Intratumoral immunotherapy: using the tumour against itself

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Summary

Diverse immunotherapy approaches have achieved success in controlling individual aspects of immune responses in animal models. Transfer of such immunotherapies to clinical trials has obtained some success in patients, with clinical responses observed or effective antigen specific immune responses achieved, but has had limited impact on patient survival. Key elements required to generate *de novo* cell-mediated antitumour immune responses *in vivo* include recruitment of antigen-presenting cells to the tumour site, loading these cells with antigen, and their migration and maturation to full antigen-presenting function. In addition, it is essential for antigen-specific T cells to locate the tumour to mediate cytotoxicity, emphasizing the need for local inflammation to target effector cell recruitment. We review those therapies that involve the tumour site as a target and source of antigen for the initiation of immune responses, and discuss strategies to generate and co-ordinate an optimal cell-mediated immune response to control tumours locally.

Keywords: chemokine; dendritic cell; immunotherapy; T cell; tumour

When would intratumoral immunotherapy be clinically applicable?

The majority of human tumours grow in an immunocompetent host and elicit minimal, if any, clinically relevant antitumour immune responses at the point at which they present. However, various tumour-associated antigens that are recognized by specific immune cells are identifiable in patients with cancer, indicating that the immune system is capable of recognizing tumours.[1–6] Evidence in animal models suggests that tumour formation occurs at a higher frequency in immunodeficient mice.[7] Animals that have a deficiency in T cells or in the interferon (IFN) pathway have a higher incidence of tumour formation when compared to controls.[8] Furthermore, those tumours that develop in immunodeficient animals are highly immunogenic when transplanted into immunocompetent hosts, suggesting that immune shaping of the tumour phenotype has not occurred in these tumours.[7,8] Circumstantial evidence that similar immune selection occurs in spontaneous human tumours, comes from studies demonstrating reduced major histocompatibility complex (MHC) class I expression,[9] deficiencies in antigen processing and presentation machinery[10] and, importantly, selection against mutations that occur in MHC-binding epitopes.[11]

Any intratumoral therapy requires access to the tumour site. Initial studies will be further limited to accessible sites in appropriate clinical scenarios. The accessibility of primary melanomas, and the standard use of surgical excision, provide interesting examples. New chemotherapy drug combinations and radiation therapy doses are beginning to show some promise.[12–18] Nonetheless, the low efficacy of traditional treatment modalities has resulted in many alternative immunotherapeutic treatment modalities being tested.[19] The most promising immunotherapeutic approach, high-dose IFN-α, is now approved by the Food and Drug Administration (FDA) for high-risk melanoma, including both thick (>4 mm) node-negative patients and node-positive patients (AJCC Stage II and Stage III, respectively). However, high dose IFN-α is not without its risks: in one study it was associated with grade III toxicity in 67% of patients; 9% of patients experienced life-threatening toxicity and two patients died from hepatotoxicity.[20] While a disease-free survival benefit was shown in studies of high-dose IFN, the initial overall survival benefit reported in the ECOG 1684 trial appears to be lost with longer follow-up. Given the conflicting data, as well as the high toxicity, there is controversy among investigators as to whether high-dose IFN should be the standard of care. In Europe, high-dose IFN is not the accepted adjuvant treatment for patients with Stage II and Stage III

disease, and among investigators, both in Europe and elsewhere, other adjuvant approaches, such as vaccines and non-toxic long-term IFN schedules, are being explored. In addition to such non-specific approaches, a number of immunodominant and commonly shared melanoma tumour antigens have been identified. Vaccine approaches using either autologous tumours or allogeneic tumour cells expressing shared antigens, or alternatively using purified defined tumour antigens or epitopes, has shown some promise in small trials.[21–24] The use of *ex vivo* cultured autologous tumour cells, which should include multiple target antigens, is highly labour-intensive and such cells are not always successfully grown from the primary tumour material. Use of defined tumour-associated antigen has been associated with a loss of specific antigen expression in tumours following repeated treatment.[25] However, for the purpose of this review, one relevant criticism of the majority of vaccine-based approaches is that they are performed at sites distant from the tumour. Antigen-directed vaccination therapies generally result in an accumulation of specific cells at the vaccination site.[26–28] Thus, intratumoral approaches are appealing because they may direct antigen-specific responses back to the tumour site, and also exploit the presence of multiple undefined tumour antigens present in the endogenous tumour. Furthermore, the local aspect of the immune reaction within the tumour would be expected to reduce systemic toxicity, analogous to the use of isolated limb perfusion compared to systemic drug delivery.[29]

As the standard of care for grading and staging in melanoma requires surgical removal of the primary lesion, typically with tumour-free margins, intratumoral therapy is currently only practical in patients with local recurrence or surgically accessible metastases. Eventually, for those therapies that look promising, it may be possible to use a neoadjuvant approach and treat the primary lesion prior to surgical excision. As systemic gene therapy improves, it may also be possible reach metastases or primary tumours that are not accessible for direct injection. We will argue that such neoadjuvant therapies would provide broadly tumour reactive, immune-mediated control of emerging tumours in patients.

Life cycle of the optimal cell-mediated immune response

From the wealth of published literature on those immune responses that are most capable of controlling tumour growth *in vivo*, we hypothesize that there are certain features that must be incorporated to initiate effective cell-mediated antitumour immunity. We also believe that there must be local provision of antigen, in a form highly accessible to immature dendritic cells.[30] This antigen must be provided in conjunction with molecules capable of activating these dendritic cells plus resident immune-modulating

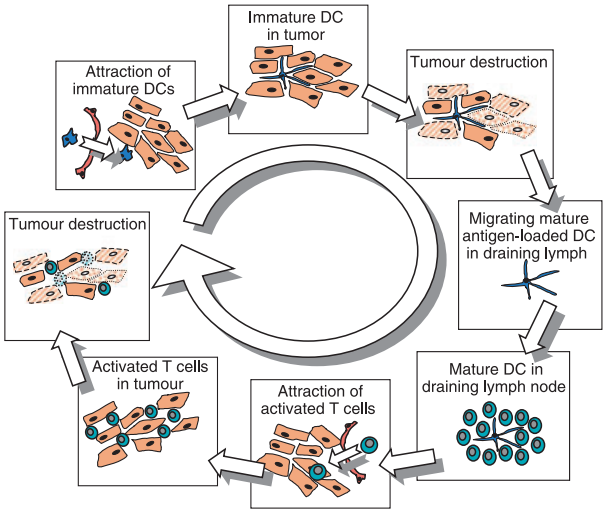

Figure 1. Life cycle of the optimal cell-mediated antitumour immune response. Local attraction of immature dendritic cells (DCs), which can subsequently be loaded with tumour antigens via cytotoxic therapy and activated to emigrate to regional lymph nodes, effectively prime antitumour effector cells and return to the tumour site for further tumour destruction.

cells, such as macrophages.[31–37] These criteria are hardly new, and are shown in Fig. 1. Almost any effective vaccination strategy of the previous 200 years incorporates antigen in some form of vehicle and accompanied by classical adjuvants, whether non-specific bacterial DNA and cell-wall products or the inherent properties of an attenuated viral vector.[38] However, one critical difference between the goals of infectious disease vaccination and tumour immunotherapy is that the former is intended to protect systemically against a subsequent potentially pathogenic challenge, whereas the latter aims to redirect immune responses to an existing tumour in a specific location, or locations, within the host. Pathogenic challenge with an infectious agent requires local immune activation, even in vaccinated hosts, to identify the infection site.[39,40] The location specificity of vaccinations is clear, particularly in classical models such as the delayed-type hypersensitivity response, where potent responses occur solely at the antigen site.[26] For these reasons we will discuss immunotherapeutic approaches that act in concert with current understanding of effective cellular immune responses, and consider the spatial regulation required of therapies to both initiate and maintain immune activity against tumours.

Lessons from tactics applied in existing tumour immunotherapies

Attracting dendritic cells to tumours

Dendritic cells have critical importance in priming effective, antigen-specific T-cell activation within the secondary

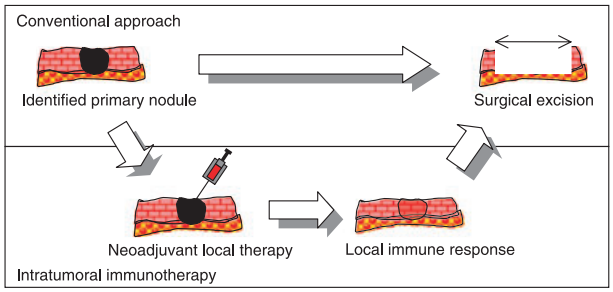

Figure 2. Example of intratumoral immunotherapy in a neoadjuvant setting.

lymphoid organs.[30] Therefore, the first step towards an optimal initiation of antigen-specific antitumour responses involves the attraction of dendritic cells to the tumour. Several studies have documented the presence and prognostic significance of dendritic cells within various human tumours.[41–43] These observations support the notion that increasing the number of dendritic cells at a tumour site could have desirable antitumour effects for a host.

Distinct from the systemic and distant vaccination applications of *ex vivo*-derived dendritic cells to initiate antigen-specific immune responses, various studies have applied such dendritic cells directly to the tumour site (Fig. 2). Table 1 lists the advantages and disadvantages of such an approach. Direct intratumoral application is particularly relevant in view of evidence demonstrating that few dendritic cells administered at vaccination sites reach the draining lymph node,[44,45] and the dendritic cells accumulate antigen-specific T cells at the site of vaccination at the expense of peripheral circulating T-cell numbers.[28] Intratumoral injection of syngeneic dendritic cells into malignant gliomas in rats led to infiltration of the tumour by CD4 and CD8 cells, and ultimately a prolongation in survival and immunity to subsequent tumour rechallenge.[46] In a murine colorectal tumour model, mice receiving primary tumour challenges followed by excision and rechallenge were better protected against rechallenge when the primary tumour was co-administered with syngeneic immature dendritic cells.[47] Significant protection against rechallenge was also observed in this model where the dendritic cells were injected into established tumours.[47] In a preliminary clinical study, a small cohort of patients with metastatic dermal or subcutaneous breast and melanoma tumours received autologous dendritic cells intratumorally. Regression of injected tumours was observed in six out of 10 patients, and biopsies of the regressing tumours showed the presence of dendritic cells.[48] To enhance the efficacy of intratumoral dendritic cells, these dendritic cells have also been engineered to express proinflammatory cytokines.[49–51] The data relating to dendritic cell transfer requires careful interpretation because it is unclear in such models whether the transferred dendritic cells are behaving exactly as anticipated. It has been shown that *ex vivo*-derived dendritic cells have poor viability *in vivo*, and that host dendritic cells are required for the therapeutic effect.[52] The mechanism of antigen transfer to host dendritic cells is at present unclear, and it is not understood whether the death of trafficking dendritic cells is a normal process in the draining lymph node.

Table 1. Advantages and disadvantages of intratumoral immunotherapy

Using the tumour as a site of vaccination	
Advantages	Disadvantages
No need to identify antigens or MHC	Limited accessibility of tumours
Ability to manipulate environment	Tolerogenic tumour environment
Identification of tumour site for effector cells	Acceptability of delay before tumour excision

MHC, major histocompatibility complex.

An alternative approach to the *ex vivo* generation of dendritic cells is to exploit the endogenous precursor population, via the chemokine-orchestrated migratory and homing properties of dendritic cells, to increase the number of endogenous dendritic cells at a tumour site.[53,54] Several options have been tested to attract endogenous dendritic cells to tumours, including the genetic modification of tumour cells and direct intratumoral injection of recombinant proteins.[55–59] Dendritic cell attraction has been proposed as a critical component of the widely studied expression of granulocyte–macrophage colony-stimulating factor (GM-CSF) in tumours.[55,56,60–65] However, it is important to note that GM-CSF is not a chemoattractive cytokine, but does induce secretion of chemokines from resident macrophages that are chemoattractive for dendritic cells.[66–68] Thus, a more direct approach is to similarly provide appropriate chemokines at the tumour site. Based on chemokine receptor expression data, it is possible to identify chemokines that will be chemoattractive for immature dendritic cells.[53,69–74] Immature dendritic cells express the chemokine receptors CCR1, CCR5 and CCR6, which bind, amongst other molecules, the important inflammatory chemokines CCL3, CCL5 and CCL20. Injection of recombinant chemokine has been shown to cause infiltration of cells into the injection site,[75,76] while constitutive expression of CCL3 has been shown to abrogate the tumorigenicity of an immunogenic tumour established subcutaneously.[77] However, in the less immunogenic B16 melanoma model, the growth of subcutaneous tumours expressing CCL3 was unaffected.[77,78] Interestingly, cells injected intravenously (i.v.) to form lung metastasis did not grow where CCL3 was expressed, suggesting that location plays a large role

in the efficacy of chemoattraction.[78] A slow-release formulation of the chemokine CCL20 using polymer rods was shown to cause accumulation of Langerhans' cells at the subcutaneous implantation site,[79] and these cells could subsequently be antigen loaded and activated to enhance antigen-specific immunity.[79] As with CCL3, described above, Crittenden *et al.*[77] demonstrated that mice challenged with immunogenic colorectal tumours, which had been genetically modified to secrete CCL20, failed to develop tumours compared with non-chemokine-expressing controls, and that expression of CCL20 was associated with a significant increase in dendritic cells at the site of tumour inoculation. Similarly, intratumoral injection of adenovirus-expressing CCL20 resulted in significant inhibition of tumour growth.[57] Intratumoral injection of recombinant CCL21 resulted in the accumulation of dendritic cells, along with T cells,[80] and inhibited growth of lung carcinoma and melanoma in a T-cell-dependent manner.[59,80] Intratumoral injection of herpes simplex virus (HSV) amplicons expressing CCL21 into established A20 and CT-26 tumours in mice caused significant infiltration of T cells and dendritic cells and resulted in CD8-dependent antitumour immunity.[58]

Based on these data, we hypothesize that the attraction of dendritic cells to a tumour site can be a vital first step in priming effective antitumour responses *in vivo*, and that chemokine gene expression can be an effective technique for using to recruit endogenous cells to the tumour without the need for *ex vivo* manipulations. However, a number of growing human tumours has been characterized to constitutively express chemokines that are chemoattractive to dendritic cells.[81–83] Similarly, in less immunogenic tumour models, expression of chemokines in tumours is insufficient to cause tumour rejection.[77] It is possible that the cells attracted to tumours by such chemokines do not take up antigen, or remain immature and function as tolerogenic antigen-presenting cells (APC).[84–87] The tumour-type dependence of the presence and prognosis of infiltrating dendritic cells probably relates to environmental factors within the tumour that regulate antigen loading and maturation of dendritic cells. For this reason we will directly address strategies to enhance these key features of the initiation of immune responses.

Antigen loading of dendritic cells in tumours

Dendritic cells, as professional APC, must obtain tumour antigen in order to initiate *de novo* antigen-specific immune responses. An extremely varied range of strategies has been applied to provide antigen to dendritic cells. The major distinction in dendritic cell loading with antigen is between *in vitro* and *in vivo* provision of antigen. For those strategies involving adoptive transfer of *in vitro*-differentiated dendritic cells, it is extremely easy to provide antigen in a suitable form *in vitro* prior to dendritic cell transfer. Antigen-presentation strategies, such as peptide antigen loading, have only a very short duration of efficacy, with epitopes rapidly lost from MHC on the dendritic cell surface and associated loss of T-cell stimulatory activity.[88] *Ex vivo* loading has been improved by conjugating exogenous protein antigen to molecules that direct it into appropriate cross-presentation pathways, such as heat shock proteins,[89] complement[90,91] and antibody.[92–94] These data translate well to *in vivo* models, where similar conjugation of antigen prior to *in vivo* administration dramatically enhances the generation of antigen-specific immune responses.[89,91,93,94] Dendritic cells efficiently present antigens coded in tumour-derived RNA,[95,96] and more stable expression of tumour antigens via infection with viral vectors generates dendritic cells capable of inducing transgene-encoded antigen-specific immunity.[97–99] In addition, dendritic cells actively phagocytose apoptotic tumour cells, and can cross-present apoptotic tumour-derived antigen on MHC class I.[100,101] Dendritic cells do not readily take up and present antigen from live cells. However, there is a growing body of literature reporting that dendritic cells can take up and present antigens incorporated in vesicular structures (called exosomes) released by live tumour cells.[102–104]

Each of these strategies has been applied via *in vitro* loading to prime immune responses *in vivo* via vaccination at distant sites. Using the tumour as the site of vaccination ensures that antigen is locally available and that the target antigens need not be defined. Nevertheless, it still remains to transfer tumour-associated antigens, whether antigen-encoding DNA or RNA, or translated proteins or digested peptides, from the tumour cell to the dendritic cell within the tumour *in vivo*. A very applicable strategy to provide endogenous tumour antigens to dendritic cells is the combination of intratumoral dendritic cells with systemic cytotoxic therapies. A number of recent reports describe the use of systemic chemotherapeutics that cause tumour cell death, combined with intratumoral dendritic cells.[105–107] In these models, the combination was significantly more effective than either agent alone[105–107] and also caused regression of distant uninjected tumours.[106] Similarly, intratumoral injection of dendritic cells was more effective in a breast tumour model when combined with agents that enhance levels of cell death in the tumour.[108] For these reasons, we believe that it is unnecessary to prepare and load dendritic cells *ex vivo*, in circumstances where dendritic cells can be efficiently loaded with relevant antigens within the tumour by combining local attraction of immature dendritic cells with tumour cytotoxicity.

Activating dendritic cells loaded with tumour antigens

Dendritic cells are highly responsive to inflammatory stimuli, such as ligands of the tumour necrosis factor

(TNF) family[30,109–111] and ligands activating Toll-like receptors on dendritic cells.[31–35] However, the uptake of cell-associated antigen can directly influence dendritic cell maturation to full T-cell priming potential. For example, apoptotic cells efficiently load dendritic cells with tumour antigen and do not cause dendritic cell maturation.[85,112,113] In contrast, antigen from non-apoptotic cells also loads dendritic cells, but causes dendritic cells to mature and up-regulate costimulatory molecules.[35,37] *In vivo* the immunostimulatory effects of antigen formulation is further confused by the presence of many other cell types. For example, macrophages are commonly present in tumours at higher levels than dendritic cells, and continuing the example from above, macrophages also actively phagocytose apoptotic cells. However, following the phagocytosis of apoptotic cells, macrophages secrete a range of anti-inflammatory cytokines, including interleukin-10 (IL-10) and transforming growth factor-β (TGF-β).[114–117] In contrast, macrophages respond to non-apoptotic cells with limited phagocytosis and the secretion of pro-inflammatory cytokines, including TNF-α and interleukin-1β (IL-1β).[114–116]

Therefore, the data on apoptotic cell vaccination must be interpreted carefully depending on whether the dendritic cells are loaded *in vitro* alone vs. *in vivo* in the cellular milieu. Similarly, it is important to distinguish between modes of death that occur *in vitro* vs. *in vivo*. Apoptotic cells that have not been efficiently phagocytosed may proceed to secondary necrosis;[118] therefore, at increased doses of apoptotic cells, where phagocytes may be overwhelmed, apoptotic cells begin to vaccinate mice against associated antigens.[119] In mice bearing mutations resulting in the defective phagocytosis of apoptotic cells, there is an enhanced tendency to develop autoimmunity.[120,121] Complement components have recently been described as important opsonins for clearance of apoptotic cells,[122,123] and there is a direct correlation between deficiency in certain complement components and the development of autoimmunity.[124] Thus, in these models it is likely that apoptotic cells which are not phagocytosed can proceed to secondary necrosis, leading to antigen presentation by, and activation of, dendritic cells. Cells that undergo a physiological non-apoptotic death, i.e. one where cells are fated to die but are not able to activate apoptotic pathways,[104,125,126] are highly immunogenic.[104,116,125,127,128]

Therefore, an interesting strategy to activate dendritic cells concomitantly with uptake of tumour antigens would involve blocking the phagocytosis of apoptotic cells, or blocking the inhibitory effects of cytokines produced following phagocytosis. Thus, it has been shown that apoptotic cells are significantly more immunogenic in mice treated with carageenan to block phagocyte function.[119] The *in vivo* tolerance that can be induced by dendritic cell uptake of apoptotic cells[85,129,130] may relate to cytokines secreted by dendritic cells in response to phagocytosis of apoptotic cells,[131] particularly IL-10[119] and TGF-β.[117] TGF-β secreted by tumour cells, or within the tumour microenvironment, has been postulated as one of the mechanisms by which tumours evade immune control, as TGF-β inhibits both dendritic cell maturation and T-cell effector function. Based on these data, we hypothesize that if tumour antigen is provided via conventional intratumoral apoptotic death,[105–107] there would be a significant therapeutic advantage generated by blocking the phagocytosis of apoptotic cells, or alternatively blocking IL-10 and/or TGF-β within the tumour environment. Thus, we maintain that in developing intratumoral therapies, dendritic cells attracted to the tumour site should be loaded with antigen via cytotoxic therapies that concomitantly activate the dendritic cell.

One interesting local therapeutic approach that is being actively pursued in the treatment of both basal cell carcinoma and melanoma involves topical application of members of the imidazoquinoline family, particularly Imiquimod. Topical treatment with Imiquimod causes the maturation of local Langerhans' cells,[132] and injection of immature dendritic cells into Imiquimod-treated skin results in the maturation of dendritic cells *in situ*.[133] Moreover, Imiquimod-mediated maturation of injected immature dendritic cells generated antitumour immune responses that were superior to similar injection of mature dendritic cells.[133] These data suggest that in addition to the phenotypic maturation status of the dendritic cell, the inflammatory status of the local environment plays an important role in the activation of cell-mediated immune responses. Topical treatment with Imiquimod has shown significant therapy in basal cell carcinoma[134] and cutaneous melanoma.[135] These data demonstrate that such local immunotherapies can provide significant benefit with minimal systemic risk in patients with normal immune status.[136] Topical immune adjuvants, such as the imidazoquinolines, could be widely applied to enhance immune responses at accessible sites.

Attraction of primed effector cells to the tumour site

The degree of lymphocyte infiltrate into the tumour is an independent prognostic marker for improved survival in specific classes of melanoma patients.[137,138] However, there is much discussion as to the functionality of those lymphocytes found within many tumour types.[139,140] Efficient priming of effector cells does not necessarily mean that antigen-specific cells can proceed to clear tumours. In a transgenic T-cell model, where the specific antigen was simultaneously expressed by normal liver cells, endogenous T cells did not cause liver pathology, even following antigen-specific vaccination *in vivo*.[141] In this model, administration of a liver pathogen was required to initiate T-cell-mediated autoimmune liver destruction.[141]

Similarly, multiple strategies have been demonstrated to generate measurable cytotoxic T-lymphocyte (CTL) responses in patients, without significant correlation to clinical responses.[142–144] The presence of large numbers of transgenic or *in vitro*-expanded tumour antigen-specific T cells does not consistently cause regression of tumours in animals or patients.[145–147]

One explanation for these data may lie in the trafficking properties of activated T cells, and in the tumour environment. Naïve T cells have 'central' trafficking properties.[53,148–153] In contrast, subpopulations of activated cells lose expression of CD62L and CCR7, gaining adhesion molecules such as CD44 and the chemokine receptor, CCR5, that enable adherence to the peripheral basement membrane hyaluronate[145,154,155] and trafficking towards inflammatory chemokines such as CCL3 and CCL5.[156–158] Such effector cells are much more capable of trafficking to peripheral sites that are the source of ongoing infections.[157,159,160] These data support the hypothesis that modulation of the tumour site will be critical to enhance the efficacy of tumour immunotherapies, particularly during the effector phase.

The tumour site is not a site of inflammation. Despite expression of a range of chemokines, and even expression of cytokines such as TNF-α,[161–163] simultaneous expression of counteractive cytokines, such as IL-10 and TGF-β, means that activated T cells are poorly attracted to the tumour site. T cells that are unresponsive to TGF-β are significantly more effective in adoptive transfer models than T cells that are inhibited by TGF-β expressed by tumours.[164] *In vivo*, radiolabelled antigen-specific T cells showed limited additional tumour-specific trafficking when compared to non-specific cells.[165] One innovative solution to this problem has been genetic modification of the T cells to express a receptor for a chemokine expressed by tumours.[166] Transfer of this receptor significantly increased T-cell trafficking to tumours and the antitumour efficacy of adoptively transferred T cells. Therefore, it could be possible to design vaccination strategies that generate T cells with a more appropriate trafficking capacity. However, using intratumoral therapies, it may be possible to incorporate features that directly modify the tumour site to increase the efficacy of effector T-cell trafficking.

A number of groups have engineered tumours to express chemokines that are known to attract effector T cells. For example, modification of tumours to express CCL3 and CCL20 has generated effective antitumour immune responses.[57–59,77,78,80] However, these chemokines are pleotropic and their receptors are also expressed on other critical cells, in this case immature dendritic cells, which were the intended target of the therapies. Thus, few experiments have assessed the effect of modified tumours specifically on the effector-cell stage of the antitumour immune response. Emigration of effector cells to the tumour site is dependent on the presence of IFN-γ,[167] which causes local expression of chemokines capable of attracting further effector cells.[40,168] Adoptively transferred effector cells require such chemokine-mediated signals in order to generate antitumour immune responses, as treatment of such cells with pertussis toxin prevented systemic therapy.[169] This observation has implications for therapy, as intratumoral injection of adenovirus expressing the chemokine CXCL10, the receptor for which is found on activated T cells, synergistically enhanced the efficacy of adoptive T-cell therapy.[170] Similarly, intratumoral injection of adenovirus expressing the T-cell-responsive chemokine XCL1 also synergistically enhanced the efficacy of adoptive T-cell therapy.[171] In addition, we have demonstrated that expression of CCL3 in tumours significantly enhances antitumour responses when combined with the adoptive transfer of activated T cells (M. J. Gough *et al.*, submitted). We hypothesize that along with intratumoral therapies that efficiently prime antitumour T-cell responses, strategies should be incorporated to identify the tumour site for effector cell trafficking, and that the combination will greatly improve their efficacy of intratumoral immunotherapy.

Co-ordination of an effective intratumoral immunotherapy

Many of the strategies advocated above to attract immature dendritic cells to the tumour site, and to mature the dendritic cells at the tumour site, would also attract any effector cells generated back to the tumour. Thus, the apparently pleotropic effects of chemokines and the common features of trafficking mechanisms, may underlie organization and cohesions in immune responses. For this reason it is likely that modification of the tumour site is critical for the initiation, execution and promotion of effective immune responses. Examining the mechanics of effective immune responses, and taking into account the results from current immunotherapies applied in animal tumour models and human clinical trials, does provide information for further research. Recruitment or provision of the appropriate APCs, and their loading with tumour antigen, is a critical target of therapies and therapeutic strategies. Comparatively neglected are strategies that target the effector arm of the immune response to the tumour site. Clearly, the generation of sufficient effector cells is a basic requirement for subsequent tumour elimination. Yet, as discussed above, the generation of large quantities of effector cells, as determined by adoptively transferred *in vitro*-generated cells, or *in vivo* vaccination procedures, does not necessarily result in tumour elimination. We hypothesize that performing the vaccination procedure within the tumour site will increase the efficacy of the antitumour therapy by simultaneously attracting any effectors generated to the site. Moreover,

we hypothesize that designing therapeutic strategies which utilize antigens present endogenously within tumour cells, through the attraction of immature dendritic cells and immunogenic tumour cell death, will provide site-targeted therapeutic immunity.

Nevertheless, difficulties will probably persist. The evolution of immune evasion during tumorigenesis complicates a number of these processes. Cytokines such as TGF-β and IL-10, within the tumour, limit dendritic cell maturation along with effector T-cell generation and function. Moreover, immune responses seem designed to be short-lived. Chronic immune activation seen in diseases such as rheumatoid arthritis is the result of ongoing responses that do not occur in tumour tissues. How these environments differ may again relate to the immunosuppressive cytokine environment, but sustaining a response will be critical to long-term tumour elimination by immunotherapies. Finally, access to the tumour site will be critical. We have provided an example, in this review, of melanoma, a tumour type with typically accessible primary tumours. However, distant metastasis in this tumour type will remain a difficult target for location-dependent therapies. Systemic delivery approaches will be required to reach these distant sites, and may yet be found in the developing field of gene therapy.

We have summarized tumour immunotherapies that target the tumour site and target the key features of optimal immune responses. We hypothesize that co-ordination of an effective immune response within tumours will provide tumour responses superior to those generated via *ex vivo* or distant vaccination strategies.

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