Main immunoregulatory mechanisms that favor breast cancer development

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Abstract

Even after the improvements made in recent years in early diagnosis and treatments, breast cancer is still the most common cancer and the leading cause of cancer death in women around the world. Several attempts to design new alternative therapies like immunotherapy have been evaluated in clinical trials, but they have shown limited efficacy. The failure of immunotherapy may be related to suppressive mechanisms in the tumor environment. Consequently, for the development of new immunotherapy based treatment strategies is very important to understand the immunoregulatory mechanisms present in the tumor microenvironment. Some of the immunoregulatory mechanisms described in breast cancer will be discussed in this review.

KEY WORDS: *Breast cancer. Immunotherapy. Immunoregulation. Immunosuppression.*

Introduction

Cancer is a disease that affects a large number of people and constitutes one of the main causes of death in the entire world. Most important neoplasms include breast cancer, which is the malignancy with the highest incidence and mortality in women worldwide[1]. In Mexico, breast cancer is the malignancy with the highest incidence and mortality[2]. Breast cancer most common type is ductal carcinoma, which originates in cells of the galactophorous ducts that carry milk from the lobules to the nipple. Breast cancer can also originate in cells of the lobules (lobular carcinoma), which are the milk-producing glands. Less often, breast cancer can originate in stromal tissues, including fatty and fibrous connective tissues of the breast[3]. Inflammatory carcinoma is an infrequent breast cancer variant that is highly aggressive, has a 5-year survival rate of 40% and affects especially young women[4,5]. Cancer cells can invade surrounding healthy breast tissue and reach axillary lymph nodes, thus obtaining a door for access to other parts of the body. In addition to histological classification, there are breast cancer subtypes depending on genetic alterations, gene differential expression and the type of leukocytic infiltrate, among other factors[6]. Together, these factors determine each tumor's biological behavior and treatment response[7].

Breast cancer treatment depends on the type of cancer and the degree of dissemination[8]. Current treatments are highly aggressive for patients and consist in surgical removal of the tumor and adjuvant therapies such as radiotherapy, chemotherapy, immunotherapy and hormone therapy. Their efficacy largely depends on an early diagnosis enabling small tumors to be eliminated by surgical or chemical methods; however, even at early stages, treatment cannot warrant tumor cell complete elimination, which results in a high rate of disease recurrence. The risk of breast cancer relapse depends both on tumor extent and biological

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characteristics. Lymph node involvement status is one of the most widely used prognostic factors, with a clear correlation existing between the number of lymph nodes involved and the risk for relapse. Recurrent cancer can appear at the same site (local recurrence), in a nearby area such as the chest wall and infraclavicular or supraclavicular lymph nodes (regional recurrence) or at distant sites such as the liver, the lung or the brain (distant recurrence)[9]. Unfortunately, recurrent cancer is highly aggressive and develops rapidly, which largely limits treatment options and efficacy. One treatment alternative that has been investigated in recent years is immunotherapy, but immune tolerance mechanisms that normally protect against the development of autoimmune diseases have been observed to be able to be used by several tumors to evade or suppress local immune response, thereby hindering the development of effective anti-tumor immunity and limiting the therapeutic effects of different immunologic strategies such as the use of dendritic cell-based vaccines[10-12]. Consequently, for the development of new immunotherapy strategies it is highly important to understand the immunoregulatory systems present in tumor microenvironment. Here, some of the immunoregulatory mechanisms described in breast cancer will be discussed.

Tumor antigens

Tumor cells can express proteins that are recognized as antigens by the immune system. These proteins can be tumor-specific antigens (TSA), such as mutated proteins, chromosomal aberrations or some viral proteins' derivatives. There are also the so-called tumor-associated antigens (TAA), which are not tumor-specific and can be found in healthy tissues, but are more commonly expressed by tumor cells than TSA[13]. TAAs may be originated by overexpression of cellular proteins that normally are not exposed to the immune system, such as embryonic proteins[14]. Most TAAs are weak immunogenes because they derive from the body's own proteins, which limits immune recognition. In addition, lymphocytes that are able to recognize these TAAs have low affinity owing to thymic tolerance processes[13]. TAAs aforementioned characteristics have hindered the search for therapeutic targets. Nevertheless, some TAAs showing some of the characteristics required to be therapeutically used in breast cancer, such as being immunogenic and being expressed in tumor cells in a significant percentage of patients, have been identified. Among them, one of the most important is human epidermal growth factor receptor 2 (HER2), which is expressed in approximately 25% of primary tumors. HER2 is a type 1 transmembrane oncoprotein that was initially described in breast cancer pathogenesis in 1987[15]. HER2 expression is associated with poor prognosis, since this kind of tumors are of rapid evolution and dissemination. Currently, monoclonal antibodies against HER2 have been generated, such as trastuzumab, which is a humanized monoclonal antibody that recognizes HER2 extracellular domain and that was approved by the US Food and Drug Administration in 1998[16]. Its clinical use has improved the survival of patients with breast cancer at early and advanced stages[17]. Trastuzumab's mechanism of action consists in preventing HER2 receptor dimerization, thereby blocking cell proliferation-promoting protein kinases signaling and activation. However, nearly 15% of treated patients relapse in a 12-month period[10,18]. The mechanism of resistance to trastuzumab is HER2 alternate dimerization with other proteins of the same family, such as HER3[19]. In view of such treatment resistance, new agents have been developed, with pertuzumab standing out, which is another anti-HER2 humanized monoclonal antibody that recognizes a different epitope than that recognized by trastuzumab. Pertuzumab prevents HER2 dimerization with HER3[20]. Pertuzumab combination with trastuzumab and chemotherapy with docetaxel has demonstrated higher efficacy in the treatment of breast cancer than either agent separately with docetaxel[18,21]. In addition, other anti-HER2 compounds have been developed, such as lapatinib and the trastuzumab-emtansine conjugate, which are mainly used as second-line treatment in case of resistance to trastuzumab in combination with docetaxel[22,23]. In addition to blocking HER2 dimerization, these antibodies activate antibody-dependent cytotoxicity[24]. Therapeutic success of these monoclonal antibodies is also due to the fact that patients with HER2-overexpressing tumors don't possess multiple immunosuppression mechanisms, as it has been observed in patients with HER2-negative tumors[25,26]. Ertumaxomab is a bivalent antibody that recognizes HER2 and CD3, which enables tumor-infiltrating lymphocytes activation, by means of which it might have higher therapeutic effect; currently, it is being assessed to find the maximum dose and clinical efficacy (ClinicalTrials.gov, NCT01569412)[26,27].

In conclusion, the use of TAA-targeted monoclonal antibodies is a highly promising therapeutic option, but it depends on specific expression of these antigens in tumor cells, as well as on the absence of immunosuppressant mechanisms that might inactivate their antibody-dependent cytotoxicity effect.

T cell-mediated immunoregulatory mechanisms

Main immunoregulatory mechanisms that have been described in breast cancer include those mediated by T cells. The presence of CD4+ T cells in tumor infiltrate has been associated with negative prognosis, unlike the presence of CD8+ T cells[28]. One of CD4 T cell populations that plays an important role in breast cancer pathogenesis is interleukin 4 and 13 (IL-4 and IL-13)-producing Th2 cells, which promote tumor development by inducing the production of growth factors that accelerate cancer cell proliferation[29-31]. In addition, both these cytokines have been observed to be involved in anti-tumor immune response local suppression[32,33], in the promotion of metastasis[34] and in tumor cells resistance to apoptosis[35]. IL-13 can be observed both in tumor and tumor adjacent tissues; however, there is higher expression within tumor tissue, which positively correlates with tumor size[36]. IL-13 tumor development-promoting function in breast cancer has been confirmed in animal models, where its biological activity is blocked[29,33]. The presence of the CCL5 chemokine has been described to promote breast cancer growth and metastasis by attracting CD4 lymphocytes, which express the CCR3 receptor, and by promoting their differentiation into Th2 cells[37-40]. By means of T cell response modulation in animal models, decreasing Th2 cell induction and favoring the presence of Th1 cells has been observed to produce an induction of CD8 cytotoxic cells able to control tumor growth[41]. This strategy is being investigated as a treatment alternative.

Other CD4 T cell population that has been associated with tumor development is regulatory T (Treg) cells, characterized by Forkhead Box P3 (FoxP3) transcription factor expression[42]. Treg cells usually participate in peripheral tolerance mechanisms by preventing autoimmune or immune hyperreactivity processes[43]. An important infiltration of Treg FoxP3+ cells within tumors and surrounding regions has been observed in different neoplasms, including breast cancer; specially, when it occurs in tumor-surrounding areas, this infiltration is associated with poor prognosis[44-48]. Breast tumor-derived Treg cells show specificity for TAA and recognize the same epitopes than effector T cells[49], which evidences their potential participation in anti-tumor response suppression. In support of the above, the ability of tumor Treg cells to suppress the cytotoxic activity of CD8+ T cells that are specific to TAAs such as HER2 has been demonstrated in animal models[50]. Treg frequency increase does not only occur at the lesion site; higher frequency of these cells in peripheral blood has also been reported in patients with breast cancer, especially at advanced stages[51]. However, in spite of the existence of an increase of Treg cells in breast cancer patients' blood, their activation status and suppressor capacity are not elevated, unlike what occurs in tumor tissue-derived cells, which show highly elevated activation status and suppressor capacity[47,52]. Treg cells are attracted to tumor cell-produced CCL-22 chemokine, which is recognized by Treg cell-expressed CCR4 receptor[32]. In addition to their migration, Treg cell accumulation in tumor microenvironment is due to in situ proliferation of these cells[52]. Molecules with regulatory function that are highly expressed in tumor Treg cells include cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), inducible T-cell co-stimulator (ICOS) and glucocorticoid-induced tumor necrosis factor receptor (GITR)[52-54]. Since these molecules are important to Treg cells functionality and are highly expressed, they have been studied as potential therapeutic targets that allow for tumor Treg cells suppressor effect to be blocked and this way enable the development of an anti-tumor response[55-57]. Currently, an anti-CTL4 antibody (ipilimumab) is used in clinical practice for the treatment of melanoma, and its effect on breast cancer is being assessed (ClinicalTrials.gov, NCT01502592). In addition, another anti-CTLA-4 antibody, tremelimumab[58], is being investigated in clinical trials (ClinicalTrials.gov, NCT02536794). Similarly, there are preclinical studies showing that GITR modulation may favor the development of anti-tumor responses[56,59,61]. The combined use of these agents might have an impact on tumor development by inhibiting one of the main suppressor mechanisms in human tumors, such as Treg cells.

Antigen-presenting cells in breast cancer

Th2 cell generation in breast cancer is induced by dendritic cells (DC) that have been conditioned by the tumor microenvironment and are characterized for expressing OX40L, which is one of the molecules directly implied in CD4+ T cells polarization towards a Th2 profile[62,63]. DC are conditioned in the tumor microenvironment by the presence of thymic stromal lymphoietin (TSLP), which is produced and secreted by tumor cells and makes for DC to express large amounts of OX40L and not to express IL-12[63]. When interacting with CD4+ T cells, tumor DC induce T cells polarization to produce IL-4, IL-13 and tumor necrosis factor alpha (TNF-α), but

not IL-10[63]; this Th2 cell variant is known as pro-inflammatory Th2 cells[64]. Furthermore, TSLP-exposed DC have been shown to acquire the ability to induce Treg cells from CD4+ T cells[65]. In addition to Th2 and Treg induction, which favor tumor development, breast tumor-isolated DC have their antigenic, phagocytic and maturation capacity decreased, as well as the expression of co-stimulation molecules, such as CD40 and the B7 family[66,67]. On the other hand, plasmacytoid DC (pDC) that infiltrate breast tumors have been observed to have deficient IFNα production caused by higher amounts of transforming growth factor beta (TGF-β) and TNF-α present in tumor microenvironment[53,68]. These cells have elevated capacity to induce and activate Treg lymphocytes, thus eliciting their accumulation and activation at the lesion site[69]. In fact, infiltration of both cell populations in breast tumors has been associated with poor disease prognosis[69-71]. Tumor pDC express elevated levels of ICOS ligand, and it is through this molecule that pDC activate Treg cells, thus causing for them to produce large amounts of IL-10, which has an immunosuppressant effect[69,70].

Breast tumors cell infiltrate also contains macrophages; these cells are known as tumor-associated macrophages (TAM)[72,73]. The presence of TAM in breast cancer is associated with poor prognosis and higher risk for the development of metastasis[74-76]. Most TAM have an anti-inflammatory M2 phenotype and express elevated levels of the cyclooxygenase 2 (COX-2) enzyme, which leads to the production of prostaglandins that, in turn, favor the generation of Th2 cells and tumor growth[77-79]. TAM have been described to favor tumor cell proliferation and survival by inducing the expression of molecules such as bcl-2[79]. TAM can induce COX-2 expression in tumor cells by means of the production of IL-1β, which induces TAM polarization in M2, thus generating a retro-stimulation system that favors tumor development[78,80]. TAM can directly affect lymphocyte activation by secreting large amounts of IL-10 and TGF-β[81], and promote tumor growth by means of the secretion of growth factors such as vascular endothelial growth factor and epidermal growth factor[82]. They are also able to produce chemokine CCL22 to attract Treg cells into the tumor microenvironment[83].

Immune system cells and tumor stroma cells interactions

Tumor microenvironment consists of cancer cells, inflammatory cells and stromal cells. The dynamical interactions of cells that form part of tumor microenvironment determine the environmental conditions under which tumor development is produced[84]. Recent experimental evidence has demonstrated that an important component of tumor stroma is mesenchymal stem cells (MSCs)[85]. MSCs, which originally were reported in bone marrow stroma, have strong attraction for tumor microenvironment and can differentiate into cells that favor the tumor niche[86-89]. These cells are able to promote tumor growth in breast and colon cancer animal models[90-93]. However, the mechanisms whereby they induce tumor development are not known, and it has been suggested that it can be partly mediated by their angiogenic and immunoregulatory properties, which have been described in vitro for bone marrow-derived MSCs[91,94,95]. One of the mechanisms is tumor cell proliferation and migration induction by means of IL-6 and IL-8 secretion[96,97]. Exposure to TNF-α, which is present in tumor microenvironment, has also been reported to induce chemokines CXCL9, CXCL10 and CXCL11 in MSCs, which promotes attraction and mobilization of tumor cells expressing the CXCR3 receptor for these chemokines[98]. Finally, MSCs have been observed to produce large amounts of TGF-β, which directly affects leukocyte functionality and induces Treg cells generation in the tumor microenvironment[99].

Blockade of immune checkpoints

A series of molecules able to suppress local immune response, known as immune checkpoints, are expressed in tumor infiltrate cells[11]. Among the most widely studied, CTLA-4 and programmed cell death 1 (PD-1) are found mainly in patients with melanoma or renal carcinoma[100-102]. For CTLA-4, there are humanized monoclonal antibodies, developed for the treatment of patients with advanced melanoma[100]. In breast cancer, two anti-CTLA-4 antibodies are currently on evaluation process, as previously mentioned. CTLA-4 block inhibits Treg cells suppressor effect, as previously discussed, but it also favors a prolonged activation of effector T cells by hindering CTLA-4-mediated negative regulation, which is expressed in activated T cells. In these, CTLA-4 uses two inhibitory mechanisms: one of them is the transmission of a negative signal through its extracellular region, and the second is competitive antagonism of the co-stimulating signal that is mediated by CD28 and competes for CD80 and CD86 during interaction with antigen-presenting cells[103-106]. PD-1 is an inhibitory receptor expressed in T cells that limits their capacity of response[107]; its ligand PD-L1 is

expressed in tumor cells and tumor lymphocytic infiltrate, and its expression is correlated with poor prognosis in some tumors[12]. In breast cancer, PD-L1 expression in tumor cells is associated with negative clinical and pathological characteristics[108-110], with metastasis[111] and with decreased frequency of CD8 T cells[112,113]. PD-L1 expression has also been observed in leukocyte infiltrate, but its clinical meaning is controversial[114-116]. Currently, different clinical trials are ongoing with anti-PD-1 antibodies in patients with breast cancer in order to know their safety and clinical efficacy[117].

Impact of chemotherapy on anti-tumor immune response

Many of the medications used in chemotherapy have been described to be able to promote anti-tumor immunity mainly due to their capability to induce tumor cell immunogenic death[118,119]. An example is anthracyclines, which cause for tumor cells to expose calreticulin on their surface, as well the release of chromatin-binding high-mobility group B1 protein (HMGB1) and adenosine triphosphate, which together induce DC activation[120-122]. In addition, doxorubicin has been observed to increase the proliferation of CD8 T cells specific for tumor antigens[123]. 5-fluorouracyl stimulates antigen capture and cross presentation in DC[124], whereas taxanes increase natural killer cells activity and favor leukocyte infiltration[124,126]. Low-dose cyclophosphamide (50 mg/day) inhibits Treg cells activity by decreasing FoxP3 transcription factor[127]; its use has been assessed in combination with trastuzumab and granulocyte and macrophage-colony stimulating factor, with longer survival being observed in those patients who developed an anti-tumor immune response[18]. Finally, trastuzumab and pertuzumab combination therapy has antibody-dependent cellular cytotoxicity as one of its main mechanisms of action[11,129].

These data clearly illustrate the importance of immune response in the treatment of breast cancer, demonstrating that therapeutic success of many compounds used in chemotherapy is largely due to their capability to stimulate an anti-tumor immune response in patients.

T cells adoptive transference

With the use of T cells with specific receptors to some tumor antigens, citotoxic cells that are able to recognize and react to tumor cells can be generated. At its first stages, this technology used lymphocytes isolated from the same tumor leukocyte infiltrate, but this has the great limitation that sufficient numbers of specific lymphocytes have to be obtained from clinical samples[130]. Subsequently, the generation of chimeric receptors that contain an antigen recognition site and an intracellular signaling domain were generated in order to guarantee lymphocytic activation when in contact with tumor cells[131,132]. The main limitation is that tumor antigens that are different to own antigens have to be found, since own antigens can elicit own material recognition and generate serious toxicity problems and adverse effects[133]. The use of this technology in breast cancer is beginning to be assessed in some clinical trials (clinicaltrials.gob, NCT01837602 and NCT 02547691).

Conclusions

Breast cancer remains a public health problem that affects a large number of women. Current treatments, both surgical and chemo- and radiotherapeutic, are highly aggressive for patients, and their efficacy is focused on disease early stages, and considerably decreases at advanced stages. These treatments do not always achieve cancer cells complete elimination and the disease can reappear in short time. In view of this panorama, immunotherapy is highly attractive for the treatment of neoplasms, due to immune response specificity, which can prevent the collateral damages observed with chemotherapy and radiotherapy, which affect both body's tumor cells and normal cells. Another important factor is immune memory, which potentially could decrease recurrence rate. However, to date, many immune treatments have shown limited effect on patient survival. This is largely due to the presence of immunosuppressant mechanisms at the lesion site, such as the presence of Treg and Th2 cells, and the production of suppressor cytokines such as IL-10 and TFG-β. Identification and characterization of immunoregulatory mechanisms that affect immune response are essential to the design of new therapeutic strategies that can impact on patient survival. Currently, one of the treatments with the highest efficacy in breast cancer is the use of anti-HER2 monoclonal antibodies (trastuzumab and pertuzumab), which in combination with chemotherapy achieve an important reduction of the disease and can in some cases eliminate the totality of tumor cells. However, it is only applicable to patients with tumors that express the HER2 protein. The efficacy of these monoclonal antibodies is a clear example of the potential of the use of immunotherapy in the treatment of cancer. Currently, several antibodies targeted against immunoregulatory molecules, such as

PD-1 and CTLA-4, are being assessed, with the idea of blocking immunosuppressant mechanisms that inhibit the generation of an anti-tumor response. The combined use of immunoregulatory molecule blockers with immune system activators, such as DC-based vaccines or transference of cytotoxic lymphocytes directed against TAA, is one of the most promising immune strategies in the treatment of breast cancer and other neoplasms. In the next few years, onco-immunology will definitively have a very important boom in the development of new treatments against cancer.

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