Effects of Adjuvants on Benzpyrene Tumorigenesis in Hamsters[1] (36939)

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A previous study (1) showed that when hamsters were injected with Freund's incomplete adjuvant (FIA) on the second day of pregnancy, their progeny, challenged at 3 weeks of age, showed greatly increased susceptibility to benzo (a) pyrene (BP) tumorigenesis (as compared with the control progeny of untreated pregnant hamsters). It was suggested that this phenomenon was in some way related to classical immunologic enhancement.

In the experiments reported here, three additional nonspecific immunoenhancing adjuvants were similarly tested: Freund's complete adjuvant (FCA) pertussis vaccine (Pert) and BCG. All three resulted in enhancement of BP-induced tumors in the progeny, comparable to that previously noted with FIA. In contrast, the direct effect of two of the adjuvants tested was inhibition of BP tumorigenesis.

Materials and Methods. Sixty random-bred Syrian hamsters (LVG-LAK, from the Lakeview Hamster Colony) synchronously exposed to pregnancy, were divided into 5 groups. Group A animals were not treated in any way. Group B, on day 3 of pregnancy were injected sc in the left lower back with 1.0 ml of FCA, in which 0.5 ml of phosphate-buffered saline was emulsified; Group C similarly, using FIA. Group D animals received 1.5 mg of live BCG (Research Foundation, 70 West Hubbard St., Chicago, ILL 60610) sc on days 3 and 10 of pregnancy; Group E, 0.25 ml of standard pertussis vaccine (Eli Lilly and Co.) sc on days 3 and 10 of pregnancy.

All progeny were left undisturbed until 3 weeks old, when males and females were caged separately, and given the first of 3 weekly sc injections of BP (3 mg dissolved in 0.25 ml of olive oil) in the right lower back. Tumor incidence and size were recorded at suitable intervals. The experiment was terminated 98 days after the first BP injection, when a number of animals with large tumors were in critical condition. At termination, all animals were autopsied, final tumor incidence in each group was noted, and all tumors were weighed.

Nine of the hamsters injected during pregnancy with pertussis vaccine, together with 13 untreated postpartum hamsters, were injected as above with BP, the first injection being given 21 days after delivery. In a second (confirmatory) experiment, 26 nonpregnant 6-week-old female hamsters were injected as above with FCA, 30 untreated animals from the same shipment serving as controls. Thirty four days later, the first of 3 weekly BP injections was given to both groups.

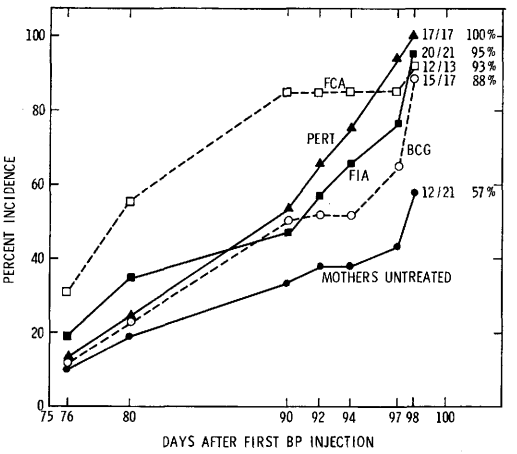

FIG. 1. Cumulative percentage incidence of tumors in the progeny of adjuvant-treated and untreated mothers. Final figures for day 98 represent autopsy observations. The apparent sharp increase on day 98 reflects the finding of tumors too small to be palpated.

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TABLE I. Vertical Effect of Maternal Adjuvant Injection on BP-Induced Tumor Formation in the Progeny.

Maternal treatment[a]	Av tumor wt (g/hamster)	Treated/ control	Av wt of tumors (g)	Treated/ control
None	2.0[b]		3.5[c]	
FCA	7.6	3.8	8.3	2.6
FIA	4.2	2.1	4.4	1.2
BCG	3.5	1.8	3.5	1.0
Pert	7.2	3.6	7.2	2.1

[a] FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; BCG, Bacille Calmette-Guerin; Pert, standard pertussis vaccine.

[b] Figures in this column were obtained by dividing the total weight of the tumors found at autopsy in each group by the number of animals in that group.

[c] Average weights of the tumors found at autopsy in each group, excluding animals without tumors.

These two experiments were terminated 101 and 112 days, respectively, after the first BR injection.

Results. Male and female offspring were found about equally susceptible to BP tumorigenesis, in contrast to the decreased susceptibility of males noted in a previous study (1). (This may perhaps be correlated with the finding that several males in each group had grossly atrophic testes, weighing less than 0.1 g, and a number of others studied microscopically showed aspermatogenesis and interstitial cell atrophy. The reason for this is unknown).

Figure 1 shows earlier development and significantly increased incidence of BP-induced tumors in the progeny of hamsters injected with each of the adjuvants, as compared with the progeny of untreated hamsters. (The increases from day 97 to day 98 reflect the discovery at autopsy of several tumors too small to be palpated). Table I shows that the average tumor weight in g per hamster was 1.8 to 3.8 times as great in the maternally treated as in the control progeny. The average weight of the tumors which occurred was significantly increased in the progeny of hamsters injected with FCA or Pert.

Table II, despite the suboptimal number of animals, suggests that Pert, which caused marked potentiation in the progeny exerted horizontal protective action against BP tumorigenesis in the "mothers", both the incidence and the average weight of the tumors being decreased. Table III shows a significant reduction in incidence of BP tumors in non-pregnant female hamsters given a single injection of FCA 34 days before the first BP treatment. The average weight of the tumors found at autopsy was not significantly altered.

Discussion. The mechanisms involved in the observed phenomena are not clear, but one may attempt an interpretation based on current concepts of the possible actions of adjuvants. Several *in vitro* studies (2) have indicated that adjuvants may stimulate activities of the thymus-derived (T) lymphocytes, which are responsible for cell-mediated immune reactions. Furthermore, a humoral sub-

TABLE II. Horizontal Effect of Pertussis Vaccine on BP Tumorigenesis in Female Hamsters.[a]

Treatment	Tumor incidence	g/hamster	Treated/ control	Av wt of tumors (g)	Treated/ control
None	10/13 (71%)	4.7[b]		6.8[c]	
Pert	3/9 (33%)	1.3	0.28	3.9	0.57

[a] First BP injection 34 days after first Pert injection.

[b,c] See footnotes in Table I.

TABLE III. Horizontal Effect of FCA on BP Tumorigenesis.[a]

Treatment	Incidence	g/hamster	Treated/ control	Av wt of tumors (g)	Treated/ control
None	24/27 (90%)	3.8[b]		4.2[c]	
FCA	13/23 (52%)	2.7	0.7	4.8	1.1

[a] FCA given sc and animals challenged with BP 34 days later.
[b,c] See footnotes in Table I.

stance may be secreted by the T lymphocytes that will enhance humoral antibody production by bone-marrow-derived (B) lymphocytes (3). Thus, tumor suppression in the mothers may be the result of an increased T lymphocyte function. This may be brought about by expansion of the number of T lymphocytes that carry receptors for tumor antigens and/or by activated macrophages resulting from a reaction between sensitized T lymphocytes and the respective antigens in the adjuvants used (3). The suppressive effect of increased T lymphocyte activity with respect to cell-mediated immune reactions would not be extended from mothers to offspring, since maternal lymphocytes do not pass the maternal–fetal barriers in the uterus and mammary glands. On the other hand, if T lymphocytes do indeed influence B cells via a humoral substance, it is conceivable that this humoral substance could cross the maternal–fetal barriers. Such a humoral substance(s) the nature of which is not yet defined, would increase the ability of the B lymphocytes in the progeny to make humoral antibodies to tumor antigens during oncogenesis. Antibodies so induced may be important in the enhancement of tumor growth.

Summary. The effects of adjuvants (Freund's complete and incomplete, BCG, and pertussis) on the development of benzpyrene-induced tumors were studied in hamsters. Two opposite effects were observed, depending on experimental design. In the first instance, prior treatment of adult pregnant or non-pregnant female hamsters with adjuvants appeared to suppress tumor development. On the other hand, prior treatment of mothers with adjuvants during pregnancy greatly enhanced the development of tumors in their progeny. A possible explanation of these antithetical results is suggested.

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