Review Article

Dendritic cells: Immunological sentinels with a central role in health and disease

LUKE H STOCKWIN,1 DENNIS McGONAGLE,2 IAIN G MARTIN3 and G ERIC BLAIR1

1School of Biochemistry and Molecular Biology, 2Department of Rheumatology, University of Leeds and 3Department of Surgery, Leeds General Infirmary, Leeds, United Kingdom

Summary Immunological effector cells must be sensitive to the antigens or environmental signals that indicate that a pathogen is present. To this end, a group of cells known as the professional antigen-presenting cells have the ability to educate T, B and NK cells as to the fingerprints of specific infections. The most adept of these cells are a closely related family termed dendritic cells (DC). A subset of these act as peripheral sentinels, specializing in the uptake, processing and presentation of antigenic material combined with an ability to detect a wide variety of ‘danger’ signals. These ‘danger’ or activation signals induce profound changes in dendritic cell physiology, facilitating the efficient stimulation of both adaptive and innate immunity. In the present review, a number of recent advances in the understanding of DC biology are discussed. These advances offer insights into the pathogenesis of a wide variety of diseases and point towards future strategies for immunotherapy.

Key words: autoimmunity, cancer, dendritic cell, immunotherapy, persistent viral infection, transplantation.

Introduction

Dendritic cells (DC) are now recognized as essential regulators of both innate and acquired arms of the immune system.1 Dendritic cells, in addition to their distinctive morphology, have a number of phenotypic and functional characteristics that make them formidable APC (Table 1). Dendritic cells bear sole responsibility for the stimulation of virgin T lymphocytes, a property that distinguishes them from all other APC (e.g. B cells).2,3 The DC are also essential accessory cells in the generation of primary antibody responses and are powerful enhancers of NK cell cytotoxicity.4 Conversely, DC are also involved in the maintenance of tolerance to antigens, with DC in the thymus contributing towards shaping of the T cell repertoire by deleting autoreactive lymphocytes.5 As a consequence of this heterogeneity in vivo, it is now acknowledged that DC are a family of cells, with each subset exerting control over a different area of immunity. In terms of origin, DC are bone marrow derived cells originating from both myeloid and lymphoid precursors.4,9 Examples of DC in vivo include the epidermal Langerhans cell, the interstitial DC (found in the heart, lungs, liver and other organs), the veiled DC found in afferent lymph, interdigitating DC (found in T cell-rich areas of lymphoid tissue) and thymic DC. Follicular DC (FDC) are an exception to this group because they are believed to be involved in the long-term maintenance of B cell memory by retaining immune complexes; these cells differ markedly from the aforementioned group and will not be discussed here (reviewed by Tew et al.10).

The main function of Langerhans and interstitial type DC is as immunological sentinels, being strategically placed to detect invading microbes. Sentinel DC remain dormant until signals in the extracellular milieu (derived either from microbes or distressed bystander cells) induce a rapid change in function, also known as ‘activation’. Activation induces a number of important changes in DC (Fig. 1), not least of which is migration into local lymphoid tissue where they communicate antigenic information to lymphocytes. Recent clarification of the biochemistry of activation signals and the effects of DC activation have prompted research into the part played by activated DC in the pathogenesis of disease and whether DC-modulating immunotherapies can be used in the treatment of a wide range of diseases, including autoimmunity, allergy, immunodeficiency, transplant rejection, persistent viral infection and cancer.

Properties of dendritic cells

Dendritic cells were initially characterized on the basis of their distinctive morphology, with numerous cytoplasmic processes giving rise to a stellate appearance. As a consequence, the DC has a high surface area permitting intimate contact with a large number of surrounding cells. As proof of this point, in vitro DC form large spherical aggregates with lymphocytes, and experimentally, only one mature DC is required to stimulate 100–3000 T cells. Dendritic cells also possess an array of mechanisms for sampling antigen. These include macropinocytosis, where fluid from the extracellular milieu is taken up into pinocytic vesicles and antigen is concentrated by expelling excess water via channels called aquaporins.11 The DC also expresses a repertoire of receptors for efficient receptor-mediated antigen uptake. Additional receptors expressed by DC include FcγRII (CD32), FcγRI...
The C3bi complement receptors

RII and the C3 bi complement receptors

CD11a

MHC-I/II

CD40

CD80

CD86

Flt-3L (early)

SCF (early)

Gp-CSF

TGF-β (LCs)

IL-4 (in vitro)

Danger Stimuli e.g.

TNF

LPS

dsRNA

HSP

Aptoptotic bodies

Immune Complexes

CpG DNA

Figure 1  Dendritic cell life history can be subdivided into a number of phases, all with discrete cellular functionality. Transition between phases is mediated by diverse signals and is accompanied by changes in expression patterns of many surface markers and secreted factors.

Table 1  Properties of dendritic cells

1. Stellate morphology and cytoplasmic processes giving rise to the characteristic ‘conker’ appearance when immature. Maturing DC develop complex veils (in afferent lymph).
2. Rapid motility, as demonstrated by time lapse photography.
3. Efficient stimulation of autologous and allogeneic T cells (naïve and mature); DC are approximately 100-fold better at stimulating an allogeneic MLR than bulk leucocyte culture. The DC are unique in their ability to stimulate naïve/virgin T cells, unlike other APC, such as B cells, which only activate memory T cells.
4. Adept environmental antigen sampling. Fluid phase endocytosis estimated at 100 times the DC volume per hour for in vitro-generated DC. Ultrastructural analysis reveals the presence of many fluid-filled endosomes. Antigen sampling is also by receptor-mediated uptake, for example Fc receptors (FcRII/CD32, FcRI/CD64), complement receptors (C3bi/CD11b) and C-type lectin receptors (MMR, DEC-205).
5. Dendritic cells are distinct from macrophages in that they do not express CD16 or macrophage characteristic antimicrobial enzymes (e.g. lysozyme and myeloperoxidase).
6. High surface density of antigen presentation molecules, for example MHC-I and MHC-II (expression 10–100-fold greater than on other APC, e.g. B cells).
7. Mature DC have a high surface density of accessory/costimulatory molecules (CD40, ICAM-1/CD54, ICAM-3/CD50, LFA-3/CD58, B7-1/CD80 and B7-2/CD86).
8. Production of large quantities of IL-12 after treatment with activation signals, including CD40L or LPS.
9. An ability to cross prime antigen into the MHC-I presentation pathway; protein from the exogenous milieu can be presented to CD8+ T cells. This allows specific class-I mediated immunity to be generated without the DC becoming infected. Cross priming is therefore of prime importance in the generation of CTL responses against pathogens that infect non-haemopoietic cells.

DC, dendritic cell; ICAM, intercellular adhesion molecule-1; LFA, leucocyte functional antigen.

CD64, FcγRII and the C3 bi complement receptors CD11b, which increase the efficiency of immune complex endocytosis. Dendritic cells express C-type lectin receptors, including the macrophage mannose receptor and DEC-205, which bind bacterial carbohydrates. Dendritic cells also express markedly higher levels of antigen presentation molecules than any other cell (e.g. CD1a, MHC-I and MHC-II). In addition to this, DC have a high surface density of accessory/costimulatory molecules, including intercellular adhesion molecule (ICAM)-1/CD54, ICAM-3/CD50, leucocyte functional antigen (LFA)-3/CD58, B7-1/CD80 and B7–2/CD86 (Fig. 2), which facilitate both the interaction with, and stimulation of, lymphocytes. An additional noteworthy property of DC is that exogenous antigen (e.g. immune complexes) can be ‘cross primed’ into the MHC class I presentation pathway. This pathway would normally only present antigen from the endogenous compartment. The DC are thought to possess a specific mechanism that allows fluid from an endocytic vesicle to gain direct access to the cytoplasm. In essence, this allows the DC to present new peptides to CD8+ T lymphocytes without themselves being infected or damaged.
Dendritic cell activation

Throughout evolution, the presence of a pathogen has been accompanied by a series of unique markers. For example, this may be dsRNA for influenza virus or LPS in the case of gram-negative bacteria. The DC are sensitive to a wide range of these stimuli that serve not only to activate innate immunity via the release of chemokines and proinflammatory mediators, but also trigger DC migration towards local lymphoid tissue in order to generate antigen-specific (adaptive) immunity.

It is hardly surprising that intact, viable microbes, such as influenza virus or mycobacteria, can be observed to activate DC. However, the question remains as to which individual molecules mediate DC activation and via which receptors. Factors specific to bacteria, such as LPS and, more surprisingly, CpG motifs in DNA, have been shown to activate DC. Lipopolysaccharide-induced activation is mediated by CD14 acting in concert with the recently described Toll-like receptors (TLR) 2 and 4. However, the receptor that mediates DNA-induced activation still remains to be identified. The ligation of Fc receptors during the uptake of immune complexes has also been shown to induce the maturation of dendritic cells. A further interesting observation is that several DC-activating factors (including LPS, TNF-α and CD40L) are able to rescue immature DC from apoptosis.

The second group of DC-activating signals is derived from distressed or dying cells. The phenomenon of Langerhans cell migration into tissue culture media after in vitro culture of skin explants for 24 h is thought to reflect inflammatory stimuli resulting from skin damage promulgating DC migration. Cells, as a consequence of infection, will release pools of pro-activating factors, which will indirectly activate DC. The action of pro-inflammatory cytokines (TNF-α) and prostaglandins (PGE₂) on immature DC produces a maturation event characterized by an increase in the levels of membrane HLA-DR (MHC-II) and a reduction in the propensity of the cells to take up soluble antigen. In addition, DC that encounter apoptotic cells undergo maturation in vitro; this maturation involves autocrine/paracrine secretion of IL-1β and TNF-α and is thought to be mediated by αβ integrin and CD36.

One of the primary events after activation is the release of chemokines, such as RANTES, monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, and MIP-1β, RANTES, MCP-3 and fMLP and an increase in the chemotactic response towards chemokines stromal cell-derived factor (SDF)-1 (CXCR4) and monocyte-derived chemokine (MDC), CCR4. Chemokines play an important role in targeting DC to lymphoid tissue. The chemokine receptor CCR7 is gradually up-regulated after activation. The ligands for this receptor, 6Ckine and MIP-3β, are chemotaxitants for DC and are produced primarily in the T cell-rich parafollicular areas of lymphoid tissue.

For the Langerhans cell, activation is also accompanied by the loss of specific markers, such as cutaneous lymphocyte antigen (CLA) and Birbeck granules, along with altered surface expression of cell adhesion molecules that facilitate movement into the afferent lymph. For example, Langerhans cells express E-cadherin, which mediates homotypic interactions with keratinocytes. The production of this molecule is down-regulated during maturation. MHC class II molecules, stored within vesicles termed MIIC, are transported to the cell surface and there is a concomitant increase in surface half-life of both MHC class I and MHC class II molecules.

Activation-induced changes serve to change the immature DC, which are adept at antigen sampling, into DC dedicated to the presentation of antigen. Immediately after exposure to an activation signal, such as TNF-α, there is a short-term increase in antigen uptake followed by an almost complete cessation in uptake preceding migration into afferent lymphatics.
In summary, DC activation is accompanied by a number of changes, including increased expression of costimulatory and antigen presentation molecules, a decrease in the propensity to capture soluble antigen, the increased stability of MHC class I and II molecules, changes in chemokine ligand and receptor expression and translocation into lymphoid tissue.

**Dendritic cells in lymphoid tissue**

Once inside the lymphoid tissue, the DC interacts with T, B and NK cells. The unique ability of DC to attract and stimulate naïve T cells at this point is still poorly understood; however, it appears that DC secrete a C-C chemokine (DC-CCK1) that selectively attracts these cells,47 while both DC and activated B cells produce a C-C chemokine ABCD-1 that attracts mature T cells.53 Dendritic cells, by virtue of their high levels of surface MHC-II and costimulator expression, readily cluster CD4+ T helper cells. Two different subsets of dendritic cells have recently been characterized that control the fate of naïve T helper cells.46 One subset, designated DC1, secretes large quantities of IL-12 that promotes the development of a Th1-type phenotype, which is important in the generation of immunity to intracellular parasites. Another subset, the DC2, promotes a Th2 pattern of cytokine production via the release of an as yet uncharacterized factor. The Th2 responses are important in the generation of immunity to extracellular infections and also in allergic responses. The interaction of the CD4/TCR complex with DC1 MHC-II has significant effects on the cytokine production of DC, one of the most important being enhancement of IL-12 production. Expression of CD40L by T helper cells further stimulates the production of IL-12 and IL-15 from DC.47,58 The secretion of these Th1-type cytokines by DC is important in enhancing the generation of cytotoxic T cells from naïve CD8+ T cells. The CD40/CD40L pathway has many other important functions in the context of DC. The interaction of DC-expressed CD40 with CD40L on NK cells has also been shown to enhance NK cell cytotoxicity and IFN-γ production.5

Dendritic cells also interact both directly and indirectly with B cells. As alluded to previously, one of the first observations made regarding DC was that they are essential accessory cells for the generation of primary antibody responses. The addition of DC to a culture of B cells promotes an LFA-1-dependent clustering, proving that DC/B cell communication is not mediated solely via Th cell intermediates.59,50 The DC stimulate the proliferation of CD40-activated B cells and also enhance maturation into IgM-producing plasma cells.51 This differentiation of naïve B cells into IgM-producing plasma cells is dependent on DC secretion of IL-12.53 In the presence of IL-10 and TGF-β, DC have also been observed to promote immunoglobulin class switching towards IgA.54

The final stage in the DC life cycle is apoptosis, mediated either by T lymphocytes or NK cells. This process makes way for the next wave of DC migrating from local tissues into the afferent lymph53,56 and provides an explanation as to why DC are never found in the efferent lymph. In summary, immature DC are activated by a number of environmental signals that are associated with cellular stress or the presence of a microbe. These signals are transduced by the DC into costimulators, cytokines and chemokines, which activate innate and acquired immunity.

**Dendritic cells and infectious disease**

Dendritic cells are commonly the first immunological cells to encounter foreign organisms. Not surprisingly, DC play an important role in the generation of protective immunity towards intracellular parasites.57,58 However, as a consequence, DC function may also be subverted as part of the life cycle of a pathogen. A number of viruses use molecules expressed by DC as receptors; examples include CD4, CCR5 and CXCR4 (HIV),59 CD13 (coronavirus and cytomegalovirus)60,61 and CD46 (measles virus; MV).62 The most extensively studied example of DC involvement in infection is HIV. This lentivirus can remain latent in DC and exploits the trafficking of DC towards lymphoid tissue as a strategy to enhance the infection of permissive CD4+ lymphocytes.63,64 The HIV appears to be activated in DC by CD40 ligation or the presence of Th cells. The activation status of the DC themselves is thought to have an impact on viral replication, with immature and cutaneous DC supporting productive infection of macrophage tropic virus, while mature DC are able to transport HIV but appear unable to replicate both T cell and macrophage tropic strains of virus.65 A further interesting observation is that viruses derived from infected DC carry T cell-specific factors that make them highly infectious.66 The mechanisms used by HIV to subvert antigen presentation by DC undoubtedly have direct parallels in other viral infections. For example, activation of MV-infected DC with CD40L or with CD4+ T cells results in a profound viral replicative event, which ultimately leads to cell death.67 The aforementioned activation of DC by CD40L occurs primarily in the lymph node and it appears that this mechanism will prevent both MV- and HIV-infected DC from successfully presenting antigen to T cells.68

Mechanisms for interfering directly with antigen presentation, such as those used by adenoviruses, EBV and CMV, may also prevent DC from effectively modulating CTL responses.59 For example, many human herpesviruses, including EBV (HHV4), encode a viral homologue of IL-10, which will suppress the ability of professional APC such as the DC to produce effective CTL responses.70 Human cytomegalovirus (HCMV) causes severe morbidity in immunocompromised patients and again subverts immunosurveillance using a latent ‘protein-free’ replicative stage. Human cytomegalovirus remains latent in the CD33+ myeloid progenitors of monocytes and dendritic cells. Reactivation has been observed when these cells are treated with cytokines that promote differentiation of DC (TNF-α, IL-4 and granulocyte–macrophage colony stimulating factor (GM-CSF)). Reactivation under the influence of these signals would be an ideal mechanism for interfering with antigen presentation. The inadequate antigen-presenting ability of Langerhans cells that express hepatitis C virus genes is thought to reflect active interference with antigen presentation by an indeterminate mechanism, perhaps involving perturbed IL-12 production.71

The picture for other intracellular parasites is somewhat similar. Effective Th1-type immune responses are essential for the generation of immunity towards parasites such as...
**Histoplasma, Leishmania** and **Mycobacterium** sp.\textsuperscript{72–74} It appears that DC secretion of Th1-promoting cytokines, such as IL-12, is inhibited by infection with these parasites.\textsuperscript{75} Other microbes, such as the sexually transmitted intracellular pathogen **Chlamydia** sp., have been detected in DC,\textsuperscript{76} although the mechanisms that afford this organism relative freedom from immunosurveillance still remain to be clarified. A **Salmonella** sp. was recently discovered to both infect and survive within DC and, as with HIV, this may be an important mechanism for disseminating the disease away from mucosal sites.\textsuperscript{77} In summary, many organisms use DC as bases for the evasion of immunosurveillance. Subversion of DC antigen presentation and activation is a newly recognized phenomenon, which helps to explain the persistent nature of some infections.

**Autoimmunity**

Dendritic cells have been studied in a number of common autoimmune conditions. Despite the diverse nature and tissue distribution of autoimmune diseases, DC share certain common characteristics irrespective of disease and sites from which they are recovered. These include increased numbers of tissue DC, particularly in a perivascular distribution, DC infiltration at the earliest stage of disease and an altered DC phenotype. Early DC infiltration contributes to the recruitment of other immune cells.

Rheumatoid arthritis (RA) is a chronic destructive autoimmune disease of poorly defined pathogenesis with a prevalence approaching 1%. For almost two decades, the helper T cell has been considered to be the mediator of the immune response leading to joint destruction. This is based on the strong HLA DR4 association with RA, on histological features of the synovium and on the trend towards a response in RA with T cell-modulating therapies. However, the primacy of T cells in RA has been challenged and several authors have investigated DC expression and function in RA. Increased numbers of DC have been shown in both the peripheral blood, synovial fluid exudates\textsuperscript{78,79} and synovial tissues in patients with RA.\textsuperscript{80}

It has been argued that RA is due to DC presentation of endogenous self peptides.\textsuperscript{81} This is a feasible hypothesis, but a more important question is: what factors result in synovial DC activation? Both TNF-\(\alpha\) and GM-CSF are abundant in the synovium, which could contribute to activation, as could mechanical factors such as trauma. Pro-inflammatory mediators, such as TNF-\(\alpha\), could be up-regulated in the synovium by rheumatoid-factor auto-antibodies directed against the Fc moiety of immunoglobulins, which could lead to macrophage activation by complement. The plethora of cellular and molecular abnormalities reported in RA could therefore be secondary to the ravages of chronic inflammation rather than being of primary pathogenic importance. Anti-TNF-\(\alpha\) therapy is an exciting development in the treatment of RA. The sustained immunomodulatory effect of anti-TNF therapy in early RA could be due to modulation of DC function down-regulating both costimulatory signals and DC trafficking.\textsuperscript{82}

Seronegative polyarthritis includes the related conditions ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis and undifferentiated arthritis.\textsuperscript{83} These diverse clinical entities are interrelated clinically by spinal inflammation and at an immunological level by the presence of the MHC class I molecule HLA B27. In the HLA B27 transgenic rat model of AS there is evidence that DC play a critical function, because disease can be transferred by bone marrow cells presumed to be DC.\textsuperscript{84}

Putative activation signals for seronegative arthritis have been identified. Bacteria may preferentially home to the joint tissues and activate an immune response.\textsuperscript{85,86} Another signal could be TNF-\(\alpha\), which can induce similar disease phenotypes in experimental models to human AS.\textsuperscript{87} The synovial inflammation seen in these conditions could be related to pro-inflammatory cytokines released from the adjacent joint capsule. Direct mechanical trauma to the tissues could also serve to initiate cell-mediated immunity in these conditions.

Dendritic cell infiltration is an early feature of islet cell autoimmunity in diabetes mellitus\textsuperscript{88} and contributes to local lymphoid tissue formation in the islets cells.\textsuperscript{89} What could be the signal for DC activation in autoimmune diabetes? The destructive processes are directed against the pancreatic \(\beta\) cell. A viral tropism for the \(\beta\) cell is the one mechanism that has been postulated to initiate autoimmunity. Clues to DC activation signals for endocrine disease in general come from studies in thyroid disease, which show that DC infiltration is preceded by metabolic abnormalities in the thyroid gland itself,\textsuperscript{90} suggesting that some types of autoimmunity are due to primary abnormalities in the tissue targeted.

Psoriasis is a common skin condition with a prevalence of about 2%. The skin has a rich source of Langerhans cells, which are thought to be important in the pathogenesis of inflammation at that site. Streptococcal bacteria and IFN-\(\gamma\) have both been implicated in the pathogenesis of psoriasis\textsuperscript{91} and will also influence the activation status of DC. A recently identified cutaneous danger signal may be IL-1. This is based on experiments that show that mechanical stressing of keratinocytes results in liberation of high concentrations of IL-1,\textsuperscript{92} which is noteworthy because the most common sites of psoriasis are the elbows and knees, where the skin is subject to considerable trauma and stretching.

Knowledge of the central role of DC in autoimmune disease is important for both determining the mechanism of action of currently available therapies and for the development of future therapeutic strategies.

Not all studies on DC support an activated DC phenotype in autoimmune disease, with some studies showing that expression of costimulatory molecules is down-regulated at the sites of disease.\textsuperscript{93,94} This could be due to an immunomodulatory response at these sites to reduce the severity of inflammation. However, these changes have been interpreted as representing a primary defect in DC function that could theoretically allow auto-aggressive T cell clones to emerge during thymic selection. For such a contention to be supported, DC abnormalities would need to be demonstrated prior to the chronic phases of the respective diseases.

**Cancer**

The cell-mediated arm of the immune system plays an important role in the detection and elimination of malignant cells. T lymphocyte responses to tumour cells will require initial antigen presentation by professional APC such as the DC. Dendritic cell infiltration into a tumour has for many years
been linked to increased patient survival and reduction in the number of metastases in a variety of malignancies (including endometrial, gastrie and lung cancers). This positive prognostic indicator is tempered by the observation that DC isolated from cancer patients show an impaired ability to present antigen as a product of decreased surface expression of the costimulators CD80 and CD86.99,100

It also appears that many sporadic tumours actively exploit immunosuppressive pathways to interfere with antigen presentation. Tumour-derived IL-10 acts directly on tissue DC to prevent maturation and therefore subsequent immunogenic presentation to T lymphocytes.101 Increased expression of FasL on tumour cells may also induce apoptosis in DC and T cell effectors.102,103 Vascular endothelial growth factor (VEGF) is produced by a majority of carcinomas and has been shown to inhibit maturation of DC within the tumour and to impair differentiation of haemopoietic progenitors into DC.104 An in vitro study using a renal cell carcinoma cell line has shown that factors are released that inhibit the differentiation of dendritic cells from CD34+ progenitors. Interleukin-6 and macrophage (M)-CSF have been subsequently identified as the factors mediating this inhibition via the down-regulation of the GM-CSF receptor.105,106 These mechanisms represent parodies of the normal processes intended to establish tolerance between a tissue and the immune system. The role of the DC in the pathogenesis of sporadic cancers is still poorly understood.

Therapeutic applications

The observation that DC play a role in the progression and severity of many diseases has led many researchers to investigate how modulation of DC function could be used therapeutically. The power of the activated DC could be used in cancer or intracellular parasite infection to redirect an immune response towards defective (malignant) or infected cells that have evaded immunosurveillance (Fig. 3). Alternatively, DC maturation could be suppressed to alleviate the symptoms of autoimmunity and allotransplant rejection.

Advances in the extraction and in vitro culture of DC have been a major driving force behind the recent increased interest in these cells and have facilitated the inclusion of these powerful adjuvants in therapeutic trials. Refined laboratory protocols are now available for either the generation of DC from a number of readily available sources113 or for the direct isolation of DC from mixed cell populations.114 Cells that have been found to yield DC, after culture in lineage-restricting cocktails of cytokines, include CD34+ stem cells115–118 and CD14+ monocytes.119–121 CD14+ monocytes are perhaps the most readily available precursors used to generate human DC, because they constitute some 7–8% of human PBMC. The simplest method of obtaining DC is by allowing PBMC to adhere to tissue culture dishes for 2 h. After several washes, the adherent fraction will be enriched for CD14+ monocytes. Immature DC can then be produced using GM-CSF + IL-4/IL-13. The GM-CSF has the effect of restricting differentiation towards a myelomonocytic lineage, while IL-4 or IL-13 inhibits the development of macrophages.122 The overall result is a forced differentiation towards a DC lineage. Cells obtained from in vitro culture with high doses of GM-CSF and IL-4/IL-13 resemble immature DC, in that they have a high affinity for soluble antigen and express lower levels of costimulatory molecules than the activated form.123 Subsequent addition of TNF-α, LPS or CD40L to a culture of these DC promotes maturation and activation, as would be expected from the discussion of DC life history presented earlier. High-purity DC cultures can also be obtained by immunomagnetic selection.124 Contaminating leucocyte subsets can be depleted with a cocktail of antibodies against T cells (CD3), B cells (CD19), NK cells (CD56), monocytes (CD14) and macrophages (CD16), leaving a fraction enriched for DC. Alternatively, DC can be positively selected from mixed cultures using antibodies against antigens that have DC-restricted expression, for example CD83 or CMRF-44.126

Dendritic cell-based immunotherapy of cancer has been demonstrated in a number of studies using murine tumours. Established malignancies ranging from melanoma to mammary carcinoma28 have all been successfully regressed using infusions of antigen-loaded, activated DC. The generation of specific tumour-specific CTL requires the introduction of antigen into the DC, thus providing a target for immunity. The DC are able to present antigen that has been introduced by a variety of methods, including plasmid, mRNA, peptide (eluted and synthetic), cell lysate and...
recombinant viral vectors, including adenovirus\textsuperscript{133} and vaccinia virus.\textsuperscript{134}

Data relating to human DC clinical trials are scarce, but certain studies have reported encouraging results. One study has used DC pulsed with tumour lysate or peptide for the treatment of patients with metastatic melanoma.\textsuperscript{135} Of the 16 patients immunized, five had objective responses (30\%) with two subjects experiencing considerable disease regression. Malignant melanoma, due to its intrinsic antigenicity, is an ideal target for a DC-based immunotherapy. Of the published studies on the use of DC for the treatment of prostate cancer, one of the most promising cites that nine of 33 patients (27\%) responded to immunization.\textsuperscript{136} Hsu \textit{et al.} treated patients suffering from follicular B cell lymphoma with DC and tumour-specific idiotype protein. This resulted in a marked antitumour immune responses in all four patients.\textsuperscript{137} For B cell lymphoma, the presence of a truly tumour-specific antigen is the ideal in terms of a DC-based immunotherapy, because it will reduce autoimmune complications arising from coexpression of antigens on normal tissue. For example, patients immunized against melanoma-derived antigens (e.g. MART-1) sometimes experienced an autoimmune vitiligo resulting from destruction of normal melanocytes by autoreactive CTL.\textsuperscript{135} It is suspected that DC immunotherapy of solid tumours will be considerably less efficacious than for melanoma or leukaemia, perhaps due to the higher incidence of HLA antigen dysregulation in these tumours and the paucity of good tumour-restricted antigens. Further increases in our understanding of how DC are activated will allow us to associate more ‘danger’ with the tumour antigens under investigation.

Culturing DC \textit{ex vivo} for tumour immunotherapy represents a considerable challenge. One alternative may be to use infusions of factors that directly promote DC differentiation, in an effort to increase the level of antitumour immunosurveillance. A case in point is the haemopoietic growth factor Flt-3. This growth factor has been shown to increase the numbers of DC and monocytes in peripheral blood without producing the serious side effects that are a consequence of using other cytokines/growth factors as adjuvants (e.g. IL-2, IFN-\(\gamma\)).\textsuperscript{138} Flt-3 is able to induce protective antitumour immunity in some animal models,\textsuperscript{139,140} which is thought to be a consequence of increased presentation of tumour antigens combined with increased NK cell activity.\textsuperscript{144} The synergy of a DC growth-promoting factor, such as Flt-3 ligand, with a DC activation signal, such as CD40L or tumour necrosis factor-related activation-induced cytokine (TRANCE), may provide additional benefits by promoting CTL generation from an already expanded pool of DC.

With respect to persistent viral disease, DC function could be modulated to reactivate silent T cells and lessen the effects of relapse. Diseases that may be amenable to this form of intervention include viral hepatitis (HBV and HCV), papillomavirus infection and chronic HCMV infection. Optimizing DC therapies for persistent viral disease is hampered by a lack of appropriate animal models. However, it is encouraging to note that in a murine model of chronic hepatitis, where mice are transgenic for hepatitis B surface antigen (HBsAg), tolerance to HBsAg can be broken by immunization with cytokine-activated DC.\textsuperscript{142} It is inferred from the earlier discussion of DC life history that, for immunotherapy of cancer and viral disease, DC activated by members of the tumour necrosis family of ligands (e.g. TNF-\(\alpha\) or CD40L) will be the best candidates for breaking immunological tolerance.\textsuperscript{143,144}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{dc_immunotherapy.png}
\caption{Dendritic cell immunotherapy of cancer. A state of immunological tolerance usually exists between the tumour and the host. Infusions of activated, tumour antigen-loaded dendritic cells (DC) stimulate the proliferation of antigen-specific anti-tumour CTL.}
\end{figure}
In situations where intracellular parasites interfere with DC function, there may be some opportunity to correct the defects. For example, DC secretion of IL-12 is inhibited by Leishmania sp. and prevents an effective Th1-type immune response, which is crucial for parasite clearance. Infusions of parasite-pulsed DC, in combination with IL-12 or other Th1-promoting cytokines, may help to reduce parasite burden by repolarizing immunity. It has been suggested that a possible therapy for HIV could involve infusions of IL-12-transfected DC in an attempt to counter the suppression of Th1 immunity that is a characteristic of this infection.

Conversely, T lymphocytes could be anergized by direct suppression of DC activation for the treatment of autoimmune diseases and for reducing the severity of allotransplant rejection. Enhancement of the ability of DC to tolerize T lymphocytes could be accomplished in a number of ways, for example by pretreatment of DC with maturation-suppressing factors such as IL-10 and TGF-β. One recent study has shown that infusions of Flt-3 ligand can increase the survival time of skin allografts when administered in combination with a factor such as anti-CD40L, which will prevent DC activation. In a further landmark study, it has been found that DC transfected with CD95L could induce antigen-specific tolerance after being pulsed with a peptide to which they had previously been sensitized. This observation provides proof that it may also be possible to delete autoreactive T cells from the repertoire using modified DC.

Conclusions

In the present review, we have described how an in-depth understanding of DC biology has provided insights into the coordination of both innate and adaptive immunity. The importance of these cells in a number of disease states is also being gradually revealed. It is therefore realistic that in the near future, once the lineage, danger and tolerance paradigms have been fully resolved, it will be possible to modulate DC function for considerable therapeutic benefit.

Acknowledgements

We would like to offer our sincere thanks to Stephen Richards, Richard Jones and Peter Cotterrell for their constructive comments regarding the manuscript. Laboratory work is supported by grants from Yorkshire Cancer Research, The Arnold T unstall Fellowship and The Medical Research Council.

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