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REVIEW

Keith Dredge · J. Blake Marriott · Stephen M. Todryk
Angus G. Dalgleish

Adjuvants and the promotion of Th1-type cytokines in tumour immunotherapy

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Abstract Immunotherapy includes both active and passive mechanisms that have the potential to treat many tumour types. Whereas monoclonal antibodies may kill cells by merely binding to them, 'cancer vaccines' involve the induction of an active immune response. The activation of tumour antigen-specific T-helper and cytotoxic T lymphocytes or non-specific macrophages and natural killer (NK) cells using immunotherapeutic approaches may lead to the subsequent destruction of tumour tissue. Administration of a tumour antigen alone is often not sufficient to stimulate an appropriate immune response. However, incorporating an immunological adjuvant into a vaccine regime often improves anti-tumour immunity. There are various types of adjuvants used in immunotherapy, ranging from microbial, chemical, and cellular components to proteins and cytokines. Previous reports have demonstrated that the induction of Th1-promoting cytokines, using specific adjuvants, can enhance anti-tumour immunity and can reduce or even prevent tumour growth. There is also increasing evidence that many adjuvants induce Th1-type cytokines, which correlates with the induction anti-tumour immunity. Th1-type responses which comprise cell-mediated immunity are characterised by the secretion of interferon-γ by T cells, which is induced by antigen-presenting cell (APC)-derived IL-12. This review describes immunoadjuvants that are currently undergoing preclinical investigation, and emerging clinical data revealing that adjuvants which induce Th1-type responses can improve the efficacy of cancer vaccines. Therefore, the use of Th1-inducing adjuvants may provide an essential strategy for the future success of immunotherapy.

Keywords Cytokine · Immune adjuvant · Th-1 immunity · Tumour immunotherapy

K. Dredge (✉) · J.B. Marriott · A.G. Dalgleish
Division of Oncology,
St. George's Hospital Medical School,
Cranmer Terrace, London SW17 0RE, UK
E-mail: k.dredge@sghms.ac.uk
Tel.: +44-208-7255841
Fax: +44-208-7250158

S.M. Todryk
Immune Regulation Research Group,
Department of Biochemistry, Trinity College Dublin, Ireland

Introduction

Over the years, a large range of putative cancer vaccines have been reported. Whole tumour cell vaccines (modified in vivo or ex vivo), recombinant and synthetic vaccines and dendritic-based vaccines (with or without adjuvants) are currently under investigation. An adjuvant is any product (or a combination of different components) which increases or modulates the immune response against an antigen. Examples include incomplete Freund's adjuvant (IFA), bacillus Calmette-Guérin (BCG), *Mycobacterium vaccae* and *alum*. Some adjuvants convey long-term presentation of the antigen (depot effect) or help to target immune cells by delivering antigens in a particulate form to antigen-presenting cells (APC).

The immune response can be broadly categorised into a cellular or humoral mediated response. The production of interferon-gamma (IFN-γ), interleukin-2 (IL-2) and IL-12 leads to a Th1-type cellular response, while production of IL-4, IL-5 or IL-10 leads to Th2-type humoral immunity. Many cancer vaccines, particularly in combination with immune adjuvants, elicit strong cellular immune responses leading to the production of Th1-type cytokines such as IL-2, IL-12, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IFN-γ [8]. There is a wealth of preclinical studies and some clinical evidence to show that the production of these Th1-type cytokines correlates with anti-tumour responses. Other factors such as the route of administration, the length of intervals between boosting, and in some cases, the amount of adjuvant can have a bearing on the outcome of the desired response. An understanding of such processes will allow tumour immunology to delineate several immune

mechanisms and use selected adjuvants to improve the treatment of many cancers. However, this review will focus on the cytokines that are produced/potentiated by adjuvant therapies, the immune responses generated and their subsequent abilities to destroy tumour cells.

Rationale for adjuvant use in tumour immunotherapy

There are a number of mechanisms and processes in immune cell/tumour cell interactions whereby adjuvants may improve an immune response culminating in the destruction of a tumour (Fig. 1). A particular adjuvant may enhance the immunogenicity of tumour cells, or it may lead to the blockade of immunosuppressive factors produced by tumour cells.

T cells require two signals from APC for activation, a specific MHC-associated antigen that interacts with the T cell receptor and a second signal from appropriate costimulatory molecules. In some situations, insufficient presentation of antigen by APC to T cells induces tolerance. Moreover, in cases where antigen/T cell interactions occur, the costimulatory molecules required for T cell activation are often absent, resulting not only in an insufficient immune response but also in the induction of anergy. For example, ligation by B7 family proteins of the CTLA-4 molecule on T cells results in anergy, while ligation of CD28 leads to T cell activation. Ensuring adequate T cell costimulation provides the appropriate cytokine stimuli for T cell and macrophage/NK activation. Macrophages can lyse tumour cells by releasing tumour necrosis factor-alpha (TNF-α), IL-1, or by the generation of reactive oxygen intermediates

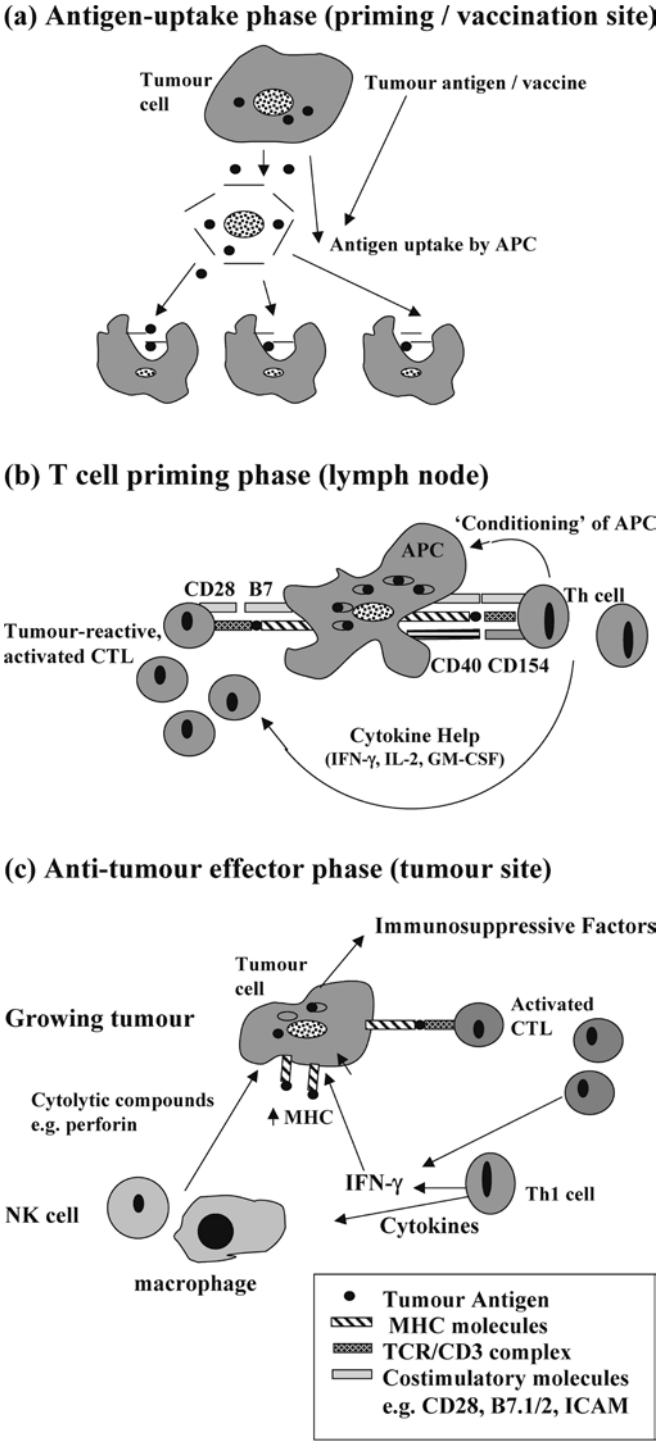

Fig. 1A–C. Phases in the generation of anti-tumour immunity during vaccination. **A** The immune system is thought to be turned on by ''danger'' signals associated with certain molecules such as cell products released from tumour cells (heat shock proteins, mitochondria, RNA and DNA). Such tumour cell components are taken up and processed by APC at the priming or vaccination site. **B** Recognition of antigenic peptides is thought to cause upregulation of costimulatory molecules (CD40, B7 family) on local APC (chiefly DC), which is the second signal in addition to antigenic peptide with MHC that is required to initiate an antigen-specific immune response [73]. This recognition occurs by APC migrating to the draining lymph nodes where DC ''conditioning'' by T-helper cells occurs resulting in priming of tumour reactive activated CTL cells which is enhanced by cytokine ''help'' from Th cells. The activation of CTL immunity to tumours occurs either via direct recognition of antigen on the tumour cells, or by the presentation of tumour antigens on host DC (''cross-presentation'') which subsequently prime CTL (''cross-priming''). This latter ability is a specific property of DC that allows them to cross-present and prime antigens derived from other cells for the stimulation of CTL [26]. The resultant cytokine milieu dictates whether Th1-type cells, which secrete IFN-γ, IL-2, IL-12 and IL-18 or Th2-type cells which secrete IL-10, IL-4 and IL-5, are activated. **C** Finally, the anti-tumour activity at the tumour site can be induced by a number of cell types. Th1 cells produce IFN-γ, known to possess anti-tumour activity by several mechanisms [2, 3, 68]. For example, IFN-γ has been shown to facilitate both antigen-dependent and antigen-independent events as a prerequisite for efficient CTL/target interactions as well as FasL upregulation and triggering of Fas-dependent and Fas-independent lysis [3]. It has also been demonstrated that tumour responsiveness is necessary for IFN-γ-dependent inhibition of tumour angiogenesis by $CD4^+$ T cells [2]. CTL (following effective cross-priming), NK and macrophages have the ability to directly kill tumour cells often by releasing cytolytic compounds such as perforin and granzymes. However, tumour cells can also release immunosuppressive factors such as TGF-β, FasL and prostaglandins. Immune adjuvants may circumvent these factors, break T cell tolerance to tumour antigens, ensure adequate T cell costimulation and/or provide signals to enhance particular cytokine responses that initiate anti-tumour immunity

(ROI), particularly hydrogen peroxide. Activation of natural killer (NK) cells potentiate the Th1-type response by secreting cytokines such as IFN-γ and destroying tumour cells with cytolytic granules similar to those found in cytotoxic T cells. Such processes may also be enhanced following therapy by immune adjuvants.

Nishimura et al. have reported that although both Th1 and Th2 cells possess the ability to completely eradicate tumours in mice, Th1 cells exhibited stronger cytotoxicity than Th2 cells [54]. Furthermore, they showed that Th1 and Th2 cells use distinct adhesion mechanisms during tumour eradication. The leukocyte function-associated antigen (LFA)-1-dependent cell-cell adhesion step appears to be essential for Th1 cell therapy but not for Th2 cell therapy, indicating that Th1 cells respond to tumour cells by producing cytokines which recruit other effector cells such as $CD8^+$ T cells, NKT or NK cells into the tumour tissue. However, the inability of Th2 cells to enter tumour tissue (due to a defect in adhesion mechanisms) suggests that they accumulate on endothelial cells around the tumour mass [54]. The mechanism of subsequent tumour necrosis by Th2 cells remain unknown. A possible mechanism is based on the ability of Th2-derived cytokines to activate other inflammatory cells such as eosinophils and macrophages that produce superoxides and nitric oxides capable of inducing tumour necrosis [30]. Further evidence shows that the therapeutic efficacy of both Th1 and Th2 cells is based on their quantitative rather than on their qualitative cytokine properties. Fallarino et al. showed that although the adoptive transfer of $CD4^+$ T cells could treat established murine tumours, and that the Th1 clone was fully capable of eradicating a tumour, the Th2 clone required repeated injections, and optimal protection was only achieved by combining Th2 transfer with the injection of peptide-pulsed dendritic cells (DC) [14]. This indicates an interaction in vivo between the transferred T cells and DC, resulting in effective host production of the Th1 cytokine IL-12.

Most tumour-associated antigens represent self-proteins and as a result of tolerance are poorly immunogenic. Cytokine-modulating adjuvants may break tolerance and allow tumour-reactive T cells to kill tumour cells. Adjuvant treatment with a toll-like receptor agent (TLR9) CpG-oligonucleotide (CpG-ODN) has recently been shown to be effective in breaking tolerance and enabling tumour elimination in mice [36]. Tolerance to carcinoembryonic antigen (CEA), which is overexpressed by the majority of lethal malignancies, has also been shown to be reversed by immunisation with a CEA-derived peptide in cancer patients [15]. In a clinical study, colon cancer patients generated anti-CEA humoral and cellular immune responses following treatment with an anti-idiotype monoclonal antibody vaccine that mimicked CEA [16]. More recently, human anti-idiotypic antibodies, including one that mimicked CEA, have been shown to be good immunogens by targeting Fc receptors on APC, allowing efficient stimulation of both helper and cytotoxic T-cell responses [13].

Adjuvants may prevent or reverse T-cell anergy. It has been demonstrated that IL-10 is a major contributor to the induction of anergy by the active inhibition of IL-2 production, thus preventing gene transcription [58]. Therefore, adjuvants that promote IL-2 or inhibit IL-10 could have the potential to overcome T cell anergy. IL-2 can enhance T cell responses in cancer patients [57], and systemic administration of IL-2 in DC-based immunotherapy has been shown to increase the induction of an antitumour effect [44]. GM-CSF appears to be the most frequently used cytokine used in humans and has been shown to enhance DTH and $CD8^+$ T cell responses against melanoma-associated peptides in cancer patients [32].

It is the cytokine milieu that dictates the appropriate activation of a specific set of immune cells that will ultimately destroy tumour cells. Adjuvant therapy along with a cancer vaccine may alert the immune system via the induction of cytokines, and also potentiate, prolong or manipulate the cytokine response required to interfere with a growing tumour. It has been postulated that there are certain events that should be induced by an ideal adjuvant in order to elicit a T cell response. Firstly, mimicking danger signals should attract APC at the site of administration of the antigen. Secondly, the targeting of the antigen to surface receptors on APC will allow endocytosis and cross-priming to occur. Thirdly, induction of APC maturation should facilitate antigen presentation to T cells, and finally costimulation of T cells [9].

Secretion of immunosuppressive factors such as TGF-β and IL-10 has been described in some cancers, notably gliomas [59]. These factors interfere with the generation of an effective immune response, such as the activation or function of CTL and $CD4^+$ T helper cells [21]. Other escape mechanisms include the expression of cell surface molecules such as Fas-L and the production of toxic oxygen and nitrogen radicals by tumour-associated macrophages. The downregulation of T cell targets such as HLA class I molecules on tumour cells also contributes to an immunosuppressive environment [9].

Adjuvants used in tumour immunotherapy

Microbial adjuvants

BCG has excellent adjuvant activity, eliciting both humoral and cell-mediated immune responses and has a long-standing safety profile which has resulted in its successful use as an antitumour agent in the treatment of both superficial bladder cancer and melanoma [1, 12]. Attempts to construct recombinant BCG (rBCG) have been made to enhance its immunostimulatory properties [18]. A further increase in IFN-γ has been observed using exogenous or endogenous IL-2 or GM-CSF (secreted from cytokine-transfected rBCG), indicating that rBCG may exert part of its antitumor action on melanoma through the induction of IFN-γ, which can be greatly

enhanced through the concomitant addition of IL-2 and/or GM-CSF. Cytokine production and proliferative responses to antigens have been found to be greater with splenocytes derived from mice injected with cytokine-secreting rBCG (IL-2, IL-4, IL-6, GM-CSF and IFN-γ) than with those from parental rBCG-immunised mice [53]. Yamada et al. constructed rBCG-secreting murine IL-2 fused with α-antigen, and found that in vitro stimulation of thioglycollate-elicited peritoneal exudate cells (PEC) with this rBCG induced more efficient cytotoxicity to the murine bladder cancer cell line (MBT-2) and increased cytokine secretion (IL-12, TNF-α, IFN-γ) in cultured PEC than did parental rBCG [79].

Immunostimulatory bacterial DNA sequences have been reported to be potent adjuvants. Nonmethylated, palindromic DNA containing CpG-ODN can activate an innate immune response by activating monocytes, NK cells, DC and B cells in an antigen-independent manner [9]. CpG-ODN acts as an adjuvant to induce the expression of Th1-type cytokines in mouse models [7], and leads to the production of IL-12, IL-18 and IFN-γ by peripheral blood mononuclear cells in humans [4]. It is hypothesised that CpG-ODN activates APC such as DC, which enhance the expression of costimulatory molecules and secrete IL-12 leading to the activation of NK cells and subsequent IFN-γ production [9]. Recognition of several microbial products is mediated by members of the Toll-like receptor (TLR) family. Activation of DC by microbial agonists of TLR2 or TLR4 results in differences in cytokine and chemokine gene transcription, which contributes to the polarisation of adaptive immunity [62]. The major role of TLR in the development of adaptive responses to microbial products is further evidenced by another study in which LPS-induced maturation of immature DC was correlated with TLR expression [77].

Peritumoural treatment with CpG-ODN resulted in complete rejection or strong inhibition in a variety of established mouse tumours including AG104A, IE7 fibrosarcoma, B16 melanoma, and 3LL lung carcinoma, whereas systemic administration had only partial effects. The CpG-ODN-induced tumour rejection was found to be mediated by both NK and tumour-specific $CD8^+$ T cells [36]. It is of interest to note that CpG motifs have been also found to inhibit metastasis in a mouse model of experimental metastasis via induction of NK/NKT cells. Neutralisation of IL-12, IL-18, or IFN-γ did not interfere with the CpG-induced antimetastatic effect. However, in sera of CpG-ODN-treated mice, high levels of IFN-α were detected, and in IFN-α/β receptor-deficient mice, the CpG-ODN-induced antimetastatic effect was strongly reduced [24]. The most recent report used CpG-ODN as an adjuvant of the CTL response against tumor-derived synthetic peptide (MART-1) to elicit a stronger systemic CTL response as compared with peptide emulsified in IFA. Moreover, CpG-ODN in combination with IFA further enhanced the CTL response. CpG-ODN by itself may be a good candidate adjuvant of the CTL response, and can also enhance the effect of classical adjuvant [50]. While not regarded as a classical Th1-type cytokine, IFN-α does have antitumour activities in an adjuvant setting using a renal carcinoma murine model [24]. Moreover, it has shown efficacy in the clinic during the treatment of metastatic renal carcinoma [34], and has been shown to act synergistically with IL-18 to induce IFN-γ production from human NK cells [55].

The adjuvant *Mycobacterium vaccae* (SRL172 from SR Pharma) has been shown to be a potent inducer of Th1 responses. In trials, patients with hormone-treated relapsed disease have shown SRL172 to be well tolerated. In a pilot study, increased Th1 cytokine production was correlated with a fall in prostate-specific antigen (PSA) levels and clinical improvement [28]. More recently, a *Mycobacterium* recombinant vaccine system has been developed which utilizes antigen delivery systems in the form of non-pathogenic *Mycobacterium* strains, genetic transfer systems in the form of cloning and expression vectors, and related technologies. This provides products containing non-toxic immuno-regulating *Mycobacterium* adjuvants, non-toxic immunostimulating exogenous antigens specific for a variety of diseases, and non-toxic amounts of cytokines that boost the Th1 pathway [41].

The *Vaccinia* virus has been used as an adjuvant in a virus-augmented melanoma cell lysate (VMO) for several reasons [37]. It binds and modifies melanoma cell membranes to augment the immunogenicity of melanoma antigens [6]. It enables the re-expression of antigens lost during differentiation [37]. Prevention of syngeneic tumor growth in mice has been shown to be effective following immunization with vaccinia virus-modulated tumor cells [78]. More recently, a study using recombinant *Vaccinia* virus encoding the influenza matrix protein as model vector found DC to cross-present *Vaccinia*-derived antigens from both apoptotic and necrotic infected cells to antigen-specific $CD8^+$ T cells. Efficient cross-presentation required uptake of necrotic cells by immature DC and exposure to maturation stimuli, especially CD40 ligand. The responding $CD8^+$ T cells secreted IL-2 and IFN-γ, proliferated and developed into cytotoxic effectors [42].

Recombinant cytokines

Recombinant cytokines such as IL-2, -4, -6 IFN-γ, TNF-α and GM-CSF have been used in cancer immunotherapy, although their use has been limited due to their systemic toxicities. However, the transduction of tumour cells with cytokine genes has allowed locally-secreted cytokines to act on tumours while minimising any systemic toxicity. Immunoregulation by IL-12 is of central importance in cell-mediated immunity (CMI) against those pathogens and tumours that are controlled by cell-mediated mechanisms [20]. Therefore, immunomodulatory cytokines are chemical adjuvants that can be useful in the development of vaccines against numerous infectious diseases and tumours. The clinical potential of

IL-12-loaded polylactic acid microspheres has been investigated in mice by intratumoural injection which promoted the development of systemic antitumour immunity that could eradicate micrometastases [63]. Huang et al. have examined whether the priming of a Th1 response by DC subsets is dependent upon the type of initial stimulus. A Th1-inducing adjuvant, heat-killed *Brucella abortus* (HKBA) was used to assess stimulation of DC subsets, relationship between antigen burden and IL-12 production, and down-regulation of DC subset IL-12 production by IL-10. The study found that the nature of the stimulating substance is important in determining which DC subsets are activated to produce IL-12 [29]. This shows the importance of selecting the appropriate adjuvant for the optimal immune response.

GM-CSF has also been used as an adjuvant in a number of studies. Kass et al. showed the effective delivery of GM-CSF to an immunisation site using a recombinant avian poxvirus in a murine model. Furthermore, the compatibility of delivering an antigen and GM-CSF in replication-defective viruses to enhance antigen-specific immunity and the combined use of recombinant avipox viruses expressing CEA and GM-CSF to generate antitumour immunity directed at a self tumour antigen was demonstrated [35]. Fifteen patients with progressive metastatic renal cell carcinoma were treated with GM-CSF and intravenous infusions of activated autologous macrophages (AAM). The combined GM-CSF and AAM treatment was well tolerated, and resulted in transitory stabilisation or partial regression in 9 out of 15 patients [43]. More recently, 10 melanoma patients immunised with N-ras peptides (thought to be involved in the development of melanoma) and GM-CSF as adjuvant mostly responded with strong delayed type hypersensitivity reactions. In 2 of the patients, an in vitro response to the vaccine could also be detected. The specificity of the reaction was confirmed by cloning of peptide-specific $CD4^+$ T cells from the peripheral blood of the patients [31].

Several cytotoxic anticancer agents have immunomodulatory effects at relatively low doses and, in combination with non-toxic doses of certain cytokines, can exert immunity-dependent curative effects in mouse tumour models [51]. For example, in the EL4 lymphoma C57BL/6 mouse model, combinations of appropriate regimens of adriamycin plus IL-2 have been found to increase survival with lifelong immunological memory [27]. In the E0771 breast tumour C57BL/6 mouse model, adriamycin plus TNF-α at doses which are without antitumour activity when given alone have resulted in complete cures of established tumours with the concomitant stimulation of CTL and NK cell responses [51].

IFN-α has been reported to be an effective adjuvant for peptide-based cytotoxic T-cell vaccines [71], and has been found to act synergistically with IL-18 to induce IFN-γ in an adjuvant setting. Continuous administration of peptide and local delivery of IFN-α was found to be important for efficient CTL induction, suggesting that IFN-α is an effective adjuvant for peptide-based vaccines [47].

Other chemical adjuvants

Some miscellaneous agents have been described in the literature, demonstrating a role for Th1-type cytokines in the anti-tumour response. IFA is now in clinical use, although peptides administered in IFA have shown improved cellular responses which are not correlated with clinical improvement. However, up to 42% of patients with advanced melanoma demonstrate a clinical response to peptide vaccination in IFA if high-dose IL-2 is added [63, 64]. A recent study has found that prolactin regulates the anti-tumour response through induction of tumouricidal macrophages and release of IL-12. Administration of prolactin in vivo inhibited the growth of Ehrlich ascites carcinoma, synergising with IFN-γ in inducing tumour killing [48]. The alkylating agent cyclophosphamide was shown to induce a switch from a Th2 to a Th1-type cytokine profile of lymphoma-bearing rats, which may be responsible for its anti-metastatic effect. The authors also found that IFN-γ was a cytotoxic factor against metastatic cells [46].

Thalidomide has been shown to be clinically useful in a number of conditions including cancer. Clinical activity in vivo is attributed to the wide-ranging immunological and non-immunological properties possessed by this drug, including anti-TNF-α and T cell costimulatory activity [45]. Thalidomide has been associated with immunomodulatory activity, providing activation signals to T cells stimulated in the absence of costimulatory signals [25]. The thalidomide-induced T cell proliferation was dependent on the autocrine production of IL-2 but not IL-4, suggesting that a Th1 phenotype occurs following costimulation. This beneficial activity may lead to possible adjuvant activity and the promotion of T cell responses in a clinical setting.

Novel thalidomide analogues optimise thalidomide's immunological and anti-cancer properties while decreasing its side effects. One such analogue (and thalidomide to a lesser extent) clinically referred to as Actimid (Celgene Corp., Warren, N.J., USA) has recently been found to provide costimulatory activity in vivo. Enhanced antitumour responses following an autologous tumour cell vaccination have been found to be mediated by the induction of the Th1-type cytokines IFN-γ and IL-2 (Fig. 2). Furthermore, the analogue has been found to costimulate naive T cells activated with immobilised anti-CD3 antibody resulting in increased IFN-γ, IL-2 and GM-CSF, while decreasing IL-4 and IL-10 concentrations [11]. Other analogues have recently shown evidence of T cell activation in a clinical setting (Marriott et al., manuscript in preparation).

Cellular adjuvants

DC pulsed with tumour antigens have been shown to be a promising adjuvant for inducing anti-tumour immunity. A recent study compared two types of DC as adjuvants for the induction of melanoma-specific T-cell responses in

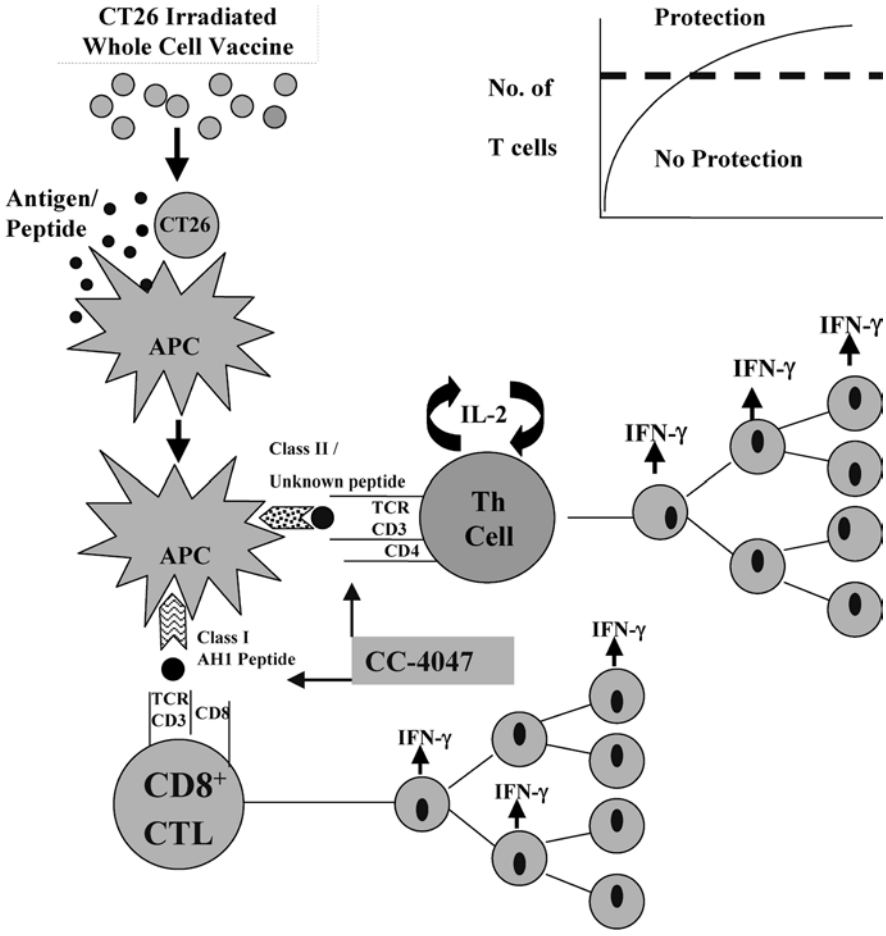

Fig. 2. A simplified diagram proposing the T cell costimulatory activity of a thalidomide analogue Actimid (CC-4047) in a murine model of colorectal cancer. Irradiated autologous tumour cells provide partial protection from subsequent challenge with live tumour cells. APC are thought to recognise, process and present peptides (including AH1) to $CD8^+$ and $CD4^+$ T cells which then become activated to provide a T-cell-mediated anti-tumour response. However, the presence of Actimid during the priming phase of the immune response provides a costimulatory signal to T cells (via IL-2) resulting in greater numbers of tumour-specific T cells, chiefly IFN-γ-secreting Th1-type cells, which correlate with enhanced protection from subsequent tumour challenge

humans following intranodal injection. Expansion of peptide-specific IFN-γ-producing $CD8^+$ T cells was observed in 5 out of 7 patients vaccinated with mature DC, but in only 1 out of 7 using immature DC. In 2 out of 4 patients vaccinated with mature DC, induction of peptide-specific cytotoxic T cells was observed as monitored by chromium release assays, whereas immature DC failed to induce peptide-specific cytotoxic T cells in the same patients [33]. It has recently been demonstrated that tumor lysate/KLH-pulsed DC can generate specific T cell responses (including IFN-γ production) and elicit regression of metastatic disease [19].

Peptide adjuvants

Immunisation with cytotoxic T cell epitope SPSYVYHQF (AH1), derived from MuLV gp70 envelope protein expressed by CT26 tumour cells, does not protect BALB/c mice against challenge with CT26 tumour cells. By contrast, immunisation with AH1 plus Th peptides OVA (323–337) or SWM (106–118) eliciting Th1 and Th0 profiles, has been found to protect 83% and 33% of mice, respectively. The results showed that tumour-related as well as tumour-unrelated but strong Th1-inducing peptides may be useful for boosting CTL responses in tumour immunotherapy [5]. K-ras mutations are frequently found in adenocarcinomas of the pancreas, and induction of immunity against mutant ras can therefore be of possible clinical benefit in patients. Data from a clinical phase I/II trial involved patients vaccinated by i.d. injection of synthetic mutant ras peptides in combination with GM-CSF. The association between prolonged survival and an immune response against the vaccine suggests that a clinical benefit of ras peptide vaccination may be obtained in this group of patients [22].

HER-2/neu peptides expressed on some tumour cells may be recognised in the context of HLA-DR molecules by $CD4^+$ Th lymphocytes on antigen-presenting cells. A recent study induced HER-2/neu peptide-specific $CD4^+$ T cell clones by in vitro immunisation with HER-2/neu peptide-pulsed autologous DC, which resulted in strong proliferation and significant levels of IFN-γ [60]. The authors concluded that the use of such a regime might be attractive for broadly applicable vaccines, and could prove useful for adoptive immunotherapy designed for breast, colorectal and pancreatic carcinomas.

Cytokine gene therapy in an adjuvant setting

DNA vaccines have the potential to elicit antitumour immunity by delivering genes encoding tumour antigens. The relatively low efficacy of these in inducing immune

responses (especially in large animal species and humans) has led to considerable efforts to improve DNA vaccine efficacy. One such strategy has been used to improve and modulate the immune response induced by DNA vaccines by supplementing with plasmids encoding cytokines [66]. DNA immunisation is an important vaccination technique that is being explored as an immunotherapeutic strategy against cancer. A DNA vaccine construct encoding for the human PSA gene was used to elicit PSA-specific host immune responses in rodent and nonhuman primate models. Cytokine gene adjuvants were also used to modulate vaccine-induced immune responses in these animal models. The authors found that coimmunisation with the IL-2 cDNA construct resulted in a significant enhancement of PSA-specific antibody responses in both mice and macaque models. In contrast, coinjection of IL-12 resulted in a reduction of antibody responses in both models. In mice, the groups coimmunised with IL-2, IL-12 or IL-18 showed a dramatic increase in Th cell proliferation compared to the results with pCPSA alone [38]. A syngeneic subcutaneous tumour model examined the antitumour activity of herpes simplex virus (HSV) vectors, including the combination of ganciclovir (GCV) and IL-12, which proved to be the most efficacious approach [72].

Suicide gene therapy and other transfections

The most common ''suicide gene'' used in cancer therapy is *Herpes simplex* virus thymidine kinase (HSVtk), which encodes an enzyme that converts ganciclovir GCV (a prodrug) into toxic metabolites which inhibit DNA replication and kill the transduced tumour cell [52]. Moreover, non-transduced tumour cells in the vicinity are also destroyed by a mechanism referred to as the ''bystander effect''. This effect is dramatically reduced in T cell deficient mice [75] as cytokine secretion involved in the cascade bias Th1-mediated immunity [76]. More recently, it has been shown that HSVtk/GCV tumour killing is more immunogenic when the cells die by a necrotic mechanism rather than by an apoptotic mechanism. A proposed ''danger signal'' that was upregulated and that alerted the immune system during necrosis was hsp70 [49]. Thus suicide gene therapy of tumours can be considered as cancer immunotherapy.

Transfer of the gene encoding the proposed danger signal hsp70 enhanced immune responses to wild-type tumours [49], and this enhancement may be due to the ability of hsp to modulate APC function [74] and/or to chaperone tumour antigens from tumours to APC for efficient T cell priming [70]. The transfer of genes encoding allogeneic major histocompatibility complex (MHC) class I molecules (HLA-B7) has been reported to cause tumour regression and the generation of immunity [61]. Allogeneic MHC molecules resemble self-MHC plus foreign peptide, and are recognised by a relatively high frequency of peripheral T cells even without prior contact with the allo-MHC. The transfer of genes encoding highly immunogenic antigens such as mycobacterial proteins [67] may also serve to provide additional epitopes for T cell help during anti-tumour CTL priming. Finally, the transduction of tumour cells with syngeneic MHC class II molecules has been performed to allow the cells to present antigens to $CD4^+$ Th cells, with some success in generating protective CTL-mediated immunity against MHC class II^- tumours [56].

More recently, using the CT26 murine colon adenocarcinoma model, unilateral intratumoural inoculation of replication-deficient HSV-1 tsK has been found to inhibit the growth of established tumours. This partial antitumour response was enhanced by a defective HSV vector, dvIL12-tk, encoding both IL-12 and HSV thymidine kinase (tk), with tsK as the helper virus. In a ''suicide gene'' strategy, GCV treatment after intratumoural inoculation of dvlacZ-tk/tsK, encoding *E. coli* lacZ instead of IL-12, resulted in enhanced antitumour activity. Antitumour activity was also enhanced by local expression of IL-12 from dvIL12-tk/tsK. The combination of IL-12 cytokine therapy with GCV treatment was the most efficacious approach, which illustrates the use of combination suicide/cytokine gene therapy as adjuvant [72]. A recent study examined the molecular adjuvant constructs encoding for IL-2, IFN-γ or IL-4 and co-administered these along with DNA vaccine constructs [encoding for HIV env/rev (pCEnv) and SIV gag/pol (pCSGag/pol) proteins]. The authors observed that coadministration of IL-2 and IFN-γ cDNA resulted in enhancement of antigen-specific T cell-mediated immune responses [39].

Adjuvants that promote Th1-type cytokines in a clinical setting

There is now evidence that the Th1 immune response is defective in multiple myeloma (MM). Frassanito et al. concluded that a dysregulated cytokine network occurs in active MM, and that increased IL-6 production by peripheral T lymphocytes contributes to the immune dysfunction, enabling tumour cells to escape immune surveillance by preventing the anti-tumour Th1 immune response [17]. More recently, thalidomide and its immunomodulatory analogue Actimid has been shown to decrease the upregulation of IL-6 and vascular endothelial growth factor (VEGF) secretion in cultures of bone marrow stromal cells, multiple myeloma cells and co-cultures containing both of these [23]. These data highlight the potential uses of such compounds in a clinical setting.

A melanoma vaccine (gp100) with or without a modified Th epitope from tetanus toxoid was evaluated in a phase I trial, and administered s.c. in either of two adjuvants, Montanide ISA-51 or QS-21, to 22 patients with high-risk resected melanoma (stage IIB–IV). While CTL responses to the gp100 peptide in peripheral blood

Table 1. Clinical trials using immunotherapy and possible Thl-promoting adjuvants

Immunotherapy	Adjuvant	Cancer type	Phase
Melanoma peptide vaccine: CTLA-4 antibody, tyrosinase, gpl00 and MART-1	IFA with or without IL-12	Melanoma (Stage III or IV)	I and II
BCG	Melanoma vaccine	Melanoma (Stage IV)	III
Tyrosinase, gp100, MART-1 and emulsified with Montanide ISA-51 and IL-12	*Alum* or GM-CSF	Melanoma (Stage II,III,IV)	II
gpl00 DNA vaccine	IL-2	Melanoma (Stage IV)	II
RAS peptides and Detox PC mixture	IL-2 or GM-CSF	Solid tumours	II
RAS peptide-pulsed DC	IL-4 or GM-CSF	Neoplasm Metastasis	I
gp100, MART-1-pulsed DC	IL-2	Melanoma (Stage III)	I/II
HSP70 vaccine	None	CML in chronic phase	I
IFN-α	Thalidomide	Metastatic kidney cancer	II
IFN-α	Thalidomide	Melanoma (Stage IV)	II
Peptide vaccine	Montanide ISA-51	CML, AML	I/II
EGRFRvIII peptide vaccine	GM-CSF or KLH	EGFRvIII-expressing tumours	I
Non-tumour cell derivative vaccine	QS21	Relapsed prostate cancer	II

Clinical trial data was obtained from the National Institute of Health service http://ClinicalTrials.gov

in 14% of patients were found, Th1-type responses to the tetanus helper peptide were detected in 79% of the patients. Data from this trial demonstrate the immunogenicity of the gp100 peptide, and suggest that immune responses may persist long-term in some patients. The frequency and magnitude of the CTL response may be improved with more aggressive vaccination regimens [69].

The use of recombinant cytokines as immunoadjuvants shows some efficacy at the clinical level. A GM-CSF-secreting melanoma cell vaccine that was engineered ex vivo with recombinant replication-incompetent adenovirus harbouring a human GM-CSF gene (Adv/hGM-CSF) resulted in increased tumour-specific CTL in some patients following vaccination. These data suggested that repeated vaccinations with irradiated autologous GM-CSF-producing tumour cells were well tolerated by patients, and led to the activation of an antitumour immune response in certain cases [40]. Another study revealed that GM-CSF had modest biological evidence of activity in prostate cancer, as manifested by the prostate-specific antigen response [10].

We have recently demonstrated in clinical study that a thalidomide analogue Revimid (CC-5013) (Celgene Corp.) could boost Th1-type cellular immunity and provide an environment for the generation of an antitumour response (Marriott et al., submitted). The induction of GM-CSF, TNF-α and IL-12 were detected in the serum of patients treated with Revimid in addition to switches in phenotype from naive to memory T cells. Table 1 summarises some other Th1-promoting adjuvants currently being assessed in ongoing immunotherapeutic clinical trials for various cancers.

Conclusions

The future success of tumour immunotherapy depends on many factors. Optimal tumour-specific immune activation is an essential process that is dependent on an appropriate cytokine milieu. In many cases, initiating a Th1-type cytokine cascade has been shown to result in effective anti-tumour responses, although the individual contribution of each of these cytokines has still not been fully elucidated. Moreover, these cytokines may not only induce tumour-specific cytotoxic activity, but also directly affect inflammatory and angiogenic mediators involved in tumour development. Preclinical studies continue to improve and delineate the effects of immunoadjuvant therapy, revealing the optimal responses required to result in tumour rejection. Ultimately, the objective of immunoadjuvant therapy is to facilitate the development and enhance the efficacy of cancer vaccines by inducing Th1-type and antitumour responses at a clinical level.

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