Review

Update on HER-2 as a target for cancer therapy
HER2/neu peptides as tumour vaccines for T cell recognition

Isabel Correa and Tim Plunkett

Imperial Cancer Research Fund, Breast Cancer Biology Group, Guy's Hospital, London, UK

Correspondence: Isabel Correa, Imperial Cancer Research Fund, Breast Cancer Biology Group, Thomas Guy House, 3rd floor, Guy's Hospital, London SE1 9RT, UK. Tel: +44 20 7955 2367; fax: +44 20 7955 2026; e-mail: i.correa@icrf.icnet.uk

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Abstract

During the past decade there has been renewed interest in the use of vaccine immunotherapy for the treatment of cancer. This review focuses on HER2/neu, a tumour-associated antigen that is overexpressed in 10–40% of breast cancers and other carcinomata. Several immunogenic HER2/neu peptides recognized by T lymphocytes have been identified to be included in cancer vaccines. Some of these peptides have been assessed in clinical trials of patients with breast and ovarian cancer. Although it has been possible to detect immunological responses against the peptides in the immunized patients, no clinical responses have so far been described. Immunological tolerance to self-antigens like HER2/neu may limit the functional immune responses against them. It will be of interest to determine whether immune responses against HER2/neu epitopes can be of relevance to cancer treatment.

Keywords: cancer vaccines, HER2/neu peptides, immunotherapy, tumour antigens

Introduction

There has been renewed interest in tumour immunotherapy during the past decade. Vaccine immunotherapy for cancer has been based on antigens against which humoral and/or cellular responses are elicited. These antigens should ideally be exclusively expressed or overexpressed by the tumour and have been termed tumour-associated antigens (TAAs). TAAs can be: products of genetic mutations; viral antigens associated with the tumour; cancer-testis antigens that are normal proteins expressed during development and then only in the testis and tumours; normal proteins that are overexpressed in tumours but restricted to specific tissues (differentiation antigens); and proteins overexpressed in tumours but present in many normal tissues. With the exception of antibodies directed against growth factor receptors on cancer cells (see the review article on herceptin in this issue [1]), antibodies have had little impact on the growth of solid tumours. Most of the efforts have therefore been focused on the cellular immune response and the identification of antigens recognized by human T lymphocytes.

Few TAAs have been identified for breast cancer, and they generally correspond to differentiation antigens or overexpressed normal proteins. Potential new target antigens have recently been described for breast cancer [2], but most of them are expressed on only a small percentage of breast cancers. One of the first TAAs described for breast cancer was HER2/neu, a 185 kDa transmembrane glycoprotein and member of the epidermal growth factor receptor family. Amplification and/or overexpression of HER2/neu have been reported in 10–40% of primary breast cancers, and also in ovarian, renal, gastric and colorectal carcinomas. In this review, we shall focus on the identification and application of HER2/neu peptides as tumour vaccines for T cell recognition.

APC = antigen-presenting cell; CTL = cytotoxic T lymphocyte; DC = dendritic cell; GM-CSF = granulocyte-macrophage colony stimulating factor; IFN = interferon; IL = interleukin; MHC = major histocompatibility complex; TAA = tumour-associated antigen; Th = T helper.

Why peptide vaccines?

Cancer vaccines can be based either on whole cancer cells or on TAAs. A recent report using autologous cancer cells fused to dendritic cells (DCs) seems promising [3], but this approach can be restricted by the low number of tumour cells obtained from some solid tumours (e.g. breast cancer), and also by the potential induction of autoimmunity against non-TAAs. The isolation of tumour cells and the generation of DCs are also both laborious and expensive.

TAA-based cancer vaccines have included recombinant virus expressing whole TAAs, or immunogenic peptides derived from the TAAs. Such recombinant viruses have generated weak responses, probably due to the presence of neutralizing antibodies against the viruses. In contrast, the use of peptide vaccines has produced significant clinical responses in patients with advanced melanoma, including total tumour regressions [4]. Peptides are relatively easy and cheap to produce in large quantities. Peptide vaccines also allow the inclusion of subimmunodominant epitopes.

Peptide-based immunotherapies include vaccine formulations to directly immunize patients, or the stimulation and expansion of peptide-specific cytotoxic T lymphocytes (CTLs) *in vitro* to be administered to patients in a method called adoptive transfer. Peptide vaccines can include the peptides for both MHC class I and class II molecules. They can be administered directly to the patient or used to pulse DCs *ex vivo* prior to re-infusion [4].

Identification of immunogenic HER2/neu peptides

$CD8^+$ CTLs and $CD4^+$ Th cell lymphocytes recognize antigens presented as small peptides in the groove of MHC molecules. Peptides bound to MHC class I and class II molecules are usually 8–10 and 15 amino acids in length, respectively. Some of the peptide residues are buried in the MHC groove and act as anchor residues, which define binding motifs specific for different MHC alleles. These binding motifs can be used to identify potential MHC-binding peptides from a given protein. Although peptides can bind MHC molecules, this does not mean that they are presented on the cell surface. Proteins are degraded intracellularly in the cytosol by the proteasome complex for presentation by MHC class I molecules, and degraded in endosomes for presentation by MHC class II molecules. Not all the putative MHC-binding peptides from a protein are generated *in vivo* and it is not currently possible to predict which peptides will be naturally processed. In the case of peptides binding MHC class I molecules, different cells (e.g. antigen-presenting cells [APCs] like DCs and tumour cells) can have different proteasome complexes that may generate different peptides from the same protein. It has been shown that some TAA epitopes are generated by tumour cells but not by DCs [5]. For these TAA epitopes, peptide-based vaccines may be the only formulation for vaccination.

Two approaches have been used to identify immunogenic peptides recognized by CTLs. First, tumour-specific CTL lines or clones have been generated using tumour infiltrating lymphocytes and autologous tumour cells. Target cells pulsed with the peptides are then used to stimulate these CTL lines or clones and identify peptide-specific T cell reactivity. This method has the advantage of there being a guarantee that peptides identified in this way are naturally processed. It is not easy, however, to obtain tumour infiltrating lymphocytes and sufficient autologous tumour cells from many tumours, including breast cancer.

The second approach of identity is known as 'reverse immunology'. In this technique, CTLs are generated by pulsing APCs with peptides. It is then necessary to determine whether the peptide-specific CTLs are able to recognize whole cells expressing the antigen. If the peptide-specific CTLs do not recognize tumour cells expressing the whole protein, this suggests that the peptide may not be naturally processed and presented. A similar approach has been used to identify immunogenic peptides binding MHC class II molecules. Using these techniques, several immunogenic peptides have been identified for the HER2/neu protein that are naturally processed and presented (Table 1).

T cell responses after stimulation with HER2/neu peptides *in vitro*

CTLs specific for HER2/neu peptides have been described, but they have not always recognized endogenously processed peptides on the cell surface [6]. Even where such reactivity has been demonstrated [7], the concentrations of HER2/neu peptides necessary for CTL recognition were at least two orders of magnitude higher than those required for viral proteins. In the case of helper T cells generated against HER2/neu peptides, only low affinity T cells have been found, even when using protocols that generated high affinity T cells for viral antigens [8]. These results suggest there could be some degree of tolerance to HER2/neu protein. Immunological tolerance to normal proteins expressed at low levels in normal tissues and overexpressed in tumours (e.g. HER2/neu) is a concern for the application of these TAAs in immunotherapy [9]. Deletion or anergy of high avidity T cells can occur and only low affinity T cells may be present. It is of interest to know whether it is possible to generate functional T cells with high affinity for HER2/neu epitopes and also whether low affinity cells are relevant for the clearance of tumour cells *in vivo*. A study has shown that whereas low avidity CTLs can be readily detected by standard immunological assays, only high avidity CTLs exert biological function *in vivo* in tumour models [10].

Table 1

Immunogenic peptides derived from HER2/neu protein that are naturally processed and presented

MHC	Peptide	Sequence	Reference
HLA-A*0201	HER2 5-13	ALCRWGLLL	[19]
	HER2 106-114	QLFEDNYAL	[20]
	HER2 369-377	KIFGSLAFL	[7,19–21]
	HER2 435-443	ILHNGAYSL	[19,22]
	HER2 654-662	IISAVVGIL	[23]
	HER2 665-673	VVLGVVFGI	[22]
	HER2 689-697	RLLQETELV	[20]
	HER2 773-782	VMAGVGSPYV	[21]
	HER2 789-797	CLTSTVQLV	[7]
	HER2 799-807	QLMPYGCLL	[7]
	HER2 835-842	YLEDVRLV	[7]
	HER2 851-859	VLVKSPNHV	[7]
	HER2 952-961	YMIMVKCWMI	[22]
	HER2 971-979	ELVSEFSRM	[7]
HLA-A*0301†	HER2 754-762	VLRENTSPK	[24]
HLA-A*2402	HER2 8-16	RWGLLLALL	[25]
	HER2 63-71	TYLPTNASL	[26]
	HER2 780-788	PYVSRLLGI	[27]
HLA-DR1, DR4, DR52, DR53	HER2 883-899	KVPIKWMALESILRRRF	[8]

MHC, Major histocompatibility complex. † Also binds to three other alleles of the HLA-A3 superfamily: A*1101, A*3101 and A*3301.

As described earlier, not all of the CTL lines or clones specific for HER2/neu peptides are able to recognize target cells expressing the antigen [6]. Once it has been demonstrated that the peptide is naturally processed and that T cells are of high affinity, a different explanation is available. This is that the conformation of the MHC–peptide complex when the peptide is loaded extracellularly to generate the CTLs is different to that of the MHC–peptide complex synthesized within the cells. For example, $CD4^+$ T cell hybridomas isolated from mice immunized with a synthetic peptide identical to a dominant and naturally processed I-A^k-restricted peptide from hen lysozyme were not able to recognize the antigen after processing by different APCs [11]. This possibility represents a concern for peptide-based immunotherapies.

Clinical trials with HER2/neu peptides

Most published clinical trials have used HER2/neu peptide HER2 369-377, which binds MHC class I molecules. All the trials have demonstrated no toxicity from treatment, but there are few data to demonstrate their effectiveness.

In a phase I study [6], four patients with metastatic breast, ovarian or colorectal cancer were immunized with peptide HER2 369-377 in incomplete Freund's adjuvant. There were no clinical responses. Peptide-specific CTLs were obtained from blood after immunization and two re-stimulations with peptide *in vitro* in 3/4 patients. However, none of these CTL lines or clones recognized tumour cells expressing HER2/neu, even when they were able to recognize target cells that had been pulsed with 1 ng/ml (10^{-9} M) peptide. A similar protocol in patients with melanoma had produced peptide-specific CTLs that were able to kill melanoma cells *in vitro* [6].

HER 369-377 peptide was administered with granulocyte-macrophage colony stimulating factor (GM-CSF) in a different phase I study. Three out of nine patients showed proliferative T cell responses to peptide *in vitro*, and 7/8 patients gave a delayed-type hypersensitivity response to peptide. No clinical responses were observed. CTL precursors were detected in only one patient who was in complete remission before the immunization, having remained free of disease 12 months after receiving a bone marrow transplant [12].

Using a different approach, six patients with advanced breast or ovarian cancer were injected subcutaneously

with DCs pulsed with HER2 369-377 and HER2 654-662 peptides [13]. After three vaccinations, HER2 369-377 peptide-specific T cells were detected in blood in two of the patients using intracellular staining for IFN-γ. These T cells were able to kill targets pulsed with the peptide, and also lysed tumour cells expressing HER2/neu. One of the two patients showed stable disease of longer than 8 months duration, having had progressive disease before the vaccination and a debulking surgery.

In two other phase I studies, longer HER2 peptides (15–18 amino acids) were used, corresponding to putative Th cell sequences [14,15]. No evidence was provided that the peptides actually bind MHC class II molecules. In one study, eight patients with stage III or IV breast or ovarian cancer were immunized with HER2/neu peptides corresponding to the extracellular domain of the protein (HER2 42-56, HER2 98-114 and HER2 328-345), or with peptides corresponding to the intracellular domain of the protein (HER2 776-790, HER2 927-941 and HER2 1166-1180). Peptides were administered with GM-CSF [14]. Proliferative responses were detected against the peptides and sometimes against the recombinant protein. No correlation between the response and the MHC haplotype of the patient was observed. Cytotoxicity or clinical responses to treatment were not reported.

In the other phase I study, 19 patients with stage IV breast or ovarian cancer were immunized with three HER2/neu peptides plus GM-CSF [15]. The peptides, corresponding to sequences HER2 369-384, HER2 688-703 and HER2 971-984, each contained a putative HLA-A2 binding motif. After immunization, 83% of the patients had proliferative responses to at least one of the peptides and some of them also showed proliferative responses to recombinant parts of HER2/neu. In some patients there was also an increase in the number of T cell precursors specific for the nonamer contained within the immunizing peptides. However, limited cytotoxicity of target cells expressing the antigen was observed (18% killing of SKOV3-A2 with clone anti-HER2 369-377 peptide, and 25% killing of Epstein-Barr virus-transformed lymphoblastoid B cells transfected with HER2/neu versus 12% in untransfected cells). Clinical response to treatment was not reported.

Clinical relevance of immune responses induced against HER2/neu peptides

The aforementioned clinical studies have shown that it is possible to induce immunological responses against HER2/neu peptides in patients with cancer, but no clinical responses have been reported. The fact that a tumour antigen elicits a tumour-specific response does not necessarily mean that the immune response will cause killing of the tumour *in vivo*. A central aim of future research must first be to establish whether those responses measured *in vitro* are of relevance for the clearance of established tumours *in vivo* and, second, to establish which, if any, parameters measured *in vitro* correlate best with a protective immunological response *in vivo*. The assessment of the immune response in immunized patients is often limited to circulating or lymph node lymphocytes rather than lymphocytes at the tumour site. In a study of patients with advanced melanoma, immunization with an anchor residue-modified peptide from gp100 stimulated strong responses in most patients, yet no clinical responses were seen [16]. In contrast, patients who also received IL-2 exhibited reduced CTL activities *in vitro* but significant clinical responses were also observed [16]. A similar observation was made in another study with melanoma patients immunized with a peptide from MAGE-3; significant tumour regression was reported in some patients but with no evidence for a CTL response in their blood [17].

Conclusion

Several immunogenic peptides derived from HER2/neu have been identified and T cells specific for these peptides have been generated *in vitro* and *in vivo*. The next steps will be to establish whether these HER2/neu-specifc T cells are of relevance for the clearance of tumours *in vivo* and, if so, what is the best immunization protocol to generate and mobilize such T cells.

Finally, we should not forget that tumour cells can down-regulate MHC class I expression [18], potentially limiting the efficacy of immunotherapies based on immunogenic peptides for T cell responses.

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