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REVIEW

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Optimizing dendritic cell–based anticancer immunotherapy: maturation state does have clinical impact

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Abstract Tumour immunotherapy using dendritic cells (DCs) is a new therapeutic approach, which has been applied to a variety of different cancers over the last 5 years. Here we discuss the clinical results of these trials in relation to the different protocols used to generate DCs, and in particular the effect that DC maturation state has had on clinical responses. In ten different melanoma trials a total of 167 patients have been treated, resulting in 9 complete tumour regressions, 24 partial regressions, 26 patients with stable disease, and 108 with progressive disease. Favourable response, defined as any outcome other than progressive disease, was not associated with previous chemotherapy, but was significantly correlated ($p < 0.001$) with the addition of TNF-α for the maturation of DCs in vitro. Hence DC maturation state has had an impact on clinical responses to therapy. However, TNF-α is not the only molecule capable of inducing DC maturation, and strategies for improving clinical responses by optimizing DC maturation are discussed.

Keywords Dendritic cell · Immunotherapy · Melanoma · Meta-analysis

Introduction

In oncology, the term immunotherapy embraces a variety of different therapeutic approaches with the shared aim of exploiting effector mechanisms of the immune system to eliminate tumour cells. Non-specific immunotherapy consists in the administration of recombinant human cytokines, principally interleukin 2 (IL-2) in order to boost existing antitumour T-cell responses [1]. Adoptive or passive immunotherapy is based on the infusion of immune effector molecules, or cells, specific for tumour antigens. Monoclonal antibodies, in particular those coupled to radioelements, have been shown to be clinically effective in this regard, but their use is currently limited to tumours with surface expression of known tumour- or lineage-specific antigens, such as the CD20 antigen expressed by non-Hodgkin's lymphoma [2].

Active specific immunotherapy, or tumour vaccination, aims to induce patient immune responses against autologous tumour. In contrast to passive immunotherapy strategies, tumour vaccination has the potential to induce long-lasting anamnestic responses and hence to prevent tumour resurgence. So far, three types of tumour vaccination have been tested in clinical trials. Firstly, tumour material obtained after surgery has been mixed with adjuvants, then reinjected into patients in the manner of a classical "inactivated pathogen" vaccination approach [3, 4, 5]. Secondly, melanoma patients have been treated with injections of peptide epitopes from tumour antigens such as gp100 and Melan-A/MART-1 [6, 7, 8]. Although both of these tumour vaccination strategies have generated encouraging results, immune tolerance to established tumour is one factor that may limit their clinical efficacy. The search for therapeutic approaches to circumvent tumour tolerance has led to the development of a third type of tumour vaccination that involves injecting dendritic cells (DCs), generated and loaded with tumor antigens ex vivo, into cancer patients.

DCs are the most effective antigen-presenting cells for T cells, and in particular they have the potential to break tumour tolerance and induce tumour-specific immune responses leading to tumour rejection. Furthermore, recent technical advances in the culture of large numbers of DCs have opened up the possibility of harnessing the unique properties of these cells for antitumour vaccination. Several research groups have therefore initiated clinical trials of DC therapy for a variety of cancers.

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To date, 306 advanced stage patients have been treated in 18 well-conducted phase I/II clinical trials (Table 1). The protocols used for the generation of DCs have been relatively diverse, and the aim of this review is to examine the relevance of these differences to the clinical outcome of treatment. Current understanding of DC lineage and the functions of DCs at different maturation stages will be briefly summarised, then we will attempt to answer two key questions. Firstly, has DC maturation state played a role in the results obtained in clinical trials of DC-based tumour therapy? And secondly, do these trials indicate what the best protocol for the generation of DCs for anticancer immunotherapy might be?

DC lineage and maturation

Peripheral blood contains two major populations of immature DCs: CD11c^{+} myeloid DCs and CD11c^{-} CD123^{+} plasmacytoid DCs, which make up 0.6% and 0.4% of PBMC, respectively [9]. Both these immature DCs have the ability to capture antigens, and both subsets undergo functional and phenotypic maturation during ex vivo culture.

Large numbers of immature DCs can also be produced ex vivo by culturing monocytes in GM-CSF plus IL-4. It is not yet clear whether ex vivo monocyte-derived DCs are functionally identical to circulating CD11c^{+} myeloid DCs, as they seem more closely related to the immature DCs resident in tissues, such as dermal DCs [10]. Nevertheless, monocyte-derived DCs share many functions with CD11c^{+} myeloid DCs, including antigen capture (phagocytosis, macropinocytosis and receptor-mediated endocytosis), antigen processing and maturation in response to inflammatory signals.

Finally, DCs can be differentiated from CD34^{+} stem cells by culture in a combination of GM-CSF and TNF-α. DCs generated under these conditions closely resemble Langerhans cells, the immature DCs of the epidermis [11]. However, this type of culture contains a mixture of DCs at different stages of maturation, and is less homogeneous than cultures of monocyte-derived DCs [12].

All these types of immature DCs undergo phenotypic and functional changes in response to inflammatory cytokines or microbial products in a process which is termed DC maturation. Until recently, DC maturation was thought of as a binary process in which immature DCs, specialized in the uptake and processing of antigens, were transformed into mature DCs with optimal T-cell stimulatory capacity. Key aspects of DC maturation include migration towards the T-cell zones of lymphoid tissue; down-regulation of phagocytosis, endocytosis and macropinocytosis; and the up-regulation of HLA class I and class II molecules, costimulatory molecules (CD80, CD86), and other DC-specific markers such as CD83 and DC-LAMP.

However, it has become clear that different types of mature DCs exist which have different functional capacities with respect to the T-cell responses they are capable of inducing. For example, depending on the timing and type of maturation stimulus, mature monocyte-derived DCs are able to drive T_H1 or T_H2 differentiation of naive T-cells [13, 14]. More strikingly, both mature plasmacytoid [15] and monocyte-derived [16] DCs have been reported to induce antigen-specific regulatory T-cell (Treg) function in CD8^{+} T cells, leading to the loss of cytotoxic activity in antigen-specific cells. Antigen-specific suppression of CTL function was also observed in two healthy subjects in vivo after subcutaneous injection of immature peptide-loaded DCs [17]. With respect to cancer immunotherapy, these results imply that incomplete, or "incorrect" DC maturation could have consequences for the clinical response. Since the goal of this type of therapy is to augment the number of tumour-specific CTL, DC preparations that are incapable of promoting the differentiation of naïve CD8^{+} T cells into effector CTL are not likely to constitute an effective therapy. Furthermore, the administration of DCs with tolerogenic functions could result in the reinforcement of tumour tolerance and exacerbation of disease. These theoretical considerations have recently been validated experimentally in a mouse model of tumour vaccination in which fully mature DCs were more effective than immature or partially mature DCs [18].

Does DC maturation state correlate with clinical outcome?

Culture conditions used to generate DCs and the reported clinical results of all stage I/II trials published to date are summarised in Table 1. Most clinical trials have used monocyte-derived DCs cultured in GM-CSF and IL-4, principally because of the relative ease with which large numbers of DCs can be generated by this technique. However, a notable exception is Engleman and Levy's group, which has used DCs derived from circulating immature DCs to treat B-cell lymphoma patients [19], and more recently, patients with advanced solid tumours [20]. The DCs that were reinfused into patients had a mature surface phenotype, but it is unclear whether they were derived from myeloid or plasmacytoid precursors, or a mixture of both. Two other clinical trials utilized DCs derived from a source other than circulating monocytes. Mackensen et al. [21] and Banchereau et al. [12] used peptide-loaded CD34^{+} stem cell-derived DCs to treat melanoma patients. In both cases the DC preparation administered to patients was not a homogeneous population, containing a variable proportion of CD1a^{+} DCs resembling Langerhans cells. Among published studies using monocyte-derived DCs, seven groups have treated metastatic melanoma patients [22, 23, 24, 25, 26, 27, 28, 29], one group has concentrated on prostate cancer [30], and four other groups have applied this therapy to metastatic malignancies expressing CEA [31], gastrointestinal tumours [32], paediatric solid tumours [33], and nasopharyngeal

Table 1 Clinical trials of DC-based antitumour immunotherapy. Clinical responses have been graded as reported by the authors of each study. However, patients who received at least one DC injection, but withdrew from trials because of progressive disease have been designated as treatment failures (i.e. PD) in all cases

Reference	Malignancy	Objective tumour response (%) CR/PR/SD/PD	Months of follow-up[a]	DC type	Injection route	Maturation stimulus
Timmerman [19]	B-cell lymphoma	16/30 (53%) [7/1/8/14][b]	12–57	$CD11c^-$ plasmacytoid and/or $CD11c^+$ circulating DC	i.v.	Spontaneous during culture
Fong [20]	Colorectal, lung	5/12 (42%) 2/1/2/7	4–10	$CD11c^-$ plasmacytoid and/or $CD11c^+$ circulating DC	i.v.	Spontaneous during culture
Murphy [30]	Prostate	8/33 (24%) 2/6/25[c]		Monocyte derived	i.v.	None
Morse [31]	Adult metastatic malignancies	2/19 (10.5%) 0/1/1/17	1	Monocyte derived	i.v.	None
Geiger [33]	Pediatric solid tumours	6/15 (40%) 0/1/5/9	1	Monocyte derived	i.d.	None
Sadanaga [32]	Gastrointestinal carcinomas	3/12 (25%)[c] 0/3/0/9	1	Monocyte derived	i.v.	None
Lin [34]	Nasopharygeal carcinoma	2/16 (12.5%) 0/2/0/14	12	Monocyte derived	Lymph node	TNF-α 50 ng/ml
Nestlé [22]	Melanoma	6/16 (38%) 2/4/0/10	3–15	Monocyte derived	Lymph node	None
Thurner [23]	Melanoma	6/13 (46%) 0/6/0/7	1	Monocyte derived	s.c./i.d. plus i.v. boost	MCM[d] plus TNF-α 10 ng/ml in some patients
Schuler-Thurner [41]	Melanoma	1/12 (8%) 0/0/1/11	1	Monocyte derived	s.c. plus i.v. boost	MCM[d]
Schuler-Thurner [24]	Melanoma	9/24 (38%) 1/0/8/15	1	Monocyte derived	s.c.	TNF-α 10 ng/ml, IL1-β 10 ng/ml, IL-6 100 U/ml, PGE2 1 μg/ml
Panelli [25]	Melanoma	1/7 (14%) 0/1/0/6	1	Monocyte derived	i.v.	None
Lau [27]	Melanoma	5/16 (31%) 1/2/2/11	10–22	Monocyte derived	i.v.	None
Chang [28]	Melanoma	4/17 (24%)[e] 0/1/3/13	1	Monocyte derived	i.d.	None
Jonuleit [26]	Melanoma	5/11 (45%) 0/3/2/6	2–15	Monocyte derived	Lymph node	TNF-α 10 ng/ml[f], IL1-β 10 ng/ml, IL-6 100 U/ml, PGE2 1 μg/ml
Smithers [29]	Melanoma	4/22 (18%) 1/2/1/18	2–40	Monocyte derived	i.d.	None
Mackensen [21]	Melanoma	8/14 (57%) 1/1/6/6	3–19	$CD34^+$ derived	i.v.	TNF-α 20 ng/ml
Banchereau [12]	Melanoma	10/17 (59%) 3/3/4/7	1	$CD34^+$ derived	s.c.	TNF-α 10 ng/ml

[a] Studies with clinical outcome graded at less than 1 month after the treatment protocol are listed as 1-month follow-up

[b] Grouped results from 10 patients with advanced disease and 25 patients in first remission after chemotherapy. Five of the latter patients had no evidence of disease at study entry, and therefore response to DC therapy could not be evaluated. Clinical response to DC therapy listed here excludes the effects of booster vaccination with recombinant idiotype protein

[c] Higher response rate if reduction in serum markers used as measure of tumour regression

[d] Monocyte-conditioned medium

[e] Three other patients treated in this trial suffered from colorectal cancer (2 individuals) and neuroblastoma (1 individual)

[f] Patients were administered both immature DCs and mature DCs loaded with different tumour antigen peptides. Immature and mature DCs were injected into separate lymph nodes

carcinoma [34]. One group has also used DC-based immunotherapy to treat renal cell carcinoma [35]. However, due to the concerns over the conduct of this trial [36, 37], we have not taken the results of this study into account.

In general a minority of patients treated in each study showed some degree of clinical response (median 38%, range 8–59%), although in some studies more than 50% of treated patients had stable disease or some degree of tumour regression. Overall, 20 complete tumour regressions (6.5%) have been reported, and nine published trials have reported at least one complete regression. The duration of the reported responses is difficult to judge, as most studies published to date only have short follow-up times (Table 1). The clinical response to DC therapy is therefore highly variable, both between studies and within individual studies.

What factors influence this variability? A formal meta-analysis cannot be performed on the entirety of these clinical reports, since they concern different malignancies which may have inherently different response rates to DC-based immunotherapy. However, more than 100 metastatic melanoma patients have been treated with DCs in 10 stage I/II clinical trials, and since most of these publications listed relevant data for individual patients, we were able to pool data from the different trials. The comparison of studies performed by different groups should be approached with caution, and a particular problem in this case is that clinical responses were graded at different times in the different trials and may not therefore be strictly comparable. Nevertheless, patients with progressive disease (PD) were rapidly identified in all of the studies, so in an attempt to minimize bias due to differences in follow-up times, clinical outcome was divided into only two categories: PD and any other outcome (including complete response *CR,* partial response *PR,* or stable disease *SD,* as defined by authors of the different studies). In addition, transient responses in studies with longer follow-up times were scored as positive responses. Furthermore, the pooled data were analysed on an intention to treat basis. That is, patients who received at least one DC injection but who withdrew from trials because of progressive disease were included, and scored as PD.

Using this approach, no significant relationship was found between favourable clinical response, designated as any outcome other than progressive disease, and patient sex, or chemotherapy pretreatment. From a molecular point of view, this is somewhat unexpected, as resistance of melanoma cell lines to anticancer agents has been related to the acquisition of resistance to apoptosis [38, 39, 40]. Theoretically, apoptosis resistance could also protect tumour cells from being killed by CTL, and therefore one might expect patients with chemoresistant tumours to respond less well to DC-based immunotherapy. However, this potential problem does not appear to have influenced short-term clinical responses to DC therapy for melanoma, which suggests that patients with chemoresistant disease may still benefit from DC therapy. Patient age was weakly correlated with response to treatment, patients older than 59 years being more likely to experience a favourable clinical response ($p<0.05$). The physiological explanation of this correlation is not clear, but one may speculate that older patients enrolled in DC therapy trials may have had less aggressive tumours than younger patients, and hence were less likely to suffer progressive disease over the short follow-up times involved in most studies.

With respect to the conditions under which DCs were generated and administered, there was no significant effect of DC dose or route of injection (Table 2) on the response to DC treatment. These two variables were not strictly independent, however, as trials administering DCs intravenously used higher DC doses (median 3×10^7 DCs per dose compared with 3×10^6 DCs per dose in trials using other injection routes). It is therefore possible that intravenous administration of DCs was relatively inefficient, but was compensated by the higher cell numbers used.

Different protocols for in vitro DC maturation have been used in antimelanoma therapies (Table 1.), however, most of these protocols involved the addition of TNF-α, either alone, or in combination with other cytokines. Protocols for the generation of DCs were therefore divided into two groups: those that involved maturation by a cytokine cocktail including TNF-α, and those that did not. Strikingly, the addition of TNF-α for the maturation of DCs before reinjection into patients was strongly correlated with favourable outcome of treatment ($p<0.001$, Table 2), as was the use of DCs derived from $CD34^+$ precursors ($p<0.01$, Table 2). These two associations are strongly confounded, however, as both studies using $CD34^+$ stem cell–derived DCs involved culture of DCs in the presence of TNF-α. Comparing $CD34^+$ stem cell–derived DCs (13 PD, 18 other outcome) to monocyte-derived DCs matured in the presence of TNF-α (22 PD, 18 other outcome), no significant difference was found ($p=0.394$, χ^2 test), suggesting that both types of DC preparation were clinically effective, and that DC lineage has not had a major impact on clinical outcome in melanoma trials to date.

Another indication of the effect of DC maturation state comes from two studies in which patients were treated with DCs matured in vitro either by monocyte-conditioned medium (MCM) alone or by MCM plus TNF-α [23, 41]. Since these two trials were conducted by the same group, inclusion and evaluation criteria were identical, as was the tumour antigen targeted by the immunotherapy. Protocols for the generation and administration of DCs were also identical, except for the DC maturation conditions. Although the authors did not comment on this difference in DC preparation, clinical responses were significantly better in those patients who received DCs matured in the presence of MCM plus TNF-α (4 PR, 1 PD) compared with those who received DCs matured in MCM alone (2 PR, 1 SD, 17 PD, $p=0{,}012$ by Fisher's exact test).

Table 2 Correlation of clinical response with patient characteristics and type of DC therapy in published melanoma trials. Data were collated from 10 published type I/II clinical trials of DC therapy for metastatic melanoma [12, 21, 22, 23, 24, 25, 26, 27, 28, 29, 41]. Individual data on patient sex, age and pretreatment were not given in references [21] and [25]. In addition, age and sex were not given for two patients treated in reference [23] and for six patients in reference [28], and individual data on patient pretreatment was not given in [29]

Variable	Clinical Response				
	PD	Other outcome (CR,PR,SD)	All outcomes	χ^2 value	p value
Sex					
F	31	24			
M	43	25		0.347	0.556
Both sexes			123		
Age					
> 59	22	25			
< 60	52	24		4.794	0.028
All ages			123		
Chemotherapy					
+	26	20			
−	32	25		0.000	1.000
Total			103		
DC dose					
$> 10^7$	37	30			
$< 10^7$	65	29		2.695	0.100
Total			161		
DC injection route					
i.v.	23	14			
Other	85	45		0.028	0.868
Total			167		
DC origin					
$CD34^+$	13	18			
Monocyte	95	41		7.433	0.007
Total			167		
DC maturation					
TNF-α	33	36			
Other	75	23		13.373	< 0.001
Total			167		

In an elegant study from a different group, the immunological efficacy of immature DCs and DCs matured in a cytokine cocktail consisting of IL-1β, IL6, TNF-α and PGE2 was directly compared [26]. Immature and mature DCs were pulsed with different tumour peptides and injected into different lymph nodes. The $CD8^+$ T-cell responses to the different peptides were then monitored after 6 and 9 weeks of therapy. Although tetramer staining showed that both immature and mature DCs induced expansion of peptide-specific $CD8^+$ T cells, mature DCs were clearly superior in their ability to induce functional CTL, as measured by antigen-specific IFN-γ ELISPOT, and target cell lysis in chromium release assays.

Hence results both from single centres that used different protocols for the generation of DCs, and from several different centres using varied techniques for DC generation, indicate that DCs matured in the presence of TNF-α were more effective in melanoma therapy than those that were not. Maturation state is therefore an important factor that influences the clinical response to DC-based immunotherapy.

What is the optimal DC maturation stimulus for anticancer therapy?

Data from clinical trials published to date imply that TNF-α should be included in protocols for the maturation of DCs for use in cancer immunotherapy. However, they do not tell us whether TNF-α should be used alone, or in combination with other maturation agents, and if so, the nature of the optimal maturation cocktail. TNF-α is one of a variety of molecules that induce DC maturation, and different maturation stimuli result in DCs with different expression profiles [42] and different functional properties [13]. In particular, TNF-α has been reported to induce a partial DC maturation, with more complete maturation only being achieved by stronger stimuli, such as LPS [43]. Hence, it is possible that stronger maturation stimuli could generate DCs with enhanced antitumour efficacy.

This raises the question of how to measure DC maturation, and how to correlate DC phenotype with in vivo antitumour efficacy. The problem is well illustrated by the results of the clinical trials reported by Thurner et al. and Schuler-Thurner et al. [23, 41]. The DCs administered in these studies were matured either by MCM alone or by MCM plus TNF-α. DCs generated by both maturation stimuli had a mature $CD86^{Hi}$HLA-$DR^{Hi}CD83^+$ phenotype, but as noted above, there were strong indications that those that had been matured in the presence of TNF-α had greater therapeutic efficacy. The markers currently used to assess DC maturation are therefore not sufficient to predict which DCs will be functionally superior in anticancer therapies.

In addition, it is not clear to what extent in vitro tests of DC function are valid as a guide for the generation of DCs for clinical use. For example, most recent clinical trials of DC therapy for melanoma have utilised DCs

matured in a cocktail of IL-1β, IL6, TNF-α and PGE2 [24, 26, 44]. This cytokine cocktail was was chosen as the optimal DC maturation stimulus because in vitro experiments had shown that DCs matured with this combination of cytokines expressed higher levels of HLA-DR, CD86 and CD83, and induced greater allogeneic T-cell proliferation than DCs matured by either TNF-α alone or a combination of TNF-α, IL-1β and IL-6 [45]. In addition, two recent reports have shown that PGE2 is essential to activate DC chemotaxis towards SLC/CCL21 and ELC/CCL19 [46, 47], which are critical chemokines for the migration of DCs into the T-cell regions of secondary lymphoid organs. The use of PGE2 in cancer therapies might therefore be expected to increase the migration and effective dose of DCs. On the other hand, we and others have shown that PGE2 inhibits the secretion of IL-12 by DCs [48, 49], and is therefore likely to decrease the efficiency of T_H1 priming in vivo. Hence it is possible to construct arguments both for and against the inclusion of PGE2 in DC-based anticancer therapies on the basis of in vitro results, but extremely difficult to predict whether the presence of PGE2 during DC maturation will increase or decrease the efficacy of antitumour therapy in vivo.

Another potential problem for the preparation of DCs for antitumour therapy is related to the phenomenon of DC exhaustion. Strong DC maturation stimuli, such as LPS, can induce high but transient levels of expression of IL-12 p70 [14]. However, after this burst of cytokine production, these mature DCs are refractory to further stimulation, and are incapable of producing IL-12 p70 during antigen presentation to T cells. This implies that "over maturaton" of DCs may not be optimal for the induction of T_H1 and CTL antitumour responses in vivo. Recent work from our group has shown that transient exposure of DCs to maturation stimuli results in the production of mature DCs that remain sensitive to signals produced by T cells [50]. In particular, these DCs retained the capacity to produce IL-12 p70 after activation with CD40L and IFN-γ. The molecular basis of this important functional difference between transiently and continually matured DCs is currently not known, and indeed these two types of mature DCs had an identical surface phenotype and showed similar levels of short-term IL-12 production.

How then can we define the optimal DCs for cancer therapy? Firstly, it is clear that there is a need to define markers of DC maturation that accurately reflect the functional capacities of these cells. Currently, only one molecule, IL-12 p70, is strongly associated with immunostimulatory T_H1 inducing DC function, and only three molecules, indoleamine 2,3-deoxygenase [51, 52], ICOS-L [53], and 87-h1 [54] have been correlated with tolerogenic DC function. If more such markers were found, it would be possible to select an optimal DC maturation protocol on a more rational basis, by maximizing the T_H1-inducing and minimizing the tolerogenic potential of DCs for clinical use. Recently, microarray experiments have shown that the expression of hundreds of genes is modulated during DC maturation [42]. In our laboratory we are currently aiming to combine the results of microarray experiments with functional studies to define genes whose expression correlates with particular aspects of DC function, in order to find new phenotypic markers of DC function which could be used to optimize DC maturation conditions.

Secondly, given the difficulty in predicting in vivo DC function on the basis of results obtained in vitro, it will be essential to define in vivo immunological markers for the response to DC therapy which correlate with favourable clinical outcome in patients, then test DCs matured under different conditions for their capacities to induce these immunological responses in healthy volunteers. The first clinical trials of DC-based tumour therapy used antigen-specific delayed-type hypersensitivity (DTH) as a measure of the immunological response to the DC treatment. This has the advantage of being convenient and simple to perform in a clinical setting, however, it gives little quantitative information, and the effector function of the T cells that infiltrate the injection site is not tested directly. More recently, T-cell responses to DC therapy have been monitored by MHC-tetramer staining to quantify the number of $CD8^+$ T cells specific for a given peptide epitope, and by ELISPOT to determine the functional response of antigen-specific cells. In this regard it is interesting to note that in two recent reports, the clinical response to DC therapy was correlated with the extent of the $CD8^+$ T-cell response. Banchereau et al. established a correlation between clinical outcome and an "immune score" based on the number of IFN-γ–secreting peptide-specific cells measured by ELISPOT [12], whereas Fong et al. correlated clinical responses to the percentage of tetramer$^+$ $CD8^+$ T cells induced after DC vaccination [20]. In particular, all of the five patients in whom $>1\%$ of circulating $CD8^+$ T cells stained positive for tetramers after therapy had a positive clinical response (CR, MR or SD] and vice versa. This level of T-cell response could therefore represent a target for tests of different DC preparations in healthy volunteers.

Finally, DC maturation protocols that appear to give stronger immunological responses in vitro and in vivo should be integrated into clinical trials of cancer patients. Ideally, patients enrolled should be randomized into two treatment arms, one receiving DCs matured by protocols including TNF-α (possibly the IL-1β, IL6, TNF-α and PGE2 cytokine cocktail, which is currently developing into a standard protocol used by several different groups), the other receiving DCs matured by the test protocol. Since treatment response rates are currently low, in particular when considering partial or complete tumour regressions, it should be possible to identify significantly more effective treatment regimes without enrolling large numbers of patients.

Conclusions

Tumour immunotherapy using DCs is a promising therapeutic approach, which has resulted in a significant number of tumour regressions in late-stage patients who had failed to respond to surgery and/or chemotherapy. Analysis of data pooled from published melanoma trials shows that response to DC therapy was not affected by prior chemotherapy. This type of immunotherapy can therefore be proposed to patients with chemoresistant disease. Furthermore, the maturation state of the DCs reinjected into patients was related to the clinical response to treatment. DC maturation state is therefore an important parameter for the clinical effectiveness of this type of therapy, which implies that DC maturation must be optimized in future trials in order to ameliorate clinical responses to DC-based immunotherapy.

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