



Review

Immune Reaction to Breast Cancer: for Better or for Worse?

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Abstract. The infiltration of breast carcinomas with lymphoid cells has often been interpreted as an indication of an active immune response against the tumour and, thus, a favourable prognostic sign. Several studies have, however, cast doubt on this assumption. *In situ* breast carcinomas are more common than invasive cancers, and it may be speculated that immune surveillance plays a role in preventing some localized cancers from becoming invasive. A secondary type of immune surveillance might be implicated in the long persistence of dormant breast carcinoma cells in the bone marrow. Breast cancer cells can carry tumor-associated antigens, particularly MUC1. These may elicit specific antibody responses, but there is less evidence for a cytotoxic T lymphocyte (CTL) response. There are indications that professional antigen-presenting cells (APC) may be present and active at the edges of breast tumours. Breast cancer cells may also interact directly with macrophages and natural killer (NK) cells. In terms of immune effector mechanisms in breast cancer, the communication with potential effector cells is likely to be often faulty because of altered expression of HLA class I molecules. Pleiotrophic cytokines are frequently present and could have a variety of effects ranging from growth inhibition to stimulated proliferation, loss of cell adhesion and activation of matrix-degrading enzymes. Fas ligand is unlikely to play a role in the immune evasion of breast cancer. There is thus evidence for a variety of immune reactions to breast cancer. It is possible that they mediate some form of surveillance, but growing, invasive tumours have escape routes and may even use cytokines to their advantage.

Key words: breast cancer; immune surveillance; tumor antigens; cytokines; Fas-FasL; immune evasion.

Introduction

Breast carcinomas frequently contain a more or less marked infiltration of lymphoid cells. It is a common textbook interpretation that a lymphoid infiltrate reflects an active response of the immune system against the cancer cells and, therefore, signifies a favourable prognosis²⁸. This has been partly based on the fact that

the medullary subtype of breast carcinomas is characterized by a marked lymphoid infiltrate and also has a better prognosis than other histological types. These two characteristics of medullary carcinomas may, of course, be totally unrelated to each other. The assumption that the immune system can eliminate malignant cells is reflected in the authors' interpretation in a remarkable case report where stage IV breast cancer re-

Abbreviations used: APC – antigen-presenting cells, CTL – cytotoxic T lymphocytes, ELISA – enzyme-linked immunosorbent assay, HLA – human leukocyte antigen, KIR – killer inhibitory receptor, MHC – major histocompatibility complex, NK – natural killer, RT-PCR – reverse transcriptase polymerase chain reaction.

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gressed following dexamethasone treatment³¹. The primary tumour was highly pleomorphic with prominent lymphoid infiltration. Analysis of blood lymphocytes and a biopsy from a subcutaneous metastasis taken during treatment showed a predominance of natural killer (NK) cells, which were then assumed to mediate the anti-tumour response. Corticosteroids are, however, commonly used in order to suppress inflammatory reactions and cell-mediated immune responses. A much-quoted review by UNDERWOOD⁵⁸ found that lymphoid infiltration was a favourable prognostic indicator. STEWART and TSAI⁵⁴ reviewed 35 publications from a wide time period and came to the conclusion that in 23 of these studies a high degree of lymphoid infiltration was correlated with poor prognosis. As pointed out by O'SULLIVAN and LEWIS⁴², some of the controversy may be due to differences in the type of analysis performed. In our own work we have shown that lymphocytes could stimulate the growth of primary breast carcinoma cells in co-culture^{43, 44}.

The concept of immunosurveillance has been in and out of favour through the years. This mechanism undoubtedly protects against the malignant transformation of virally infected cells, but there is no real evidence for a viral etiology of human breast cancer. Immune surveillance may contribute to the Darwinian selection process that leads to the development of a clone of malignant cells with a growth advantage in colorectal cancer⁶. In the case of breast cancer, it has been shown that occult *in situ* breast cancer lesions are found at autopsy in 18% of young women and 80% of elderly women, a far higher proportion than are ever diagnosed with clinical breast cancer³⁹. This type of finding has been interpreted as evidence for active immunosurveillance¹⁹. The remarkable recent paper by SHANKARAN et al.⁵³ has now provided compelling evidence that an intact immune system protects against the development of mammary carcinomas. They showed that mice that were made genetically deficient both in lymphocyte antigen receptor function and responsiveness to IFN- γ developed spontaneous adenocarcinomas in the intestines and mammary glands at the age of 12–15 months whereas control mice remained free of malignant tumours at that age. Tumours that were elicited in the mutated mice were highly immunogenic and were rapidly rejected upon transfer to immunocompetent mice of the same strain. In marked contrast tumours that arose in the immunocompetent mice were poorly immunogenic, implying that the intact immune system had selected a phenotype that escaped immune detection.

Breast cancer is in some respects unusual among

adenocarcinomas. The prognosis with appropriate treatment can be regarded as good, but the disease can recur more than a decade after apparently successful treatment and eradication of the tumour. Tumour cells can be identified in the bone marrow but they can remain dormant, possibly even for the remaining life of the patient⁷. This could be interpreted as reflecting the activity of secondary surveillance mediated by the immune system. These tumor cells are not eliminated, but somehow kept in check.

With regard to the normal physiological function of the mammary gland, it has to be kept in mind that it forms one part of the mucosa-associated lymphoid system. Breast tissue always contains IgA-producing cells¹⁵, and the presence of lymphoid cells does not, therefore, necessarily imply that they are reacting against something present in the tissue or expressed by the cells.

The interactions between breast cancer and the immune system, therefore, present paradoxes and questions. It is likely that some of the apparent paradoxes arise from the fact that breast cancer is a very heterogeneous disease. Do breast carcinomas elicit immune reactions and are such reactions then capable of restricting tumor growth and killing malignant cells, or can they, on the contrary, promote tumor cell proliferation and invasion? In this review I shall briefly examine immune reactions in breast carcinoma in terms of the different phases of the immune response, i.e. recognition, activation and effector mechanisms, as well as possible escape routes. Finally, some clinical implications will be considered.

Recognition and the Initiation of Immune Response Against Breast Cancer

In order to elicit an immune reaction, the breast cancer cells would first have to express a tumor-specific or tumor-associated antigen that is then processed by an antigen-presenting cell (APC) for the stimulation of specific T cells or B cells. Another possibility is that the cancer cells express ligands that are recognized directly by macrophages or NK cells. Antigens of the MAGE family were the first tumor-associated antigens to be described in human tumors. MAGE type genes have been found to be activated and expressed in a variety of malignant cells but not in normal tissue, with the exception of male germ cells. The mechanism of gene activation is DNA de-methylation¹². The MAGE antigens are typical T cell-stimulating antigens, intracellular proteins expressed as processed peptides in the

appropriate context of histocompatibility molecules, but they are only weakly immunogenic²⁵. MAGE gene expression has been described in breast carcinomas, but in a relatively low proportion of tumours, around 20–30%^{13, 52}. The newly described expression of the SART-1 tumour rejection antigen in breast carcinomas may present a more likely candidate for HLA class I-restricted activation of cytotoxic T lymphocytes in breast cancer²⁴. Epithelial mucins are highly expressed on the surface of breast carcinoma cells and were first known as the targets of mouse monoclonal antibodies to carcinoma cells⁵⁵. The MUC1 membrane mucin is expressed on the apical surface of normal mammary gland epithelial cells, but it is expressed in an aberrant form by carcinoma cells that can also show cytoplasmic expression³³. On breast cancer mucin, the glycosylation is abnormal, thus exposing protein epitopes that are masked in the normal mucin⁵⁵. MUC1 mucin can elicit IgG and IgM class antibody responses in breast cancer patients and a recent study has indicated that the presence of such anti-MUC1 antibodies is associated with a significant benefit in disease-specific survival⁶¹. MUC1 has been shown to inhibit T cell proliferation¹, and the natural immune response to mucins is predominantly a B cell response. Using conjugated MUC1 peptides to immunize breast cancer patients, it has, however, been possible to generate a HLA class I-restricted cytotoxic T cell response⁴⁸.

The mechanisms of immune activation by tumour-associated or tumour-specific antigens has obviously been studied mostly within the context of tumour vaccine development to create cytotoxic T cells. These efforts have focussed attention particularly on dendritic cells and surface signalling through CD40⁴⁰. Dendritic cells are the most professional APC and are derived from cells that take up antigens in the tissues, such as epidermal Langerhans cells and tissue macrophages³⁴. Their full activation into APC requires exposure to cytokines such as TNF- α and a maturation signal through CD40 delivered by its ligand (CD40L) on a T cell^{34, 40}. Interactions between CD40 and CD40L, are best known as mediators of growth stimulatory and differentiating signals from T cells to B cells, that are almost universally CD40 positive. It is now realized that CD40 can be expressed on a variety of cells, and was, in fact, first discovered on bladder carcinoma cells. CD40 expression has been described on breast cancer cell lines and, in contrast to the maturation and differentiation effects on B cells or dendritic cells, CD40L delivers a death signal to these and other cancer cells^{2, 64}.

How could the mechanisms described above operate naturally in the patient to elicit a response to a tumour-

-associated antigen? As a first step, these antigens would have to be taken up and processed by professional APC. Before that can happen, the antigens will have to penetrate through the basement membrane into the stromal extracellular matrix. It can be envisaged that this starts occurring when potentially malignant cells lose their polarity and cell-cell and cell-matrix adhesions become weaker, e.g. through alterations in E-cadherins and integrins^{4, 27}. As mentioned above, MUC1 loses its apical localization in breast cancer cells. For a more advanced stage in tumor development, BELL et al.⁵ have recently demonstrated the presence of immature dendritic cells imbedded inside breast carcinomas in all 32 samples studied, whereas mature dendritic cells were found around the peripheries of 2/3 of the tumours. In some of the cases these peritumoral dendritic cells had clusters of T cells around them, indicating an active immune response at the edge of the growing tumor.

The possibility of interactions with non-specific effector cells of the immune system should not be forgotten. Interestingly, it has recently been demonstrated that sialoadhesin, an adhesion molecule on macrophages, binds specifically to sialic acid residues on MUC1, thus providing a potential mechanism for macrophage infiltration in breast carcinomas³⁷. A variety of surface receptors has been described on NK cells that mediate inhibitory signals upon binding to major histocompatibility molecules of class I, the killer inhibitory receptors (KIR)⁵⁰. Since breast carcinomas frequently show altered expression of HLA class I (see below)^{8, 18, 26}, they can then become targets for NK cells through the more recently described natural cytotoxicity receptors³⁶. Breast carcinomas frequently contain active NK cells that are active *in vitro* against breast cancer cell lines¹⁸.

To summarize at this point, breast cancer cells can carry tumor-associated antigens that may elicit specific immune responses, such as anti-MUC1 antibodies. There is less evidence for a cytotoxic T lymphocytes (CTL) response. There are indications that professional APC may be present and active at the edges of breast tumours. The relevance of CD40 expression on breast cancer cells is speculative. Breast cancer cells may also interact directly with macrophages and NK cells.

Immune Effector Functions against Breast Cancer and Escape Mechanisms

The most frequently studied immune effector function in malignancies is probably specific Tcell-mediated cytotoxicity, since the generation of this type of

immunity is the goal of tumour vaccine development. As indicated above, there is not much evidence for such a response occurring naturally in breast carcinomas, although the cytotoxicity of specific autologous T cells against cultured breast carcinoma cells has been demonstrated⁵⁹. Such cytotoxicity was dependent on expression of the cell adhesion molecule ICAM-1 and MHC class I by the tumour. It has now become abundantly clear, particularly through extensive studies on tumour vaccines for melanoma, that the requirement for effector CTL to see the antigen on the tumour target in the context of the appropriate MHC class I molecule is absolute^{20, 51}. Altered expression of HLA class I antigens has been observed in a variety of human tumours and indeed presents a major problem for tumour vaccine development. Breast carcinomas are no exception in this respect, and a complete or partial loss of HLA class I molecules is seen in around 80% of breast carcinomas^{8, 18, 26, 63}. Tumour cells that have completely lost surface expression of HLA class I molecules should be targets for NK cells, according to the missing self hypothesis²³. Even partial expression of HLA class I molecules can still activate the KIR and thus protect cells from NK-mediated cytotoxicity. GARRIDO et al.¹⁴ have put forward a model of tumour progression involving a stepwise selection of variant cells that become T cell resistant through loss of one HLA class I specificity, but still escape killing by NK cells by virtue of retaining some HLA alleles. Such tumours would then be invisible to specific and non-specific immune mechanisms. We have found that a mixed expression of HLA class I is present in over 1/3 of breast carcinomas, and such tumours were significantly more likely to recur than either HLA class I positive or negative tumours^{17, 43}.

Immunohistochemical and flow cytometric studies have shown that several phenotypes of lymphocytic cells are represented in the lymphoid infiltrates in breast carcinomas. The majority of the infiltrating cells are T cells, with a higher proportion of CD8-positive (cytotoxic type) than CD4-positive cells (T helper type)^{22, 30, 63}. NK cells are usually also present, but in small numbers²². The specific cytotoxic capacity of tumour-infiltrating T cells appears to be limited^{35, 57}, but NK activity is present¹⁸ and non-specific lymphocyte-activated killer (LAK) cells can be generated from breast tumour-infiltrating lymphocytes that are active against autologous or heterologous breast cancer cells²¹. The limited specific T cell cytotoxic activity against breast cancer may, of course, be related to the lack of HLA class I expression described above as well as other escape mechanisms (see below). Macrophages are not often specifically mentioned in studies of lymphoid in-

filtration in breast cancer, but these cells can, in fact, constitute the majority of the infiltrating leukocytes⁴².

Cytokine production in breast cancer has been studied using a variety of techniques detecting either proteins by immunohistochemistry, flow cytometry or ELISA, or mRNA by *in situ* hybridization or RT-PCR. The source of these cytokines are T lymphocytes, but probably to an even larger extent macrophages⁴². Pleiotropic cytokines, such as TNF- α , TGF- β and IL-6, are more prominent than immunostimulatory cytokines such as IFN- γ ^{4, 9, 32, 60, 66}. These cytokines may have a direct effect on the tumor cells. Thus, IL-6 causes loss of cell-cell adhesion of breast cancer cells through down-regulation of E-cadherin⁴, and cytokines such as TNF- α can stimulate the production of metalloproteases¹⁰. The cytokine environment in breast cancer may thus favor invasive growth of the tumour. Immune responses have been observed to stimulate tumour growth *in vivo* in experimental animals⁴⁶, and we have shown enhanced growth of primary breast carcinoma cells *in vitro* in the presence of lymphocytes⁴⁴. The cytokines responsible for this growth stimulation were not identified. It has been reported that IFN- γ can inhibit tumour growth in mice, possibly partly through an anti-angiogenic effect¹¹. This takes on a new relevance with a recently published study demonstrating that the intracellular mediator of IFN- γ activity, STAT-1, interacts with the product of the breast cancer susceptibility gene, BRCA-1. It was furthermore shown that, in cells homozygous for a BRCA-1 mutation, IFN- γ could no longer cause transcription of cell cycle control genes⁴⁵. It is thus possible that one reason for the accelerated breast cancer development in carriers of BRCA-1 mutations is an escape from IFN- γ -mediated growth inhibition. In this context it is interesting to note that BRCA-1-mutated breast carcinomas have been described to have significantly more lymphocytic infiltration than sporadic breast cancers²⁹. The recent results of SHANKARAN et al.⁵³ suggest that IFN- γ may also act through enhancing antigen display on MHC class I molecules.

As indicated above, tumour-infiltrating lymphocytes often appear to have impaired functional capacity. One proposed explanation is that the tumour micro-environment is hostile, and soluble immunosuppressors have been demonstrated in breast cancer. The cytokine(s) responsible for this effect was not identified, but TGF- β was ruled out⁶⁷. A proposed mechanism for reduced T cell activity in cancer patients is a defect in the T cell receptor signalling mechanism caused by loss of the ξ -chain⁶². It would appear, however, that this is not common in breast cancer patients³⁸. Humoral immunity

receives relatively little attention in terms of tumour immunology, but one potential mechanism for breast cancer cells to escape immune attack is through surface expression of complement regulatory proteins^{16, 56}. A possible escape route that has been popular in the literature for the last 5 years is down-regulation in the tumour cells of the Fas death receptor along with up-regulation of surface-expressed Fas ligand (FasL), which could then kill activated T cells⁴¹. We have found that both normal and malignant breast epithelium co-expresses Fas and FasL, but FasL is only expressed intracellularly and, thus, unable to kill neighbouring cells⁴⁷. In a recent commentary, RESTIFO⁴⁹ has concluded that this attractive model of tumour escape has, in fact, not stood the test of time.

Summarizing briefly the operation of immune effector mechanisms in breast cancer, communication with potential effector cells is likely to be often faulty because of altered expression of HLA class I molecules and, perhaps, defective signal transduction through CD3. Pleiotrophic cytokines are frequently present and could have a variety of effects, ranging from growth inhibition to stimulated proliferation, loss of cell adhesion and activation of matrix-degrading enzymes. FasL is unlikely to play a role in the immune evasion of breast cancer.

Concluding Remarks

Returning to the question posed in the title we can first ask whether a reaction of the immune system against breast cancer might be beneficial to the patient. It can certainly be argued that immune surveillance might play a role by the fact that not all pre-cancerous lesions of the breast or *in situ* carcinomas develop into invasive carcinomas. The long latency of potentially metastatic tumour cells may also be an indication of immune surveillance particularly in view of the observation that tumour cells with a mixed HLA phenotype, which are invisible to the immune system, have a higher rate of recurrence.

Is it possible that in other situations immune reaction might, on the contrary, aid in the growth and spread of the tumour? Favouring this notion are data indicating that lymphoid infiltration may be a poor prognostic sign. We also have evidence that cytokines can act as growth factors for breast cancer cells. Cytokines released during an immune reaction may lead to increased production of matrix metalloproteases and, thus, play a part in tumour invasion and metastatic spread as well as angiogenesis.

In terms of practical clinical considerations, we are no closer to a conclusion on the prognostic significance of the frequently observed lymphoid infiltration in breast cancer than when this subject was reviewed by O'SULLIVAN and LEWIS⁴². The answer may well be that it is not possible to come to one conclusion on this subject since there are wide variations both in the composition of the lymphoid infiltrate on the one hand and the phenotype of the tumour cells on the other. The final outcome of the interaction between breast cancer and the immune system will always depend on the HLA class I expression of the tumour cells. If it holds true that tumours with a mixed HLA class I phenotype have an increased capacity to avoid immune attack and thus a higher recurrence rate, mixed expression of HLA class I antigens might be an additional indication for adjuvant therapy.

Immunotherapy has so far shown limited success in breast cancer¹⁹. MUC1 is certainly a potential candidate for a tumour vaccine antigen. The possibility of inducing the production of cytokines with undesired stimulatory effects on the tumor cells will, however, have to be kept in mind.

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