Reactive Splenomegaly and Variation of Lymphoid-Plasma Cell Population Associated with a Mouse Pituitary Thyrotropic Tumor*

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SUMMARY
A transplantable thyrotropic pituitary tumor, subline L24a, which grew after 13 months of dormancy in an intact mouse was associated in its early passage with a variety of lymphoid tumors. On subsequent passage, slow growth over a 6- to 12-month period was regularly accompanied by striking splenomegaly without lymphoid or thymic neoplasia. The enlargement was due to a great increase in cells of the lymphoid series in the red pulp without loss of splenic architecture. In thyroidectomized mice plasma cells with many Russell bodies were especially numerous, and these cells were found in hypertrophied lymphoid follicles within other organs. It is suggested that the splenomegaly and, in earlier passage the lymphoid tumors, may represent an exaggerated antibody response to a tumor's having unusual or abnormal hormonal activity. The transplanted pituitary tumors, in turn, showed severe hemorrhagic degeneration with peripheral small lymphocyte stasis in the lymphatics.

Initial observations concerning pleomorphic reticulum cell- and lymphosarcomas or lymphoid hyperplasias regularly accompanying a thyrotropic mouse pituitary neoplasm, subline L24a, have been reported (22). The original L24 pituitary tumor was developed by Dr. Jacob Furth by 131 thyroid ablation. At the outset of these experiments this tumor was an autonomous growth but highly responsive to suppression by thyroid hormone; implants into intact mice failed to progress or remained dormant for approximately 13 months (27). Growth of one tumor, designated L24a, having a latent period of over a year, in a thyroid-intact mouse was marked by pronounced lymphogenenic or hyperplasia of cells of the lymphoid group in all its subsequent passages. The present report concerns the behavior of this tumor during continued passage, with detailed study of the cell types affected under altered hormonal conditions.

MATERIALS AND METHODS
Tumors.—The association gains interest with the findings in the third transfer of subline L24a. In accord with the behavior of many animal tumors having specific function, continued passage has yielded a more autonomous growth having reduced or altered specific activity (7). With the use of an older tumor as donor tissue it was possible to follow the effects of a less active growth for a period of 12 months after implantation.

A second thyrotropic neoplasm, 17, also obtained through the courtesy of Dr. Jacob Furth, was studied through repeated passage for purposes of comparison. This tumor has maintained uniform behavior with rapid autonomous growth, active secretion of TSH, and a sex dependence which was lacking in the L24 line. A mamnotropic tumor, 38, from Dr. Furth's collection was carried in similar manner to provide further comparison of the effects on mouse lymphoid tissues of a nonthyrotropic pituitary tumor. This neoplasm grew slowly and irregularly during the period of this study but maintained its hormonal effect on the mammary gland.

Technical procedure.—All experiments were conducted on (C57L X A)F1 hybrid female and male mice (hereafter called LAF1), obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine. The technical details of thyroid ablation, tumor transfer, autopsy procedure, tissue section preparation, and multiple stains were essentially as previously described (22), with some variations. Tumors were implanted either intramuscularly into the thigh or into the dorsal subcutaneous tissue. The fixative for all organs contained 85 per cent ethyl alcohol, 5 per cent acetic acid, and 40 per cent formaldehyde, a modification by Lillie of Carnoy's solution which was especially successful in maintaining nuclear fine structure (17). Cytoplasmic RNA is best preserved if the tissues remain in Lillie's fixative for not more than 2 hours and are then transferred to 80 per cent alcohol.

Wright-Giemsa differential stain was applied to imprints of the splenic cut surface. Imprints were necessary for more accurate identification of cell types but required

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correlation with sections to locate the various changes. In later studies more uniform smears of spleen cells have been prepared with a fine camel’s hair brush and dextran. Methyl green pyronine stain\(^1\) was used to facilitate the identification of plasma cells in imprints and in sections. Less variegation and greater intensity of cytoplasmic staining follows the substitution of thionine for pyronine in combination with methyl green.\(^2\) This preparation was applied to tissue sections and smears in the later studies.

**Protocol of major experiment.**—The subline L24a thyrotropic tumor had been carried through two passages as reported (22). The mice in the third passage were organized into three groups:

- **Group A:** 30 mice radiothyroidectomized with 50 \(\mu\)C \(1^31\) and given injections intramuscularly of tumor.
- **Group B:** 30 intact mice also receiving tumor.
- **Group C:** 30 control mice kept through the year of this experiment.

Equal numbers of male and female mice comprised each group. All were about 8 weeks of age and were maintained on Rockland Farm rat diet and tap water except prior to thyroidectomy, when Remington low iodine diet\(^3\) and distilled water were substituted.

**RESULTS**

The L24a tumor grew more slowly than previously with a latent period of 4–8 months, appearing first in intact male mice. “Reverse responsiveness” to the target organ hormone has been previously noted (14). Eventually, all but nine of the 60 tumor grafts progressed, but those persisting 6–10 months after implantation were often hemorrhagic and in good part necrotic. There were tumor cells which appeared viable in histologic sections, persisting 6–10 months after implantation were often hemorrhagic and in good part necrotic. There were tumor cells which appeared viable in histologic sections, but the amount of active tumor in the large hemorrhagic masses could not be evaluated.

From the general pattern of behavior it was clear that the L24a thyrotropic tumor was autonomous but still influenced by thyroid and ovarian hormone secretion. TSH secretion was greatly reduced. Nevertheless, the grafted tumor was regularly accompanied by splenomegalgy of 10–30 times the normal size, with a disproportionate moderate response in lymph nodes and lymphoid nodules of organs (Figs. 1, 2). Splenic enlargement occurred in all mice bearing tumors, with or without the thyroid gland, and failed to develop in the few mice given inoculations in which tumor did not grow. There was no correlation with size of the tumor, since, in a few instances, tumor nodules were found only after careful sectioning of the host’s thigh muscle. Comparable observations are made in regard to human endocrine tumors; minute occult nodules may have striking systemic effect of lasting nature. A few mice of each group died spontaneously and were discarded. The remainder were permitted to live until moribund and were bled from the right heart or vena cava under ether anesthesia prior to the autopsy. All organs were weighed and sectioned.

The spleen weights of control mice in Group C, examined mainly 9–12 months after the beginning of the experiment, were close to 100 mg., with the exception of one instance of spontaneous myeloid leukemia (Chart 1). Most of the tumor grafts grew to lethal size earlier in intact mice (Group B), with another peak in tumor growth in the later months. Thyroidectomized mice (Group A), examined largely in the 6- to 8-month period after tumor implantation, developed the largest spleens—several above 2,000 mg.

**Histologic and cytologic character of the splenopathy.**—

- **Group A:** Radiothyroidectomized, tumor-bearing mice maintained the usual splenic architecture even in the most enlarged organs (Fig. 3). The lymphoid follicles were large and poorly demarcated, owing to an increase in reticulum cells, large lymphocytes, and especially plasma cells in the perifollicular zone. These cells were also prominently increased throughout the red pulp, accounting in good part for the splenic enlargement. Mast cells were also increased 3–4 times over the number in control spleens.

Plasma cells appeared in several stages of maturity, recognized by their large or small, coarse, round eccentric nucleus and abundant pyrininophilic cytoplasm. The spleens of mice bearing tumors of long duration frequently

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1 The most successful methyl green pyronine was obtained from Chroma-Gesellschaft Schmid Co., distributed by Roboz Surgical Instrument Co., Washington, D. C.

2 Contributed by personal communication from Dr. A. Roqué, Roswell Park Memorial Institute, Buffalo, New York.

3 Obtained from the Nutritional Biochemicals Corp., Cleveland 28, Ohio.


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**CHART 1.**—Spleen weights of LAF1 mice with L24a thyrotropic tumor.
were populated by plasma cells with large protoplasmic masses which were pyronine-positive and stained moderately with periodic acid-Schiff reagent, indicating their glycoprotein nature. Similar masses were also found extracellularly and were recognized as Russell bodies, which have been variously interpreted as the result of secretion or degeneration of plasma cell cytoplasm (26) (Fig. 3).

Group B: The follicular and vascular architecture also persisted in the enlarged spleens of intact tumor-bearing mice, but the cell population was more uniform and differed from that in Group A. Plasma cells were less numerous, and small lymphocytes predominated in the red pulp cords (Fig. 4). About four to eight mitotic figures occurred per high-power field. The lymphoid follicles were enlarged and poorly defined. Germinal centers were not distinct, but areas of lymphoblastic or reticulum-cell proliferation appeared in some follicles. One reticulum-cell sarcoma developed in a mouse in the older age group. This was believed to have been a spontaneous occurrence.

Group C: No abnormalities in the pattern or cell distribution were found in the spleens of control mice of comparable age, with the exception of the one instance of myeloid leukemia. Erythropoiesis was usually at a normal level of activity, but in experimental as well as in control animals individual mice had an increase in nucleated red cells in the splenic red pulp. It was not possible to correlate the degree of hematopoiesis with the severity of hemorrhage in tumors or with hormonal state of the mice in this study. Myelopoiesis proceeded at the usual rate in control mice but was relatively inconspicuous in the enlarged spleens of tumor-bearing animals.

Table 1 shows the relative number of plasma cells counted in the red pulp of the spleens. Although there was great individual variation, both tumor-bearing groups showed an increased accumulation over that in the normal spleens. The preponderance of the plasma cell in radiothyroidectomized mice was highly significant.

<table>
<thead>
<tr>
<th>Per cent of plasma cells</th>
<th>Group A, thyroidect., tumor (No. mice)</th>
<th>Group B, intact, tumor (No. mice)</th>
<th>Group C, intact, no tumor (No. mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Above 50</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15-50</td>
<td>5</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Below 15</td>
<td>2</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td>Total no. mice:</td>
<td>20</td>
<td>25</td>
<td>29</td>
</tr>
</tbody>
</table>

Effect of 50 μc. I\(^{131}\) alone.—Four mice surviving 1 year after thyroid ablation have been autopsied. All had large, hemorrhagic pituitary tumors. The thymus and spleen were atrophic, indicating that the isotope alone had not stimulated hyperplasia of lymphoid cells.

Endocrine organs.—The pituitary glands in Group A mice regularly showed a threefold enlargement in both sexes following ablation of thyroid tissue. The appearance of "thyrotrophs" (Fig. 10) attested to the lack of regulating thyroid hormone, and sections of the thyroid area failed to reveal follicular tissue, although the parathyroid glands were often recognized in collapsed stroma of the thyroid gland. The pituitary glands of intact mice were small, 2 mg. or less, and contained uniform basophilic cells. They have not been studied with differential stains.

The average weights of ovaries were 19.0 ± 3.4 and 18 ± 2.1 mg. in the experimental groups, compared with a mean of 13.1 ± 2.3 mg. in control mice. Histologically, tumor-bearing mice showed a moderate increase in follicular and luteinizing activity, as previously described. The testes were not significantly altered in weight or appearance. No variation in average adrenal gland weight or structure was found when tumor-bearing mice were compared with controls of the same sex. The adrenals of females of all groups were larger than those of the male.

The thyroid glands of intact tumor-bearing mice failed to develop a significant increase in mean weight, as compared with the glands of untreated mice. The histologic appearance of the glands suggested a proliferative hyperactivity, since the lining cells of the follicles were cuboidal rather than flat and colloid storage was reduced (Fig. 11). The glands, however, were not as congested as those associated with highly active thyrotropic tumors.

When animals were sacrificed at widely spaced times, the opportunity to pool the blood of individual mice of each group for TSH determination was forfeited. Bioassays by the McKenzie method (13) were performed at intervals. The paucity of control mice for each assay, necessitated by the small quantity of blood serum from
single mice, precludes acceptance of the results as significant; nor does averaging the figures in each group appear to be a legitimate procedure, since the tests were performed at intervals of months. The only further indication of possible thyrotropic stimulation of the thyroid gland can be observed in the liver, which showed moderate hepatic cell hypertrophy and hyperplasia with less lymphoid hyperplasia in the intact tumor-bearing mice (Fig. 12). The appearance of the liver cell may reflect a continued mild stimulus to the thyroid gland (4). Thyroidectomized mice had smaller hepatocytes but greater lymphoid hyperplasia in portal areas (Fig. 8), which canceled out any significant difference in total organ weight in the two groups.

The histologic appearance of the pituitary tumor has remained as described previously except for excessive hemorrhage and necrosis. There was variable lymphocytic and plasma-cell response at the periphery of the tumor. Stasis of small, deeply stained lymphocytes without visible cytoplasm, in lymphatic channels, was observed here as well as in some thymus gland, spleen, and lymph node sections.

Subsequent behavior of L24a tumor.—The growth vigor of this tumor on continued passage has diminished to the point of impracticability for comparative or hormonal studies. In this laboratory mouse-to-mouse continuity of this tumor on continued passage has diminished to the point of impracticability for comparative or hormonal studies. In this laboratory mouse-to-mouse continuity was lost. The excess tumor from that providing the grafts for this study was frozen in liquid nitrogen for a year. On subcutaneous inoculation into 4-day-old mice, only two tumors have grown after 8 months in those given subcutaneous hydrocortisone through the suckling period and for 3-day periods at monthly intervals thereafter. These two mice had only moderately enlarged spleens of about 500 mg., and the increase could be accounted for in part by congestion and erythropoiesis. Lymphoid follicles in the spleen were reduced to half size and were widely separated by congested red pulp with both erythro- and increased lymphocytes and reticulum cells. It is not possible to conclude that hydrocortisone was responsible for suppression of the pronounced lymphoid and plasma cell hyperplasia with two animals alone. The remaining mice are being observed for further developments. On continued passage, the autonomous L24a tumor recovered from the above hydrocortisone-treated suckling mouse grows more rapidly, with commensurate moderation of splenic cellular response. The tumor is now being developed for physiologic studies.

Splenomegaly with thyrotropic tumor 17.—Conclusions cannot be drawn from comparison of the effects of tumors having widely different hormonal activity. However, the behavior of another pituitary TSH-secreting tumor, described in detail elsewhere,4 may contribute some support for considerations expressed in the discussion.

Thyrotropic tumor 17 is an autonomous "reversely responsive" growth with a short incubation period, and it is fatal within about 2–4 months. There is evidence of active TSH secretion in an enlarged, congested thyroid gland histologically very hyperplastic and vascular; yet the tumor does not grow in thyroidectomized mice. Further evidence that the thyroid gland, stimulated to great hyperplasia by Tumor 17, is associated with little hyperthyroidism may be found in the prominent lutein and follicular hyperplasia of the ovaries and the severe involution of the thymus possibly attributable to ovarian hyperactivity. Both gonadal enhancement and thymic atrophy are opposed by normal thyroid hyperactivity, which is assumed to be minimal in these experimental animals. The tumor does not grow in intact male mice.

The spleens of mice bearing Tumor 17 are enlarged only 3–5 times, with cellular increase as well as congestion and erythropoiesis. Congestion in all organs indicates a hypervolemic basis, at least in part. The cause of erythropoietic activity in these mice is not understood. Lymphocytes and plasma cells are moderately increased in the red pulp, and no instance of the striking splenomegaly accompanying L24a tumor has appeared. However, the short course of Tumor 17 is an inevitable result of rapid tumor growth, which may be facilitated by the lesser degree of lymphoid reaction.

Mammotrophic tumor 38, studied briefly in this laboratory, has presented exaggerated myelopoiesis throughout the red pulp, with moderate erythropoiesis and lymph follicle enlargement. This is essentially the response to the presence of a variety of transplanted tumors and probably has a complex origin not specifically related to the behavior of one tumor cell type. An exaggerated reversible myelo-leukemoid reaction to a transplanted squamous-cell carcinoma has been described in detail (10).

DISCUSSION

The lymphoid response to the presence of thyrotropic tumor L24a has been designated as hyperplasia rather than neoplasia, since architectural derangement of enlarged spleens and organ infiltration were minimal. Pleomorphic cell types and variation in plasma-cell differentiation also suggested a nonmalignant state. It has not been possible to obtain progressive growth by the transfer of lymph nodes or spleen to adult or suckling homologous mice. The former were followed for a year. The mice given injections of enlarged spleen in the neonatal period were sacrificed at 10 months. Only one tumor, an unusual reticulum-cell sarcoma, with many large multinucleated cells of histiocytic appearance, was found in the mesentery. This may be regarded as fortuitous or as an indication that the spleen implanted was pre-malignant. However, the distinction between prominent hyperplasia and the neoplastic state, classified as either lymphosarcoma or leukemia, is not a clear one. A period of hyperplasia with normal organ pattern precedes and merges imperceptibly with the more diffuse spread of spontaneous lymphoma (18). Plasma-cell granulomas and plasmacytomas develop under related but varied conditions, and it is presumed that the former eventually may lead to the neoplastic growth (19).

It is possible that growth of the pituitary tumor has augmented a low genetic tendency to develop lymphomas. The LAF hybrid is a vigorous strain of uniform genetic state. These mice have been utilized in radiation longevity and disease incidence studies because of their long life and large healthy litters. The incidence of spontaneous lymphomas is approximately 13 per cent in females and

4 Data of one of the authors (E. S.) to be published.
6 per cent in males living beyond 18 months (25). A higher incidence was reported by Nowell et al. in long-term experiments with LAF$_1$ mice (16). These authors found an over-all incidence of 22 per cent nonthymic lymphatic leukemia in 601 males and females kept through life. Pulmonary adenomas appeared in 24 per cent and ovarian tumors in 11 per cent of the females. During the 12 months of our experiment (mice up to 13.5 months of age) adenoma of the lung appeared in two mice, and no ovarian tumors were seen. Only one leukemia, presenting a contrasting picture of diffuse organ infiltration and generalized lymph node enlargement, was noted in the 30 control mice. The leukemia of strain A mice, the male parent of LAF$_1$ hybrids, does not characteristically involve the thymus and lymph nodes (7).

It is also possible that activity of a latent virus may have been facilitated in some manner by the presence of this pituitary tumor. We have not been able to support such a concept by transfer of the spleen, perhaps because the growth was in a preneoplastic state. If virus is eventually recovered from the spleen, the initial stimulus will still not be established. Virus has been isolated from radiation-induced lymphomas in mice (11) and contributed to lymphomogenesis with chemical carcinogens (8).

The present experiments do not support the hypothesis, previously presented, that high thyrotropin stimulus alone, or in combination with somatotropin or radiation, was responsible for associated lymphoid growth (22). The present tumor has low thyrotropic hormonal activity, as evidenced by the appearance of the thyroid gland. However, a long period of mild thyrotropin hyperactivity may have played a role. In addition, a thyrotropin analog or another form of thyroid-stimulating hormone may be a product of the pituitary tumor. An entity distinct from thyrotropin has been recognized in the plasma of patients with Graves’ disease (1). The substance manifests its presence in a delayed peak of $^{131}$I release from the thyroid in mouse bioassay. Antiserum against bovine or human TSH does not neutralize long-acting thyroid stimulator present in serum of a thyrotoxic patient (2). Long-acting thyroid stimulator is believed to have a site of origin other than the normal pituitary gland (6, 13) but could conceivably be present in pituitary tumor cells. Evidence for the existence of such a delayed thyrotropic effect was reported in our bioassay of the blood of mice developing lymphomas while bearing the L24A tumor in its earlier passages (22).

The concept of an abnormal or unusual hormone secreted by pituitary tumor cells of the mouse leads, on theoretical grounds, to the possibility that lymphoid and plasma-cell hyperplasia with numerous Russell bodies in L24A tumor-bearing mice represented response to a prolonged antigenic stimulus. It should be recalled that this tumor emerged after 13 months of dormant state in an intact mouse, presumably suppressed by thyroxine antagonism. This autonomous, long-dormant growth may have a cell population derived from a minority pituitary cell type of distinct specific activity, ordinarily repressed. Other transplanted pituitary tumors with more physiologic secretion and, hence, less antigenicity and shorter course, may call forth relatively less cellular response. A milder plasma cell or lymphoid response in regional lymph nodes and spleen has been described by Baruah and others during the progressive growth of a variety of transplanted tumors (3, 20).

Metcalf has called attention to an increased incidence of reticular tumors in C3H mice subjected to prolonged antigenic stimulation and suggested that random neoplastic mutations from increased mitotic activity or activation of a latent tumor virus may have been the mechanism of origin of the tumors (15). Malignant lymphoid tumors appeared with the original growth of L24a tumor in our studies (22). These lymphomas may have represented the ultimate effect of such a prolonged antigenic stimulus. The hemorrhagic necrotic state of the pituitary tumors with stasis of small dark lymphocytes at the periphery is further evidence in favor of intense antibody activity.

The explanation for greater numbers of plasma cells with Russell bodies in response to tumors in thyrotoxicized mice, as compared with intact mice, is not clear. Two possibilities suggest themselves: Unopposed TSH may have had a direct effect on the lymphocyte, effecting transformation to the plasma cell. Alternately, radiation in the form of 50 $\mu$c. $^{131}$I may have augmented the tendency in this direction.

The association in man of lymphoid hyperplasia and lymphoma with hyperthyroidism, especially Graves’ disease, has been discussed by Ultmann and associates (24). Concurrent hyperthyroidism and an unusual lymphomatous disease associated with intracytoplasmic crystals in lymphoplasmacytoid cells has also been recorded by Goldberg, who regarded the cells as akin to those seen in some cases of Waldenström’s macroglobulinemia (9). Thomson reports acute leukemia developing in a patient treated with $^{131}$I for hyperthyroidism but exonerates the isotope, since chromosomal aberrations were not found (23).

REFERENCES

2. Adams, D. D.; Kennedy, T. H.; Purves, H. D.; and Sirett,
Fig. 5.—Increased plasma cells in medulla of lymph node of Group A thyroidectomized mouse. There was no disturbance of architecture. X 400.

Fig. 6.—Normal thymic pattern and cell content in L24a tumor-bearing mouse with splenomegaly. X 200.

Fig. 7.—Perivascular lymphoid-plasma cell hyperplasia in lung of Group A thyroidectomized mouse with L24a tumor. X 400.

Fig. 8.—Periportal lymphocyte proliferation and normal hepatocytes in Group A thyroidectomized mouse bearing L24a tumor X 400.
Fig. 9.—Perivascular lymphocyte hyperplasia in kidney in Group B intact L24a tumor-bearing mouse. × 400.

Fig. 10.—“Thyrotrophs” in pituitary gland of Group A thyroidectomized mouse. × 400.

Fig. 11.—Mild hyperplasia of thyroid gland in Group B intact mouse bearing L24a tumor (11a) as compared with normal gland of control mouse (11b). × 400.

Fig. 12.—Periportal lymphoid nodule and hyperplasia of hepatocytes in Group B intact mouse bearing L24a tumor. × 400 (compare with that of thyroidectomized mouse, Fig. 8.).
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