

Current issues in dendritic cell cancer immunotherapy

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Current Opinion in Molecular Therapeutics (2002) 4(1):
© PharmaPress Ltd ISSN 1464-8431

Dendritic cells (DCs) initiate and direct the immune response. Their inability to detect danger signals from transformed cells and to generate an effective immunological response may allow cells with a malignant phenotype to evolve into cancers. This defect can be corrected for many cancer types and the immune response boosted to eliminate malignant cells by means of DC-based vaccines/therapies. Rapid advances in our understanding of basic DC physiology and improved methods for DC isolation have made clinical application of DC therapy practical and encouraging phase I/II results are emerging.

Keywords Cancer, clinical trials, dendritic cells, immunotherapy, vaccine

Introduction

Dendritic cells (DCs) have unique properties as antigen presenting cells (APCs), and are therefore a logical, and perhaps optimal, vehicle for generating cancer vaccines/therapies. Information regarding DC subsets, phenotypes, and their function is increasing rapidly and its translation into improved clinical practice is imminent. Several practical factors, including the description of methods to generate DC-like cells from monocytes *in vitro* in 1994 [REF?], have also encouraged a dramatic increase in clinically directed DC research. In this review, we will discuss some basic issues pertaining to DC immunotherapy and some of the most recent clinical articles on DC cancer immunotherapy.

DC as danger sensors

Elimination of cells with abnormal growth characteristics should be achieved by the coordinated function of the innate and adaptive arms of the immune system detecting altered 'non-self' antigens and 'danger signals' from pre-malignant cells, which are not present on normal 'self' cells and to which the immune system is tolerized (the nature of these signals and their implication for tolerance was reviewed recently in [1]). As initiators of the immune response, DCs are natural sensors of the danger signals [2] which prompt the cascade of events linking the innate and the adaptive immune systems [3]. Stress induced by extreme exercise and surgery rapidly increases the number of circulating DCs in blood, possibly as a result mobilization from marginated or splenic sites [4], and this too may be part of the natural response to pathogens.

Abnormal DC function in cancer

The induction of the immune response specific for altered self-antigens appears to be hampered by abnormal DC physiology. Evidence for dysfunction at various levels has

been presented: we demonstrated the presence of minimally activated DCs within kidney, bladder and prostate cancers [5-7], and these findings have since been confirmed by us [8] and others [9] in breast cancer. Although reduced blood DC counts have been described in cancer patients [10], this may vary with the stage of disease. DC counts appear to be preserved in patients with stage I and II breast cancer [Ho *et al*, manuscript in preparation]. DC function may be affected locally at the tumor site, but systemic defects have also been described, eg, DC capacity to upregulate critical costimulatory molecules appears to be defective in multiple myeloma [11]. Recent reports point to a dysfunction in DCs evaluated from lymph nodes (LNs) draining cancerous lesions, where an abnormally high number of immature DCs were observed [12]. A subset of myeloid cells with a less mature phenotype but capable of inhibiting antigen-specific recognition by DCs was reported to be increased in the blood of cancer patients [13]. These data suggest that an efficient immunotherapy program should be capable of 'correcting' the variety of defects observed in cancer patients and elicit a strong tumor-specific response. Furthermore, the seminal work conducted in mice (reviewed in [14]) indicating the curative potential of DC immunotherapy has provided a second impetus to develop DC-based clinical immunotherapy.

DC subsets and subtypes

At present no DC-specific molecular markers have been identified, and our current understanding of the DC population is based on the mutually exclusive expression of various surface markers [15]. Human blood DC subsets have been classically defined as expressing CD11c or CD123 markers and named 'myeloid' or 'lymphoid', respectively (reviewed in [16] and [17]). Alternatively, the expression of immunoglobulin-like transcript (ILT) molecules defines an ILT3+/ILT1+ subset of mainly CD11c+ cells or an ILT3+/ILT1- subset, corresponding in the main to the CD123+ subset. These two populations are found in peripheral blood and in lymphoid tissues, although different migration pathways for them have been described. New reagents and information regarding DCs are accumulating under the auspices of the DC Section of the Leucocyte Differentiation Antigen Workshop (www.hlda8.org). Recently described antibodies, which identify novel DC-specific molecules, demarcate novel DC subpopulations [18]. The function of the various subsets appears to be different and, therefore, a good understanding of the DC subset constitution used in a vaccine is essential.

Choice of a DC preparation for immunotherapy

DCs originate from the bone marrow and migrate via the blood to the tissues, where they function as immunological sentinels. Subsequent to exposure to relevant antigen, myeloid DCs migrate towards lymphoid tissues (node, spleen and perhaps liver), where in a supportive microenvironment they interact with naïve T-lymphocytes to initiate the immune response. Considering that tissue DCs are not readily accessible, the DC (or its precursors) present in blood represent the best source for clinical purposes.

There are three options: 'DC-like' cells may be obtained by *in vitro* transformation of a number of cell types: (i) CD34+ (CD34+DC) precursors; (ii) monocyte (Mo-DC) in the presence of cytokine mixtures; alternatively, (iii) natural blood DC (BDC) or precursors may be harvested directly from the blood without cytokine treatment. *In vitro* transformation of precursors, notably monocytes in the presence of cytokines, allows the highest yields, hence these protocols are more commonly adopted for immunotherapy. All three preparations take up antigen effectively, express co-stimulatory molecules, act as efficient APCs in mixed leukocyte reaction and generate MHC-restricted class I/II antigen-specific responses [19]. However, differences between *in vitro* generated DC preparations have been described [20]; moreover, we have observed differences between BDC and Mo-DC in their expression of important surface molecules such as DC-SIGN, DEC-205 and the mannose receptor [21,22]. More importantly, BDCs appeared to be more efficient in the induction of *in vitro* primary immune responses [Osugi *et al*, submitted], as well as *in vitro* antigen uptake and processing [23].

CD34+-derived cells

CD34+ hematopoietic stem cells (HSCs) isolated from bone marrow, peripheral or cord blood can be transformed into a DC phenotype *in vitro* in the presence of granulocyte/macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor (TNF α). This technique yields DCs that have been compared with the two subsets described in skin; one that resembles Langerhans cells expressing CD1a, Birbeck granules, CD207 (langerin) and E-cadherin; the second resembles interstitial (dermal) DC and lacks Birbeck granules (reviewed in [24]). This procedure can be performed on apheresis harvests, which collect CD34+ cells after mobilization, thereby generating sufficient DCs for immunotherapy. Bone marrow and blood stem cell transplant protocols utilizing immunoselected CD34+ precursors have paved the way and two clinical trials utilizing CD34+-derived DCs have been published (see Table 1).

Mo-DCs

The most widely used preparation strategy transforms or differentiates monocytes into a DC-like cell *in vitro* in the presence of GM-CSF and IL-4. The number of CD14+ cells in peripheral blood (monocytes can be obtained from apheresis products) makes this method an attractive option to generate large numbers of DCs. The resulting Mo-DCs have an 'immature' phenotype and can be 'matured' in the presence of additional stimuli, including TNF α , LPS, CD40L, monocyte-conditioned media or a cocktail of various cytokines (typically, TNF α , IL-1 β , IL-6 and prostaglandin E2). Monocytes were first purified by adherence, some now utilize immunoselected CD14+ cells and commercial interests are developing these strategies. Alternatively, a procedure that avoids the separation of monocytes benefits from the presence of lymphocytes during the differentiation procedure may generate higher DC yields [Au: I don't understand this sentence]. This too is now available as a good manufacturing practice (GMP) system and the commercial version replaces the more conventional IL-4 with IL-13 [25]. One risk involved in this procedure is that Mo-DCs may revert from a DC phenotype to a macrophage phenotype in the absence of the exogenous cytokines, and

lose the extraordinary antigen presenting capacity of DCs. Furthermore, DCs placed in an IL-10-rich environment are considered likely to drive a tolerogenic response [26]. The recent description of two individuals in whom established influenza responses were abrogated by immature Mo-DC [27] vaccination emphasizes that this is cause for concern.

Blood DCs

Although blood is an attractive source of DCs in transit to the tissues, the low numbers of DCs in blood (approximately 1% PBMCs) has been seen as a challenge to harvesting sufficient quantities of DC. BDCs have been isolated from blood using density gradients such as Percoll, Nycodenz and metrizamide [28]. These methods have been adapted for clinical protocols, and a commercially available GMP version now includes sequential gradients of buoyant density solutions followed by short *in vitro* culture in serum-free medium and in the absence of cytokines (reviewed in [29]). A detailed analysis of DC counts on apheresis products obtained using Cobe Spectra equipment and automated software has provided considerable reassurance regarding the availability of BDCs, and established that sufficient numbers of DCs can be obtained (minimum of 30 million from a 10 l procedure) for immunotherapy programs, and further that the product preserves the original DC subset distribution [López *et al*, manuscript submitted]. A method for enrichment of BDCs based on the use of the CMRF-56 mAb specific for an activation marker on DCs [30] allows the isolation of high yields of BDC [López *et al*, manuscript in preparation].

Blood DC mobilization

Flt3- ligand (Flt3L) increases the number of circulating DCs, resulting in mobilized BDCs that may be very useful in therapeutic applications. Initial clinical trials have increased levels of BDCs by 20-fold both in cancer patients and healthy individuals [31]. Early data suggest that they are not activated by the procedure [32]. The Flt3L/G-CSF hybrid protein, also mobilizes BDCs, but the subset constitution is probably different. Although reasonable BDC counts seem to persist after chemotherapy, these mobilization options may be invaluable for myelosuppressed patients [33].

Tumor-associated antigen loading

The source of antigen used for immunization varies widely, from well-defined HLA-restricted tumor-associated antigen (TAA) CTL epitopes in the form of synthetic peptides (or tumor eluates), the use of whole proteins (eg, immunoglobulin idiotype or recombinant TAA) to the use of whole tumor cells either as cell lysate or intact (irradiated) cells. While the use of a less defined material may improve the coverage of the epitopes presented, thus minimizing the risk of immune evasion, the risk of additional autoimmune phenomena remains a concern. Certainly, the use of more defined epitopes allows a more formal evaluation of the immune response, which may in turn expedite interpretation of clinical trials.

Virus carrying TAA and infecting DCs have been studied as effective antigen delivery systems. Retroviral vectors have been used [35] to present class I and II epitopes. Adeno [36] and canarypox [37] viruses known to replicate poorly in humans have also been used. However, safety issues may limit their use in wider clinical practice.

Table 1. Reports on the use of DCs for cancer immunotherapy.

Type of cancer	Antigen	DC type	R ¹	N ²	Response reported	Reference
Non-Hodgkins lymphoma	Idiotypic	Imma Mo	iv > sc	4	One CR, one PR; Ag-specific proliferation	[65]
Multiple myeloma (follow up)	Idiotypic protein + KLH	Blood	iv	26	24/26 KLH-specific, 4/26 Id-specific proliferation (CR)	[72]
Multiple myeloma	Idiotypic protein	Imma Mo	iv	1	Ag-specific proliferation response (Ab)	[80]
Multiple myeloma	Idiotypic protein + KLH	Imma Mo	iv	6	Increase in CTL proliferation in 3/6 patients; Ab and Id proliferation; IFN γ production detected by ELISA	[81]
Multiple myeloma	Idiotypic protein + KLH	Blood	iv	12	11/12 KLH-specific, 2/12 Id-specific proliferation (CR)	[82]
Chronic myeloid leukemia	Leukemic DC (Ph1+)	Ma Mo	iv	1	Ph1-specific CTL	[83]
Melanoma	Melan A or MAGE-1	Imma Mo-DC versus Mat Mo-DC	in	11	Three PRs, two SDs (of eight evaluable)	[49]
Melanoma	MelanA/MART-1, MAGE-1	Ma, Imma Mo	in	11	5/7 CTLs using Ma Mo, and 1/7 CTL with Imma	[49]
Melanoma	MAGE A1/A3 peptide	Imma Mo	iv > sc	24	Increase in peptide-specific CTLs in 14/24 patients	[70]
Melanoma	Melan A, gp100	CD34+	iv	14	Peptide DTH, met regression and vitiligo	[69]
Melanoma	Tyrosinase gp100 peptides	Imma Mo	iv	16	One CR, two SDs, 5/16 patients produced IFN γ response as determined by ELISA	[71]
Melanoma	Lysate/peptide mix + KLH	Imma Mo	in	16	DTH to peptide and KLH, two CRs, three PRs and met regression	[66]
Melanoma	MAGE 3A1 HLA A1 peptide	Ma Mo	sc, id > iv	11	Peptide-specific CTLs in 6/11 patients; met regression in 6/11	[67]
Melanoma	MAGE 3A1 HLA A2 peptide	Ma Mo	sc, id > iv	8	Peptide-specific CTLs in 8/8 patients	[84]
Melanoma	Tumor/Allo PBMC hybrid	Act. PBMC	sc	16	One CR, one PR and five SD	[85]
Melanoma	Mart-1; gp 100 peptides	Imma Mo	iv	10	Increased CTLs in 1/5 patient, one PR and met regression	[86]
Melanoma	MelanA, tyrosinase, MAGE-3 + flu MP	CD34+	id	18	Ag-specific ELISPOT in 16/18 patients; met regression and delayed disease progression	[68]
Breast	Tumor lysate	Mo	in	1	Met regression	[87]
Prostate	PMSA P1 and P2 peptides	Imma Mo	iv	37	One CR, ten PRs	[74]
Prostate (follow up)	PMSA P1 and P2 peptides	Imma Mo	iv	37	DTH and cytokine production, which related to clinical response	[75]
Prostate	Fusion protein GMCSF + PAP	Blood	iv	13	Peptide-specific proliferation of T Ab PAP in 20/31 patients, and delayed disease progression	[76]
Prostate	PSMA P1 and P2 peptides	Imma Mo	iv	51	Seven PRs, peptide-specific CTLs	[88]
Prostate	Fusion protein GMCSF + PAP	Blood	iv	31	Proliferation to GMCSF and PAP	[89]
RCC	Tumor/ AlloDC hybrid	Ma Mo	iv	17	Four CRs, met regression, one mass reduction >0%	[41]
RCC	Cell lysate + KLH	Ma Mo	iv	12	RCC, normal kidney and KLH proliferation	[90]
RCC	Cell lysate + KLH	Ma Mo	iv	4	One PR ; KLH IFN γ responses detected	[91]
Metastatic CEA expressing	CAP-1 peptide	Imma Mo	iv	21	One SD	[92]
Metastatic CEA expressing	CAP-1 peptide/RNA	Imma Mo	iv	17	CEA-specific CTL sequel response in peptide and RNA cohorts	[93]
Breast/ovarian	Her 2/Neu MUC-1 Peptides	Mat Mo	sc	10	5/10 patients demonstrated CTLs	[94]
Glioma	Acid eluted peptide	Imma Mo	sc	7	CTLs infiltrated tumor and prolonged survival	[95]
Glioma	Tumor/Auto DC fusion	Ma Mo	id	8	Two PRs	[96]
Gastrointestinal	MAGE peptide	Imma Mo	iv	12	Peptide-specific CTLs in 4/8 patients, and reduced tumor marker in 7/12.	[97]
Solid tumors (pediatric)	Tumor lysate + KLH	Imma Mo	id	8	Tumor regression onbserved	[98]
Pancreas.	Tumor lysate + KLH	Ma Mo	sc	1	Vaccine DTH, proliferation, decreased marker; met regression	[99]

¹Route of immunization

²Number of individuals immunized

CAE carcinoembrionic antigen; CAP peptide fragment of CAE; CR complete remission; DTH delayed type hypersensitivity; flu influenza MP HLA A2.1 peptide 55-66; id intradermal; Id Idiotypic; il intralymphatic; in intranodal; iv intravenous; Ma Mature; Imma Immature; Mo MoDC; Met metastasis; PAP Prostatic acid phosphatase; Ph1 Philadelphia chromosome; PR partial remission; proliferation lymphocyte proliferation; sc subcutaneous; SD stable disease

Another novel approach to antigen delivery is the use of tumor-DC fusion cells. The injection of DC-tumor hybrids in mice generated cure of advanced metastatic disease [38]. Tumor fusions have now been generated with human cells, and hybrids produced in the presence of polyethylene-glycol (PEG) yielded measurable specific responses against breast cancer cells [39,40]. Moreover, striking clinical responses were reported with the use of electroporated hybrid preparations [41]. Further detailed studies on the quality of the cells injected and the nature of the immune response generated are eagerly anticipated. A number of clinical trials using both allogeneic and autologous DC are currently underway in many centers and the initial results should emerge soon [Walden P, personal communication].

DCs can also be loaded with whole tumor or specific TAA, DNA or RNA, the latter of which is very effective in mouse models and is also capable of eliciting human CTL-specific responses *in vitro* [42] and electroporated RNA may be superior to DNA plasmid expression of RNA [43]. Clinical trials in patients with prostate cancer using this technique are underway.

Optimal stimulatory signals for presentation

Various stimuli may prompt DC activation/'maturation' or differentiation and generate significant antigen presenting capacity. Often this activation is the result of the initial encounter with pathogens that alert the immune system [44], but clearly not all stimuli are equal [45]. The use of immature Mo-DCs is no longer an option because of their undesirable inhibitory effects on established CTL responses [27]. Immature DCs can elicit regulatory CD4+CD25+ T-cells [46,47] that actively suppress naïve and memory T-cells [48]. A head-to-head comparison of immature and mature DCs found that intranodal injection of mature Mo-DCs was superior in generating CTLs specific for melanoma antigens compared with immature Mo-DCs [49].

More work is required to determine the optimal activation/'maturation' or differentiation state for Mo-DCs. Various agents have been used to maximize activation assessed by increases in the expression of the co-stimulatory molecules CD80 and CD86; activation markers CD40, CD83 and CMRF-44; the adhesion molecule CD54 and the MHC class II antigens. The highly repeated DNA sequence containing bacterial-type CpG motifs has been shown to induce efficient maturation of DCs [50], particularly driving the so-called 'plasmacytoid' (CD123+) DC subset via the induction of IL-12 production [51,52]. Equally, products of bacterial origin such as the outer membrane proteins (Omp) from Gram-negative bacteria, eg, OmpA from *Klebsiella pneumoniae* are potent inducers of DC maturation [53]. In current prostate and kidney cancer trials, BCG and purified protein derivative (PPD) are being used to mature DCs. Other alternatives to induce maturation include exposure to CD40L, and a variety of cytokine cocktails including TNF α , IL-1 β , IL6 and PGE2. Calcium ionophores are also being investigated actively. Monocyte conditioned media has also been shown to be effective in the maturation of DCs [54], as has T-cell-conditioned media but the use of these less defined products in clinical practice may generate regulatory concerns.

Cryopreservation

The evidence to date suggests that several immunizations will be required for initial therapy, and further that these may need to be repeated in the long term. The doses used vary between 1 and 30 million DCs, and the frequency of the injections from 1- to 6-week intervals. Mo-DC preparations can be safely frozen while maintaining their functional capacity [55-57], and BDCs can also be cryopreserved [unpublished].

Routes of administration

Comparative studies in mice suggest that subcutaneous injection, rather than other routes of administering antigen-pulsed DCs, produce better outcomes. A recent paper [58] compared responses in patients to BDCs loaded with recombinant mouse prostatic acid phosphatase injected intravenously, intradermally or into cannulated lymphatics. Only the latter two routes resulted in T-lymphocyte IFN γ production and the intravenous route resulted in new antibody production; as such, clinical trials are moving away from the intravenous route of DC administration. Some investigators still prefer the intranodal injection; however, injection into the skin (subcutaneous or intradermal) is becoming the preferred route of administration.

Migration studies

Success in generating an efficient and long-lasting immune response requires an optimal interaction between DCs and memory T-lymphocytes. Central and effector memory T-lymphocyte pools have been characterized based on the expression of isoforms of the chemokine receptors CCR7 and CD45 [59]. The location of the central memory pool T-lymphocytes in the lymph nodes (LNs) means that ideally DCs should be administered so that they reach LNs. DCs activated in the tissue reach the LNs via the lymphatics. Cells from the blood can only enter the LNs via high endothelial venules, and mature DCs lack the relevant CD62L and CCR5 molecules essential for migration to the LNs. Careful evaluation of the fate of injected Mo-DCs in monkeys showed that they are cleared from the dermis injection site within 36 h, and also that the mobilization appeared to be more efficient for immature DCs [60]. In humans, similar studies were conducted using indium-labeled Mo-DCs injected intravenously, intradermally or subcutaneously, which demonstrated that best LN localization of injected DCs was obtained by intradermal injection [61]. Intravenously administered DCs are transiently taken up by the lung, and then localize to the spleen and liver for at least 7 days, whereas DCs injected into a lymphatic vessel in the dorsal foot rapidly migrate to the draining lymph nodes where they remain for > 24 h [62].

Monitoring the immune response

The availability of surrogate markers with which to monitor the immune response after DC administration is just as vital to DC-based immunotherapy as it is other forms of cancer immunotherapy. While there is general agreement that Th1 response is most desirable, direct correlates of clinical efficacy and immunological parameters are still to be defined. Based on animal models, an efficient immunization is expected to elicit IFN γ -secreting CD8+ lymphocytes that recognize and lyse the original tumor cells. Antigen-specific CD8+

lymphocytes can now be identified using MHC/peptide tetramer complexes, although it should be noted that an increase in CD8+ does not necessarily relate to functional activity. The detailed evaluation of responses to melanoma-derived peptides demonstrates that tetramer analysis needs to be combined with assays for measuring cytokine production, such as the enzyme-linked immunospot assay (ELISPOT), flow cytometry-based intracellular cytokine staining, and, more specifically, the sorting of viable antigen-specific cells for further characterization [63,64], including cytotoxic function. An active delayed-type hypersensitivity response (DTH) to TAAs, like the CTL response, appears to either correlate with responder status or have some other predictive value in some clinical trials. It is now common to include surrogate foreign model antigens in the vaccine preparation to control the efficacy of the immunization; these antigens include MHC class II-restricted keyhole limpet hemocyanin (KLH) and MHC class I-restricted influenza matrix protein. A more defined (and agreed) panel of controls, which would allow some comparison of protocols between centers, would be a step forward.

DC phase I/II clinical trials

There have now been several phase I/II studies of DC cancer immunotherapy published and many others are underway, and these have taught researchers a number of important lessons. The first clinical trials in relapsed low-grade non-Hodgkin's lymphoma (NHL) [65] used BDCs infused intravenously and produced very encouraging clinical and laboratory results. Perhaps the most significant feature was that DC vaccination (and now GM-CSF/KLH conjugated idiotypic) can generate T-cell reactivity to idiotypic, whereas this was not achieved with previous idiotypic vaccinations. The second study reported used Mo-DCs injected intranodally under ultrasound guidance in advanced metastatic melanoma [66], the excellent clinical results of which have been a stimulus to several trials in this disease. These and other clinical studies published to date are listed in Table 1 (an updated version is maintained at <http://www.mmri.mater.org.au>).

The interest in immunotherapy for advanced melanoma reflects the current lack of options for stage III/IV disease, and also the clear evidence that this is a disease that does respond, at least in a proportion of individuals, to immunotherapy. In addition, there are also some well-defined melanoma TAAs. Mo-DCs matured with monocyte-conditioned medium (MCM) subcutaneously and intradermally induced specific CTL expansion in eight out of 11 HLA/A1 patients, but it was notable that these responses declined after further intravenous injections [67]. Regressions of individual metastases occurred in six out of 11 patients. Notably, the same group has reported poor clinical results (no regressions in eight evaluable patients) in HLA-A2 individuals using the same MAGE-3 antigen. Curiously, the latter patient group had active melanoma peptide-specific IFN γ effector CD8+ T-cells present (confirmatory cytotoxic studies were not performed) and received DCs by the possibly more optimal subcutaneous protocol.

A protocol using CD34+-derived DCs administered subcutaneously enhanced immunity in at least one assay (ELISPOT or proliferation) in 16 out of 18 patients in which

DTH responses were independent of blood assay results [68]. During a 10-week evaluation, four patients with multiple lesions experienced regression at one or more disease sites and three patients with limited disease showed evidence of clearance of disease. In broad terms, the immunological responses of patients were good predictors of disease response. Another trial made use of CD34+-derived DCs administered intravenously: the monitoring of immunological responses was limited, but these patients had a lesser clinical response, again suggesting that perhaps the intravenous route is less effective [69].

The use of immature Mo-DCs may be associated with a less beneficial clinical outcome [69,70]. Again, immunological responses appeared to predict which groups experienced a clinical response [71]. The formal comparison of immature versus mature Mo-DCs used different DC preparations and antigens in the same patients; it was not possible to allocate the positive clinical outcomes to a particular preparation although, as commented earlier, differences in T-cell responses were seen.

DC-based idiotypic vaccination for low-grade NHL and multiple myeloma has also provided some insights [Au: INTO WHAT? INSIGHTS INTO MECHANISM OR EFFICACY?]. Multiple myeloma patients may be more immunosuppressed than is usual for cancer patients, and idiotypic determinants of multiple myeloma may be weak TAAs. These factors, in addition to intravenous route of administration of DCs in multiple myeloma trials, may account for the relatively low frequency of anti-idiotypic responses. In one trial of idiotypic vaccination, four out of 26 patients developed an anti-idiotypic response, whereas 24 out of 26 patients generated a response to the immunogenic carrier protein KLH [72]. Interestingly, 75% of the patients who generated an anti-idiotypic response study were in complete disease remission. Another study performed in the post-transplant setting [73] remains to be fully reported, but it too suggests responses may occur in the minimal residual disease setting after autologous stem cell transplant for multiple myeloma. A remarkable nine out of 18 clinical responses (five complete responses and four partial responses) were seen in this context [Valone FH, personal communication].

Results in renal cell carcinoma appear to be promising, but perhaps more unexpected have been the responses noted in patients with brain tumors. However, as gliomas break the classic blood/brain barrier, the ability to deliver effector T-lymphocytes to this site is probably unimpaired [Au: Unclear what point is being made here].

As prostate, breast and bowel cancer account for the majority of conventional cancer treatment activity, the impact of DC immunotherapy in these diseases is being followed with keen interest. Prostate cancer has attracted the most attention, partly because organ-specific antigens (eg, prostate-specific membrane antigen (PSMA) and prostatic acid phosphatase (PAP)) can also be considered as TAAs. Mo-DCs used in conjunction with PSMA peptides have been used in a phase II trial, which resulted in one complete and ten partial responders amongst 37 patients with presumed local recurrence [74]. There was a strong association between clinical responders and preexisting immune

competence [75]. A protocol which used BDCs and the novel DC activating GM-CSF-PAP compound generated T-cell responses to PAP and also showed a decrease in circulating PSA [76] stimulated a phase III study of patients with progressive metastatic disease. It remains to be seen whether the anecdotal reports listed in Table 1 for the clinical benefits of immunotherapy in breast and bowel cancer will be the harbingers of more impressive clinical data.

So, what lessons have been learnt? As stated earlier, extensive immunological monitoring to establish the surrogate markers is now mandatory. It appears that the immunosuppressed patient with advanced cancer is not a good candidate for immunotherapy. Other tentative conclusions that may be drawn from the studies conducted so far include: (i) immature Mo-DCs should not be used; (ii) intravenous administration may be less favorable than other routes; and (iii) follow up vaccination may be essential (and an ethical obligation) in responding patients.

DC phase III studies

A phase III study of the DC therapy APC-8015 (Provenge; Dendreon Corp) in prostate cancer has been completed in patients with hormone refractory progressive metastatic prostatic cancer and is about to undergo its first analysis [73]. A second phase III trial in hormone-sensitive patients is being initiated. Further phase II studies are being explored prior to initiating a phase III study in multiple myeloma [73]. The phase III study in melanoma will compare Mo-DC vaccination with melanoma peptides with conventional treatment with dacarbazine. Other phase II studies are establishing protocols, which will encourage phase III studies. It is hoped that clinicians and scientists alike will encourage and support these cooperative studies.

Side effects

Fortunately, as hoped for, adverse events to DC therapies have been limited. Minor discomfort at injection sites is formally reported, in addition to minor febrile reactions and myalgia. No definite treatment-related hematological hepatic or renal toxicity has resulted. As most vaccinations to date have used antigen preparations with highly specific TAA peptides autoimmune effects were anticipated. Vitiligo occurs as an appropriate response to melanoma therapies but other autoimmune diseases have been notably absent despite animal model predictions [77]. Immature Mo-DCs might generate more autoimmune phenomenon but this has not been tested formally. As most TAAs generate low-affecting CTL responses, it is argued that this may be protective [78]. Clearly, certain at-risk autoantigens do not generate autoimmune responses, probably due to deletional tolerance [79].

Conclusions

The rapidly growing literature reporting the use of DCs for cancer immunotherapy is highly encouraging. Results never achieved with other therapies for advanced cancer are now being reported on a case basis more frequently. Data emerging from phase I/II trials encourages the view that improvement of DCs preparations, their delivery and the optimal activation of DCs will increase the efficacy of this immunotherapeutic approach. Ultimately, the greatest advance will be to apply DC therapy earlier in patients who

have minimal residual disease and are less immunosuppressed. As we progress, the design of more standardized protocols become more critical for the objective comparison of results. The results of the current and hopefully new phase III studies, are necessary for deciding the role this therapy should play in modern cancer clinical practice.

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