Oncogenic proteins as tumor antigens
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Human immune responses to oncogenic proteins have been reported and are continuing to be defined. Immunity to nonmutated overexpressed oncoproteins as well as to mutated or unique cancer-specific oncoproteins has been identified. Immune system based treatments targeting oncogenic proteins have shown therapeutic efficacy in animals models and are currently being translated into human clinical trials.

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Abbreviations
APC antigen-presenting cell
CTL cytotoxic T lymphocyte
DC dendritic cell
LHRH luteinizing hormone releasing hormone

Introduction
Oncogenes encode proteins involved in normal cell division and differentiation. Proteins expressed by 'activated' oncoproteins are involved in the transformation of normal cells into malignant cells. Oncogenes become 'activated' by a variety of mechanisms including gene amplification, mutation, and translocation. Each mechanism of activation can result in the expression of proteins with the potential to serve as tumor antigens and as targets for immunotherapy. Animal models have validated the fact that immunity to a wide variety of oncogenic proteins can be elicited by vaccination. In humans, many patients with cancer develop immunity to the oncogenic proteins expressed by their own cancers. In 1996, the issue of whether oncogenic proteins can be tumor antigens is no longer an open experimental question.

This article will review current publications concerning oncogenic proteins as tumor antigens. Mutant and translocated segments of oncogenic proteins are expressed only in cancer cells. Overexpressed oncogenic proteins are expressed in both normal and cancer cells. The publications reviewed here help to validate the claim that mutant and translocated segments of oncogenic proteins can serve as tumor specific antigens. Studies to be reviewed here also demonstrate that overexpressed oncogenic proteins can serve as tumor antigens.

Immunity to mutated oncogenic proteins
Immunity to p53 and Ras oncoproteins has received the most attention, in part because mutations in these proteins are exceedingly common. Mutations in p53 and Ras are present in about 50% and 15% of human malignancies, respectively. Ras is a mutated oncogenic protein. The Ras protein becomes 'activated' by a single nucleotide substitution. Ras DNA, containing a point mutation, will encode a mutated protein with a single substituted amino acid. Mutated Ras proteins play a role in the creation of a malignant phenotype in a cancer cell, in part, by contributing to increased signal transduction resulting in uncontrolled cell growth. Mutations in Ras protein impart an abnormal function to this normal cellular protein. By contrast, p53 is a tumor suppressor gene. Mutations resulting in the generation of abnormal p53 proteins cause inactivation of the function of normal p53. p53, as a normal cellular protein, plays a role in mediating the natural death of a cell. Mutations which inactivate the normal function of p53 help 'immortalize' cancer cells. p53 is viewed as a possible immunotherapeutic target because mutant p53 protein accumulates in the cancer cell cytosol and nucleus, due to altered intracellular trafficking. Thus, mutant p53 protein is cancer specific and potentially available in increased abundance to serve as an immunotherapy target.

The p53 gene has multiple mutational 'hot spots'. Thus, mutant p53 proteins can be expressed in a wide variety of mutant and truncated forms. Previous studies in animal models have demonstrated that immunity to particular mutant segments of p53 can be elicited by immunization against peptides spanning the mutant segment [1]. Studies in humans demonstrated that antibodies to p53 can often be detected in patients bearing cancers that express mutant p53 [2]. The p53 antibody response found in these patients is directed to regions of p53 uninvolved by mutations, and represent areas of 'self' [3].

Due to the heterogeneity of p53 mutations, antibody responses are easier to evaluate than T cell responses and have therefore received more attention. A current study evaluating preoperative sera from a well defined group of 255 patients with colorectal cancer confirmed an incidence of p53 antibodies of 25% [4]. The presence of antibodies correlates with poor prognosis, particularly in early stage patients. Data concerning p53 mutations and/or expression, however, was not presented. Thus, it is unknown whether the presence of antibodies correlates directly with poor prognosis or merely serves as a secondary marker for the expression of mutant p53, which is known to correlate with decreased survival.

Antibody responses to oncogenic proteins can occur early in the course of cancer and may predict undetected malignancy or premalignancy. Two recent reports give credence to the possibility that the detection of immunity might be used to screen for malignancy. The first report
were diagnosed with lung cancer [5°°]. The second report describes the detection of a higher incidence of antibodies to p53 in women without breast cancer who have a significant family history of breast cancer (11%) compared with controls (1%) [6]. These provocative results should stimulate further studies to evaluate antibodies to cancer related proteins as tumor markers.

The majority of p53 antibodies detected are IgG which predicts the coexistence of helper-T-cell responses. A recent report of three breast cancer patients with antibody responses to p53 described a lymphoproliferative-T-cell response to wild-type p53 protein. All three patients demonstrated an accumulation of p53 in primary tumors [7°]. Thus, T cell responses might occur in response to mutations in p53 protein, but the responses may be directed to nonmutated portions of the protein. No information is available concerning the frequency of T cell responses and whether responses correlate with outcome. In animal models, helper-T-cell responses to tumor antigens can be effective in tumor therapy. Antigen targets for helper-T-cell therapy, however, need to be available to the immune system in adequate quantities. It is unknown whether p53, as an intracellular protein, is available in the extracellular cancer environment in adequate quantities to serve as an immunotherapeutic target for antibody and helper-T-cell responses.

Vaccine and cytotoxic T lymphocyte (CTL) therapy directed against mutant p53 protein has been proposed. CTLs specific for mutant p53 peptides can lyse transformed cells [1]. Thus, animal models are being used to learn how to best immunize against p53 peptides. One such study has shown that the addition of interleukin (IL)-12 to a p53 peptide vaccine results in the regression of established Meth A sarcoma expressing p53 [8].

Vaccine and CTL therapy directed against normal p53 has also been proposed. CTLs against normal p53 would not be cancer specific but might selectively lyse cancer cells, due to the relative increased accumulation of p53 protein in the cytosol of cancer cells. The assumption is that p53 accumulation in the cytosol can relatively increase the quantity of p53 presented by MHC class I molecules of cancer cells. Recent studies have demonstrated that CTLs specific for normal p53 peptides can be generated from human peripheral blood leukocytes, but have not yet validated that human p53 peptide specific CTLs can lyse cancer cells [9,10]. Studies in a unique animal model, however, have validated that p53 is processed by the MHC class I antigen processing pathway of cancer cells and that murine CTLs specific for normal human p53 peptides can selectively lyse human cancer cells [11°°]. Mice transgenic for HLA-A2 were immunized with human p53 peptides. Murine CTLs specific for the peptides and restricted by HLA-A2 were elicited. The p53-specific CTLs lysed a wide variety of human cancer cell lines, but did not lyse nontransformed human cells. This model provides a method to generate CTLs specific for human proteins and restricted by human MHC molecules, but without the confounding influence of normal human tolerance to self proteins.

Mutations in Ras are less complex than in p53 and thus are easier to evaluate. Mutant Ras proteins usually have single amino acid substitutions at residues 12 or 61. Previous studies showed that T cell immunity to Ras could be elicited by immunization to Ras peptides spanning the mutant segment [12]. Current studies in animal models have demonstrated that immunization with mutated Ras peptides can result in the generation of CD4+ CTLs capable of lysing cells transformed by Ras [13]. Other investigators have validated that immunization with mutant Ras proteins can elicit CD8+ CTLs and protect from challenge with tumors expressing mutant Ras [14]. Ras-specific antibodies, helper T cells and CTLs have now been identified in humans with cancer. Utilizing a panel of recombinant Ras proteins with the commonest mutations, serum antibodies specific for Ras were detected in 32% of patients with colon cancer and 3% of controls [15]. Antibodies reactive to Ras proteins with specific mutations were detected in some patients. The antibodies responded, however, to both wild-type and mutant Ras in the majority of patients. Ras-specific antibodies were directed largely to epitopes near the carboxyl terminus, an area unaffected by mutation. The presence of Ras mutations in patient tumors and the correlation of antibodies with outcome were not evaluated.

Helper T cells specific for mutant Ras have been identified in patients with gastrointestinal cancer [16-18]. In one patient with colon cancer, both CD4+ and CD8+ T cell responses to the same mutant were identified [16,17]. The corresponding mutation was not detected in the primary tumor, however, implying that the immune response eliminated cancer cells expressing the mutant Ras or alternatively that the observed immunity was unrelated to the cancer. Another study detected Ras peptide- and protein-specific helper-T-cell responses to the commonest Ras mutation in several patients [18]. The demonstration of antibody and T cell responses to mutant Ras protein strongly implies that patients can be primed by the mutant Ras protein expressed in autochthonous cancer.

Evaluation of human CTL responses to mutant Ras is more difficult and less is known. In one study, human-peptide-specific CTLs could be generated to a common mutant Ras peptide [19]. The mutant-peptide-specific CTLs, however, showed no lytic activity against tumor cells bearing the mutant protein. In another study, CTL clones specific for a Ras peptide derived from a single patient lysed a colon cancer cell line containing the same
mutant Ras is a good model for the testing of peptide-CTL therapy. Although sparse, these studies set the stage for further attempts to target mutant Ras for vaccine and CTL therapy.

Mutant Ras is a good model for the testing of peptide-based cancer vaccines [21**]. Ras peptides loaded onto antigen-presenting cells (APCs) derived from leukopheresis have been reinfused into pancreas cancer patients [22,23**]. Leukopheresis is a process by which APCs can be removed from the patient’s bloodstream and the rest of the blood components can be returned into the patient’s circulation. Techniques, such as leukopheresis allow the collection of large numbers of immune cells without adverse effects to the human body. Two out of five patients developed peptide-specific proliferative T cell responses after vaccination, but responses to Ras proteins were not evaluated.

Immunity to translocated oncogenic proteins

Chromosomal translocation can result in the generation of fusion genes expressing chimeric proteins. Targeting the joining region segment of chimeric proteins is conceptually the same as targeting the mutant segment of oncogenic proteins. The first fusion gene discovered was Bcr-Abl in chronic myelogenous leukemia. Mice immunized with joining region segment peptides of Bcr-Abl can develop peptide-specific helper T cells that respond to the Bcr-Abl protein [24]. Human studies have determined that Bcr-Abl peptides can bind to particular MHC class I molecules [25] and that both CTLs and helper T cells specific for Bcr-Abl peptides can be generated [26*,27]. It has not yet been validated, however, whether human Bcr-Abl peptide-specific T cells can lyse leukemia cells or respond to the Bcr-Abl protein.

Chimeric proteins are very common in human malignancy and often play a major role in early malignant transformation [28]. Thus, many potential chimeric protein targets exist. Evaluation of immunity to Bcr-Abl represents only inaugural attempts. It is highly likely that particular chimeric proteins will prove to be processed and presented correctly for CTL targeting. Unfortunately, deriving the essential information requires empiric experimentation individualized for each chimeric protein. Progress will be slow.

Immunity to overexpressed oncogenic proteins

Much of the detectable response to mutant oncoproteins is directed against the normal ‘self’ portions. Many other studies have detected immune responses to self proteins such as tyrosinase and MelanA/MART in cancer patients (reviewed elsewhere in this section). Detection of responses to self proteins has resulted in a ‘paradigm shift’ in tumor immunology [29**]. It has become accepted that a large set of antigenic determinants on self proteins have not induced self tolerance; immunity to self can be induced and might translate into effective cancer therapy.

Overexpressed oncogenic proteins represent a subset of self antigens with several advantages as tumor antigens. Immunity to self tumor antigens might result in the destruction of normal tissue. Greater abundance of overexpressed oncogenic proteins in cancer cells versus normal cells should provide for an increased therapeutic ratio. A major problem with any form of specific immunotherapy is the development of negative antigen variants, cells which no longer express the immune target. Cancer cell variants escaping from immunotherapy through the loss of overexpressed oncogenic proteins are likely to express a less aggressive phenotype.

Among the most extensively studied overexpressed oncogenic proteins is HER-2/neu, a growth factor receptor overexpressed in 30% of breast and ovarian cancers and in a wide variety of other adenocarcinomas. Previous studies have shown that HER-2/neu-specific antibody and T cell responses can occur in patients with HER-2/neu-positive cancers [12]. Immunization to HER-2/neu seems to occur in some patients by virtue of overexpression in their own tumor. Recent publications have validated that HER-2/neu-specific CTLs can recognize and lyse HER-2/neu-positive cancer cells and have identified additional CTL epitope targets [30-32].

Detection of existent antibody, helper-T-cell and CTL immune responses against HER-2/neu provides evidence that immunomodulation is possible. The problem is to discern what type of immunity will have a therapeutic antitumor effect and how to safely generate or augment the resultant immune response. Several groups have begun to explore therapeutic regimens in animals and in humans.

In the most compelling animal studies, infusion of HER-2/neu-specific antibodies into HER-2/neu-transgenic mice prevented the development of breast cancer in 50% of mice destined to develop HER-2/neu-overexpressing tumors [33**]. HER-2/neu-specific antibodies are known to downregulate HER-2/neu expression in vitro. Importantly, breast cancer cells from treated mice displayed markedly reduced phosphorylation. Thus, HER-2/neu-specific antibodies can affect cancer cell development and growth in vivo and result in improved long-term survival.

HER-2/neu-specific murine monoclonal antibodies with tumorstatic function have been humanized [34] and are currently being studied in clinical trials alone and in combination with chemotherapy; results have not yet been published. In a published Phase I clinical trial, patients with far advanced cancer were treated with an antibody bispecific for HER-2/neu and FcyRII (CD16) [35]. Substantial toxicity and several tumor responses were observed. The toxicity was presumably due to the activation and/or the destruction of CD16-positive cells. Of note, bispecific antibody therapy induced immunity against HER-2/neu in some patients (JR Gralow et al.,
unpublished data). These studies validate the suggestion that immunity to HER-2/neu can be elicited in humans. The use of bispecific antibodies as a strategy to induce immunity to HER-2/neu, however, might be limited by toxicity.

Methods to generate immunity to ‘self’ tumor antigens are not well established. If tolerance is directed against only a few epitopes, and hence a large set of antigenic determinants on self proteins have not induced self tolerance, it is likely that immunity to oncogenic self proteins can be induced by immunization to subunit peptides. Accordingly, antibody and T cell immunity to rat HER-2/neu (89% homologous to HER-2/neu) can be elicited by immunization to groups of peptides from the intracellular or the extracellular domains of HER-2/neu [36]. These studies point to an immunization strategy that might be effective in human vaccines.

Methods for inducing immunity to peptide-based vaccines are also not well established. In vitro, dendritic cells (DCs) can stimulate T cell responses to HER-2/neu peptides [37]. In vivo, DCs pulsed with tumor associated CTL peptide epitopes can induce tumor regression in mice bearing C3 sarcoma and Lewis lung carcinoma (3LL) [38]. Thus, potent APCs, such as DCs, can be valuable reagents for oncoprotein vaccines as well as being stimulators for the generation of oncoprotein-specific T cells in vitro, therefore providing a prelude to adoptive immunotherapy.

Many other overexpressed proteins are associated with malignancy. Attempts to target such proteins are in their infancy. Vaccine therapy might be effective for overexpressed oncogenic proteins with a limited tissue distribution, such as HER-2/neu. For overexpressed oncogenic proteins that are present as functional cell surface receptors, monoclonal therapy might be effective. In the latter category, epidermal growth factor receptor (EGFR) has received much attention [39]. Antibodies specific for EGFR have been shown to inhibit EGF binding and block the activation of receptor tyrosine kinases [40]. Incubation of tumor cells with this antibody in vitro results in cellular apoptosis. Such promising in vitro studies set the stage for human therapy trials.

**Immunity to other proteins involved in oncogenesis**

The publications reviewed above focused on immunity to proteins expressed by genes known to be oncogenes and tumor suppressor genes. Other recently identified tumor antigens may fit into the same category, but the cancer related function of the genes encoding the tumor antigens is less clear. Two examples of this are MAGE and connexin. Immunity to MAGE has been detected in malignant melanoma (reviewed elsewhere in this section). Genes of the MAGE family are normally expressed only in the testis and may play a role in early spermatogenesis [15].

Members of the MAGE family are expressed so commonly in cancer that in all likelihood they will eventually prove to play a role in malignant transformation and/or maintenance of the transformed state.

Connexin proteins are involved in gap junctional intercellular communication between cells. A mutant connexin 37 octamer peptide has been shown to be the target of CTLs against murine 3LL [41] and studies using the mutant peptide have validated the protective and therapeutic effects of a peptide-based vaccine [41]. Connexins have been proposed to serve as tumor suppressors and connexin expression is often aberrant during carcinogenesis. It is possible that the mutant segment of connexin 37, first identified as a tumor antigen, is also involved in oncogenic transformation. Furthermore, identification of other tumor antigens in cancer patients might lead to the identification of additional categories of oncogenic proteins.

Finally, once the issue of immunizing against self proteins is raised, there may be other categories of potential targets for cancer immunotherapy. The process of neoplasia from transformation to metastasis is exceedingly complex and has many stages. The concept of immunotherapy against oncogenic proteins might be broadened to include self proteins that are essential for invasion, metastasis and hormone regulation. Proteins involved in each step are potential targets. As one example, transformed cells are often dependent upon a normal hormonal environment during the early stages of cancer. Normal hormones such as luteinizing hormone releasing hormone (LHRH) have been targeted in contraceptive vaccines. Immunization of the normal male against LHRH is possible and induces a form of ‘immunological castration’ and sterility. Prostate cancer in early stages is sensitive to hormone environment. Immunization against LHRH can inhibit the growth of androgen-dependent prostate cancer in animals [42]. It is likely that LHRH vaccines would mediate similar effects in humans.

**Conclusions**

The field of tumor immunology has spawned many theories concerning interactions between the host immune system and cancer progression. The demonstration that many patients with cancer have detectable immunity to oncogenic proteins should finally allow a better understanding of the interactions, both positive and negative, between immunity and cancer. There are examples of cancer vaccines being successful in individual patients, however, in most circumstances the nature of the antigens in the vaccines have been ill defined. The development of vaccine and T cell therapy against defined oncogenic proteins will allow researchers to understand why therapy works when it works and to determine why it fails when it fails. Further studies of immunity to biologically relevant oncogenic proteins should finally provide therapeutic vaccine strategies of which the results are predictable and able to be reproducibly analyzed.
References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


A detailed report of the p53 antibody response detected in two heavy smok- ers who had no evidence of malignancy at the time of the initial antibody determination. Subsequently, the patients developed p53 lung cancers. This study suggests the use of an antibody response to p53 as a potential cancer diagnostic and candidate biomarker for lung cancer.


Three breast cancer patients who were identified who had the mutated p53 gene in their tumor and accumulated p53 protein. These patients also had an antibody response specific for p53. Analysis of the T cell response in these individuals revealed proliferation in response to wild-type p53. This study provides evidence of the importance of the role of immune recognition of self epitopes even in mutated cancer-specific oncoproteins.


Investigations outlining a novel technique for the determination of antigenic epitopes. HLA-A2-transgenic mice were immunized with putative A2-binding peptides and peptide-specific CD8+ T cells were generated. These were then tested against a panel of p53-expressing HLA-matched targets. This is a unique system for defining potential human epitopes that may be biologically relevant.


Excellent overview of the preclinical studies leading up to human clinical trials of a Ras-based vaccine for the treatment of malignancy.


After an extensive study of the binding of peptides derived from oncogenic fusion proteins to various HLA-types [26], the authors succeeded in generating peptide-specific T cells that were able to proliferate in response to one or more synthetic peptide coated targets. The peptides were derived from the Bcr-Abl fusion protein. This study provides the foundation for the potential generation of leukemia-specific immunity in humans.


Comprehensive theoretical overview of the immune response to self cancer proteins. The review includes relevant references and discusses similarities in the study of autoimmunity and cancer immunity.

recognized by ovarian tumor-specific cytotoxic T lymphocytes.


