Vaccines for Tumours: Melanoma

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Abstract

This case commentary concerns a 56-year-old patient with stage III malignant melanoma which was removed but within 7 months the patient developed secondary tumours in his vertebrae. A multi-epitope vaccination was given after which the patient developed vitiligo and posterior uveitis. The topics addressed include the different types of antigens that can be expressed by malignant melanoma, how immune surveillance fails, the immunological mechanisms involved in the side effects of vaccination, and a comparison of peptide vaccination with dendritic cell immunotherapies. This commentary was created in 2015 as part of the masters programme in Molecular and Cellular Biochemistry at the University of Oxford.

Case

A 56-year-old barrister had a stage III malignant melanoma removed from his hand but within 7 months had developed secondary tumours (metastases) in his vertebrae. He joined a clinical trial of experimental multi-epitope vaccination and developed pale non-tumorous skin patches (vitiligo) and autoimmune eye disease (posterior uveitis).

Commentary

Vaccines have been hailed as one of the most effective health interventions ever created, however their impact thus far has been largely confined to infectious diseases. Recent dramatic results using immunotherapy have reignited hopes for harnessing the power and specificity of the immune system in tackling cancer (Chapman et al., 2015).

Discuss the different types of tumour antigens that can be expressed by malignant melanoma cells. How does the immune surveillance fail in such patients?

An antigen is a substance which the immune system can develop an immune response towards. There was once scepticism towards the existence of tumour antigens, perhaps because tumours are derived from self and immune tolerance therefore exists to most of the proteins tumours express (Hewitt et al., 1976). However many tumour antigens have now been identified and they can be grouped into five major categories: 1) tumour specific shared antigens, 2) differentiation antigens 3) mutated genes 4) overexpressed proteins and 5) viral antigens (Coulie et al., 2014).

Tumour specific antigens include the cancer germline or 'cancer testis' antigens which are expressed almost exclusively in tumours in adults. They are also expressed in male germline and trophoblast cells however as these cells do not normally express HLA (MHC) molecules there is no risk of cross-reactivity. This group

includes the MAGE (melanoma antigen-encoding gene) A, B and C family of genes, which were the first tumour antigens to be identified in 1991. These families include a total of 24 genes which are encoded for in three different regions of the X chromosome. MAGE-A3 is one of the most widely expressed tumour antigens with 74% of metastatic melanoma samples positive for it (Van Der Bruggen et al., 2002). Other antigen families in this class include BAGE, GAGE, SSX, and LAGE (NY-ESO-1) (Giavina-Bianchi et al., 2015; Coulie et al., 2014). The re-expression of these antigens in tumour tissue is possibly due to the demethylation of their promoters (Grunau et al., 2005). A large clinical trial of a MAGE-A3 peptide vaccine failed to meet its primary endpoint but is continuing for a secondary endpoint of disease free survival in a sub-population with a genetic signature (GlaxoSmithKline).

A second class of tumour antigens are differentiation factors, which although are also expressed in some normal tissues can nonetheless become the target of an immune response. For melanoma this group includes proteins which are involved in melanin production such as tyrosinase, gp100, tyrosine related protein 1 & 2, and melan-A. A phase III clinical trial using gp100 peptide vaccination in combination with IL-2 showed improved responses compared to IL-2 alone (Schwartzentruber et al., 2011)

The high genomic instability and mutation in cancers leads to creation of new antigens (neoantigens) through multiple mechanisms. Chromosome translocations can create fusion proteins which have novel protein sequences at the junction of the fusion (Palanisamy et al., 2010). As well as simple amino acid substitution due to point mutations, frame shift mutations and mutations in splice sites can result in a stretch of novel amino acid sequence being produced. Melanoma has one of the highest mutation rates of cancer and hence produces many neoantigens (Schumacher and Schreiber, 2015; Linnemann et al., 2015). Some mutations in melanoma occur at high prevalence in the same gene such as *BRAF* and *NRAS*. However, most mutations are unique and hence therapy would require personalised development (Schumacher and Schreiber, 2015). Despite this, personalised vaccination for melanoma has recently been demonstrated in a phase I clinical trial (Carreno et al., 2015).

Mutations can also affect regulatory sequences which can be manifested as protein overexpression. As T cells require a threshold level of antigen expression for recognition, a tumour specific response can be developed to proteins which are overexpressed in tumours. For example PRAME, survivin, and telomerase are all overexpressed in many melanoma tumours but not in normal tissues (McKenzie and Grossman, 2012; Huang et al., 2013).

Although many cancers are caused by viruses and hence express viral antigens which can be used in vaccination to protect against tumours, there is little evidence that this is the case for melanoma. Although some human endogenous retroviral antigens have been detected in some tumours (Tuttleton Arron et al., 2011).

Despite the presence of antigens and cytotoxic T cells that can recognise them, in many cases the tumour nonetheless progresses (Germeau et al., 2005). This phenomenon can be understood through the concept of immunoediting in which tumours progress through three stages in relation to the immune system: elimination, equilibrium, and escape.

In the elimination phase the immune system destroys newly transformed cells through recognition of tumour antigens as evidenced by the higher incidence of cancer in immunodeficient mice (Vesely et al., 2011).

However, in some cases the tumour mutates and enters an equilibrium stage in which its growth is controlled by the immune system but it progressively accumulates immune evasion mechanisms through mutation under the selective pressure of the immune system (DuPage et al., 2012). Striking evidence of this process can be found in transplant patients (undergoing immunosuppressive treatment to prevent rejection) who develop cancers due to a previously immune suppressed tumour in the transplant (MacKie et al., 2003).

Eventually the tumour may exhaust the immune system's tumour suppression mechanisms and escape, resulting in cancer.

There are many mechanisms by which the tumour can evade immune surveillance including reducing its antigenicity as well as decreasing the immunogenicity of the microenvironment. For example tumours have

lower MHCI antigen presentation levels caused by mutations in the MHCI α and β-2-microglobulin subunits themselves, or in genes involved upstream in antigen presentation such as TAP-1 & 2, the LMP immunoproteasome subunits, and the signalling pathways that induce the expression of these genes (including JAK, STAT and the interferon gamma receptor). Mutations in the tumour antigens themselves can also cause loss of antigenicity.

Tumour cells can also reduce immunogenicity through modification of their own surface molecules as well as secreting immunosuppressive factors. For example through expression of PD-L1 which increases apoptosis in T cells while suppressing apoptosis in Tregs. Furthermore, prolonged antigen expression results in the expression of CTLA4 on antigen presenting cells (APC) which negatively regulates T-cell proliferation and function. Blocking these pathways with monoclonal antibodies such as Pembrolizumab and Ipilimumab has shown promise in clinical trials (Callahan et al., 2015).

Expression of Indoleamine 2,3-dioxygenase results in tryptophan depletion which prevents T-cell proliferation (van Baren and den Eynde, 2015). Cancer-associated fibroblasts produce extracellular matrix and CXCL12 which physically excludes T cells from the tumour microenvironment. Additionally, expression and secretion of vascular endothelial growth factor (VEGF) inhibits dendritic cell (DC) maturation and differentiation. Transforming growth factor beta (TGFβ) secretion suppresses T-cell activation, proliferation and differentiation as well as NK function and DC activation at the same time as inducing Treg cells. Treg cells can mediate peripheral tolerance by suppressing DC and T cells for example by producing IL-10. Myeloid-derived suppressor cells also produce TGFβ as well as arginase and iNOS which results in nitric oxide production. This then results in the nitration of CCL2 which traps T cells in the stroma surrounding tumours, hence preventing infiltration. FasL expression is induced by TGFβ, IL-10, and VEGF and results in low T cells but abundance of Treg cells possibly due to high levels of c-FLIP, an apoptosis inhibitor (Joyce and Fearon, 2015).

Through all these mechanisms and more the immune system fails to prevent tumours from progressing (figure 1).

Explain the immunological mechanisms involved in the side effects of multi-epitope vaccination in this patient.

The occurrence of autoimmunity in melanoma patients after cancer immunotherapy (or spontaneously) is well documented (Teulings et al., 2015). Vitiligo is due to a loss of melanocytes and hence pigment in the skin. Posterior uveitis is inflammation of the uvea which is the pigmented layer situated between the retina and sclera in the eye. The common factor in both of these conditions is autoimmunity towards pigmented cells. Vaccination has triggered an immune response to tumour antigens (as intended), however these antigens include differentiation factors such as tyrosinase which are also expressed in other non tumorous tissue. The immune response to the tumour has cross-reacted with other pigmented cells.

There are two main immunological mechanisms involved in these side effects, firstly the breaking of immunological tolerance that previously existed and secondly the destruction of melanocytes by cytotoxic T cells.

Previous to vaccination both central and peripheral tolerance existed towards the differentiation antigens. Central tolerance is due to the negative selection of developing T lymphocytes in the thymus. If developing T cells have a high affinity to self-peptide-MHC complexes then they are deleted from the repertoire.

This central tolerance is incomplete and some low affinity self-reactive T cells can still exit the thymus. However, in the absence of inflammation, only low levels of co-stimulatory molecules are expressed on APCs such as DCs and their interaction with T cells results in tolerance to the presented molecules in the form of either T-cell anergy, deletion, or iTreg conversion (Bluestone, 2011).

In the case of this patient the vaccination regime caused this tolerance to be broken though the recognition of tumour antigens presented on DC cells by T cells in an inflammatory setting. This causes co-stimulatory

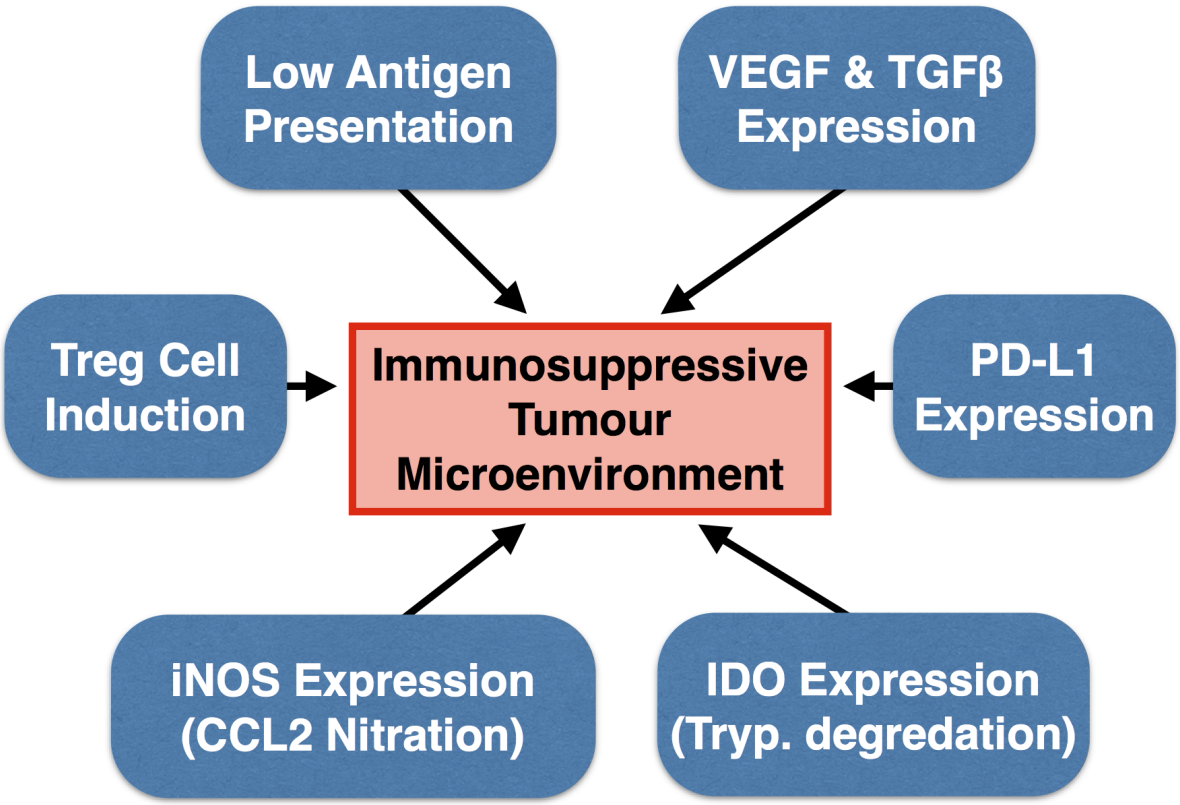

Figure 1: Summary of mechanisms which create an immunosuppressive tumour microenvironment

molecules such as CD80/86 to be expressed and provide the second signal required for T-cell activation.

This enabled cytotoxic T lymphocytes (CTL) that recognise melanocytes to proliferate and destroy these pigmented cells. The mechanistic basis for this cytotoxic activity includes the release of perforin which mediates the delivery of granzyme to the target cell through an immune synapse resulting in apoptosis. Evidence to support this model includes that CD8+ T cells from melanoma patients can kill non-cancerous melanocytes. Furthermore clonotypically identical T cells can be found in both tumours and areas of vitiligo (Byrne and Turk, 2011). Hence side effects such as vitiligo are actually signs that the therapy is working — indeed a 'clear survival benefit' is seen in such patients (Teulings et al., 2015).

Compare peptide vaccination with dendritic cell-based immunotherapies for tumours of this kind.

Dendritic cells are professional antigen presenting cells (APC) which play a key role in initiating CTL responses. They acquire antigens at the site of infection and travel to the local secondary lymphoid organs for presentation to T cells to activate them. For tumour antigens this requires cross presentation i.e. exogenous antigen presentation in the context of a MHC class I complex. DCs are the most potent APC and so using them should achieve the highest response. One DC based immunotherapy has been licensed against prostate cancer by the FDA: Sipuleucel-T (Kastenmüller et al., 2014).

Large numbers of immature DC cells can be generated by ex vivo culture of monocytes or CD34+ cells with

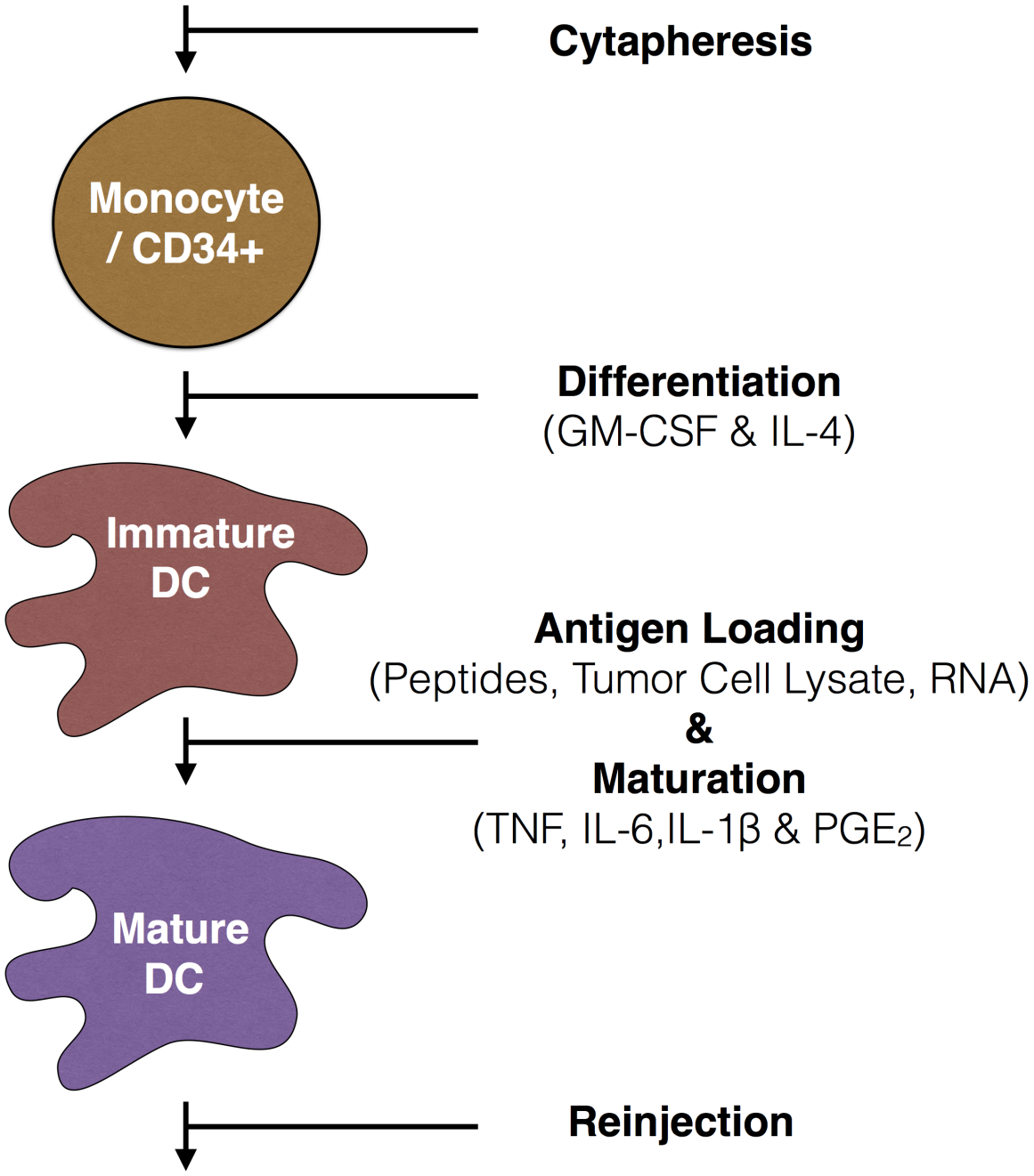

Figure 2: Production of an ex-vivo DC vaccine

GM-CSF and IL-4. These can then be loaded with antigens and matured with cytokines such as IL-1β, TNF, IL-6 and PGE_2 and reintroduced into the patient (figure 2). The advantage of this process is that the DC can be loaded with optimal amounts of tumour antigens in a highly immunogenic environment (for example in the presence of TLR ligands like CpG) (Sabado and Bhardwaj, 2015). This is in comparison

to peptides alone which if introduced into the immunosuppressive / noninflammatory tumour environment could induce tolerance (H. Yi and Appel, 2013). Furthermore the antigens can be loaded in their optimum form and time. DNA or RNA transfection could be more immunogenic than peptides as the resulting proteins can be presented through both MHC class I and II pathways and CD4+ cells can help the CTL response. Additionally peptides are degraded faster and are subject to HLA restriction.

The biggest disadvantage of the ex vivo DC approach is the time and expense required to generate the personalised vaccines. In contrast a peptide vaccine can be manufactured in one batch and administered to many patients. However it is also possible to target DCs *in vivo* for example by ligating the antigen to an antibody or ligand which will bind to DCs. Targeting different receptors can produce different immune responses. For example, some receptors lead to early endosomes which favours MHCI presentation and CD8+ T cells, whereas late endosomes favour MHCII presentation and CD4+ T cells. Furthermore different subsets of DC express different receptors and elicit different immune responses. For example CD205+ DCs present antigens on both MHC class I and II whereas CD8- 33D1+ DCs are specialised in MHC II presentation (Palucka and Banchereau, 2012). In comparison naked peptide vaccination is not able to bias immune responses by specific targeting.

Although DC based vaccines have not yet been shown to be broadly superior to peptide vaccines, they are much more complex and so there are many areas that could potentially boost their efficacy. For example although maturation induces increased expression of CCR7 receptor so that DC can migrate along CCL19/21 gradients towards lymph nodes, recent studies have shown that pretreating the injection site with tetanus/diphtheria toxoid can improve migration (Mitchell et al., 2015).

Manuscript History

This case commentary was originally produced as part of the masters programme in Molecular and Cellular Biochemistry at the University of Oxford (MBiochem, Part II) in 2015. The case, and the commentary questions were set by the course organisers. Additionally, the following limitations were imposed: The word count must not exceed 2000 words (excluding the case, questions, citations, and figure captions), the number of references must not exceed 30, and no more than three figures or tables may be used.

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