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Studies in Leukemia: XI. Active Immunization of C3H x 101 Mice Against Induction of Leukemia.* (24302)

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Our previous reports have described induction of leukemia in C3H mice by means of cell-free brain filtrates(1). Experiments were devised to study the possible immunologic properties of cell-free brain filtrates exposed to ultraviolet light. The present report gives the results of experiments regarding active immunization of mice against leukemia.

Materials and methods. C3H x 101 mice, from 8 to 10 weeks old, served in the experiments. The mice were obtained from Cumberland View Farms, Clinton, Tenn. Cell-free brain filtrates (CFBF) were prepared by method previously described(2). Tumor-cell suspensions were prepared by grinding mesenteric tumor in ground glass tissue mill and suspending the cells in a 0.9% solution of sodium chloride, buffered at pH 7.4, to a concentration of about 10^6 cells/cm^3. Brains of 5 leukemic C3H x 101 mice were pooled; 1000 cc. of CFBF was prepared. A control filtrate was similarly prepared with brains of non-leukemic C3H x 101 mice. The filtrates were stored for 48 hours at −15°C, then thawed 24 hours at 4°C. After filtrates had thawed, the non-leukemic CFBF was filtered through a coarse and a fine glass-sintered filter; the leukemic CFBF was filtered through the same filters. Samples of leukemic CFBF taken before and after this filtration retained their leukemogenic properties when later tested. The filtrates were irradiated with ultraviolet light (UVI) in a Spinco centrifilmer irradiation machine at 1750 rpm at full power and rate of flow of 200 ml/minute.† When irradiation was accomplished, the filtrates were stored in 5 ml and 10 ml vials at −60°C until used. All experimental mice were immunized with ultraviolet irradiated cell-free brain filtrate (UVI-CFBF) by subcutaneous inoculations of 0.1, 0.25, and 0.5 cc at 48-hour intervals. The mice were challenged 10 days after last inoculation, by intraperitoneal (IP) injection of 0.5 cc CFBF or tumor-cell suspension prepared from leukemic C3H x 101 mice. The mice were killed when they appeared terminally ill and autopsies performed. In mice described as positive, both gross and microscopic data regarding leukemia were similar to those described elsewhere(3) for the Swiss strain. Ten C3H x 101 mice were

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immunized with UVI leukemic CFBF and 10 with UVI non-leukemic CFBF. This experiment was repeated with a different source of leukemic CFBF for challenging. In addition, 2 groups of 10 C3H x 101 mice each, were similarly immunized and challenged with tumor-cell suspension.

Results. All animals that evidenced leukemia did so from 2 to 4 weeks after challenging. For our purpose, mice that survived 20 weeks after having been challenged, were considered negative. No negative animals subsequently showed evidence of leukemia.

Table I summarizes the effect of immunization with UVI leukemic CFBF when mice are challenged with leukemic CFBF. The differences between duplicate experiments are not statistically important; therefore, they are grouped together for final analysis. Protection afforded by immunization with UVI leukemic CFBF is highly significant.

The results of active immunization with UVI-CFBF when challenged with tumor cells are summarized in Table II. Protection is not afforded against a high concentration of tumor cells.

Discussion. Under conditions of our experiment, ultraviolet irradiated cell-free brain filtrate (UVI-CFBF) actively immunizes mice against the leukemogenic agent in leukemic CFBF. That this immunity is not a result of a non-specific mouse brain effect is shown by failure of UVI *non-leukemic* CFBF to confer any significant immunity. The incidence of leukemia in this control group is similar to that reported previously in non-immunized C3H mice(1).

Immunity may not be absolute and may represent only a prolonged latent period; however, in all positive animals, leukemia developed within 4 weeks of challenge whereas the remaining animals survived over 20 weeks. This suggests an absolute immunity at time of challenge.

The failure of UVI-CFBF to protect against tumor cells may be the function of several factors: (1) a possible quantitative relationship of antibody level to the concentration of tumor cells; (2) the inaccessibility of the active agent in tumor cells to the antibody; (3) possible so-called autonomy of the established tumor cells.

TABLE I. Effect of Active Immunization on Induction of Leukemia with *Cell-Free Brain Filtrate.*

Immunized with	No. leukemic / No. immunized		Total
UVI non-leukemic CFBF	8/10	9/10	17/20
UVI leukemic "	2/9 *	1/10	3/19*

CFBF = Cell-free brain filtrate
UVI = Ultraviolet irradiated

Corrected CHI $square_{(1)} = 14.44^{***}$

* One mouse in this group died of trauma during challenging inj. For statistical analysis, this animal was considered positive and the group restored to 10 animals.

TABLE II. Effect of Active Immunization on Induction of Leukemia with Tumor Cell Suspension.

Immunized with	No. leukemic / No. immunized
UVI non-leukemic CFBF	10/10
UVI leukemic "	10/10

CFBF = Cell-free brain filtrate
UVI = Ultraviolet irradiated

Summary. 1. Leukemic and non-leukemic cell-free brain filtrates were irradiated with ultraviolet light. C3H x 101 mice were actively immunized by subcutaneous inoculations. 2. Active immunization with ultraviolet irradiated leukemic cell-free brain filtrate gave significant protection against challenge of leukemic cell-free brain filtrate. 3. Active immunization with ultraviolet irradiated cell-free brain filtrates did not protect against challenge of a high concentration of tumor-cell suspension.

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