Tumor regression at an untreated site during immunotherapy of an identical distant tumor
(hepatoma/"immune" RNA/fibrin fragment E)

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ABSTRACT The effects of two immunotherapy regimens on the development of an untreated, uniformly lethal transplantable line-10 hepatoma in strain 2 guinea pigs were monitored during treatment of an identical tumor 10 cm away. Line-10 cells were injected intradermally simultaneously at each of two sites. When one site was treated 6 and 16 days later with rabbit antibody against guinea pig fibrin fragment E, the complete regression of the treated tumor, a 25–30% depression in the development of the untreated tumor, and an increased survival time were observed. In another group of animals, when one site was treated 5 days after tumor challenge with syngeneic nonsensitive "tumor-immune" RNA in a regimen including syngeneic nonsensitive lymphoid cells and tumor-specific antigen, all animals survived after complete and apparently specific regression of the tumors at both the treated and untreated sites. For the RNA regimen, we have shown that immunotherapy of an intradermally established line-10 tumor results in complete abrogation of both the treated and a distant untreated tumor; i.e., demonstrating a systemic effect.

We have previously reported the development of two unique models of tumor immunotherapy which consistently result in the complete abrogation of a uniformly lethal intradermally established tumor—the transplantable line-10 ascites variant of a diethylnitrosamine-induced hepatoma in strain 2 inbred guinea pigs (1, 2). One model involves the use of syngeneic or xenogeneic "tumor-immune" RNA-rich extracts injected subcutaneously under a tumor bleb induced 5 days previously by injecting a uniformly lethal dose of 10⁶ line-10 cells intradermally. The RNA was injected as part of an immunotherapeutic regimen which included syngeneic nonsensitive peritoneal exudate cells (NS-PEC) and tumor-specific antigen (TSAg); this regimen resulted in complete regression of the tumor in all 24 animals treated as well as immunity to subsequent tumor challenge, and was apparently tumor-specific (1). The rationale for the RNA therapeutic regimen (see legend, Fig. 1) was as follows: to circumvent the possible deficiency of immunocompetent host lymphoid cells in the tumor area (3), we injected normal NS-PEC first under the growing tumor; to "convert" the NS-PEC to a specific state of immunologic sensitivity to the line-10 TSAg, the RNA was then injected immediately into the same site (4); to attempt an amplification effect (i.e., increased blast transformation and cell proliferation) of lymphoid cells that might have been "converted" by the RNA, line-10 TSAg was injected later as a specific immunologic stimulus. The second model made use of rabbit antibody against guinea pig fibrin fragment E (GpFFE) injected 6 and 16 days after the injection of a lethal dose (10⁶ cells) of line-10 tumor (2). The antibody was injected subcutaneously directly below the intradermally growing tumor (2); this resulted in the complete regression of the tumor in all 18 animals treated as well as immunity to subsequent tumor challenge, and was apparently not tumor-specific (2). The rationale for the anti-FFE therapy was to inhibit the formation of the tumor-associated fibrin matrix and/or to induce an inflammatory cell infiltrate at the tumor site (2). For both immunotherapy models, we now report the effect of treating one tumor site on the development of an identical tumor at an untreated second site 10 cm away.

The RNA extracts used in this study were obtained by phenol extraction (5) of the lymphoid tissues from strain 2 inbred guinea pigs or Rhesus monkeys immunized against the line-10 tumor (1, 4). When strain 2 guinea pigs were injected simultaneously with 10⁶ line-10 tumor cells at each of the two sites 10 cm apart and only one site was treated 5 days later with the RNA therapeutic regimen, complete local tumor regression was observed at both sites (Fig. 1). These animals survived tumor-free and were resistant to subsequent challenge with 10⁶ line-10 tumor cells. Since no significant difference was observed when the animals were treated with syngeneic or xenogeneic RNA (five animals each in the treated group), all 10 animals are considered together in Fig. 1. All the control animals treated with saline developed growing tumors at both injection sites and died within 55 days (Fig. 1).

The anti-FFE preparation used in our second model was obtained by immunizing rabbits with an extract of fibrin fragment E prepared from guinea pig plasma and precipitating the resulting rabbit antiserum with (NH₄)₂SO₄ (2). When strain 2 guinea pigs were injected simultaneously with 10⁶ line-10 tumor cells at each of two sites 10 cm apart and only one site was treated 6 and 16 days later with the anti-GpFFE IgG preparation, complete local tumor regression was observed at the treated (1°) site (Fig. 2). Progressive tumor growth was noted at the 2° untreated site although this development was depressed by approximately 25–30% in comparison to the control untreated animals (Fig. 2). In addition, the survival time of the treated animals was prolonged over the controls (97 days in comparison to 55 days) (Fig. 2). No significant anti-tumor effect at either site was noted when 1.0 mg of normal rabbit IgG (from the same rabbit) or 0.5 ml of saline was injected subcutaneously under the tumor at the 1° site (Fig. 2).

In our previous work, although the line-10 tumor was injected at one site and treated at a time when significant regional metastases are known to frequently occur (6), all treated animals survived tumor-free, suggesting that both the RNA and the anti-GpFFE therapeutic regimens might...
have systemic effects (1, 2). Our present data indicate a marked systemic effect of RNA immunotherapy in comparison to a lesser systemic effect observed with anti-FFE. These differences may be due to the mechanisms by which the RNA and the anti-FFE lead to the development of immunity to the line-10 tumor. In the RNA regimen, a large number of additional immunocompetent lymphoid cells were supplied to the tumor site; these cells were then presumably converted to a state of specific immunity for the tumor by the RNA and activated to undergo proliferation due to stimulation by the TSAg (1, 4). On the other hand, the only source of tumor-sensitized cells using the anti-FFE regimen was host cells that were probably recruited to the tumor area as a result of the formation of anti-FFE/FFE antibody-antigen complexes and/or the breakdown of the tumor fibrin matrix (2). The appearance of immunocompetent cells at the tumor site in the anti-FFE system is probably slower since it depends only on the course of events associated with cell-mediated tumor immunity in the host. The effective systemic immunity observed with the RNA therapy, therefore, could be due to the presence of a much larger number of lymphoid cells specifically sensitized to the tumor which could enter the circulation and reach the second untreated site.

The systemic response against the line-10 tumor is apparently specific in the RNA regimen (1). The use of NS-PEC, RNA obtained from a guinea pig immunized to an antigenically distinct tumor (line-1), and line-1 TSAg resulted in progressive growth of the line-10 tumor and death in all animals (1). Furthermore, if the NS-PEC, "line-10-immune" RNA, or line-10 TSAg were omitted from our therapeutic regimen, or if Escherichia coli RNA was used with NS-PEC, little or no tumor regression was observed (1). However, when NS-PEC and "line-10-immune" RNA were injected without subsequent injection of line-10 TSAg, a 30% depression in the growth of the tumor was noted (1). This indicates that the specific "tumor-immune" RNA is an essential component of our immunotherapy regimen. On the other hand, the anti-FFE therapy is presumably nonspecific since the anti-FFE probably acts by inhibiting the formation or causing the disruption of a tumor-associated fibrin matrix and by inducing an inflammatory cell infiltrate at the tumor site (2). Indeed, this is one of the possible advantages of anti-FFE therapy since it might then be applicable against a variety of mammalian tumors. Furthermore, anti-FFE might also be applied diagnostically to detect high concentrations of fibrin associated with neoplastic cells (2, 7–13).

The results we reported previously for both immunotherapy regimens were highly significant in that they were both more effective in treating an intradermally established line-10 tumor than bacillus Calmette-Guérin (BCG) therapy, the most effective nonspecific immunotherapeutic agent used against this tumor up to that time (6, 14). The data presented here not only confirm our previous findings for regression of local tumors (1, 2), but indicate that at least the RNA regimen has systemic therapeutic effects as well, in contrast to BCG therapy which has not been effective in causing the regression of a line-10 tumor with which it was not in intimate contact (6, 14, 15). In fact, with the RNA regimen, we demonstrate that immunotherapy of one intradermally established line-10 tumor resulted in the complete abrogation of both the treated and a distant untreated tumor. In neither form of tumor immunotherapy have we yet had the opportunity to develop optimal conditions for therapy or to develop combined therapeutic regimens. This may take some time because the limited in the availability of strain 2 guinea pigs limits the rate at which extensive and comprehensive protocols can be translated into completed experiments. The adaptation of our immunotherapy models to other tumors and in other species is being investigated.

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