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ORIGINAL PAPER

Exploiting dendritic cells for active immunotherapy of cancer and chronic infections

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Abstract Dendritic cells (DCs) are important antigen-presenting cells (APCs) that can prime naive T cells and control adaptive immune responses with respect to magnitude, memory and self-tolerance. Understanding the biology of these cells is central to the development of new generation immunotherapies for cancer and chronic infections. This review presents a brief overview of DC biology and of the preparation and use of DC-based vaccines.

Keywords Dendritic · Vaccine · Antigen presenting · Cancer

DC biology

Protective immunity results from the concerted action of innate and adaptive immune systems [1]. The innate immune system includes phagocytic cells, natural killer (NK) cells and complement, and has evolved to respond rapidly to pathogens to protect the host early in infection. The adaptive immune system, which consists of B and T lymphocytes, is required for the eventual clearance of many infections and for the generation of immunologic memory. Recent evidence from a number of laboratories indicate that both innate and adaptive immunity function to protect against the development of malignant tumors [2]. Antigen-presenting cells (APCs) form an important link between innate and adaptive immunity. APCs process intracellular and extracellular proteins into antigenic peptides, which are then presented on cell surface MHC molecules to cells of the adaptive immune system [3]. Because of their ability to express co-stimulatory molecules and cytokines, APCs can stimulate the expansion of lymphocytes that recognize the displayed peptides, initiating an adaptive immune response. Although monocytes, macrophages, B cells and dendritic cells (DCs) can all function as APCs, DC are thought to be the principle initiators of adaptive immune responses [4, 5]. In culture with lymphocytes, relatively few DCs and very little antigen are needed to stimulate T cell responses, and primary responses to antigens may be achieved using DCs in long-term culture [6, 7]. In both in vitro and in vivo assays, DCs are by far the most potent APCs [8].

DC subtypes

DCs are bone marrow-derived cells that are present in trace amounts in the blood (<0.1% of blood mononuclear cells) and virtually every tissue. Cell surface phenotyping has shown that in mice and apparently in humans there are as many as five distinct subpopulations of DCs [9–12]. It is not completely clear, however, if all of these represent distinct cell lineages or are a reflection of functional plasticity. In humans, the three best-characterized DC subsets may be derived by culturing precursor cells obtained from the blood [9]. Cells closely resembling epidermal Langerhans cells may be obtained from CD34+ hematopoietic progenitor cells (HPCs) cultured with granulocyte/macrophage colony stimulating factor (GM-CSF), tumor necrosis factor α (TNFα) and transforming

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growth factor β (TGF-β). Cells resembling so-called dermal or interstitial DCs (also known as DC1) may be obtained by culturing monocytes in GM-CSF and interleukin-4 (IL-4) followed by stimulation with proinflammatory cytokines such as TNFα or with microbial products such as lipopolysaccharide (LPS). Circulating CD11c- BDCA2+ so-called ''plasmacytoid'' DC precursors in the blood may be differentiated into a third type of DC (plasmacytoid DC, or DC2) following exposure to viruses or bacterial (unmethylated CpG motif) DNA [13]. Plasmacytoid DCs are unique, that they travel to the lymph nodes directly from the blood (instead of through the lymphatics), and that upon stimulation they produce very high levels of type I interferon (interferons α and β) [9]. Although, it has been thought that most DCs are of myeloid origin, the precise origin of the different DC subtypes is not completely clear [14]. In mice, which have been studied in more detail than humans, there is evidence that all DC types can be derived from both common myeloid and common lymphoid progenitors [15–17], as well as from a third progenitor cell type that does not have myeloid or lymphoid potential [18].

Antigen uptake and maturation

DCs exist in a resting state in virtually every tissue and are recruited to sites of inflammation by chemokines such as MIP1α (CCL3), MIP1β (CCL4) and RANTES (CCL5). In tissues, DCs and DC precursors capture antigens from a wide range of sources including bacteria, viruses, dead or dying cells and extracellular proteins, peptides and immune complexes. DCs have a host of different antigen uptake receptors for this purpose, including scavenger receptors [19], Fc receptors [20, 21] and C-type lectins [22, 23]. Some of these receptors induce simultaneous uptake and stimulatory signals, whereas others are inhibitory receptors that induce suppressive signals upon antigen uptake.

DCs continuously sample their environment for antigens, but they must be stimulated or ''matured'' before they can become strong stimulators of the immune system [8]. In fact, there is accumulating evidence, that in the absence of maturation stimulus DCs function to maintain immunologic tolerance to captured antigens [24–26]. DC maturation is induced by two classes of stimuli or ''danger'' signals termed exogenous and endogenous [27–29]. Exogenous stimuli are associated with microbial infections and are mediated by signaling through DC pattern recognition receptors such as Toll-like receptors (TLRs) [8, 28, 30, 31]. There are at least 10 different TLRs, each recognizing different sets of pathogen-associated molecules. For example, TLR3 recognizes double-stranded RNA, TLR4 recognizes lipopolysaccharide and TLR9 recognizes bacterial CpG motif DNA. Myeloid DCs express TLRs 2 through 8, whereas plasmacytoid DCs express TLRs 7 and 9, so these different DC subtypes respond differentially to different TLR ligands. DCs may secrete a number of important inflammatory cytokines following TLR stimulation, including high-levels of IL-12, interferon α and TNFα, but the specific cytokine profile induced depends upon the subtype of DC stimulated as well as the nature of the stimulus. For example, plasmacytoid DCs secrete abundant interferon α upon maturation, whereas myeloid DCs does not.

Endogenous maturation stimuli originate from inflammatory molecules produced by cells of the host immune system or by damaged tissues [8, 29], and stimulate signaling through specific receptors on the DC. Examples of endogenous stimuli include TNFα and related molecules as CD40L and TRANCE expressed on activated lymphocytes such, proinflammatory cytokines such as IL-1, or molecules released from damaged cells such as uric acid [32].

DC maturation is characterized by decreased phagocytic capacity, enhanced processing and presentation of antigens, induced ability to migrate to T cell areas of lymph nodes, and increased ability to stimulate T cell proliferation and cytokine production [33]. In culture, it is at this stage that DCs acquire their characteristic dendritic appearance, with numerous cytoplasmic processes or ''veils.'' Maturation is accompanied by phenotypic changes that include increased cell surface expression of MHC and co-stimulatory molecules, including members of the TNF receptor (CD40), TNF (OX40L, CD27L) and B7 (CD80 and CD86, B7-H3) families. There is down-regulation of chemokine receptors such as CCR2 and CCR5 that direct DCs to sites of inflammation (via MCP1, MIP1α, MIP1β and RANTES) and up-regulation of CCR7, which targets the DC to lymphatic vessels and lymph nodes via interaction with CCL19 (MIP-3β) and CCL21 (SLC).

Antigen processing is coordinately regulated by maturation. For example, following receipt of a maturation signal, DCs reduce the pH of endosomes (this facilitates processing of exogenously acquired antigens through activation of endosomal proteases), remove invariant chain from the antigen binding pockets of MHC class II molecules (so that processed peptides can access the empty pockets), and exocytose peptide-bound MHC class II molecules to the cell surface together with co-stimulatory molecules. Maturation also up-regulates the activity of certain proteasome members to create ''immunoproteasomes'' which enhance the processing of antigens that access the MHC class I pathway by altering the way in which they are cleaved [34].

The process of maturation is also accompanied by up-regulation of adhesion molecules such as CD54 (ICAM1), cytokines (e.g., TNFα, IL-12, IL-18) and chemokines (RANTES, MIP1, IP-10). The latter enable the recruitment of T cells, monocytes and other DCs into the

local environment. In their mature state DCs express other markers such as CD83 (a molecule involved in thymic T cell selection and DC-DC interactions) and DC-LAMP, a lysosomal protein, that distinguish can mature from immature DC. In the T cell areas of lymphoid organs mature DCs live for only 1–2 days [35], but their life span can be prolonged if they encounter T cells that are activated and expressing membrane-bound activators such as CD40L and TRANCE [8].

Antigen presentation

Through their T cell antigen receptor (TCR), T lymphocytes specifically recognize peptide antigens bound to the highly polymorphic MHC molecules on the APC surface. MHC class I molecules present peptide antigens to CD8+ T cells, whereas MHC class II molecules present peptide antigens to CD4+ T cells. In addition, other less polymorphic antigen-presenting molecules structurally similar to MHC class I are also found on DCs. This would include CD1 molecules, which function to present microbial lipids to antigen-specific T cells [36, 37]. As with TLRs and endocytic receptors, different DC subtypes have different sets of CD1 molecules. For example, CD1a is found on epidermal Langerhans cells, whereas CD1b and CD1c are found on dermal dendritic cells. CD1d presents specific glycolipids (galactosyl ceramides) to NKT cells, cytokine secreting cells that are important mediators of T cell immunity [38, 39]. CD1 molecules have not been found on plasmacytoid DCs.

Infection of host cells with viruses results in the processing of viral peptides onto MHC class I via the so-called endogenous pathway [33, 40]. DCs and most other cell types can process peptides this way from endogenously synthesized cytoplasmic proteins. In this pathway, viral proteins are ubiquitinated and degraded by the proteasome, and the resulting peptides are transported via transporters for antigen presentation (TAP) molecules into the endoplasmic reticulum (ER), where they are loaded onto MHC class I. The MHC class I/peptide complexes exit the ER via the trans-Golgi network and are transported to the cell surface, where the bound peptide is presented to the TCR on CD8+ T cells.

In contrast, processing of extracellular protein antigens commonly results in presentation of molecules onto MHC class II [33, 40]. MHC class II/peptide complexes are formed through endocytosis of extracellular sources of protein. Endosomes containing the ingested protein mature and fuse with lysosomes, where acid proteases degrade the proteins into peptide fragments that are then loaded onto MHC class II molecules. The MHC class II/peptide complexes are transported to the cell surface within specialized vesicles, where the bound peptide is presented to the TCR on CD4+ T cells.

DCs can also present extracellular antigens onto MHC class I through a process known as cross-presentation [33, 40–42]. Cross-presentation permits DCs to elicit CD8+ T cell responses to exogenous as well as endogenous antigens (known as ‘‘cross-priming’’) [43]. CD cross-priming has been shown to be necessary for the induction of T cell immunity to viruses or other intracellular pathogens that infect other cell types but that don’t infect DCs [41]. Dead cells and immune complexes can specifically target the cross presentation pathway through dedicated receptors on the DC surface such as the Fcγ receptor for immune complexes and LOX1 for necrotic cell debris [20, 21, 44, 45]. Cross-presentation of antigens can be enhanced by stimulation of certain TLRs or by type-I interferon [46–48].

Lymphocyte activation

DCs can migrate very rapidly to lymph nodes, where they can dynamically interact with many T cells at a time [49, 50]. In the lymph node, triggering of a T cell response is dependent upon the intensity and length of the DC-T cell interaction [51, 52]. Activation requires two types of signal at the immunological synapse between a DC and a T cell, the first between the MHC/peptide complex on the DC and the TCR, and the second through assorted co-stimulatory molecules and cytokines. Through these signals mature DCs can induce T cells to expand clonally and to differentiate into memory and effector cells. There is a plasticity of this response that is dependent on many factors, including the antigen dose, the nature of the DC maturation stimulus and the state of maturation of the DC. All of these influence the secreted cytokine profile of the DC and the polarization of responding T helper (Th) cells in the form of a Th1 or Th2 response [53–56]. DC maturation is critical for this induction of immunity. Immature DCs that circulate through lymph nodes in the steady state can induce T regulatory responses and are important at maintaining immune tolerance toward self-antigens [25, 26, 57].

The generation of effector CD8+ T cells (cytotoxic T lymphocytes, or CTLs) is particularly important in establishing immunity to tumors and intracellular infections, but the type of T helper response is important as well, especially for maintaining immunologic memory [58, 59]. Th1 cells, which produce interferon γ(and TNFα, support CTL responses, whereas Th2 cells (which produce IL-4, IL-5 and IL-13) support humoral immunity and down-regulate Th1 responses. Th1 polarization is potently induced by DC secretion of IL-12, although IL-12 is not an absolute requirement for this, perhaps because of other DC-generated cytokines such as IL-23 or IL-27 [35, 60]. In addition to activating T cells, it is now known that DCs can also directly interact with and activate B cells [61, 62], NK cells

[63] and NKT cells [38]. All these cells may play a critical role in the induction of anti-tumor immunity.

Ex vivo manipulation of DCs for active immunotherapy

Vaccines against microbial pathogens are traditionally prepared by isolating an attenuated or killed version of the pathogen and mixing it with an adjuvant that serves to boost the immune response. The success of this type of vaccine often depends on its ability to stimulate the production of neutralizing antibodies [64]. For a number of chronic intracellular infections such as HIV, hepatitis C, tuberculosis or malaria, however, this approach has not proved sufficient to generate protective immunity. To control these disorders, the generation of Th1 and CTL responses appears to be very important [65, 66].

The induction and maintenance of CTL-mediated immunity also appears to be of great importance for immunotherapies for patients with cancer [67–69]. By studying cellular immune responses in cancer patients, a variety of tumor-associated antigens have been identified that are recognized by T cells. These antigens can be used to ''vaccinate'' individuals against their own tumors [70–72]. Attempts to vaccinate cancer patients with killed tumor cells, cell lysates or tumor antigen proteins or peptides have produced immunologic and clinical responses, some of them are complete and long-lasting [73–78]. However, it remains to be demonstrated in large, prospective, randomized trials that these antigen/adjuvant preparations can provide a clinically-significant benefit in the form of improved survival [76, 79, 80].

Cancer vaccine adjuvants such as QS-21, GM-CSF or Incomplete Freund's Adjuvant (IFA, or Montanide) have all been shown to boost CTL responses to tumor-associated antigens, but these responses are often weak and require in vitro restimulation of T cells to be detected [75, 81]. Because of their potent immunostimulatory capacity and their ability to prime naïve T cells, the manipulation of DCs as a ''natural'' vaccine adjuvant has generated great interest [82]. To date, the most common approach has been to isolate large numbers of DCs by culturing bone marrow-derived progenitors ex vivo in the presence of cytokines, loading the DCs with antigens and re-injecting them back into the subject. In mice, bone marrow-derived DCs loaded ex vivo with tumor antigens by a variety of methods have been shown to induce potent antigen-specific CTL responses. These vaccines can protect mice from challenge with tumors bearing the antigenic peptide in association with cell surface MHC, and can cure animals bearing established tumors [83–86].

In human subjects, vaccination of healthy volunteers with peptide-pulsed DCs is well tolerated and clearly induces antigen-specific T cell immunity in the form of CTL and Th1 responses [87, 88]. CTL responses and occasional tumor regressions have been observed in a number of small clinical-studies with cancer patients vaccinated with DCs loaded using a variety of tumor-associated antigens. Clinical and immune responses have been reported in patients with metastatic melanoma [89–92], metastatic renal cell carcinoma [93, 94], B cell lymphoma [95, 96], prostate cancer [97–101], breast and ovarian cancer [102], and colon and lung cancer [103]. Larger trials have also shown some promise [104, 105].

Three general methods have been described for the preparation of DCs from human subjects for use in clinical trials. The methods differ in the starting population of blood cells used and in the different mixtures of cells obtained in the finished product. The methods involve, respectively: (1) differentiating DCs from non-proliferating monocyte precursors, (2) differentiating DCs from proliferating CD34+ hematopoietic progenitor cells, or (3) directly isolating DCs from peripheral blood. The optimal route of administration for the cells has not yet been determined. They are injected typically, intradermally, subcutaneously or intravenously. Other routes such as direct injection into lymph nodes or tumors have also been described.

Monocyte-derived DCs (Mo-DCs)

By far the most common method is to prepare DCs from blood monocytes. Mo-DCs can be generated from peripheral blood mononuclear cells (PBMCs) obtained from whole blood, although to obtain yields sufficient for the production of multiple vaccines, PBMCs are usually obtained by leukapheresis. To prepare Mo-DCs, CD14+ monocytes are first selected from PBMCs, using either a simple plastic adherence step (monocytes adhere to plastic, whereas lymphocytes do not), or by large-scale cell sorting using immunomagnetic beads. The monocytes are induced to differentiate into immature CD14- CD83- DCs by culturing for several days in the presence of IL-4 and GM-CSF. The DCs are then typically stimulated to mature by culturing for an additional period of time (1–2 days) in the presence of proinflammatory cytokines, usually a cocktail of IL-1β, IL-6, TNFα and prostaglandin-E2 (PGE_2) [106]. Mature Mo-DCs are large, CD14- CD83+ cells that express high levels of MHC class I and II and co-stimulatory molecules such as CD40, CD80 and CD86. Peptide antigens may be loaded or ''pulsed'' onto the DCs either before or after maturation. Cell lysates or purified or recombinant proteins are typically loaded just before the maturation stimulus. Viruses or RNA may be added either before or after maturation.

DCs derived from CD34+ hematopoietic progenitor cells

This method begins with the collection of CD34+ proliferating progenitors from the peripheral blood [107, 108]. This requires mobilizing CD34+ progenitor cells from the bone marrow by treating patients with granulocyte colony stimulating factor (G-CSF) prior to leukapheresis [81, 89]. The cells are expanded in culture for a week or more in the presence of GM-CSF and TNFα. The final product is a more complex mixture of cells than Mo-DCs. It includes a population similar to Mo-DCs and another population phenotypically identical to epidermal Langerhans cells. Differentiation may be skewed toward Langerhans cells by adding TGFβ to the culture. A fairly large percentage of the final product consists of myeloid cells at varying stages of differentiation including some CD14+ cells. These do not function as APCs but do not appear to interfere with the potency of the vaccine, although this has not been well studied. CD34+ progenitor cell-derived DCs may be matured and loaded with antigens similarly to Mo-DCs.

DCs enriched from peripheral blood

In the first clinical trial to use DC-based immunotherapy for cancer, DCs were purified directly from PBMCs by a series of density gradient centrifugation steps [96]. In this procedure, the PBMCs were first depleted of monocytes by centrifugation through discontinuous Percoll gradients. The monocyte-depleted PBMCs were then cultured for 24 h in the presence of antigen, and then the DCs were separated from lymphocytes by sequential centrifugation through 15% and then 14% metrizamide gradients. The low-density fraction containing the DCs was then cultured overnight again in the presence of antigen, washed and injected back into the patient [96]. The final product contained between 50% and 90% DCs, and in subsequent studies it was found that although the DCs obtained at the time of leukapheresis had an immature phenotype, by the end of the 2-day procedure they express maturation markers such as CD80, CD86, CD83 and CCR7 [103]. DC preparations based on this method have been used in phase III trials for the immunotherapy of prostate cancer [104, 109].

The yield of DCs obtained by this procedure has been significantly enhanced by stimulating the patient with Fms-like tyrosine kinase-3 ligand (Flt3 ligand, or FL) prior to leukapheresis [103]. FL mobilizes and expands peripheral blood DCs, and increases the final DC yield approximately 20-fold. Taking advantage of the increased numbers of DCs obtained in the leukapheresis product following administration of FL, a modification of the DC isolation procedure has been described that omits the initial Percoll gradient and the 14% metrizamide gradient (only the 15% metrizamide gradient is used) [103]. Unfortunately, pharmaceutical grade FL is no longer commercially available. Still, the development of commercial closed systems that take advantage of cell separation technology based on immunomagnetic beads and the short preparation time involved may make the enrichment of DCs directly from blood an attractive option in the future.

Choice of cell type

All three types of DC preparation have been shown to stimulate proliferation and cytokine production of antigen-specific T cells, and have been associated with clinical responses in trials involving human subjects with cancer. However, no direct comparisons of these cells have been performed in human subjects. In vitro data from one group has indicated an advantage of CD34+ cell-derived DCs over Mo-DCs in the presentation of peptides [110], but Mo-DCs have many advantages for clinical use. They are considerably simpler to prepare and are a well-characterized, homogenous population of cells. In addition, patients do not need to be pre-stimulated with cytokines prior to leukapheresis to prepare Mo-DCs.

Ex vivo maturation of DCs

Whatever the method used to prepare the DCs, it is clearly important that the DCs are matured prior to clinical use [57]. Clinical studies with DC-based vaccines indicate that DCs that have been matured ex vivo more effectively stimulate T cell responses [111], and there is evidence that the use of antigen-loaded immature DCs in vaccines actually leads to immune tolerance, perhaps due to anergy or T regulatory cell mechanisms [24, 25, 112].

In early work on DC-based vaccines, DCs were matured using supernatants from cultured monocytes (monocyte-conditioned medium, or at MCM) as a source of proinflammatory cytokines [113–115]. This has been largely replaced by a cocktail of three cytokines IL-1β, IL-6 TNFα and PGE_2 that mimics the effect of MCM on DC maturation [106]. One concern with this method is that DCs matured in the presence of PGE_2 do not secrete IL-12 [116]. However, the addition of PGE_2 is important for inducing DC migratory ability [117], and DCs matured with this cocktail still strongly stimulate CTL responses both in vitro and in vivo. This may be due to the action of other IL-12-related cytokines such as IL-23 and IL-27 [60].

Loading DCs with antigens ex vivo: peptide antigens

There are many ways to load DCs with antigens. One of the more commonly used methods is to co-culture them directly with peptides [89, 90]. This has been made possible because

of progress in identifying immunodominant peptide epitopes for tumor-associated and microbial antigens that are recognized by T cells [70, 72]. Use of peptides requires knowledge of a patient's HLA type and the existence of relevant MHC-restricted peptide epitopes. HLA-A2-restricted peptides are commonly used, often in combinations, although other MHC class I-restricted epitopes and even MHC class II-restricted T helper epitopes may be used as well. Altered or enhanced peptide antigens have also been used to boost immunity to less immunogenic self-antigens [64, 103]. Peptides from microbial antigens such as influenza matrix protein are frequently included to test for recall immune responses. Peptides may be loaded onto DCs either before or after DC maturation. In our hands, pulsing peptides onto mature DCs has resulted in somewhat better specific T cell stimulation in vitro.

The optimal peptide dose with which to load DCs is not entirely clear. Intuitively it might be thought that increasing the concentration of peptide would result in better antigen loading and more potent stimulation of T cells. The opposite, however, appears to be true. Alexander-Miller et al. showed that APCs loaded with very low concentrations of peptide (as little as 0.1 nM) stimulate high-avidity T cell clones that much more effectively recognize and lyse antigen-expressing target cells. [64, 118]. For clinical use it will be very important to load DCs with a peptide dose that stimulates expansion of high-avidity T cell clones. Most protocols currently pulse DCs with peptide concentrations of 1–10 μM, although the optimal dose will most likely need to be determined through dose escalation studies in human subjects.

Proteins and cell lysates

One disadvantage of using peptide-pulsed DCs is that the dominant epitopes of the antigen of interest must be known for the HLA type of the patient. This often restricts peptide vaccination studies to individuals with common HLA types. Alternatively, DCs can be loaded prior to maturation with purified or recombinant proteins [95], or tumor cell lysates [92, 119–121]. Loading with whole proteins allows host HLA molecules to select epitopes from an antigen's entire sequence.

The immunogenicity of protein-loaded DCs can be enhanced by using proteins coupled to cytokine or carrier protein sequences [95, 122], or using proteins of xenogeneic origin [99]. Keyhole limpet hemocyanin (KLH), a powerful immunogen derived from a marine mollusk, is one such protein that is commonly used to non-specifically boost the immunogenicity of a vaccine. It is also added as a control to test for the ability of a vaccine to prime T helper responses to a neoantigen [4, 40]. One disadvantage of loading DCs with purified protein antigens is that the MHC class I pathway is not specifically targeted. Pulsing DCs with crude protein mixtures such as tumor cell lysates may bypass this limitation by stimulating cross-priming via chaperone proteins [121, 123, 124].

DNA, RNA and viruses and bacteria

Loading DCs with antigens by DNA transfection has been successfully performed [125, 126], however it is often associated with a significant amount of cell death. DCs tolerate RNA transfection well, however, and this has been used to successfully load DCs with RNA encoding specific antigens or even whole tumor RNA [97, 127, 128]. Typically, DCs are transfected by RNA electroporation prior to maturation. DCs can also be loaded with antigens by infecting with non-replicating viral vectors such as recombinant adenovirus or pox viruses [129–133]. As with loading DCs with proteins or RNA, this method allows vaccines to be generated for patients of any HLA type, since the encoded proteins are cleaved and processed onto MHC molecules within the host cell. Viral, and even bacterial vectors such as Listeria, have the additional advantage of providing endogenous TLR agonists to potentiate DC maturation [134, 135].

Apoptotic cells, immune complexes and other methods

Apoptotic tumor cells can be used as an antigen source to take advantage of cross-presentation of extracellular antigens onto MHC class I [92, 136, 137]. Cross presentation of specific protein antigens may also be targeted by loading DCs with IgG immune complexes of those antigens, which are taken up via Fc receptors on the DC [20, 21, 44]. A number of other methods have also been employed to manipulate DCs ex vivo to create cellular or cell-derived vaccines. Cell fusions between DCs and tumor cells create heterokaryons that can stimulate anti-tumor T cell responses in vitro, in laboratory animals and in humans with cancer [138, 139]. DCs can also be used as a source of exosomes, antigen-presenting vesicles which can be loaded with antigens and used to stimulate T cell immunity [140–142].

Treatment strategies for the use of dendritic cell vaccines

Although it has been established that antigen-loaded DCs generated ex vivo by a number of different methods can induce antigen-specific immunity in clinical studies, numerous technical variables need testing before this approach is optimized, and more controlled studies are needed to prove efficacy in the treatment of cancer or

serious chronic infections. In addition, most of the reports on the therapeutic use of DC vaccines published to date have been with patients with advanced cancer. Although occasional dramatic clinical responses have been observed, it is likely that DC immunizations may be most effective in the adjuvant setting when the patient is in remission or the tumor burden is low but risk of disease progression and death is high. The use of DC vaccines in combination with other novel therapies such as antibodies that target tumor antigens, anti-angiogenesis agents [143], or agents that target molecules that inhibit the immune response such as CTLA-4 and PD-1 [144–150] also needs to be addressed.

A significant drawback of DC vaccines is that the ex vivo production of individually tailored cellular therapies is both laborious and expensive. For this reason the use of in situ approaches that take advantage of the biological properties of DCs in vivo has generated great interest. Approaches that can mobilize DCs to an accessible site where they can be matured and pulsed with antigens in vivo are being developed that may hopefully lead to potent therapies that do not required expensive facilities or labor intensive cell processing [8]. Such approaches include the systemic mobilization of DCs using Flt3 ligand, the local injection of chemokines such as MIP-3β, the use of DNA vaccines containing bacterial CpG motifs (which can target DCs and B cells via TLR9), or the use of topical compounds such as Imiquimod (a TLR7 agonist) [31, 151–153]. As more is discovered about the biological properties of DCs, we should be better able to manipulate these important cells to take advantage of their immunoregulatory properties for the treatment of human disease.

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