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Developing an effective breast cancer vaccine: Challenges to achieving sterile immunity versus resetting equilibrium

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A B S T R A C T

Introduction: Evading immune destruction is an emerging hallmark of cancer. Immunotherapy of cancer is categorized as either specific stimulation of the immune system by active immunization, with cancer vaccines, or passive transfer of humor or cellular materials, such as, tumor specific antibodies (including immunomodulators) or adoptive cell therapy that inhibit the function of- or directly kill tumor cells. Modulation of immune response in cancer patients is the result of a balanced activity of T regulators and T effector cells.

Methods and results: We will present the current information and the prospects for the future of immunotherapy in patients with breast cancer including tumor antigens for vaccines and targets for monoclonal antibodies and adoptive T-cell therapy.

Discussion: Active immunotherapy in breast cancer and its implementation into clinical trials has largely been a frustrating experience in the last decades. After many years of controversy, the concept that the immune system regulates cancer development is experiencing a new resurgence. It is clear that the cancer immunosurveillance process indeed exists and potentially acts as an extrinsic tumor suppressor. It has been also clear that the immune system can facilitate tumor progression by sculpting the immunogenic phenotype of tumors as they develop. Cancer immunoediting represents a refinement of the cancer immunosurveillance hypothesis and resumes the complex interaction between tumor and immune system into three phases: elimination, equilibrium, and escape.

Conclusion: What do we know about tumor immunogenicity and how might we therapeutically improve tumor immunogenicity? The first vaccine and the first immunomodulating agent were recently approved by the US Food and Drug Administration (FDA) for the treatment of prostate cancer (sipuleucel-T) and melanoma (ipilimumab), respectively. The success of future immunotherapy strategies will depend on the identification of additional immunogenic antigens that can serve as the best tumor-rejection targets. Therapeutic success will depend on developing the best antigen delivery systems and on the elucidation of the entire network of immune signalingsignaling pathways that regulate immune responses in the tumor microenvironment.

Introduction

The primary concept of active immunotherapy in breast cancer is to enable the immune system to detect neoplastic growth and to either prevent carcinogenesis and/or reject transformed cells with a potential for malignant tumor growth. Taking into account the dramatic impact of vaccines against infectious diseases (including those preventing cancer), such an approach has the potential of not only successfully treating cancer patients but also preventing recurrences for an extended period of time. Potential side effects (especially in comparison to chemotherapy) are limited, and the approach has a particular appeal to patients and also to physicians. Immunotherapy has become a clinical reality for a number of both haematological malignancies and solid organ cancers [1]. However, with the exception of antibody-based HER2 targeting, immunotherapy in breast cancer and especially the implementation of active immunotherapy into clinical trials (specifically in the adjuvant setting) has largely been a largely frustrating experience over the last two decades. Recent advances in clinical and basic research lead to a new understanding of not only cancer immunology but also of breast cancer as a heterogeneous disease. Chemotherapy and targeted treatments can modulate the immune system. Immune response to cancer is a dynamic process that can lead to the rejection of cancer

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but can also have regulatory effects that promote tumor growth. This concept of immunoediting in cancer immunology has profound impact on how we today understand some of the long-term efficacy of chemotherapy and radiotherapy today; treatments formally formerly believed to be highly immunosuppressive. The recognition of breast cancer heterogeneity and the qualitative differences in tumor biology help to focus immune related therapies to entities that have a fair chance of treatment response. It is likely that the combined understanding of tumor and host response may lead to an extension of the immunotherapeutic advances to breast cancer. Despite immune surveillance solid tumors are usually weakly immunogenic and do appear to have somehow managed to avoid detection by the various effector cells of the immune system or have been able to limit the extent of immunological surveillance and killing, thereby evading eradication. Mechanisms involved in immune evasion are several and balance between immune and autoimmune response needs to be investigated [2,3]. The field of cancer immunology simplify tumor-host immunological interactions, as highly immunogenic cancer cells may well evade immune destruction by disabling components of the immune system that have been dispatched to eliminate them. More complex and sophisticated mechanisms operate through the recruitment of inflammatory cells that are actively immunosuppressive, including regulatory T cells (Tregs). They can suppress the actions of cytotoxic lymphocytes [4,5]. Mechanisms underlying the dynamic interplay between immune cells and tumor progression are currently under investigation. The accumulated data indicate that the success of an immune response toward a tumor can be determined by the type of immune response elicited. A tumor-directed immune response involving cytolytic CD8+ T cells, T helper 1 (Th1) cells, and NK cells appears to protect against tumor development and progression, while the immune response that involves B cells and activation of humoral immunity and/or a Th2 polarized response, can promote tumor development and progression. This balance between a protective cytotoxic response and a harmful humoral or Th2 response can be regulated systemically by the general immune status of the individual [6]. As a consequence of this mechanism, immuno-evasion operate through the recruitment of inflammatory cells that are actively immunosuppressive, including Tregs that can suppress the actions of cytotoxic lymphocytes.

Regulatory T cells in breast cancer

Forkhead box P3 (FOXP3)+ Tregs cells usually maintain immune tolerance and prevent inflammatory diseases [7]. Tregs phenotype is characterized by expression of several functional antigens. Specifically a Tregs expresses FOXP3, Forkhead/winged-helix transcriptional factor, critical for TRegs activity and development [7]. It also express CTLA-4 (cytotoxic T lymphocyte Ag-4) that binds to B7s on antigen resenting cells (APC) and acts as co-stimulatory molecule for TRregs (blocking CTLA-4 inhibits TRegs and upregulate immune response) [8]. Treg cell phenotype and comparison with T effectors cell is reported in Figs. 1 and 2. TRegs cell-deficient mice and humans carrying non-functional alleles of the FOXP3 gene are affected by the severe systemic autoimmunity and lymphoproliferative disease. The impaired function of TReg cells is implicated in the development of several common autoimmune and inflammatory diseases, including type 1 diabetes, rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus [9–12]. TRegs function allows protective antitumor and antipathogen immunity, but preventing inflammatory disease by restraining aberrant responses to self or to innocuous antigens. TReg cells are crucial for the induction and maintenance of peripheral tolerance to self-antigens. While exerting their function TReg cells can also suppress immune responses to: tumor antigens, alloantigens and

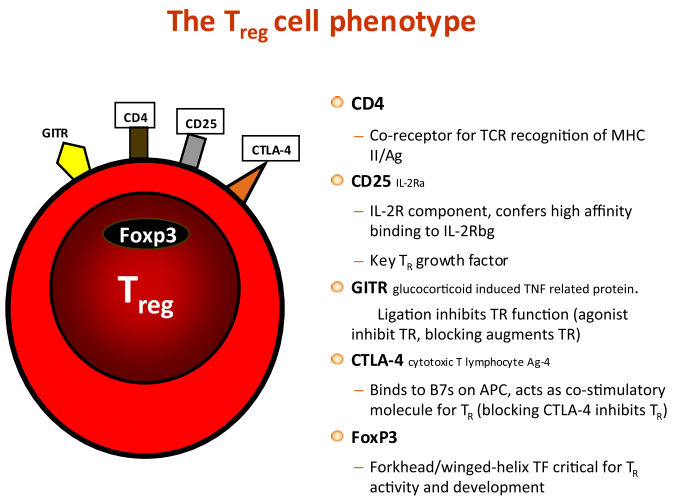

Fig. 1. Treg cell phenotype.

allergens. The modulation of the immune response by Tregs and the balance with T effector cells enhance immune suppression or immune evasion and exerts a fundamental impact in processes as self tolerance, in organ transplantation or in response to allergens.

The prognostic importance of FOXP3 expression in patients with breast cancer has been investigated [13]. FOXP3 expression in breast tumors was associated with worse overall survival probability and the risk increased with increasing FOXP3 immunostaining intensity. FOXP3 was also a strong prognostic factor for distant metastases-free survival but not for local recurrence risk [13]. In multivariate analysis FOXP3 resulted an independent prognostic factor and the hazard ratio of FOXP3 expression and of lymph node positivity were similar. In the Milan 3 trial, the probability of 10-year survival in node-negative subgroup was 100% for FOXP3-negative and 82% for FOXP3-positive patients; in node-positive subgroup 82% for FOXP3-negative and 41% for FOXP3-positive.

Patients [13]. According to these data Tregs may play an important role in breast cancer immunopathology due to their potent suppressive activity of both T cell activation and effector function. Comprehensive analysis of immune effector functions at different stages of tumor metastasis is fundamental to the design of effective immune intervention. Research is needed about the predictive potential of host factors and their potential role in breast

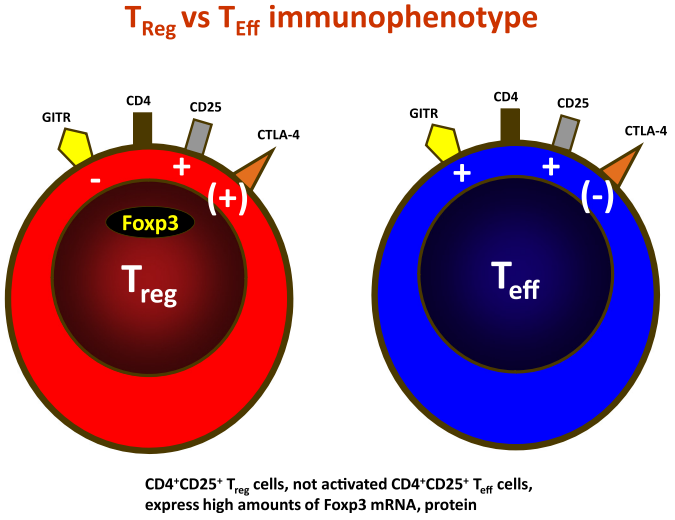

Fig. 2. Treg versus T effectors immunophenotype.

cancer pathogenesis. Treg cells can avoid the anti-tumor activity of immune effector cells in breast cancer tissue, resulting in poor prognosis of breast cancer patients.

Potential clinical application of therapeutic cancer vaccines

The identification of immunological and genetic features affecting immune response in patients with minimal tumor burden are the optimal background for development of clinical studies in the adjuvant setting. An active immunization has the potential advantages of a non-toxic therapeutic modality capable of inducing antitumor immune responses in patients with tumors [14]. Induction of strong immunity by cancer vaccines is expected to lead to the establishment of immunological memory, thereby preventing tumor recurrence. Induction of strong immunity by cancer vaccines is expected to lead to the establishment of immunological memory, thereby preventing tumor recurrence. In order to optimize the immunological response to a vaccination strategy we need first to identify the target antigen and the patient population to be targeted.

Research on tumor associated antigens (TAAs) has identified a large collection of peptide epitopes that have been and are being used for vaccination of cancer patients [15]. Several potential advantages of using peptide-based vaccines include 1) easy and relatively inexpensive production of synthetic peptides; 2) the easy administration of peptides in a clinical setting; 3) the possibility of treating only those patients whose tumors overexpress the target antigens and 4) the availability of in vitro or ex vivo assays that can assess patients' immune response to vaccine epitopes [15]. These antigens are down-regulated in somatic adult tissues while become aberrantly re-expressed in various malignancies. TAAs expression is associated with a poorer outcome and is more prevalent in higher grade and advanced-stage tumors. An intensive research into their possible use as therapeutic vaccines is actually ongoing due to their potent immunogenicity. The aim of future studies will be to assess the immunoreactivity of several antigens in a large series of breast cancer samples classified in according to molecular subtypes. Identification of potential target in subpopulations of patients with breast cancer may allow identification of patients who are potential candidates for adjuvant therapeutic vaccines. It is our current thinking that patients with minimal residual disease after preoperative chemotherapy are the ideal setting to test the efficacy of a vaccination strategy. To date, vaccines for breast cancer have been mainly used in end-stage disease. Several clinical studies have been completed with vaccines against antigens such as MUC1, CEA, HER2 and the carbohydrate antigens with varying results [15]. TAAs antigens offer a novel opportunity for fostering vaccine development and therapy. Vaccination in patients with breast cancer could induce an expansion of CD8+ cytotoxic T lymphocytes (CTLs) capable of rejecting tumor cells via recognition of tumor-associated antigenic (TAA) epitopes presented on the surface of cancer cells in association with human leukocyte antigen (HLA) class I molecules. An ideal and successful vaccine should have: a target antigen on tumor cells to direct the immune response; a platform to present the vaccine-derived antigen to immune system; an adjuvant to enhance immune stimulation, and appropriate monitoring techniques [14]. Results on vaccination trials are not exciting [15]. These negative results can be related to the selection of a population of metastatic patients that is characterized by large tumor burden; as a consequence we observe the ability of large tumors to escape the immune system and the difficulty to break immune tolerance [16]. Therapeutic cancer vaccines will probably be more active in patients with minimal residual tumor burden, but most of the trials so far have been conducted on metastatic patients and limit the success of phase I/II trials [16,17]. The design of a clinical trial in patient with breast cancer should identify the better population candidate to a vaccine trial and, primarily, the better antigen. Large population analyses on specific subtypes of breast cancer are necessary in order to select patients who have higher probability to express that specific antigen. First select the patient, then drive the design of the clinical trial. In order to design a 'second generation' immunotherapy protocols we should highlight 3 issues [1]: the ability to initiate tumor-specific immunity, either directly by providing tumor associated antigens or indirectly, by favoring the cross-presentation of endogenous tumor antigens [2]; the capacity to recruit effector immune cells within the tumor site, by increasing tumor visibility [3]; the ability to preserve immune cell functionality within the tumor microenvironment through the subversion of immune-escape mechanisms. It is becoming clear that these three features cannot be provided for by a single modality and combined therapies should be proposed. Prediction of clinical efficacy based on immunologic monitoring is crucial for the rational design of cancer vaccination studies. Doses, immunization schedules, methods of administration, timing of vaccinations and of following boosts to maintain a durable immune response need to be addressed in prospective clinical trials. Optimal combination vaccine therapy with a variety of novel approaches (e.g., monoclonal antibody as trastuzumab or tyrosine kinase inhibitors) is a great promise but it also requires evaluation in clinical trials to assess its benefit. More focused developmental guidelines are needed to address characteristics of therapeutic cancer vaccines.

Future perspectives and critical hurdles in breast cancer immunotherapy

With increased understanding of the importance of multiple immune effector mechanisms for tumor elimination and of the immunosuppressive forces that influence these mechanisms in the tumor microenvironment, it has become clear that both passive and active immunotherapies depend on the patient's immune system for long-term tumor control or complete tumor elimination. Several questions are still critical and should be addressed: What are the most important determinants of tumor immunogenicity? What are the possible mechanisms by which tumor immunogenicity is regulated? How might we therapeutically modulate tumor immunogenicity and what do we need to understand to move forwards? Should all cancer patients be treated with an active immunotherapy approach or only individuals potentially more "responding"? How can we predict that the individual will develop an immune response against a particular antigen used in the vaccine formulation? Is there any genetic signature predicting response to immunotherapy? Can we combine vaccine therapy with therapeutic monoclonal antibodies or small target oriented molecules? Continued basic research into the molecular mechanisms regulating carcinogenesis and immunosurveillance/tolerance will identify new potential target introducing vaccine therapy in prevention trials for patients at high risk for developing cancer [18]. Target antigens that are directly involved in promoting the neoplastic process can induce an optimal antibody response. As preventive vaccines operate during the early phases of carcinogenesis, effective inhibition of the specific targets will arrest the whole. As for all preventive medicine, an extremely low incidence of adverse effects will be a prerequisite of preventive cancer vaccines. The translation of preclinical data into preventive treatments requires more attention since the plan is to vaccine a healthy individual. There is no doubt that the findings reported in cancer prevention vaccination trials open a new field at the interface of basic science, clinical medicine, public health, and public policy. The first vaccine and the first immunomodulating agent were recently approved by the US Food and Drug Administration (FDA) for the treatment of prostate cancer (sipuleucel-T) and melanoma (ipilimumab), respectively. The success of future immunotherapy

strategies will depend on the identification of additional immunogenic antigens that can serve as the best tumor-rejection targets. It is also important to target antigens that are biologically important to tumor progression. Therapeutic success will also depend on developing the best antigen delivery systems and on the elucidation of the entire network of immune signaling pathways that regulate immune responses in the tumor microenvironment. Challenges for the future are also related to identification of critical hurdles that can significantly delay clinical translation of advances in immunotherapy in cancer patients care. Some of these critical hurdles include a) Complexity of cancer, tumor heterogeneity and immune escape; b) Lack of definitive biomarker(s) for assessment of clinical efficacy of cancer immunotherapies; c) Definition of new conventional clinical response criteria that take into consideration differences between response patterns to cytotoxic agents and immunotherapies; d) Insufficient exchange of information critical to advancing the field. Addressing these hurdles, will facilitate and improve the translation of novel immunotherapies to patients with cancer.

Conflict of interest statement

No conflicts of interest to declare.

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