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Improving Tumour Immunity

Dr Kostas Kosmatopoulos of Vaxon-Biotech and Dr Jean-Pierre Abastado at the Laboratory for Tumour Immunology, Singapore Immunology Network, approach the promising future of antitumoural immunity

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Prophylactic vaccines against infectious diseases are one of the most brilliant achievements of modern medicine and immunology. Harnessing the immune system in a similar way to fight cancer has been the holy grail of tumour immunologists since Coley's initial attempts in the late 19th century. Unfortunately, the course of cancer immunotherapy research has, until now, been marked by more disappointments than successes.

However, several significant achievements have rekindled optimism in recent years. Encouraging clinical results have been obtained not only with immunostimulants such as anti-CTLA-4 monoclonal antibody (mAb) (1) and ODN-CpG (CpG7909) (2) that non-specifically stimulate strong immune responses, but also with active- and adoptive-specific immunotherapy. Firstly, certain vaccines (BLP-25, Provenge) (3,4) have been shown to enhance overall patient survival in randomised Phase IIb/III clinical studies. Secondly, potent immune responses were shown to correlate with a survival benefit in vaccinated patients participating in recent clinical trials (5,6). Thirdly, spectacular and long-lasting clinical responses (complete and partial regression of primary tumours and metastases) have been obtained in metastatic, previously lymphodepleted melanoma patients adoptively transferred with large numbers of high-affinity anti-melanoma cytotoxic T lymphocytes (CTL) amplified in the laboratory (7). All of these observations show that triggering a potent and high-affinity antitumoural immune response is a precondition for clinical efficacy. This is now the key challenge in tumour vaccine research.

CYTOTOXIC T LYMPHOCYTES (CTLs)

CTLs are currently considered to be the main effectors of antitumoural immunity, and tumour vaccination therefore aims to generate large numbers of high-affinity CTLs. CTL stimulation requires two distinct signals. Signal one is delivered through the T cell receptor (TCR) after its engagement with an antigen presented in association with class I human leukocyte antigens (HLA-I) expressed by dendritic cells (DCs). Signal two is delivered through receptors, such as CD28, that are expressed by CTLs after their interaction with costimulatory molecules expressed by DCs (B7 family) (see Figure 1). DCs must first be activated to express these costimulatory molecules, and this can be achieved by the engagement of CD40 molecules or toll-like receptors (TLR), for example.

ADJUVANT POTENCY

The magnitude of the immune response to a vaccine depends on the target antigen and on the potency of the adjuvant. Enormous efforts are being made to develop adjuvants that can bolster the weak immunogenicity of tumour antigens. Such adjuvants variously stimulate DC through TLR (ODN-CpG for TLR-9 and monophosphoryl lipide A for TLR-4) (8), CD40 (CD40 ligand and anti-CD40 moAb) (9), or HLA-II (soluble LAG-3 ligand) (10); others specifically target DCs *in vivo* (antigens fused to

Figure 1: Antigen presentation and activation of cytotoxic T lymphocytes

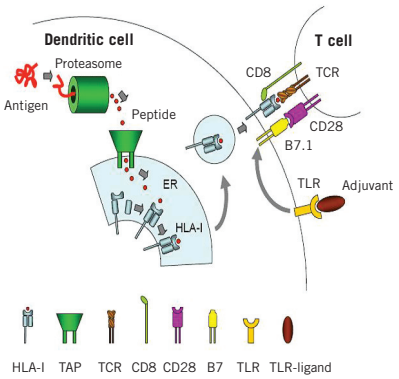
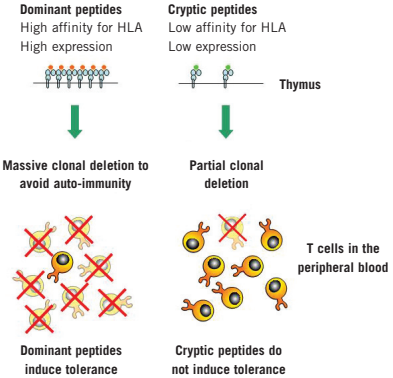


Figure 2: Tolerance of CTL respertoire specific for dominant and cryptic self peptides



chemokines, immunoglobulin Fc fragment or anti-DEC205 and anti-CD40 antibodies) (11), or facilitate antigen delivery into DCs *in vivo* (virosomes, liposomes, detoxified bacterial toxins and viral vectors) (12,13).

The efficiency of an antitumoural immune response depends not only on its magnitude but also on its duration. In chronic diseases like cancer, a moderate but sustained immune response might be more beneficial than a strong but transient response. Several strategies can be used to prolong immune responses. One is to inhibit costimulatory molecules such as CTL antigen 4 (CTLA-4) (14) or programmed cell death protein 1 (PDCD1) (15), which deliver a negative signal that attenuates the expansion of recently activated T cells. Another approach consists of removing immunosuppressive Treg cells by using cytotoxic agents such as cyclophosphamide or toxins fused to IL-2 (16). A third possible approach is to interfere with immunosuppressive molecules induced by tumours, such as TGF-beta (17).

ACTIVATING ANTITUMOURAL CTLs

However, even with a potent adjuvant and inhibition of the negative feedback of the immune response, strong antitumoural immunity will only be obtained if the target antigen itself is immunogenic. The immunogenicity of tumour antigens presented by the HLA-I system depends on their affinity to HLA-I and on the quality (affinity and frequency) of the specific CTL repertoire available for mobilisation.

Antitumoural CTLs recognise short peptides (9-10 amino acids) derived from endogenous tumour antigens and associated with HLA-I at the surface of tumour cells. These peptides are produced during intracellular processing of the relevant antigen, bind to HLA-I molecules, and are exported to the cell surface (see Figure 1). Binding affinity for HLA-I depends on the overall peptide sequence, and particularly on the presence of 'anchor motifs' (primary and secondary), that differ for each HLA-I allele (18). On peptides presented by most HLA-I molecules, primary anchor motifs are situated at the extremities, at positions two and nine or 10. Secondary anchors and residues, which contact the TCR and confer antigenic specificity, are often located in the middle of the peptide. Not all peptides produced during intracellular antigen processing are equally presented on the surface of tumour cells. Schematically, these peptides fall into two groups: dominant and cryptic peptides. This distinction is based on the peptide density at the cell surface, which depends mainly on HLA-I affinity. Dominant peptides exhibit strong HLA-I binding affinity, are abundantly expressed at the cell surface, and are usually strongly immunogenic. Dominant peptides can be specifically targeted by vaccination with the whole antigen. By contrast, cryptic peptides exhibit weak HLA-I binding affinity, are weakly expressed at the tumour cell surface, and are essentially non-immunogenic. However, although unable to initiate an immune response by stimulating naïve T cells, cryptic peptides can stimulate activated T cells and thereby maintain an ongoing immune response. Relative to naïve T cells, activated T cells require far less exposure to HLA-I/peptide complexes in order to be stimulated (19).

TUMOUR ANTIGENS

Apart from those derived from oncogenic viruses (HPV, EBV and so on), tumour antigens are 'self' (host) proteins. They can be divided into two main groups: tumour-specific mutated self antigens (frequently patient-specific) and shared non-tumour-specific, non-mutated self antigens that are common to most patients with a given type of cancer. Mutated self antigens result from somatic mutations in normal genes, reflecting the genetic instability of tumour cells. However, identification and isolation of mutated self antigens, although technically feasible, is of limited practical value because these antigens are usually patient-specific. Vaccination with defined and well-characterised shared antigens is clearly the method of choice. However, as shared antigens correspond to normal proteins expressed not only by tumour cells but also by normal cells and tissues, they are generally tolerated by the immune system, albeit to variable degrees (20).

Self tolerance is a phenomenon by which autoreactive T cells are inactivated or eliminated in order to prevent autoimmune disease. Deletion of autoreactive T cells mostly takes place in the thymus, when immature T cells encounter self antigens presented by thymic DCs or by medullary epithelial cells (21). Inactivation of autoreactive T cells occurs in the periphery and is mediated by several mechanisms, such as inappropriate or suboptimal presentation of antigens, and immune suppression by regulatory T cells and immunosuppressive cytokines (22). However, tolerance of self antigens, and thus of shared tumour antigens, is not absolute. The residual antitumoural T cell repertoire, which remains available for antitumour reactivity, depends on the efficiency of antigen presentation and on TCR affinity (23). As a result, the T cell repertoire against poorly presented cryptic peptides escapes tolerance induction mechanisms and contains numerous T cells with high affinity, whereas the T cell repertoire against dominant peptides is composed of only a few T cells with low affinity (see Figure 2). This has been confirmed in experimental models (24-26).

Likewise, in humans, a negative correlation has been shown between the HLA-I affinity and specific CTL affinity of several peptides derived from tumour antigens (27). This tolerance to dominant tumour peptides explains why, for example, vaccination of mice with autologous tumour antigens such as gp100, tyrosinase, p53 and HER-2/neu fails to induce an immune response (28,29).

CIRCUMVENTING SELF TOLERANCE

Overcoming or circumventing self tolerance of shared tumour antigens is thus a major goal of cancer vaccine research. The most

Table 1: Definitions

Active immunotherapy	Manipulations aimed at activating the patient's own immune system, such as vaccines
Adoptive immunotherapy	Administration of immune substances or cells that have been produced outside of the patient's own body, such as antitumour monoclonal antibodies or tumour infiltrating lymphocytes
Specific immunotherapy	Active immunotherapy targeting defined antigens
Non-specific immunotherapy	Active immunotherapy aiming at non-specifically stimulating the immune system

promising way of circumventing self tolerance is to use cryptic tumour peptides, whose specific T cell repertoire is relatively intact. This approach was first proposed by Sercarz *et al* more than 10 years ago (30). The main problem, of course, is how to elicit an immune response to peptides that, by definition, are not inherently immunogenic. One possibility is to enhance the HLA-I affinity of the target cryptic peptide. As mentioned earlier, HLA-I affinity depends on the presence within the peptide sequence of primary and secondary anchor motifs specific for a given HLA-I allele. In theory, HLA-I affinity can be enhanced by identifying unfavourable primary and secondary anchor motifs and replacing them with more favourable residues. However, replacing selected residues, especially in secondary motifs, may lead to a shift in the conformation (shape) of the peptide moiety that contacts the TCR and confers antigenicity.

A general approach that enhances the affinity of cryptic peptides presented by HLA-A*0201, the most frequent HLA-I allele (40-45 per cent of the population), was recently described (31). It consists of replacing the first residue, whatever its nature, by a tyrosine. Cryptic peptides 'optimised' in this way bind efficiently to HLA-A*0201 and have been shown to induce strong antitumoural immunity in mice (26). Excitingly, one optimised cryptic peptide derived from the universal tumour antigen hTERT was found to induce a strong and broad immune response in cancer patients, which appeared to correlate with a clinical response (32,33). The capacity of optimised cryptic peptides to induce antitumoural immunity has now been demonstrated in several experimental models (34-36). Taken together, these observations support the view that optimised cryptic peptides are more promising than dominant peptides as candidates for tumour immunotherapy.

THE NEXT GENERATION

Having established the therapeutic potential of optimised cryptic peptides, the optimal vaccination protocol remains to be determined. The main question is whether these cryptic peptides should be used throughout the vaccination protocol, or solely to prime antitumoural immunity that can then be maintained by boosting with the corresponding native cryptic peptide. When this question was addressed, it was shown that induced CTL had much higher affinity for the native peptide when the latter strategy was used. This also explains why repeated vaccination of melanoma patients with an optimised tumour peptide induced CTL that failed to recognise tumour cells (37).

Because of the disappointing results so far obtained with most tumour vaccines, which have been based on dominant peptides, research has tended to shift towards ways of stimulating the immune response through the use of new adjuvants, immunostimulants and delivery vectors. However, while these aspects are clearly crucial, the way forward seems to lie in the use of optimised cryptic peptides derived from shared tumour antigens, combined with a potent adjuvant and a method for inhibiting negative feedback of the immune response. Encouraging preliminary clinical results suggest that a new series of products effective against most cancers may be within our grasp. ◆

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