Bacterial Polysaccharide, Cortisol, and Acute Leukemia in the Rat.* (25310)

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Murphy and Sturm(1) described a transplantable lymphosarcoma originally induced by 1, 2, 5, 6 dibenzanthracene in the rat. When the tumor cells were injected subcutaneously, a lymphosarcoma developed at that site, while their intraperitoneal injection resulted in rapidly fatal leukemia. Like other malignant neoplasms in rodents(2-6), the Murphy-Sturm lymphosarcoma undergoes necrosis following injection of polysaccharide complexes of Gram-negative bacteria. In previous studies(7,8) necrosis and regression of the Murphy-Sturm lymphosarcoma has been induced by *Klebsiella pneumoniae* polysaccharide. The present study concerns effects of *K. pneumoniae* polysaccharide in the Murphy-Sturm leukemia, inhibition of anti-leukemic effect of bacterial polysaccharide by cortisol administration, and tumor resistance following successful treatment of leukemic rats.

Materials and methods. Young, male Sprague-Dawley (Holtzman) rats, weighing 125-135 g, were maintained on Purina laboratory chow, lettuce and water. Except for normal controls, each rat received approximately 100,000 cells from a Murphy-Sturm lymphosarcoma, suspended in 0.85% NaCl and culturally free of pleuropneumonia-like organisms and bacteria. Part of the animals received tumor cells intraperitoneally and the remainder, intravenously. Body weights and peripheral blood leukocyte counts were determined daily and erythrocyte counts and hematocrits were done at various intervals during experiment. Haptenic polysaccharide, non-lethal in control rats(9), was prepared by alkaline extraction and ethanol precipitation from *Klebsiella pneumoniae* as previously described(10). Except for leukemic controls, rats from both intraperitoneally and intravenously implanted groups were given 4 daily consecutive injections by tail vein, 0.1 mg/100 g body weight/injection, of the polysaccharide prepared as 0.1% solution in 0.85% NaCl. Animals in the intraperitoneally implanted group were given the first polysaccharide injection when peripheral blood contained lymphoblasts and total leukocyte count exceeded 20,000/mm^3. Intravenously implanted rats received the initial polysaccharide injection either upon first decline in growth rate or 1 or 2 days later upon first elevation of white blood cells. Beginning 2 days prior to the polysaccharide injections daily subcutaneous injections of cortisol acetate, 2.5 mg/100 g body weight/day, were given to part of the rats in intravenously and intraperitoneally implanted groups and at about the same time (5 to 8 days after tumor cell implantation) in the leukemic control rats. Cortisol administration was continued until early death of leukemic rats or for 1 week after last polysaccharide injection in surviving animals. To evaluate the effects upon body weight, one group of normal rats was given daily injections of cortisol acetate and another group, the 4 daily injections of polysaccharide. One month after recovery from leukemia, part of the polysaccharide-treated rats were reinjected by tail vein with a suspension of lymphosarcoma cells and body weight and white blood counts determined every few days for 3 months. Autopsies were performed and microsections of various tissues prepared from animals after polysaccharide treatment and from untreated leukemic rats 1, 3, 5, 7 and 9 days after implantation of tumor cells. Smears of femoral bone marrow were prepared with Wright's stain.

Results. Leukemia developed in all untreated animals receiving lymphosarcoma cells by either intraperitoneal or intravenous route. No spontaneous remissions were observed and all untreated animals died with evidence of leukemia, anemia and sometimes with hemorrhages in and about the eyes. Cessation of normal growth and decline in body weight regularly preceded by 1 or 2 days the elevation of leukocytes in peripheral blood (Fig. 1, 2). Seven days after injection of tumor cells,

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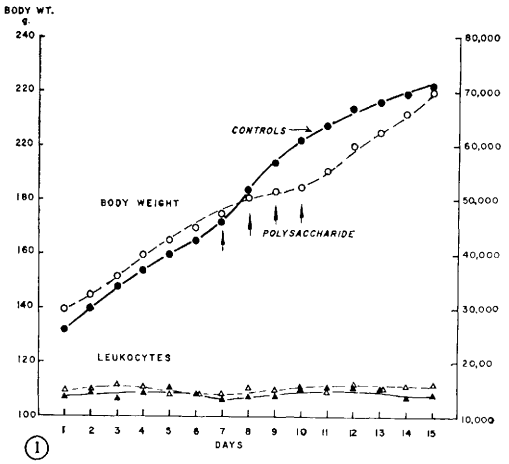

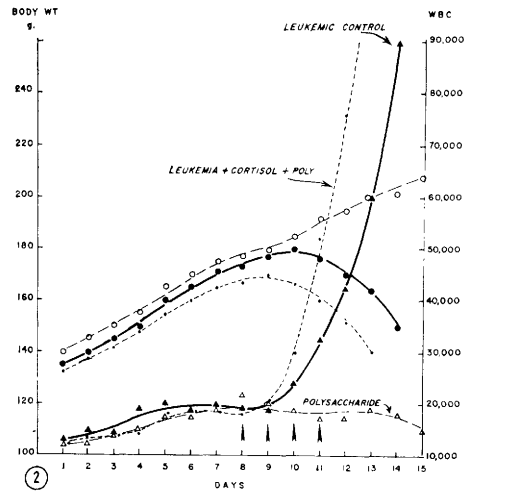

FIG. 1. Effect of 4 injections of *K. pneumoniae* polysaccharide (0.1 mg/100 g body wt) upon body wt and leukocytes of peripheral blood in non-leukemic, young, growing rats (leukocyte counts 20 hr after inj. of bacterial polysaccharide). Normal controls: body wt ●——●, WBC ▲——▲. Polysaccharide injected: body wt ○--○, WBC △--△.

FIG. 2. Absence of anti-leukemic effect of bacterial polysaccharide in cortisol-treated rats. *K. pneumoniae* polysaccharide inj. in rats beginning 8 days after intrav. transplantation of tumor cells and with leukemic infiltration of marrow, liver and spleen but without elevation in white blood count. Leukemic controls: body wt ●——●, WBC ▲——▲. Leukemia + polysaccharide: body wt ○--○, WBC △--△. Leukemia + polysaccharide + cortisol: body wt ●- - -●, WBC ▲- - -▲.

body weight no longer increased and histologic sections revealed infiltration of large lymphoblastic cells into bone marrow, liver and spleen. The rats died within 1 to 3 days following onset of leukocytosis. During earliest phase of leukocytic increase, mature cells of granulocytic series were prominent but subsequently the large lymphoblasts predominated, constituting 85-97% of cells in blood, femoral marrow, and markedly infiltrating liver and spleen. Animals dying with leukemia following intravenous implantation were similar to those after intraperitoneal implantation except that some of the latter had jaundice and bile duct obstruction due to tumor tissue about the porta hepatis and small bowel mesentery.

Cortisol administration to control rats did not alter their growth rate. Cortisol did not modify the time of appearance of leukemia, but rats so treated died with marked elevation in white blood cells and pronounced anemia about one day sooner than the untreated leukemic animals.

The 4 injections of bacterial polysaccharide slightly decreased the weight increase in healthy, growing rats (Fig. 1); none of the rats died. When polysaccharide injections were given to leukemic rats with marked elevation of their white blood count, 60% died after first or second injection, while the remaining 40% showed a transient decrease in white blood count but died with leukemia 4 to 5 days after the rats in the untreated leukemic group. When the polysaccharide was injected in rats in the aleukemic phase of their acute disease, *i.e.*, with a decreased growth rate and leukemic infiltration of tissue but without change in leukocytes of peripheral blood, 80-90% of the animals recovered and showed no subsequent evidence of the disease (Fig. 2, Table I). Half of the recovered animals were reinjected with tumor cells but failed to develop leukemia or lymphosarcoma.

The anti-leukemic effects of bacterial polysaccharide were markedly reduced by daily injections of cortisol (Table I).

Discussion. The present data indicate that

TABLE I. Effect of *K. pneumoniae* Polysaccharide and Cortisol upon Survival of Leukemic Rats (10 Rats/Group).

Route of inj. of tumor cells	Treatment: Control	Cortisol	Cortisol + poly.	Polysac.
Intraper.	0	0	10%	80%
Intrav.	0	0	0	90%

the Murphy-Sturm leukemia, like the lymphosarcoma produced by the same cells(7), regresses upon injection of polysaccharide from *K. pneumoniae*. There are other similarities between the Murphy-Sturm leukemia of the rat and experimental malignant tumors in rodents: (a) the enhanced lethal effect of bacterial products in animals with large tumors(7) and with advanced leukemia, (b) decreased therapeutic effectiveness of bacterial polysaccharide in rats with large tumors and in advanced leukemia, (c) resistance or "immunity" to tumor cells following regression of the lymphosarcoma(11) and of the leukemia, (d) inhibition of anti-leukemic and tumor-necrotizing effects (unpublished studies) of bacterial polysaccharide by cortisol administration. Studies by Stoerk(11) and unpublished studies by us indicate that resistance of rats with regressed tumors to reinoculation disappears with cortisone administration. Corticosteroid administration also permits transplantation of tumor to heterologous hosts(12).

The various considerations above may be correlated by postulating an indirect mechanism of tumor injury by bacterial polysaccharide, wherein the opposing effects of cortisol and polysaccharide, as well as acquired tumor resistance, are mediated through non-neoplastic tissues. Even the simplest scheme includes lymphoid tissue, cytotoxins, plasma proteins, tumor tissue, pituitary and adrenal. Lymphoid tissue is adversely affected by corticosteroids(13) and the lymphocyte is implicated in host resistance to tumor transplants(12,14, 15). Tumor necrotizing(16) and ACTH-releasing(17) properties as well as other effects of bacterial products are diminished by plasma proteins. Bacterial polysaccharide transiently increases adrenal corticosteroid production in the guinea pig(18) and probably in the rat(19) thus implying that the injurious effects upon tumor tissue are opposed, at least transiently, by the effects upon the pituitary and adrenal. Maintenance of adrenal weight with reduction in splenic and thymus weight in rats with large tumors(7) may reflect accompanying functional changes in these tissues, thereby explaining why the bacterial polysaccharide is not only more lethal but, in surviving animals, is less effective in inducing regression of the neoplastic process in rats with large tumors or advanced acute leukemia.

The various aspects briefly outlined above are only suggestive of the complex inter-relationships requiring experimental evaluation before the mechanism of induced tumor-necrosis is understood.

Summary. *K. pneumoniae* polysaccharide, injected intravenously, cures rats with leukemia due to Murphy-Sturm lymphosarcoma cells. Cortisol administration markedly inhibits the anti-leukemic effect of the bacterial polysaccharide. Resistance to tumor cells follows the polysaccharide-induced recovery from leukemia.

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