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Targeting the dendritic cell: the key to immunotherapy in cancer?

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Immunotherapy denotes a strategy for manipulating a patient's immune response [1]. In cancer or infectious disease the approach is designed to boost the patient's response to tumour antigens or pathogens. Conversely, immunotherapeutic strategies in autoimmunity or allergy are designed to silence the patient's response to autoantigens or allergens. Two principal approaches to immunotherapy, modulation of the immune system *de novo* by therapeutic vaccination or administration of exogenous reagents, such as cytokines and antibodies, in order to boost endogenous immune function, have been described [2].

The use of immunotherapy intended to enhance anti-tumour immunity has been established since William Corey in the 1890s achieved some success by administering bacterial extracts to cancer patients [3]. Since that time thousands of experimental models of and hundreds of clinical trials of immunotherapy have been reported. The strategies employed have been either nonspecific (e.g. administration of immunological adjuvants such as IL-2 and lymphokine-activated killer cells) [4] or antigen-specific (e.g. immunization with genetically modified tumour cells; vaccination with tumour peptides or DNA-encoding tumour antigens) [5]. Although each of these approaches has been shown to boost anti-tumour immunity in animal models none has yet proved of consistent benefit in the clinic. This has focused attention in several different cancers on the dendritic cell (DC) as a critical initiator and regulator of T cell responses.

DC are the most potent APC and unique in their capacity to initiate a primary T cell immune response [6]. DC resident in peripheral tissues have an immature phenotype, characterized by the capacity for avid antigen sampling but limited ability to activate naïve T cells resident in draining lymph nodes. The full antigen presenting potential of DC is only apparent after receipt of maturation signals, which direct migration of DC from the peripheral tissue to the draining lymph nodes. There is a consensus that effective cancer vaccines will need to elicit both CD4+ IFN-γ producing and CD8+ cytotoxic T cell responses [7]. Successful anti-tumour immunity will therefore depend on receipt by DC of maturation signals, which drive differentiation of naïve CD4+, and CD8+ T cells into Th1/Tc1 effector cells.

Early vaccine trials, in which immature and mature DC were loaded with tumour antigens and infused into patients, demonstrated that only mature DC induce effective anti-tumour immunity [8]. Maturation signals stimulate up regulation of costimulatory molecules on DC and production of inflammatory cytokines, which counteract the tolerance inducing properties of immature DC [9]. Cross-linking of the costimulatory molecule, CD40, by CD40L expressed by CD4+ T cells has the additional effect of priming for CD8+ T cell memory, a mechanism that may be essential for achieving long-term tumour immunity [10].

Unravelling the contribution of different stimuli to maturation of DC has been greatly aided by the observation by Sallusto and Lanzavecchia that peripheral blood monocytes, exposed to a combination of the inflammatory cytokines, GM-CSF and IL-4, differentiate into immature DC [11]. This has permitted exploration of the characteristics of an easily accessible, homogeneous population of DC, which can be manipulated for clinical application. A cocktail of cytokines, IL-1β, IL-6 and TNF-α, together with PGE_2 is conventionally used to induce maturation of monocyte derived DC (MDC) [12]. The optimal combination, however, of maturation stimuli for the best possible anti-tumour response is as yet unclear. Recent studies have revealed the potent maturation effects of type I IFNs on MDC [13] and subsequent enhancement of cytotoxic T cell responses [14]. In addition to effects on maturation, Renneson *et al.* [15] in the present issue have demonstrated that monocytes differentiated in the presence of IFN-β in combination with the survival factor, IL-3, are effective inducers of antigen-specific cytotoxic T cells. The optimal timing of exposure to Type I IFNs during

differentiation and maturation of DC has still to be resolved, but these studies complement trials demonstrating the clinical promise of type I IFNs as therapies for selected malignancies [16].

Most currently published trials of DC vaccines have utilized MDC or DC derived from CD34 + haematopoietic progenitor cells loaded with tumour antigen, peptide, idiotype or tumour cells [17]. Most studies demonstrated some degree of enhanced systemic anti-tumour immunity and critically, the absence of serious side-effects. Bhardwaj and colleagues have suggested that the most impressive clinical response was associated with immunization with whole protein, killed tumour cells or tumour lysates [12]. These sources of antigen can target the MHC class II pathway and, via cross presentation, the class I pathway, and thus generate both Th1 and Tc1 immunity. There is, however, limited evidence relating to migration of DC to the tumour site or the presence of cytotoxic T cells infiltrating the tumour [18,19]. It is probable that both these criteria must be fulfilled for successful clearance of tumour load. Moreover, limited access to central lymph nodes probably accounts for the relative lack of efficacy of DC vaccination in controlling solid tumours such as lung cancer [17]. In addition, because of technical difficulties in isolating sufficient numbers of DC directly from peripheral blood, the potential therapeutic benefits of such DC populations have only begun to be evaluated [20].

Since DC are critical for induction of tumour immunity the reasons for their failure to eliminate tumour burden must be considered. Firstly, tumour-derived inhibitory factors such as IL-10, TGF-β, VEGF and prostaglandins have all been implicated in the down regulation of DC function [21]. Secondly, T cells expressing a CD4+CD25+ regulatory phenotype (T_{reg}), which can suppress DC function, have been detected in cancer draining lymph nodes [22,23]. Thirdly, DC may fail to be recruited in adequate numbers to draining lymph nodes or intratumorally [24]. Therefore, procedures such as depletion of T_{reg} cells, neutralization of inhibitory factors, and gene targeting of tumours and cancer draining lymph nodes with DC chemoattractants should be considered as adjuvant therapies prior to vaccination.

Direct enhancement of DC competence at the site of the tumour and in cancer draining lymph nodes represents an alternative to therapeutic vaccination. This strategy may be advantageous as mature DC derived *ex vivo* secrete low levels of IL-12 [9]. Methods such as injecting Bacillus-Calmette-Guerin into tumours or systemic administration of DC growth and maturation factors such as GM-CSF and CD40 ligand have not convincingly demonstrated either expansion or maturation of tumour infiltrating or draining DC populations [25]. Novel approaches to immunotherapy are therefore urgently required. Srivastava and colleagues have demonstrated that heat shock proteins (HSP)-peptide complexes isolated from a specific tumour can be used to elicit prophylactic and therapeutic immunity against the cancer from which the immunizing preparation was isolated [26]. Internalization of HSP-peptide complexes generates extremely efficient access to the MHC class I pathway and activates DC competence in spite of exogenous administration of the complex. Steinman *et al.* [27] have reported that targeting antigen to the DEC-205 endocytic receptor on DC mediates antigen uptake and presentation to CD4+ and CD8+ T cells which, in conjunction with CD40 ligation, induces maturation of DC and strongly activating signals to CD8+ T cells.

Two main dendritic cell populations have been identified in humans; myeloid DC, which include interstitial DC, and plasmacytoid DC, which are primarily located in blood and secondary lymphoid organs [28]. Most forms of therapy designed to boost DC function have been, whether intentionally or not, directed to the myeloid subset. Plasmacytoid DC have recently been reported to prime tumour-specific CD8+ T cells and to infiltrate tumour tissues [29,30]. As plasmacytoid DC are the principal type I IFN-producing immune cell they represent an extremely attractive therapeutic target. Plasmacytoid DC of human origin specifically express the toll-like receptor, TLR 9, one of a family of receptors which recognize components of microorganisms and thus transmit a 'danger' signal to DC in peripheral tissues [31]. Ligation of TLR9 by unmethylated CpG oligodinucleotides (CpG) has been shown to generate potent anti-tumour immunity including reversal of impaired DC function and elimination of cancerous cells in lung tumour-bearing mice [32]. These effects were optimal when DC were stimulated in conjunction with neutralization of IL-10. CpG conjugated to tumour peptide or in conjunction with tumour peptide are now subject to trial in several forms of cancer and initial published results are eagerly anticipated.

DC are an attractive target for therapeutic manipulation of the immune system in cancer. However, the lack of generally accepted protocols for conducting and evaluating the results of clinical trials is hindering the progress of immunotherapy. This is probably inevitable at present, considering the multitude of parameters, an indication of which is given in this review, whose rational manipulation will be required to optimize design of intervention strategies. It is probable that no single approach, therapeutic vaccination, boosting the immunostimulatory profile of endogenous DC or neutralization and depletion of inhibitory factors and cells, will be sufficient to provide long-lasting antitumour immunity. A multitargeted approach based on a greater understanding of DC biology will be required to attain the goal of effective immunotherapy in cancer.

References

1 Wickelgren I. Immunotherapy. Wielding worms at asthma and autoimmunity. Science 2004; **305**:170–1.

2 Steinman RM, Mellman I. Immunotherapy: bewitched, bothered and bewildered no more. Science 2004; **305**:197–200.

3 Hall SS. A commotion in the blood 1st Edn. New York: Henry Holt, 1998.

4 Kimura H, Yamaguchi Y. Adjuvant immunotherapy with interleukin-2 and lymphokine activated killer cells after non-curative resection of primary lung cancer. Lung Cancer 1995; **13**:13–31.

5 Pardoll DM. Cancer vaccines. Nat Med 1998; **4**:525–31.

6 Holt PG, Stumbles PA. Regulation of immunologic homeostasis in peripheral tissues by dendritic cells: the respiratory tract as a paradigm. J Allergy Clin Immunol 2000; **105**:421–9.

7 Steinman RM, Dhodapkar M. Active immunisation against cancer with dendritic cells: the near future. Int J Cancer 2001; **94**:459–73.

8 Jonuleit H, Gieseke-Teutenberg A, Tuting T *et al.* A comparison of two types of dendritic cell as adjuvants for the induction of melanoma-specific T cell responses in human following intranodal injection. Int J Cancer 2001; **93**:243–51.

9 Viera PL, de Jong EC, Wierenga EA, Kapsenberg ML, Kalinski P. Development of Th1-inducing capacity in myeloid dendritic cells requires environmental instruction. J Immunol 2000; **164**:4507–12.

10 Shedlock DJ, Shen H. Requirement for CD4 T cell help in generating functional CD8 T cell memory. Science 2003; **300**:337–9.

11 Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumour necrosis factor alpha. J Exp Med 1994; **179**:1109–18.

12 O'Neill DW, Adams S, Bhardwaj N. Manipulating dendritic cell biology for the active immunotherapy of cancer. Blood 2004; **104**:2235–46.

13 Nagai T, Devergne O, Mueller TF, Perkins DL, van Seventer JM, van Seventer GA. Timing of IFN-beta exposure during human dendritic cell maturation and naive Th cell stimulation has contrasting effects on Th1 subset generation: a role for IFN-beta-mediated regulation of IL-12 family cytokines and IL-18 in naive Th cell differentiation. J Immunol 2003; **171**:5233–43.

14 Le Bon A, Etchart N, Rossmann C, Ashton M, Hou S, Gewert D, Borrow P, Tough DF. Cross-priming of CD8+ T cells stimulated by virus-induced type I interferon. Nat Immunol 2003; **4**:1009–15.

15 Renneson J, Salio M, Mazouz M, Goldman M, Marchant A, Cerundolo V. Mature dendritic cells generated in the presence of IFN-β and IL-3 prime functional antigen-specific CD8+ T cells. Clin Exp Immunol 2005; **139**:468–75.

16 Blattman JN, Greenberg PD. Cancer Immunotherapy. a treatment for the masses. Science 2004; **305**:200–5.

17 Davis ID, Jefford M, Parente P, Cebon J. Rational approaches to human cancer immunotherapy. J Leuk Biol 2003; **73**:3–29.

18 Thomas R, Chambers M, Boytar R, Barker K, Cavanagh LL, MacFadyen S, Jenkins M, Andersen J. Immature human monocyte-derived dendritic cells migrate rapidly to draining lymph nodes after intradermal injection for melanoma immunotherapy. Melanoma Res 1999; **9**:474–81.

19 Mukherji B, Chakraborty NG, Yamasaki S *et al.* Induction of antigen-specific cytolytic T cells in situ in human melanoma by immunization with synthetic peptide-pulsed autologous antigen presenting cells Proceedings Natl Acad Sci USA 1995; **92**:8078–82.

20 Reichardt VL, Okada CY, Liso A *et al.* Vaccination with idiotype using dendritic cells after autologous peripheral blood stem cell transplantation for multiple myeloma–a feasibility study. Blood 1999; **93**:2411–9.

21 Wang T, Niu G, Kortylewski M *et al.* Regulation of the innate and adaptive immune responses by Stat-3 signalling in tumour cells. Nat Med 2004; **10**:48–54.

22 Viguier M, Lemaire F, Verola A *et al.* Foxp3 expressing CD4+CD25 (high) regulatory T cells are overrepresented in human metastatic melanoma lymph nodes and inhibit the function of infiltrating T cells. J Immunol 2004; **173**:1444–53.

23 Rosenberg SA, Dudley ME. Cancer regression in patients with metastatic melanoma after the transfer of autologous anti-tumour lymphocytes. Proc Natl Acad Sci USA 2004; **101**:14639–45.

24 Vicari AP, Vanberyleit B, Massacrier C *et al.* In vivo manipulation of dendritic cell migration and activation to elicit anti-tumour immunity. Novartis Found Symp 2004; **256**:241–54.

25 Paczesny S, Ueno H, Fay J, Banchereau J, Palucka AK. Dendritic cells as vectors for immunotherapy of cancer. Seminars Cancer Biol 2003; **13**:439–47.

26 Srivastava P. Role of heat shock proteins in innate and adaptive immunity. Nat Rev Immunol 2002; **2**:185–94.

27 Bonifaz LC, Bonnyay DP, Charalambous A *et al.* In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. J Exp Med 2004; **199**:815–24.

28 Banchereau J, Paczesny S, Blanco P, Bennett L, Pascual V, Fay J, Palucka AK. Dendritic cells: controllers of the immune system and a new promise for immunotherapy. Ann NY Acad Sci 2003; **987**:180–7.

29 Hartmann E, Wollenberg B, Rothenfusser S *et al.* Identification and functional analysis of tumor-infiltrating plasmacytoid dendritic cells in head and neck cancer. Cancer Res 2003; **63**:6478–87.

30 Rothenfusser S, Hornung V, Ayyoub M *et al.* CpG-A and CpG-B oligonucleotides differentially enhance human peptide-specific primary and memory CD8+ T-cell responses in vitro. Blood 2004; **103**:2162–9.

31 Medzhitov R. Toll-like receptors and innate immunity. Nature Rev Immunol 2001; **1**:135–45.

32 Vicari AP, Chiodoni C, Vaure C *et al.* Reversal of tumour-induced T cell paralysis by CpG immunostimulatory oligonucleotide and anti-IL-10 receptor antibody. J Exp Med 2002; **196**:541–9.