Immunological Factors in Human Sarcomas and Melanomas: A Rational Basis for Immunotherapy

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THE CONCEPT that immunity to cancer might be acquired in the same manner as immunity to infectious diseases is not new. This view was very popular during the early 1900's when remarkable immunity was induced against transplantable neoplasms of laboratory rodents. However, it soon became evident that this tumor immunity was, in reality, homograft immunity directed against normal histocompatibility antigens in the tumor. Thereafter, interest in cancer immunology rapidly declined and little progress was made in this field until the work of Foley [8] in 1953 and Prehn and Main [31] in 1957. These investigators conclusively demonstrated tumor specific antigens in methylcholanthrene induced sarcomas of mice. This observation stimulated new interest in cancer immunology and during the past decade there has been tremendous progress in this field.

Tumor specific antigens capable of inducing a host immune response which specifically retards the growth of neoplastic cells have been demonstrated in a wide variety of viral [28] and carcinogen induced neoplasms,[14] as well as in certain spontaneous tumors of laboratory rodents.[10] Recently, sensitive immunological technics have been used to detect antibodies reactive with antigens of autologous tumor tissue in the sera of patients with Burkitt's lymphoma [13, 15, 29] malignant melanoma,[17, 18, 22, 25, 27] neuroblastoma,[11] skeletal and soft tissue sarcomas [4, 20, 21] colonic neoplasms [9, 12] and other tumors.[1, 7, 12] Thus, there is increasing evidence that human cancers like animal neoplasms, contain tumor specific antigens which are immunogenic in the autologous host. Therefore, tumor antigens can no longer be regarded as a laboratory curiosity and now must be seriously considered in relationship to human neoplastic disease.

This report will review some of our previous immunologic studies with human melanomas and sarcomas and describe our recent attempts at immunotherapy in these diseases.

Immunologic Studies with Human Malignant Melanoma

We have previously suggested that certain clinical features of malignant melanoma indicate the importance of immunologic factors in this disease.[22] Examples of host immunity in malignant melanoma include the unexpectedly high rate of spontaneous regression,[5] observation that transfusions of blood from patients with spontaneous regressions sometimes induced regression of melanoma in recipients of the transfusions,[34, 35] reports that therapeutic cross-transplantation of tumors and sensitized leukocytes is sometimes successful [26] and the demonstration of cytotoxic effects of autologous serum against melanoma cells in short-term tissue culture.[17] Additional experimental evidence to support the presence of immunological factors in malignant melanoma was provided by our immunofluorescence studies which demon-

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strated the presence of a common tumor antigen in malignant melanoma which was immunogenic in patients with this disease.[22] Since then, additional reports have appeared which confirm and extend our earlier immunofluorescent studies.[18, 27, 32] In addition, the existence of cellular immunity is suggested by the demonstration of delayed cutaneous hypersensitivity reactions to autologous extracts of malignant melanoma.[6]

Recently, a complement fixation technic has been developed for the study of human tumor antigens and antibodies. This technic has been applied to our studies of immunologic factors in malignant melanoma. The complement fixation technic, method of antigen preparation and complement source have been previously described.[4] The HuMel-1 melanoma cell line used for most of these studies was derived from an amelanotic melanoma which did not form melanin in tissue culture.

The distribution of antibody in the serums of patients with various stages of malignant melanoma to the HuMel-1 melanoma antigen is shown in Table 1. Using four units of antigen (determined by box titration with autologous serum) 67% of the patients with malignant melanoma had detectatble antimelanoma antibody. This was significantly higher than 22% incidence of antibody in the normal blood bank donors. It will be noted that patients with localized melanoma (89%) or those undergoing spontaneous regression of their melanoma (100%) had a significantly higher incidence of antimelanoma antibody than those with advanced metastatic disease. The incidence of antibody in the latter group did not differ significantly from the incidence of antibody in the normal sera. A study of serial serum specimens on melanoma patients at various stages of their disease revealed a drop in antibody titer to undetectable levels as patients developed widespread metastatic disease.

TABLE 1. *Correlations between Incidence of Antimelanoma Antibody Detected by Complement Fixation and Extent of Disease in Patients with Malignant Melanoma*

Type of Serum	# Pos./ # Tested††	% Pos.
All melanoma patients	42/63*	67
Patients with localized disease	31/35*	89
Patients with unusual host resistance	5/5	100
Patients with metastatic disease	6/23	26
Normal blood donors	11/50	22

†† Antigen was HuMel-1 diluted 1/8, positive sera had titer 1/8 or >.

* Values differ significantly ($p < 0.01$) by X^2 from normal blood donors.

Thus, these complement fixation studies have revealed a good correlation between the presence of antibody to a common melanoma antigen and the extent of the patients disease.

Immunologic Studies with Human Sarcomas

Recent immunologic studies have revealed a high incidence of antibody to sarcoma specific antigens prepared from a human liposarcoma in the serum of patients with various types of skeletal and soft tissue sarcomas.[4, 20, 21] The sarcoma specific antigen was found in tissue culture cells drived from different histologic types of sarcomas but not in normal fibroblasts obtained from sarcoma patients nor in cells from nonsarcomatous malignancies. The purpose of this study was to determine whether there was any relationship between the antisarcoma antibody titer and the course of the patient's disease. For these studies, serum samples were obtained preoperatively and at various intervals during the postoperative period in patients with skeletal and soft tissue sarcomas undergoing definitive resection of their neoplasms. A total of 21 patients have been studied to the present time but the results of the group are typical of those illustrated

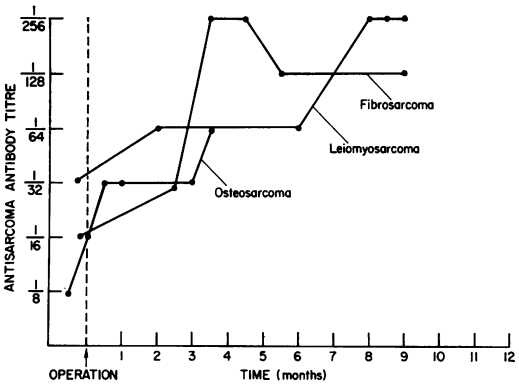

FIG. 1. Antisarcoma antibody titers determined by complement fixation against the HuSA-1 liposarcoma antigen. Serial serum samples obtained following resection of the primary sarcoma in patients who remained free of disease.

in Figures 1 and 2. Note that the antibody titer usually increases following surgical resection of the tumor mass and remains elevated in patients who remain free of disease (Fig. 1). However, all patients who developed recurrent disease with pulmonary metastases almost simultaneously were noted to have a declining level of antisarcoma antibody which dropped to 0 with progressive disease (Fig. 2). Thus, there is an extremely good correlation between recurrent disease and declining antisarcoma antibody titer. Furthermore, patients who have remained tumor free for several years postoperatively have been found to maintain their antisarcoma antibody at relatively stable levels.

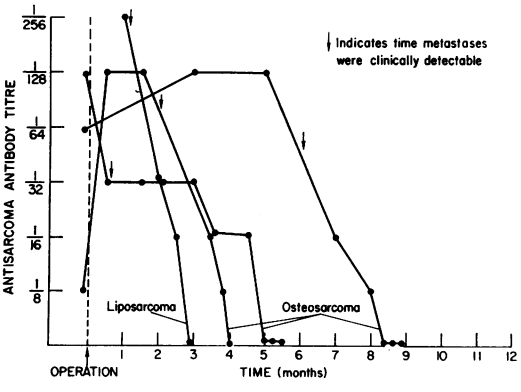

FIG. 2. Antisarcoma antibody titers determined by complement fixation against the HuSA-1 liposarcoma antigen. Serial serum samples obtained following resection of the primary sarcoma in patients who developed recurrent disease with pulmonary metastases. The time at which pulmonary metasases were detected on the chest x-ray is indicated by an arrow.

The remarkable correlation between the antitumor antibody titer and the course and extent of disease in patients with malignant melanoma, skeletal and soft tissue sarcomas suggested to us that the antigens involved might be important for the patient's immune host response against his tumor. Therefore, it appeared logical to attempt to increase the patients immune response against these antigens by immunotherapy.

Immunotherapy Studies with Guinea Pig Sarcomas

Prior to attempts at immunotherapy of human neoplasms, experiments were performed in inbred guinea pigs using a methylcholanthrene induced liposarcoma MCA-A. The experimental model used was similar to that recently described by Kronman and associates.[16] In these experiments, living tumor cells were injected intramuscularly in the leg at a cell dosage (1×10^5 cells) previously found to induce 90 to 100% tumor growth. Immunization was then carried out simultaneously or up to 10 days later with multiple intradermal innoculations of lethally irradiated tumor cells (15,000 rads) or a small dose of living tumor cells mixed with bacille Calmette Guérin (BCG). BCG was used as immunological adjuvant in these studies since it is capable of inducing a heightened immune response against a wide variety of animal neoplasms.[19, 30, 36]

The results of one experiment are summarized in Figure 3. It will be noted that both living tumor cells and irradiated tumor cells mixed with BCG significantly inhibited growth of the intramuscularly injected tumor as compared to controls. However, living cells appeared to be significantly more effective than irradiated

cells. The increased effectiveness of living cells probably relates to the quantity of antigen present in the replicating tumor cells. It should be emphasized that quantitative factors are very important in these experiments because no protection against tumor growth was observed if the challenge dose in the leg was increased to 1×10^6 cells. To avoid the danger of tumor growth at the immunization site, we decided to use irradiated tumor tissue plus BCG for our studies of human immunotherapy.

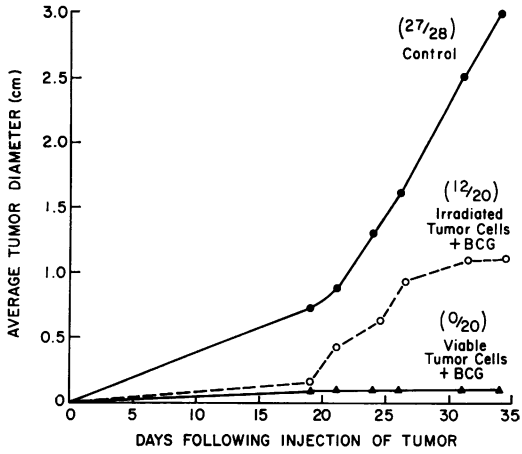

FIG. 3. Immunotherapy experiments with a transplantable liposarcoma in syngenic strain 2 guinea pigs. 1×10^5 liposarcoma tumor cells were innoculated intramuscularly into the leg and immunotherapy initiated intradermally in four sites on the back with 1×10^6 living or 1×10^7 irradiated tumor cells mixed with BCG.

Immunotherapy in Patients with Advanced Malignant Disease

During the past 2½ years, 12 patients with advanced malignant melanoma, skeletal and soft tissue sarcomas have received immunotherapy. The eight patients with malignant melanoma have been previously recorded [23] and will be briefly summarized here. All of these patients had advanced disease with multiple subcutaneous metastasic melanoma nodules which were directly injected with BCG (.1 ml. of BCG vaccine per nodule). This therapy produced temporary tumor regression of injected nodules in five patients and two patients had regressions of melanoma nodules that were not injected with BCG. The regression of uninjected melanoma nodules in one of these patients was temporary, lasting only 2 months, whereas, a complete regression of 2½ years' duration has occurred in the other patient who has no evidence of disease at this time.

There was a good correlation between the patient's immunologic competence at the beginning of immunotherapy and the therapeutic result. All patients who could be sensitized to dinitrochlorobenzene (DNCB) [3] or tuberculin developed a fourfold rise in antibody and had some response to immunotherapy whereas, patients who could not be sensitized to DNCB or tuberculin failed to respond to BCG therapy.

Four patients with sarcomas (two osteosarcomas, one fibrosarcoma and one rhabdomysarcoma) who had multiple bilateral pulmonary metastatic lesions unresectable at the time of exploratory thoracotomy have received immunotherapy. These patients have been treated with innoculations of BCG into the intrapulmonary metastatic nodules and intradermal immunization

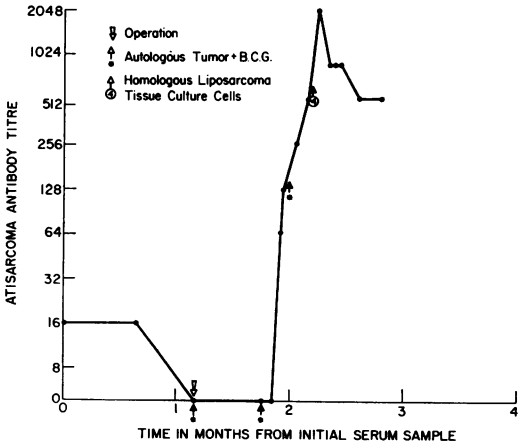

FIG. 4. Antisarcoma antibody titers determined by complement fixation against the HuSA-1 liposarcoma antigen on serial serum samples following immunotherapy in a 16-year-old boy with unresectable pulmonary metastases from a rhabdomysarcoma of the chest wall.

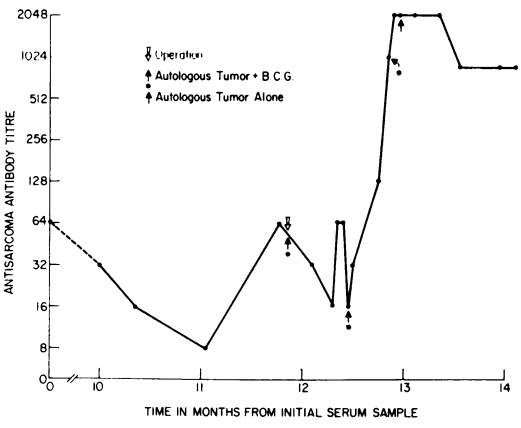

FIG. 5. Antisarcoma antibody titers determined by complement fixation against the HuSA-1 liposarcoma antigen on serial serum samples from a 14-year-old boy with a primary osteosarcoma of the right femur who developed pulmonary metastases 10 months after resection of the primary for which a left pneumonectomy was performed and immunotherapy initiated.

with irradiated tumor cells mixed with BCG. The immunization procedure consisted of the injection at multiple intradermal sites of 50–75 million tumor cells irradiated with 15,000 rads and mixed with two ampuls of BCG.

The results of immunotherapy in these patients have been disappointing since they all have subsequently died of the disease. However, a temporary decrease in the growth rate of pulmonary metastatic lesions was observed in two patients and a rising titer of antisarcoma antibodies occurred following immunization in all patients. Since most of these patients did not have detectable antibody at the beginning of immunotherapy, the rising titers were particularly impressive as illustrated in Figure 4. Note that the large increase in antibody titer did not occur until following the second immunization in this instance. No patient had growth of tumor at the immunization sites.

Immunotherapy as an Adjunct to Surgical Therapy

After we had established the safety of immunotherapy in patients with advanced disease, we began to study those with somewhat earlier disease. However, all patients in this group have had extensive disease which would ordinarily be considered incurable by surgical therapy alone, e.g., bilateral pulmonary metastases. Eight patients (four with melanoma and four with sarcoma) have received immunotherapy following surgical resection of all gross metastatic lesions.

The results of immunotherapy in patients in this group cannot be evaluated at the present time because of a short follow-up period. However, all of these patients have had an impressive increase in their antisarcoma antibody titers following immunotherapy as illustrated in Figure 4. In addition, some of these patients developed cutaneous hypersensitivity reactions to their tumor extracts following immunotherapy.

Discussion

These studies have revealed a remarkable correlation between the incidence and titer of antitumor antibodies detectable by complement fixation and the extent of disease in patients with malignant melanoma, skeletal and soft tissue sarcomas. Patients with localized disease were more likely to have antitumor antibodies than those with advanced disease. A rising titer of antisarcoma antibody was consistently observed following tumor resection in patients with skeletal and soft tissue sarcomas. Furthermore, study of serial serum specimens in patients with melanomas and sarcomas who developed recurrent disease have revealed a progressive decline in their titers of antitumor antibody with advancing disease.

There are several possible explanations for this correlation between the incidence and titer of antitumor antibody and stage of malignant disease. It is possible that the antitumor antibody is produced at a relatively constant rate but is constantly being absorbed from the circulation by a growing tumor mass. Therefore, one would expect removal of the tumor to result in an

increase in the titer of antitumor antibody and regrowth of tumor to lower the titer of antibody. Since antibody cannot penetrate into the interior of a living cell, this would suggest that the effective antigens reactive with the antitumor antibody are located on the cell surface. Some support for this hypothesis comes from the observation that low titers of antisarcoma antibody have been eluted from the sarcoma tissues of two patients, thus suggesting *in vivo* absorption of antibody to tumor cells had occurred.

However, another explanation for these findings is that the growing tumor mass produces specific immunosuppression due to the induction of high doses tolerance and therefore the dropping antibody titer in patients with recurrent disease is due to decreased production of antibody. Removal of the tumor would then remove the large antigenic mass and permit a return of immunologic competence to the sarcoma specific antigens. There is no evidence to support this hypothesis and, in fact, the observation that antibody production can be stimulated in patients with advanced disease by immunization with autologous tumor suggests high dose tolerance may not be a suitable explanation for these findings.

Immunotherapy studies with methylcholanthrene sarcomas in syngenic guinea pigs have demonstrated the effectiveness of immunotherapy using active immunization with irradiated tumor cells and BCG by the intradermal route. The effectiveness of active immunization in this experimental model indicates that a growing tumor does not induce a maximum immune response in the host and provides a theoretical basis for active immunization in the treatment of malignant disease. However, the importance of quantitative factors is apparent since this procedure was not effective in animals receiving large tumor cell doses.

A preliminary evaluation of immunotherapy using active immunization with BCG and irradiated autologous tumor has demonstrated that a heightened immune response can be elicited in patients with melanomas and sarcomas. Although the results of immunotherapy in patients with advanced disease have not been very impressive, one significant remission has been achieved in malignant melanoma.

The poor results observed in patients with advanced disease might have been expected because of previous animal studies indicating that immunity against cancer is relative rather than absolute. The importance of quantitative factors observed in our immunotherapy model using guinea pig sarcomas are consistent with Southam's study on the autotransplantibility of cancer in man which indicates that innoculation of 10^8 cells almost uniformly results in tumor growth at the innoculation site.[33] A neoplasm only one centimeter in diameter contains approximately 10^9 tumor cells. Therefore, by the time most tumors are clinically detectable, they have already outgrown the patient's immune defenses, and it is unlikely that immunotherapy alone will ever bolster host defenses sufficiently to reverse this process in the patient with advanced disease. For these reasons, it is unlikely that immunotherapy alone will ever play the major role in the treatment of cancer.

However, immunotherapy is a logical adjunct to definitive surgery for several reasons.[24]

1) Patients who have only a small foci of cancer cells remaining after surgical removal of the bulk of tumor are those most likely to benefit from immunotherapy because the tumor mass which must be destroyed by host responses is smallest at that time.

2) The specificity of the immune response for cancer cells provides us with a possible therapeutic tool which will have selectivity for small foci of cancer cells not possible with other presently available treatment modalities such as chemotherapy and irradiation.

3) Surgical patients are most likely to respond from the immunologic standpoint to any immunotherapeutic maneuvers, since the cancer patients' general immunological competence is greatest in the stage of localized cancer and progressively declines with advancing disease.[2, 3]

4) Immunotherapy would be expected to complement rather than interfere with other currently available methods of managing cancer recurrences following operation such as irradiation and chemotherapy. Since both irradiation and chemotherapy are immunosuppressive agents, immunotherapy should logically precede these other treatment modalities.

At the present time, immunotherapy should be regarded as an experimental method of therapy which may eventually be useful as adjunct to other methods of cancer treatment. Preliminary evaluation of a method of immunotherapy of proven effectiveness in a model system using a guinea pig sarcoma has been encouraging. A heightened immune response can be induced in patients with melanomas and sarcomas following immunization with irradiated tumor and BCG. We are presently evaluating his procedure in patients who have recurrences following definitive cancer surgery. If the safety and effectiveness of this method of immunotherapy can be established in these patients, it is likely that immunotherapy will prove useful as an adjunct to definitive cancer surgery.

Summary

Immunologic studies have revealed a remarkable correlation between the patient's immune response to his tumor and the extent and progression of malignant disease in melanoma and sarcoma. A rising titer of antitumor antibody was consistently observed following surgical removal of the tumor mass in patients with skeletal and soft tissue sarcomas. Whereas, all patients with recurrent disease were found to have a progressive decline in their titers of antitumor antibody with advancing disease.

A preliminary evaluation of immunotherapy in 20 patients with advanced melanoma and sarcoma has revealed that active immunization with BCG and autologous tumor cells induced a heightened immune response against the tumor antigens. The results of immunotherapy using this technic have been encouraging and hopefully will eventually lead to the development of immunotherapy for use as an adjunct to definitive cancer surgery.

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Discussion

Dr. Edward T. Krementz (New Orleans): I would like to report on some of the work that was started by Dr. Creech in our department at Tulane 5 years ago.

The first slide shows a patient with melanoma who Dr. Creech had been studying for several years. He was a 43-year-old man who had cutaneous and subcutaneous metastases over the lower abdomen and in the amputation site following