*Acta Oncologica* Vol. 34, No. 5, pp. 647–650, 1995

# INTERACTIONS BETWEEN THE IMMUNE SYSTEM AND BREAST CANCER

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**The functional and prognostic significance of lymphocytic infiltration of breast carcinomas has remained unclear. Using primary cultures we have demonstrated that lymphocytes could stimulate the growth of breast cancer epithelium in about half of the cases tested. The growth stimulation was subsequently shown to be strongly correlated with expression of MHC class I by the tumour cells. Furthermore, preliminary data suggest that carcinomas with a mixed population of MHC class I-positive and -negative cells were associated with a higher incidence of lymph node metastases and increased relapse rate compared with tumours that were homogeneously MHC class I-positive or -negative. The interactions between breast cancer and the immune system are clearly complex and the results suggest that the nature of these interactions can to some extent be determined by the level and pattern of MHC class I expression by the tumour cells.**

The normal breast is a composite organ made up of glandular epithelial cells of two types, the luminal and basal or myoepithelial cells, and stroma containing fibroblasts, adipocytes, blood vessels as well as cells of the immune system. During each menstrual cycle, hormonally regulated changes take place involving proliferation and secretory activity of the epithelium but increases in the amount of stroma and accumulation of lymphocytes also occur. Unless pregnancy occurs this phase is then followed by cell death and a reduction in the stroma. The breast is part of the mucosal immune system. Lymphoid cells are therefore always present in the breast and there is constant release of IgA (1).

This basic composition is to some extent repeated in carcinomas of the breast. In addition to the malignant epithelium, these tumours thus contain normal glandular epithelium as well as stromal fibroblasts, blood vessels and cells of the immune system. The proportional contribution of each component is quite variable between individual patients. In the so-called scirrhous breast carcinomas, a hard fibrous stroma predominates whereas in some cases the lymphocytes may be so numerous that the tumour resembles a lymphoma.

The functional and prognostic significance of lymphocytic infiltration of breast carcinomas has remained unclear despite decades of research. Although it is generally assumed that this indicates some kind of protective involvement of the immune system, there have been several reports suggesting a correlation between lymphoid infiltration and poor prognosis. Thus Underwood (2) concluded that lymphocytic infiltration was a favourable prognostic sign. On the other hand, Stewart & Tsai (3) found in a review of 35 studies that in 23 of these a higher degree of lymphocytic infiltration was correlated with poor prognosis. They concluded that in a subset of breast carcinoma patients the tumour cells may be stimulated to grow by infiltrating lymphocytes.

Received 1 November 1994.
Accepted 26 March 1995.
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Presented at the 5th Scandinavian Breast Cancer Symposium, May 28–June 1, 1994, Reykjavik, Iceland.

## Material and Methods

*Cell lines.* The breast cancer cell lines MCF-7, T-47D, ZR-75-1 and MDA-MB-231 were maintained in RPMI 1640 medium (Gibco) supplemented with HEPES 0.01 M, glutamine 0.2 M, penicillin 50 U/ml and streptomycin 50 μg/ml, 10% fetal calf serum and, in the case of T-47D and MCF-7, insulin 0.2 IU/ml.

 ISSN 0284-186X

*Primary cultures.* Fresh samples of breast carcinomas and unaffected tissue from the same breasts were kindly provided by the Department of Pathology. Following digestion of finely minced tissue overnight with collagenase I (Sigma) cultures were established and maintained for up to two weeks in a specifically designed highly supplemented serum-free medium (4, 5).

*Effector cells and partner cells in co-cultures.* Lymphocytes were isolated as mononuclear cells from peripheral blood on Ficoll/Hypaque gradients. The blood samples were collected from healthy blood donors (for culture with cell lines) and breast cancer patients, so that co-culture experiments with primary cultures were generally performed in an autologous as well as heterologous combination. There were no marked differences between results obtained in autologous compared with heterologous co-cultures. For experiments with cell lines, peripheral blood mononuclear cells were separated into adherent cells (monocytes) and non-adherent cells (lymphocytes), and the non-adherent cells were further fractionated into NK-cell-enriched and NK-cell-depleted fractions on Percoll gradients (6). Fibroblast cultures were derived from normal skin biopsies.

*Estimation of growth and proliferation.* Proliferation of cell lines was estimated by direct cell counts and uptake of $^{3}$H-thymidine. For primary cultures a semi-quantitative system for continuous assessment of growth during the culture period was established using a combination of colony counts and estimation of colony size, expressed as a growth score. For comparison of responses in co-culture experiments the cultures within each experiment were ranked for growth score (see 6 for detailed description).

*Immunohistochemistry.* MHC class I expression was detected using a rabbit polyclonal antibody against $\beta$-2-microglobulin and a mouse monoclonal antibody (LB-2) was used for the detection of ICAM-1. Staining was visualized using LSAB kits from DAKO.

## Results

### *In vitro studies of cellular interactions in breast cancer*

*Studies using cell lines.* Dickson & Lippman (7) proposed a model of growth regulation in breast cancer involving hormones and peptide growth factors, autocrine pathways as well as 'cross-talk' between stroma and the malignant cells. This model is based on extensive studies on breast cancer cell lines that demonstrated the hormone-regulated release by these cells of growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor $\alpha$ and $\beta$ (TGF$\alpha$ and TGF$\beta$). In our own studies we found that four breast cancer cell lines were growth-inhibited to different degrees by unfractionated human peripheral blood lymphocytes at an effector:target ratio of 15:1. T-47D and MCF-7 showed 77% and 72% inhibition of uptake of $^{3}$H-thymidine respectively and were more sensitive than ZR-75-1 and MDA-MB-231, which were inhibited by 56%. This inhibition was mostly mediated by natural killer cells but the T-47D cell line was also very sensitive to growth inhibition by monocytes. At the lower effector:target ratio of 1:1 the cell line MDA-MB-231 was not affected whilst in the case of MCF-7 a slight growth-stimulatory effect (10–20%) was observed (Guðmundsdóttir & Ögmundsdóttir, unpublished results).

*Studies using primary cultures of breast carcinomas.* Most of the commonly used breast cancer cell lines are derived from very advanced cancer such as pleural effusions, and they have been maintained in culture for many years. The question therefore arises how representative such cells are of clinical human breast cancer. The biology and physiology of normal tissues is preferably studied in primary cultures or short-term cell lines in order to minimize erroneous results caused by artificial in vitro conditions. The use of primary cultures in breast cancer research has been hampered by problems in culturing these cells. Petersen & van Deurs (4) developed methods and media that do support the short-term growth of malignant epithelial cells from breast carcinomas. We have used these methods in co-culture experiments in order to study the effects of lymphocytes and fibroblasts on the growth of mammary carcinoma cells (5). We obtained epithelial growth that was sufficient for experimentation and evaluation in 75% of cases. Based on morphological criteria, we estimated that at least 35% of the cultures contained proliferating malignant epithelium. Later chromosome studies have suggested that this may be an underestimate (8).

The effects of lymphocytes and fibroblasts on the growth of mammary carcinoma in primary culture are summarized in Table 1. The lymphocytes were in most cases autologous and the fibroblasts were derived from skin biopsies of healthy donors. Each sample was set up in five parallel cultures and these were ranked for growth as described in Material and Methods. The Table thus shows how often the highest growth score was obtained in the co-culture with lymphocytes and similarly for fibroblasts and control cultures without partner cells (5). It can be seen that the highest growth score was most commonly

**Table 1**

*The effect of lymphocytes and fibroblasts on the growth of breast carcinoma cells in primary culture*

| | *n* |
|---|---|
| Highest growth score in culture with | |
| Lymphocytes | 11 |
| Fibroblasts | 6 |
| No effect of partner cell | 3 |
| Total | 20 |

seen in co-cultures with lymphocytes. The mean growth score for all 20 cases cultured with lymphocytes was 100.6 compared with 74.9 for the control cultures. Comparative studies on uninvolved breast tissue revealed less variation in growth, and in 5 of 17 such cultures optimal growth was obtained in the presence of lymphocytes.

*Growth responses to lymphocytes related to tumour expression of MHC class I and ICAM-1*

It is well known that tissue cells are required to express major histocompatibility antigens of class I in order to interact with cells of the specific immune system. Vánky et al. (9) have shown that T-cell-mediated killing of breast carcinoma cells occurs if the tumour cells express MHC class I and the adhesion molecule ICAM-1. We have therefore analysed breast carcinomas for the expression of these two surface molecules and compared the results with the responses observed in the co-culture experiments described above. Out of 59 breast carcinomas 57.6% showed some staining with anti-$\beta$-2-microglobulin-antibody, indicating expression of MHC class I, and a slightly lower proportion, 44.1%, expressed ICAM-1. Expression of these two markers was correlated. The relationship between presence of these membrane markers and growth responses of primary breast carcinoma cultures to lymphocytes is shown in Table 2. It can be seen that growth stimulation in response to lymphocytes was significantly associated with the expression of MHC class I by the tumour cells. The same trend was evident for ICAM-1, but this association did not reach a level of significance.

*Tumour expression of MHC class I related to clinical history*

On analysing the data presented in the preceding paragraph, we had noticed that the expression of MHC class I

**Table 2**

*Growth responses of breast carcinomas to lymphocytes related to expression of MHC class I and ICAM-1*

| | Growth in presence of lymphocytes | | |
|---|---|---|---|
| | Stimulated | Not stimulated | Total |
| MHC class I | | | |
| Positive | 12 | 2 | 14 |
| Negative | 3 | 7 | 10 |
| Total | 15 | 9 | 24 |
| $\chi^2 = 5.53$, $p < 0.05$ | | | |
| ICAM-1 | | | |
| Positive | 9 | 3 | 12 |
| Negative | 5 | 6 | 11 |
| Total | 14 | 9 | 23 |
| $\chi^2 = 1.05$, not significant | | | |

**Table 3**

*Pattern of MHC class I expression related to lymph node status and relapse rate*

| | MHC class I expression | | |
|---|---|---|---|
| | Positive | Mixed positive/ negative | Negative |
| Lymph node metastases | 58% | 71% | 32% |
| Relapse | 8% | 41% | 11% |

*Figure.* Heterogeneous expression of MHC class I antigens in an infiltrating ductal breast carcinoma. Original magnification: × 125.

by the tumour cells was frequently heterogeneous and, furthermore, that the positive growth response to lymphocytes was most strongly correlated with heterogeneously positive tumours rather than those that were uniformly positive. We therefore compared the pattern of MHC class I expression with two clinical parameters, presence of lymph node metastases and relapse rate. The results are presented in Table 3 and they are based on findings for 48 patients and a follow-up period of 2 to 5 years. It can be seen that patients with tumours showing heterogeneous expression of MHC class I were most likely to have lymph node metastases and to experience recurrence of their disease. These differences were statistically significant at $p < 0.05$ using chi squares but the numbers are low and follow-up not very extensive. Similar studies are therefore now in progress that will include 207 patients diagnosed during 1981–1984. The Figure shows an example of heterogeneous expression of MHC class I in a breast carcinoma.

## Discussion

The concept of a growth-stimulating and tumour-supportive function of lymphocytes is contrary to the classical view of immune surveillance. The interactions between breast cancer and the immune system are obviously quite complex and subject to individual variation. The data presented here and the review by Stewart & Tsai (3)

referred to above suggest that in a significant subgroup of breast cancer patients tumour-infiltrating lymphocytes might create a favourable environment for the malignant cells. Our results indicate that the nature of the interaction between breast carcinoma and lymphocytes in each patient is determined by the pattern in which the tumour expresses MHC class I. It can be envisaged that MHC class I-positive tumour cells might trigger T-lymphocytes to release cytokines that could in turn stimulate the growth of MHC class I-negative cells in the same tumour, the latter cells being 'invisible' to the T-cells. Further studies are needed in order to substantiate the proposed association between pattern of MHC class I expression and disease status and prognosis. Attempts at using various types of immunotherapy have not been rewarded with much success in breast cancer (10, 11). Better understanding of the mechanisms governing the interactions between lymphocytes and breast cancer should in future provide a more rational basis for evaluation of the involvement of the immune system in the patient and might then suggest approaches and patient subgroups that could be suitable candidates for some form of immunotherapy.

## ACKNOWLEDGEMENTS

This work was supported by grants from the University of Iceland Research Fund and The Icelandic Science Fund. The assistance of Kristrún Ólafsdóttir and Jón Gunnlaugur Jónasson of the Department of Pathology with the selection, sectioning and staining of paraffin-embedded tissues is gratefully acknowledged.

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