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Review Article

The immunology and immunotherapy of breast cancer: an update

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Abstract

Adenocarcinomas of the breast behave clinically and epidemiologically in ways that show host resistance factors are important for outcome in addition to grade and stage of malignancy. Immune reactivity to autologous tumors is indicated by the general presence of lymphoid infiltration (LI) and regional lymph node changes; however, these changes predict favorable outcome only in non-metastatic disease. LI is characterized by $CD4^+$ and $CD8^+$ tumor infiltrating lymphocytes reflecting latent cell-mediated immunity (CMI). CMI and humoral immune reactivity have been demonstrated to autologous tumor and a variety of tumor-associated antigens (TAA) have been implicated including CEA, HER-2/neu, MAGE-1, p53, T/Tn and MUC-1. Immune incompetence involving CMI is progressive with the stage of breast cancer and is prognostically significant. Immunotherapy of several types has been designed to address this immunodeficiency and the TAAs involved.

Animal models have employed drug therapy, cytokine transfection, vaccines with autologous tumor, cytokines like interferon alpha (IFN-α) and interleukin-2 (IL-2), TAA tumor vaccines, and immunotoxins with evidence of tumor regression by immunologic means.

Immunotherapy of human breast cancer is a rapidly growing experimental area. Positive results have been obtained with natural IFN and interleukins, particularly in combination strategies (but not with high dose recombinant IFN or IL-2), with autologous tumor vaccine (but not yet with transfected autologous tumor); with a mucin carbohydrate vaccine (Theratope™) in a combination strategy (but not with mucin core antigen) and with several immunotoxins. Combination strategies involving immunorestoration, contrasuppression, adjuvant, and immunotoxins are suggested for the future. © 1999 International Society for Immunopharmacology. Published by Elsevier Science Ltd.

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1. Introduction

Virtually all breast cancers are adenocarcinomas. The major subtypes are infiltrating ductal carcinomas (81%), medullary carcinoma (6%), and lobular carcinoma (5%) (Fisher, Gregorio & Fisher, 1974). Survival in breast cancer correlates with the original size of the tumor, the extent of regional spread, and the histological features of malignancy. Alone, and in combination, these are important prognostic variables; however, it has been long known that breast cancer is a disease with extreme variability. From an actuarial standpoint, breast cancer survival curves, depending upon the stage of the disease and the form of treatment, take up to 10 years to plateau, indicating variable rates of recurrence irrespective of the form of treatment. The survival curves for most other malignancies reach a plateau within two, and certainly five, years. Breast cancer is an exceptional disease amongst cancers. It has been long thought that this variability results from important host factors independent of the grade of malignancy and the extent of local-regional invasion.

It is the purpose of this article to summarize the immunologic studies in human breast cancer which indicate that breast cancers exhibit several antigens, are reacted to by the host's immunity, overcome progressively these defenses which then capitulate in generalized immunodeficiency and, finally, can respond to various forms of immunotherapy with regression.

This article is abbreviated and the reader is referred to Hadden (1995) for full referencing.

There are a number of unique epidemiological features of breast cancer which reflect these host factors. The incidence of non-invasive malignancies determined by mammography is in excess of predicted clinical frequency (Fisher, 1980a). Histological detection of additional foci of tumor cells in the presence of a malignancy in a pathological specimen has a significant frequency (Baak, Van Dop, Kurver & Hermans, 1985); however, it is extremely rare to have two clinical tumors in the same breast. The co-existence of carcinoma in situ in a patient with breast cancer appears to have a 'surveillance effect' and to be associated with significantly improved survival and is thought to reflect immunity to breast cancer antigens (Black, Zachray, Hankey & Feuer, 1996).

Also, it is not uncommon to find at autopsy, foci of malignancy that never made clinical disease. In the estimated 40% of patients with involved lymph nodes not removed by surgery, the recurrence rate is not 40% but only 15%, indicating that these obviously malignant lesions either failed to progress or regressed (Fisher, 1980b). Finally, monoclonal antibodies detect a higher frequency of tumor cells in regional nodes and bone marrow than would be expected on the basis of recurrence rates (Cote, Rosen, Lesser, Old & Osborne, 1991; Osborne & Rosen, 1994). Thus, persistent tumor cells can exist but may not cause recurrence. Such findings indicate that 'all cancers do not progress to overt lesions' (Fisher, 1980b). This assertion reflects the notion that the host provides an important component to the biology of breast cancer and the actuaries further reflect this fact.

If one superimposes the disease-free survival rates and overall survival rates in women with breast cancer treated with surgery and radiation, it is clear that it takes seven years to define the plateau in disease-free survival and nine years to define overall survival rates. If one compares these superimposed curves, it is apparent that once recurrence takes place, the time between recurrence and death progressively increases only a small amount, indicating that the late recurring tumors are only slightly less aggressive than those recurring earlier. These data indicate that different growth rates of the same type of cancer cannot explain the period of clinical latency of up to seven years. This period presumably results from host resistance to tumor growth.

Fisher (1980a) has pointed out a serious dilemma in considering chemotherapy after surgery and radiation: if, as collected studies indicate, 70–80% of patients will remain free of disease as a result of surgery plus radiation, should all patients be exposed to a toxic, expensive therapy for the benefit of 10–15% of the patients? While chemotherapy can delay recurrence in early breast cancer, it has only relatively recently been demonstrated to increase modestly 10 year survival (Anonymous; Early Breast Cancer Trialists Collaborative Group, 1992); it has only palliative value in advanced metastatic breast cancer. Recognizing that breast cancer chemotherapy is immunosuppressive (Zielinsji, Muller, Kubista, Staffen & Eibl, 1990) and will surely impact negatively on host resistance mechanisms, one must ask if immunotherapy can enhance host resistance factors and delay or prevent recurrence.

2. Lymphoid infiltration and nodal reactivity in breast cancer

Moore and Foote (1949) noted that medullary carcinoma of the breast is generally associated with marked lymphoid infiltration and has a more favorable prognosis. These observations with medullary carcinoma are generally accepted and this lymphocyte reaction appears to reflect a prominent role of humoral immunity with plasma cell infiltration around the tumor and follicular (B cell) hyperplasia in regional lymph nodes. (Fisher, Gregorio, Redmond, Dekker & Fisher, 1976; Hsu, Raine & Nayak, 1981)

Black, Kerpe and Speer (1953) were the first to observe a correlation between the survival of patients with breast cancer and sinus histiocytosis in the axillary nodes. This favorable prognosis was irrespective of histology of the primary tumor, the presence of nodal metastases, or age. In these studies, 61% of the patients with various forms of breast cancer were noted to have sinus histiocytosis. Several studies confirmed the association of sinus histiocytosis (Hadden, 1995) with favorable prognosis and noted further that chronic inflammatory changes with hyaline deposition and fibrosis follow sinus histiocytosis. This latter inflammatory condition is associated with nodal depletion and increased metastasis tendency.

Hamlin (1968) formally postulated the importance of host resistance factors in which cellular infiltration in or around the tumor and lymph node changes of follicular hyperplasia, sinus histiocytosis, and other reactive changes were focused upon. Sixty-three percent of the 272 patients were considered to be positive for host-defense factors and showed a significant improvement of survival compared to those considered negative. Tsakraklides, Olson, Kersey and Good (1974) presented a retrospective series of 277 cases in which lymph nodes regional to breast cancer were designated lymphocyte predominant, germinal center predominant, lymphocyte depleted, or unstimulated representing 54, 17, 4, and 25% of the tumors, respectively. Ten year survival rates were 75, 54, 33, and 39%, respectively, indicating that both T and B lymphocyte predominance favored survival.

A subsequent multicenter prospective study (Fisher et al., 1976) of 303 patients concluded, based upon only 20 month survival data, that, other than sinus histiocytosis, no nodal changes predicted short-term treatment failure. This publication marked the end of attempts to make meaning of the morphology of nodes regional to breast cancer and today seldom are references made to node findings.

What can be drawn from the collected findings? Lymph nodes regional to infiltrating ductal

carcinomas show lymphocyte dominance (>70%) vs unstimulated (<30%). Despite the lack of adequate control data on axillary nodes unrelated to breast cancer, these findings suggest that a preponderance are lymphocyte reactive, this implying immune mechanisms. The findings of sinus histiocytosis, which is marked in up to one-third of patients and present in the majority, is a finding on which pathologists must vary considerably. While it probably predicts survival when present in uninvolved nodes, it is a minor factor compared to tumor malignancy grade and the extent of nodal involvement and is not useful as a predictor on an individual patient basis. Unfortunately, from an immunological standpoint, it does not infer any particular mechanism, only reactivity. These collected studies, based upon morphology alone, strongly suggest that the reactivity represents immunologic response to tumor-associated antigens (TAA).

Lymphocyte infiltration (LI) was noted in human breast cancers in a number of early studies before 1974, but it is difficult to determine whether medullary carcinoma was distinguished from other forms. Of 13 studies reviewed by Underwood (1983), the majority reported a positive prognostic association. Clearly, a sharpening of focus occurred during this period and it became apparent that while medullary cancer is associated with diverse aggregates of lymphoid cells, the majority of the tumors (i.e. infiltrating ductal carcinomas) have scattered cells in the stroma surrounding the tumor (tumor-associated lymphocytes [TALs]), and to a lesser extent within the tumor (tumor-infiltrating lymphocytes [TILs]). As interest in and as more careful attention to LI grew, its incidence grew. The most extensive studies report 76% positive for LI (Fisher et al., 1974; Aaltomaa et al., 1992).

While the perception and incidence of LI grew in breast cancer of all types, whether LI is a favorable prognostic sign continues to be controversial (cf. Hadden, 1994; O'Sullivan & Lewis, 1994). While several reports suggest LI may not be prognostically significant (Rosen et al., 1989; Tang et al., 1990; Scholl et al., 1994; Ogmundsdottir, Petursdottir & Gudmundsdottir, 1995), and even associated with poor prognosis, recent multivariate analyses (Rilke et al., 1991; Aaltomaa et al., 1992; Nistico et al., 1997), show that LI is positively correlated with axillary node status, tumor diameter, and histological variables, all negative correlates themselves. Nevertheless, LI predicates recurrence-free survival (RFS) and overall survival in rapidly proliferating erbB/HER-2/Neu positive, node-negative tumors. In contrast, highly curable, small, slowly proliferating tumors show little or no infiltrate. As with sinus histiocytosis, it seems that tumor-associated lymphocytes are a predictor of survival only when the grade and extent of tumor are controlled for as variables. In highly malignant, oncogene-positive tumors they may inhibit growth early but, once overcome, the otherwise poor prognosis associated with the degree of malignancy becomes the overriding variable.

As the LI phenomenon became documented, attention turned to the type of cell involved in the infiltrates. Studies (Hadden, 1995; O'Sullivan & Lewis, 1994; Schondorf et al., 1997) showed these tumor-associated cells to be T-lymphocytes in the majority with a few B-lymphocytes and granulocytic cells including natural killer (NK) cells. Both $CD4^+$ and $CD8^+$ mononuclear cells are present in approximately equal numbers. Both $CD4^+$ T-cells and macrophages predominate in aggregates and $CD8^+$ T-cells in the isolated stromal cells (Bahn & Desmaraus, 1983; Chin et al., 1993; O'Sullivan & Lewis, 1994; Grekou et al., 1996). Significant numbers of $CD4^-$, $CD16^+$ macrophages have been observed (Claasen, van Ravenssway, Kluin & Fleuren, 1992; Pupa, Menard, Andreola & Colnaghi, 1993; Scholl et al., 1994), particularly with erb 2 colony-stimulating factor-1 (CSF-1) positive tumors.

The controversy around the prognostic significance of LI in breast cancer has produced two postulates: one that LI merely reflects an inflammatory reaction resulting from tumor-derived cytokines and the other that LI represents a defense reaction overridden by metastatic disease. Likely, both explanations are true and that tumor-derived cytokines contribute to the overriding of effective resistance mechanisms.

Studies on major histocompatibility complex-related antigen expression (HLA/class I or HLA-DR/class II) on tumor-associated lymphocytes and on the tumor itself show that, in contrast to non-malignant breast tissue, less than half of tumors had high expression of class I or II antigens (Maiorana et al., 1995). Loss of MHC antigens would favor escape from tumor surveillance immune responses. Some tumor-associated lymphocytes are positive for class I, class II antigens and interleukin 2 receptors (IL-2r), indicating activation. Variable correlations exist for lymphocyte infiltration and MHC antigen expression (Hadden, 1995).

Analysis of T-cell receptor (TCR) expression of TALs show most to be an $\alpha\beta$ TCR-positive; however, γd TCR-positive T cells have been recently observed (Bank et al., 1993); these γdT-cells may be cytotoxic but may also contribute to immune suppression (Seo & Esawa, 1995). Analysis of the $\alpha\beta$ TCR spectrum indicates polyclonality (Durie, George, Campbell & Damato, 1992) i.e. reactivity to multiple antigens. Analysis of cytokine mRNA expression showed low level expression which increases with the intensity of infiltration and is as high as 30% in mucin-positive tumors (Vitolo et al., 1992). These data indicate mixed T-cell infiltrates occur in a high percentage of breast cancers and that the T cells involved show some evidence of activation.

TILs/TALs from other cancers in general share low level interleukin-2 (IL-2) expression, little or no reactivity to T-cell mitogens like phytohemagglutinin (PHA), or in mixed leukocyte culture (MLC), and little or no cytotoxicty. These cells, when grown in IL-2, restore their proliferative and cytotoxicity responses. To the extent studied, TALs from breast cancer are similar in showing little or no killer cell reactivity, and low PHA and MLC reactivity (Hadden, 1995; O'Sullivan & Lewis, 1994; Hudson et al., 1998). In two studies, breast cancer had TILs which showed increased cytokine expression when cultured with autologous tumor cells (Ruppert et al., 1991; Schwartenruber et al., 1992). TALs from lymph nodes regional to breast cancer also show reduced CD4 : CD8 ratio, increased macrophages, depressed mitogen responses, variable cytokine expression, and variable cytotoxic responses (Horny & Horst, 1986; Alam, Clark, George & Campbell, 1993). Nodal TALs have cells which suppress normal lymphoproliferative responses (Vose & Moore, 1979). Autologous tumor reactivity of nodal lymphocytes are manifested by increased clumping with tumor cells, enhanced cytokine secretion, proliferation, and cytotoxicity with in vitro addition of tumor cells (Hadden, 1995).

TILs from breast cancers cloned with IL-2 and, in some cases, with autologous tumor, have yielded predominantly $CD4^+$ T cells with variable but generally low cytotoxicty for autologous tumor (Whiteside, Miescher, Hurlimann, Moritta & von Heidner, 1986; Belldegrun, Kasid, Uppenkamp, Topalian & Rosenberg, 1989; Radrizzani, Gambacorti-Passerina, Parmiani & Fossati, 1989; Balch et al., 1990; Skornick, Topalian & Rosenberg, 1990; Yanelli et al., 1996). In other studies, T cells cloned from peripheral blood, regional lymph nodes, or pleural effusions with IL-2 and, in some cases, with autologous tumor, yielded $CD8^+$ T lymphocytes with highly specific MHC-restricted and unrestricted killing of autologous tumor targets (Vose & Bonnard, 1982; Sato, Sato, Takahasi, Koshiba & Kikuchi, 1986; Roberts, Shipton & Moore, 1987; Jerome et al., 1991; Jerome, Domenech & Finn, 1993; Baxevanis et al., 1994; Dadmarz, Sgagias, Rosenberg & Schwart-

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