Immunologic Acceleration of Death in Animals with Transplanted Tumors

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Summary

In a previous publication, some prolongation of life was reported when DBA/1 mice were inoculated with Dbrb tumor 10 days after receiving an implant of a Millipore chamber containing rat lymph nodes sensitized against Dbrb tumor. In contrast, similar experiments with C57 mice and C-1498 tumor indicated that sensitized rat lymph tissue at times shortened survival while inhibiting tumor growth. The present studies with DBA/1 mice and also C3H mice and H-2712 tumor were done in an effort towards clarification of these findings. It appeared that a shortened survival by an accelerated death could be produced: (a) by increasing the amount of sensitized lymph nodes, (b) by hypersensitization of the rat to the mouse tumor before removing lymph tissue, or (c) by simultaneous challenge of the test animals with tumor inoculation and sensitized nodes. It is suggested that the heterologous transplantation of specifically sensitized lymph nodes caused a release of antibodies through the Millipore chamber for a brief period. An excessive antigen-antibody reaction then contributed to an accelerated, allergic type of death of the inoculated host. Gross and microscopic study of the tumors and major organs at autopsy tend to confirm this explanation.

Introduction

In a previous publication (2) we described a method that appeared to establish a temporary state of “adoptive immunity” in mice challenged with a specific transplantable tumor. In this work, originally based on the observations of Billingham et al. (1) and Mitchison (6), lymph node tissue from rats previously sensitized against the mouse tumor, were enclosed in Millipore filter chambers and these were implanted back into healthy mice that were then challenged with the tumor. These experiments were designed to test the hypothesis that the rat lymph nodes would produce anti-mouse tumor antibodies, and that these antibodies released through the filter chamber would inhibit the growth of the challenging tumor.

We reported that there was some prolongation of life of DBA/1 mice inoculated with Dbrb tumor 10 days after they had received a millipore chamber containing minces of rat lymph nodes sensitized against the Dbrb tumor. However, the growth of the tumor in these mice was not inhibited. In contrast, the results of similar experiments using C57 mice and C-1498 tumor indicated that the sensitized rat lymph nodes did not prolong the life of the mice, and indeed sometimes shortened their lives, but did inhibit the rate of growth of the tumor.

It is reasonable to expect that, in general, the greater the growth rate of a tumor, the shorter will be the time of survival. The apparent exceptions to this general principle in our experiments have led us to investigate the possibility that an immunologic reaction may contribute directly as a cause of death in certain malignancies, and to speculate whether a neoplasm itself may not induce a similar immunologic mechanism as a form of graft vs. host reaction in some clinical conditions.

Materials and Methods

EXPERIMENT 1. DBA/1 mice from 6 to 9 weeks of age were injected with a 0.1-ml suspension of isologous Dbrb tumor, prepared as described below, s.c., in the right rear thigh. Eight to 10 days later, they were killed and the tumor was aseptically removed. Tumor tissue was forced through a tissue press with a pore size of 1 mm. To the fragments of tumor thus obtained, sufficient sterile normal saline solution was added, so that the suspension would readily pass through a No. 18 hypodermic needle. Sprague-Dawley rats weighing between 150 and 180 gm were sensitized with a single inoculation of 0.1 ml of the tumor suspension in the lower lip, and the cervical lymph nodes were removed from the rat 10 days later. Then 125 mg of the rat lymph node tissue, finely minced, were enclosed in 1 Millipore chamber3 (following the method by Castellanos and Sturgis (3)) and implanted in the peritoneal cavity of healthy DBA/1 mice. Each mouse was then inoculated in the right posterior thigh with 0.1 ml of tumor suspension immediately after the lymph node implantation. Twenty-five mice received lymph node and tumor and 25 control mice received tumor alone. The duration of survival of the 2 groups was compared.

EXPERIMENT 2A. Experiment 1 was repeated, except that 250 mg of sensitized lymphoid tissue, twice the amount, was implanted in chambers, and the duration of survival compared with controls inoculated with the mouse tumor alone.

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2 Spontaneous mammary adenocarcinoma. Host DBA/1J mice (100%). Jackson Memorial Laboratory, Bar Harbor, Maine.

3 Millipore filter: TWWP, 0.45 μm pore; (100 μ, 10 μ thin). Millipore Filter Corp., Bedford, Massachusetts.
EXPERIMENT 2B. In order to study histologic changes associated with implantation of the mouse tumor and sensitized rat tissue, 20 DBA/1 mice as controls received tumor alone, and 20 experimental animals received 250 mg of sensitized rat nodes in Millipore chambers at time of challenge with the tumor. In this experiment, the rats were hypersensitized with 2 injections of mouse tumor 2 weeks apart, and the cervical lymph nodes removed 16 days following the 2nd injection. When the mice of both groups began to show evidence of disease by the 4th day, they were all sacrificed, and the vital organs weighed before the preparation of microscopic sections.

EXPERIMENT 3. C3H mice from 6 to 9 weeks of age were injected with a suspension of isologous H-2712 tumor. Because of impending necrosis, the tumor was removed on the 7th day for injection into rats. The preparation of tumor suspension was carried out as described above. Each rat was hypersensitized by 2 injections, 2 weeks apart, of 0.1 ml of the tumor suspension, and the cervical lymph nodes were removed 2 weeks following the 2nd injection of tumor. Each mouse was implanted with a Millipore filter chamber containing 250 mg of lymph node tissue, and 0.1 ml of the tumor suspension was injected in the right posterior thigh immediately after implantation.

EXPERIMENT 4. Experiment 3 was repeated, except that the experimental group received hypersensitized rat lymph nodes, but was not challenged with the mouse tumor. Control animals received tumor alone.

EXPERIMENT 5. In order to test the specificity of the mouse tumor as an “antigen,” 30 C3H experimental animals were implanted with 250 mg of rat lymph nodes hypersensitized by 2 injections, 2 weeks apart, of mouse muscle (a mouse muscle suspension was prepared in a similar fashion to that of the tumor suspension). Immediately after implantation, they were challenged with the tumor. Thirty control animals received tumor alone. As in all previous experiments, the survival time of the 2 groups was compared.

Results

EXPERIMENT 1. The results are shown in Chart 1. When the experimental animals were challenged with the tumor, on the same day of the lymph node implantation, no extension of life was observed as compared with controls. This survival curve is

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Chart 1. Survival curve of an experimental group of DBA/1 mice injected with Dbrb tumor and receiving on the same day 1 Millipore chamber containing 125 mg of lymph node tissue from a rat sensitized to the mouse tumor 10 days earlier. Control mice received tumor but no lymph nodes. L.N., lymph node; T, tumor. Survival for both groups was similar.

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![Survival curve chart](chart1.png)

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Chart 2. Survival curves of mice injected with tumor and receiving on the same day 1 Millipore chamber containing 250 mg of lymph nodes from a rat sensitized to the mouse tumor 10 days earlier, and control mice receiving tumor but no lymph nodes. In the 1st 8 days the experimental group showed an accelerated death rate.

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EXPERIMENT 2. In order to study histologic changes associated with implantation of the mouse tumor and sensitized rat tissue, 20 DBA/1 mice as controls received tumor alone, and 20 experimental animals received 250 mg of sensitized rat nodes in Millipore chambers at time of challenge with the tumor. In this experiment, the rats were hypersensitized with 2 injections of mouse tumor 2 weeks apart, and the cervical lymph nodes removed 16 days following the 2nd injection. When the mice of both groups began to show evidence of disease by the 4th day, they were all sacrificed, and the vital organs weighed before the preparation of microscopic sections.
substantially different from the 1 previously reported (2), where the tumor was injected 10 days after lymph node implantation.

Chart 2 (Experiment 2A) shows the result of increasing to 250 mg the amount of sensitized lymph nodes obtained after a single inoculation of tumor. The experimental animals, challenged with the tumor on the same day of lymph node implantation, showed an early acceleration of death, in the 1st 8 days. No extension of survival was observed.

The results of Experiment 2B are shown in Table 1 and in Figs. 1–4. Comparison of the mean organ weights in the mice that received sensitized lymph nodes and tumor, with organ weights in the mice that received only tumor, showed, by the 4th day, that the spleen weighed less and the lung weighed more in the experimental animals. This difference was statistically significant. No significant difference was observed in the weights of liver and brain.

Examples of the microscopic findings at the site of tumor injection are shown in Figs. 1 and 2. The mouse with sensitized rat lymph nodes shows small foci of tumor with extensive central necrosis (Fig. 1). The tumor is limited to the fibroadipose tissue surrounding the nerve trunks. Many cells have small picnotic nuclei and shrunken cytoplasm. In the mouse without sensitized rat lymph nodes (Fig. 2), the foci of tumor cells are larger and extend in some areas through the perimysium and form infiltrating cords surrounding individual fibers, some of which are atrophic. In neither case is there any definite cell reaction at the margin of the tumor.

The cellular changes in the liver of a mouse receiving sensitized lymph nodes is shown in Fig. 3 (upper). There is a swollen, finely vacuolated pale cytoplasm and picnotic nuclei. No similar findings are observed in mice which did not receive sensitized rat lymph nodes (Fig. 3, lower).

Microscopic changes in the spleen of mice which received sensitized lymph nodes are shown in Fig. 4 (upper). Small ischemic infarctions with extensive coagulative necrosis is observed, with no reaction in the surrounding parenchyma. These changes are not observed in mice which received no sensitized rat lymph nodes (Fig. 4, lower).

Chart 3 (Experiment 3) shows the results obtained in C3H mice when the sensitization of the lymph node from the rat was increased, and 250 mg were enclosed in the Millipore filter chamber. As can be seen, the experimental group (lymph node plus tumor) challenged with the mouse tumor on the same day of lymph node implantation, died in an accelerated fashion. By the 8th day after implantation, of 130 mice, 103 had already died. In the control group only 5 animals of 100 had died at this time. By the 12th day, when only 2 experimental animals remained alive, 38 control mice were still living; and by the 16th day when all experimental animals were dead, 26 control mice were still surviving.

Chart 4 shows the results obtained in Experiment 4, when 40 mice were implanted with 250 mg of hypersensitized rat lymph nodes, but received no tumor. Only 11 had died 33 days later; the remaining 29 remained alive until sacrificed 120 days later. The control animals receiving tumor alone died as usual within 17 days.

Chart 5 (Experiment 5) shows the results when mice were implanted with rat lymph nodes hypersensitized to mouse muscle and challenged with the tumor immediately after implantation. The rates of death of both, experimental and control mice, were similar.

Discussion

The method of study employed in this and in our previous report (2) rests upon the assumption that heterografts of sensitized lymph nodes enclosed in Millipore filter chambers continue to liberate antibodies for a demonstrable period of time. Other studies (5) tend to support this assumption. A recent observation (4) also indicates that lymphocytes under such conditions can undergo active proliferation.

In the course of the present experiments, it became evident that survival of mice inoculated with a lethal tumor could be
CHART 4. Survival curve of mice receiving 250 mg of hypersensitized rat lymph node in 1 Millipore filter chamber, but were not injected with tumor. Control mice received only tumor. By the 33rd day only 11 experimental animals had died, and 29 remained alive until sacrificed 120 days later.

CHART 5. Survival curve of mice receiving 1 Millipore chamber containing 250 mg of lymph nodes hypersensitized to rat muscle. Tumor was given on the same day. Control mice received only tumor. The rates of death of both experimental and control mice were similar.
shortened by 3 different modifications of the technic: (a) by implanting the lymph node into the test animal on the same day of tumor inoculation, (b) by increasing the amount of sensitized lymph node implanted, and (c) by hypersensitizing the rat from which the lymph nodes were obtained. The use of any of these 3 means of increasing the effectiveness of the sensitized rat lymph nodes resulted in an accelerated death of the animals. In contrast, the survival of the animals appeared to be enhanced, in our previous report (2), when a smaller amount of sensitized rat lymph node tissue was used, and when there was a longer interval between lymph node implantation and the time of tumor inoculation.

The accelerated death, which was observed in Experiments 2 and 3 when we employed the above mentioned means of increasing the immunologic effectiveness of the lymph nodes, resembled death from allergic causes. On the 3rd to the 5th day following tumor inoculation and node implantation, the animals appeared disoriented and their breathing became labored. Bloody pleural effusions and bloody ascitic fluid were almost invariably present. Apparently neither infection nor the spread of the tumor were involved in the death of these animals. The histologic changes at autopsy tended to confirm our impression that death was caused by allergic phenomena. Because such accelerated death was not observed in control animals receiving the sensitized lymph nodes only, and not inoculated with tumor (Experiment 4), nor when the rat nodes were sensitized against mouse muscle (Experiment 5), this must be considered a specific graft vs. host reaction involving the tumor itself, rather than merely a rat vs. mouse death. We suggest that a lethal factor results from the antigenism between the sensitized lymph nodes and their specific antigen, the tumor. When autopsy was performed in the 11 animals that died after receiving hypersensitized lymph nodes, but no tumor (Chart 4), death was apparently due to other causes, as intestinal obstruction. The viscera of these animals revealed none of the histologic changes described in mice receiving lymph nodes and tumor.

The possibility that an adverse reaction between specifically sensitized lymph nodes and the tumor may contribute to the cause of death in the experimental animals, draws attention to the mechanism of death of the control animals that received tumor alone and no sensitized lymph nodes. It is tempting to speculate that the actual cause of death in these tumor-bearing animals is the same as that in animals receiving sensitized lymph nodes; that is, the possibility that the animals' own lymph nodes became sensitized to the tumor and a lethal antigen-antibody reaction is induced. The difference in survival in Chart 3 could represent the interval of time required for auto-sensitization of the "control" animals after their tumor inoculation, a time interval which was unnecessary in those implanted with previously sensitized lymph nodes. Such an explanation for a contributing cause of death from cancer is not inconsistent with those frequent occurrences in clinical medicine, in which patients die with relatively small tumors that have not demonstrably interfered with vital organ functions.

In view of the present results, it is necessary to amplify our earlier observation (2) that the implantation of smaller amounts of sensitized lymph nodes did provide some inhibition of tumor growth and prolongation of survival. Our hypothesis in the previous study that the antibodies against the tumor retarded its growth would not be opposed to the present observation that larger amounts of antibody at the time the tumor is introduced caused an accelerated death.

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References

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