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Lymphoreticular infiltrates in invasive ductal breast cancer

A histological and immunohistological study*

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Summary. Fifty-two invasive ductal breast cancers were investigated histologically and immunohistologically to assess localization and composition of the lymphoreticular infiltrates. The tumour-infiltrating cells were mainly located in the intervening stroma, whereas tumour foci often exhibited lower numbers of lymphoreticular cells. Macrophages (Mono 1+ and KiM 6+) and helper/inducer cells bearing the T4 surface antigen (Leu-3a+) regularly constituted the majority of the tumour-infiltrating lymphoreticular cells. In more than 80% of cases large numbers of macrophages were found, and many T4 cells occured in about 60%. Next in frequency were the T lymphocytes (Leu-1+) which were mostly observed in high (46%), or in moderate (39%) numbers. In about 2/3 of the cases moderate numbers of T8 (suppressor/cytotoxic) lymphocytes (Leu-2a+) were detected. B lymphocytes (T0 15+) and natural killer cells (Leu-7+) were generally encountered in very low numbers, while eosinophilic granulocytes were virtually absent from the lymphoreticular infiltrates. Tissue mast cells and plasma cells were present in very low numbers in about one half of the tumours but cases with low, moderate or – rarely – even high numbers of infiltrating cells also occured. It must be emphasized that an in situ histomorphological analysis of the cellular part of the stromal reaction of invasive ductal breast cancers allows only limited conclusions concerning the functional properties of the tumour-infiltrating lymphoreticular cells. From the present study, macrophages and T4 cells but also T8 lymphocytes might be of significance in immunooncological reactions "against" clinically detectable stages of invasive breast cancer.

This work is dedicated to Prof. Dr.Dr.h.c. K. Lennert in honor of his 65. birthday.

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Introduction

Lymphoreticular infiltrates represent an essential part of the stromal reaction to a malignant tumor (Hamperl 1956). According to Holmes (1985), proliferative and cytotoxic activities of tumour-infiltrating cells in patients with lung cancer were markedly reduced suggesting some form of immune paralysis evolving from the microenvironment of the tumour. Converse findings, however, also have been published. Vose (1982) reported on a concentration of reactive, interleukin-2 responding lymphocytes in various malignant tumours (lung, breast, colon) indicating a selective trapping or homing. These tumour-infiltrating lymphocytes showed a predominance of cytotoxic T8 cells and a corresponding reduction in T4 cells. Moreover, in various malignant human tumours a positive correlation has been found between the density of the lymphoreticular infiltrates and the prognosis of the patient. This is true of invasive ductal breast cancer in general (Hamlin 1968; Black et al. 1975), but especially of a distinct histological entity designated medullary breast cancer with lymphoid stroma (Bässler et al. 1981). In the present study, we investigated 52 cases of invasive ductal breast cancer histologically and immunohistologically, to determine localization, size, and composition of the lymphoreticular infiltrates. The detailed evaluation of the tumour-infiltrating cells was performed to gain information on the in situ histomorphology of host-tumour interactions.

Materials and methods

Between February 1983 and July 1985, 52 females (median age: 54 years) with invasive ductal breast cancer entered the study. None of these patients underwent any form of tumour-specific therapy prior to operation.

Diagnosis of cancer was routinely established in frozen sections taken from different parts of the excised tumour. Paraffin section diagnosis in nearly all cases showed typical invasive ductal carcinomas with strands and columns of neoplastic cells and varying amounts of intervening connective tissue. Subsequently, the mastectomy specimen and the axillary lymph nodes were carefully prepared to determine the stage of the disease according to the TNM classification (Table 1).

One part of the tumour was snap-frozen in liquid nitrogen and stored in a refrigerator at −80° C. 6 μm thick serial frozen sections of the tumours were immunostained using the

Table 1. Separation of the breast cancers ($n = 52$) according to the TNM classification

Tumour stage (T)	Number of cases
T1	24
T2	23
T3	1
T4	4

Table 2. Monoclonal antibodies used to determine the composition of the lymphoreticular infiltrates in invasive ductal cancer of the female breast

Monoclonal antibody	Specifity	Source/reference
Leu-1	T lymphocytes	BD
Leu-3a	Helper/inducer cells	BD
Leu-2a	Suppressor/cytotoxic lymphocytes	BD
T0 15	B lymphocytes	Stein et al. (1982)
Leu-7	Natural killer cells	BD
Mono 1	Monocytes, macrophages	BRL
KiM 6	Macrophages, plasmocytoid T cells	Laboratory Radzun, Kiel

BD, Becton Dickinson Heidelberg, FRG; BRL, Bethesda Research Laboratories Eggenstein, FRG

method previously described by Stein et al. (1982). The remaining tumour tissue was fixed in formalin, embedded in paraffin, and the 4 μm thick sections were subject to the following dyes and enzyme-reactions: haematoxylin and eosin, GIEMSA and naphthol AS-D chloroacetate esterase.

Localization (intervening stroma, tumour foci) and frequency distribution of the lymphoreticular infiltrates were evaluated with a panel of monoclonal antibodies (Table 2), GIEMSA stains, and the naphthol AS-D chloroacetate esterase reaction. In each tumour, the number of T lymphocytes, T4 cells, T8 lymphocytes, B lymphocytes, natural killer cells, monocytes/macrophages, plasma cells, tissue mast cells and eosinophils was calculated semiquantitatively using the following four-grade scale: absent/very low – low – moderate – high. Cellular infiltrates in the immediate vicinity of foci of tumour necrosis were neglected. Naphthol AS-D chloroacetate esterase was used precisely to determine the number of mast cells, which were labelled bright red (Leder 1964). In the GIEMSA stain the secondary granules of the eosinophils appeared orange-red, while the cytoplasm of plasma cells exhibited typical basophilia with a pale paranuclear area.

The chi-square test was applied for the statistical analyses.

Results

Regularly, the lymphoreticular cells accumulated preferentially in the intervening stroma, while the tumour cell nests were infiltrated to a considerably lesser extent. The mastopathic lesions in the vicinity of the tumours contained varying amounts of "inflammatory" cells.

In most tumours *monocytes/macrophages* (Mono 1+) were detected in large numbers (Table 3). The macrophages preferentially infiltrated the stroma but were often closely attached to or infiltrating in the tumour foci (Fig. 1). While immunostaining with Mono 1 yielded a high degree of infiltration in nearly 83% of the cases, the macrophage-specific antibody KiM 6 detected comparable cell numbers only in about one half ot the tumours.

Intrastromal *T4 cells* (Leu-3a+) also built up a major component of the lymphoreticular infiltrates and were generally found in high (59.6%) or moderate (25.0%) numbers (Table 3). Like the macrophages, most T4 cells were encountered in the stroma. Immunostaining revealed that a minor

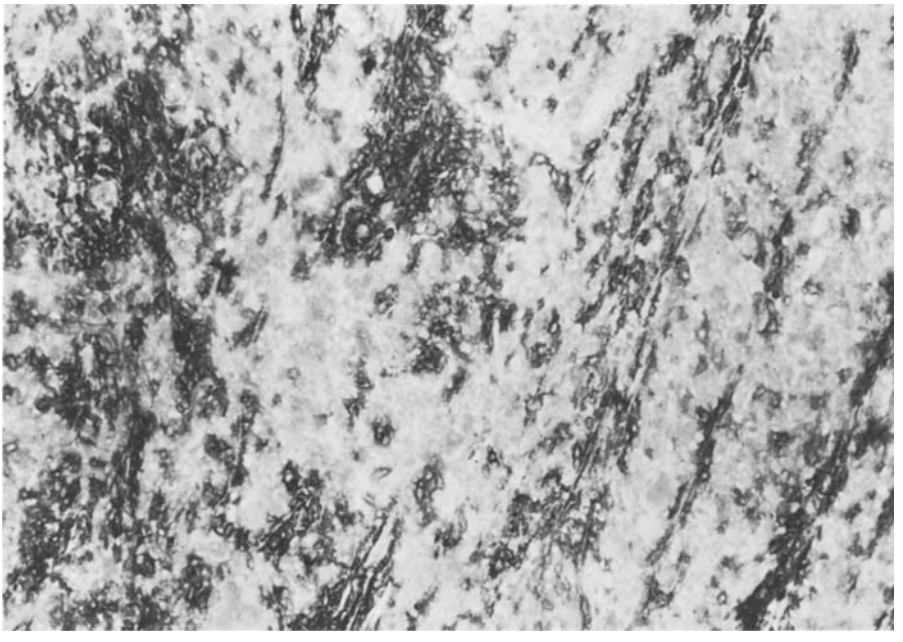

Fig. 1. Invasive ductal breast cancer. Extensive infiltration of the tumour by sheets and strands of monocytes and macrophages. Mono 1, ×140

Table 3. Frequency distribution of lymphoreticular infiltrates in 52 invasive ductal breast cancers. Degree of infiltration: 1, absent/very low; 2, low; 3, moderate; 4, high

	1	2	3	4
	T/S (%)	T/S (%)	T/S (%)	T/S (%)
Monocytes/ macrophages	1.9/1.9	9.6/0.0	46.2/15.4	42.3/82.7
T4 cells	11.5/1.9	23.1/13.5	34.6/25.0	30.8/59.6
T lymphocytes	23.1/5.8	44.2/9.6	25.0/38.5	7.7/46.1
T8 lymphocytes	19.2/7.7	40.4/5.8	32.7/67.3	7.7/19.2
B lymphocytes	84.6/69.2	15.4/26.9	0.0/3.9	0.0/0.0
Plasma cells	100.0/55.8	0.0/23.1	0.0/11.5	0.0/9.6
NK cells	80.8/84.6	17.3/13.5	1.9/1.9	0.0/0.0
Mast cells	100.0/50.0	0.0/28.8	0.0/15.4	0.0/5.8
Eosinophils	100.0/98.1	0.0/0.0	0.0/1.9	0.0/0.0

T, infiltration of tumour foci; S, infiltration of intervening stroma; NK cells, natural killer cells

portion of the positive cells exhibited a fusiform "monocytoid" appearance (Fig. 2).

Intrastromal *T lymphocytes* (Leu-1+) were mainly observed in high (46.1%) or moderate (38.5%) numbers, but cases with a low (9.6%) or very low (5.8%) degree of infiltration also occured (Table 3). The typical

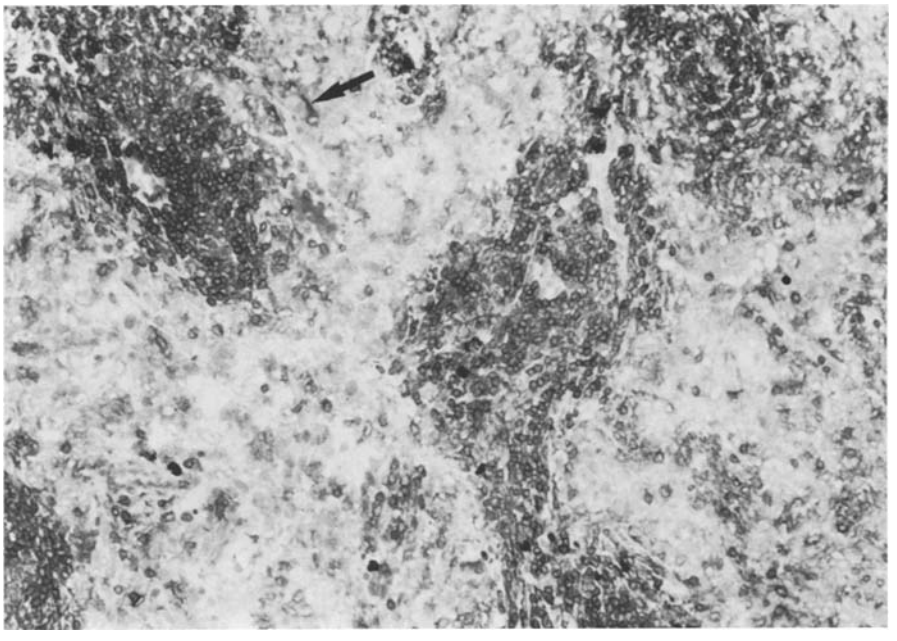

Fig. 2. Invasive ductal breast cancer. Immunohistology reveals extensive infiltration of the tumour by sheets of T4 cells. Most of the stained cells are of lymphocytic appearance, but few spindle-shaped cells (*arrow*) are also stained. Leu-3a, ×140

immunohistologic aspect is shown in Fig. 3. The difference between the amount of T lymphocytes in the stroma and in the tumour foci was more pronounced than it had been seen for macrophages and T4 cells (Fig. 3a, b).

In about two thirds of the cases *T8 lymphocytes* (Leu-2a+) were found in moderate numbers (Fig. 4a, b), but tumours with a high (19.2%), and also very low or low (13.5%) degree of stromal infiltration were not infrequent (Table 3). In about 70% of all tumours the T4 cells outnumbered the T8 lymphocytes considerably, whereas in only 10% were T8 lymphocytes slightly more frequent. One fifth of cases showed almost equal numbers of both cell types.

In contrast to the T lymphocytes and the main subsets of T cells, the *B lymphocytes* (T0 15+) were never seen in comparably large numbers (Fig. 5). In the majority of cases (69.2%) intrastromal B cells could not be detected in the lymphoreticular infiltrates, and in further 26.9% the number was low (Table 3). Rare tumours exhibited a moderate degree of infiltration (3.9%) while high numbers of B lymphocytes never were found in our material (Table 3). Altogether, in more than 90% of the cases the number of T lymphocytes exceeded that of the B cells, while only in one exceptional tumour the latter were more frequent.

Even more infrequent than B lymphocytes were the *natural killer cells* (Leu-7+). 84.6% of the tumours contained virtually no NK cells, and in further 13.5% the degree of intrastromal infiltration was low (Table 3, Fig. 6).

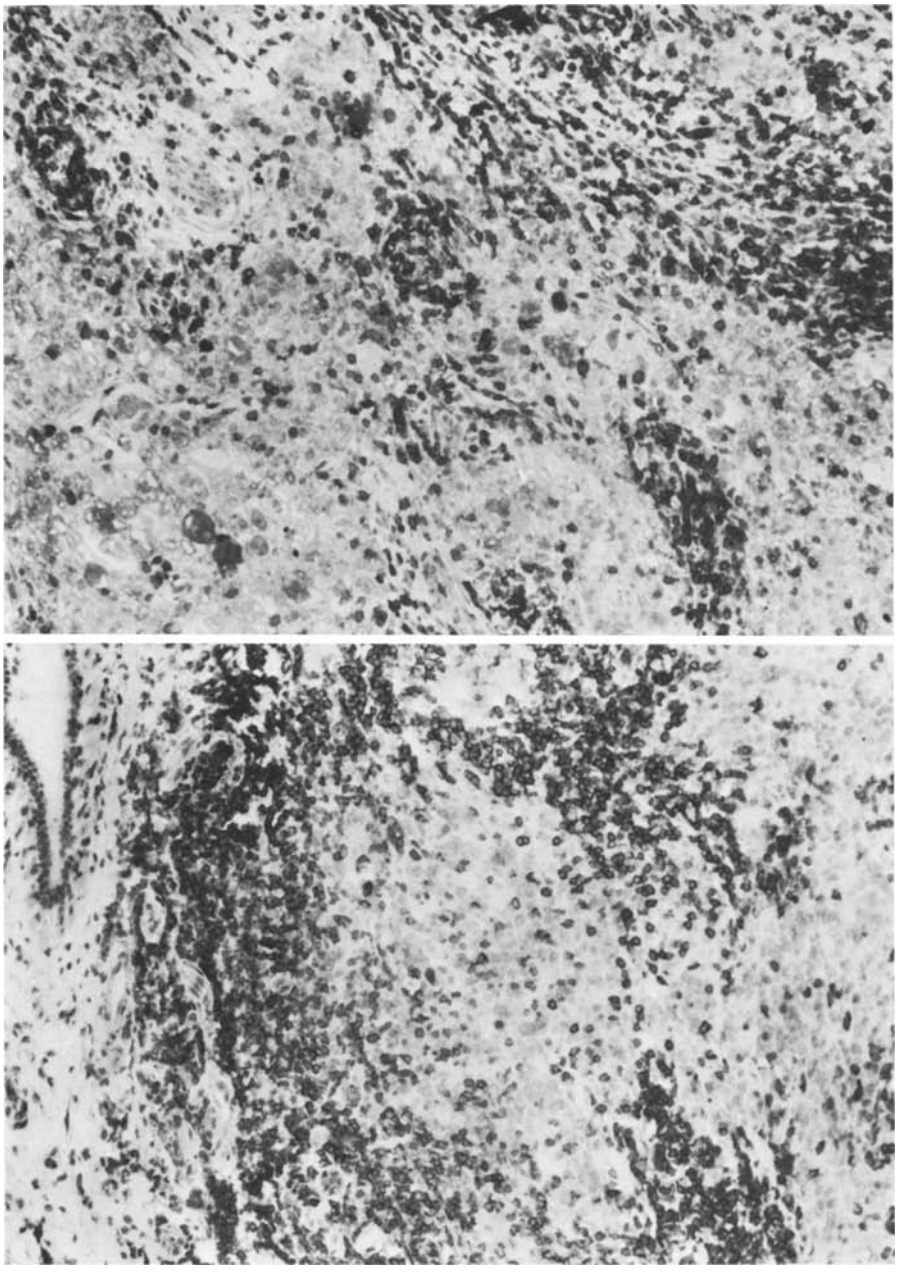

Fig. 3a. Invasive ductal breast cancer. Numerous T lymphocytes mainly accumulating in the stromal bands (*top right*) infiltrate the tumour. Bizarre pleomorphic tumour cells are apparent (*bottom left*). Leu-1, ×140. **b** Invasive ductal breast cancer. Edge of the tumour at *left*. Large clusters of T lymphocytes are located in the marginal zone. Leu-1, ×140

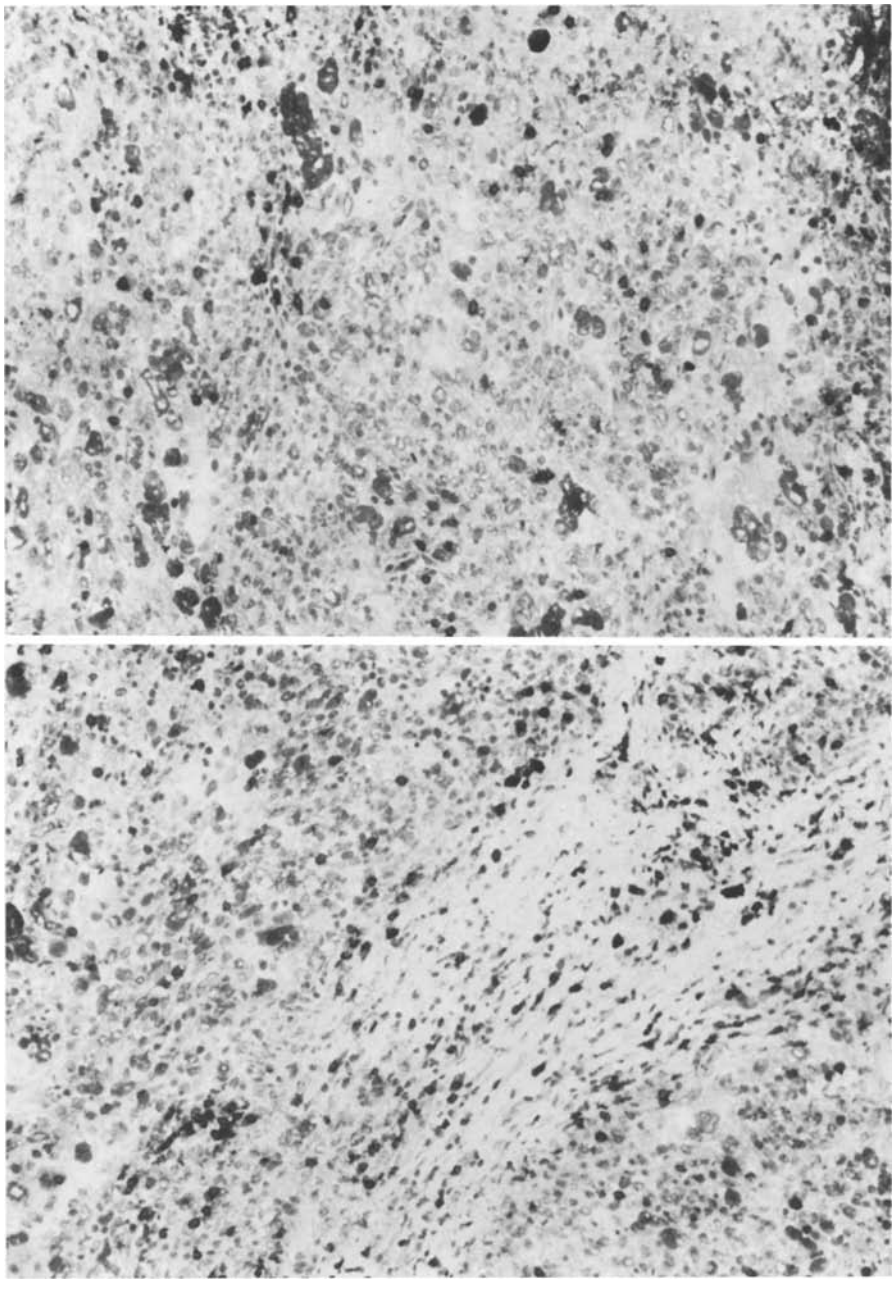

Fig. 4a. Invasive ductal breast cancer. Immunohistology demonstrates a moderate number of T8 lymphocytes between tumour cells which exhibit extremely pleomorphic nuclei. Leu-2a, ×140. **b** Invasive ductal breast cancer. The T8 lymphocytes accumulate predominantly in the broad intervening stromal bands. Leu-2a, ×140

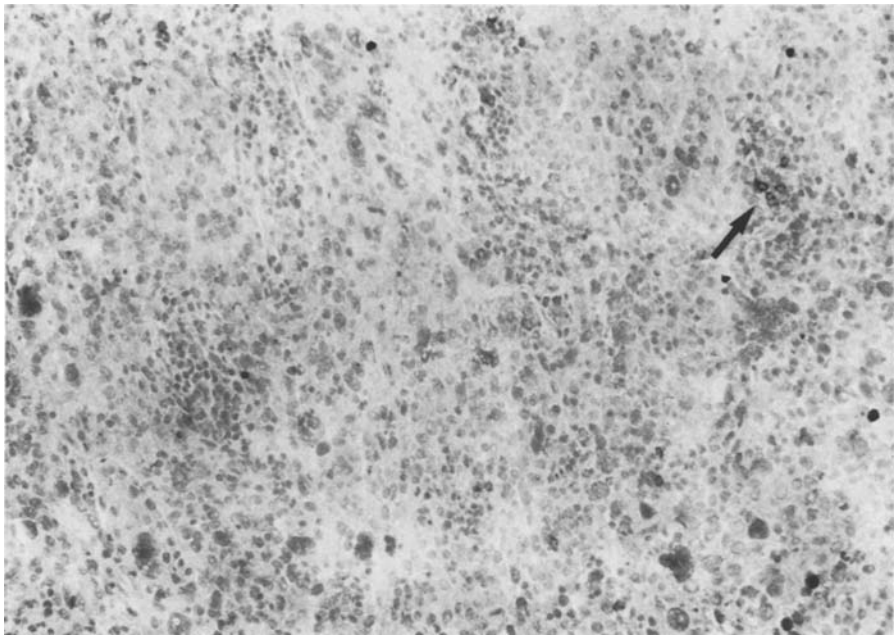

Fig. 5. Invasive ductal breast cancer. A small group of B lymphocytes (*arrow*) is shown but most of the tumour is devoid of stained cells. T0 15, ×140

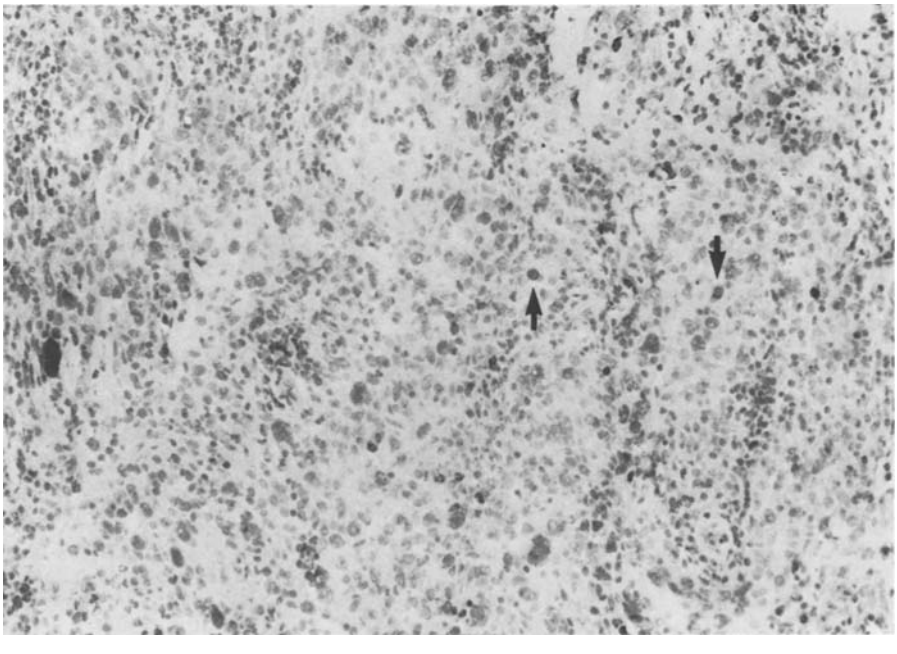

Fig. 6. Invasive ductal breast cancer. Very few natural killer cells (*arrows*) in a representative area of the tumour. Leu-7, ×140

All cells which could be identified histologically (GIEMSA and naphthol AS-D chloroacetate esterase) were found in the tumour stroma almost exclusively. While the breast cancer tissue was nearly devoid of *eosinophils, tissue mast cells* and *plasma cells* could be observed in varying numbers. In about one half of the tumours, mast cells and plasma cells were almost absent from the lymphoreticular infiltrates, but low, moderate, or even high numbers of both cell types were encountered in the other half (Table 3).

Regarding the extent and composition of the lymphoreticular infiltrates between the various tumour stages, no significant differences could be found.

Discussion

The present combined histological and immunohistological study clearly shows that the composition of the lymphoreticular infiltrates in invasive ductal breast cancer exhibits some general features. Macrophages, T lymphocytes, and T4 cells regularly constituted the major components, but T8 lymphocytes often also represented a considerable portion of the tumour-infiltrating cells. In contrast, the amount of B lymphocytes and natural killer cells was generally low, in many tumours both cell types nearly were absent from the lymphoreticular infiltrates. The same holds for eosinophils, while plasma cells and tissue mast cells were encountered in varying, often low or very low but sometimes also moderate or even high numbers. All lymphoreticular cells preferentially accumulated in the intervening stromal bands while the tumour foci mostly contained considerably lower numbers of infiltrating cells. Regarding the amount of tumour-infiltrating cells, no significant differences could be detected between the tumours of the different stages, especially between T1 and T2 tumours.

Marked differences are observed comparing the extent of the cellular stromal reaction in conventional histology and the corresponding immunohistological findings. This is especially true of the tumour-infiltrating macrophages which cannot be calculated with certainty in GIEMSA stains. In conventional histology, the amount of lymphoreticular cells is determined mainly by the number of round lymphoid cells. Thus, the best correlation between GIEMSA and immunohistology is achieved when the number of (Leu-1+) T lymphocytes is taken into consideration, especially because the B lymphocytes in breast cancers constitute only a small or negligible portion of the tumour-infiltrating cells.

Obviously, the lymphoreticular infiltrates in a malignant tumour can be regarded as an expression of interactions between host defense and tumour. Whether the composition of the lymphoreticular infiltrates is specific for invasive ductal breast cancer, or represents the general histomorphology of cellular immunooncologic reactions in human cancer cannot be stated with certainty. It should be emphasized that an analysis of the lymphoreticular infiltrates in axillary lymph node metastases of breast cancer yielded results comparable to the findings in the primary tumour (Horny and Horst 1985). Immunohistology of infiltrating adenocarcinoma of the large bowel also showed similar findings, especially with regard to the localization and

organisation of the tumour-infiltrating lymphoreticular cells. Histological evaluation with GIEMSA staining showed differing results regarding the number of plasma cells, eosinophils, and mast cells (unpublished observations).

Our findings only corroborate in part with other immunohistological studies on invasive breast cancer. There is general agreement that benign mastopathic lesions contain lower amounts of "inflammatory" cells than infiltrating tumours (Giorno 1983; Rowe and Beverley 1984). Furthermore, in non-cancerous breast tissue the B lymphocytes outnumber the T cells, while an inverse relation is observed in invasive carcinoma (Shimokawara et al. 1984). Also in suspensions of human breast cancer tissue a predominance of T lymphocytes has been found immunocytologically (Eremin et al. 1982).

It seems to be established that in overt malignancy of the female breast the infiltrating lymphoreticular cells accumulate predominantly in the stromal bands and at the edge of the tumours, while the tumour foci generally show a considerably lower degree of infiltration (Svennevig and Svaar 1979; Göttlinger et al. 1985). While most investigators found that B lymphocytes and natural killer cells were rarely encountered in invasive ductal breast cancer (Bhan and Des Marais 1983; Hurlimann and Saraga 1985), the T lymphocytes often represented a major fraction of the infiltrating cells (Schoorl et al. 1976; Rowe and Beverley 1984).

Discrepancies, however, exist concerning the amount of macrophages and the ratio of T4:T8 cells. While our analysis revealed that macrophages represented the overwhelming majority of the infiltrating cells in most tumours, according to Whitwell et al. (1984), and Hurlimann and Saraga (1985) macrophages were rarely detectable in most of their breast cancer cases. Göttlinger et al. (1985), however, also observed large numbers of macrophages in the tumour stroma, a great portion of these cells bearing the T4 surface antigen. Rowe and Beverley (1984), and also Hurlimann and Saraga (1985) reported on a predominance of T8 lymphocytes as compared with T4 cells, while in our study the number of the T4 cells in most cases exceeded that of the T8 lymphocytes by far. Whitwell et al. (1984) also found more T4 cells when compared with T8 lymphocytes in 12 of their 17 breast cancers. Whether the conflicting immunohistological findings result from the application of different monoclonal antibodies which detect different subsets of monocytes/macrophages cannot be stated with certainty.

Regarding the frequency distribution of eosinophils, plasma cells, and mast cells in invasive breast cancer, relatively few reports exist. Already Böhmig (1930) underlined the paucity of breast cancer tissues in eosinophils. Lauder et al. (1976) found a high plasma cell-content in 38 (76%) out of 50 cases with invasive breast cancer, while our analysis yielded that most tumours contained low or even negligible amounts of plasma cells. The role of mast cells in host-tumour interactions has been a matter of discussion since the early observation of Ehrlich (1879), who found that various tumours contain mast cells. Hartveit (1981) studied 50 consecutive nodal-

negative females with invasive breast cancer and found varying numbers of mast cells at the periphery of the tumours, whereas the central parts in more than 50% of the cases contained no mast cells.

The present findings revealed large numbers of macrophages and helper/inducer cells in most of the tumours analyzed. These cell types could obviously be of special significance in host-tumour interactions whereas natural cytotoxicity does not seem to be of great importance in the clinically detectable stages of invasive breast cancer – judged by the low amounts of natural killer cells. Correspondingly, a lack of killer cell-activity and very low levels of natural killer cell-activity of lymphocytes infiltrating human breast cancers have been demonstrated by Eremin et al. (1981) in cytotoxicity assays. The pluripotency of macrophages (production of monokines, cytostatic and cytoxic effects) allows these cells to be regarded as possibly powerful antitumoural effectors; but promoting and accelerating interactions between macrophages and tumour cells have also been described (De Baetselier et al. 1985; Kopper and Lapis 1985). Though the significance of T4 cells in the control of tumour growth has been substantiated in a mathematical model (De Boer et al. 1985) the preferential intrastromal accumulation of most lymphoreticular cells does not seem to be indicative of an intensive interaction between host defense and the malignant tumour.

In conclusion, it has to be emphasized that despite the preferential intrastromal accumulation and the reduced tumoricidal capacity of tumour-infiltrating lymphoreticular cells found in immunocytological studies (Tötterman et al. 1978), there is sufficient evidence that the degree of "leucocytic" infiltration in a malignant tumour may be regarded as an indicator of prognosis (Hamlin 1968; Underwood 1974). A combined histomorphologic and functional study on the lymphoreticular cells in the same tumour tissue possibly could resolve this enigma.

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