Introduction

In animal models, malignancy can be treated by vaccine and T cell therapy. In humans, one of the major obstacles in developing cancer vaccines has been the lack of defined target antigens. Recently, however, several groups have identified proteins in human malignancy that can be recognized by the patient immune system [1]. Identification of human tumor antigens is an important step towards extrapolating successful animal studies into immune therapies for human malignancy. One ploy to identify tumor antigens has been to characterize the antigens on autologous tumors that are recognized by autologous T cells [3]. Antigens identified by such studies have largely been "self" proteins expressed on normal as well as malignant cells [1-5]. These proteins may be expressed during fetal development, but present in adults in only a limited number of tissues, (e.g., MAGE), or may be related to the normal function of the now malignant cell, (e.g. tyrosinase). In some circumstances, the proteins identified are involved in malignant transformation or the maintenance of the transformed state (e.g., HER-2/neu or ras). Immune responses directed against each of these proteins have been detected in patients with a variety of cancers.

The realization that the immune systems of patients with cancer can generate responses against proteins expressed on their own malignancy, even self proteins, has intensified interest in the development of an effective cancer vaccine. However, methods to generate immune responses to "self" tumor antigens are not well defined. A key to inducing an immune response to self proteins may lie in the use of peptide based vaccines. In several model systems immunity to self or transgene encoded proteins has been elicited by immunization with peptides [7, 8]. T cells recognize antigen in the context of processed peptides that bind in the MHC molecule. Current theories of self recognition suggest that immunodominant epitopes of self proteins elicit tolerogenic responses [7]. Other potentially immunogenic epitopes, functionally defined as subdominant epitopes, are "ignored" by the immune system when in the protein, but are immunogenic as peptides. If subdominant epitopes could be identified for "self" tumor antigens, peptide vaccines might be most appropriate for generating responses.

Peptide vaccines have several advantages. Peptides are relatively easy to construct and produce and may retain chemical stability over time, decreasing lot to lot variation. Vectors with infectious or oncolytic potential are not necessary. Perhaps the most important advantage is the theoretical ability to manipulate the immune response with defined peptide epitopes. Protective immunity generated by vaccination depends on elicitation of immune responses to the generation of T cell function thus allowing a

Results and Discussion

The HER-2/neu oncogen of a peptide vaccine. T cell protein with homology to HER-2/neu overexpressed in 20-40% of disease, and is an independent HER-2/neu may also be expressed in 30-60% of ductal carcinoma.

Studies from our HER-2/neu overexpression system [6]. Of 50 patients with HER-2/neu protein, only 4% of 145 patients had specific for the HER-2/neu protein [5, 6]. In 7 patients T cell responses directed against peptide epitopes derived from domains of the protein, indicating the immunization in rats and mice and immunization in intact her responses.

The finding that some patients who did not have toxoid used as a control.

To determine whether immunization was necessary, rats were immunized to peptide based on the acid sequence of the rat HER-2/neu. Immunization elicited IgG.
In humans, the generation of defined animal epitopes that may identify tumor antigens involves methods that are largely based on the recognition of proteins involved in the identification of self-tolerogenic epitopes. A key to peptide based vaccines is the generation of proteins that are recognized as "self" tumor antigens, with the most important epitopes that depend on the elicitation of immune responses with appropriate function. Peptide vaccines will allow the generation of T cell populations specific for defined epitopes and with defined function thus allowing a directed and effective immune response.

Results and Discussion

The HER-2/neu oncogenic protein is a good model system in which to assess the efficacy of a peptide vaccine. The HER-2/neu oncogene (c-erbB-2) encodes a transmembrane protein with homology to epidermal growth factor receptor. HER-2/neu is amplified and overexpressed in 20-40% of invasive breast cancers, is associated with aggressive disease, and is an independent predictor of poor prognosis in subsets of patients [9]. HER-2/neu may also be related to cancer formation with overexpression being detectable in 50-60% of ductal carcinomas in situ [10].

Studies from our laboratory showed that some breast cancer patients with HER-2/neu overexpressing cancers have a preexistent immune response to the protein [5, 6]. Of 50 patients with HER-2/neu positive tumors, 42% had an antibody response to the protein. Only 4% of 145 normal blood donors had detectable antibody responses. CD4+ T cells specific for the HER-2/neu protein and peptides have also been detected in cancer patients [5, 6]. In 7 patients with HER-2/neu positive breast cancers, 3 had proliferative T cell responses directed against the protein and/or peptides. All three responded to peptide epitopes derived from both the intracellular (ICD) or extracellular (ECD) domains of the protein. One patient responded to the peptides, but not to the intact protein, indicating the intact protein may suppress an effective immune response. The 4 patients who did not have a response to the protein or peptides did not respond to tetanus toxoid used as a control.

The finding that some patients had an existent immune response to HER-2/neu was surprising in that others had previously attempted and failed to immunize rats to rat neu protein expressed by a recombinant vaccinia virus [11]. Although mice immunized with the same vectors developed vigorous immune responses to rat neu, when rats were immunized they did not develop detectable antibody or delayed type hypersensitivity responses to the protein. The conclusion was that mechanisms of tolerance prevented immunization in rats and would prevent immunization in humans. Presumably, immunization with intact HER-2/neu protein would be ineffective in generating immune responses.

To determine whether a peptide vaccine could elicit immunity to HER-2/neu, rats were immunized to peptides derived from the homologous rat protein, neu. The amino acid sequence of the rat neu protein was analyzed using a sequence motif searching program, "TSites", that incorporates several algorithms to distinguish peptide epitopes appropriate for class II MHC restricted T cell responses [12]. A panel of potential peptide epitopes was identified and constructed. Rat immunization studies validated that peptides identified in this manner can elicit both T cell and antibody immunity [13]. Rat neu and human HER-2/neu are 89% homologous. Rats were immunized with combinations of ECD and ICD peptides that are highly homologous or 100% homologous between rat and human neu proteins. Complete Freund's adjuvant was used. Immunization elicited IgG antibody responses that were specific for both rat and human...
protein. Peptide immunizations also elicited CD4+ T cell proliferative responses specific for rat neu protein. Thus, the concept that HER-2/neu peptides can be used in vaccines to circumvent T cell and antibody tolerance is valid. Of note, there was no evidence of toxicity or autoimmunity through a five month observation period.

Immunogenic peptides identified in studies such as these may be directly applicable to the design of a vaccine for use in human cancers. Rat "self" peptides, rather than foreign peptides, were used to immunize rats so that the results might be extrapolated to the use of human peptides in humans. The peptides used were homologous to the human HER-2/neu sequence and the antibodies generated were specific for both rat and human protein. Thus, the results predict that immunization of humans with the same peptides is likely to generate similar immune responses. The induction of an immune response to rat neu in rats lays the foundation for testing peptide based vaccines in humans for generating immune responses to HER-2/neu protein in patients with HER-2/neu positive cancers.

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References