Chapter 8

Antigen-Presenting Cell/Tumour Cell Hybrid Vaccines in Cancer Immunotherapy

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Additional information is available at the end of the chapter

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Abstract

In recent years, there has been a considerable interest in the development of immunotherapeutic approaches for treating cancers, including strategies for inducing antigen-specific cytotoxic T cells (CTLs) capable of killing tumour cells *in situ*. These approaches include both the active induction of CTLs by vaccination of tumour bearing patients, and the *ex vivo* expansion of tumour-specific CTLs for adoptive cellular transfer. One promising approach has been through the generation of hybrid cells, formed by fusion of professional antigen presenting cells (pAPCs) with tumour cells expressing relevant tumour associated antigens. Dendritic cells (DCs) represent the most potent form of pAPCs, and have been widely used in the generation of APC/tumour cell hybrid vaccines, in the context of a range of tumour types. Studies of fusion cell vaccines in animals have demonstrated not only the induction of tumour-specific CTLs, but also protection against subsequent tumour challenge and regression of established tumours. Results of clinical trials in patients have been less dramatic, but have shown the ability of hybrid vaccines to induce tumour-specific T cell responses, in some instances associated with disease stabilization or tumour regression. In addition to dendritic cell fusion vaccines, a number of non-DC fusion vaccines have been described.

Keywords: antigen-presenting cell, cancer, tumour, hybrid, fusion, vaccine

1. Introduction

Recently, there has been significant interest in the development of immunotherapeutic approaches for cancer management. This has been strengthened by the approval of the first therapeutic dendritic cell-based vaccine explicitly prepared for the management of cancer by the Food and Drug Administration (FDA) in 2010 [1]. Many experimental cancer immunotherapy

studies depend on the use of professional antigen-presenting cells (pAPCs), such as dendritic cells, as inducers of tumour-specific immune responses, in particular for inducing tumour antigen-specific cytotoxic T-lymphocytes (CTLs) capable of targeting and killing tumour cells. One such strategy has been the development of APC/tumour fusion cells as candidate cancer vaccines. The approach was first described by Guo and colleagues [2], who showed that a vaccine made by fusion of hepatoma cells and activated B-cells protected rats against subsequent tumour challenge, and induced rejection of established tumours, by a mechanism that was mediated by CD4+ and CD8+ T-cells. In this chapter, we shall review the status, prospects and limitations of APC/tumour cell fusion vaccines for immunotherapy of cancer.

2. The concept of APC/tumour cell hybrids

The idea behind APC/tumour cell fusion hybrids as immunotherapeutic agents is relatively straightforward (**Figure 1**). Tumour cells express mutated proteins or overexpress proteins that the immune system recognizes as antigenic, and which differentiate them from normal somatic cells. However, they fail to present these to the host immune system in a way that elicits an effective anti-tumour immune response. In addition, many tumours evade immune responses [3] by a number of mechanisms, including downregulation of antigen processing, reduced or failure to express major histocompatibility complex (MHC) molecules, and failure to express co-stimulatory molecules. By contrast, professional APCs are potent inducers of antigen-specific T-cell responses, due to a high level of expression of MHC class I and MHC class II molecules, efficient antigen processing and expression of T-cell co-stimulatory molecules. *In vitro* fusion of tumour cells and professional APCs produces hybrid cells that express tumour-associated antigens (TAAs), and process and present them in a way that induces tumour-specific immunity (**Figure 1**). Fusions of tumour cells and APCs therefore represent potential agents for cancer immunotherapy, as they express multiple tumour antigens, process and present them to CD4+ and CD8+ T-cells, and provide effective T-cell co-stimulation [4].

3. Sources of tumour antigens—why use whole tumour cells

The first step in developing a tumour vaccine is to provide a source of tumour-specific antigens. There are a variety of tumour antigen sources, including peptides, exosomes, dead or dying tumour cells, recombinant viruses, DNA or RNA transfection or whole tumour cells. The latter represents an effective way for pAPC to present the entire range of antigens expressed in a given tumour, stimulating anti-tumour responses against a broad array of antigens, including mutations relevant to the oncogenic process [5]. Dendritic cells (DCs) represent the most potent form of pAPCs, and DCs pulsed with whole tumour cells or their derivatives have been used in clinical trials of cancer immunotherapy. Unlike vaccines using known tumour-associated peptides or antigens, whole tumour cell-derived vaccines may also present undefined tumour-specific antigens, extending the range of potential targets for the

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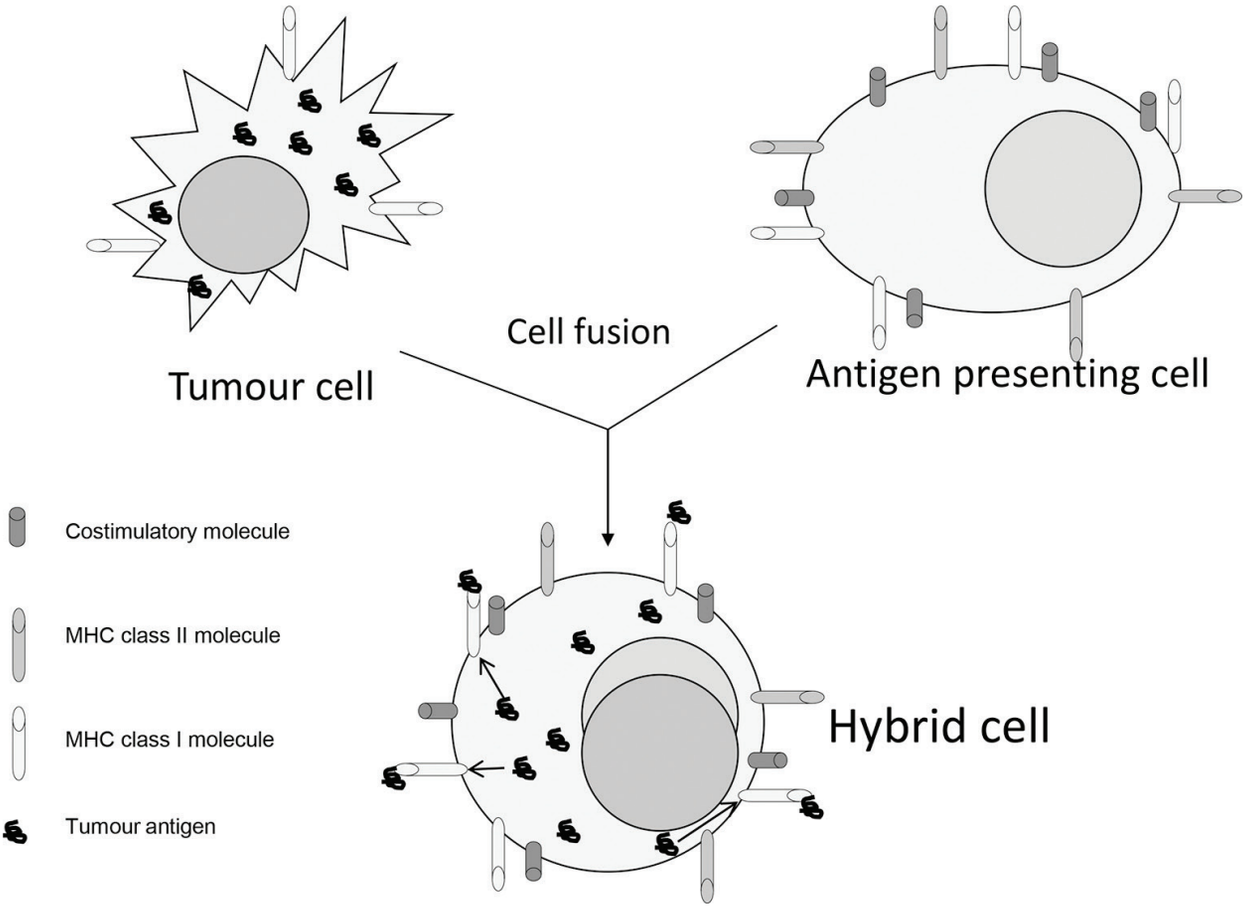

Figure 1. Principle of APC/tumour cell hybridization. (Figure adapted with permission from Ref. [4].).

immune system, resulting in the polyvalent stimulation of both CD8+ CTLs and CD4+ T-cells against a range of tumour antigens. Thus, by using whole tumour cells as a source of tumour antigens, a multi-antigenic response will be produced, and the probability of tumour escape *via* loss of antigens should be reduced.

4. Choice of pAPCs—the role of dendritic cells

Dendritic cells are the most potent antigen-presenting cells for naive T-cell activation. To understand the therapeutic use of DC vaccination strategies, it is important to understand the biology of DCs and how they regulate the innate and the adaptive immune systems—particularly in the context of the tumour microenvironment [6].

DCs are bone marrow-derived cells, which are found in a resting or immature state in non-lymphoid tissues, where they capture antigens. Stimulation of the immature DCs with a range of factors, including microbial products, inflammatory cytokines or cognate receptor-ligand interactions, induces the DCs to undergo maturation, resulting in increased antigen presentation, increased expression of MHC and co-stimulatory molecules, and migration to

secondary lymphoid organs, where they present the antigens to naive, antigen-specific T-cells [7]. DCs present the captured antigen to the T-cells in the form of peptide bound to self-MHC molecules in lymphoid tissues. DCs are the most potent type of pAPC, and can elicit immune responses even where very low numbers of antigen-specific T-cells are present. In mice and humans, there are two major subsets of DCs: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). The majority of studies of cancer immunotherapy have focussed on the use of mDCs.

DCs capture antigens in the periphery by a variety of mechanisms. The DCs then migrate into the lymph nodes (LNs), whilst processing the protein antigens into peptides that bind to MHC class I and MHC class II molecules. Antigens can also reach DCs resident in the lymphoid tissues through the lymphatic system [8].

On interaction with DCs, naive CD4+ and CD8+ T-cells (expressing appropriate T-cell receptors, with specificity for the peptide-MHC complex presented by the DCs) are activated to differentiate into effector T-cells with a variety of functions. Depending on additional signals that they receive, CD4+ T-cells can differentiate into helper T-cells with different patterns of cytokine release (TH1, TH2, TH9 or TH17 cells), or into T-follicular helper (TFH) cells that help B-cells to differentiate into antibody-secreting cells, or regulatory T (T_{Reg})-cells that have suppressive effects on the functions of other lymphocytes. Naïve, antigen-specific CD8+ T-cells differentiate into effector cytotoxic T-lymphocytes on activation. The nature of the T-cell response produced is dependent at least in part on the subset and differentiation status of DCs presenting the antigen [9].

DCs also play a role in controlling antibody responses. They do so by interacting both directly with B-cells and indirectly by activating cytokine-releasing CD4+ helper T-cells. The mechanism of direct presentation of (unprocessed) antigens by DCs to B-cells is incompletely understood [8]. Through these properties of DCs, activating both T-cell and B-cell arms of the immune response, DCs and their derivatives represent ideal candidates for cancer vaccines [9].

5. DCs in the tumour microenvironment

DCs are found in most tumours in humans and mice. Tumours, however, can avert antigen presentation and the establishment of tumour-specific immune responses through a variety of mechanisms, causing an imbalance between immunity and tolerance [10]. By switching the differentiation of monocytes to macrophages, rather than DCs (through the interplay of interleukin (IL)-6 and macrophage colony-stimulating factor; [11]), tumours can prevent the induction of tumour-specific T-cell responses. In addition, tumour glycoproteins such as carcinoembryonic antigen (CEA) and mucin 1 (MUC1) are endocytosed by DCs into early endosomes, bypassing the normal pathway of processing and presentation of antigens to T-cells [12]. Tumours also interfere with DC maturation. Firstly, they can inhibit DC maturation through the secretion of IL 10 [13], leading to antigen-specific anergy. Secondly, tumour-derived factors may subvert the normal maturation of mDCs, in ways that lead to the promotion of tumour growth ('pro-tumour' DCs). For example, tumour-derived TSLP induces OX40 ligand expression by DCs, which supports the differentiation of CD4+ T-cells

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into TH2 cells, and promotes tumour development through the secretion of IL 4 and IL 13, inhibiting tumour cell apoptosis and stimulating tumour-associated macrophages to secrete factors that promote tumour growth, such as epidermal growth factor (EGF) [14, 15].

Thus, DCs can have direct pro-tumour effects by promoting the survival and progression of tumour cells in a variety of ways [16]. The complex interactions between DCs and the tumour microenvironment can lead to the dysfunction of endogenous DCs in cancer-bearing patients. However, culture conditions have been defined by which DCs can be differentiated *in vitro* to optimize their APC functions, allowing such cells to be used effectively as cancer-immunotherapeutic agents.

6. *In vitro* differentiation of DCs for use as vaccines

The goal of cancer immunotherapy is to elicit tumour-specific CD8+ T-cell-mediated immune responses that will be sufficiently robust and long-lasting to generate durable tumour regression and/or eradication. The application of *ex vivo*-educated DCs emerged in an effort to avoid possible interferences in therapeutic efficacy due to the dysfunction of endogenous DCs commonly observed in cancer patients [17]. *Ex vivo* DCs are mainly generated through *in vitro* differentiation of peripheral blood mononuclear cells (PBMCs) in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 or IL-13 [18]. DC-based vaccines should present a 'mature' phenotype in order to activate an antigen-specific immune response upon T-cell encounter. This differentiated state is characterized by the expression of several co-stimulatory molecules (such as CD80 and CD86, CD40, CD70, or inducible T-cell co-stimulator ligand), the necessary activating 'second signals' in the immunological synapse [19]. Mature DCs also have high levels of expression of the antigen-presenting molecules, MHC class I and MHC class II (and CD1 for presentation of lipid antigens). In addition, a third signal is required to trigger an efficient $CD8^+$ T-cell response, which is the presence of an immunostimulatory cytokine profile [17]. This process is accompanied by an augmented chemokine-driven migratory capacity, with increased chemokine receptor 7 (CCR7) expression, which favours lymph node homing and T-cell encounter, and allows antigen presentation and T-cell activation [20]. This complex context has required the exploration of various strategies. A 'standard' maturation cocktail, composed of tumour necrosis factor (TNF)-α, IL-1β, IL-6, and prostaglandin E2 [21], has been extensively used to develop conventional DCs. This 'standard' mature DCs acquire an activated phenotype, respond to LN-homing signals and secrete moderate amounts of T helper (Th)1 cytokine IL-12p70, but with low immunoregulatory cytokine production [21]. Targeting the innate danger signal pathway of Toll-like receptors (TLRs) improved migration, cytokine profiles and immune responses [22]. Alternative approaches use type-1 polarized DCs, generated in the presence of interferon (IFN)-γ, which show a mature state with IL-12 release, chemotactical response to the LN-homing chemokine CCL19 and generate Antigen-specific T-effector cells [23]. Alternative strategies for the production of 'clinical grade' DCs include '*Fast* DCs', which are generated in a 3-day culture, show similar performance [24, 25], and DCs derived from CD34+ blood progenitor cells [26]. Taken together, considerable progress has been made over the years in

generating DCs suitable for use as cancer-immunotherapeutic agents, although the potential impact of *ex vivo*-generated DCs on immunotherapy requires additional studies to be fully understood.

7. DC/tumour cell fusion approaches

Several approaches have been used to generate APC/tumour cell hybrids, including electroporation, chemical fusion using polyethylene glycol or viral fusion. Fusion efficiencies can vary greatly. Other limitations of DC/tumour cell hybrids include a lack of replicative capacity of the fusion cells, and poor standardization of the resulting fusion products [27]. Methods of separating heterokaryons of the APC and tumour cells from unfused cells and cellular debris are an important consideration following the fusion. In many studies describing DC/tumour fusion vaccines, no definitive evidence of heterokaryonic fusion cell formation was given, and the effects described cannot, therefore, be directly ascribed to the hybrid cells themselves. Corroboration of this conclusion comes from reports that fusion hybrids generated from autologous (syngeneic) and allogeneic DCs displayed equivalent immunological function and therapeutic effects *in vitro* and *in vivo*. This suggests that at least part of the therapeutic effect of the DC/tumour fusion vaccines in these studies may depend on tumour antigen scavenging and presentation by antigen-presenting cells of host origin within the vaccine preparation. In support of this, a recent study showed that the presence of unfused (syngeneic) DCs in the vaccine preparation enhanced the immunogenicity of the vaccine, possibly by a combination of uptake and processing of necrotic tumour cells by the DCs and their differentiation to mature DCs following the electrofusion process [28].

8. Dendritic cell/tumour fusion hybrids and their utility in cancer immunotherapy

DC/tumour hybrid fusion cells may be more effective in cancer immunotherapy than other DC-based vaccine approaches. DC-tumour cell fusion potentially confers not only the DCs' professional APC capacity but also the endogenous expression of a range of TAAs for processing and MHC-restricted T-cell sensitization. Many investigators have shown, in animal models, that vaccination with DC/tumour fusion hybrids protected against challenge with the relevant tumour and mediated the regression of established tumours of a wide range of tumour types, including renal, colon, lung, breast, hepatic and cervical carcinomas, melanoma, sarcoma, neurological and haematological tumours [29–35]. In addition, studies in tumour-prone mouse strains vaccinated with fusion cell vaccines showed protection against, or delay in the development of, tumours [36–38]. Both syngeneic and allogeneic DCs were shown to be effective as APCs for fusion hybrids for vaccination, and the mechanisms of protective immunity induced by DC/tumour fusion vaccines depended on their ability to induce both CD4+ and CD8+ T-cells, with CD8+ antigen-specific CTLs representing the major mediators of tumour rejection [30, 31, 33, 34, 37].

Appropriate antigen loading is a crucial parameter for optimizing the efficacy of anti-tumour immunotherapy. Using a murine colon cancer model, Yasuda and his colleagues evaluated

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the anti-tumour efficacy of four different preparations of DC vaccines, including DCs pulsed with tumour lysate, DCs pulsed with necrotic tumour cells, DCs pulsed with apoptotic tumour cells and DC/tumour fusion hybrid cells. Their results showed that DC/tumour cell fusion hybrids and DCs pulsed with apoptotic tumour cells induced stronger anti-tumour protection than DCs pulsed with necrotic tumour cells, whilst vaccination of DCs pulsed with tumour lysate failed to elicit any anti-tumour effect [35]. DC/tumour fusion hybrid cells induced the most effective anti-tumour response in animals receiving higher doses of tumour-cell challenge. DC/tumour cell fusion hybrids also induced the strongest cytotoxic T-lymphocyte activity and *in vitro* production of IFN-gamma of the preparations tested. These results suggest that DC/tumour fusion hybrids are stronger stimulators of protective immunity against solid tumours than other antigen-loading strategies using whole tumour cell materials [35]. Furthermore, DC/tumour cell fusion hybrids have been shown to demonstrate superior efficacy for the treatment of murine tumour models than other DC-based vaccination strategies in other studies [39–43]. Parameters that may require further adjustment to maximize the anti-tumour effect of DC/tumour cell fusion hybrids include the DC maturation state, fusion efficiency between DC and tumour cells, and the use of appropriate adjuvants.

In clinical trials for patients with a variety of metastatic diseases, fusion hybrid vaccines were well tolerated, but the overall objective response rate was less might have been expected from the animal studies. For example, in a study of DC/tumour cell hybrid vaccination in patients with stage III/IV melanoma, Trefzer et al. reported 1 complete clinical remission, 1 partial response and 6 cases of disease stabilization in 17 patients studied, with 11 of 14 patients analysed demonstrating T-cell responses to tumour-associated T-cell epitopes [44, 45]. Similarly, in a study of 21 renal cell cancer patients vaccinated with autologous tumour/allogeneic dendritic cell fusions, 2 showed partial clinical responses and 8 showed disease stabilization [46]. In this study, of the 21 patients included, 10 showed increased anti-tumour immune responses in response to the vaccine, with increased CD4 and/or CD8 T-cell expression of interferon-gamma on stimulation of cells with tumour cell lysate [46]. Avigan showed disease regression in 2 patients with breast cancer, and disease stabilization in 6 more of the vaccinated patients, in a study of 23 patients with breast or renal cancer, vaccinated with autologous DC/tumour cell fusions [47]. Finally, in 17 patients with multiple myeloma, immunized with autologous DC/tumour cell fusion vaccines, T-cell responses to autologous tumour cells was seen in 11 patients, with disease stabilization seen in the majority of evaluable patients [48]. Although the clinical responses seen in these phase I/II clinical trials have been less dramatic than the responses seen in the animal studies, the vaccines have proved to be safe, and larger, placebo-controlled studies are needed to demonstrate whether these DC/tumour cell vaccines offer significant therapeutic benefit.

9. Future cancer regimens using DC/tumour fusion cells

Effective and selective targeted therapies with little toxicity are urgently needed for patients with advanced cancer. Treatment of cancer patients with DC/tumour fusion cells alone may be limited by the induction of immunosuppressive mechanisms. DC/tumour fusion cells can induce not only antigen-specific CTLs but also Tregs, which may counteract their

therapeutic effects [49]. Some chemotherapeutic agents, such as cyclophosphamide and gemcitabine, can activate anti-tumour immunity by depleting Tregs and myeloid-derived suppresser cells (MDSCs) [50], leading to improved clinical outcomes. Recent reports have shown that CTLs induced by vaccination may express the marker programmed death 1 (PD1, and that its ligand, PD-L1, is upregulated in tumour cells by IFN-γ produced by activated CTLs) [51]. The interaction of PD1 (on the CTL) and PD-L1 (on the tumour) leads to impaired CTL function. In a recent preclinical study, it was shown that the use of an anti-PD1 antibody was associated with enhanced CTL activity, and decreased Tregs [52]. Moreover, inactivation of CD4+CD25+Foxp3+ Tregs by an anti-CD25 antibody following DC/tumour fusion cell vaccination significantly improved anti-tumour immunity in a murine model [53]. Therefore, the inhibition of immune checkpoint blockade may enhance CTL activity and reduce induction of T-cell anergy in DC cancer vaccination strategies, and a therapeutic regimen combining DC/tumour fusion hybrid cells, chemotherapy, Treg depletion and/or antibody blockade of PD1-PD-L1 signalling may have potential in advanced cancer patients [54]. Further work will be required to identify which combinations of such strategies will provide optimum benefit, and in which patients and tumour types [55].

10. Fusion of DCs and cancer stem cells

It is well accepted that cancer stem cells (CSCs) are resistant to standard therapies, such as chemotherapy and irradiation [56]. Therefore, small populations of chemoresistant CSCs may result in tumour relapse and growth, following conventional cancer therapies [57]. Importantly, chemoresistant CSCs preferentially express stem cell markers, including OCT3/4, ABCG2, nestin, SOX2, Bmi-1, Notch-1, CD44, CD133 and CD177 [56]. CSCs also overexpress a range of known tumour-associated antigens, such as survivin, MUC1, hTERT, HER2, CERP55, COA-1 and WT1 [58]. In addition, MUC1 expression is upregulated in chemoresistant CSCs which are efficiently lysed by MUC1-specific CTLs in mice [59].

Thus, CSCs remain potential targets for cancer vaccines, and the success of cancer vaccines may at least partly depend on the efficient induction of anti-CSC immunity. Fusions of DCs with pancreatic tumour cells with CSC characteristics were shown to process and present multiple endogenous CSC-specific antigenic peptides on MHC class I and II molecules, and to induce CSC-specific CTL responses [57]. Moreover, fusions of DCs and both CD133+ and CD133- glioma tumour cells were equally effective at inducing cytotoxic anti-tumour immunity against autologous glioma cells [60]. These data suggest that fusion cells generated with DCs and CSCs (DC/CSC-FCs) can process and present CSC antigens, and induce CSC-specific CTLs, without the need to identify CSC-specific antigens. Therefore, DC/CSC fusion cell vaccines may provide an approach capable of eliminating residual, chemoresistant CSCs that would otherwise result in disease relapse following conventional cancer therapies.

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11. Other APC/tumour cell hybrids

Most studies of hybrid cell vaccines have used autologous or allogeneic DCs as the APC to fuse with tumour cells. However, non-DC APCs have also been used to generate hybrid cell vaccines. As mentioned above, Guo et al. used activated B-cells as APCs in their study of fusion cell vaccines against hepatocellular cancer in rats [2]. Several phase I clinical trials have been reported using non-DC APCs as fusion cell vaccine partners, including activated autologous B-cells, and activated allogeneic peripheral blood lymphocytes [61, 62]. As with the clinical trials using DC/tumour fusion cell vaccines, clinical or immunological responses were reported in individual patients, and the approaches were safe with minimal toxicity.

11.1. The use of EBV B-lymphoblastoid cells as pAPC

It is important that the hybrids express multiple tumour antigens in the context of MHC class I and/or class II molecules as well as co-stimulatory molecules essential for T-cell activation, and that careful characterization of the vaccine cell lines should be carried out prior to their use as immunotherapeutic agents. To address the limitations of poor standardization and replicative capacity of DC/tumour cell fusion hybrids, we have used an EBV B-lymphoblastoid cell line (B-LCL) as APCs in generating APC/tumour hybrid cell lines. EBV B-LCLs show many of the characteristic features of professional APCs, including high levels of expression of MHC class I and class II molecules, and important T-cell co-stimulatory molecules, such as CD80, CD86 and CD40 [63], and are immortalized for growth in cell culture. They therefore represent an attractive alternative to DCs as the APC partner in APC/tumour hybrid vaccine cells. The LCL that we have used (HMy2; [64]) has been modified to allow for double chemical selection of the fusion cells, facilitating the selection of stable, self-replicating LCL/tumour hybrid cell lines following fusion. Fusion of HMY2 with a range of haematological tumour cells and cell lines resulted in hybrid cell lines that expressed high levels of MHC class I and class II molecules, as well as relevant T-cell co-stimulatory molecules [27, 63, 65, 66]. Interestingly, these hybrid cell lines expressed TAAs not only associated with haematological malignancies, including TAAs that are commonly expressed in solid tumour cells, and widely expressed tumour antigens such as hTERT and survivin [27, 63, 65, 66; Khalaf et al., unpublished]. Stimulation of peripheral blood T-cells from both healthy donors and tumour-bearing patients *in vitro* using LCL/tumour hybrid cell lines induced tumour antigen-specific CTLs that secreted interferon-gamma and killed tumour cells presenting the relevant antigen(s), demonstrating the potential of these hybrid cell lines to induce tumour-specific immune responses in humans, *in vitro* at least [27, 66, 67; Khalaf et al., unpublished]. An important feature of the HMy2/tumour cell fusion system is that it produces stable hybrid cell lines, which proliferate spontaneously in tissue culture. This means that detailed phenotypic and antigenic characterization of the hybrid cells can be carried out, and that large numbers of standardized cells can be produced. So far, however, there have been no clinical trials of LCL/tumour hybrid cell vaccines.

12. Adoptive immunotherapy

An alternative use of APC/tumour fusion cells in cancer immunotherapy is as *in vitro* stimulators of tumour-specific CTLs for adoptive cellular immunotherapy [27, 48, 68]. Adoptive CTL therapy has been used with clinical benefit in a range of malignancies, including haematological and non-haematological tumours [69–71]. As outlined above, we have shown that LCL/tumour hybrid cell lines can induce tumour antigen-specific CTLs in PBMCs from both healthy individuals and tumour-bearing patients [27, 66, 67]. Given some of the current uncertainties of APC/tumour cells as therapeutic cancer vaccines in humans, and the demonstrated ability of APC/tumour hybrid cells to induce tumour antigen-specific CTL *in vitro*, [27, 66, 72–76], the use of APC/tumour hybrid cells as inducers of tumour-specific CTLs for adoptive immunotherapy merits further investigation.

13. Future challenges and directions

Many animal studies over the previous two decades have shown the ability of APC/tumour cell fusion vaccines to protect against tumour challenge, to eliminate established tumours, and to prevent tumour development in genetically prone animals. Phase I/II clinical trials have shown that vaccination with APC/tumour hybrid vaccines is both safe and well tolerated, although the efficacy of the approach in human subjects remains to be established. Outstanding questions remain in relation to the optimization of the approach for use in humans, the nature of the APC used for the production of hybrids, tumour types where it may be effective, and whether it should be used in conjunction with other forms of cancer therapy. Further investigation is required to address these questions. In addition, APC/tumour fusion cells have been shown to induce tumour antigen-specific CTL *in vitro*, and in this capacity they may have a role in generating antigen-specific effector T-cells for adoptive T-cell cancer immunotherapy [4].

The limited efficacy of DC/tumour cell hybrid vaccines in clinical trials may be due to a number of factors. Firstly, genuine DC/tumour fusion cells need to be verified and isolated or selected. A number of clinical studies did not demonstrate clear verification of DC/tumour fusion cells, making assessment of resultant clinical impact difficult. Secondly, the optimal-dosing schedule and number of fusion cells per injection remain uncertain. This may differ in patients with different tumour types and burdens, and immunological status. Furthermore, the site of vaccine delivery may affect the treatment response. In a clinical study, the intradermal injection of DC-tumour hybrid vaccine resulted in superior antitumour response compared to other routes [77]. Other pre-clinical data suggest that the provision of a third signal with the hybrid vaccines may generate a better response rate [78, 79]. Finally, the use of DC/tumour fusion vaccines may demonstrate significant efficacy when combined with other treatment modalities. There is evidence that radiation as well as chemotherapy combination synergizes with immunotherapy vaccines [80]. Moreover, targeting the

immunosuppressive immune mechanisms such as regulatory T-cells and myeloid-derived suppressor cells may also improve the vaccine efficiency [81]. The immune system, and its interaction with normal and tumour cells, is complex and the advances that are being made in immunotherapy field are numerous. Although these advances have been fewer to date in the field of cancer vaccination than in other forms of cancer immunotherapy, such as monoclonal antibodies against immune checkpoint control molecules, the potential of harnessing and directing the immune system to eliminate tumours through antigen-specific immunotherapy remains the goal of many researchers. APC/tumour fusion cells represent a promising approach to realizing this goal.

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