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Pre-Treatment General Immune Competence and Prognosis in Breast Cancer. A Prospective 2-Year Follow-Up.

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Abstract—*A prospective study of pre-treatment general immune competence in breast cancer patients has been instituted to determine its relationship to recurrence and its clinical value in predicting prognosis. A 2 yr follow-up has been completed on 198 patients.*

The tests of immune competence, all completed before treatment was commenced, were total WBC, absolute and percentage lymphocyte counts, serum immunoglobulins (IgG, IgA and IgM), lymphocyte transformation with PHA and delayed cutaneous hypersensitivity responses to tuberculin PPD and dinitrochlorobenzene (DNCB).

None of these pre-treatment tests was predictive for recurrence or prognosis when the effects of stage-related immunosuppression were excluded by analysing the results within each clinical stage. Hence for those patients who developed recurrence within 2 yr, pre-treatment immunological status gave no additional prognostic information over conventional clinico-pathological data.

INTRODUCTION

IT IS now widely recognised that the behaviour of cancer of a particular organ is heterogeneous and unpredictable in the individual patient. Early assessment of prognosis is possible to some degree from clinical and histological features of the tumour and regional lymph nodes, together with additional aids, such as liver and bone scintigraphs. These methods are in regular use to "stage" cancer patients so that appropriate treatment modalities can be employed. However, with the possibilities of effective adjuvant chemotherapy and immunotherapy regimes, there is a pressing need to determine with greater precision the likely prognosis for individual patients within any one stage group. Otherwise unnecessary, toxic and potentially harmful therapy may be given to good prognosis patients, to ensure adequate treatment for those with a bad prognosis.

One of the hopes accompanying the resurgence of tumour immunology has been that a quantitative assessment of host–tumour interaction might help provide such prognostic precision. General immune competence has been extensively studied in cancer patients as one determinant of this equation, but assessment of its practical clinical significance is dependent on such factors as homogeneous patient groups, use of a spectrum of immunological tests and a prospective, pretreatment study with prolonged follow up. To determine its clinical value we have instituted a prospective study of immune competence in breast cancer patients. Multiple tests have been employed in an effort to improve predictive value, and all tests have been performed before treatment and at regular intervals afterwards, to avoid confusion between tumour related effect and effects of treatment.

We have previously shown [1] that while pre-treatment immune competence is generally related to clinical stage, this is only significant in the case of the DNCB response and now report the two-year follow-up of the same group of breast cancer patients, with an analysis of the relationship of pre-treatment immune competence to clinical outcome at this time.

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MATERIALS AND METHODS

1. *Patients*

Consecutive and unselected patients, who were suspected of having breast cancer on clinical grounds, were referred from an outpatient clinic for immunological testing. Informed consent was obtained and venepuncture and DNCB sensitisation were then performed. Appropriate investigations, including mammography, chest X-ray, skeletal radiology and bone scans were performed, prior to admission 12–21 days later. DNCB and Mantoux challenge were then carried out and read before definitive treatment.

Patients who had previous malignancy at any site were excluded. One hundred and ninety-eight female patients were later confirmed to have adeno-carcinoma of the breast, on reliable histological criteria. Individual tests were lost in a small number of patients for a variety of technical reasons. The PHA results are reported in only the last 73 patients, because the technique used earlier was not considered sufficiently accurate and reproducible.

All patients remaining alive have been followed for 2 yr from the date of primary treatment, at intervals of 3 months or less with special reference to the development of local or distant recurrent disease, or death attributable to malignant disease. Recurrence in stage IV patients refers to clinical reactivation of disease following initial systemic therapy. The distribution of patients according to stage of disease and outcome at 2 yr is shown in the accompanying Tables 1–4.

2. *Tumour staging*

Patients were allocated into one of 4 stage groups, after investigation and surgery, based on the TNM classification, namely:

Stage I	$T_{1-2}\ N_{0-1a}\ M_0$
Stage II	$T_{1-2}\ N_{1b}\ M_0$
Stage III	$T_{3-4}\ N_{0-2}\ M_0$
Stage IV	$T_{1-4}\ N_{0-3}\ M_1$

Patients with tumours of less than 5 cm diameter were allocated to stage III if their nodal status was N_2 and patients whose nodal status was N_3 were allocated to stage IV.

Patients with surgically amenable disease were treated primarily by simple or radical mastectomy. Histological assessment of lymph node involvement was obtained in all patients undergoing radical mastectomy and where possible, in those undergoing simple mastectomy by biopsy of a low axillary node.

3. *Immunological tests*

(a) *Total and differential WBC.* An automatic counter recorded total WBC. Absolute and relative lymphocyte counts were calculated from the differential WBC after examination of a Romanowsky-stained film.

(b) *Serum immunoglobulin levels.* IgG, IgA and IgM levels were measured by the radial immunodiffusion technique of Mancini *et al.* [2] using Behringwerke Ag Tri-Partigen plates, allowing the diffusion to go to completion. Comparability of results throughout the study was ensured by testing, with each set of determinations, a portion of a pool of normal serum stored at −20°C. The results for the pool did not vary significantly.

(c) *Lymphocyte response to PHA.* For the last 73 patients, lymphocyte transformation in medium containing foetal calf serum using a microtest technique with 3 concentrations of PHA was adopted [3]. The test was performed in triplicate for each concentration of PHA (0.3, 0.8 and 4.0 μg/ml) and the counts from the unstimulated cultures were subtracted from the test counts. Results were expressed as mean disintegrations per minute after uptake of tritiated thymidine.

(d) *Mantoux test.* One tenth of a millilitre of 1:1000 purified protein derivative (PPD) equivalent to 10 units of old tuberculin, was injected intradermally unless there was a history of tuberculous infection or close contact with the disease, when 1:10,000 PPD was used. The diameter of induration was measured at 72 hr and the responses were graded as follows:

Negative	Grade 0	0– 4 mm.
Positive	Grade 1	5–10 mm.
	Grade 2	11–20 mm.
	Grade 3	>20 mm.

When there was a positive response to 1:10,000 PPD, the response was regarded as one grade higher than an equivalent response to 1:1000 PPD.

(e) *DNCB response.* The DNCB sensitisation and challenge tests were performed essentially as described by Aisenberg [4] but using a lower sensitising dose. In brief, 2000 μg DNCB in 0.1 ml acetone was applied within a plastic ring of 2.2 cm diameter to the anterior aspect of the upper arm. At the same time 100 μg DNCB in 0.1 ml acetone was applied to the

volar surface of the forearm to detect prior sensitisation. These sites were allowed to dry and occluded by dressing for 48 hr. No patient developed an early delayed hypersensitivity reaction to the 100 μg dose. However, a considerable number of patients exhibited a delayed hypersensitivity response at this site 11–14 days later, indicating reaction to residual DNCB bound to epidermal protein. Previous experience had shown that excessive local response occurred in such patients if the standard challenge doses were also applied. Therefore, these patients were regarded as strong positive responders and were not subjected to challenge doses. Those who did not show this reaction to the initial 100 μg were challenged with doses of 50 and 100 μg DNCB 12–14 days after sensitisation. Responses were read at 72 hr, a positive response comprising erythema and induration at the test site. The responses were graded as follows:

Negative	Grade 0	negative to both 50 and 100 μg.
Positive	Grade 1	negative to 50 μg but positive to 100 μg.
	Grade 2	positive to both 50 and 100 μg.
	Grade 3	positive to the initial 100 μg applied at the same time as the sensitising dose.

4. *Statistical methods*

Patients were considered in each of four categories according to disease outcome 2 yr following presentation, i.e. no recurrence, local recurrence only, distant recurrence and death due to cancer. Additional analyses (survivors versus those dying of breast cancer, and those with recurrent disease versus those free of disease) were also performed.

Quantitative variables were analysed by standard methods including the *t*-test. Mean values in each recurrence category were compared using the standard deviation of the total number of patients in the group. A correction factor [5] was applied to adjust for the differing proportions of each stage group within each recurrence category.

The total WBC and absolute and percentage lymphocyte counts were subjected to logarithmic transformation prior to analysis in order to preserve the purely multiplicative relationship between these variables. In the case of the PHA responses a log transformation was again used and the results were expressed in the corresponding form as this variable was extremely skew and had a large coefficient of variation. The DNCB and Mantoux skin tests were analysed in two ways—simply as positive or negative by the use of the chi-square test and also according to grades of response after computing mean scores and standard deviations.

RESULTS

For each individual test the results are represented in Tables 1–4 as mean values in each patient group; standard deviations are given for the totals only. The results of lymphocyte responses to PHA are shown only at one concentration (0.3 μg/ml) as the results with the other two concentrations were qualitatively similar.

In the 198 breast cancer patients studied, when the effects of stage-related immunodepression were excluded by analysing results as described, there was no significant correlation (i.e., at the 5% level) between the results of any pretreatment test and disease outcome.

DISCUSSION

The relationship of general immune competence to cancer behaviour has been the subject of much work and a very large number of papers over the last 30 yr. Results of early studies using a variety of recall antigens and non-specific "host resistance" factors can best be described as confusing. Although there has been general agreement in more recent papers that immune competence tends to diminish in patients with advanced disease [6, 7], it is difficult to differentiate the direct tumour-related effects from those of general ill health.

Some correlation between immune competence and malignant disease behaviour has also been demonstrated [8–14] but in general these studies have involved tests carried out at varying times in the course of the disease in heterogeneous groups of patients with a variety of solid tumour types. More recently, the importance of a number of aspects of quality control has been appreciated including the use of sufficiently large groups to allow meaningful analysis, the use of homogeneous tumour populations [15] and consideration of the effects of age, ill health [16] and primary treatment. None of the very large number of studies in this field, with the exception of this present one, has conformed to these require-

Table 1. Total white cell count and lymphocyte percentage

	WBC × 10^3/mm^3					Lymphocytes, %				
Stage group	I	II	III	IV	Total	I	II	III	IV	Total
No recurrence	(53) 6.35	(24) 7.03	(24) 7.22	(6) 7.08	(107) 6.74 ±1.97	(50) 30.3	(23) 30.1	(22) 25.5	(6) 21.5	(101) 28.7 ±11.4
Local recurrence only	(5) 7.06	(3) 8.07	(5) 5.86	(2) 8.75	(15) 7.09 ±1.85	(4) 34.0	(3) 24.7	(5) 29.2	(2) 34.0	(14) 30.3 ± 8.9
Distant recurrence	(4) 7.13	(7) 6.54	(12) 7.37	(6) 5.84	(29) 6.85 ±2.87	(4) 33.3	(7) 27.3	(11) 27.6	(6) 30.7	29.0 ± 7.6
Death due to breast cancer	(1) 6.70	(4) 6.80	(18) 7.07	(20) 7.00	(43) 7.00 ±1.69	(1) 39.0	(4) 21.0	(16) 30.1	(19) 23.3	(40) 26.1 ± 9.8
Total	(63) 6.47 ±1.91	(38) 7.00 ±1.93	(59) 7.09 ±1.90	(34) 6.93 ±2.05	(194) 6.84 ±1.94	(59) 30.9 ±12.0	(37) 28.0 ±10.2	(54) 27.6 ± 8.8	(33) 24.9 ± 9.0	(183) 28.3 ±10.4

Patient numbers are shown in brackets.
Mean values are given in each stage group.
Standard deviations are shown for the totals.

Table 2. Serum immunoglobulin levels (mg/100 ml)

	IgG					IgA					IgM				
Stage group	I	II	III	IV	Total	I	II	III	IV	Total	I	II	III	IV	Total
No recurrence	(55) 1291	(24) 1174	(19) 1234	(5) 1216	(103) 1250 ±329	(55) 212	(24) 236	(19) 207	(5) 214	(103) 217 ±121	(54) 180	(24) 142	(19) 144	(5) 190	(102) 165 ±80
Local recurrence only	(5) 1280	(3) 1686	(5) 1314	(2) 1199	(15) 1362 ±329	(5) 204	(3) 190	(5) 182	(2) 297	(15) 206 ±80	(5) 200	(3) 206	(5) 143	(2) 89	(15) 167 ±95
Distant recurrence	(4) 1326	(7) 1519	(11) 1284	(5) 1418	(27) 1376 ±464	(4) 209	(7) 209	(11) 171	(5) 274	(27) 205 ±109	(4) 155	(7) 212	(9) 172	(5) 175	(25) 177 ±86
Death due to breast cancer	(1) 1340	(4) 909	(18) 1182	(21) 1382	(44) 1256 ±663	(1) 172	(4) 217	(18) 323	(21) 229	(44) 265 ±125	(1) 72	(4) 139	(18) 141	(21) 146	(44) 142 ±72
Total	(65) 1293 ±339	(38) 1250 ±434	(53) 1234 ±361	(33) 1351 ±714	(189) 1278 ±448	(65) 211 ±129	(38) 225 ±95	(53) 237 ±124	(33) 238 ±116	(189) 226 ±119	(64) 179 ±85	(38) 159 ±71	(51) 147 ±87	(33) 154 ±69	(186) 161 ±81

Patient numbers are shown in brackets.
Mean values are given in each stage group.
Standard deviations are shown for the totals.

Table 3. Lymphocyte response to PHA (0.3 μg/ml) (DPM expressed as log value)

Stage group	I	II	III	IV	Total
No recurrence	(27) 4.692	(7) 4.330	(8) 4.357	(2) 4.320	(44) 4.556 ±0.742
Recurrence	(4) 5.365	(9) 4.897	(9) 4.321	(7) 3.789	(29) 4.515 ±0.890
Total	(31) 4.779 ±0.734	(16) 4.649 ±0.927	(17) 4.336 ±0.650	(9) 3.907 ±0.590	(73) 4.540 ±0.798

Patient numbers are shown in brackets.
Mean values are given in each stage group.
Standard deviations are shown for the totals.

ments. Hence it is disappointing that the present study has shown no relationship between immune competence and prognosis over and above that which could be predicted from standard clinicopathological staging.

Other workers have reported similar results to our own in patients with operable breast cancer [17, 18] and the study closest to ours is that of Stein [19] and his co-workers who investigated a similar number of early breast cancer patients using multiple tests of cell-mediated immunity, including lymphocyte count, lymphocyte response to PHA and cutaneous response to PPD and DNCB. However, instead of testing patients before operation, they carried out their tests 4 weeks after mastectomy. Their findings were in agreement with ours in that they found no relationship between the postoperative immunological status and the subsequent course of disease in patients with operable breast cancer followed for 2 yr.

What then can we conclude about the relationship of immune competence to breast cancer behaviour? There seems little doubt that the bulk of the evidence, especially that deriving from careful studies, has shown no relationship between immune competence and cancer behaviour, except in patients with terminal disease. Yet the question of the relevance of general immune competence in cancer patients cannot be dismissed completely and two aspects of the question deserve attention. Firstly, there is still a considerable amount of indirect evidence which would suggest that at least some tumours are under partial immunological control by their host. All that can be said at the present time is that this control does not seem to be reflected by the well-defined and widely accepted parameters of cellular immunity discussed in this paper. However, there may be other parameters of host resistance which are as yet undefined, and further advances are more likely to come from studies in basic immunology than through continuing investigations of the clinical tumour–host interaction.

Secondly, it must be emphasised that no really long term studies in this field have yet been presented. Whilst we have shown no relationship between immune competence and recurrence of breast cancer within a period of 2 yr, it must be accepted that those patients with early breast cancer who recur within this period of time constitute a subgroup of patients with aggressive tumours, and perhaps these tumours are least likely to be affected by a rather weak host resistance. The host–tumour equation may be much more evenly balanced in those patients whose disease recurs after this time, and it is therefore important that patients with early breast cancer continue to be studied to determine this relationship in the large group of patients who will recur between 2 and 10 yr. This will be the subject of continuing study and a further report.

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Table 4. Skin tests

	DNCB					Mantoux				
Stage group	I	II	III	IV	Total	I	II	III	IV	Total
No recurrence	(42/50) 2.02	(20/24) 1.92	(22/24) 2.09	(4/6) 1.17	(88/104) 1.96 ±1.07	(30/53) 1.07	(14/25) 0.87	(14/23) 1.13	(1/6) 0.17	(59/107) 0.99 ±1.06
Local recurrence only	(5/5) 2.60	(3/3) 2.33	(5/5) 2.40	(2/2) 3.00	(15/15) 2.53 ±0.64	(4/5) 1.20	(2/3) 1.33	(4/5) 1.60	(2/2) 1.50	(12/15) 1.40 ±0.99
Distant recurrence	(3/4) 2.00	(5/6) 2.00	(11/12) 1.92	(4/6) 1.50	(23/28) 1.86 ±1.08	(1/4) 0.25	(5/7) 1.29	(7/12) 1.17	(5/6) 2.00	(18/29) 1.24 ±1.15
Death due to disease	(1/1) 3.00	(2/4) 1.25	(17/18) 2.22	(12/21) 1.10	(32/44) 1.68 ±1.23	(1/1) 2.00	(3/4) 1.75	(13/20) 1.31	(5/19) 0.53	(22/44) 1.02 ±1.18
Total	(51/60) 2.08 ±1.08	(30/37) 1.89 ±1.10	(55/59) 2.17 ±0.89	(22/35) 1.29 ±1.23	(158/191) 1.93 ±1.10	(36/63) 1.05 ±1.08	(24/39) 1.08 ±1.08	(38/60) 1.24 ±1.10	(13/33) 0.79 ±1.14	(111/195) 1.07 ±1.10

The proportion of patients showing a positive response to the antigen is indicated in brackets.
Mean grades of response are given in each stage group.
Standard deviations are shown for the totals.

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