Dendritic cell immunotherapy: clinical outcomes

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The use of tumour-associated antigens for cancer immunotherapy studies is exacerbated by tolerance to these self-antigens. Tolerance may be broken by using ex vivo monocyte-derived dendritic cells (DCs) pulsed with self-antigens. Targeting tumour-associated antigens directly to DCs in vivo is an alternative and simpler strategy. The identification of cell surface receptors on DCs, and targeting antigens to DC receptors, has become a popular approach for inducing effective immune responses against cancer antigens. Many years ago, we demonstrated that targeting the mannose receptor on macrophages using the carbohydrate mannan to DCs led to appropriate immune responses and tumour protection in animal models. We conducted Phase I, I/II and II, clinical trials demonstrating the effectiveness of oxidised mannan-MUC1 in patients with adenocarcinomas. Here we summarise DC targeting approaches and their efficacy in human clinical trials.

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In the last two decades, dendritic cells (DCs) have surfaced as powerful cells, to target antigens to initiate T-cell immunity by efficient uptake, processing and presentation of endocytosed antigens.1 Many of the DC cell surface receptors have been identified, including mannose receptor (MR), DC-SIGN, L-SIGN, LSECtin, CIRE, Langerin, MGL, Dectin-1, Dectin-2, DNGR-1, MICL, CLEC2, CLEC12B, LOX-1, DCIR, BDCA-2, DEC205, scavenger receptor, DC-ASGPR, FIRE, DC-STAMP and Toll-like receptors (TLRs).2 Targeting of these receptors is becoming an efficient strategy to improve the immunogenicity of antigens in DC-based anticancer immunotherapy, especially in pre-clinical animal models and in vivo DC antigen presentation and T-cell stimulation assays. A major challenge for vaccine design is targeting antigens to DCs in vivo in humans, facilitating cross-presentation, and conditioning the microenvironment for Th1- and Th2-type effective T-cell immune responses. Here we present DC immunotherapeutic approaches in preclinical stages with emphasis to those in human clinical trials.

IMMUNE RESPONSES BY TARGETING THE MR

The MR is a carbohydrate (mannose, fucose, glucose, maltose, GlcNAc) binding C-type membrane lectin expressed by DCs and macrophages (Table 1). We had demonstrated over 20 years ago that antigen uptake via the MR results in processing and presentation of peptide epitopes via the MHC class I and class II pathways.3–7 These studies were the first to indicate the MR to be a viable DC cell surface receptor for antigen delivery for vaccine development. In addition, peptides and proteins conjugated to mannose have been shown to stimulate MHC class II-specific T cells with 200–10 000-fold higher efficiency as compared to non-mannose conjugated antigens.8 The MUC1 antigen conjugated to oxidised mannan (poly-mannose, comprising aldehydes) leads to rapid and 1000 times more efficient MHC class I presentation to CD8+ T cells with a preferential T1 response, compared to MUC1 antigen conjugated to reduced mannan (no aldehydes).3 Both oxidised and reduced mannan stimulate bone marrow-derived DCs and enhance OTI/OTII T cells in vitro, and in vivo they induce a mature phenotype of lymph node and splenic DCs.9 Oxidised and reduced mannan stimulate interleukin (IL)-1beta and tumour necrosis factor-alpha, with oxidised mannan stimulating interferon (IFN)-gamma and IL-12p40 cytokines, and reduced mannan stimulating IL-4, IL-10 and IL-13.9 The stimulation of DCs was demonstrated to be TLR-4 dependent.9–11 Further, ex vivo targeting of macropahges or DCs with oxidised mannan-MUC1 and re-injection into mice induce T-cell responses and protect against MUC1 tumour challenge.11 Moreover, oxidised and reduced mannan complexed to DNA via poly-l-lysine induces cellular and humoral immune responses in mice,12,13 and mannosylated dendrimers are endocytosed by bone marrow-derived and Flt3-L DCs, stimulate CD4 and CD8 T cells and protect against tumour challenge.10,11 Studies by others have also demonstrated the efficacy of MR targeting by the use of mannan-coated cationic liposomes (nanoparticles) incorporating HIV-1 DNA, or with mannosylated anionic liposomes having increased interaction to murine and human DCs.16 Mannosylated liposomes incorporating ErbB2 T-cell epitopes and TLR agonists induce strong immune responses, and bind to immature DCs.18 Mannan-coated poly(o, ε-lactide-co-glycolic acid) nanoparticles have also been noted to induce strong T-cell responses.19 Mannosylation of chitosan microspheres (MCMs) incorporating Bordetella bronchiseptica
antigen induces IgA antibody responses in mice. In addition, HER2 protein complexed to the cholesteryl group bearing mannan induces CD8+ T cells and rejects HER2+ tumours in mice. 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self-antigen. Clearly, targeting the MR on DCs shows promise and oxidised mannan-MUC1 warrants inclusion as a harmless adjuvant therapy in the current management of patients with breast cancer. However, the number of patients in this study is small, and a large Phase III trial needs to be done in this cohort of patients. It is important to understand the status of disease of the patients at the time of immunotherapy treatment, as immunotherapy may not be clinically relevant in advanced cancer patients as opposed to early-stage patients. Interestingly, using oxidised mannan-MUC1 does not require further manipulation in order to activate and mature DCs. In fact, oxidised mannan not only binds to the MR, but also activates DCs via TLR-4; hence, mannan plays a dual role in this vaccination strategy. It is clear that targeting cell surface receptors on DCs, such as the MR, leads to immunity against otherwise poorly immunogenic self-antigens and has implications for enhancing the efficacy of cancer immunotherapy studies using self-antigens.

**EX VIVO DC CULTURE AS VACCINE APPROACHES**

Use of ex vivo-grown monocyte-derived DCs has recently been popular for cancer vaccine studies, with the large amount of DCs that could be generated with ease and not requiring large volume of cells. Ex vivo culture of monocyte-derived DCs, pulsed with p53 and re-injected into head and neck squamous cell carcinoma patients, demonstrated an increased T-cell frequency in 11/16 patients, IFN-gamma secretion by T cells in 4/16 patients and decrease in T regulatory cells in most patients. It was speculated from these studies that stronger DC maturation stimuli are required to improve vaccine efficacy. In addition, six uterine cancer patients injected with autologous ex vivo-grown DCs and electroporated with Wilm’s tumour antigen 1 showed HLA-A2-restricted T-cell responses. We have demonstrated that ex vivo culture of human monocyte-derived DCs and pulsing with oxidised mannan-MUC1 and re-injection into patients with adenocarcinoma resulted in strong cellular immune responses (IFN-gamma-secreting T cells), delayed type hypersensitivity responses and clinical responses, and was well tolerated in all 10 patients. DCs matured with lipopolysaccharide and IFN-gamma and pulsed with six HLA-A2 + HER-2/neu peptides in 27 in situ ductal carcinoma patients demonstrated T-cell immunity against the peptides that were present 1 year post immunisation. Likewise, immunisation with IFN-gamma and DCs pulsed with HLA-A2 + prostate cancer antigen peptides demonstrated stable disease in 4/12 patients, a significant slower rise in prostate serum antigen levels and stimulation of cytotoxic T cells.

The use of autologous tumour lysates to pulse ex vivo-grown DCs represents a powerful approach, as numerous tumour epitopes could be presented simultaneously by the DCs. Indeed, maturation and pulsing of CD14 + DC precursors with autologous tumour lysates resulted in delayed-type hypersensitivity responses and Th1 cytokine secretion, and increased the number of NK cells and CD8 + IFN-gamma + cells in the blood of immunised double-negative breast cancer patients; however there was no difference in overall survival among the patients receiving or not receiving DC vaccine. A number of animal studies and human clinical trials have been completed using ex vivo-grown monocyte-derived DCs with promising results.

**ANTI-DC CELL SURFACE RECEPTOR MONOClonAL ANTIBODY TARGETING**

Recently, several studies have been reported that target antigens to DC receptors in clinical trials. Two phase I clinical trials were conducted in advanced epithelial carcinoma patients injected with CDX-1307 (recombinant human choriionic gonadotropin beta-chain (hCG-beta) fused to anti-MR monoclonal antibody, with or without the addition of GM-CSF and TLR-3 and TLR7/8 agonists). Superior humoral and T-cell responses and the longest duration of stable disease were noted when GM-CSF and TLR agonists were included, and anti-hCG-beta antibodies had tumour-suppressive functions in vitro. This vaccination regimen is currently in Phase II clinical trials in newly diagnosed, resectable hCG-beta-expressing bladder cancers, where low tumour burden and early intervention may provide greater potential for benefit, as demonstrated in our 16.5-year follow-up study. Similarly, NY-ESO-1, a cancer-testis antigen widely used in melanoma studies, fused with either anti-MR or anti-DEC205 antibodies, leads to stimulation of CD4 + and CD8 + T cells from peripheral blood mononuclear cells of cancer patients, as compared to only CD4 + T-cell stimulation when NY-ESO-1 is used without antibody conjugation. Thus, targeting either the MR or DEC205 on DCs is a promising vaccination strategy to induce strong cellular immune responses. Indeed, in a Phase I clinical trial using CDX-1401 (NY-ESO-1 fused to anti-DEC205 and mixed with TLR-3 and TLR-7/8 agonists) in 45 advanced cancer patients, 13 demonstrated stabilisation of disease with a median duration of 6.7 months and 2 patients showed tumour regression.

**FUTURE DIRECTIONS**

A strategy to improve the immunogenicity of antigens is by ‘antigen targeting’. DCs are a family of professional antigen-presenting cells that play a major role in the initiation of innate and adaptive immune responses against pathogens and tumours. Understanding the role of DC cell surface receptors aids in the understanding of the mechanism underlying their potent antigen-presenting capacity. An array of DC cell surface receptors have been discovered in the last 5–10 years, which function in inducing immune responses and individually shows promise as targets for vaccine delivery. Although all are in pre-clinical stages, we await data from clinical trials on the effectiveness of targeting different DC cell surface receptors. In addition, the discovery of TLRs and stimulation of DCs via TLR ligands has opened new avenues for designing DC-based cancer immunotherapeutics. It remains to be determined whether TLR-targeted approaches will result in enhanced immunogenicity in humans, as primarily seen in animal models. Moreover, chemokine receptors present on DCs such as CCR1, CCR2, CXCR4, CCR5, CCR6 and CXCR1 have been shown to generate enhanced immune responses in vitro and in animal models, as well as bacterial toxins, DC-binding peptides, internalisation peptides (Int) and Fc receptors. DC receptor targeting of antigens is a promising approach for cancer immunotherapy, and in particular targeting the MR on DCs results in protection of recurrence in breast cancer patients up to 16.5 years later. We await the next 5 years of new clinical data from immunotherapy studies targeting an array of DC cell surface receptors, including MR, DEC205, DC-SIGN, scavenger receptor, TLR, and so on, all of which show promise for stimulating strong immune responses and with potentially strong clinical outcomes. In light of the encouraging results regarding anti-CTLA-4 antibody (ipilimumab), now approved for metastatic melanoma, it will be interesting to see if the combination of antigen-specific vaccines with check-point inhibition will result in improved outcomes of cancer vaccines.

**CONFlict of interest**

The authors declare no conflict of interest.
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