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A Pneumotropic Line of Lymphocytic Leukemia from Inbred Princeton Mice. (25954)

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Lymphocytic leukemia (lymphosarcoma) occurs naturally in adult Princeton mice (Pr, PRI, or P) from the random-bred colony maintained at the Rockefeller Institute. The disease is sporadic with a morbidity rate of 3.1% in 1050 discarded breeders, 10 to 12 months old, killed and autopsied during the past 7 years. Six of the 33 cases of leukemia (2.7%) were recorded in 215 males and 27 (3.2%) in 835 females. A branch of the Princeton strain with a high rate of leukemia, 80 to 90% in old adults, was established by Dr. Clara Lynch in 1946(1). This branch, designated PL or PrL, was inbred for more than 20 generations. In connection with other studies on murine leukemia it was of interest to determine the behavior of the PrL line in random-bred Princeton mice. Differences in activity of neoplastic cells from the 2 lines, one with low morbidity under natural conditions and the other high, were brought out in transmission experiments and are presented here. The naturally acquired leukemia of adult mice from the random-bred Princeton colony is characterized chiefly by marked enlargement of regional lymph nodes and spleens. The disease is reproducible in weanlings from the same colony by intraperitoneal injection of organ suspensions from naturally affected adults and is transmissible by serial passage. On repeated passage in recently weaned mice hepatitis virus may appear and alter the leukemic reaction.

Materials and methods. Random-bred female weanlings under 15 g in weight, usually 10 to 12 g, were used in most of the following experiments. The PrL line was discontinued by Dr. Lynch in 1951 but a subline was maintained by Dr. Wilhelmina F. Dunning at Univ. of Miami, Fla. We are indebted to her for a supply of young PrL mice with transplanted leukemia. Suspensions for intraperitoneal injection were prepared from pooled mesenteric lymph nodes and spleens of 2 or more leukemic mice. A bulky but unmeasured tissue mince, finely cut with scissors, was added to 2 or 3 ml of saline. After standing for a brief period the turbid supernatant was removed and constituted the inoculum. Similar suspensions were prepared from lungs and thymus for nasal instillation. Lymphocytes were verified by phase microscopy. In the presence of hepatitis virus liver minces were homogenized in a glass tissue grinder with sufficient saline to make a concentration of about 10%. Testing of suspensions was carried out in groups of 5 or 6 mice. Intraperitoneal injections were made with an inoculum of .1 to .15 ml and nasal instillation, following ether anesthesia, with about .05 ml. The injected mice were usually held under observation until one or more deaths had occurred. At this time all survivors that showed signs of illness were killed with ether and autopsied.

Results. A passage series begun in weanlings with an organ suspension from a random-bred leukemic adult was maintained for 40 transfers in 240 mice. The interval between injection and onset of external signs varied from 25 days in the first passage to 7 days after continued transfer. Mortality rate

was 25% and morbidity rate 88%. At autopsy regional lymph nodes, particularly the mesenteric, were enlarged and generally colorless. Spleens were hyperplastic and often pallid. Involvement of liver, kidneys and ovaries also occurred but was more variable. Free fluid was occasionally present in the peritoneal cavity. Lungs were consistently normal and thymus pale. The invasive cell in sections of leukemic organs was a lymphocyte. All passage series made with similar suspensions during the past 7 years have conformed to this general pattern.

None of the mice in this series showed any evidence of murine hepatitis. The number of passages which may elapse before appearance of the causal virus is unpredictable and highly variable. In a later series necrotic lesions were detected in the liver after 8 passages and a virus of low virulence recovered. Unlike the virus reported in 1952 (MHV Pr) this strain was rarely lethal on intraperitoneal injection in weanlings(2). In presence of the virus the morbidity rate from leukemia was not appreciably affected but the pathologic reaction was less marked and somewhat delayed.

A similar passage series was begun in random-bred weanlings with a spleen and lymph node suspension from 2 leukemic mice of the inbred PrL branch. Thirty-six successive transfers were made in 216 females at intervals of 6 to 8 days. Morbidity and mortality rates were 94 and 31%, respectively. The macroscopic pathologic response was noticeably different from that of the preceding series. Hyperplasia of regional lymph nodes was accompanied by congestion resulting in a pink or light red coloration. In some mice the axillary and inguinal nodes were surrounded by a wide red zone of subcutaneous injection. Spleens were commonly enlarged and somewhat darker in color than normal. Involvement of livers, kidneys and ovaries also occurred but was not constant. Lungs were usually affected and showed pink areas of stippling or streaking. This reaction was best observed in the gross with a low power dissecting microscope.

Proliferation of the neoplastic cells in the lungs was clearly indicated on histological examination. Infiltration of the alveoli by the leukemic lymphocytes resulted in formation of discrete compact islands (Fig. 1). Perivascular and peribronchiolar cuffing also occurred. Interstitial tissue was somewhat distended by erythrocytes and lymphocytes. Erythrocytes and, less regularly, fluid deposits were present in some of the alveoli. In more advanced cases, characterized by areas of frank consolidation, the normal architecture of the lung was obliterated by extensive sheets of closely packed tumor cells.

In the 36th passage necrotic foci appeared in the liver of one mouse and hepatitis virus of low virulence was subsequently recovered. Ten additional spleen passages were made. Results were variable but indicative of interference in development of the leukemic cells. Mortality rate of 60 mice was 42%. Thirty of the 34 survivors, killed on the 7th day after injection, showed liver lesions (necrotic foci, hemorrhages and pits) pathognomonic of murine hepatitis. Twenty-one of the survivors also showed leukemic manifestations which were somewhat less advanced than those observed prior to onset of hepatitis. A liver section in which an extensive area of necrosis is adjacent to a cluster of the leukemic cells is presented in Fig. 2.

Macroscopic appearance of lungs from the 2 leukemic PrL mice was suggestive of the pulmonary reaction produced in mice by viruses of grey lung pneumonia and wild rat pneumonia(3,4). The resemblance was subsequently shown to be purely superficial by histological examination but it prompted injection of lung suspensions by the nasal route. Cell-free supernatants were innocuous on nasal instillation but whole suspensions resulted in pulmonary as well as generalized leukemic lesions. Forty passages were made in 218 random-bred weanlings. The respective morbidity and mortality rates were 82 and 38%. The interval between injection and onset of leukemic symptoms was longer and more variable than that following intraperitoneal injection. Average interval was 16 days, with a minimum of 11 and a maximum of 29 days. Ninety-two mice were killed and autopsied

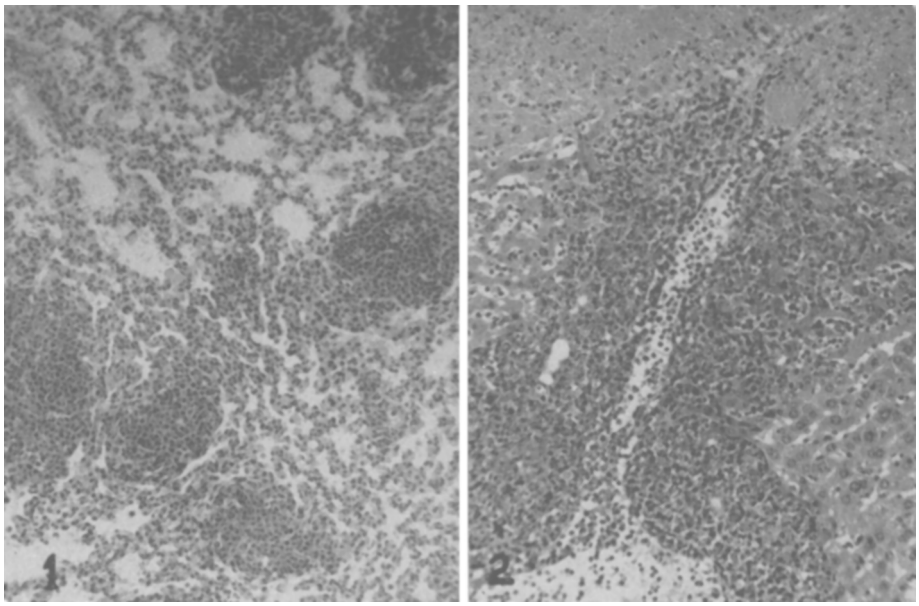

FIG. 1. Photomicrograph of lung removed on 7th day from a Princeton mouse inj. intraper. with leukemic cells of PrL line. Compact groupings of neoplastic cells and interstitial thickening. HE 125×.

FIG. 2. Photomicrograph of liver removed on 7th day from Princeton mouse inj. intraper. with spleen suspension of PrL line. The margin (upper) of a large necrotic focus is bordered by infiltrating leukemic cells and a small area of normal liver parenchyma. HE 125×.

shortly after outward signs of illness were observed. Pulmonary involvement was constant and usually accompanied by hyperplasia of spleens and lymph nodes. It may be noted that Princeton mice from our colony are free from intercurrent respiratory disease. The red coloration of all leukemic organs except the kidneys was particularly striking in the mice of this series.

None of the mice injected intranasally showed any indication of hepatitis at autopsy. Following appearance of the causal virus in the mice of the preceding intraperitoneal series a second one was begun with a lung suspension from the 20th nasal passage. Sixty-eight successive spleen and lymph node transfers have now been made in weanlings in absence of hepatitis. Free fluid containing the leukemic cells was first observed in one mouse of the 36th passage and by the 50th one was a nearly constant finding. In the presence of fluid, leukemic response of the abdominal organs was noticeably retarded.

Attempts to induce pulmonary lesions with the low leukemic line of random-bred Princeton mice were uniformly unsuccessful. In these experiments suspensions of lungs or spleens and lymph nodes from mice of the intraperitoneal series were injected intranasally. Lungs of the injected mice, some of which were held for 2 months, were regularly normal. Nasal instillation of one spleen and lymph node suspension was attended, however, by leukemic involvement of regional and abdominal lymphoid organs. The neoplastic cells were maintained for 2 additional nasal passages but were not demonstrable thereafter.

Discussion. In reviewing the earlier work on lymphocytic leukemia in mice Dunn stressed the individual and recurring pattern of organ involvement and noted that the lungs were sometimes extensively invaded(5). The observations of Furth, Seibold, and Rathbone and of Furth on pulmonary infiltration in leukemia are noteworthy(6,7). Furth appears to have been the first to induce tumor growth in lungs of mice by nasal instillation of neoplastic cells(7).

Our autopsy records of the low level leukemia in random-bred Princeton mice number 33 natural cases and some hundreds of experimental ones. In no instance was a pul-

monary reaction observed macroscopically at autopsy. The pathogenicity and invasiveness of the neoplastic cells were evidently altered during their residence in inbred mice. How or when these changes occurred is a matter of speculation. On transmission to random-bred mice the altered cellular behavior was indicated by an increased toxic reaction in lymphoid tissue and by regular proliferation in the lungs. The latter change was so constant on both intraperitoneal and intranasal injection that the term pneumotropic line seemed appropriate.

In view of the present emphasis on viruses in the etiology of certain murine tumors including the leukemias the possible role of the respiratory tract in outward dissemination of these agents may be worthy of consideration.

There was no apparent difference between the 2 leukemic lines in regard to their effect on emergence of murine hepatitis virus in Princeton weanlings following intraperitoneal injection. Similar viruses were obtained with both lines. There was reason to believe, however, that nasal instillation of the high level leukemic cells provided a means of avoiding the liver lesions produced by these latent agents.

Summary. 1) Lymphocytic leukemia, of sporadic occurrence in random-bred Princeton mice (3.1% in 1050 adults), was transmitted to weanlings by intraperitoneal injection of lymphoid organ suspensions and maintained by serial passage. Spleens and regional lymph nodes were commonly enlarged but lungs were not involved. 2) A high level leukemia line from inbred Princeton mice, originally established by Dr. Clara Lynch, was similarly maintained in random-bred weanlings. Neoplastic cells also proliferated in lymphoid tissue, though with evidence of increased toxicity, and also invaded the lungs. Pulmonary lesions as well as generalized ones were regularly produced by intranasal injection of cells from high leukemia line but not from the low level one. 3) Necrotic lesions of liver appeared in one passage series with each of the 2 lines and murine hepatitis viruses of low virulence were recovered.

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Overwintering of Western Equine Encephalitis Virus.* (25955)

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The mechanism of maintenance of Western equine encephalitis virus in nature during non-epidemic periods and particularly during cold winter months has puzzled many investigators. This virus has been isolated from many species of birds(1,2,3) and a chronic viremia has been produced in experimentally infected wild birds from 1 to 10 months duration(4). This finding has suggested the possibility of an overwintering reservoir for the virus. In studies of 1503 birds, Kissling *et al.* (5) failed to isolate virus from wild birds either in winter or spring, but during the summer when mosquitoes were active virus infected birds were present. Although WEE virus has been isolated repeatedly from pools of mosquitoes collected during practically every month of the year in temperate cli-

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