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EVIDENCE FOR IMMUNE FACILITATION OF BREAST CANCER GROWTH AND FOR THE IMMUNE PROMOTION OF ONCOGENESIS IN BREAST CANCER

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Summary Autoimmune diseases have been extensively studied in man and experimental animal models and salient points are reviewed, as a clear understanding of the immune mechanisms involved is essential if one is to understand the potential of immune interactions with established cancer cells or in the premalignant period of hyperplasia. Such reactions may be of benefit to the host, with down regulation of tumor growth, or unfavourable, with facilitation of oncogenesis and cancer growth. In particular, evidence is cited that supports a beneficial effect of the host response to non-small-cell lung cancer and the association of a poor prognosis in established breast cancer caused by a heightened immune response to the tumor. Histologic evidence supports these conclusions, as do studies of specific and nonspecific immune reactivity in breast cancer patients. The potential for cytokines to stimulate breast cancer growth, increase angiogenesis and decrease cell adhesion is reviewed, also recent evidence for autologous lymphocyte stimulation of breast cancer. Parallels between immune promotion of breast cancer in mice, caused by the mouse mammary tumor virus, and the development of breast cancer in women are also reviewed. If the mouse model has relevance for human breast cancer, one could predict that there would be a reduced incidence of breast cancer in a population of chronically immunosuppressed women following organ transplantation. Such is the case. This finding, plus the fact that all treatments that have shown efficacy in breast cancer have one thing in common, they are immunosuppressive, strongly support the role of immune facilitation of breast cancer growth and immune promotion of oncogenesis in breast cancer in a substantial number of women.

Probably the most important thing in science is to ask the right question. I think, therefore, that it might be of interest to outline the events that led to the question «could there be immune facilitation of breast cancer growth?» that appeared in an editorial[1] published in 1992.

In the late 1950's and early 60s there was great excitement in Medicine as evidence rapidly accumulated that autoimmune disease was a reality. This was seen vividly in the thyroid clinic, Grave's disease was found to be the result of stimulation of thyroid function by antibodies[2] and Hashimoto's thyroiditis resulted in destruction of the thyroid by immune cellular mechanisms of the patient[3]. An occasional patient would show features of each disease with a period of stimulation of function in an otherwise dying gland. As a young physician one became comfortable with the possibility that the immune system could stimulate or depress function. As will be shown later this feature is seen again and again when the interaction of immune function and the growth of cancer cells is examined; examples of stimulation and instances of down regulation.

Animal models were developed to study the mechanisms involved in the initiation of autoimmune diseases. In several models a specific disease could be induced by inoculating the animal with the appropriate organ antigen, autologous or allogeneic in origin, homogenized in Freund's

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complete adjuvant (FCA). In 1959 and, again, in 1962 Waksman[4, 5] reviewed some 600 published reports. An important generalization was made: the severity of the induced disease correlated very well with the intensity of a delayed hypersensitivity reaction (DHR) to the organ antigen in the animal in which the disease was produced. Severe disease was accompanied by an intense DHR, mild disease with a weak DHR. For example Brent et al[6] showed that the tempo of a graft rejection was reflected in the DHR expressed by the recipient animal when challenged by injection of soluble histocompatibility antigens of the donor animal. Where the barrier was strong, rejection was rapid and the DHR intense. Where the histocompatibility barrier was weak, there was a sluggish prolonged rejection and a weak DHR to the antigen. In autoimmune disease a correlation with the severity and tempo of the immune reaction also correlated well with the DHR to the appropriate antigen.

A further correlation could be made. In rapid graft rejection or severe autoimmune disease the stromal infiltrate of the organ with round cells would be marked. In contrast in sluggish, prolonged rejection, or mild disease, the infiltrate was much less pronounced.

In 1964 a paper was published[7] that was the first to show that 27% of 50 patients with cancer showed a positive DHR when skin tested with acellular extracts of their own tumors. This experiment was done following a suggestion by Medawar, who thought it would be interesting to ascertain if there was such evidence of an autoimmune reaction to cancer in humans. This result was intriguing. On returning to Ottawa, from a postgraduate year in the University Hospital in Ann Arbor, Michigan, in the unit of Bill Beierwaltes, funds were found to repeat this experiment. From 1966 to 1969 some 144 patients were tested with extracts of their own tumors[8, 9]. The results of Hughes and Lytton[7] were confirmed: 26% of patients had such a reaction. Further, it was discovered that the strongest reaction was induced by cell membranes and that there was a low incidence of a positive reaction where no lymphocytic or round cell infiltrate of the tumor stroma was seen. Only 5.8% of such patients were positive, compared with a much greater chance of seeing a positive reaction where the infiltrate was marked, 44.4%, or very marked, 100%.

These were exciting findings. They suggested that there was an autoimmune reaction to autologous tumor in one of four patients with a solid tumor. Would it be of benefit to patients to strengthen this reaction using the methods that successfully induced autoimmune disease in animals, vaccination with membrane antigen homogenized in Freund's complete adjuvant? It was known that several solid tumors had a very much more favourable prognosis following curative surgery when the stroma of the primary tumour showed a marked infiltrate of round cells. As examples, in post gestational choriocarcinoma, a tumor of fetal tissue proliferating in the maternal host (an F1 hybrid) a significant survival advantage was observed for those women showing a marked infiltrate of the tumor stroma when compared with those whose tumors had a mild infiltrate[10]. In 179 patients with testicular seminoma[11], 56% of patients survived more than 10 years if the tumor had a definite lymphoid stroma, and only 29% where such an infiltrate was absent. In colon cancer, a highly significant 5 year survival advantage was seen for 78 patients who had a local round cell reaction to their tumor[12] compared to 70 where this was absent. Spratt et al[13] studied 1,137 patients with colon cancer, followed for 10 years and showed that an absence of such an infiltrate carried a very ominous prognosis. In non-small-cell carcinoma of the lung improved prognosis has been associated with the degree of lymphocytic infiltration of the tumor[14], particularly in the case of patients with squamous cell carcinoma of the lung[15]. In medullary carcinoma of the breast, a tumor that makes up about 5% of breast cancers in the western world, a marked survival advantage was seen in 104 such cases of a cohort total of 1,411 cancers followed for 20 years[16]. Seventy-four percent of medullary breast cancers were alive, compared with 14% of cases with similar stage non-medullary breast cancer. A striking feature of medullary cancer is the rich stromal infiltrate by lymphocytes. Improperly treated medullary cancers had a median survival of 2.2 years suggesting that such tumors are potentially highly malignant. The authors, Bloom et al[6] stated that «their biological potential is countered by an effective host resistance».

Thus it was evident that in some tumors, a host immune reaction to the tumor gave a survival advantage. Could one increase the intensity and frequency of such favourable reactions in the adjuvant setting following «curative» surgery? The prospect of taking cell membrane tumor antigens, homogenised with Frend's complete adjuvant (FCA), and using such an homogenate to vaccinate patients intradermally held promise but had potential dangers. If normal tissue antigens were present there was potential danger of inducing severe autoimmune disease, as had been shown in men[17] where severe acute orchitis was induced following autovaccination of an homogenate of normal testicular tissue with FCA, prior to completing the orchidectomy as treatment for prostate cancer. Today such a clinical experiment would have difficulty in passing an Ethics Review Board!

So, before starting a potentially dangerous experiment it was thought prudent to examine the survival experience of the largest cohort of tumor patients that had been skin tested with extracts of their own tumors, female breast cancer. There were 56 such women of whom 12 had shown a delayed hypersensitivity reaction to extracts of their tumors. Although the longest follow-up was only six years I was confident that we would see a survival advantage in these 12 women. The results of this study were published[18] in 1971 and, shockingly, were exactly the opposite of my prediction. There were three major findings. *First*, of 52 patients tested and followed for at least 2.5 years, 40 had a negative DHR to their own tumor; of these 31 (77.5%) were alive, 9 (22.5%) had died of the cancer. Of 12 patients showing a positive DHR to their tumors 5(41.5%) were alive, 7(58.5%) were dead. The difference between the two groups was statistically significant. *Second*, a significant association was found between a positive DHR and the degree of nuclear differentiation, seen only twice in 21 moderate to highly differentiated tumors but in 10 of 35 patients with anaplastic tumors, a finding that had been predicted by Maurice Black[19]. *Third*, antigens from one patient gave a positive reaction in another with breast cancer, suggesting common antigens. Two conclusions were suggested in the discussion; that we were seeing immune facilitation of tumor growth, and that a viral influence might be present in the origin of breast cancer.

In 1971, I abandoned any further study of breast cancer. For the next 20 years, I collaborated with Ariel Hollinshead of George Washington University, Washington DC, in the study of immune reactivity of non-small-cell lung cancer patients[20]. In 1973, we started a trial of specific active immunotherapy of such lung cancer in patients having had curative surgery. Over the years our initial positive findings of improved survival[21] in those patients who had received adjuvant vaccination were independently confirmed by Takita et al[22] in a study of squamous cell lung cancer patients and eventually, in the company of Pittsburgh, in a large Canadian-US multicenter trial. However, this last trial overall gave a negative result, a cause of much tribulation. In the paper[23] describing this study three pages were used to describe the massive protocol violations that were recognized.

By 1991, we had accumulated 6 cases in which metastases regrew many years following curative resection of lung cancer, with no new primary tumor discovered[24,25] an exquisitely rare observation in lung cancer. On the strength of these observations the first international workshop on tumor dormancy[26] was held in Ottawa in October 1991. In preparation for this workshop, two meta-analyses were performed. The first[27] examined the effect of alkylating agents given as adjuvant therapy for non-small-cell lung cancer in 10 randomized trials. A significant worsening of survival was seen in patients receiving such therapy compared with controls. This finding has been confirmed in a much larger meta-analysis[28] published in 1995, with a combined hazard ratio of 1.15 ($P = 0.005$). The second meta-analysis[29] showed a highly significant survival advantage in patients effectively immunized, showing a DHR to tumor antigen at one year postsurgery greater than ≥ 2 cm compared to nonimmunized randomized controls who did not show this reaction.

Taken together one concluded that boosting the autoimmune reactivity to lung cancer was beneficial in the adjuvant setting, whereas the use of strongly immunosuppressive alkylating agents worsened the prognosis by disarming such helpful reactivity. In the adjuvant treatment of breast cancer such immunosuppressive drugs as melphalan or, more convincingly C,M,F (Cyclophosphamide, methotrexate, 5 fluorouracil) were beneficial[30]. The use of cortisone in advanced

lung cancer was detrimental in a randomized trial[31],.compared to controls (P = <0.02), causing a more rapid death due to lung cancer growth, whereas in advanced breast cancer cortisol can be beneficial[32] whilst nonspecific immunostimulation was shown to be detrimental[30]. Thus, the question of possible immune facilitation of growth of established breast cancer was then raised. What strategies should be used to find evidence that this was so? These are listed as follows, and I will say a few words on each.

1. Read the extensive publications by Richmond Prehn on immunostimulation of tumor development.

2. Examine the literature that has sought evidence for immune reactivity in breast cancer by pathologists and surgeons, correlated with prognosis.

3. Examine the literature that has tried to link specific and nonspecific immune reactivity in breast cancer patients with prognosis.

4. Perform a literature search for evidence that cytokines can stimulate breast cancer growth, increase angiogenesis, and decrease cell adhesion.

5. Review evidence that autologous lymphocytes can stimulate in vitro growth of breast cancer.

6. In terms of immune promotion of oncogenesis in breast cancer see if there are parallels between the immune promotion of breast cancer in mouse models and in women.

1. The reader may consult some key reviews by Prehn, to obtain an understanding of the origination and development of evidence supporting the role of the immune system in stimulating tumor development and growth[33,34,35,36,37]. The literature is large and convincing.

2. The association of stromal cell stimulation and worsening of prognosis in a subset of patients with breast cancer was reviewed in detail[38] in 1993. Published articles in the literature are divided into three groups. Those that showed a good prognosis when the stromal infiltrate is intense, 9 articles, of which 6 were descriptions of medullary cancer. Two articles concluded that there was no relationship with prognosis. Twenty-three articles showed a worsening of prognosis where the stromal infiltrate was intense. Eleven papers described the stromal infiltrate in inflammatory breast cancer, which has an apalling prognosis. Of the remaining 12 publications, 6 are particularly impressive; Champion et al[39], Fisher et al[40], Meyer and Hixon[41], Fisher et al[42], Parkes et al[43] and Rosen and Groshen[44]. Overall one may conclude that the immune system may down regulate tumor growth in medullary cancer, or stimulate growth of other breast cancers. A particularly telling case for the latter was provided by Kurtz et al[45]. They studied 18 factors in 496 stage I-II duct cancers treated by conservative surgery and radiotherapy. Multivariate analysis showed that a major lymphocytic stromal reaction was the factor most strongly correlated with recurrence in younger women. The authors concluded that the intensity of the cellular reaction may reflect a cancer-host response that favours rather than impedes cancer growth.

A retrospective study[46] of 1,200 cases of breast cancer showed that the overexpression of the c-erb B2 oncogene indicated a bad prognosis in node positive patients. The oncogene overexpression was strongly associated with the presence of a lymphoplasmacytic infiltration in the tumor ($P = 10^{-6}$). Length of follow-up was 19 years. In a study of 106 primary breast cancer by Tang et al[47] samples were analyzed for the oncogenes C-erb B2, Int-2, and C-myc. A very strong association was found between oncogene amplification and dense lymphocyte infiltration of the tumor, becoming highly significant in those tumors presenting high level amplification of C-erb B2 and Int-2 (P = 0.0007). The authors concluded that cytokine production may be associated with paracrine immunological phenomena which are themselves associated with a poor prognosis. A recent extensive review[48] of the literature (94 references) concluded that Her-2/neu overexpression (C-erb B-2) is an independent indicator of increased risk of recurrent disease in women with node-negative cancer. A strong negative association was found between high levels of C-erb B2 amplification and absence of estrogen receptor or progesterone receptor.

3. Specific and non-specific immune reactivity in breast cancer patients is associated with a poorer prognosis. Non-specific stimulation of breast cancer patients using the method of Bacil-

lus Calmette Guérin (BCG) inoculation or scarification as an adjuvant therapy significantly worsened the prognosis[30] by 20%.

In a study[49] of 134 patients with breast cancer and 63 patients with benign breast lesions assessing immune responses in vitro by lymphocyte stimulation, «a striking and paradoxical finding was the demonstration that patients with low risk overall had markedly and significantly lower responses to the battery of stimulating mitogens and antigens than patients with high or intermediate risk disease».

A series of 77 patients with inflammatory breast cancer were treated with radiotherapy and chemotherapy[50]. Half the patients were randomized to receive BCG. A paradoxical finding was that patients who were tuberculin negative on skin testing survived significantly better than those who were tuberculin positive.

A study was designed[51] to show that inflammatory breast cancer in Tunisia was the result of failure of immune surveillance. This aggressive tumor accounts for 58% of all breast cancers in that country. An unexpected finding was that women with this tumor had a delayed hypersensitivity reaction to soluble breast cancer antigen with a frequency three times greater than that shown in women with other forms of breast cancer. Tunisian adults were shown to have increased immune reactivity when compared to a comparable cohort of adult Americans[52].

In another study[53] the thymidine labelling index (TLI) was studied in the primary invasive carcinoma of 133 patients. Operable patients with indices above the median had a significantly higher rate of recurrence than those with indices below the median. Regression analysis showed a significant linear increase of log TL1 with increasing degrees of inflammatory cell reaction at the margin of the breast cancer ($P < 0.001$). The authors stated, «this anomalous association of a risk factor for early relapse with features that imply a good prognosis invite an attempt at explanation».

Cannon et al[54] studied the lymphoproliferative responses of peripheral blood mononuclear cells in 96 stage I and II breast cancer patients and 36 lung cancer patients in the early postoperative period following mastectomy or full or partial lobectomy. The test used was a one-way mixed leukocyte culture (MLC) against a pool of mitomycin-C treated lymphocytes from 6 allogeneic donors. Depressed lymphoproliferative responses were associated with a significantly longer disease-free interval in breast cancer patients and a significantly shorter disease-free interval in lung cancer patients. The MLC measures recognition by T cells of alloantigens determined by the major histocompatibility complex present on the lymphocyte surface membrane.

Using S1 mapping, RNA blotting and *in situ* hybridization Parkes et al[55] analyzed material taken from 34 patients who had been operated for primary breast cancer in 1976 and followed for 11 years. Plasma cells were found in tumors from 84% of women who had relapsed and died (19 patients) whereas plasma cells were detected in only one tumor of 15 women who had survived (6%), a case of medullary carcinoma, free of disease. The authors concluded that the presence of plasma cells in infiltrating duct carcinoma and mixed infiltrating duct and lobular carcinoma is associated with a poor prognosis.

Using the Prausnitz-Küstner reaction, Grace and Dao[56] found that a patient's antibodies may be responsible for inflammation seen in inflammatory breast cancer. The C-erb-2 onco protein is amplified and overexpressed in inflammatory breast cancer and noninflammatory breast cancer with positive axillary nodes[57]. Antibody responses have been identified in the sera of patients having breast tumors having this oncoprotein, in 647 cases which also had a lymphoplasma cell infiltrate[58], and in 11 of 20 premenopausal breast cancer patients[59]. One patient with the greatest antibody response also showed a significant proliferative T cell response to the HER-2/neu protein and peptides. The authors of this paper caution that immunity to this protein could possibly lead to tumor stimulation by release of inappropriate cytokines at sites of tumor deposition. In a recent study[60] of a newly isolated human breast cancer cell line it was shown that a monoclonal antibody that binds to the extracellular domain of erb B-2 is a potent growth factor for these cells. The conclusion reached was that at least some breast cancer cells are stimulated by such antibodies. Com-plimenting this finding was an earlier study by Stancovski et al[61]. These authors showed that monoclonal antibodies to the extracellular portion of the ERBB2 protein of human breast cancer cell lines could either inhibit or

strongly stimulate the growth of these cancers in athymic mice. They also caution that antibody therapy could accelerate tumor growth.

4. The effect of cytokines on breast cancer growth, angiogenesis and cellular adhesion have been the subject of much investigation.

Tumor infiltrating lymphocytes (TIL) in human breast cancer have been extensively studied for their capacity to produce cytokines[62,63,64]. It is hoped that down regulation of tumor growth and killing of tumor cells in vivo might be accomplished if appropriate cytokines could be identified and delivered to breast cancer cells in the host. The published literature on cytokines is already vast and is growing rapidly, and is impossible to review in-toto. Two reviews[65,66] show clearly that cancer infiltrating mononuclear cells produce cytokines that can either stimulate or down regulate cancer growth. This is an important conclusion that must be emphasized as it is not universally appreciated. For macrophages, that represent a major component of the lymphoreticular infiltrate of tumors, promotion of growth of tumor cells in vitro is best seen at low effector to target cell ratios[67]. Rubbert et al[62] found that TIL could produce tumor necrosis factor (TNF) when autologous tumor cells were added to lymphocyte cultures. Mitogen stimulated TIL produced low levels of IL-2, TNF and IFNα. Vitolo et al[64] studied the mRNA expression for cytokines in TIL. In ductal breast cancers which contained intracellular or intraductal mucous up to 30% of lymphoid cells in the tumor stroma were positive for IL-2, TNFα, IFNα and IL-2R. Schwartzentruber et al[63] showed that TIL stimulated by autologous breast cancers secreted TNFα, GM-CSF and IFNα.

It is therefore reasonable to cite evidence that specific cytokines can stimulate breast cancer growth, promote angiogenesis, and affect tumor cell adhesion. Peoples et al[67] have shown that TIL produce heparin-binding epidermal growth factor-like growth factor (HB-EGF) and basic fibroblast growth factor (bFGF) in vitro under nonspecific conditions and in vivo in tumors by immunohistogical staining. HB-EGF and bFGF derived from TILs directly stimulated breast cancer cells to grow in vitro and also stimulated vascular smooth muscle cells whilst bFGF displayed angiogenic properties. Another potent angiogenic factor[68], vascular endothelial growth factor, (VEGF) is produced by TIL in situ at bioactive concentrations in human prostate and bladder cancers. Recent data[69] has shown that human breast cancer cell lines are stimulated to grow by VEGF. This is so for hormone dependent and independent cells.

Tumor necrosis factor (TNF) has been shown to stimulate DNA synthesis[70] of primary epithelial cells cultured from normal or lactating mouse mammary glands and stimulated the growth of two mouse breast tumor lines and inhibited the growth of a third line, derived from the same primary tumor as one of the tumor lines that was stimulated by comparable ranges of TNF. Enhanced tumor progression in vivo has been shown when TNFα or interferon is given to mice bearing preneoplastic breast lesions[71]. This data suggests that enhanced tumor progression is mediated by natural killer cells and T cells. TNF is a potent inducer of angiogenesis in vivo[72,73], and is produced by macrophages. The importance of tumor angiogenesis and metastasis in invasive breast carcinoma has been emphasized by Folkman et al[74,75].

Interleukin-6 can inhibit the proliferation of several duct carcinomas of breast in vitro but at the same time it enhances motility of breast cancer cells and causes increased cell-cell separation with decreased adherence type function formation, reviewed by Sehgal and Tamm[76]. Human mammary epithelial cells transfected with the int-2 gene are stimulated to grow by IL-6[77].

Colony-stimulating factor-1 (CSF-1) has been suggested as a key mediator of breast cancer invasion and metastasis[78]. Autocrine growth stimulation due to the combined expression of CSF-1 and its receptor in breast cancer cells is considered probable. Paracrine stimulation of tumor cell growth is suggested by the finding of oncogene amplification and correlates with a dense lymphocytic infiltration in human breast cancers[47].

Scholl postulates that breast cancers express receptors for several growth factors and can, at least potentially, be stimulated to proliferate in the presence of the corresponding ligands, produced by infiltrating monocytes. Direct evidence for such stimulation has been published, reviewed in the preceding paragraphs.

5. *Autologous lymphocyte stimulation of breast cancer in vitro*

In 1974, Fidler et al[79] reported that there was stimulation of tumor growth in vitro by low ratios of sensitized lymphocytes to tumor cells. Of the 13 dogs used in these studies two had breast cancer; a minature poodle and a beagle. The addition of autologous serum to the lymphocyte-tumor cultures potentiated the stimulation of growth. These authors concluded that their results supported the work and hypothesis of Prehn.

More recently, Ögmundsdottir et al[80], were able to establish primary cultures of breast cancer and unaffected tissue in the serum-free hormonally defined highly supplemented growth medium CDM3 of Petersen and van Deurs[81]. Cocultures of fresh samples of breast carcinoma and autologous lymphocytes were sucessful in 20 cases. The highest growth score was seen with lymphocytes in 11 such cultures. In 5 of 17 cocultures of uninvolved breast tissue and lymphocytes optimal growth was seen with lymphocytes. Growth stimulation in response to lymphocytes was significantly associated with the expression of MHC class I by the tumor cells, with a not significant trend to the expression of the adhesion molecule ICAM-1.

At this point it is reasonable to conclude that there is immune facilitation of tumor growth in a substantial subset of women with established breast cancer. Reinforcing the conclusion is the fact that all treatments that have given positive results in breast cancer have one thing in common, they are immunosuppressive to a greater or lesser degree. This topic has been reviewed in detail by Stewart et al[82] and only salient points will be emphasized here.

a) In patients having metastatic breast cancer treated with corticosteroids, median response rates of 30% have been seen, lasting 3-14 months. Additive effects are seen when corticosteroids are combined with endocrine therapy. In vitro studies have shown that corticosteroids can facilitate the growth of breast cancer cells, cause inhibition of growth or show a dose dependent biphasic response. The immunosupressive effects are quite clear cut, where a clear dose response exists for suppression of Interleukin 1 (IL-1), IL-2 production by macrophages and lymphocytes, and suppression of IL-6 and TNF production by human monocytes.

b) Adjuvant chemotherapy with cyclophosphamide, methotrexate and fluoruracil (CMF) is immunosuppressive and causes prolonged impairment of certain aspects of B, T and NK cell function. Zielinski et al[83] reviewed their studies showing a depression of antibody production following vaccination, prolonged impairment of mitogen-induced soluble interleukin-2 receptor production, and a decrease in proliferation of peripheral blood mononuclear cells following phytohemagglutinin stimulation. The depressed activity was seen up to 3 years following cessation of CMF therapy.

c) Escalating doses of chemotherapy with allogeneic or autologous bone marrow transplantation causes marked and prolonged immunosuppression lasting up to 2 years[84,85]. Defective production of IL-2 is seen up to 18 months following high-dose cyclophosphamide and whole body irradiation.

d) Locoregional radiotherapy following a mastectomy for breast cancer has been found to confer a systemic benefit for survival, reviewed by Stewart[86]. One explanation for improved survival is that there is a significant T-cell lymphopenia and decrease in T-cell responses that can persist for as long as 11 years in women so treated for breast cancer.

e) Robinson et al[87] found that in women treated for bilateral breast cancer and now without evidence of disease, NK cell activity was higher than in normal controls and declined on long term tamoxifen therapy. Tamoxifen also reduced CD4+ T cells and the CD4/CD8 ratio in such patients.

6. There is evidence for immune promotion of oncogenesis. Much of the evidence cited to support the concept of immune facilitation of established breast cancer was provided by S. C. Jane Tsai, a co-author of several papers on this topic. Over a three-year period she educated me on the mouse model of breast cancer caused by the mouse mammary tumor virus; MMTV. It is now sixty years since Bittner[88] observed that mice with a high incidence of spontaneous mammary cancer passed this disease to their offspring through nursing. This led to the discovery of a milk factor, a retrovirus. The usefulness of this mouse mammary tumor model is reviewed by Wei et al[89].

Distinct preneoplastic lesions precede the tumor, the most common of which is the hyperplastic alveolar nodule. NK cells and macrophages infiltrate this preneoplastic lesion. Stimulation of the NK cells by polyinosinic-polycytidylic acid or IL-2 enhanced tumor progression with a decrease in the latency period of mammary adenocarcinoma and an increase in its incidence. In contrast, various immunosuppressive manoeu-vres such as neonatal thymectomy and administration of anti-lymphocyte globulin or antiasialo-GM1 (which reduces NK cell activity) decreased the incidence of tumors. Wei et al[89] conclude that the stimulation of oncogenesis in the mouse is a byproduct of the immune reaction to the superantigen of the mouse mammary tumor virus. There is data that shows 38-40% of human breast cancers contain gene sequences homologous to the MMTV *env* gene that are absent from other tumors and tissues[90].

If a sizeable subset of women share a pattern of immune promotion of neoplastic progression or oncogenesis, similar to that observed in mice carrying the MMTV, then one might expect women who are chronically immunosuppresseed to show a low population incidence of de-novo breast cancers. Such a predition was made in Brugge[91], April 1994, at the Lancet conference "The Challenge of Breast Cancer". To investigate this hypothesis the incidence of breast cancer was assessed in just such a population female transplant recipients. Results were based on data provided to the Collaborative Transplant Study in Heidelberg[92] since 1983 and published in September 1995.

The overall incidence of breast cancer was significantly lower amongst 25,914 transplant recipients than would be expected from background rates. During the follow-up period of 1-11 years, 86 cases were observed compared with 113.8 expected (X_1^2 = 6.75, p = 0.009). Incidence was particularly low in the first transplant year with relative risk 0.49 (95% CI 0.64 to 1.03). A subset of 13,003 women received a combination of cyclosporin, azathioprine and steroids (CSA), the remainder only one of two of these drugs. There were 30 cases of breast cancer in the 13,003 patients, 53.8 expected, and 56 cases in 12,911 patients treated otherwise, 60 expected, (RR 0.58, 95% CI 0.36 to 0.93, p = 0.011).

Furthermore the CSA group had a very low rate after the first year, with 24 observed cases rather than 39.8 expected, giving an SIR of 0.60. The low breast cancer rate applied only to kidney recipients particularly in 8,166 North American women where the incidence was halved. The number of heart recipients was small, 2,185, and the results are less reliable since the sample size is small. All other major cancers had higher than expected incidence, in some cases substantially with a marginal increase in others.

The finding of a decreased incidence in transplant recipients supports the notion that, in a subset of women who develop breast cancer, there is immune promotion of cancer. At least a decade ago an appealing concept was offered to explain why some women are at high risk for breast cancer namely, failure of immmunosurveillance. Natural killer (NK) cells are a subpopulation of large granular lymphocytes which are thought to be involved in immunosurveillance against tumor cells and defence against virus infections. Pross[93] et al studied NK function over 5 years in 155 such women at high risk and, contrary to all expectations, they found higher NK activity in women with benign breast syndrome (BBS) than in those without BBS or Stage I cancer. Women with BBS had atypical lobular hyperplasia either diffuse or localized. In patients with no evidence of BBS at the initial evaluation but who subsequently developed BBS, the NK cells became more reactive. Pross and co-workers suggested that the increased activity could be due to viral stimulation of interferon production or that it might reflect the systemic effect of hormonal status. They cited seven other studies that measured immunocompetence in patients with benign breast disease and increased risk for developing cancer. In all these other investigations, immunoreactivity in such patients was higher than in healthy cohorts. Of particular relevance to the MMTV model was the observation[94] that women with hyperplastic benign breast disease had a higher frequency of enhanced MMTV antigen leucocyte migration (a test of cell-mediated immunity) than women without. Such hyperplastic lesions were judged homologous to the hyperplastic alveolar nodule in mice, which is likewise associated with enbanced migration[95].

Thus there is a clear parallel between increased NK cell activity in the preneoplastic lesions in mice and the increased immune reactivity of women at high risk for developing breast

cancer, more particularly increased NK activity. The effects of immunosuppression on mice bearing preneoplastic lesions and in women immunosuppressed for organ transplantation strongly suggest that a viral cause of breast cancer in a subset of women should be considered[90]. If true, then future strategies could be developed that could reduce the incidence of this pandemic cancer.

Conclusion

The evidence reviewed provides strong support for immune facilitation of the growth of established breast cancers in women. The parallel between the effects of immunosuppression of mice infected with the mouse mammary tumor virus and in women chronically immunosuppressed following organ transplantation suggests that immune promotion of breast cancer oncogenesis is a reality in a substantial subset of women and raises the serious possibility that a retrovirus may be involved in the development of breast cancer in such women.

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Resumen

Inmunopromoción del cáncer de mama e inmunofacilitación de su crecimiento

Se recalcan los mecanismos inmunes involucrados en las enfermedades autoinmunes en el hombre y en modelos experimentales con el fin de comprender el potencial de las interacciones inmunológicas con las células cancerosas o durante el periodo de hiperplasia premaligna. Estas reacciones pueden llegar a ser beneficiosas para el huésped con inhibición del crecimiento tumoral, o perniciosas con facilitación de la oncogenesis y crecimiento tumoral. En particular, se presenta evidencia del efecto beneficioso de la respuesta inmune del huésped al cáncer de púlmon no a células pequeñas, en contraste con la asociación de mal pronóstico en cáncer de mama debido a una respuesta inmune aumentada. Estas conclusiones encuentran apoyo en observaciones histológicas de reactividad inmunológica específica e inespecífica en pacientes con cáncer de mama. Sigue una revisión acerca del potencial de las citoquinas para estimular el crecimiento de este tipo de tumores, incrementar la angiogenesis y disminuir la adhesión cellular, así como tambien las recientes evidencias que muestran la capacidad de los linfocitos autólogos en la estimulación del cáncer de mama. Se describe tambien la existencia de un paralelismo entre la promoción inmune del cáncer de mama en el ratón causado por el virus del tumor mamario murino y el desarrollo del cáncer de mama en la mujer. Si el modelo murino tuviera relevancia para la mujer, se podría predecir que habría una reducción en la incidencia del cáncer de mama en la población de mujeres crónicamente inmunosuprimidas después de un trasplante de órgano. En efecto, esto es lo que ocurre. Este hallazgo, junto con el hecho que todos los tratamientos que han resultado efectivos en combatir el cáncer de mama comparten el hecho de ser inmunosupresores, apoyan fuertemente el rol de la inmunofacilitación en el crecimiento de los tumores de mama y la promoción inmune de la oncogenesis en el cáncer de mama en un número apreciable de mujeres.

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