Short Communication

Progressive Atrophy of the Thymus in Mice Carrying Lewis Lung Carcinoma Grafts*

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Summary. A rapid and progressive thymus atrophy in B6D2 mice following transplantation of Lewis lung carcinoma cells is reported. Spleen weight, however, increased in tumor-carrying animals. Tumor extracts failed to induce these alterations. The mechanism of these perturbations of the immune system in tumor-carrying animals is discussed.

Introduction

Nonspecific depression of the immune system has been reported in a variety of human cancers (Lehane and Lane, 1974; Bolton et al., 1975; Eilber et al., 1975; Harris and Sinkovics, 1976). This suppression is most pronounced in advanced cancer patients (Bolton et al., 1975; Eilber et al., 1975), detectable in a proportion of patients at early stages of the disease, and associated with a poor prognosis (Hersh et al., 1976; Pinsky et al., 1976).

In animals carrying experimental tumors nonspecific immune depression has been reported in both mice (Plescia et al., 1976; Haba et al., 1976; Hamaoka et al., 1976) and rats (Schumm and Billmire, 1976). This depression has been shown to affect mainly the T-cell compartment of the immune system, whereas T-independent responses are less affected.

In this report these observations are extended to include thymus atrophy and splenomegaly in mice carrying Lewis lung carcinoma grafts.

Materials and Methods

Mice. B6D2F1/BOM mice [F1, hybrids between C57 (♀) and DBA (♂)] aged 6–7 weeks were used throughout the study (about 20 g). Only males were used.

Evaluation of Tumor Growth and Effect on Thymus and Spleen. At harvest the total body weight of the mice was measured together with the weight of thymus and spleen after desanguination by cardiac puncture. The weight of the primary tumor was determined by weighing both hind legs and subtracting the weight of the left hind leg from that of the right. Each experimental group consisted of at least 5 animals.

Tissue Homogenates. Tumor homogenates were prepared from 2-week-old tumors that then weighed 5–10 g. Isolated tumor tissue was homogenized under sterile conditions at room temperature in a glass homogenator in saline, one part tumor tissue to two parts saline (wt/vol). The homogenate was then spun at 1800 g at 4 °C for 30 rain. The supernatant was stored frozen at -20 °C until used. Liver extracts were prepared in an identical manner. Every 48 h, 0.5 ml extract was injected intraperitoneally. This was the highest dose that the mice tolerated.

Protein concentrations were measured according to the standard Lowry-Folin method (Lowry et al., 1951), and in tumor and liver extracts were 43 mg/ml and 33 mg/ml, respectively.

Determination of Corticosterone and Leucocyte Levels. At the end of each experiment, as indicated above, blood was collected by cardiac puncture, centrifuged at 1800 g for 20 min, and the plasma stored frozen at −20 °C until tested for levels of corticosterone. Before centrifuging, small portions of each blood sample were taken for leucocyte and differential counting. Total leucocyte number was counted with a celloscope (Coulter Counter, Model DN). Differential counting followed standard procedures with May-Grfinwald Giemsa staining. Corticosterone levels were measured with a Cortipac kit cortisol CPB assay using labeled selenium. For corticosterone, 75% cross-reactivity correction with cortisol standards was used.

Histologic Examination. Thymus and spleen from the various experimental groups were prepared by a modified Maximow technique
Results

Effect of Tumor on Thymus Weight. As shown in Figure 1, a rapid and progressive loss of thymus weight was observed in tumor-carrying animals. After 3 days there was significant thymus atrophy with a decrease of about 30 mg. However, this reduction was apparently not related to the tumor-carrying state, as it was also observed in animals receiving tumor extracts or liver extracts. It may therefore be related to the handling of the animals and change of environment. However, no further significant decrease was observed in animals receiving liver or tumor extracts. Thymus atrophy in tumor-carrying animals, however, progressed, and thymus weight had reduced to about 6 mg (less than 10% of original weight) at 17 days.

Since this thymus atrophy might have been secondary to metabolic competition by the tumor cells, the effect of starvation was examined. Mice were given sucrose water (0.7% NaCl, 1.4% glucose) only. As shown in Figure 1, thymus atrophy in these animals was even more profound than that observed in tumor-carrying animals, reaching a thymus weight of only 7 mg after 8 days. A good correlation was found between net body-weight reduction in tumor mice, i.e., total body weight minus tumor weight, and thymus atrophy in tumor-carrying animals as well as starved mice. Histologic examination of thymuses from the various groups showed a normal histologic picture in mice receiving tumor extract and liver extract, similar to that found in control mice. In tumor-bearing animals and starved animals, however, a marked atrophy was observed, predominantly in the cortical areas. The medulla revealed a normal picture, except for slight fibrosis.

Alterations in the Spleen. As shown in Figure 2, spleen weight increased rapidly in tumor-carrying animals, while only a slight increase was observed in animals receiving liver and tumor extracts. By day 13, hypertrophy of the spleen in tumor-carrying animals was three times that in control animals. In starved animals, however, spleen weight decreased markedly to about one-fifth of that in control animals.

Histologic examination of spleens from tumor-carrying animals showed that hypertrophy in these animals was located in the red pulp, which revealed signs of increased hematopoiesis, while the white pulp was normal or somewhat atrophic.

Corticosterone Plasma Levels. Tumor-carrying animals showed a moderate decrease in corticosterone levels in the plasma (90 ± 1 ng/ml at day 17, vs. 140 ± 3 ng/ml in control animals). In contrast, starved mice showed a marked increase in corticosterone levels (510 ± 8 ng/ml on day 8).

Leucocytes in Plasma. The tumor-carrying animals showed a marked increase in total leucocyte number to about 5 times (29.7 ± 4.4 × 10⁶/ml) the control level.