Influence of Preinfection of C57BL/6 Mice with Graffi Leukemia Virus on 3-Methylcholanthrene-Induced Subcutaneous Sarcoma[1] (37660)

CARRIE E. WHITMIRE AND RONALD A. SALERNO[2]

Department of Viral-Chemical Oncology, Microbiological Associates, Bethesda, Maryland 20014

The interaction of virus and chemical or physical carcinogens has been reported as cocarcinogenic (1–3), anti-carcinogenic (4–6) or without effect (7, 8). The importance of the interaction of the experimental host, the viral and chemical carcinogens on co-, anti-, or nil carcinogenic effects has been demonstrated (9). Few studies have questioned the influence of two oncogenic agents which induced two different types of neoplasms (10, 11). Dimethylnitrosamine, which induced benign and malignant liver and lung tumors, had no effect on the development of viral-mediated lymphoma in the AKR mouse (10). Other reports suggest that different neoplasms are mutually exclusive (12–14). Lilly and Duran-Reynals (15) reported that 3-methylcholanthrene (MCA) induced skin carcinogenesis and spontaneous viral leukemia in the AKR mouse did not occur together at the expected frequency. We found that the occurrence of spontaneous leukemia and/or MCA-induced subcutaneous sarcoma in AKR mice was dependent on the latency period required for each neoplastic type to develop (16). Leukemia and sarcoma often developed in the same animal when the latency period for MCA sarcoma development coincided with the natural latency period for leukemia development. Recently Chen *et al.* (17) have demonstrated that one spontaneous tumor does not exclude the development of a second tumor and suggest that transformation events may occur simultaneously and independently in two different types of tissues and organs.

The present study on the combined effects of the Graffi leukemia virus and MCA investigates the mutual influences of virus-induced leukemia and chemically-induced sarcoma in C57BL/6 mice and examines further the latency period as a determining factor in the co-occurrence of induced neoplasms differing in cell type.

Materials and Methods. Virus. Graffi type C RNA virus (18), which induces lymphatic leukemia in mice, was maintained in our laboratory by passing extracts of virus-induced neoplastic thymus, spleen, and mesenteric lymph node tissues into C57BL/6Cum mice. For these experiments a pool of 10% infected tissues was lyophilized and preserved at 3°. The virus was titrated for infectivity by the spleen antigen test in which C57BL/6Cum newborn mice less than 72 hr of age (NB) were inoculated intraperitoneally (ip) with 0.05 ml/log dilution of virus; two litters were used for each dilution and the spleens were harvested individually for half the mice at 27 days and for the remaining mice at 42 days. Indication of infection was determined by testing the spleens in the complement fixation test for the gs antigen of type C RNA virus (19). These tests indicated a virus dose producing infectivity in 50% of the animals (ID_{50}) of $10^{2.4}$ at 27 days and $10^{3.3}$ at 42 days per 0.05 ml. Intraperitoneal inoculation of $10^{-2.5}$ log/0.05 ml dilution induced 12–21% leukemia and $10^{-1.5}$ log/0.05 ml dilution induced 100% leukemia in NB or 1-week-old mice in the 8-month observation period (Figs. 1 and 2). A partially purified,

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[2] Present address: Division of Virus and Cell Biology Research, Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486.

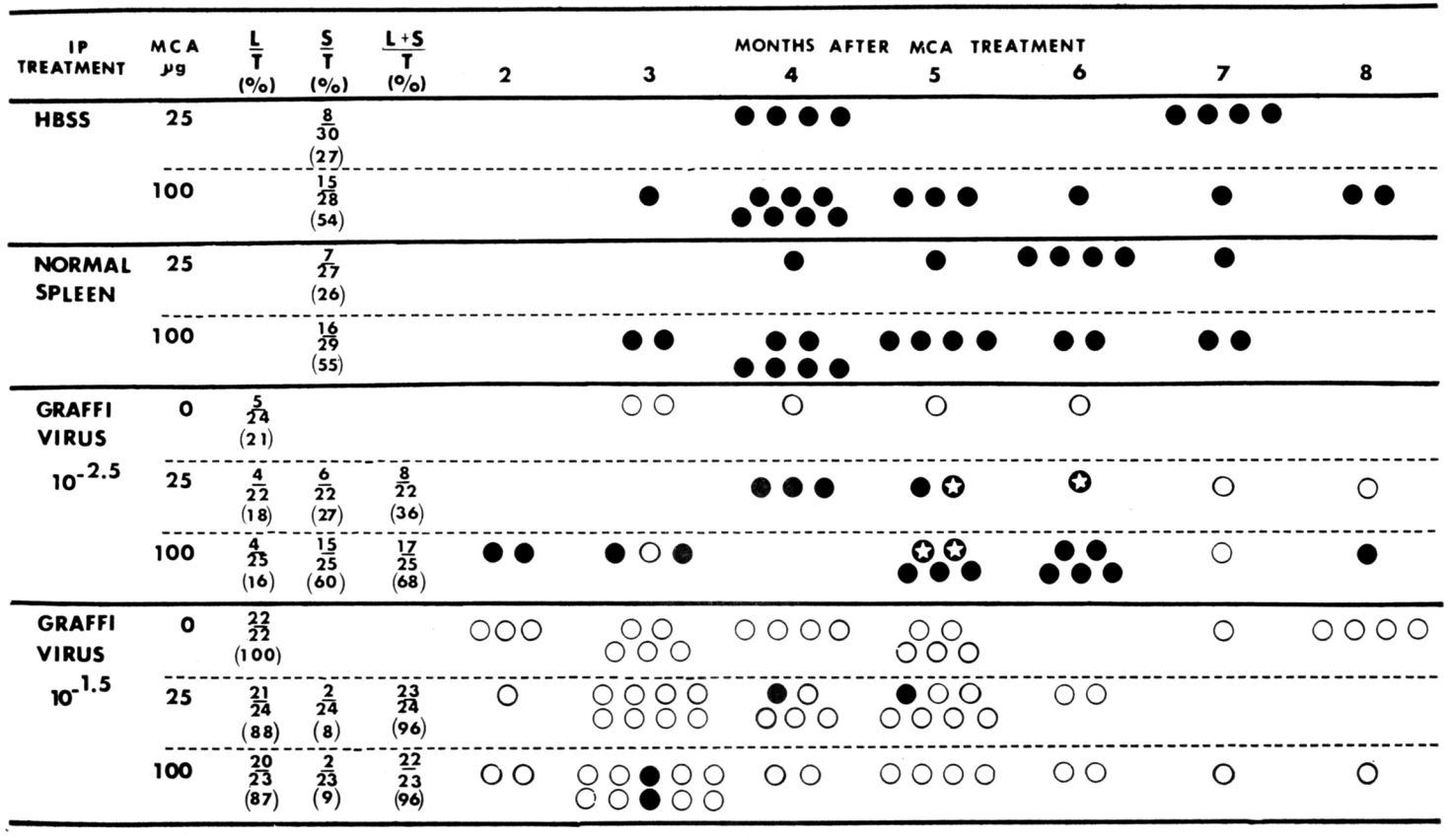

FIG. 1. Incidence and type of neoplastic expression when C57BL/6 mice were infected with Graffi virus as newborns and given MCA as weanling mice. L/T: Incidence of leukemia/total mice at risk for 8 months; S/T: Incidence of sarcoma/total mice at risk for 8 months; and L + S/T: Total incidence of neoplasia/total mice at risk for 8 month. (●) Sarcoma; (○) Leukemia; (✪) both sarcoma and leukemia.

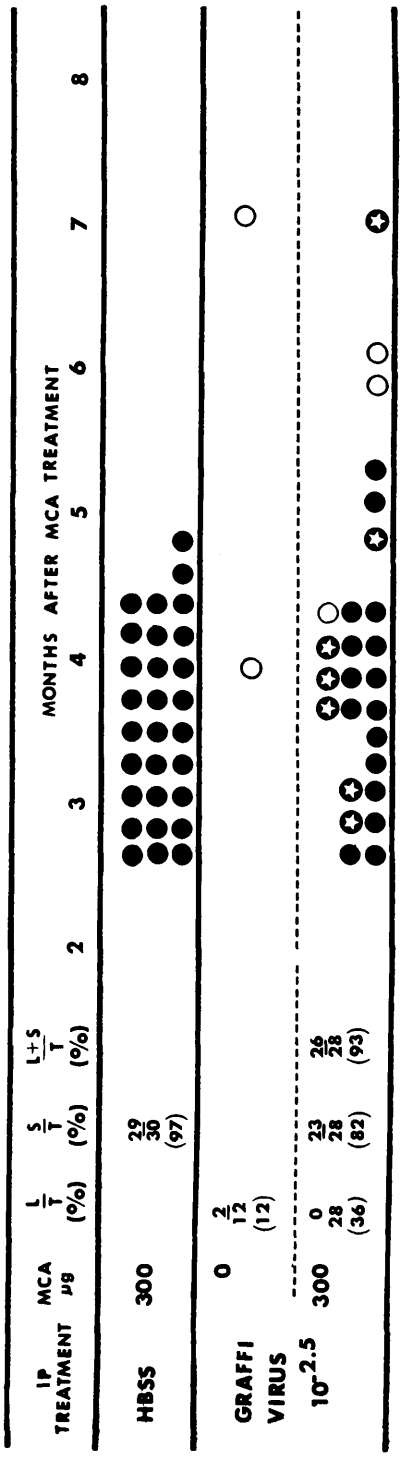

FIG. 2. Incidence and type of neoplastic expression with C57BL/6 mice infected with Graffi virus at one week of age and given MCA at two weeks of age.

noninfected preparation of normal spleens from 4–6 week old C57BL/6Cum mice was prepared using a modified Moloney purification procedure (20) and was used as a control inoculum at $10^{-1.5}$ log/0.05 ml.

Chemical carcinogen. Trioctanoin and MCA were obtained from Eastman Organic Chemicals, Rochester, New York. Doses of 25, 100, or 300 µg MCA/0.05 ml trioctanoin were inoculated subcutaneously (sc), as described previously (21).

Treatment. In Experiment 1, NB mice were randomly divided into 4 groups of about 90 mice (45 males and 45 females) each and received one of the following treatments: (1) 0.05 ml Hanks' Balanced Salt Solution (HBSS), (2) 0.05 ml of $10^{-1.5}$ normal C57BL/6Cum spleen preparation, (3) 0.05 ml $10^{-2.5}$ Graffi virus preparation, (4) 0.05 ml $10^{-1.5}$ Graffi virus preparation. All dilutions were made in HBSS and inoculated ip. When the mice were 4 weeks old, each group was divided into 3 subgroups of 30 mice and inoculated sc with either trioctanoin, 25 or 100 µg MCA.

In Experiment 2, male and female 7-day-old mice were inoculated ip with 0.05 ml $10^{-2.5}$ Graffi virus preparation or HBSS and sc with 300 µg MCA or trioctanoin at 14 days of age. There were about 30 mice per group.

Autopsy. Mice were sacrificed when subcutaneous tumors were 1.5–2.0 cm in size or when the mice were moribund with leukemia. Leukemia was confirmed by standard histological procedures as lymphatic leukemia; subcutaneous tumors were fibrosarcomas.

Analysis of data. Eight months after mice were given MCA, the tumor incidence and latency periods were computed by methods described previously (21). Latency for tumor development was based on the time from MCA treatment for tumors to develop to 1.5–2.0 cm in size. Coexpression of leukemia and sarcoma was based on the histological diagnosis of leukemia at the time mice were sacrificed with sarcoma. The ID_{50} dose was determined by the method of Reed and Muench and expressed as the antilog of the log ID_{50} (22).

Results. Experiment 1. When mice were

infected with a high dose of Graffi virus ($10^{-1.5}$) the predominant neoplastic expression was leukemia. MCA treatment (25 or 100 μg) did not significantly effect the incidence of Graffi leukemia, however the incidence of MCA-induced sarcoma was significantly reduced ($p < 0.01$) from that induced in uninfected mice or mice receiving normal spleen placebo (Fig. 1).

When mice were infected with a low dose of Graffi virus ($10^{-2.5}$) the incidence of leukemia was low (5/24, 21%) in the 8-month observation period. MCA treatment combined with this virus dose increased the total incidences of neoplasia although the incidence of either leukemia or sarcoma was not influenced. Several animals developed both sarcoma and leukemia however the general rule was development of either leukemia or sarcoma. The incidence of sarcoma was MCA-dose dependent (Fig. 1).

Experiment 2. Sarcoma was made the predominant neoplastic expression by giving a high dose (300 μg) of MCA. In order to reduce the incidence of Graffi leukemia, a low dose of virus ($10^{-2.5}$) was given at 7 days of age instead of during the first 72 hr after birth. Although the results in Experiment 1 (Fig. 1) failed to demonstrate any effect of MCA on the incidence of leukemia induced with this dose of virus, the results of Experiment 2 (Fig. 2) demonstrated an overall increase in the incidence of leukemia when 300 μg MCA was given 7 days after virus. This increase in the incidence of leukemia was due to co-occurrence of the two neoplastic expressions diagnosed at necropsy. MCA alone does not produce leukemia in 8 months when given sc.

Discussion. The development of Graffi virus leukemia and/or MCA sarcoma in C57BL/6 mice receiving both virus and MCA was dependent on the dose of each carcinogenic agent administered. When a high dose of virus was given which induced 100% leukemia in 8 months, a low dose of MCA did not appreciably alter the incidence of leukemia, however, the incidence of sarcoma was significantly reduced (Fig. 1). If, however, a low dose of virus was combined with a high dose of MCA which induced virtually 100% sarcoma, the incidence of leukemia was increased (Fig. 2). When a low dose of virus and a low dose of MCA were combined, the incidence of leukemia or sarcoma was not altered (Fig. 1).

Why was one neoplastic expression favored over the other based on the dose of each carcinogen? A primary reason one neoplastic expression is favored over another is that in both chemical- and viral-induced neoplasia the latency period is inversely related to the dose of carcinogen given. With 25, 100, and 300 μg MCA, the average latency for sarcoma development was 24, 20, and 14 weeks, respectively. Graffi viral leukemia was induced with $10^{-2.5}$ and $10^{-1.5}$ virus dilution in 18–20 and 17 weeks, respectively. If a high dose of leukemia virus ($10^{-1.5}$) is combined with a low dose of MCA (25, 100 μg), then leukemia would precede sarcoma induction, thereby reducing the population at risk at the time of sarcoma development. This is similar to the findings of Figge (13) and Squartini (14) in the development of leukemia and mammary tumors. Figure 1 demonstrates the occurrence of high incidences of leukemia during the 2nd and 3rd months while sarcoma development was delayed until the 4th and 5th months with 25 and 100 μg MCA. On the other hand, a high dose of MCA (300 μg) given to 14-day-old mice induced sarcoma during the 3rd and 4th months which more closely coincides with the time leukemia develops with $10^{-2.5}$ dose of Graffi virus. This would account for the development of leukemia and sarcoma in the same animal receiving both virus and chemical with these doses. There is an apparent increase in the incidence of leukemia and an acceleration of its development in these animals indicating a cocarcinogenic effect; however, Graffi virus failed to increase the incidence of MCA induced sarcoma under any of the conditions studied.

As shown above, the combination of a low dose of virus ($10^{-2.5}$) and low doses of MCA (25 and 100 μg) did not alter the incidence of leukemia or sarcoma. Although the incidence of leukemia and sarcoma were small there appears to be a change in the average latency periods for neoplastic development to oc-

cur. In these mice receiving virus and 25 μg MCA, the latency period for leukemia was delayed by 14 weeks (32 vs 18 weeks) while sarcoma induction occurred during a 7-week period, 14–21 weeks post-MCA treatment, with an average latency period of 17 weeks. When 25 μg MCA was given alone, sarcomas developed over a 10-week period, 19–29 weeks post-MCA treatment, with an average latency of 24 weeks. Similar effects on latency were observed between RadLV-induced leukemia and MCA-induced sarcoma, however, with RadLV a significant decrease in sarcoma induction occurred (23). These influences seen with RadLV and MCA were postulated to be related to a host immunological response to a common viral genome antigen in the chemically-induced sarcoma and viral-induced lymphoma. A similar immunological response bringing about a delayed development of leukemia may be responsible for the alteration in Graffi virus leukemia latency period. No reduction in incidence of sarcoma was seen, however, and may indicate a difference in antigenic components of RadLV and Graffi leukemia viruses.

From these studies and other studies (16, 21, 23–26) we have concluded that two neoplastic expressions (induced or spontaneous leukemia and MCA sarcoma) in the same mouse can influence each other. This influence is dependent on the latency period for development and frequency of occurrence of each type of neoplasm. Both latency and incidence factors are related to the dose of each carcinogen in the case of viral or chemical induced carcinogenesis. In both viral and spontaneous neoplasm the stage of neoplastic development at the time the chemical carcinogen is administered is also an important factor (16). The exclusion of one type of neoplasm by another is a function of life span of the animal (16, 25, 26) or possibly to cross immunological influences (6, 23). Although MCA may increase the incidence of leukemia under certain circumstances, we have not succeeded in increasing the incidence of sarcoma by combined treatment. On the contrary, RadLV-induced leukemia inhibited MCA sarcoma induction (23). For co-occurrence of two different neoplastic expressions in the same animal, they must have similar latency periods.

Summary. When C57BL/6 mice were given both Graffi leukemia virus and 3-methylcholanthrene the development of either leukemia and/or sarcoma was dependent on the dose of each carcinogen given. A high dose of virus reduced sarcoma induction because the survival time of the mice was less than the average latency period required for sarcoma induction due to the high incidence of leukemia. A high dose of 3-methylcholanthrene (300 μg) increased the incidence of leukemia induction by a low dose of virus without affecting the incidence of sarcoma. This occurred since the latency period for sarcoma and leukemia coincided and 25% of the mice developed both leukemia and sarcoma. The combination of a low dose of virus and a low dose of 3-methylcholanthrene did not alter the incidence of leukemia or sarcoma; however, with this combination of virus and chemical carcinogens, the average latency period for the development of leukemia was delayed and the average latency period for sarcoma induction was accelerated. Graffi virus failed to increase the incidence of MCA induced sarcoma under the conditions studied.

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