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Enhancement of Spontaneous C3H/HeJ Mammary Tumorigenesis by Long-Term Polyadenylic·Polyuridylic Acid Therapy

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SUMMARY

Female C3H/HeJ mice received weekly s.c. injections of 2 mg polyadenylic·polyuridylic acid. Therapy was initiated at either 2 or 9 months of age. In both cases, polyadenylic·polyuridylic acid-treated animals developed the spontaneous mammary carcinoma associated with this strain more rapidly. Because the opposite result was formerly observed for AKR spontaneous leukemia, the data indicate the polyadenylic·polyuridylic acid has no generalized antineoplastic effect upon spontaneous tumors genetically associated with specific murine strains.

INTRODUCTION

Previous reports that the synthetic polyribonucleotide, poly(A)·poly(U),[1] could inhibit the development of spontaneous neoplastic disease have become the basis for the potential use of this agent in man. For example, weekly injections of poly(A)·poly(U) inhibited tumorigenesis in AKR mice resulting in a 4.5-month increase in median survival time (5). In a more recent study (8), poly(A)·poly(U) decreased postsurgery metastasis in the C3H/HeJ mammary carcinoma model. The present study was designed to test whether or not long-term poly(A)·poly(U) therapy could temporally inhibit the development of the spontaneous mammary carcinoma characteristic of C3H/HeJ mice, as previously observed for the AKR strain.

MATERIALS AND METHODS

Mice. Female C3H/HeJ mice were obtained from the The Jackson Laboratory, Bar Harbor, Maine. This strain is characterized by a spontaneous mammary carcinoma which arises in 60 to 100% of female mice at approximately 8.8 months of age, resulting in a mean life-span of 11 months (11, 13). Evidence indicates that a mammary tumor virus is the etiological agent in this strain (6).

Poly(A)·poly(U). Both polyadenylic acid and polyuridylic acid were obtained from P-L Biochemicals, Milwaukee, Wis. The double-stranded macromolecular, poly(A)·poly(U), was prepared by mixing equimolar NaCl solutions of poly(A) and poly(U) and by allowing annealing of the complementary strands to proceed for 1 hr at 37°. An increased viscosity confirmed that the annealing of the 2 single-stranded polynucleotides to the double-stranded form had occurred, and this may be additionally checked by comparison of melting point curves in which a hyperchromic shift is observed for the double-stranded molecules (2). A final concentration of 10 mg poly(A)·poly(U) per ml was used throughout, and the s.c. injection of 0.2 ml weekly resulted in the administration of 2 mg poly(A)·poly(U) per injection.

Experimental Design. Mice were fed *ad libitum* with Purina NIH Open Formula Mouse Ration 5018. All s.c. injections were made in the right flank. No tumors arose at the site of injection. Autopsies were performed on all animals that died.

RESULTS AND DISCUSSION

Two experiments were performed to assess the effect of poly(A)·poly(U) on the development of the spontaneous mammary carcinoma of C3H/HeJ mice. In the 1st study, retired breeder females were randomized into 2 groups, 1 of which received weekly s.c. injections of poly(A)·poly(U) (2 mg) beginning at 9 months of age. Chart 1 demonstrates that poly(A)·poly(U) therapy enhanced the development of tumors in these mice. When the number of survivors over the course of the experiment was evaluated by the Student *t* test (12), a p value of <0.001 was obtained. Weight changes were comparable for both groups of mice, and therefore this parameter could not explain the altered incidence of tumor. Furthermore, the decrease in longevity could not be explained by poly(A)·poly(U) toxicity since younger mice tolerated a similar long-term regimen with no apparent problem. Autopsies revealed the presence of gross mammary tumors in essentially all cases. The data show that poly(A)·poly(U) therapy decreased the latent period for the development of and resulting death due to spontaneous tumor. The median survival time of approximately 14 months for control mice was somewhat longer than normally observed and resulted from the use of 9-month-old animals for the study. This represented a population selected in favor of survival because those mice of similar litters that died between birth and 9 months of age due to increased susceptibility to the tumor or due to other genetic factors were not a part of the study.

[1] The abbreviation used is: poly(A)·poly(U), the double-stranded macromolecule polyadenylic·polyuridylic acid.

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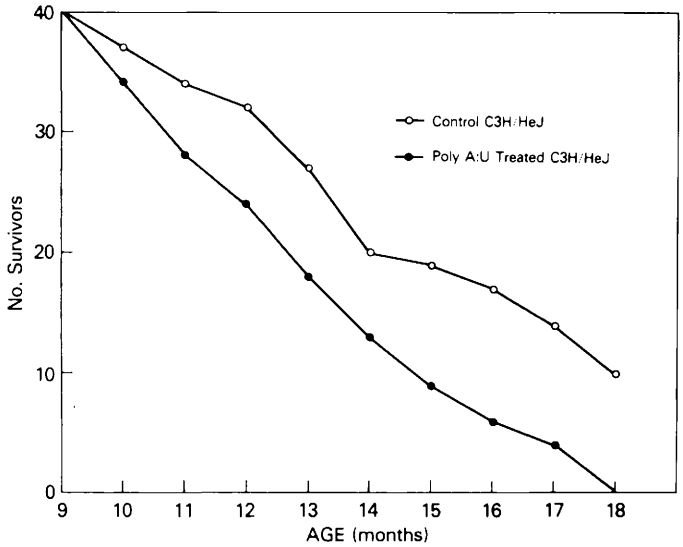

Chart 1. Survival of C3H/HeJ mice following poly(A)·poly(U) injections beginning at 9 months of age.

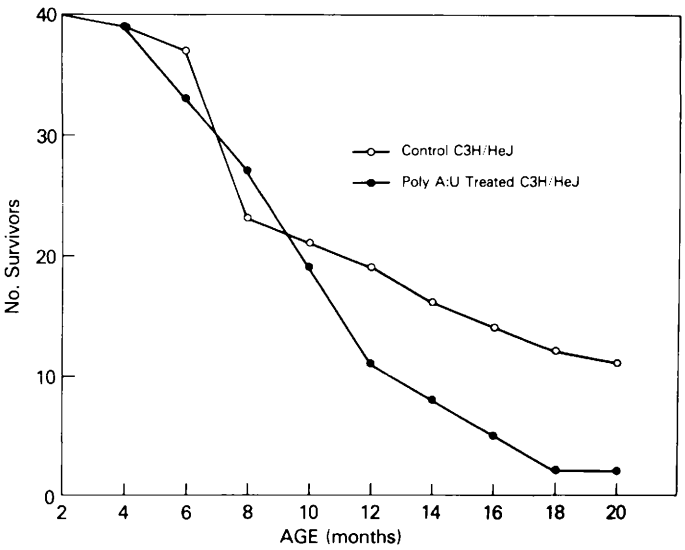

Chart 2. Survival of C3H/HeJ mice following poly(A)·poly(U) injections beginning at 2 months of age.

Because the initial study utilized 9-month-old mice, it was highly probable that poly(A)·poly(U) administration was begun subsequent to virus-induced neoplastic transformation. In such a case, poly(A)·poly(U) would have to operate immunologically or otherwise against the transformed cells in order for any effect to be observed. In order to test whether or not poly(A)·poly(U) might inhibit virus-induced cell transformation instead, 2-month-old C3H/HeJ mice were treated weekly with 2 mg poly(A)·poly(U). Such mice are free of clinical evidence of neoplastic disease which otherwise arises at about 8.8 months (11, 13). Chart 2 indicates that treatment with poly(A)·polyU) did not begin to alter survival patterns until 10 months of age. At this point, poly(A)·poly(U) therapy again enhanced spontaneous tumor development resulting in the decreased longevity compared to controls. As with the initial study using 9-month-old animals, weight changes between the control and treated groups were comparable, and autopsies revealed that essentially all mice died of spontaneous mammary carcinoma.

Lacour *et al.* (8) demonstrated that poly(A)·poly(U) therapy decreased the incidence of metastasis in C3H/He mice following surgical removal of the spontaneous tumors. In that study, C3H/He mice received weekly injections of 250 μg poly(A)·poly(U) i.v. following mastectomy. Poly(A)·poly(U) prolonged survival time when compared to surgery alone. More recently, Lacour *et al.* (7) reported that the i.p. injection of newborn C3H/He mice with 15 μg poly(A)·poly(U) on Days 1, 3, and 5 after birth decreased the eventual incidence of mammary tumors. Two points relative to their data should be made. Although the previous results show a decreased incidence of tumor among poly(A)·poly(U)-treated mice, Lacour's Table I (7) indicates that survival was better for nontreated animals [43 of 127, (33%) *versus* 22 of 83 (26%) for the poly(A)·poly(U)-treated mice]. In addition, the termination of their study at 13 months may have been premature since, as seen in Charts 1 and 2, the greatest difference in survival rates occurs after 10 to 13 months.

There are no solid data on the mechanism by which poly(A)·poly(U) affects the development of spontaneous neoplasia. Although it is clear that polynucleotides can inhibit the replication of C-type viruses *in vitro* (1, 14), the diversity of effects attributable to poly(A)·poly(U) in various models (3, 4, 10) has made it difficult to define its precise mode of action *in vivo*.

When compared to the previous observation of a temporal inhibition of spontaneous AKR leukemia, the present demonstration of poly(A)·poly(U)-mediated enhancement of tumorigenesis in the C3H/HeJ strain underscores the difficulty of predicting the efficacy of poly(A)·poly(U) on experimental tumors. Such discordant results are characteristic of immune adjuvants generally (9) and indicate the need to prove the effectiveness of such agents in animal tumor models analogous to specific human cancers before widespread clinical application is attempted.

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