Tumour-infiltrating CD4 and CD8 T lymphocytes in breast cancer

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SUMMARY

Lymphocytes are cells circulating between the blood and tissues. It has been stated that a correlation exists between immune infiltrate and breast cancer. These tumours are infiltrated by T cells, B cells, natural killer cells and macrophages. The infiltrating T cells are of helper ($CD4^+$) and cytotoxic ($CD8^+$) phenotypes. Specific immunity mediated by cytolytic T lymphocytes is suspected of playing an anti-cancer role. It is widely known that regional lymph nodes are an important immunological defence or "barrier" against tumour expansion. Some authors have reported that in cancer patients natural killer (NK) cells and $CD8^+$ T cells are diminished in regional lymph nodes, particularly those involved by the tumour.

In the presented study the authors review current knowledge on this problem and the possibility of using successful immunotherapy with monoclonal antibodies for breast cancer.

KEY WORDS: breast cancer, $CD4^+$, $CD8^+$

Lymphocytes are mobile cells, continuously recirculating between the blood and tissues, returning to the blood via the lymphatic system. It has been stated that peripheral blood contains approximately 2% of all lymphocytes. The rest of these cells can be found in lymphatic organs, namely the tonsils, spleen, young thymus and, especially, lymph nodes [1].

The correlation between immune infiltrate and breast cancer has been widely investigated since the early work of Black [2]. Several studies have stated that these tumours are infiltrated by a heterogeneous population of immune cells, namely T cells, B cells, natural killer (NK) cells and macrophages [3]. Tumour infiltrating lymphocytes (TIL) are of helper ($CD4^+$) and cytotoxic ($CD8^+$) phenotypes, and express activation markers such as CD25 and the transferrin receptor [4]. Tumour-infiltrating lymphocytes are able to produce and release vascular endothelial growth factor (VEGF) and basic fibroblastic growth factor, which can induce lymphangiogenesis and angiogenesis and consequently could permit the dissemination of cancer cells through lymphatics to regional lymph nodes [5]. Antitumour effects are mediated through the induction of a $CD8^+$ T cell response against a specific antigen. Cytotoxic $CD8^+$ lymphocytes and NK cells bind with the Fas ligand and cause tumour cell death. TIL can interact with tumour antigen and cause direct or indirect cell lysis by releasing cytokines such as: interferon γ (INF-γ), tumour necrosis factor α (TNF-α), and granulocyte colony-stimulating factor (GM-CSF).

Functionally T cells lack antibody-dependent cellular cytotoxicity and show no or low natural killer (NK) activity [6]. A quantitative assessment of cell-mediated immunity is the measurement of subpopulations of T-helper and T-suppressor lymphocytes. Impaired cellular immunity can be associated with a depressed CD4/CD8 ratio. The exact relation between the efficacy of T cell-dependent immune mechanisms or between the extent and type of lymphocyte infiltration and tumour progression in carcinoma of the breast is a matter of debate [7,8]. This interrelation is suspected of determining the influence of the immune system on the pathogenesis and progression of breast cancer [9]. Activity of tumour-infiltrating lymphocytes is impaired

by inhibitory cytokines, increased regulatory T lymphocyte reaction, tumour cell MHC molecule alterations, and aberrant Fas ligand expression [10].

Adaptive immunity is mediated by antibodies and by $CD4^+$ and $CD8^+$ T cells, and it usually exploits an indirect pathway, termed cross-priming, to achieve initial recognition of cancers [11]. Specific immunity mediated by cytolytic T lymphocytes is suspected of playing an anticancer role. Nevertheless, tumours have developed various mechanisms such as downregulation, mutation, or loss of HLA class I molecules to escape from T-cell-based immune surveillance [12]. It is widely known that regional lymph nodes are capable of being an important immunological defence or "barrier" against tumour expansion [13]. Several studies have reported that removal of uninvolved lymph nodes might have a devastating effect on the immunological defence of the host against systemic metastasis or tumour recurrence [14]. Some authors have reported that in cancer patients natural killer (NK) cells and $CD8^+$ T cells are diminished in regional lymph nodes, particularly in the lymph nodes with tumour involvement [15, 16]. Nodal status as determined by pathological examination of lymph nodes has been commonly shown to be the single most important predictor of survival in breast cancer [17, 18]. A direct relationship between the risk of distant recurrence and number of involved axillary nodes has been established [19]. Wong et al. [20] demonstrated that the tumour-infiltrating lymphocytes of patients with positive lymph nodes had greater tumoricidal activity.

In an analysis of the peripheral blood and axillary lymph nodes of 40 breast carcinoma patients, Whitfort et al. [15] demonstrated that while there was little difference in the overall proportions of T and B lymphocytes, there was a larger $CD4^+$ helper T cell population in regional nodes that had been invaded by cancer. Rubbert et al. [16] also showed a decline of $CD8^+$ T cells in regional lymph nodes, particularly in lymph nodes with cancer involvement in breast carcinoma patients. These data indicate that draining lymph nodes of cancer patients may host specific or non-specific suppression of NK cells and CD8+ T cells, which seems to be responsible for their poor immunological activity. Battaglia et al. [21] tried to determine the distribution of a diverse lymphocyte population in normal human lymph nodes in comparison with corresponding peripheral blood. In their study they examined uninvolved lymph nodes in an early stage of cervical or endometrial cancer and benign diseases. The authors found that $CD4^+$ lymphocytes were equally represented in both lymph nodes and peripheral blood, whereas $CD8^+$ lymphocytes were much less numerous in lymph nodes. Consequently the $CD4^+/CD8^+$ ratio in lymph nodes was almost twice that of peripheral blood. Natural killer cells were $<2\%$.

Georgiannos et al. [22] noted marked variation in intensity of the infiltrate between individual cancers of 60 examined patients with breast cancer with over 90% of tumours having a moderate or intense infiltrate. T-helper ($CD4^+$) and cytotoxic T cells ($CD8^+$) were found in all primary tumours, but without visible distribution dependency within individual tumours. The infiltrate changed from one that was $CD4^+$ predominant to one that was cytotoxic $CD8^+$ predominant. Analyzing the association between the characteristics of the immune infiltrate and established markers of prognosis they reached significance only in the intensity of the infiltrate of TIL cells and the number of positive lymph nodes and with ER (oestrogen receptor) expression, but not with PR (progesterone receptor) expression.

Dadmarz et al. [23] separated tumour infiltrating lymphocytes from breast tumours, metastatic lymph nodes and malignant pleural effusions from 34 patients with breast cancer. In addition to screening bulk TIL cultures, cells were separated into $CD4^+$ and $CD8^+$ subsets and extensively studied. Three $CD4^+$ TIL lines were found to secrete significant amounts of GM-CSF and TNFα. In contrast to the $CD4^+$ T cells, the $CD8^+$ TIL cells did not secrete TNFα, but they did secrete GM-CSF when cultured with autologous tumour without presence of other stimulators. In the study of Kohrt et al. [24] percentage analysis of nodes involved by breast tumour and magnitude of CD4 and CD8 changes did not show a statistically significant relationship. These observations oppose a simple linear relationship between immune alterations and tumour

invasion, but suggest that actually tumour invasion can be preceded by dynamic changes in the immune profile within tumour-draining lymph nodes.

Macchetti et al. [9] used flow cytometry to analyse the tumour-infiltrating leukocytes of breast cancer patients with T1 to T2 breast tumours in an attempt to correlate phenotypical markers of tumour-infiltrating leukocytes with axillary lymph node status. The surface characteristics were determined by using preparations of monoclonal antibodies which allowed the lymphocytes to be phenotyped as T cells, B cells, $CD4^+$ T cells, $CD8^+$ T cells, or NK cells respectively. In patients with lymph node metastasis, an increased mean percentage of tumour-infiltrating $CD4^+$ T cells, but not $CD8^+$ T cells, was observed which was correlated with worse prognosis.

Although TIL are activated in most breast cancers, implying recognition of tumour antigen, cell-mediated immunity is obviously incomplete. This may emphasize the potential role of immunotherapy for breast cancer and suggest that it may succeed in targeted patients, especially those with minimal residual disease. Lindencrona et al. [25] showed that HER-2 specific tumour immunity after DNA vaccination by plasmid (HER-2 and CM-CSF) relied completely on both CD4 and CD8 T cells, and that anti-HER-2 antibodies were not necessary to elicit a protective antitumour immune response.

Successful immunotherapy as a form of adjuvant therapy for breast cancer relies on the demonstration that cell-mediated immunity against tumour cells remains functional [26]. The antitumour effects in therapy by cytokines (GM-CSF) were mediated through the induction of a $CD8^+$ T cell response [27]. However, it is not certain that this infiltrate is necessarily present in an anti-neoplastic role. Presence of class II antigens in tumour cells could enhance the immune response against the tumour through the ability to present antigens, or by stimulating the proliferation of allogenic lymphocytes through the generation of allospecific cytotoxic T lymphocytes. It is now possible to manipulate immunological effector cells or antigen-presenting cells ex vivo in order to induce an effective antitumour response. Numerous attempts using autologous breast cancer cells and tumour infiltrating/associated lymphocytes (TIL/TAL) have been made to identify breast cancer associated antigens but they were not fully successful [28].

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