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HER-2/neu immunotherapies

Take two – get more: optimization strategies for HER-2/neu-based immunotherapies inhibiting carcinogenesis

Barbara Seliger and Rolf Kiessling

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The identification of a set of tumor-associated antigens (TAA) which are recognized by T lymphocytes and therefore represent tumour vaccine candidates has rekindled interest in using immunotherapy strategies for the treatment of cancer patients. The T-cell-based immunotherapies currently employed include allogeneic stem cell transplantation, the use of genetically modified tumour cell lines, peptides derived from tumour antigens, TAA-specific DNA, RNA or proteins, as well as dendritic cells pulsed with relevant peptides. The vast majority of TAA identified so far are of intracellular origin and therefore sequestered from antibody recognition. There is, however, an increasing perception that the most powerful antitumor effects are those mediated by an 'integrated' immune response, where antibodies, $CD8^+$ and $CD4^+$ T cells act in concert. Examples of tumor antigens that elicit this type of orchestrated immunity are the cell surface-bound receptors, such as the tyrosine kinase receptors, which are accessible for both antibodies as well as T cells. Of these, the HER-2/neu oncogene has lately attracted attention among clinicians as well as tumor immunologists.

HER-2/neu (HER-2) codes for a 185 kDa transmembrane receptor with tyrosine kinase activity. It exerts a number of biological functions including regulation of the cell cycle, differentiation, angiogenesis, apoptosis, as well as cellular transformation. Several properties make HER-2 an interesting tumor vaccine candidate. Gene amplification and/or overexpression of HER-2 frequently occurs in a variety of human tumors of distinct origin and in some cases appear to be associated with the clinical outcome of the disease. The critical role for HER-2 in epithelial oncogenesis was originally demonstrated by transfection experiments and lately also confirmed by antisense and RNAi technologies.[1] These studies not only established the fact that HER-2 is essential for tumor growth and survival, but even stresses its implementation as a tumor vaccine since it minimizes the risk of HER-2-negative escape variants. A further proof of the concept that HER-2/neu loss variants will not grow *in vivo* was observed in the HER-2/*neu*-transgenic model of mammary carcinomas. In this model, transgenic mammary carcinoma cells lacking the expression of the $p185^{neu}$ tyrosine kinase receptor were unable to form tumors because $p185^{neu}$ expression is a prerequisite for their tumorigenicity.[2]

In the past few years, several *in vitro* and *in vivo* studies in rodents and humans demonstrated that HER-2 is immunogenic, thereby eliciting antibody, CTL as well as T-helper cell responses in subjects with HER-2/neu-overexpressing tumors.[3] In addition, murine neu-transgenic mouse models were employed for studying the natural and adaptive immunity on HER-2/neu carcinogenesis.[4] These studies have illustrated that HER-2/neu-based vaccines can induce tumor protection. However, immune responses against HER-2 are typically weak and rapidly disappear following cessation of booster immunizations, since HER-2/neu is a 'self' tumor antigen, which is also expressed at low levels on epithelial surfaces. The challenge of overcoming the immunological lethargy to self tumor antigens has been the 'Holy Grail' for clinicians and tumor immunologists, and led to the development of some innovative immunizing strategies. In general, these immunizing strategies have demonstrated greater promise in a prophylactic setting where vaccinized animals were protected against a subsequent tumor challenge rather than a therapeutic setting with the aim of abrogating an established tumor.

The preventive potential of specific immunoreactivity has drawn major attention to the treatment of tumors. In order to develop an efficient tumor prevention vaccine, cells, proteins, peptides and DNA have been employed. Recently, Quaglino *et al* have described a new approach to improve immunogenicity against HER-2/neu, which recently appeared in the Journal of Clinical Investigation (2004). In their report it even prevents the progression of precancerous lesions in a HER-2/neu mammary carcinogenesis model. It was based on the fact that activation of T-cell memory by 'priming' with a DNA vaccine followed by a second protein-based 'boost' will often enhance the efficacy of DNA vaccination. Therefore, the authors employed a combination of a plasmid DNA coding for the extracellular and transmembrane domain (ECD-TM) of the rat p185neu, followed by a boost with $p185neu^+$ allogeneic cells engineered to secrete IFN-γ. Their choice of a cellular 'boost' was based on their earlier results demonstrating that tumor cells secreting IFN-γ were particularly immunogenic and that allogeneic vaccines could markedly enhance the recognition of p185 neu. This novel approach resulted in a high percentage of tumor-free mice in their 'BALB-neuT' transgenic model. The findings represent a breakthrough since it represents a very aggressive model of mammary carcinogenesis with a mutated rat neu gene driven by the MMTV promoter inducing atypical hyperplasia as early as 6 weeks of age, carcinomas *in situ* at week 15 and palpable tumors already appearing at the 22nd week of age.

The challenge in this model has been the induction of a sustained memory response. Earlier efforts to halt the development of preneoplastic lesions was only successful by repeated vaccinations with the ECD-TM plasmid starting at week 6.[6] The prime-boost approach taken by Quaglino *et al* appears to at least partially overcome the difficulty of inducing a strong sustained memory response as the cellular vaccine boost

was given at 13 weeks of age and yet half of the mice remained tumor-free until the experiment ended at week 32. Even more surprising was their findings from the morphologic analysis demonstrating that the neoplastic lesions from week 18 shrank markedly in the vaccinated mice. This implied that the implementation of a combination of pDNA/cellular vaccine is a powerful tool of breaking tolerance, inducing a protective memory response to the neu protein in this transgenic model. The possibility of inducing an even stronger and more durable immune response that leads to a higher proportion of tumor-free mice by repeating this prime-boost procedure is currently under investigation by the same group.

A humoral response was previously demonstrated to be central for impeding tumor growth in this BALB-neuT model (Cappello *et al*, 2003).[7] Here, Quaglino *et al* extend their previous observations by establishing the important role of antibodies in tumor protection through an elegant combination of gene expression analysis and use of B-cell knockout mice. Comparison of the mRNA profile of mammary glands from 10-week-old untreated mice with that of 22-week-old prime-boost vaccinated ones identified 17 differentially expressed genes which are all related to antibody responses. In addition, the authors also demonstrated that prime-boost vaccination induced high antibody titers directed to p185neu, which correlates with the downregulation of p185neu in the cells of the mammary lesions.

The prominent role of antibodies in tumor protection in this model is not surprising since p185neu represents a surface receptor involved in cell growth regulation. This effect may therefore be analogous to the growth-inhibitory effect of the 'humanized' monoclonal antibody Herceptin or Trastuzumab, which has been tested in several clinical trials and found to be an effective adjuvant therapy for HER-2-positive breast cancer patients. This antibody inhibition of neoplastic progression is different from direct immunological destruction of malignant cells. However, does this rule out a direct role of cellular immunity in protection against HER 2/neu-expressing tumors? Most likely this appears not to be the case both in their model and in the resistance against human HER-2/neu-expressing tumors: (i) A direct effect of T cells producing IFN-γ was also implicated in tumor resistance in their transgenic model. (ii) One important anti-tumor mechanism mediated by antibodies including Herceptin is the antibody-dependent cell-mediated cytotoxicity involving Fc receptor-expressing effector cells (reference to Fc receptor ko article). (iii) Although the BalbneuT mice may not be a suitable model to study the elimination of neoplastic cells through direct killing, cytotoxic T lymphocytes (CTLs) have been demonstrated earlier by others to have the potential of recognizing both murine and human HER-2-expressing tumors.[3]

The critical question that remains as yet unanswered is whether the significant results of this thought-provoking study could be directly extrapolated to the clinical setting, an issue also cautiously commented on by the authors. Could the progression of human carcinoma *in situ* of the breast be halted by an effective HER-2/neu-based tumor vaccination, causing a 'Herceptin'-like effect? As the frequency of patients responding to Herceptin is limited and the majority of patients develop resistance within a year of treatment, a more efficient and preferable approach represents vaccination strategies based on the concerted immune response with HER-2-specific T cells concurrent to antibody responses. Furthermore, the characterization of a distinct set of differentially expressed genes/proteins by cDNA technology or proteomics might even help to improve tumor classification and the identification of targets/epitopes, resulting in the design of novel treatment strategies. Combination immunotherapy, like the combination of chemotherapy regimens that are currently in the clinical practice, may represent the future treatment of choice for adjuvant therapy of breast cancer. ■

Barbara Seliger is at the Institute of Medical Immunology, 06112 Halle/Saale, Germany and Rolf Kiessling is at the Cancer Center Karolinska, Stockholm, Sweden.
E-mail: Barbara.Seliger@medizin.uni-halle.de
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