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Tolerogenic dendritic cells and their potential applications

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Summary

Dendritic cells (DCs) play a pivotal role in regulating the balance between immunity and tolerance of the immune system. Recent advancements in DC biology and techniques for manipulating the function of these cells have shown their immense therapeutic potential for treating a variety of immune disorders. Theoretically, antigen-specific tolerogenic DCs can be generated *in vitro* and delivered to patients to correct the dysfunctional immune responses that attack their own tissues or over-react to innocuous foreign antigens. However, DCs are a heterogeneous population of cells with differences in cell surface makers, differentiation pathways and functions. Studies are needed to examine which subset of DCs can be used for what type of applications. Furthermore, most of the information on tolerogenic DCs has been obtained from animal models and translational studies are needed to examine how a DC therapeutic strategy can be implemented clinically to modulate immunity.

Keywords: dendritic cells; immune responses; T cells; therapy; tolerance

Introduction

Dendritic cells (DCs) are a heterogeneous group of cells that are specialized in the regulation of immune responses. Although DCs were discovered by Steinman in his pursuit to understand immunogenicity,[1] these cells play an equally important role in maintaining immune tolerance.[2] Despite being discovered relatively late compared with other immune cells, such as T cells and B cells, the importance of DCs in regulating the immune system led to an intensive quest for knowledge on these cells over the past few decades. As a result, a huge body of information was generated regarding their development, functions and cell surface marker expression. Several recent articles provide detailed information on DC development and function.[3–5] In this review, we will focus on the role of DCs in peripheral tolerance and potential applications of tolerogenic DCs. Most studies in this area were conducted with mouse models, so this review will discuss mainly work carried out with mouse DCs.

Bone-marrow-derived murine DCs can be classified into three subpopulations based on their lineages during development, classic DCs (cDCs), plasmacytoid DCs (pDCs) and tumour necrosis factor and inducible oxide synthase-producing DCs (TipDCs). All these subpopulations originated from a common myeloid progenitor cell called monocyte, macrophage and DC precursor.[4,5] The monocyte, macrophage and DC precursor can be differentiated into common DC precursors in the presence of fms-like tyrosine kinase 3 ligand (Flt3L) or monocytes in the presence of macrophage colony-stimulating factor. In bone marrow, common DC precursors give rise to pDCs or pre-cDCs that travel through blood to peripheral destinations to differentiate into cDCs. Under inflammatory conditions, monocytes can differentiate into TipDCs. Based on cell surface markers and anatomic locations, cDCs can be further divided into subsets. For example, in lymphoid tissues such as the mouse spleen, two subsets of cDCs, $CD8^+$ $CD205^+$ and $CD8^-$ $33D1^+$, are localized in different cell zones.[6] In non-lymphoid tissues, such as lung and liver, there are also two subsets of cDCs,[7] $CD103^+$ $CD11b^+$ and $CD103^-$ $CD11b^{hi}$. Unlike the cDCs, pDCs are relatively long-lived DCs and they produce type I interferon in response to viral infection.[8,9] The

monocyte-derived TipDCs also play an important role in enhancing host immune responses to infections through the secretion of tumour necrosis factor-α and nitric oxide, reminiscent of activated macrophages.[10–13]

Langerhans cells in the skin epidermis are functionally related to DCs and have been considered as another DC subpopulation.[14–18] Accumulating evidence suggests that Langerhans cells originate from an embryonic precursor that migrates into the epidermis during late embryonic development.[19] Langerhans cells can renew themselves locally, independent of adult circulating precursors, under steady-state conditions, whereas under severe inflammatory conditions, they can be repopulated by circulating blood precursors.[20]

DC functions

Generation of antigen-specific immune responses

The important role of DCs in immunity was first recognized by Steinman *et al.* using mixed leucocyte reaction assays[21,22] and the splenic DCs were found to be the major cell type required for the induction of lymphocyte responses. The DCs are located in both lymphoid and non-lympoid tissues.[7,23,24] They take up antigenic materials from internal and external sources, such as infected host cells or infectious microorganisms, and process and present them to naive T cells. Upon interacting with DCs, naive T cells can be differentiated into helper T cells to regulate the function of other T cells or B cells, or into cytotoxic T cells to eliminate the infected cells or further into memory T cells to defend the body from future infection by the same source. For a naive T cell to differentiate, its T-cell receptor must be engaged and co-stimulatory pathways must be activated. In addition, a particular cytokine environment is also needed to programme the differentiation of a particular T-cell response. Dendritic cells express high levels of MHC class I and II molecules on the cell surface and these molecules bind processed antigens for presentation to T cells. Under certain conditions, such as inflammation, co-stimulatory molecule expression is also elevated in DCs. In addition, DCs can secrete cytokines that can influence the direction of T-cell differentiation. For instance, a naive $CD4^+$ T cell can be differentiated into antigen-specific T helper type 1, type 2 or type 17 cells depending on the cytokine environment.[25,26] Although other cells, such as B cells and macrophages, can also present antigens, DCs are the most efficient professional antigen-presenting cells.

Generation of antigen-specific tolerance

Dendritic cells are important not only in the generation of T-cell immune responses, but also in immune tolerance. As antigens can be derived endogenously from the host cells or externally from foreign entities, the immune system has to be able to distinguish between innocuous and harmful antigens to avoid autoimmune diseases or undesired immune responses. Therefore, immune tolerance has to be in place to maintain homeostatic balance. The antigen-specific immune tolerance can be generated in thymus or peripheral tissues. T cells are developed in the thymus from their progenitors, which enter the thymus through blood vessels near the corticomedullary junction. Within the thymus, T cells go through positive and negative selection processes in anatomically different locations to shape the entire peripheral T-cell repertoire. New developments regarding T-cell selection and generation of immune tolerance in the thymus has recently been reviewed by Klein *et al.*[27] It is clear that in addition to thymic epithelial cells, DCs in thymus play an important role in the generation of central immune tolerance.[27,28] The central tolerance is achieved by deletion of potentially self-reactive antigen-specific T cells as well as by the generation of regulatory T (Treg) cells.[29] Antigen-specific Treg cells generated in the thymus are $CD4^+$ $CD25^+$ $Foxp3^+$ and also called natural regulatory T cells or nTreg cells. These cells exert their tolerogenic effects on other T cells in a contact-dependent fashion along with secretion of anti-inflammatory cytokines such as transforming growth factor-β (TGF-β) and interleukin-10 (IL-10).[30]

Peripheral tolerance can be generated by DCs via several mechanisms, including generation and expansion of Treg cells, T-cell deletion or induction of anergy.[31–33] $CD4^+$ $CD25^+$ $Foxp3^+$ Treg cells can develop in the periphery under subimmunogenic antigen presentation, during chronic inflammation or during normal homeostasis of the gut.[34] These induced regulatory T cells are also called iTreg cells. In addition, there are other types of peripheral Treg cells, such as Th3 ($CD4^+$ TGF-β^+Foxp3^+) and Tr1 ($CD4^+$ IL-10^+ $Foxp3^-$).[35] Dendritic cells are shown to be essential for the development of these Treg cells both *in vitro* and *in vivo*.[36] In addition to the generation of Treg cells, presentation of antigens from dying cells by DCs[37] or low doses of intact soluble proteins targeted to DCs in the steady state,[2] can lead to antigen-specific tolerance through deletion of corresponding T cells. Finally, T-cell anergy (partial or total unresponsiveness) is an important part of peripheral tolerance[38,39] and DCs are required for generation of T-cell anergy.

Modulation of functions of other cells

In addition to the antigen-specific activation of T cells for the development of immunity and tolerance, DCs can influence the functions of other immune cells.[3] First, DCs are shown to play an important role in B-cell activation.[40,41] Although DCs are known to process internalized

antigens to present degradative products on MHC for T-cell receptor recognition, they can retain antigens in their native form for the engagement of B-cell receptors on B cells. Second, DCs are involved in priming and proliferation of natural killer (NK) cells.[42,43] Natural killer cells play an important role in host defence against viral infection; IL-15 is required for NK development[44,45] and DCs are required to trans-present IL-15 on their high affinity IL-15 receptor to activate NK cells[42] and to produce IL-15 for NK proliferation.[43] Third, DCs are required for myeloid homeostasis; depletion of murine DCs in mice leads to systemic myeloid expansion.[3] The elevation of the serum levels of Flt3L because of the lack of DCs is probably responsible for the myeloid expansion. Finally, DCs are required for implantation during embryonic development.[46] It has been shown in a transgenic mouse model allowing conditional ablation of uterine DCs in a spatially and temporally regulated manner that depletion of uterine DCs resulted in a severe impairment of the implantation process, leading to embryo resorption.[46]

In vitro generation of tolerogenic DCs

Following the establishment of protocols for the generation of DCs from murine bone marrow[47,48] or human peripheral blood,[49] the potential of DCs for clinical applications has been under intensive investigation.[2,50,51] As DCs are involved in the regulation of both immunity and tolerance, they have, conceptually, a variety of clinical applications for treatment of diseases that result from immune deficiency or imbalance. Over the past two decades, a large body of information has been accumulated regarding DC development,[3–5] maturation,[51–53] and migration,[54,55]as well as *in vitro* manipulation.[47,48,56,57] This accumulated body of knowledge is useful for therapeutic development using antigen-loaded tolerogenic DCs.

Growth factor-engendered immature DCs

Since immature DCs play a key role in maintaining self-tolerance under steady-state conditions, considerable effort has been made over the last decade to use immature DCs generated *in vitro* for tolerance induction.[58,59] The most commonly used growth factors are IL-10, TGF-β, hepatocyte growth factor and vasoactive intestinal peptide.[60–62] Dendritic cells generated under these conditions typically present low numbers of self-peptide–MHC complexes (signal 1) coupled with limited co-stimulatory molecule expression (signal 2) and pro-inflammatory cytokine production (signal 3), leading to T-cell anergy and apoptosis.[63] Upon adoptive transfer, antigen-specific tolerance induction could be achieved in a variety of animal models. More encouragingly, Lutz and colleagues have shown that immature DCs can be generated from human bone marrow by low doses of granulocyte–macrophage colony-stimulating factor (GM-CSF) in the absence of IL-4 under good manufacturing practices conditions. Dendritic cells generated in such a way are resistant to maturation in response to Toll-like receptor agonists or CD40 ligation,[64] though their ability to retain an immature state *in vivo* following adoptive transfer remains elusive.

Genetically modified immature DCs

One attempt to maintain the immature phenotype following *in vivo* delivery is to genetically modify DCs with viral vectors that express anti-inflammatory cytokines such as IL-10, IL-4 or TGF-β.[65,66] Presumably, continued exposure to these cytokines secreted by DCs themselves will prevent them from undergoing maturation. Furthermore, these cytokines may provide additional help to facilitate the generation of regulatory T cells or deviate from the T helper type 2 response during DC–T-cell interaction.[67–70] This gene-transfer approach has also been extended to encode other transgenes such as pro-apoptotic molecules (e.g. CD95 ligand and tumour necrosis factor-related apoptosis-inducing ligand) and immunoregulatory proteins (e.g. Indoleamine 2,3-dioxygenase and cytotoxic T lymphocyte antigen 4), which directly eliminate effector T cells or suppress their functions.[71–75] In addition to viral vectors, recent development of small interference RNA technology allows silencing of pro-inflammatory cytokine, such as IL-12, to reduce DC immunogenicity.[76]

Immunosuppressive drug-induced tolerogenic DCs

Immunosuppressive drugs such as corticosteroids, cyclosporine, tacrolimus, rapamycin, deoxyspergualin, vitamin D3, mycophenolate mofetil and sanglifehrin A have been used to modulate DC differentiation and function. Lee *et al.*[77] showed that cyclosporine A or tacrilumus could inhibit DC maturation through blocking nuclear factor-κB. This effect could be overcome, at least in part, by IL-4 but augmented by TGF-β. Generation of tolerogenic DCs by LF15-0195, a potent deoxyspergualin, appears also to be through suppression of nuclear factor-κB signalling and these DCs are refractory to tumour necrosis factor-α or lipopolysaccharide-induced maturation, retaining a stable immature state.[78]

Both vitamin D3 and dexamethasone can influence DC differentiation by down-regulating their capacity for IL-12p70 secretion. The resultant DCs are able to convert $CD4^+$ T cells into IL-10-secreting Treg cells, potently suppressing the proliferation of responder T cells. However, up-regulation of PD-L1 was noted only on vitamin D3-treated DCs but not dexamethasone-treated DCs and blockade of PD-L1 abolished the regulatory capacity of vitamin D3-DCs.[79] Different from other immunosuppressive drugs, rapamycin appears to have a more versatile effect on DC differentiation and function. It has

been reported that rapamycin suppresses the functional activation of bone-marrow-derived DCs that can induce the production of $CD4^+$ $CD25^+$ $foxp3^+$ Treg cells, both *in vitro* and *in vivo*, and promote organ transplant tolerance.[80–82] Others reported that inhibition of mammalian target of rapamycin (mTOR) during differentiation did not affect the acquisition of a DC phenotype but instead promoted apoptosis of monocyte- or CD34-derived DCs.[83,84] However, a recent study showed that rapamycin increased pro-inflammatory cytokines produced by Toll-like receptor-activated myeloid DCs, blocked their IL-10 production and signal transducer and activator of transcription 3 activity, and significantly increased the T-cell allostimulatory potential of myeloid DCs.[85] Similarly, rapamycin has been linked to enhanced antigen presentation through induction of autophagy in DCs.[86] Collectively, mTOR exerts divergent immunoregulatory functions during DC activation and differentiation depending on the DC type, and the dose and duration of rapamycin exposure.

Potential applications of tolerogenic DCs

In addition to *in vitro* manipulation of DC phenotype and functions, strategies to target immature DCs *in vivo* have also been investigated. For instance, targeting of DEC-205-expressing $CD8\alpha^+$ DC with an anti-DEC-205 antibody–peptide fusion molecule results in efficient deletion of the corresponding peptide-specific T cells, and induction of FoxP3-expressing Treg cells.[87,88] An additional example is the tolerogenic influence of apoptotic cells on immature DCs.[89–93] Together, these *in vitro* and *in vivo* approaches offer potential applications of tolerogenic DCs in autoimmunity, acquired immune diseases, gene and cell therapy, and transplantation.

Autoimmune diseases

Autoimmune diseases occur when immune responses of patients are directed against antigens produced from their own cells. There is a long list of autoimmune diseases, such as rheumatoid arthritis, psoriasis, multiple sclerosis, type 1 diabetes and systemic lupus erythematosus, all of which are caused by dysfunctions of the immune system. Despite a variety of therapeutic strategies being proposed,[94–99] many of these diseases are still managed with non-specific medications such as corticosteroids or chemotherapeutic drugs, which are associated with considerable adverse events.[100] Because DCs regulate both central and peripheral tolerance, they have been explored for treating autoimmune diseases using animal models.[101–104] The rationale of using DCs for treating autoimmune diseases has further been supported by the recent finding that constitutive ablation of DCs results in spontaneous fatal autoimmunity.[105] As the antigens involved in some of these diseases are known, it is feasible to generate antigen-specific tolerogenic DCs to treat these diseases. If antigen-specific tolerance can be established, there should be no major impact on host immune responses against tumour cells or microbial and viral infections.

Hypersensitivity diseases

Hypersensitivity diseases are also caused by unbalanced immune responses, but to foreign antigens, these diseases can be classified into type I, II, III and IV based on the types of antibodies or cells involved in mediation of the diseases. Because of space limitation, only type I hypersensitivity diseases will be used as an example to discuss the possible therapeutic application of tolerogenic DCs. A wide range of commonly occurring diseases, such as asthma and allergies, belong to type I hypersensitivity diseases. Patients with type I hypersensitivity over-react to certain common antigens in the environment by producing high levels of IgE antibodies although these antigens do not cause immune responses in healthy individuals. The immune responses to allergens in these patients are deviated towards T helper type 2 responses. Therefore, antigen-specific tolerance can be potentially generated with tolerogenic DCs.

Gene and cell therapy

Gene and cell therapies hold great potential for treatment of human diseases.[106–108] For gene therapy, host immune responses against gene therapy vectors are one of the barriers to its clinical applications. Potentially, therapeutic gene products can be immunogenic as well. Viral vectors are in general more efficient for gene delivery. Although most advanced viral vectors do not express viral genes, repeated administration still leads to a decrease in transgene expression and an increase in the immune responses against the vector. Hence, there is a need to induce antigen-specific tolerance towards viral vectors without compromising immunity towards wild-type virus. A general tolerance induction is not safe because patients need immunity against infection and tumour formation. The induction of vector-specific tolerance will be a major breakthrough because it will mitigate the current problem associated with re-administration of gene therapy vectors. For cell therapy, if non-self cells are used, alloantigens from donor cells will induce immune responses. Even if autologous cells are used, if cells need gene-correction, the therapeutic gene may cause problems. Therefore, antigen-specific tolerance can be useful for enhancing cell therapy.

Transplantation

In transplantation, alloantigens from donor cells can be presented to recipient T cells in at least three different

pathways, direct, indirect and semi-direct.[109,110] The direct pathway involves donor DCs in grafts presenting antigens to recipient T cells whereas the indirect pathway involves recipient DCs presenting donor antigens to recipient T cells. Donor MHC complexes can be transferred from donor DCs to recipient DCs through exsomomes or membrane contacts and presentation of alloantigens in this way is called a semi-direct pathway.[109,111] Although there are no human studies reported for the successful application of DCs in transplantation, many transplantation studies using rodent models showed that modified DCs can efficiently enhance graft survival.[111–117] The potential of using tolerogenic DCs in transplantation is enormous, but more research is needed to translate the success of using tolerogenic DCs in rodent models into human applications.

Challenges to the therapeutic development

There are many hurdles to be overcome before tolerogenic DCs can be used in clinical applications. These challenges include defining the nature of DCs to be used for a particular application, standardizing protocols for their generation, identifying the optimal route for their delivery, and translating the success obtained with rodent models into human applications. However, none of these challenges are insurmountable.

First, to achieve consistent tolerogenic effects for a particular application, it will be important to define qualitative and quantitative characteristics for a type of DCs to be used for a particular application. As DCs are heterogeneous and display different cell surface markers in different tissues, it is unlikely that a single set of surface markers can be used to standardize the tolerogenic DCs for different applications. It is generally believed that immature DCs induce tolerance. However, how to sustain the immature state of DCs, especially after *in vivo* delivery, remains a challenge. Therefore, it is important to identify experimentally the type of DCs with the best tolerogenic ability for a particular application and to define their surface markers qualitatively and quantitatively. Currently, little has been done in this area.

Second, the methods for generation of tolerogenic DCs have to be standardized. The DCs can be generated *ex vivo* from progenitor cells of different tissue sources such as bone marrow, peripheral blood and perhaps induced pluripotent stem cells[118] as well. In addition to the source of progenitor cells, there are different protocols using different growth factors for generating DCs. Traditionally, most studies use GM-CSF and IL-4 to generate DCs from precursor cells from bone marrow or blood[56] whereas newer protocols use the Flt3L.[57] Furthermore, the amount of antigens to be loaded should also be optimized because this may affect the behaviour of DCs. For some applications, the antigens may need to be identified first. Without the precise information on antigens, it is unlikely that antigen-specific tolerogenic DCs can be generated.

Third, methods for delivery of tolerogenic DCs have to be defined. Although it is known that DCs can be delivered though different routes, such as intravenous or subcutaneous injections into mice[28,119] or humans,[120] the efficiency of the delivered DCs reaching their destinations is very low. When DCs are delivered intravenously into mice, both mature and immature DCs can reach the spleen at the efficiency of 1–1·5%.[28] Interestingly, the delivered cells can home to the thymus, but at a much lower efficiency. A large portion of DCs delivered do not reach their destinations, so their effect on the immune system needs to be clarified. It is likely that DCs delivered through different routes will show differences in homing efficiency to different tissues. This will influence their tolerogenic effects on different organ systems. Information on DC migration *in vivo* is scarce and little is known about which route of DC delivery should be used for which type of application. In addition, the amount of DCs to be used for a particular application to achieve the optimal effect remains to be determined. DCs in tissues are likely interacting with other cells. It is also not known what changes might occur when DCs are delivered.

In addition, most studies on tolerogenic DCs have been performed in rodents and more studies are needed to translate the experimental success in rodent models into human applications. Although rodents and humans share great similarities in immunology, there are major differences in the cell biology of organs. For example, the lung airway epithelium in mice and humans is quite different. Mouse airway epithelium contains a large population of non-ciliated, non-mucous secretion cells, called Clara cells, which secrete a 10 kDa molecular weight protein (CC10) that has anti-inflammatory effects.[121,122] On the other hand, the human lung airway contains only a small percentage of Clara cells located at the terminal bronchioles.[123] As this difference in cell biology is expected to affect the local cytokine environment, it will influence the innate and adaptive immune responses locally. Therefore, therapeutic strategies for human applications have to reflect this type of difference.

Finally, commercialization of tolerogenic DCs could also be a challenge because this type of medication will be expensive to manufacture. However, as DC biology advances and protocols for producing tolerogenic DCs become standardized, hospital laboratories may be able to provide the service as long as the cost can be absorbed by the health-care system of the jurisdiction.

Concluding remarks

Considerable progress has been made in our understanding of DCs: their development, behaviour and function. Importantly, DCs can be generated in culture from

precursors of different tissue sources. Studies in animals have demonstrated the enormous potential of using tolerogenic DCs for treating a variety of immune-imbalanced diseases as well as for enhancing future gene and cell therapies. This potential can be realized only when all the major challenges are overcome with more intensive experimental investigations.

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