Radioiodinated Antibody in the Regression of Subcutaneous Ehrlich Mouse Carcinoma*

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The application of immunological principles to the treatment of malignant tumors depends on the preparation of specific antisera against tumor cells, the administration of such antisera or fractions therefrom to animals bearing the homologous tumor, and localization in the tumor mass of the greater part of the antibodies present in the general circulation. At least in theory, it thus becomes feasible to utilize tumor-specific antibodies as immunological deterrents of tumor growth or as specific radioactivity carriers for therapeutic radiation of tumors. Antisera of varying specificity, against several tumors, have now been prepared and studied by several workers (1, 11, 15). Major problems have been encountered in the purification of antibodies to a point at which cross-localization is minimal with normal tissue components and maximal with cellular components of the tumor site; several attempts have been made to improve this situation (2, 3). Possibly as a result of preoccupation with the important and fundamental problems connected with specific localization, few studies (15) have appeared that directly test the feasibility of final objectives—for example, the use of radioactive tumor antibody in attempts to promote tumor regression. This report presents findings on the effect of a radioiodinated antibody, made against suspensions of Ehrlich mouse carcinoma cells, on the growth of the latter in solid (subcutaneous) form and attempts to shed some light on the mechanisms by which regression, induced by radioactive antibody, may occur.

MATERIALS AND METHODS

Immunization of rabbits. Tumor cell antigen.—The procedures for collection and washing of Ehrlich ascites tumor cells and details of the immunization procedure were the same as previously reported (13).

Preparation of normal and immune \( \gamma \)-globulin.—Pooled sera from six nonimmunized rabbits and from seven immunized rabbits were used for the preparation of normal and immune \( \gamma \)-globulins, respectively, by the method of Deutsch (4). The preparations were dialyzed free of salt and dried by lyophilization.

Radioiodination of \( \gamma \)-globulin.—The globulin fractions were labeled, essentially by the method described by Rajam and Knorpp (14), except that glycine buffer (pH 8.5) was used and mixing was carried out by counterstream ejection through a capillary. This method of mixing increases uniformity of labeling and is based on the jet-iodination method of McFarlane (10). Removal of unbound radioiodine was effected by ion exchange on Dowex 2-200-400 anion exchange resin. This method, properly carried out, yields a product with less than 2 atoms of iodine per protein molecule. The importance of limiting iodine substitution on the molecule, relative to efficiency of localization in the tumor site, has been stressed (12). Both normal and immune globulins were labeled to a specific activity of approximately 100 uc/mg. To avoid self-radiation effects, animals were given injections within 2 hours following preparation.

Demonstration of post-regression refractory state: influence of cortisone.—Twenty-five Swiss mice were given bilateral inoculations, by the subcutaneous route, of \( 1 \times 10^6 \) washed Ehrlich ascites tumor cells suspended in 0.1 ml. Alsever’s solution. After 40 days the mice were palpated for tumors, and those with small residual tumors (incomplete regression) were discarded. Of the remainder, twelve were given bilateral inoculations, exactly as before, in fresh sites; ten were given bilateral inoculations and placed on a cortisone administration regimen as follows: \( 2.5 \) mg. cortisone acetate per animal for 2 days following inoculation; \( 1.25 \) mg. for the 3d and 4th days, and \( 0.05 \) mg/day thereafter. Cortisone was administered subcutaneously. All animals were palpated for tumor growth on the 14th day after inoculation. An additional group of ten normal mice was

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inoculated and palpated at the same times as the cortisone-treated group.

**Effect of radioiodinated immune globulin on tumor growth.**—Seventy Swiss mice were given bilateral inoculations of tumor cell suspension. After 3 days, the animals were divided into six groups of ten to twelve animals each. The several groups were treated as follows: group 1 received no further treatment; group 2 received cortisone acetate according to the schedule previously detailed; group 3 received 0.1 mg. immune, nonradioactive globulin per animal; group 4 received 0.1 mg. radioactive normal globulin per animal; group 5 received 0.1 mg. radioactive immune globulin per animal; group 6 received the same treatment as group 5, and, in addition, animals were simultaneously placed on a cortisone regimen identical with that used for group 2.

Cortisone acetate was administered subcutaneously, and the proteins were injected intravenously in a volume of 0.1 ml.

Tumor sizes were measured 7, 14, and 20 days post-therapy.

**Statistical evaluation.**—Significance of differences between average sizes of tumors in the various groups was evaluated by the Student-Fisher test.

**RESULTS**

When animals with regressed tumors were given inoculations of fresh tumor cells, none of the 22 inoculated sites developed tumors by the 14th day. The administration of cortisone to animals with regressed tumors, reinoculated as before, had no effect in promoting tumor growth; of twenty inoculated sites, none showed tumor growth by the 12th day and were entirely comparable with the group that had not received cortisone. The control group, consisting of normal animals given inoculations and palpated with the other groups, showed positive tumor growth at all sites given inoculations.

In studying the effect of radioiodinated immune globulin on tumor growth, the several experimental and control groups were palpated for tumor size on the 7th, 14th, and 20th days. Results may be summarized as follows: all six groups developed tumors of approximately comparable size by the 7th day. Differences became apparent by the 14th day, when tumors in groups 1, 2, and 6 attained maximum size, the average size in group 6 being greater than in groups 1 and 2, which were comparable. Groups 3, 4, and 5 showed definite evidence of regression: average tumor size in group 3 was significantly less than in group 1 (P > 0.01); in group 4, significantly less than in group 1 (P > 0.01), but not significantly different from group 3 (P < 0.05); and markedly and significantly less in group 5 than in group 4 (P > 0.01). By the 20th day, average tumor sizes in most groups had diminished slightly (groups 1 and 6), remained approximately constant (groups 2 and 3), or diminished to sizes compatible with almost complete regression (group 5). These results are summarized graphically in Chart 1, which also indicates the standard deviation from the mean, for each group.

**DISCUSSION**

The possibility that a tumor-bearing host may develop antibody-like factors against the growing
tumor has been suggested by several workers. That such factors can be demonstrated is shown by the work of Mitchison and his co-workers (8, 9), and additional evidence for their existence has been adduced by demonstrations of retardation or regression of a second implant of viable tumor, or by elicitation of an anaphylactic reaction in animals previously sensitized by tumor growth (5). It has also been shown that x-radiation, administration of cortisone in sufficient amounts, or a combination of these two agents, results in marked potentiation of tumor growth. These latter findings are presumably due to inhibitory effects on antibody-producing mechanisms (6). Thus, observed growth of a tumor in an experimental animal may be regarded as the outcome of a balance of factors, alterable in favor of the host by the use of agents which increase the formation of tumor antibody, and particularly the penetration of such antibody into the tumor bed.

The demonstration, in mice, of a refractory state following regression of the Ehrlich mouse ascites carcinoma suggests that, in this system, antibody against the tumor may develop in the course of tumor growth. It was further shown that marked tumor regression follows the administration of radioactive antibody. This regression would appear to be associated with a radiation effect, since nonradioactive antibody from the same antibody pool, administered in identical amounts, produced a comparatively slight and transient effect on regression, at least under the conditions of these experiments. While it is logical to believe that regression associated with radioactive antibody is due to radiation death of multiplying tumor cells, certain observations tend to significantly modify this view. Thus, tumors in all groups grew at approximately the same rate over the first week following therapy; differences in growth became apparent only after 7–10 days. While the concept of latency of radiation effect can be invoked, it is equally possible that the first 7 days represented a period of increasing antibody formation, by the animal, against components of the growing tumor. The action of localized radiation can be regarded as due to the production of centers of inflammatory response in the tumor mass with an increase in vascular permeability, thus enhancing the penetration of antibody, formed by the host, into the tumor bed. Thus, the observed increase in speed and extent of regression might be a result of this penetration, a viewpoint which is in harmony with concepts, already cited, of antibody formation during tumor growth.

Two observations made during this study lend considerable support to this view. The administration of radioactive normal (nonimmune) globulin produced a distinct increase in speed of tumor regression, an effect as great as that produced with nonradioactive immune globulin; the effect observed with the latter material may be ascribed to some degree of specific cytotoxic action, while that of the former is attributable to a radiation effect, largely independent of specific cytotoxicity. Further, the administration of cortisone (in doses sufficient to inhibit antibody production (7) and, presumably, to increase capillary permeability) along with radioactive immune globulin completely abolished the effect of the latter on regression.

Objections to this interpretation can be raised on the ground that cortisone administration may have resulted in increased catabolic rate of the injected radioactive globulin or have decreased normal capillary permeability; both of these effects would diminish the amount of radioactivity localized at the tumor site. However, when cortisone was administered to animals that had undergone tumor regression and were reinoculated with tumor cells, no effect on the refractory state could be demonstrated.

It may be concluded that, while the several aspects of regression induced by radioactive tumor antibody undoubtedly bear a complex relation to one another, a major factor in regression by this means may be an indirect effect, mediated by radiation and closely associated with antibody formation by the host.

SUMMARY

1. The effect of radioiodinated γ-globulin, prepared from rabbit antiserum against cells of the Ehrlich mouse carcinoma, on rate of regression of the subcutaneous form of this tumor, has been studied.

2. Under standardized conditions, the rate of tumor regression was greatest with radioactive immune γ-globulin, and considerably less marked with radioactive normal γ-globulin and with nonradioactive immune γ-globulin.

3. Cortisone administration completely inhibited the increase in regression rate obtained with radioactive immune globulin.

4. A major factor in regression induced by radioactive immune globulin may be an indirect effect mediated by radiation, involving increase in capillary permeability and closely associated with antibody formation by the host.

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