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Suppression of the Tuberculin Response in Malignant Disease

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Recently there has been an increasing interest in immunological aspects of cancer. Evidence for the presence of some degree of host control over certain tumours has been accumulating (Black and Speer, 1959), and results of treatment of experimental animal and human tumours by immunological methods raise hopes of useful advances to come (Woodruff and Nolan, 1963). However, less work has been done on the competence of the lymphoreticular system in established cases of human cancer than on seeking cancer-specific antigens and associated antibodies. It has recently been shown that the circulating antibody response to challenge with tetanus toxoid is reduced in cancer patients to an extent not found in patients who are debilitated by advanced non-malignant disease (Lytton *et al.*, 1964). At the same time there is evidence of a delayed hypersensitivity reaction to a cell-free microsomal fraction of autologous cancer tissue in 25% of cancer patients, indicating attempted cellular rejection of the tumour (Hughes and Lytton, 1964). For this reason, in cancer patients cellular immune reactions may warrant more attention than circulating antibody response. We have made an attempt to investigate the competence of the cellular immune mechanism in cancer patients before the onset of general debility by studying the incidence and degree of tuberculin sensitivity in matched cancer and control patients.

Methods

Cancer Patients.—We studied 122 patients with malignant disease admitted to King's College Hospital Group. Lymphoreticular neoplasms were excluded and the primary sites were as follows: breast, 43; colon, 32; lung, 14; stomach, 11; skin melanoma, 4; oesophagus, 3; thyroid, 3; pancreas, 3; parotid, 3; others, 6. Patients with evidence of general debility, such as marked loss of weight, fever, anaemia, and weakness, were excluded, but otherwise there was no selection of cases. Some cases later proved to have widespread disease, but in all instances the general state of the patient was regarded as good. The spread of the tumour was classified into grade A, localized neoplasm; grade B, spread to local lymph nodes, but curative surgery still possible; grade C, spread beyond local lymph nodes. It is realized that such a classification is arbitrary, and of limited usefulness, especially as histology on the lymph nodes was not available in all cases. Twenty patients who had been tested pre-operatively were followed up and retested, when possible, 18 months after surgical removal of their tumour.

Control Patients.—Patients with various non-malignant conditions admitted to the surgical wards of the same hospitals were used as controls. For each cancer patient a control was chosen in the same five-year age group, but no selection was exercised apart from that required to match age. In both cancer and control groups a history of active tuberculosis and radiological evidence of active tuberculosis was sought, but these were found so infrequently in both groups as to be of no significance.

Tuberculin Testing.—Tuberculin sensitivity was measured by means of the multiple puncture test described by Heaf (1951). A single batch of P.P.D. made for Heaf testing was used for all tests. A platinum loop of fluid was placed on the skin, spread over an area of 1 cm. in diameter, and punctured by a Heaf gun calibrated to a depth of 2 mm. Care was taken to avoid using the same site again. The response was read routinely at 72 hours but on occasion at 48 or 96 hours. Experience showed that the results were the same within this period. Responses were graded negative and positive 1–4 as recommended by Heaf (1951) and set out in Table I. Papule formation rather than erythema was used as the criterion of response, and in doubtful cases assessment was made after stretching the skin. The relationship of any surgical operation to skin-testing was recorded to allow assessment of a possible non-specific depressant effect of operation on tuberculin-testing.

TABLE I.—*Grades of Tuberculin Response*

Grade	Response
Negative	No response
Positive 1	Discrete papules at the puncture sites
Positive 2	Coalescence of papules to form a ring
Positive 3	Central area raised
Positive 4	Surrounding erythema and weal formation

Effect of Cortisone.—Since the work of Citron and Scadding (1957) on the effect of cortisone on the depressed tuberculin response seen in sarcoidosis further tests have been carried out with the Mantoux method in some patients who were tuberculin-negative to the Heaf test. 0.05 ml. of old tuberculin containing 50 T.U. was mixed with either 0.05 ml. of saline or 0.05 ml. of cortisone acetate containing 1.25 mg. of cortisone; 0.1 ml. of each mixture was then injected intradermally into the patient, and the reactions were read at 48 hours.

Results

Tuberculin Results in All Patients.—The results of the Heaf test in all the patients studied are given in Table II. They show a depression of delayed hypersensitivity response among the cancer patients, of whom only 52% gave a positive result, compared with 81% positive results among control patients. These results have been analysed by the χ^2 test for linear trend in proportions (Armitage, 1955), and this gives $\chi^2=37.61$, which is highly significant ($P<0.001$).

TABLE II.—*Tuberculin Response in All Patients*

	Total	Negative	Positive				
			1	2	3	4	%
Cancer Patients	122	59	32	19	11	1	52
Control Patients	122	23	25	31	34	9	81

Tuberculin Response in Relation to Disease.—The tuberculin response has been investigated in relation to spread of disease, and the results are given in Table III. Five early cases were

TABLE III.—*Tuberculin Response and Spread of Tumour*

Tumour Group	Total	Negative	Positive				
			1	2	3	4	%
A	33	8	11	10	3	1	76
B	36	19	9	7	1	0	47
C	48	31	9	7	1	0	35

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not classified. There is an increasing degree of immunological depression with spread of the tumour. When cases of groups A and B carcinoma of the breast are compared with their corresponding control patients, it is clear that even in these early cancers, where the tumour mass is small, where no secondary infection is present, and where there is no change in any of the vital functions of the body, there is still depression of the delayed hypersensitivity response (Table IV).

TABLE IV.—*Tuberculin Response in Early Breast Cancer*

	Total	Negative	Positive				
			1	2	3	4	%
Cancer Patients	27	10	10	7	0	0	63
Control Patients	27	1	4	7	11	4	96

Tuberculin Response and Operation.—The possibility that surgery might have a non-specific effect on the tuberculin reaction was considered. In a group of 10 patients who had tests before and after operation only minor differences were obtained (Table V). When the overall results are analysed according to the relation of testing to operation, they are essentially the same for those tested pre-operatively and post-operatively in both the cancer and the control group, apart from a slight depression in the post-operative controls (Table VI). Later all tests were performed before operation was carried out. The finding of a lower incidence of positive reactions in the post-operative control group is not significant, because of the small number of patients studied.

TABLE V.—*Tuberculin Response and Operation*

Test	Total	Negative	Positive			
			1	2	3	4
Pre-operative	10	0	2	6	2	0
Post-operative	10	2	1	5	1	1

TABLE VI.—*Tuberculin Response and Operation*

Patients	Total	Negative	Positive				
			1	2	3	4	%
Pre-operative Cancer ..	70	33	15	13	8	1	54
Post-operative Cancer ..	52	26	17	6	3	0	50
Pre-operative Control ..	93	12	17	28	27	9	87
Post-operative Control ..	29	11	8	3	7	0	62

Effect of Cortisone on Negative Heaf Response.—The effect of local cortisone on the tuberculin reaction is shown in Table VII. When a positive response was present with 50 T.U.+ saline, the reaction with tuberculin + cortisone was always smaller. Thus the effect of cortisone was to depress the tuberculin reaction under the conditions of this experiment.

TABLE VII.—*Effect of Cortisone on Tuberculin (Mantoux) Reaction in Cancer Patients*

Case No.	Skin Response	
	Tuberculin + Saline	Tuberculin + Cortisone
59	15 mm.	8 mm.
75	Negative	Negative
79	15 mm.	7 mm.
91	6 mm.	Negative

Follow-up of Patients Tested Before Operation.—Of the first 72 patients tested only 20 were tested before operation. The remaining pre-operative cases have all been tested in the last 12 months. The initial response in the 20 pre-operative patients tested one year ago is given in Table VIII. Cases tested post-operatively have not been considered in this part of the study because of the possibility of a non-specific depressing effect of the operation on the Heaf response. These 20 patients have been followed up and retested where possible: 11 died in 18 months; nine of them had been tuberculin-negative. Of the nine survivors five had been initially Heaf-positive grades 2–3, and of these only one had had a recurrence in 18 months. Two others were unchanged—a negative and a positive grade 1 response. The histories of the remaining two patients are summarized below: these show a return of strong tuberculin reactivity after removal of the tumour.

TABLE VIII.—*Pre-operative Tuberculin Response in Patients Followed Up*

Total Patients	Negative	Positive			
		1	2	3	4
20	11	4	3	2	0

Case 1.—Man aged 52. 1951: Pulmonary tuberculosis. 1962: Carcinoma of bronchus; pre-operative Heaf test negative; pneumonectomy. 1964: Clinically well; no evidence of recurrence; chest *x*-ray, no recurrence; Heaf test positive grade 3.

Case 2.—Man aged 47. 1962: Carcinoma of bronchus; pre-operative Heaf test positive grade 1; pneumonectomy. 1964: Clinically well; no clinical or *x*-ray evidence of recurrence; Heaf test positive grade 3–4.

Discussion

Ideally, the ability of the patient to respond to an antigenic stimulus which has not been previously encountered should be examined. However, such stimuli that produce delayed hypersensitivity are less readily available than those producing circulating antibodies. The classical example of induction of delayed hypersensitivity is B.C.G. vaccination, but most patients of the cancer age group have encountered mycobacterial antigens through natural infection. Good *et al.* (1962) have used allergenic chemicals to induce a skin sensitivity which closely resembles the classical tuberculin reaction, but we have hesitated to use these chemicals in normal control patients. We have had little success in inducing delayed hypersensitivity reactions in patients by using diphtheria toxoid-antitoxin floccules (Uhr *et al.*, 1957). We have therefore chosen a less direct method of studying delayed hypersensitivity in malignancy, and investigated depression of a pre-existing immunity. By utilizing adequate control groups the expected incidence of tuberculin sensitivity in the cancer and control groups should be the same; any difference in reactivity found in cancer patients should be related to the development of the cancer.

The tuberculin response may be affected by many factors (Pepys, 1963). Patients show a decline in tuberculin sensitivity with advancing age, with regard to both the incidence and the insensitivity of the reaction (Johnston *et al.*, 1963). Even hypnosis may influence the macroscopic response (Black *et al.*, 1963). We have been careful to avoid any known fallacies, and the Heaf test was chosen because of its simplicity and ready standardization of technique. A single standard test was performed in all cases, and no attempt was made to determine an absolute state of reactivity by using larger doses of antigen, such as the Mantoux test with 100 T.U.

The depression of tuberculin response in Hodgkin's disease, both early and late, is well recognized (Good *et al.*, 1962). Several workers (Parker *et al.*, 1932; Logan, 1953; Graham and Graham, 1964) have recorded the incidence of tuberculin sensitivity in patients with malignant disease. These authors agree that there is no change in the incidence of positive reactions in early cancer, but patients with late disease show progressive depression which is attributed to the accompanying debility rather than to the neoplasm itself. Fairley and Matthias (1960), using 10 T.U. and 100 T.U. tuberculin, showed a decrease in tuberculin sensitivity in 43 cancer patients, but the spread of the tumour is not discussed. Lamb *et al.* (1962)

found little difference between the incidence of anergy in a group of normal patients and in a group of cancer patients in good health when tested against seven different antigens. There was a marked increase in anergy in a group of debilitated cancer patients. Extremely stringent criteria of anergy were required, and it is unlikely that small differences in reactivity would have been demonstrated.

Our findings in the cases with early breast cancer show an undoubted depression of response in the early stage of the disease long before general debility has occurred. This change in early malignancy differs from the results found with humoral antibody production in cancer patients where the depression of activity did not occur until late in the disease (Lytton *et al.*, 1964). The depressed cellular immunity has been demonstrated only for tuberculin, but it would seem likely that this represents a general change in reactivity rather than specific anergy to a single antigen. It is extremely interesting that in two cases the tuberculin response has become strongly positive after what would appear to have been curative surgery for lung cancer. Cortisone is seen to have no enhancing effect on the tuberculin response in malignant patients. This differs from the findings in sarcoidosis, where anergy is often decreased when tested with cortisone and tuberculin intradermally (Citron and Scadding, 1957).

The nature of the depression of the cellular immune mechanism is uncertain. Although other workers have shown that the tuberculin reaction is depressed in critical illness (Furcolow *et al.*, 1948) and in starvation (Seeberg, 1951), it does not follow that the depression seen in cancer patients is entirely due to such secondary complications.

Vandeputte *et al.* (1963) and Miller *et al.* (1964) have shown in experimental animals that tumours induced by carcinogens and by the polyoma virus are more common in neonatally thymectomized mice than in normal control mice. It has been suggested that human cancer may occur as a result of immunological unresponsiveness. Our work suggests that this is not so in human cancer, as there is an increasing depression of tuberculin response with spread of the tumour, and, secondly, removal of the tumour may be followed by a return of immunological reactivity. These findings also are against the hypothesis that the carcinogen which induced the cancer may also have depressed the antibody-producing mechanism, as may occur in mice (Berenbaum, 1964).

The tumour may depress the lymphoreticular system either directly or by alterations in the hormone balance. We believe that, though the effects of starvation and debility may be seen late in the disease, the early depression is due to a specific immunological depression. This may be caused by the uptake by the tumour of some essential metabolite of the lymphoid cells, or the tumour may produce a substance which depresses the lymphoreticular system. Graham and Graham (1964) have demonstrated a "tolerance agent" in certain tumours which prolongs the rejection time of mouse-skin homografts.

It is seen that as the cancer becomes more widespread, so the depression of immune responses increases. In addition to the effects of debility this may be due to the liberation of increasing amounts of a tolerance agent from the tumour or to bacteria or viruses finding an ecological niche in the tumour (Kampschmidt and Schultz, 1963).

Finally, the depression of the immune mechanism may be due to antigenic competition. The patient may be responding so well to a cancer-specific antigen in the tumour that he is unable to respond adequately to further exogenous stimulation.

Whatever the mechanism underlying these findings of a depressed cellular immune reaction, a greater understanding of the relationship between malignancy and the host-immune response will facilitate possible immunotherapy of human tumours, which may depend on an efficient antibody response by the cancer patient.

Summary

A study of tuberculin response in cancer patients and controls has shown a marked depression of response in cancer patients.

This is present in some early cancers, but increases with spread of the tumour.

This depression of the immune mechanism may be reversed in certain cases by successful curative surgery.

The importance of these findings is discussed.

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