Effect of Moloney Virus on Protein-Bound Iodine Levels in CFW Mice. (32491)

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(Introduced by H. G. Steinman)
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The literature is replete with evidence for and against the thyroid being a contributory factor in the development of experimental lymphomas or leukemias. Thyroxin-treated animals develop hyperplasia of lymphoid tissues(1,2,3); experimentally-induced thyrotoxicosis may enhance lymphoid tumor development(4) and shorten the survival time of animals bearing these malignancies(5). However, other investigators could not demonstrate that administration of thyroid hormone affects the rate of induction of lymphoid tumors by irradiation(6) nor that thyroxin influences the survival of animals with transplanted lymphatic leukemia(7).

In man, investigators have reported that the erythrocyte *in vitro* uptake of I^{131} tagged triiodothyronine from human subjects with lymphomas is significantly reduced suggesting hypofunction of the thyroid gland(8). Scott, Reilly, and Searle(9) noted elevated plasma I^{131} and decreased thyroid I^{131} levels in patients with thymomas and lymphosarcomas. However, Davis has concluded that the protein-bound iodine (PBI) is normal in leukemic patients(10). Uptake of radioactive iodine (RAI) by the thyroid has also been reported to be normal in 9 of 10 patients with chronic lymphocytic leukemia(11).

The present experiment was undertaken to investigate the effect that an induced lymphoid neoplasm may have on thyroid function. In previous studies, (cf. reference 1-7) thyroid hormone was administered and the effect on lymphoid tissue and tumors studied. Since the administration of exogenous hormone may alter thyroid function, the effect of lymphoid tumors on thyroid activity cannot be evaluated. We report here the results of injecting Moloney Leukemia Virus into mice and its effect on thyroid function as measured by the PBI.

Material and methods. Two-hundred grams of fresh spleen were obtained from Balb/c mice with Moloney Leukemia. The following steps were performed with reagents and material at 4°C. The spleens were placed in one liter of Dulbecco's phosphate-buffered saline without bicarbonate (Gibco Cat. No. 404: Grand Island Biological Co., Grand Island, N. Y.(12) and containing 100 units of penicillin and 0.1 mg streptomycin per milliliter. The spleens were homogenized for 4 minutes in a blender and an additional liter of phosphate-buffered saline added and homogenization continued for an additional minute. The final suspension was made up of 10% weight/weight leukemic Balb/c spleen. Viral titers were $10^{-5.5}$ spleen-enlarging dose (50%)(13).

Four-week-old male and female CFW mice (Carworth Farms, Inc., New City, N. Y.) were kept separated except for a small number which were allowed to breed. Control mice were kept isolated from inoculated animals. Mice were fed only Wayne-Lab Blox (Allied Mills, Inc., Chicago, Ill.) and given tap water from the same source *ad libitum*. Half of the population was inoculated with 0.2 cc of the 10% spleen extract. Both control and inoculated mice were fed 2½ food pellets each, every 2 days, and the amount remaining after each feeding period noted for each mouse. The mice were weighed at bimonthly intervals. Eleven weeks after inoculation, both the inoculated mice and their controls were sacrificed by etherization. Blood was collected *via* an incision into the brachial plexus of vessels. Microtiter PBI determination were performed by Bio-Science Laboratories, Van Nuys, Calif. Necropsy was performed on each mouse and hematoxylin and eosin stained sections prepared of the spleen and thymus of each animal.

Results. Seventy-one CFW mice, 30 of which were inoculated 11 weeks prior to being studied and 41 of which served as controls, were sacrificed at 15 weeks of age. (This

TABLE I. Student T Test Comparisons Between Various Sub-Groupings of Moloney Virus Inoculated and Uninoculated CFW Mice.

	No.	Mean	Variance	T ratio	Degree of freedom	P
Inoc male and virgin female	30	3.32	.485	1.80	69	>.05
Uninoc male and virgin female	41	3.09	.160			
Inoc virgin female	23	3.28	.613	1.083	38	>.05
Control virgin female	17	3.06	.149			
Inoc male	7	3.46	.07	.57	28	>.05
Inoc virgin female	23	3.28	.61			
Inoc male	7	3.46	.07	2.08	29	<.05
Control male	24	3.11	.17			
Control male	24	3.11	.174	.386	39	>.05
Control virgin female	17	3.06	.149			
Control postpartum	7	3.49	.04	1.8	18	>.05
Inoc postpartum	13	3.14	.22			
Inoc virgins	23	3.28	.613	.570	34	>.05
Inoc postpartum	13	3.15	.244			
Control postpartum	7	3.49	.038	2.64	22	<.05
Control virgins	17	3.07	.155			

group of mice had not been allowed to breed). The mean PBI of the inoculated animals was 3.2 μg percent and that of the uninoculated ones was 3.09 (Table I). The difference was tested by a Student T test which assumed equal variances (CEIR Program—Stat 02***) and was found not to be statistically significant. Twenty postpartum mice, 13 of which had been inoculated at 4 weeks of age, were also studied. These mice were 10-18 days postpartum at time of sacrifice. Control postpartum mice had a significantly higher PBI at the 5% level than the virgin controls. Further analysis was performed to determine whether there were any correlations between PBI level and sex or pregnancy that might be masking a true difference between inoculated and uninoculated animals. Comparison of inoculated males and control males showed that the former had a higher PBI. The difference (3.46 — 3.11 = .35) was just significant at the 5% level. The program (CEIR Program-Stat 03***) which does not assume equal variances still found this difference to be significant at the 5% level (T = 2.548 on 15.856 D.O.F.). While inoculated females tended to have higher PBIs (mean = 3.28) than normal female mice (mean = 3.06), the difference was not significant. The difference in mean PBI between control male and female CFW mice was almost non-existent, whereas that between inoculated CFW males and females was larger but still not significant. Although these tests indicated that there was probably no relationship between the level of PBI and the sex of the host, a Chi-square test was also performed to ensure that the PBI was truely independent of the sex of the host. The PBIs of Moloney-inoculated males and females were compared to the expected PBIs if sex were not a factor influencing the PBI level. A non-significant Chi-square value of .27 was obtained indicating that there was not a sex-related difference in the PBIs.

Hematoxylin and eosin sections of the spleen and thymus of every CFW mouse were examined under the light microscope. A grading system of 0 to +++ (0 = normal, + = atrophy of thymic cortex or general hyperplasia of the spleen, ++ = neoplastic cells resembling lymphoblasts interspersed with macrophages (starry-sky) and loss of normal architecture, and +++ = invasion of the capsule in addition to changes noted in ++). Degrees of change were used to rank the specimens and a correlation coefficient run among the 3 variables, spleen, thymus and PBI. The correlations between the spleen and the PBI (—.324) and the thymus and the PBI (—.388) were significant at the 5% level (Table II). These variables were inversely related as indicated by the negative

TABLE II. Correlations Between PBI, Spleen and Thymus

	Spleen	PBI	Thymus
Spleen	1	—.342	.776
PBI	—	1	—.388
Thymus	—	—	1

sign, *i.e.*, the greater the splenic or thymic involvement, the lower the PBI value. As noted previously, the mean PBI of the leukemic group was higher than the control value. The correlation between thymus and spleen (.776) was significant and positive at the 1% level and indicated that the involvement in the spleen was directly proportional to the degree of involvement in the thymus.

Discussion. In mice, the Moloney Leukemia Virus induces a disease marked by a generalized lymphocytic neoplasia. Invariably, these animals exhibit marked splenomegaly and massive involvement of peripheral and mesenteric nodes. In the majority of mice, the thymus gland is greatly enlarged(14). Microscopically, lymphoblasts interspersed with macrophages are seen; the capsule is invaded and the normal architecture of the thymus or spleen is lost(15).

All 4-week-old CFW mice develop clinical evidence of leukemia in 3 to 4 months following our inoculation procedure. Table III indicates the degree of splenic and thymic involvement seen in the Moloney Virus-treated mice 11 weeks following inoculation. At this time, only about 1/5 of the animals had developed frank microscopic evidence (++ or +++) of leukemia. No pathological changes were noted in the hematoxylin and eosin sections of the thyroid.

Thyroid function varies markedly among strains of mice(16,17,18). We chose a random-bred strain, CFW, because of its hardiness and moderately large blood volume. Variance in the PBI value of the control mice was relatively small suggesting uniform thyroid function in these animals. Our CFW mice were maintained until a few of the animals developed frank microscopic evidence of leukemia. They were sacrificed at this point to avoid the possibility of: (1) infection complicating the leukemic process, and (2) a reduced food intake secondary to their disease. The PBI was chosen as a sole parameter of thyroid activity to be measured. The PBI is statistically as good a measure of thyroid function as the radioactive iodine(19,20,21, 22).

Our results indicate that the mean PBI of the inoculated mice, considered as a group, tended to be higher than controls, but this difference was not statistically significant. Within the inoculated groups, comparison of the degree of splenic or thymic leukemic involvement with the PBI revealed a statistically significant relationship in that higher PBI values were associated with mice who demonstrated no microscopic evidence of leukemia (0 and +). Lower PBI values were characteristic of mice who evidenced leukemic involvement (++ and +++); that is, the greater the leukemic involvement of the spleen and thymus in the CFW mouse, the greater the probability that this mouse would have a PBI near the lower PBI values found in our series. The lower the PBI, the greater the chance that an inoculated animal would have thymic or splenic evidence of leukemia.

Interpretation of our PBI findings is difficult as there are many factors which influence the level of protein-bound iodine: (1) Our findings may be species-specific; the variation in the PBI levels between species has been mentioned(16,17,18). (2) Severe caloric restriction or inanition may lead to significant decreases in PBI levels in man(23, 24,25) and may inhibit leukomogenesis in experimental animals(26,27). Caloric intake was monitored in our CFW mice; there was no significant difference between the two experimental groups, at any time, in food consumption or in body weight at the 1% level. (3) Severe chronic illness may lower the PBI (28). At time of sacrifice, our CFW mice appeared "clinically" normal; and at post-mortem, we found no evidence of pyogenic infection in the animals. A possibility that we could not evaluate because of the insufficient blood available from the mice is that Moloney-induced leukemia may alter the thyroxin-binding protein levels which would change our interpretation of the PBI values.

The control CFW mice, which were from 10 to 18 days postpartum, had a statistically

TABLE III. The PBI, Thyroid and Splenic Involvement of Inoculated Mice.

Mouse Indentification	μg %	Splenic involvement	Thymic involvement
A2 female	3.4	0	0
A3 "	3.2	++	++
A4 "	3.7	0	0
A5 "	3.6	+	+
B1 "	3.0	0	0
B2 "	3.2	+	+
B3 "	2.0	++	++
B4 "	2.7	0	0
C1 "	3.6	0	0
C2 "	3.1	+	++
C3 "	3.3	+	+++
C4 "	3.3	+	+++
C5 "	2.9	0	+
D1 male	3.3	0	+
D2 "	3.8	0	0
D3 "	3.7	+	+
D4 "	3.4	0	+
D5 "	3.5	0	0
F1 "	3.0	0	+
F3 "	3.5	0	0
G1 post partum	3.0	+	+
G2 " "	2.4	+	+
G3 " "	3.6	+	++
G4 " "	3.0	+	+
G5 " "	3.2	+	+
H1 female	3.7	0	0
H2 "	2.8	+	++
H4 "	3.0	0	0
H5 "	3.9	0	0
I1 "	2.9	0	+
I2 "	3.1	0	0
I3 "	2.5	+	+++
J1 "	3.0	0	0
J2 "	3.3	0	0
K1 post partum	3.3	0	0
K2 " "	3.1	0	0
K3 " "	2.1	+	+
K4 " "	3.4	+	+
K5 " "	3.4	+	++
K6 " "	3.7	0	0
K7 " "	3.7	0	0
K8 " "	3.0	0	0

higher PBI at the 5% level when compared with nonpregnant female control animals (Table I). However, we could not demonstrate a significant difference between inoculated virgins and inoculated postpartum animals and, also, between control postpartum and inoculated postpartum mice. In the human female, the elevated PBI of pregnancy decreases fairly rapidly after delivery and becomes normal from a few days(29) to 6 weeks after delivery(30). Whether our postpartum CFW mice had elevated protein-bound iodine levels during pregnancy was not determined.

Summary. Protein-bound iodine levels were determined on CFW mice inoculated with Moloney Leukemia Virus. When frank microscopic evidence of leukemia appeared in several animals, all inoculated animals and their controls were sacrificed. Hematoxylin and eosin sections were made from the spleen and thymus of each animal, and the degree of microscopic involvement with leukemia noted. The difference in the mean PBI between virgin controls and virgin inoculated mice was not significant at the 5% level. Even though the mean PBI between the control animals and the inoculated animals as a group was not significant, a significant inverse correlation at the 5% level was demonstrated between the amount of leukemic involvement of the spleen or thymus and the PBI. The maximum increase in PBI occurred prior to microscopic involvement with leukemia and trended lower as microscopic evidence of splenic and thymic involvement appeared.

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Enhancement of Vaccinia Virus Plaque Formation by Trypsin.* (32492)

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The purpose of this report is to describe a phenomenon which was observed when we attempted to characterize a viral inhibitor. Trypsin was found greatly to enhance virus plaque formation when it was included in the medium.

Materials and methods. Media and cell cultures. Growth medium for the establishment of chick cell cultures consisted of Gey's balanced salt solution (BSS) with 5% calf serum, 0.1% lactalbumin hydrolysate, 0.1% proteose peptone, and 0.06% sodium bicarbonate. Maintenance medium used for virus assays with vaccinia virus consisted of BSS with 0.11% sodium bicarbonate, 0.1% proteose peptone, 0.1% lactalbumin hydrolysate, and 0.1% yeast extract. Chick embryo cell cultures were prepared as previously described (1).

Virus. Vaccinia virus, strain NY 914, was grown on the chorioallantois of 10 to 11-day-old developing chick embryos. Infected membranes were removed 48 hours after inoculation and triturated with maintenance medium. Virus preparations were centrifuged at 800 *g* for 30 minutes to remove cellular debris and were stored in glass ampules at −60°. Virus was assayed according to the method described by Lindenmann and Gifford(2).

Trypsin and soybean trypsin inhibitor. Trypsin, 2.5% solution, was obtained from the Grand Island Biological Co., Grand Island, N. Y. Crystalline soybean trypsin inhibitor was obtained from Mann Research Laboratories, New York. Both substances were diluted in maintenance medium.

Results. Trypsin was added to a preparation of a viral inhibitor in order to study its susceptibility to this enzyme. After a period of incubation soybean trypsin inhibitor in approximately equimolar concentrations was added and residual antiviral activity was measured in chick embryo cultures challenged with vaccinia virus by the method of Lindenmann and Gifford(2). Surprisingly, the numbers and size of vaccinia plaques which developed over the next 48 hours were greatly increased when compared to control cultures. The phenomenon was further studied since it increased the efficiency of quantifying this virus and may have applications to other viruses. The antiviral agent originally employed was found to be irrelevant in demon-

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