Introduction

The dendritic cell (DC), an uncommon type of bone marrow-derived leukocyte, is widely acknowledged as the most effective antigen presenting cell (APC) and has the unique capacity to initiate and control immune responses against naïve and tumour antigens. These cells have generated intense interest in the scientific community because of their potential use as “autologous adjuvants” in cancer vaccination.

DC biology

DC differentiate from early myeloid and lymphoid progenitors. In man at least two blood subsets are described – the CD11c+/CD123+ “myeloid” DC and the CD11c+/CD123- “lymphoid” DC. They have different properties. Current suggestions are that the CD11c+/CD123+ DC provide epithelial and non-epithelial surveillance and migrate to regional lymph nodes through the afferent lymph. The CD11c-/CD123+ DC migrate directly to the lymph node from the blood stream via high endothelial venules. Monocytes may contribute directly to both populations.

DC integrate signals from the environment to link the innate and cognate (T cell and antibody) immune responses. In their role of immunosurveillance, CD11c+ DC encounter and take up antigen from the tissues. Exogenous and endogenous protein antigen (the handling of the latter is better characterised than other forms of antigen) is processed by the proteasome into short peptide sequences. Peptide epitopes are then complexed to MHC class I and II molecules and transported to the cell surface. Peptide epitope antigen (the sequence of which is specific for a particular MHC allele) can only be recognised by corresponding CD4 or CD8 T cell receptors when it is presented in conjunction with the respective MHC class II or I molecule. Danger signals upregulate DC antigen uptake and processing, migration (controlled by chemokines), costimulatory membrane molecule receptors and cytokine release. Their complex control of gene products and functions allows for great DC diversity, specialisation and their fine control of immune responses, including self-tolerance.

DC in cancer

Abnormalities in cancer

An effective immunologic response to tumour is reliant on a coordinated immune response, a major component of which is the tumouricidal role played by CD8+ cytotoxic T lymphocytes (CTL). In a normal individual, tumour antigen from transformed cells is taken up by DC, processed, and presented to T and B lymphocytes in secondary lymphoid tissues. The ensuing interaction between DC and an antigen-specific naïve CD8+ T cell results in clonal proliferation and expansion of CD8+ effector and memory T cells. Effector CTL are then able to recognise and kill antigen-bearing tumour cells in the periphery, preventing the establishment of the tumour. Once a malignancy becomes established, it is clear that DC, along with the immune system, have failed in their role of immunosurveillance. There is a multitude of reasons for this to have occurred. Abnormal DC numbers have been described in some cancer patients and abnormal DC function has been noted in breast adenocarcinoma, renal cell carcinoma, prostatic adenocarcinoma, colonic adenocarcinoma, basal cell carcinoma, multiple myeloma, melanoma, and transitional cell carcinoma of the kidney and bladder.

DC function can be suppressed by the release of tumour-derived inhibitory factors. Interleukin (IL)-10 release from melanoma, multiple myeloma and bronchogenic carcinoma may impair DC function. IL-6 and macrophage colony stimulating factor (M-CSF) released by renal cell carcinoma inhibited the differentiation of CD34-derived DC from CD34+ progenitors. Vascular endothelial growth factor (VEGF) release from human tumours can alter dendritic cell maturation, and high serum levels of VEGF have also been associated with the presence of immature myeloid cells in the blood, which in turn were closely correlated with the stage and duration of clinical neoplastic disease.

In multiple myeloma, DC numbers are relatively normal but CD80 induction is reduced. In colorectal carcinoma, there was low or absent expression of the costimulatory molecules CD80 and CD86 on DC obtained from the tumour site. In addition, data from analyses of breast carcinoma, renal cell carcinoma, transitional cell carcinoma and prostatic adenocarcinoma have revealed that DC are not effectively recruited and activated in tumour tissue. In melanoma, DC appear to be able to present tumour antigen to T cells, however they are not able to induce immunity, as evidenced by the presence of anergic melanoma-specific T cells. The notion that DC abnormalities in cancer may have clinical consequences is supported by the fact that low DC numbers in tumour biopsy specimens were associated with a poor prognosis in bowel adenocarcinoma, cutaneous T cell lymphoma and prostatic adenocarcinoma.

In addition to immune evasion, as a result of abnormalities in DC priming of CTL, impaired immune responses to tumours may occur due to intrinsic defects in neoplastic cells themselves. Down-regulation of MHC molecules and antigen processing into tumour cell surface complexes prevents effective recognition by effector CTL, and has been described in many tumours. Abnormal tumour antigen expression can allow “immune escape” in an analogous fashion to the acquisition of metabolic alterations by malignant cells, which confers chemotherapy resistance.

DC in immunotherapy

Rationale

The use of DC in cancer immunotherapy is based on two main principles. The first is that cancers express either unique tumour-specific antigens or self-antigens that are abnormal in quantity or quality. These antigens are processed by the tumour cell and expressed in conjunction with self-MHC
molecules, providing the immune system with a recognisable therapeutic target. The second premise is that DC function is abnormal in vivo and can be normalised in an ex vivo setting, free from the negative influences of the tumour. The appeal of laboratory-based preparation of DC vaccines is that, in contrast to direct in vivo peptide vaccination, the process can be performed in a defined manner with specific control over such factors as DC activation and antigen loading.

**DC preparations**

There are three main preparations currently in use for DC immunotherapy programs.

The most commonly used preparation is the monocyte-derived DC (MoDC)\(^3\). Large numbers of these DC-like cells can be generated from peripheral blood samples or apheresis products and for this reason, they have become the most common form of DC to be used in laboratory and clinical research. After approximately five days of culture with GM-CSF and IL-4, monocytes differentiate into a cell with an immature DC-like phenotype, with loss of CD14, low CD40, CD80 and CD86 expression but no CD83. MoDC can be activated to a mature phenotype with various cytokine cocktails\(^3\) prior to antigen loading and vaccination.

CD34 cells can be differentiated into DC\(^5\)-\(^8\) and have been used in vaccination studies\(^9\), but are extremely cumbersome to produce and are seen as a less popular alternative.

Blood DC precursors (BDC), obtained by immunomagnetic selection from leucapheresis products, are the focus of increasing attention\(^9\). Production does not require a long period of culture or exogenous cytokines and can be undertaken following good manufacturing practice (GMP) guidelines\(^10\)-\(^13\). It is also tempting to speculate that BDC can be harvested at an appropriate stage of differentiation for antigen loading and vaccination.

**Tumour antigens**

In addition to the choice of DC preparation for vaccination, the selection of tumour antigen for vaccination is of great importance. The ideal tumour antigen for vaccination is one that is highly specific for tumour tissue and not expressed on normal tissues. It should be processed by the tumour cell and contain immunogenic epitopes that are presented in conjunction with MHC molecules. It should be stable and not down-regulated with disease progression. Although not entirely necessary, targeting a tumour antigen that is of vital functional importance to the tumour would be a considerable advantage. Unfortunately, at our current level of knowledge, we are only able to identify tumour antigens that fulfil some of these ideals.

The form of antigen preparation is also of vital importance. There is a spectrum of options from peptide antigen encoding a single MHC restricted epitope to various forms of whole tumour antigen, which encompass a range of epitopes specific for multiple different MHC alleles. As the range of antigen and epitope coverage increases, so too does the likelihood of autoimmune injury after vaccination. Despite the theoretical risks, there is relatively little evidence to suggest that autoimmunity will pose a major impediment to anti-tumour vaccination\(^14\).

**Clinical trials**

Since the first clinical trial of DC immunotherapy\(^14\) was published in 1996, a large number of trials in many different diseases has been undertaken. Virtually all of these trials have been phase II trials and only recently have phase III studies commenced (for a review of current clinical trials see reference 3). Perhaps the most common malignancy to be examined thus far is melanoma. Although comparatively few of these studies have been formally published, trials using MoDC\(^15\)-\(^17\) and CD34DC\(^18\) have generated encouraging results with objective clinical responses in 20-30% of patients with late stage disease. Some of these responses have been complete and long-lasting. Associations have been noted between the clinical response (including cutaneous vitiligo) and immunologic response. In one trial\(^17\), in which subjects were vaccinated with CD34DC pulsed with four melanoma-derived peptides, clinical responses (albeit at an early stage of follow-up) were correlated with the number of peptide-specific immunologic responses. The clinical response rate of DC trials in melanoma certainly seems encouraging and warrants further investigation by randomised controlled trial, particularly in early stage disease.

Another tumour in which promising results has been seen is renal cell carcinoma. This tumour has been historically associated with responses to non-specific immunologic therapies such as IL-2\(^2\) and lymphokine activated killer cells\(^19\) (LAK). Excellent results were achieved (4/17 attained complete remission) in renal cell carcinoma patients through vaccination with allogeneic DC:tumour hybrids\(^20\). Characterisation of the cell preparation used in this trial is under further study.

Multiple myeloma\(^21\) and non-Hodgkin’s lymphoma\(^22\) are promising candidates for immunotherapy, particularly in the setting of minimal residual disease post-autologous transplant. A recently reported study of follicular non-Hodgkin’s lymphoma patients showed impressive results after vaccination with idiotypic protein pulsed DC\(^23\). Even more impressive than the 20% complete response rate after one dose was that patients with disease progression after the first vaccination subsequently responded to further treatments. The identification of more broadly applicable tumour antigens than immunoglobulin idiotype will provide increased impetus to study DC vaccination in these diseases.

Prostate carcinoma has been evaluated in a number of trials but clinical responses are inconsistent\(^16\)-\(^17\), mainly being restricted to stabilisation of tumour marker levels. CTL responses after vaccination with MoDC have been noted in vivo in breast, ovarian\(^17\) and colonic adenocarcinoma\(^24\) amongst others, demonstrating the feasibility of this approach.

**Vaccination protocols**

One of the problems with assessment of the accrued data is that all the reported trials have studied small numbers of patients treated with multiple different vaccination protocols. As a consequence, a consensus on the optimal choice of DC preparation, state of activation, dose, route and frequency of vaccination and timing of antigen loading has not been reached. One of the reasons for this is that direct correlations between clinical responses and peripheral blood immunological responses have not been forthcoming, although the vast majority of clinical responses occur in patients who have an immunological response\(^1\). This may be due to the fact that the immune response in the peripheral blood does not always reflect that seen in the local tumour environment.

Some tentative conclusions regarding DC preparation can be made. It appears that BDC, MoDC and CD34DC are all feasible alternatives for an immunotherapy program. No head-to-head comparisons have been made in an in vivo setting, and there is only limited in vitro data\(^25\) to support one preparation over another. Available evidence suggests that immature MoDC may lead to antigen-specific tolerance\(^26\), clearly an undesirable outcome for tumour immunotherapy. Certainly, accumulating data suggest they may be less efficacious.
Data on DC dose has not been established, however some information regarding route of administration is coming to light. In one study it appeared that intradermal (id) or intralymphatic (il) administration was preferred over intravenous (iv), as the id and il routes produced interferon-γ (IFN-γ) responses while iv vaccination was only able to elicit an antibody response\(^\text{31}\). Trials examining direct intranal injection of DC under ultrasound guidance have also shown clinical and immunological responses\(^\text{31}\).

Vaccination frequency is another contentious issue. Most current schedules involve weekly vaccination, however this has been based largely on data obtained from experience with infectious disease. Whether this data will translate to the field of tumour immunotherapy remains to be seen. In tumour immunotherapy, particularly in phase I/II trials, the subject is vaccinated in a state in which there is a large antigenic load of tumour, in contrast to preventive vaccinations in infectious disease. It is suggested that tumour immunotherapy will be more effective if administered at a stage of minimal residual disease. Vaccination on a high frequency schedule may be detrimental\(^\text{31}\).

**Complications**

To date, relatively few complications have been seen with DC immunotherapy. By far the most common events are low-grade fever or the occurrence of a local injection site reaction. Biopsy-proven delayed type hypersensitivity is usually regarded as a good sign and indicates an immunologic response. There is one report of an allergic reaction to bovine serum albumin in a DC vaccine\(^\text{31}\). As most tumour antigens are self-antigens, there is always the potential for devastating autoimmune consequences after vaccination. This appears to be less commonly seen than intuitively expected (reviewed in reference 41). To date, the only commonly seen autoimmune condition after DC vaccination is vitiligo – the expected outcome. This has been noted in up to 43% of vaccinated patients with metastatic melanoma (F O Nestle – unpublished observation). Despite the safety data noted in reported trials thus far, it would be prudent at this early stage to avoid vaccination of patients with a tendency to autoimmune disease.

**Monitoring**

Monitoring the response of a DC vaccination subject should involve two aspects. Obviously, clinical parameters are of prime importance and the success of DC immunotherapy will ultimately be judged in this regard in large phase III trials. Efforts are also underway to define immunologic “surrogate” parameters that may be useful in predicting clinical responses. Immune responses in the peripheral blood, at the vaccination site, DLN and within the tumour tissue are being assessed. The most accessible tissue for monitoring is obviously the peripheral blood, however immunohistochemical and histologic analysis is also being used to define the nature of a response at the vaccination site, DLN and within tumour tissue. Techniques such as the “chromium release cytotoxicity assay using CTL isolated from peripheral blood are specific indicators of tumour killing but are very insensitive as the in vivo frequency of tumour-specific CTL is usually very low (<1% PBMC). Fluorochrome-conjugated MHC:peptide tetramers are highly sensitive and bind TCR-specific for a given peptide antigen. Unfortunately, not all tetramer-positive CTL have the capacity to produce IFN-γ and induce cell lysis. IFN-γ ELISPOT, intracellular staining for IFN-γ and IFN-γ secretion assays are also very sensitive markers. The latter assay has the advantage that it can be used alone or in combination with tetramer analysis and fluorescence-activated cell sorting to produce populations of antigen specific CTL for use in cytotoxicity assays. Expansion of IFN-γ secreting CTL populations is being explored for adoptive immunotherapy.

Other cytokine staining techniques can elucidate the nature of a Th1 or Th2 immunologic response. With all of these methods, considerable expertise is needed for accurate detection of low frequency tumour-specific CTL. Now that the technology and tumour peptides are more refined, serious analysis of CTL correlations can begin. These staining techniques are also applicable to tumour fine needle biopsies.

**Future directions**

Paradoxically, the future of DC immunotherapy requires some backtracking in the laboratory. A plethora of clinical trials have been completed with great initial enthusiasm, however it is now becoming apparent that use of inappropriate DC preparations may be quite detrimental. Clearer characterisation of the in vitro conditions required to break tolerance to self-antigen is critical. This is obviously not a minor undertaking. Future directions include targeting antigen to various cellular compartments, including helper epitopes or constructs, improving activation markers and adding migratory signals to improve in vivo induction of immune responses. The promising beginning indicates that the effort expended in optimising the many aspects involved may yield great dividends.

**References**
