Progress of Immunotherapy in the Treatment of Malignant Melanoma

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Evidence has accumulated since 1967 indicating the importance of the immune system in malignant melanoma. Morton et al.'s initial work described tumor-associated antigens in human malignant melanoma using immunofluorescent techniques (1). These were soon confirmed and extended by others.

Further work showed that these tumor-specific antigens elicit the production of circulating humoral antibodies in 60% of patients with malignant melanoma (Table 1). Only 20% of normal patients had these antibodies, which may have been the result of their surveillance mechanism identifying and destroying these tumors. Surveillance is the mechanism by which the host immune system, either by the T-cell lymphocytes or by circulating antibodies, seeks out and destroys what it identifies as foreign to the host. Furthermore, there appeared to be a correlation between the clinical status of the melanoma patients and the incidence of anti-melanoma antibodies in their sera. Patients with localized melanoma were more likely to have antibody in their sera than those patients with disseminated disease (2). Some patients who were observed through their course had the antibody disappear from their sera as the disease progressed. Transfusions from patients with spontaneous regression have been reported to cause regression of malignant melanoma in certain cases.

To understand immunotherapy, we must first explain that the immune response can be separated into an antibody and a cellular response (Fig. 1). The antibody response can be seen to result in opposing forms: the emergence of cytotoxic antibodies can be shown to kill tumor cells when incubated in vitro with complement, whereas enhancing "antibody," or "blocking factor," is believed to be a complex formed by the binding of fragments of tumor antigen to circulating tumor-specific antibodies (3). This blocking of the immune mechanism is thought to be directed at the host's lymphoid cells by masking its recognition of tumor or preventing its cytotoxic effects against the tumor. It can be seen that both responses may react inversely with the removal of tumor.

On the other hand, tumors may also elicit a cellular response through the lymphocytes and macrophages (Fig. 1). The lymphocytes can become immunized and demonstrate their killing potential when cultured in vitro with the tumor cells. This mechanism, called cytotoxicity, is employed to test the immunologic competence of the individual. Macrophages can also become immunized to release their toxic contents to kill tumor cells. There may also be an interaction between immunized lymphocytes and macrophages to potentiate this effect, as shown by in vivo studies in animals.

However, these immunologic responses to tumor are in dynamic motion and are changing constantly. This can be exemplified by folding and superimposing these two circles (Fig. 2). One must appreciate the fact that the immune response of any individual to the tumor is a multiplicity of actions that make up the whole immu-
nologic response. There is a delicate balance, the end result of which is a reflection of whatever mechanisms become more dominant in the individual, to cause either growth or death of the tumor.

There is evidence to suggest that the manipulation of the immune system may favorably affect the clinical course of patients (Fig. 3). The immune response may be specifically augmented by passive transfer of leukocytes from patients recovered or cured of a melanoma (4, 5). Another way would be to pass "transfer factor" from immune patients (6). A similar response may follow the use of anti-HL-A serum, which seems to increase the survival time of immune lymphoid cells. Active immunity, on the other hand, may be potentiated by altering or increasing the anti-

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**TABLE 1**

| Human Anti-Melanoma Antibody$^a$ |
|----------------------------------|
| 27 patients with MM—61% had anti-MM Ab |
| 25 normal patients—20% had anti-MM Ab |
| Transfusion of blood from spontaneous regression induced regression of MM |

$^a$From Ref. (1).
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FIG. 2. Superimposition of the antibody and cellular responses of the immune reaction.

genicity of tumor by a number of reactions to remove the "coating" of the tumor cells, such as acetylation (7), concanavalin A (8), and neuraminidase (9, 10).

Nonspecific augmentation utilizes agents to increase the response of the lymphoid cells, in general, to react and kill tumor cells. DNCB has been applied topically to squamous cell carcinoma of the skin to cause a delayed hypersensitivity reaction and thereby destroy adjacent tumor cells as well (11). This mechanism may also employ the formation of hapten conjugates. Other agents may be used to increase the im-

FIG. 3. Techniques of immune system manipulation.
mune response nonspecifically, such as smallpox and BCG vaccines. BCG may also be used to increase systemic immunity.

The use of smallpox vaccine injected directly into melanoma skin nodules was shown in a small series to result in regression of satellite nodules and to prolong survival in a few cases (Table 2) (12). All had severe systemic reactions to the virus. However, no response could be demonstrated with repeated injections in patients who had been immunized to the vaccine recently or in visceral metastases. Again, this reflected a nonspecific response to activation of the immune mechanism.

The use of BCG to stimulate the immune response was reported by Morton et al. in 1970 in a small series of eight patients treated by direct intralesional injections with the vaccine (13). Approximately 90% of the melanotic nodules regressed in those patients who were immunologically competent (as measured by delayed hypersensitivity reactions to DNCB) (Table 3). Moreover, occasional nodules at sites distant from the BCG inoculations also regressed in some patients. In fact, one patient remained completely free of tumor for 2 yr.

The potential dangers of this type of therapy, however, must be emphasized. The intralesional injections of BCG frequently result in fevers of up to 103–105°F, chills, abscesses in the local areas of injections, and sinuses that have drained for months (14). Regional lymphadenitis has been frequent, and occasionally a systemic infection has occurred with associated granulomatous hepatitis. Although not observed in Morton et al.'s series, fatal anaphylactoid reactions have been reported (but unpublished) following repeated administrations of large doses of BCG vaccine.

In contrast, the multiple intradermal scarification technique has been tolerated well with only mild fever and malaise for short periods following vaccination (14). When the two modes of administration of the vaccine were compared among patients in Uganda, where there is a high incidence of melanoma, the remission following surgical removal and vaccination was longer among those that were treated by scarification than by intralesional injections (15). This technique has been presently modified to the multiple intradermal punctures of the Tine technique, as employed with tuberculin tests.

The largest series of immunotherapy in malignant melanoma comes from UCLA, where Morton et al. recently reported their results in 151 patients over the past 7 yr (14). In an uncontrolled series, patients with Clinical Stage III melanoma treated

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**TABLE 2**

| Regression by Attenuated Virus Vaccinea |
|----------------------------------------|
| 1960–1965, 30 cases                    |
| Smallpox vaccine injected directly into lesions |
| Most had some regression of tumor      |
| Four cases had significant prolongation of life |
| All patients had severe systemic and local reactions to virus |

aFrom Ref. (12).

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**TABLE 3**

| BCG Regression of Melanomasa |
|-------------------------------|
| 8 patients—DNCB—5 positive, 3 negative |
| 3 negative—no regression of 219 nodules |
| 5 positive—90% of 184 nodules complete regression, 1 patient completely free for 2.5 yr |

aFrom Ref. (13).
with BCG immunization may have a lower recurrence rate and longer survival rate than those treated elsewhere without BCG immunization. In Stage II melanoma, moreover, where immunotherapy with BCG was begun 3–6 weeks following the surgical removal of the disease, the overall tumor-free rate for this group of 67 patients treated with BCG immunization is higher at all points in time when compared to 34 patients seen at UCLA during the same interval who did not receive BCG immunotherapy.

Nevertheless, patients with subcutaneous or visceral metastases did not respond as well (30%) as those with intradermal metastases (91%) (14). Rarely was there evidence of regression of uninjected or grossly visible visceral metastases in these patients. In general, patients with large, bulky lesions or visceral metastases did not benefit significantly from the BCG immunotherapy alone.

The explanation for the differences in response between these two types of patients is not completely clear at the present time. There are several possibilities, however, that deserve consideration.

First, there may be some biological difference between the behavior of melanoma metastatic to skin and to visceral organs. However, this may be more quantitative than qualitative and may reflect a later stage of the disease, because patients who present with intracutaneous metastatic disease almost invariably develop visceral metastases within the first year and eventually die of their disease.

This is compatible with the second observation, that the lack of immunocompetence is a manifestation of the tumor burden. The patients who are most frequently anergic as judged by the hypersensitivity parameters are those who have visceral and subcutaneous metastatic lesions. Therefore, it is not surprising that these patients would not respond well to the intradermal injections of BCG.

Finally, and more probably, the skin nodules may respond to the vaccine more intensely because the skin is the site of the most intense hypersensitivity reaction. The best way to immunize an animal is to incorporate an antigen in an inflammatory focus, usually into the skin, and invariably in skin where it is drained by regional nodes. It may have something to do with the rich lymphatic supply of the skin. It has been shown experimentally that tumor growth is potentiated in animals if the tumor is grown on a vascular island pedicle of skin that has been elevated and deprived of its lymphatic drainage (16). This is called a “privileged site” (Fig. 4).

As to the mechanism of tumor regression, it is possible that the melanoma cells
are destroyed by the immunologic reaction against the BCG-associated antigens as a result of an intense inflammatory reaction by lymphocytes and macrophages. Intradermal lesions have been shown to be infiltrated by macrophages, causing the death of tumor cells after BCG injection without phagocytosis of the tumor cells (5). Electron microphotographs have shown BCG-immune macrophage cleaving the membrane of the tumor cell, causing its degeneration as indicated by its dark appearance, but again no phagocytosis of the tumor cell was seen (17).

We have investigated the role of BCG in our laboratory to determine the mechanism of tumor cell death. The injection of two similar but antigenically distinct tumor types into an animal immune to only one of the tumors resulted in significant necrosis of nearby macrophages leading to the death of adjacent tumor cells of both types (18). However, the cells were not killed if the macrophages had not been previously immunized or “activated.” The suppression of the growth of tumor cells was demonstrated only when macrophages activated by BCG immunization were added to the inocula of tumor-immune lymphocytes and tumor cells into normal animals (19). Merely adding BCG to the inocula did not suppress the growth of tumor cells.

Macrophages are rich in lysozymes, and it is suggested that the BCG immunization increases the potential of the macrophages to kill the tumor cells, which it may do by two possible routes (Fig. 5). It may “activate” the macrophages to kill the tumor with or without the presence of tumor-immune lymphocytes; in the latter

![Diagram](attachment:fig5.png)

**FIG. 5.** Activation of macrophages by BCG, which then become cytotoxic in the presence of tumor-immune lymphocytes and tumor antigens.
case we shall say the role of the immune lymphocyte may be merely to recognize the tumor and "fire" the activated macrophage to release its toxic enzymes. Alternately, BCG may "arm" the macrophages, which are not cytotoxic as yet, but require the presence of tumor antigen and tumor-immune lymphocytes to be made cytotoxic against tumor cells (18, 19). In either event, the macrophages are caused to release their cytotoxic enzymes killing adjacent tumor cells.

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