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Macrophage infiltration of breast tumours: a prospective study

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SUMMARY In 50 cases of infiltrating breast cancer investigated in a prospective study the number of macrophages within each tumour was assessed. The macrophages were identified by their cytoplasmic acid phosphatase activity. The number of lymphocytes and plasma cells within the tumours were graded by a scoring technique. Significantly fewer cases with metastases were found among those with high macrophage and plasma cell scores. There was no correlation between lymphoreticular infiltration and the degree of tumour differentiation, but in cases without metastases the lymphoreticular infiltration between tumour cells was nearly always only slight when the macrophage score was low.

Mononuclear cells within human tumours in various sites have been noted by many authors (Underwood, 1974). Attempts have been made to correlate the presence of such infiltrates with improved prognosis. In breast carcinoma a positive prognostic association with the infiltrate has been reported in most studies (Black *et al.*, 1955; Berg, 1959; Hamlin, 1968), although in some series no such association was apparent (White, 1927; Champion *et al.*, 1972). In one particularly interesting multicentre study Morrison *et al.* (1973) reported a negative association from Boston and Tokyo and a positive association from Glamorgan in Wales. Most of the studies have been retrospective and lymphocyte and plasma cells have usually received the attention, although sometimes the less committal term 'round cell' has been preferred (Greenough, 1925; Hultborn and Törnberg, 1960; Champion *et al.*, 1972).

The macrophage has been neglected despite the fact that it can be demonstrated in large numbers in experimental animal tumours (Evans, 1972; Eccles and Alexander, 1974a). Eccles and Alexander (1974b) found the incidence of metastases reduced in animal tumours heavily infiltrated by macrophages. Monis and Weinberg (1961) demonstrated stromal macrophages in human tumours by a cytochemical method, and, more recently, Underwood and Carr (1972) have used the electron microscope. Neither tried to make any correlation with prognosis. In 1973 we began a prospective study of breast carcinoma cases in which we identified macrophages by a histochemical technique. This is a preliminary report on the cases studied in the two-year period 1973-4.

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Patients and methods

Fifty consecutive cases of infiltrating breast carcinoma (mean age of patients 56·2 years) were studied until the end of 1974. All were diagnosed by the frozen section method using cryostat sections of freshly removed tumour tissue. In each case the diagnosis was later confirmed with paraffin-embedded material. There were no cases of circumscribed carcinoma. Nine patients (mean age 35·2 years) with benign breast lesions (1 fibroadenoma, 8 benign mammary dysplasia) were also studied as controls. All the cancer cases were treated by simple mastectomy without radiotherapy. Biopsy at operation was done on only clinically suspicious lymph nodes.

Stromal macrophages were identified by their intracytoplasmic acid phosphatase activity. Blocks were taken from the periphery of the tumour and others were taken at random from its centre. The number and size of the blocks were proportional to the amount of tissue available but the maximum size was 19 mm and up to 2 mm in thickness. The tissue was fixed in methanol free formalin buffered in Millonig's buffer pH 7·2-7·4 (Pease, 1962) at 4°C for 18-21 hours. The tissue was then washed several times in cold 0·2 M sucrose in Millonig's buffer pH 7·2 for

24 h. Sections were then cut on a Dittes cryostat at 10μm and mounted on Chrom alum-gelatin coated slides. Acid phosphatase activity was measured by the method of Burstone (1958) using naphthol AS-TR as substrate and fast red violet LB as the diazonium salt.

After the sections had been cut the remaining tissue was fixed in formalin and after routine processing 5-μm sections were stained with haematoxylin and eosin and methyl-green pyronin. Additional sections were prepared from tissue not subjected to cryostat sectioning. The sections were examined by one of us (IL) in complete isolation from any details of the patient's subsequent progress. The number of acid phosphatase-positive stromal macrophages and of infiltrating lymphocytes present were scored on a four-point scale, as follows: (1) none, (2) few, (3) moderate number, (4) many.

The number of plasma cells were scored on a three-point scale: (1) none, (2) moderate number, (3) many.

As well as differences in the numbers of mononuclear cells we noticed, as a separate phenomenon, that the distribution pattern of these cells varied from case to case. In some the infiltrate was arranged round small blood vessels or as groups of cells round the periphery of the tumour, *external* to it. In other cases the infiltrate was distributed *internally*, investing and insinuating among the tumour cells. It seemed reasonable to suppose that the latter pattern might be more effective than the former. We therefore graded the degree of intercellular mononuclear cell distribution on a four-point scale from 1 in which it was minimal up to 4 in which it was pronounced.

Also of interest was whether the degree of tumour differentiation might affect the extent of the various cellular infiltrates. All the tumours were therefore graded into three grades according to the 1968 WHO scheme—grade 1, low malignancy; grade 2, intermediate malignancy; and grade 3, high malignancy (Scarff and Torloni, 1968).

All of the scoring procedures except for the tumour differentiation were undertaken on three separate occasions without knowing the previous results. Finally, the mean of the three scores was calculated. When the assessment was completed the patient's case history was consulted and the following noted: age at diagnosis; alive or dead; survival in months; histologically proved involvement of axillary lymph nodes; other evidence of metastases (necropsy (3), liver biopsy (2), radiological, and cytology of effusions). There were 13 cases of lymph node metastasis, eight of which exhibited other evidence of metastasis. There were four cases of metastasis without proved lymph node involvement. The data were transferred on to punched cards and analysed using a SPSS (statistical package for the social sciences) program on the university's IBM 370 computer.

Results

Before analysis the cases were allocated into high- and low-score groups for macrophages, lymphocytes, and plasma cells, as shown in Tables 1 and 2. For macrophage and lymphocyte grading 'low' was defined as a mean score of 1·00-2·49 and 'high' as 2·50-4·00. For plasma cells 'low' was defined as a mean score of 1·00-1·99 and 'high' as 2·00-3·00. Fisher's exact test for statistical significance was used for all the tables.

Table 1 *Macrophage and lymphocyte scores in 9 control and 50 cancer patients*

Mean score	*Macrophages*		*Lymphocytes*	
	Cancer patients (No.)	*Controls (No.)*	*Cancer patients (No.)*	*Controls (No.)*
Low: 1·00-2·49	24 (48)	9 (100)	11 (22)	9 (100)
High: 2·50-4·00	26 (52)	0	39 (78)	0
Significance	P = 0·003		P = 0·000013	

Figures in parentheses indicate percentage of vertical column total

Table 2 *Plasma cell scores in 9 control and 50 cancer patients*

Mean score	*Plasma cells*	
	Cancer patients (No.)	*Controls (No.)*
Low: 1·00-1·99	12 (24)	7 (78)
High: 2·00-3·00	38 (76)	2 (22)
Significance	P = 0·003	

Figures in parentheses indicate percentage of vertical column total

In Tables 1 and 2 the cancer patients and the controls are compared. No control patients had high scores for macrophage or lymphocyte while over half the cancer patients had high scores for both. The differences in both cases were statistically highly significant. The difference in the plasma cell scores was also significant although here two of the control patients had high scores (Table 2).

The cases of breast cancer were allocated to two groups—namely, those with metastases and those without. Tables 3 and 4 show that there were more low macrophage and plasma cell scores among those with metastases then among those without metastases. Among the latter there were more cases with high scores. The lymphocyte scores showed a similar distribution in both groups but there was no statistically significant difference.

Table 3 *Macrophage and lymphocyte scores in breast cancer cases with and without metastases*

Mean score	*Macrophages*		*Lymphocytes*	
	Metastases present (No.)	*Metastases absent (No.)*	*Metastases present (No.)*	*Metastases absent (No.)*
Low: 1·00-2·49	12 (24)	12 (24)	4 (8)	7 (14)
High: 2·50-4·00	5 (10)	21 (42)	13 (26)	26 (52)
Significance	P = 0·018		P = 0·272 NS	

Figures in parentheses indicate percentage of total number of patients within that group

Table 4 *Plasma cell scores in breast cancer cases with and without metastases*

Mean score	*Plasma cells*	
	Metastases present (No.)	*Metastases absent (No.)*
Low: 1·00-1·99	7 (14)	5 (10)
High: 2·00-3·00	10 (20)	28 (56)
Significance	P = 0·038	

Figures in parentheses indicate percentage of total number of patients

Table 5 shows the distribution of cases with either high or low scores for macrophage, lymphocyte, and plasma cell infiltration, allocated to two grades of tumour differentiation in cases without metastases. WHO grades 2 and 3 were compounded because of the small number of cases. No statistically significant differences were detected although there is a suggestion that macrophages and plasma cells may be more frequent in the less well differentiated tumours. A similar negative finding was observed when WHO grades 1 and 2 were compounded and compared with the grade 3 cases. Table 6 shows the situation in patients with metastases. A highly significant result was obtained with the plasma cell scores. The Table shows that there were no well differentiated (grade 1) tumours with low plasma cell scores.

Table 5 *Cell scores in breast cancer patients without metastases grouped according to tumour's WHO histology grade*

WHO histology	*Macrophages*		*Lymphocytes*		*Plasma cells*	
	Low (No.)	*High (No.)*	*Low (No.)*	*High (No.)*	*Low (No.)*	*High (No.)*
Grade 1	6 (18)	6 (18)	2 (6)	5 (15)	3 (9)	2 (6)
Grades 2 and 3	6 (18)	15 (46)	10 (30)	16 (49)	9 (27)	19 (58)
Significance	P = 0·141 NS		P = 0·314 NS		P = 0·195 NS	

Figures in parentheses indicate percentage of total number of patients within each 'cell box'

Table 6 *Cell scores in breast cancer patients with metastases grouped according to tumour's WHO histology grade*

WHO histology	*Macrophages*		*Lymphocytes*		*Plasma cells*	
	Low (No.)	*High (No.)*	*Low (No.)*	*High (No.)*	*Low (No.)*	*High (No.)*
Grade 1	4 (24)	8 (47)	1 (6)	3 (18)	0	7 (41)
Grades 2 and 3	3 (18)	2 (11)	6 (35)	7 (41)	7 (41)	3 (18)
Significance	P = 0·2555 NS		P = 0·353 NS		P = 0·006	

Figures in parentheses indicate percentage of total number of patients within each 'cell block'

The degree of intercellular infiltration was analysed by defining two groups, the first being a compound of grades 1 and 2 and the second a compound of grades 3 and 4. The results of the macrophage, lymphocyte, and plasma cell scores in patients without and with metastases are shown in Tables 7 and 8 respectively. Table 7 shows significant differences in macrophage and lymphocyte scores but the differences in plasma cell scores are not statistically significant. Significant results in the macrophage and lymphocyte scores are due to the presence of very few cases with low macrophage and lymphocyte scores within cases showing marked intercellular infiltration (grades 3 and 4). The Table indicates a similar situation within the plasma cell scores, but here the overall distribution of the cases is not statistically significant. Table 8 shows

Table 7 *Cell scores in breast cancer patients without metastases grouped according to degree of intercellular lymphoreticular infiltration*

Intercellular infiltration	*Macrophages*		*Lymphocytes*		*Plasma cells*	
	Low (No.)	*High (No.)*	*Low (No.)*	*High (No.)*	*Low (No.)*	*High (No.)*
Grades 1 and 2	11 (33)	9 (27)	7 (22)	13 (39)	5 (15)	15 (46)
Grades 3 and 4	1 (3)	12 (37)	0	13 (39)	0	13 (39)
Significance	P = 0·006		P = 0·018		P = 0·065 NS	

Figures in parentheses indicate percentage of total number of patients within each 'cell box'

Table 8 *Cell scores in breast cancer patients with metastases grouped according to degree of intercellular lymphoreticular infiltration*

Intercellular infiltration	*Macrophages*		*Lymphocytes*		*Plasma cells*	
	Low (No.)	*High (No.)*	*Low (No.)*	*High (No.)*	*Low (No.)*	*High (No.)*
Grades 1 and 2	7 (41)	1 (6)	4 (24)	4 (24)	4 (24)	4 (24)
Grades 2 and 3	5 (29)	4 (24)	0	9 (52)	3 (17)	6(35)
Significance	P = 0·163 NS		P = 0·029		P = 0·302 NS	

Figures in parentheses indicate a percentage of the total number of patients within each 'cell block'

that in patients with metastases the lymphocyte scores are highest in patients with marked intercellular infiltration. The results for macrophages and plasma cells were not statistically significant.

Using the punched card data all possible combinations of the variables, taken two at a time, were computed using the SPSS program for scattergram analysis. The statistically significant results from this analysis are shown in Table 9. All three of the cell types graded—that is, lymphocytes, macrophages, and plasma cells—were statistically interrelated. They were also all significantly related to the degree of intercellular infiltration. The WHO histological grade and the age of the patient were not significantly associated with any of the other variables.

Table 9 *Statistically significant results obtained from SPSS scattergram program*

Vertical axis	*v*	*Horizontal axis*	*Correlation coefficient (R)*	*Significance (P value)*
1 Macrophages	*v*	Lymphocytes	0·3399	0·007
2 Macrophages	*v*	Plasma cells	0·2999	0·01
3 Macrophages	*v*	Intercellular infiltration	0·3368	0·007
4 Lymphocytes	*v*	Plasma cells	0·5035	0·00007
5 Lymphocytes	*v*	Intercellular infiltration	0·7047	0·00001
6 Plasma cells	*v*	Intercellular infiltration	0·3783	0·003

Discussion

Our principal finding was that high macrophage and plasma cell scores seem to be associated with a reduced risk of developing metastases. Evans (1972) found that up to 50% of the cells within certain experimental tumours are macrophages. Subsequently Eccles and Alexander (1974b) showed, in a chemically induced rat fibrosarcoma, that infiltration by macrophages was associated with an effective immune response by the host to the tumour. They demonstrated a correlation between macrophage content and a reduced rate of spontaneous metastasis of six different rat sarcomas grown in normal syngeneic recipients. Several authors have confirmed by electron microscopy that macrophages are present in human tumours. Underwood and Carr (1972) showed that macrophages were present in all the non-lymphoid human malignant tumours that they examined. Some macrophages contained degenerate whole cells while others invested the tumour cells by cytoplasmic processes. The incidence of metastases and the duration of survival were not reported.

The mechanisms by which macrophages reduce the risk of metastasis is not clear. Experimentally it has been shown that macrophages, either by themselves or in association with lymphocytes, are cytotoxic to tumour cells (Evans and Alexander, 1970). A soluble product, possibly of lysosomal origin, has been suggested as the active agent (Currie and Basham, 1975). Evans and Alexander (1972) described a specific macrophage arming factor (SMAF) that is released by sensitised lymphocytes in the presence of antigen. This factor is said to confer specific antitumour cytotoxicity to the macrophages. Cytophilic antibodies, particularly of the IgG type, have also been suggested as a means of rendering macrophages specifically cytotoxic.

Although these cytotoxic mechanisms seem well established experimentally we have been unable consistently to relate the presence of macrophages to foci of tumour cell necrosis in the corresponding areas. A similar observation was made by Underwood and Carr (1972). Perhaps the cytotoxicity is responsible for eliminating bloodborne tumour cells and the presence of large numbers of macrophages within the tumour is simply a reflection of large numbers of similar cells within the blood and other areas of the mononuclear phagocyte system.

Another possible mechanism whereby metastases may be prevented is mechanical interference with the spread of the tumour. The close contact of macrophages and tumour cells and the investment of the latter by macrophage cytoplasmic processes may be enough to prevent or retard the emigration of tumour cells. Such a mechanism would also be consistent with our finding that pronounced intercellular infiltration was associated with high macrophage and lymphocyte scores in cases without metastases. By definition, our pronounced intercellular infiltrates

were those exhibiting the closest contact between lymphoreticular cells and tumour cells.

Active phagocytosis of tumour cells could reduce the risk of immunological tolerance by lowering the amount of soluble antigen released into the circulation. Moreover, phagocytosis and the consequent processing of antigen could lead to an increase in the potential antigenicity of the tumour. The matter is complicated still further by the fact that macrophages themselves may be engulfed by tumour cells in the more aggressive tumours (Goldenberg *et al.*, 1969). If this latter process were widespread a correlation between tumour differentiation and macrophage content might be expected, but we could establish no such correlation.

A direct effect of macrophages on the rate of tumour cell proliferation is another factor that has to be considered. Krahenbuhl and Lambert (1975) showed experimentally that macrophages can inhibit DNA synthesis in tumour target cells. Tumour injection with bradykinin produces both an increase in the number of macrophages and a reduction in tumour growth rate (Krahenbuhl and Lambert, 1975; Koppelmann *et al.*, 1975). This latter study has possible therapeutic implications since it also showed that a similar increased macrophage infiltration occurred in non-injected tumours after an injection of the main tumour mass.

Our results indicate a similar correlation between plasma cell infiltration and a reduced incidence of metastasis. Berg (1959) showed that a plasma cell infiltrate around the periphery of the tumour is associated with a good prognosis. Possibly this is due to local antibody production (Roberts *et al.*, 1973). The exact role of the lymphocyte is more problematical. While most authors have suggested that a lymphocyte infiltrate indicates a more favourable prognosis, Champion *et al.* (1972) were unable to show any such correlation. Indeed, their findings hinted at an inverse correlation. Even more intriguing is the report by Morrison *et al.* (1973) that a positive correlation of lymphoid infiltration and good prognosis was observed in Glamorgan but not in Japan or Boston. We have been unable to show any association between lymphocyte or macrophage score and the degree of tumour differentiation although, curiously, in patients with metastases the poorly differentiated tumours contained fewer plasma cells. Although lymphocytes may not directly affect the frequency of metastasis they almost certainly do so indirectly—for example, by inducing the macrophage infiltrate at the site of the tumour (Eccles and Alexander, 1974b; Lejeune, 1975).

We have not drawn any conclusions about prognosis. That is the subject of another prospective study. As yet only 8 of the 50 patients have died. Although our results have not indicated an association between lymphocyte score and a reduction in the incidence of metastasis an improvement in survival times in the group with high scores is still possible. At least two studies have indicated that pronounced lymphoreticular infiltrates are associated with a good prognosis even when axillary node metastases are present (Black *et al.*, 1955; Hamlin, 1968). At present we cannot predict what the long-term effect of the current reduction in metastasis will be: it could be merely transient with no concomitant increase in survival time. We hope that follow-up and the inclusion of more cases will resolve these problems.

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