Tumor-Directed Cellular Immunity in Malignant Melanoma and Its Modification by Surgical Treatment

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There is abundant evidence for tumor-directed cellular immunity in a variety of human tumors (1–7). The continuing high mortality from cancer indicates, however, that the capacity of host mechanisms of this kind to limit the growth of established tumors and inhibit metastasis formation is strictly limited. Considerable effort is presently being expended in numerous centers in an effort to devise methods which will improve the level of such responses. By corollary the identification and avoidance of techniques and materials which might reduce host responsiveness seems a worthwhile aim which might yield practical results in the short term.

It is well-known to surgeons that cancer surgery in an unpredictable minority of patients may be followed by rapid development of metastases. In this context the demonstration of depressed lymphocyte function, as evidenced by a reduced capacity to transform on exposure to phyto- mitogens (8–10) was of considerable interest. We have recently reported a (11) temporary loss of tumor-directed cellular immunity in patients undergoing surgery for malignant melanoma or breast carcinoma in the immediate postoperative period. This paper describes the present status of this continuing study in the patients with malignant melanoma.

MATERIALS AND METHODS

The patients all attended hospitals in the Glasgow area and all had histologically proved malignant melanoma.

The technique employed to assay cellular immunity and the method of preparing tumor extracts for use as antigens and statistical methods employed have been previously described in detail (11).
RESULTS

Summary of Findings in a Study of Cellular Immunity to Malignant Melanoma

It is not the purpose of this paper to give a detailed account of cellular immunity in melanoma patients, but to set the main findings in context we have included Table 1. The salient features are:

1. Malignant melanoma extracts inhibited the migration of autologous leukocytes in 8 of 10 cases studied.
2. In homologous combinations, the leukocytes of 33 of 55 melanoma patients showed inhibition on contact with melanoma extracts.
3. There is a progressive diminution in the frequency of migration inhibition with advancing clinical stage.
4. Melanoma patients' leukocytes were infrequently inhibited by contact with extracts of neoplastic and nonneoplastic breast conditions.
5. Control donors' leukocytes showed almost no migration inhibition when exposed to melanoma extracts.

Patients Studied While Undergoing Operation and in the Postoperative Period (Table 2)

Twenty-two patients undergoing operation for malignant melanoma were studied. The operations were for excision of a primary tumor (18), of an enlarged tumor containing lymph nodes (2), and for removal of subcutaneous recurrent tumors (1). The operations were on two patients undergoing a total of three operations.

TABLE 1

| Cell donor                     | Melanoma extracts | Breast extracts |
|-------------------------------|-------------------|-----------------|
| Malignant melanoma (autologous)| 8/10 (80)         |                 |
| Malignant melanoma (homologous)| 88/55 (60)        | 1/12 (8)        |
| Malignant melanoma (primary)  | 24/37 (65)        |                 |
| Malignant melanoma (lymph nodes)| 7/15 (47)     |                 |
| Malignant melanoma (blood-spread) | 0/6 (0)          |                 |
| Control donors                 | 9/63 (14)         |                 |

* Frequency of individuals with a positive reaction/total number of individuals tested. Figures in parentheses are percentages.

TABLE 2

| Patients tested in | Preoperative period | Immediate postoperative period | Later postoperative period | Time to return of reactivity (days) |
|--------------------|---------------------|--------------------------------|---------------------------|-----------------------------------|
| +ve                | 12/16               | 1/22                           | 15/19                     | 10.6                              |
| -ve                | 4/16                | 21/22                          | 4/19                      | 5-22                              |

* Patients with positive or negative reactions/total patients tested at each stage.

Days 1-4 after operation.
nODULES (2). They lasted from 30–90 min. The premedicating agents were usually morphine and scopolamine; anesthetic agents included nitrous oxide, halothane, and thiopeptone sodium sometimes in association with suxamethonium.

Sixteen patients were tested before operation with autologous and/or homologous melanoma extracts and in 12 cases the migration of peripheral blood leukocytes was inhibited. All 22 patients were tested in the immediate postoperative period and in all but one there was no migration inhibition on contact with melanoma extracts. In the single exceptional case a preoperative test was negative, a test on the second postoperative day was positive, and one 42 days after operation was negative. Nineteen patients were tested at various times after the immediate postoperative period in 15 of whom a positive reaction returned, usually in the second postoperative week (mean 10.6 days), but with a range of 5–22 days postoperatively. One patient (not shown in the table) had three successive operations at weekly intervals. This patient gave a positive reaction before the first operation but successive tests were negative until 7 days after the third operation when positive reactivity returned and remained during subsequent tests. One patient was observed over three operations and gave a positive reaction before each. Reactivity was lost in the immediate postoperative phase but was observed on the sixth postoperative day on two occasions. Of the four patients who did not show a return of reactivity two are alive, well, and apparently tumor-free 8 and 6 months after operation, one was tested for only a short time after operation and one died of disseminated malignant melanoma 2 months after operation.

Comparison of Blood Cortisol Levels and Variations in Migration Inhibition

Blood cortisol levels were assayed for four patients. The expected rise was observed in all four, developing during the operation and persisting for approximately 2 days. In all four patients cortisol levels had returned to normal by Day 3 after operation. In all patients there was a postoperative loss of leukocyte-migration inhibition observed preoperatively. Inhibition comparable to that observed preoperatively was observed in two patients on Day 5 after operation and in one on Day 8 after operation. In the fourth patient there was no return of inhibition and the patient died of disseminated melanoma 2 months after operation.

DISCUSSION

These results extended our previous observations (11, 12) of (usually) transient postoperative loss of tumor-directed cellular immunity to a larger series of patients. Further interest in this phenomenon falls broadly into attempts to assess its significance and attempts to analyze its mechanism.

In theory it is possible that this loss of cellular reactivity, at a time when small numbers of tumor cells may be disseminated to a greater or lesser degree by operative trauma, may be highly relevant to the processes of metastasis formation. In the absence of effective cytotoxic lymphoid cells such scattered tumor cells could become coated by noncytotoxic antibodies which would effectively protect the tumor cells when potentially cytotoxic lymphoid cells were restored to normal function. It is difficult to test such a hypothesis in man and we have, therefore, instituted animal studies designed to answer these questions.

Gershon and his associates have also described a situation in which tumor removal in animals is followed by a loss of demonstrable tumor-directed cellular im-
munity (13–15). These findings were, however, confined to a relatively limited period after tumor transplantation and the loss of reactivity was stable although it could be overcome by rechallenge with tumor. We thus believe that our observations represent a separate, though perhaps related, phenomenon. Barski and Youn (16) reported on the effect of tumor excision on tumor-directed cellular immunity in mice and found that while reactivity was not demonstrable immediately preoperatively or in the first postoperative week it could be shown to be present when mice were examined 7–21 days after operation. While the great majority of patients in our present study behaved otherwise, there were four individuals who gave a negative reaction preoperatively and in the immediate postoperative phase but who gave a positive response in later tests.

The question of mechanism is also unsettled. Obviously the medication administered before and during anesthesia and the multiple metabolic changes consequent upon operative trauma must be investigated.

The anesthetics and premedicative materials used on the patients of this series are commonly employed agents, there are, however, a number of reports that anesthetic agents, including halothane and even nitrous oxide, may have transient but significant depressive activity against lymphocytes (17–20).

Preliminary results with blood leukocytes obtained during operation, when anesthetic concentrations are maximal, are conflicting. In a small number of patients with breast carcinoma examined at this time we have not demonstrated leukocyte-migration inhibition on contact with autologous and homologous tumor extracts. In studies using Purified Protein Derivative (PPD) and leukocytes taken from PPD-sensitive donors, however, inhibition comparable in degree to that seen preoperatively has been observed (Lindop and Bancewicz, unpublished data). These latter observations, while suggesting that the presence of a high concentration of anesthetic does not directly reduce migration inhibition, do not, however, exclude a delayed effect of anesthetic agents as the cause of the postoperative loss of migration inhibition.

The postoperative phase is characterized by numerous alterations in the biochemistry of the blood and tissue fluids. Many of the materials present in altered concentration, hormones, enzymes, products of tissue breakdown are highly active pharmacologically and could individually or collectively alter lymphocyte function. It is also clear that only a transient rise in these components may bring about a relatively long-lasting alteration in lymphocyte function. We have examined the well-known postoperative rise of adrenal cortical steroid levels in the body, as a possible mechanism for the observed phenomenon. The kinetics of this rise and of the loss of migration inhibition suggest that if this is indeed the mechanism, the effect of steroids on lymphocytes must be of rather long duration. The complex mechanism of steroid-mediated influences on the leukocyte-migration test is suggested by Stevenson (21) who showed both direct and indirect effects on cell motility, probably mediated by a lymphocyte–lymphokine type of mechanism.

**SUMMARY**

In a study using the leukocyte-migration test the leukocytes of a majority of melanoma patients were inhibited by contact with autologous or homologous melanoma extracts. Repeat testing of the same patients' leukocytes during the first 4 days after operation showed a complete loss of reactivity in all but one individual. Reactivity returned to preoperative levels in most patients 7–10 days after opera-
tion. The mechanism and significance of this transient loss of cellular immunity are discussed.

ACKNOWLEDGMENTS
The studies here described were supported by funds from The Secretary of State for Scotland and The McMillan Research Funds of The University of Glasgow.

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