supplemented instead with horse serum in which culture growth becomes stationary (3). Blood serum was added at various concentrations (0.001 to 10%) to the stationary cultures which were then incubated at 37° for 3 days. The cell populations were determined daily with an electronic cell counter (Coulter). The ability of various sera at progressively lower concentrations to stimulate the growth of the cultures was taken as a measure of the relative lymphoma cell growth-promoting activity of the sera. Samples were assayed at least in duplicate; the results of replicate cultures were within 3% of the mean.

RESULTS AND DISCUSSION

Lymphoma Growth in Vivo. Chart 1 shows the different growth rates of the Nb 2 node lymphoma transplants in normal, estrogenized, and pituitary tumor-bearing Nb rats. The lower points in the chart indicate the times taken for the transplanted lymphoma to become palpable, and the upper points indicate the times taken for the tumors to reach a weight of 10 g as estimated from their size (see "Materials and Methods"). In rats bearing a 10-mg pellet of 90% estrone and 10% cholesterol (Chart 1B), the tumors became palpable much earlier than in nonestrogenized males (Chart 1D) and also grew faster. The growth of the lymphomas was also accelerated in animals bearing a 10-mg pellet containing only 20% estrone (Chart 1C) and in this case was intermediate between the growth in nonestrogenized animals (Chart 1D) and those bearing a 90% estrone pellet (Chart 1B). The lymphoma grew particularly rapidly when transplanted into rats which were already bearing a growing transplanted pituitary tumor, as shown in Chart 1A. The 8 different pituitary tumors used all accelerated the growth of the lymphoma to about the same extent. In contrast, the growth of the lymphoma was not stimulated when it was transplanted into male rats bearing a rapidly growing mammary carcinoma.

Lymphoma Cell Growth-promoting Activity of Sera. Sera from normal, estrogenized, and pituitary tumor-bearing rats were examined for their ability to stimulate the growth of cultures of Nb 2 node lymphoma cells. These cultured cells enter a phase of stationary growth if transferred to medium which contains horse serum instead of fetal calf serum and in this condition can be used to detect and assay growth-promoting activity as may be present in some types of serum (3). As shown in Charts 2 and 3, the stationary lymphoma cell cultures were stimulated to active growth by the addition of serum from both normal and estrogen-treated male Nb rats. The serum from the estrogenized animals (90% estrone-10% cholesterol pellets for approximately 3 weeks), however, was much more potent. A comparison of the growth-promoting activities of these sera at the 1.0 and 0.1% levels (Chart 3) indicates that serum from estrogenized rats was at least 10 times more "active" than was serum from untreated males. The data show that the stimulation of the growth of the lymphoma cell cultures by serum from estrogenized animals correlates with the acceleration of the growth of the lymphoma by estrogen in vivo (Chart 1). However, the elevated levels of growth-promoting activity in serum from estrogenized rats were due to factor(s) other than estrogens per se, since addition of estradiol or estrone over a wide concentration range to stationary cultures failed to stimulate their growth (Chart 2).

The serum from rats bearing pituitary tumors was particularly active in stimulating the replication of the lymphoma cells as shown in Chart 3. Even at a dilution of 1 part of serum in 100,000 parts of medium (0.001% level), several samples still had significant growth-promoting activity. Indeed, at this low concentration, the samples were more active than were sera from estrogenized and untreated rats at the 0.1 and 1% levels, respectively. The high growth-promoting activity of the sera from these pituitary tumor-bearing rats correlates with the rapid growth of the lymphoma in such animals (Chart 1). The findings suggested that the lymphoma cell growth-promoting activity in the sera might include factor(s) of pituitary origin.

No significant differences were found between the growth-promoting activity of plasma (heparinized) and serum from the same blood samples, indicating that the activity in the sera was not an artifact related to blood clotting.

Effect of Hypophysectomy on the Levels of Serum Activity. Evidence that the pituitary has an important role in the growth of the lymphomas in the rat and in the stimulation of their growth by estrogen has been obtained by examining the effect of hypophysectomy on the levels of lymphoma cell growth-promoting activity in the serum. In these experiments, it was convenient to use Sprague-Dawley rats instead of Nb rats since hypophysectomized animals of the former strain were commercially available and the blood of normal Sprague-Dawley rats, like that of Nb rats, was found to contain lymphoma cell growth-promoting activity which increased when the animals were estrogenized. As shown in Chart 4, there was a substantial rise in the activity of the serum within 4 days of implantation.
of an estrogen pellet into normal males; the activity reached a plateau in about 8 days. In sharp contrast, serum from hypophysectomized animals (at the 5% level) was completely without growth-promoting activity and did not show even the low levels of activity found in the serum from normal, nonestrogenized males. Furthermore, estrogen treatment of hypophysectomized rats for 8 days failed to produce a rise in the activity of the serum. One animal, which had been partially hypophysectomized (about one-half of the anterior lobe remained), when treated with estrogen showed a serum activity intermediate between that of normal estrogenized rats and that of the completely hypophysectomized animals (Chart 4). The results indicate that the growth-promoting activity in the serum is quantitatively related to secretion by the pituitary gland. The absence of any growth-promoting activity in the serum from rats which were estrogenized after hypophysectomy strongly suggests that the acceleration of lymphoma growth by estrogen in the intact animal is an indirect effect which is mediated by the pituitary.

The present study has shown that there is a striking parallel between the stimulation of the growth of the Nb 2 node lymphoma cells in vitro by serum from estrogenized or pituitary tumor-bearing rats (Chart 3) and the acceleration of lymphoma growth in the host (Chart 1). In view of this, the cultured cells appear to be very useful for investigating the role of hormones and other factors in the growth of the lymphoma in the animal.

While the levels of lymphoma growth-promoting activity in the blood of the rats have been found to be subject to control by the pituitary, the chemical nature of the factor(s) has not yet been defined. There are indications, however, that a specific pituitary hormone, prolactin, is involved. For example, as described in an accompanying paper (3), purified rat prolactin has been found to stimulate the growth of Nb 2 node cell cultures at extremely low concentrations. Furthermore, it has been found that the lymphoma growth-promoting activities of sera from normal, estrogenized, and pituitary tumor-bearing rats (Chart 3) are paralleled by the amounts of prolactin present in the sera as measured by radioimmunoassay. It is also known that administration of estrogen can result in elevated levels of prolactin in the blood through stimulation of the pituitary gland (4, 11). These observations suggest that prolactin, secreted by the pituitary under the influence of estrogen, may be responsible for the stimulation of the growth of the lymphoma in vivo.

Our findings raise the possibility that the pituitary may have a more important role than has hitherto been recognized in the development and growth of certain malignant lymphomas. They also suggest that in such cases hypophysectomy or treatment with agents which interfere with the secretion or action of
pituitary hormones might be useful for controlling hormone-dependent cancers. In view of the regulatory action of the hypothalamus on the secretion of hormones by the pituitary, the question may also be asked, "Can higher brain centers acting on the hypothalamus indirectly influence lymphogenesis?"

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REFERENCES


Fig. 1. Lymph node involvement in an estrogenized Nb rat bearing primary 2 node lymphoma.
Fig. 2. Nb 2 node lymphoma transplant showing moderately well-differentiated reticulum cells. H & E. × approximately 500.
Fig. 3. Cultured Nb 2 node lymphoma cells. Wright's stain, × 550.
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