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Review

Dendritic cells: In the forefront of immunopathogenesis and vaccine development – A review

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Abstract

Dendritic cellls (DCs) comprise an essential component of the immune system. These cells, as antigen presenting cells (APCs) to naïve T cells, are crucial in the initiation of antigen specific immune responses. In the past years, several DC subsets have been identified in different organs which exert different effects in order to elicit adaptive immune responses. Thus, identification of such DC subsets has led to a better understanding of their distribution and function in the body. In this review, several key properties of the immunobiology, immunopathogenesis and vaccine strategies using DCs will be discussed.

Review

Dendritic cellls (DCs) are a complex, heterogeneous group of multifunctional APCs. DCs are leukocytes, distributed throughout lymphoid and non-lymphoid tissues, in peripheral blood and afferent lymph vessels [1]. It has been shown that DCs after activation with different stimuli achieve maturation, where they express high levels of several molecules on the cell surface such as MHC class I and II, accessory molecules CD40, CD80, CD86 and early activation markers such as CD83. These cells do not proliferate and after a certain time course they undergo apoptosis and will be replaced by a new pool of cells [1]. Functionally, DCs exert various effects on other immune cells, particularly in secondary lymphoid organs; DCs present non-self peptide-MHC complexes to naïve and memory T lymphocytes to mobilize specific immunity [1-4]. By contrast, in order to induce T cell-tolerance in the thymus, DCs present self peptide-MHC complexes to thymocytes [5]. The capacity of DCs to initiate primary immune responses is due to their ability to deliver specific costimulatory signals which are essential for T cell activation from the resting or naive state into distinct classes of effector cells. These immunogen-specific immune responses are critical for example, to tumor resistance, prevention of metastasis, and blocking infections. DCs also can alter the function of regulatory T cells that control activated T cells through their suppressive signals. In addition, DCs play an important role in innate immunity by secreting cytokines, e.g. IL-12 and Interferon classes I and II, involved in host defense. Moreover, DCs activate Natural killer cells (NK) and NKT cells that rapidly eradicate select targets [1]. Such diverse functions of DCs has begun to shed light on their pre-eminent role in immunological events. In this review we highlight several critical aspects of DCs in order to better understand host-pathogen interactions.

Origin and developmental processes of dendritic cells

DCs originate from hematopoietic stem cells in the bone marrow. Recently, there have been great insights into the origins of DC subsets [6,7] and their modulation by distinct cytokines of neighboring cells [8,9]. Progenitors of DCs in bone marrow migrate via the blood stream and home to peripheral tissues where they encounter several essential growth factors such as GM-CSF, IL-4, IL-15, TNF-α, TGF-β, and IL-3 secreted by various cell types including endothelial cells, Mast cells, keratinocytes and fibroblasts in the microenvironment (Figure 1). Such growth factors determine the fate of the progenitors to differentiate into immature Langerhans DCs, interstitial DCs or plasmacytoid DCs (Figure 1).

One of the hallmarks of DC progenitors is their capacity to migrate [10]. Cutaneous and nonlymphoid DC populations migrate to T-cell areas (Figure 2). For example, cutaneous interstitial DCs enter mesenteric lymph nodes [11]. Liver $OX62^+$ DCs, which reside in the portal triads [12] and along the sinusoids [13], migrate into hepatic lymph and subsequently to the celiac lymph nodes [14]. Experimentally it has been shown that isolated DCs from several organs that were reinfused into animals, within 24 hrs, home to the T cell rich area of the draining lymph nodes. Homed DCs sample and select very rare antigen specific primary T cells from the recirculating stream [15].

In addition, DC subsets are ready to confront invading pathogens [1]. In such environments DCs ingest antigens via several mechanisms including phagocytosis [15] and receptor-mediated endocytosis [16]. For example Langerhans DCs phagocytose, process, and present immunogenic peptides to T cells [1,16,17].

Antigenic infectious agents including vaccines induce pro-inflammatory cytokines (e.g., TNF-α). These cytokines promote Langerhans DC maturation in lymphoid organs where they home to the T cell rich area [18]. Langerhans DCs undergo phenotypic and functional changes during their maturation and migration. These cells, which are now loaded with antigenic peptides on MHC class II, down-regulate CD1a, CCR6, and E-cadherin, and lose the capacity to capture foreign antigens [9,18]. Mature DCs are an end stage of differentiation, and they can not be converted into either macrophages or lymphocytes.

DCs in general present marked heterogeneity in phenotype and function, which relate to their precise localizations within different tissues in the body. However, DCs do not express phenotypic markers of T lymphocytes (e.g. CD3, CD16, CD19, CD28), B cells (Ig and CD19, CD20), or NK cells (CD 16, CD56, CD57). In some instances, DCs express molecules that are also expressed on macrophages, and while the phenotypic distinction between DCs and macrophages is not always clear; studies with respect to their immunostimulatory functions (e.g., primary Mixed Lymphocyte Reaction) provide clear evidence between these two types of antigen presenting cells. In addition, DCs also express surface molecules which are specifically expressed on T cell subsets (e.g., CD4), and a DC subset residing in murine lymphoid organs express CD8α marker [7].

Functions of dendritic cells

The role of DCs has been repeatedly highlighted in cancer and infectious diseases [1]. Human $CD14^+$ progenitor DCs cultured in GM-CSF+IL-4 are equivalent to interstitial DCs (e.g., dermal DCs) and express CD1a, CD64 and Factor III a [16]. By contrast, monocytes cultured with M-CSF convert to a monocyte/macrophage phenotype [18]. These myeloid DCs home within lymphoid follicles, where they reside as germinal center DCs [19]. In this area, germinal center DCs establish the contact between T- and B-cells, which may lead to the stimulation of an active immune response [20]. DCs present processed antigenic peptides on MHC class II molecules to $CD4^+$ T cells [21], which will be activated in conjunction with co-stimulatory signals (e.g., CD40, CD86) delivered from DCs in lymphoid organs. Several receptors and their ligands are involved in the T cell/DC dialogue, e.g., CD40/CD40L [22]. For instance, up-regulation of CD40L on T cells facilitates DC maturation [23]. Activated DCs then release cytokines such as IL-12, which modulate and stimulate the production of IFN-γ from T cells [24]. Activated DCs can either prime naive $CD8^+$ T cells, or they undergo apoptosis *in situ* [25]. Activated T cells migrate to the area of the B-cell follicles via activated adhesion molecules [26-28]. There they interact with naïve antigen-specific B cells [29]. T- and B-cell interaction results in the clonal expansion of B cells, which takes place in the plasma foci of the T cell rich area [30] and in the germinal centers [31]. T- and B-cell dialogue in the germinal center might be influenced by germinal center DCs [20] and follicular DCs [30] (Figure 3).

Dendritic cells and T-cell tolerance

T cells before they encounter immunogenic antigens must undergo a step where the T cell repertoire is tolerized to self-antigen. This process when it occurs in the thymus is called central tolerance. It occurs by deletion of developing T lymphocytes; in the lymphoid organs, it is called peripheral tolerance by probably eliminating or anergizing committed mature T cells. In both situations, as discussed before, DCs not only induce primary antigen specific T cell immune responses but these cells also appear to induce tolerance of T cells to self-antigens. DCs present self-antigen via MHC class molecules in the thymic medulla. Experimentally it has been shown that if

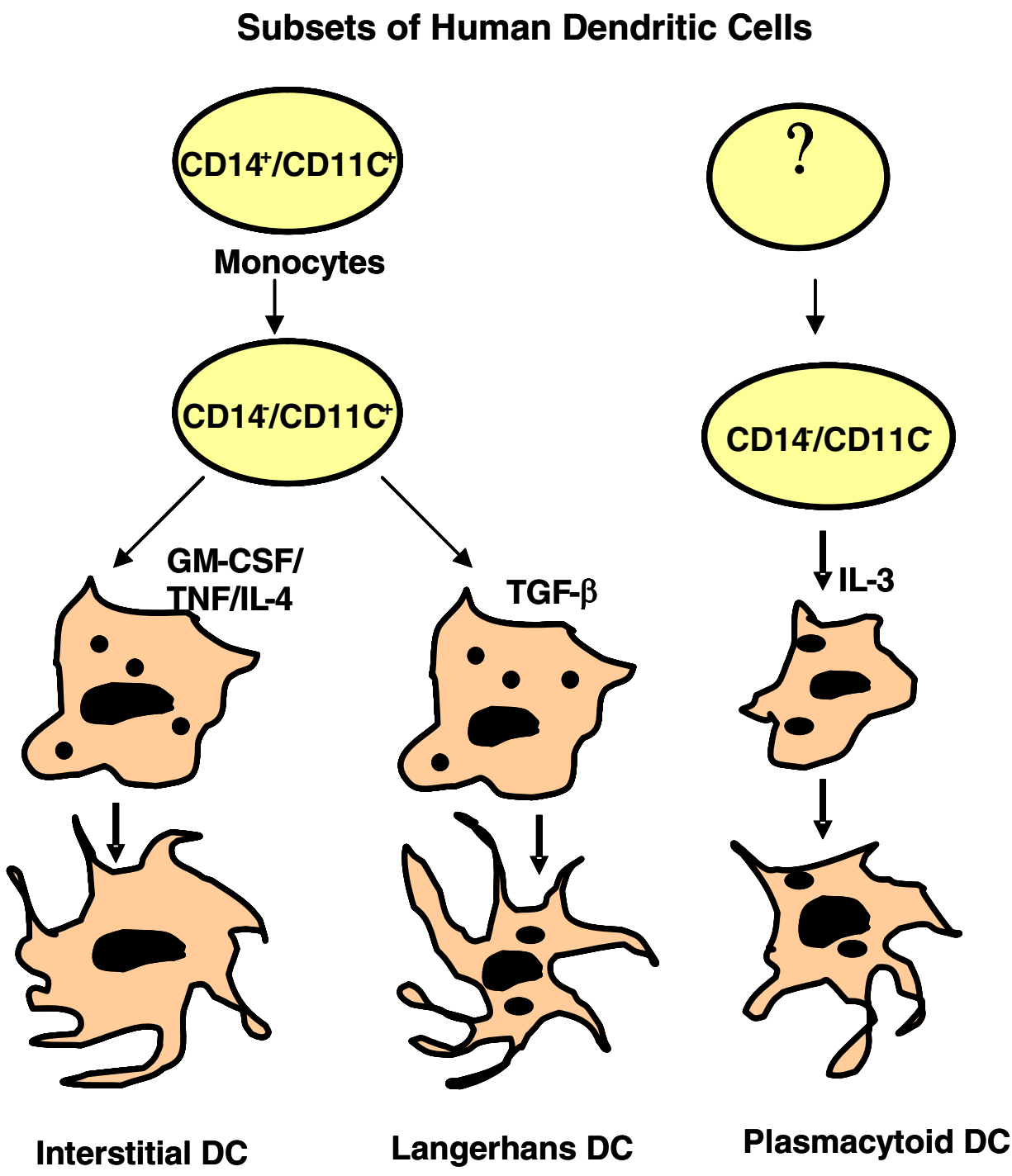

Figure 1
Human DC subsets. DC progenitors migrate from the bone marrow in the periphery and several different tissues. There they encounter various growth factors which determine the fate of these cells to differentiate into immature DC subsets.

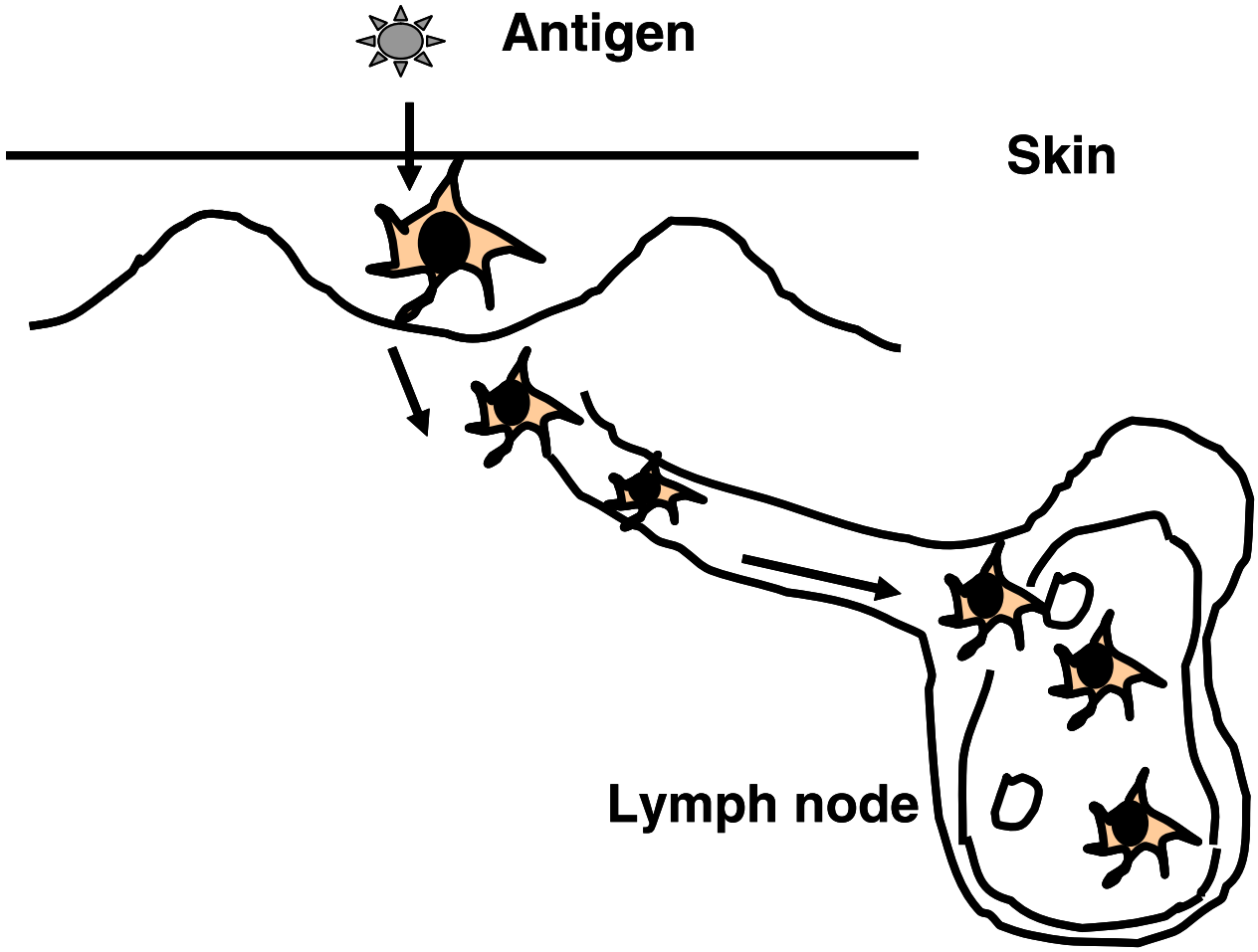

Figure 2
Migration of immature DCs into lymphatic organs. Skin surrounded by various immunogen antigens that can penetrate the epidermis. These antigens can be captured by immature Langerhans DCs, and processed. Cutaneous DCs will then be activated, migrate, and home to the lymph nodes. Matured DCs present processed antigen to antigen specific T cells inducing specific immunity.

antigen-loaded DCs are administrated to developing or fetal thymus reactive T lymphocytes, they will be deleted indicating that DCs play a critical role in this process.

Moreover, in the cortical area of the thymus, although macrophages phagocytize dying T cells which did not undergo positive selection these cells seem not to be involved in deleting auto-reactive T cells. Studies show that if MHC class II molecules are solely expressed by the cortical epithelium and not by DCs residing in the medulla, there is a higher probability towards an autoimmune disease. These results highlight the critical role of DCs in educative processes of thymic T cells to self-antigens. In addition, DCs play a critical role in peripheral tolerance by presenting self-antigen to T cells residing in specialized tissues such as the pancreas [32,33]. Presentation of processed self-antigen as peptides by DCs ensures T cell tolerance probably through T cell deletion or anergy [32-34].

The role of dendritic cells in clinical diseases

Recent studies shed light on the role of DC involvement in various diseases such as autoimmunity, allergy, transplantation, infection and cancer. For example, studies showed that DCs differentiated *in vitro* express very important co-stimulatory molecules, e.g. CD40, which allow these cells to approach T cells and deliver signals to them [22,23]. With respect to that phenomenon,

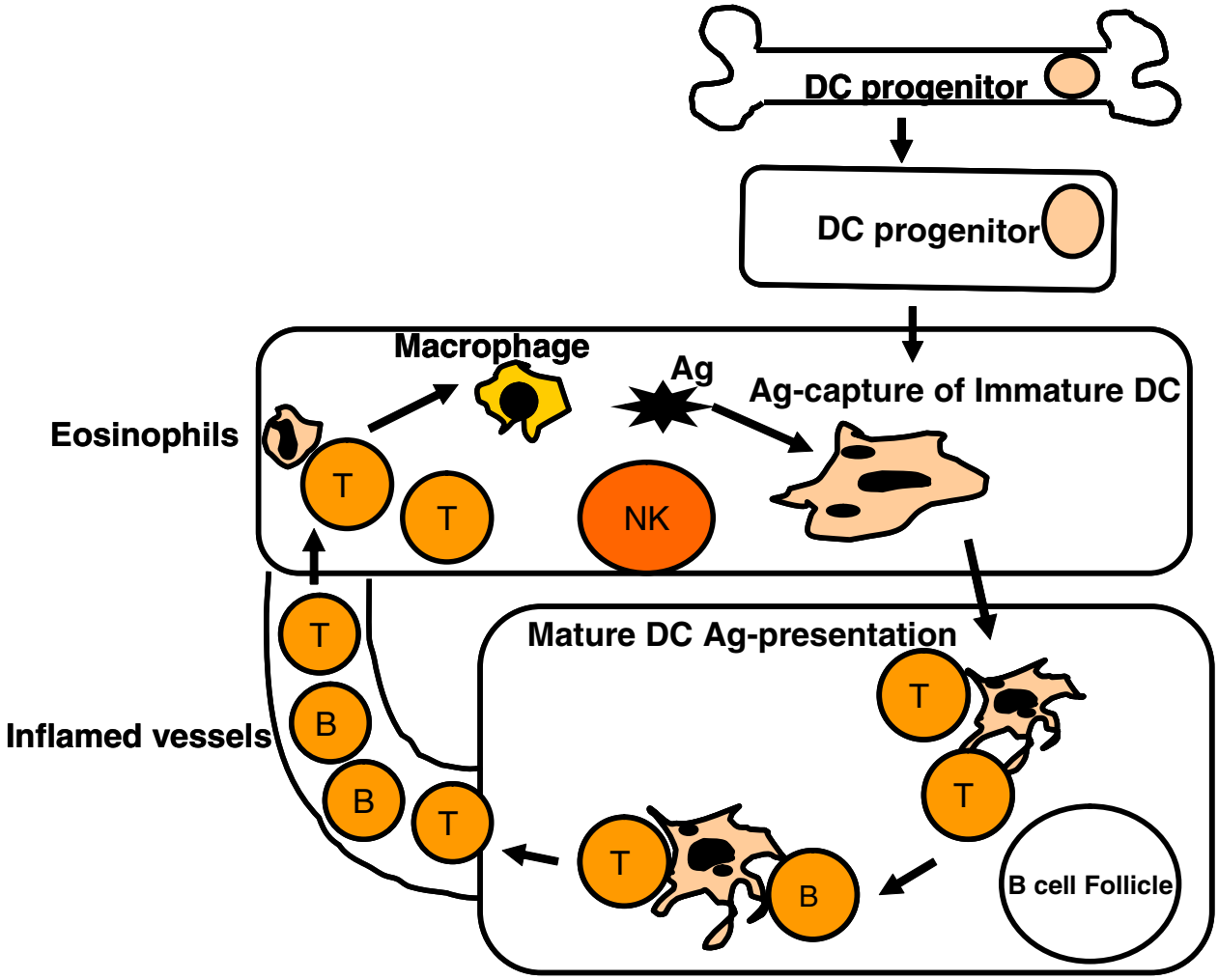

Figure 3
Induction of primary immune responses by DCs. The DC lineage comprises cells at different stages of differentiation and development in different tissues. The currently accepted scheme suggests that DCs from bone marrow move via the blood into non-lymphoid tissues. In these organs they undergo different changes with respect to shape, functions. In these organs DCs induce primary T cell immune responses.

cytokines (e.g., GM-CSF, TNF-α) produced by keratinocytes affect DC differentiation dramatically [35]. Moreover, DCs alone produce essential cytokines (e.g. IL-1β, TNF-α, IL-6), and chemokines MIP-1α, MIP-1γ, IL-15 and IL-8 [9,36-39]. Some of these cytokines contribute directly to the DCs ability to attract and recruit T cells in sites of inflammation. A number of autoimmune diseases (rheumatoid arthritis) or skin psoriasis demonstrates the accumulation of DCs in diseased tissues [40]. This evidence suggests that DC enrichment within the cytokine-rich synosium or epidermis undergo phenotypic and functional maturation *in vivo*. Furthermore, it seems that the ligation of CD40 with DCs can enhance the antigen presenting capacity of these cells [22]. It has recently been reported that rheumatoid arthritis synovial T lymphocytes express CD40L at a low level. These molecules can be dramatically upregulated when T cells are activated. In this context, stimulation of self-reactive T lymphocytes in the synosium will be induced through GM-CSF and TNF-α along with $CD80^+$ $C086^+$ DCs [41].

As mentioned above, cytokines can control the development and differentiation of DCs. For example, the combination of GM-CSF and TNF-α can promote differentiation of $CD34^+$ blood stem cells into DCs in humans [6]. Phenotypically these cells are $CD4^+CD11C^+$ since Langerhans DCs and other DC family numbers express CD4 molecules that can bind to the HIV surface envelope protein gp 120 [42]. This makes a possibility stronger that DCs may contribute to HIV pathology. On one hand, in vivo and in vitro experiments indicate that the replication of HIV-1 virus occurs during cognate $CD4^+$ T cell activation through DCs. On the other hand, there is evidence that the features of HIV pathology are an accumulation of HIV virus in the germinal centers, which is T cell rich and where a novel DC population has recently been identified [20]. Both the APC function of DCs and their close interaction with $CD4^+$ T cells suggests that germinal centers of lymph nodes may provide an additional site for HIV viral replication [42-44].

Moreover, DCs in transplanted organs are involved and they represent potent "passenger leukocytes" that sensitize host graft antigens and trigger rejection [45]. Studies have shown that the depletion of DCs from mouse islets or thyroid tissue prolonged survival in allogeneic recipients [45]. Other studies on the function of DCs after transplantation of skin and heart tissues to allogeneic recipients have shown that soon after grafting, DCs enter the recipient's lymphoid tissues [46]. Thus, there appears to be a sensitization of host T cells which occurs primarily in these tissues when they encounter the graft-derived, allogeneic DCs. Austyn et al. showed recently that host DCs can also present graft antigens to host T cells [46]. In this process it seems that host DCs bearing graft molecules would migrate into the secondary lymphoid organs to sensitize and activate T lymphocytes and induce graft rejection.

It is clear now, that cancer cells can express tumor associated antigens, which are recognized by host T cells. These T cells may not be able to reject tumor cells. These molecules, then, are not immunogenic. In order to become immunogenic they must be processed and presented by professional antigen presenting cells (APC). Since DCs possess relevant features, e.g. a) internalizing of immunogenic antigen through endocytosis, b) phagocytosis for subsequent processing and presentation of several antigens to T cells, and c) migration capability, they could acquire tumor antigen.

In the past few years the role of DCs in cancer has been suggested. There is evidence that DCs can induce immunity to tumors if they are administrated to animals or exposed to tumor associated antigen (TAA) before or when the tumor is inoculated into animals [47-49]. For example, Boczkowski et al. [50] conducted several elegant experiments to demonstrate that DCs pulsed with synthesized chicken ovalbumin (OVA) RNA were more effective than OVA peptide-pulsed DCs in activating primary OVA specific-CTL responses *in vitro*. This finding shows that the amplification of antigens from a small number of tumor cells is feasible, thus increasing the possibility of utilizing RNA-pulsed DC based vaccines for patients bearing very small tumors [50].

Studies demonstrate that when DCs are pulsed with tumor antigens in *ex vivo*, and these cells subsequently readministrated, specific immunity is established [51]. In addition, several studies showed that tumor-specific $CD8^+$ cytotoxic T lymphocytes (CTL) constitute an important effector arm of the anti-tumor immune response [52,53]. In this context to elicit specific immunity against tumor cells, DCs were pulsed with protein or peptide in the presence of lipid [54] or transfected with DNA [55] were capable of eliciting primary CTL responses *in vitro*.

Although prior investigations have established that targeting immune cells to tumors may improve immunity [47-55], in the case of DCs, however, it has been shown [56-62] that the tumor microenvironment is detrimental to DC function, and in fact may condition DCs to induce a T cell response that anergizes or suppresses tumor-specific immunity [56]. Thus, targeting DCs directly to tumors, as demonstrated by several studies, may be inefficient. Therefore, methods should be developed in order to target DCs by immunogenic TAAs outside the tumor microenvironment to improve immunity.

Vaccine design by targeting dendritic cells

Given the central role of DCs in controlling immunity, has brought a scientific focus to the critical role of DCs as an efficient vector in vaccine technology. Several approaches to target DCs efficiently have been designed.

There is a large body of literature involving experimental animal models and for tumors and infection in which DC subsets pulsed with TAAs or subunits of the pathogens such as HCV or HIV are to induce protective immunity against tumors. However, it is even more important to create novel strategies by targeting immunogenic antigens or immune regulatory agents specifically to DCs without impairing the functional properties of DC subsets and in this way modulate the immune responses *in vivo*.

These novel strategies must not be too costly, not immunopathogenic, but specific in order to overcome anergy established through negative signals which may be provided by immune component cells including DCs to the microenvironment.

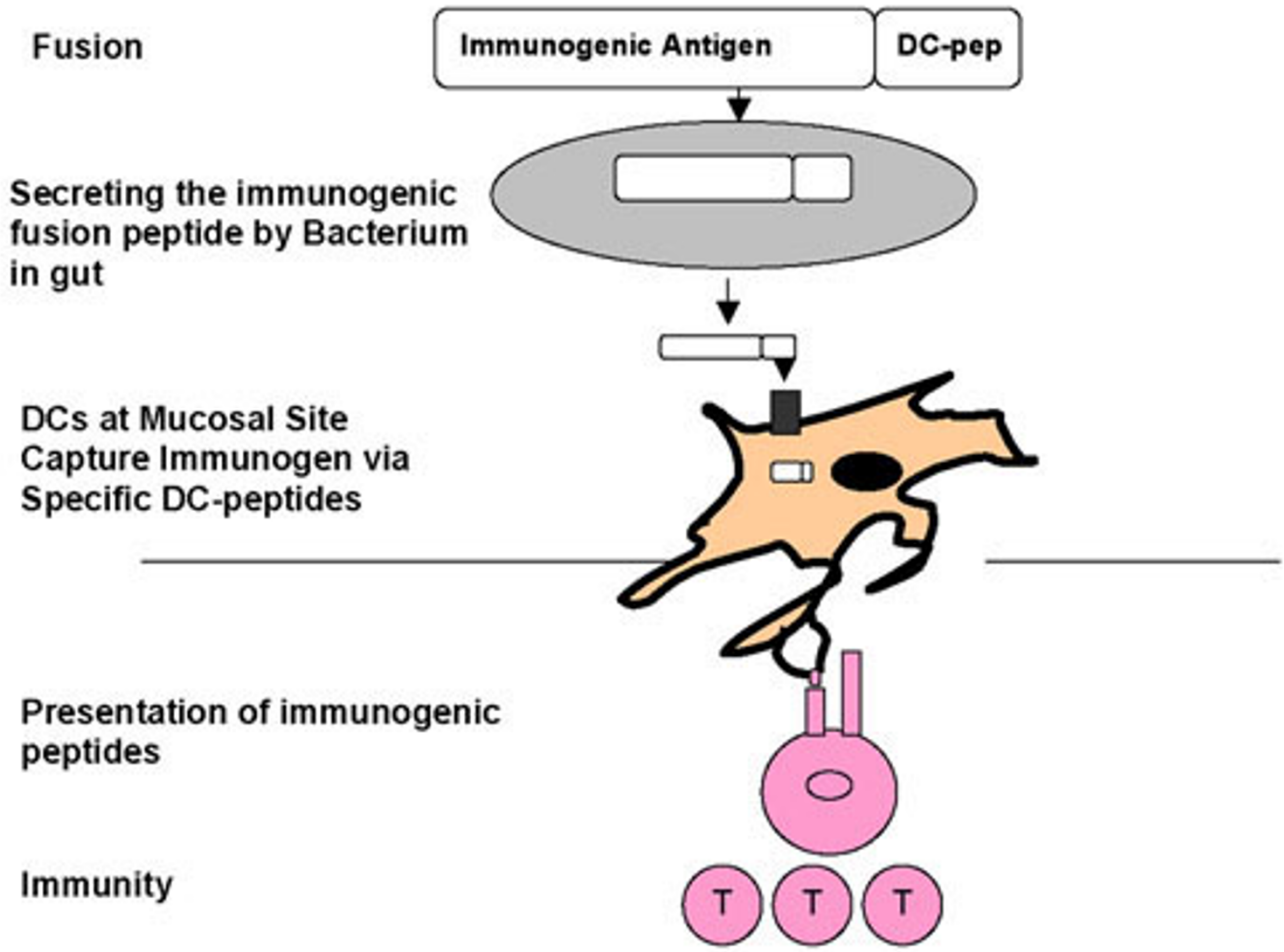

Figure 4

Delivery of immunogenic antigen to DCs by probiotic microorganisms. DNA encoding sequences of DC-binding peptides and immunogenic subunit of any pathogen will be expressed in Gram positive bacteria including *Lactobacillus*. *Lactobacillus* will be orally administrated. These bacteria colonize the gut and express and release the immunogen in the intestine. DCs in the mucosal site will then capture the immunogen via DC-binding peptide motifs. They internalize the immunogen, process and present it to T cells inducing specific responses against released immunogen.

One possible strategy is to target novel molecules expressed on the cell surface of DCs. In this, we and others utilized phage display peptide library to generate small peptides which solely bind to DC subsets and not other cells. DNA sequences encoding DC-peptides can then be fused genetically with TAA coding regions or with the subunit of the pathogen of interest. Immunogenic fusion proteins can be then expressed by probiotic microorganisms such as *Lactobacilli* or attenuated strains of Salmonella *in vivo* (Figure 4). Such novel vaccine strategies should take advantage of mucosal sites in the body, as well as the skin in order to be delivered specifically to DC subsets *in vivo* (Figure 5).

The Peyer's patch is the primary mucosal site for antigen processing in the intestine. Recent *in vivo* studies provide evidence that DC network in the subepithelial dome of Peyer's patches is a critical component in the uptake and processing of luminal antigens. Such uptake may occur by endocytosis or by phagocytosis after passage of antigen through M cells. The DCs then present the processed antigen to $CD4^+$ or $CD8^+$ T cells in the subepithelial dome, or after maturation and migration, to the interfollicular regions where antigen is presented to $CD4^+/CD8^+$ T cells [63]. In this regard, immunohistologic analysis of DC subsets including LCs in Peyer's patch has revealed that the unique microanatomical localization of DC subsets

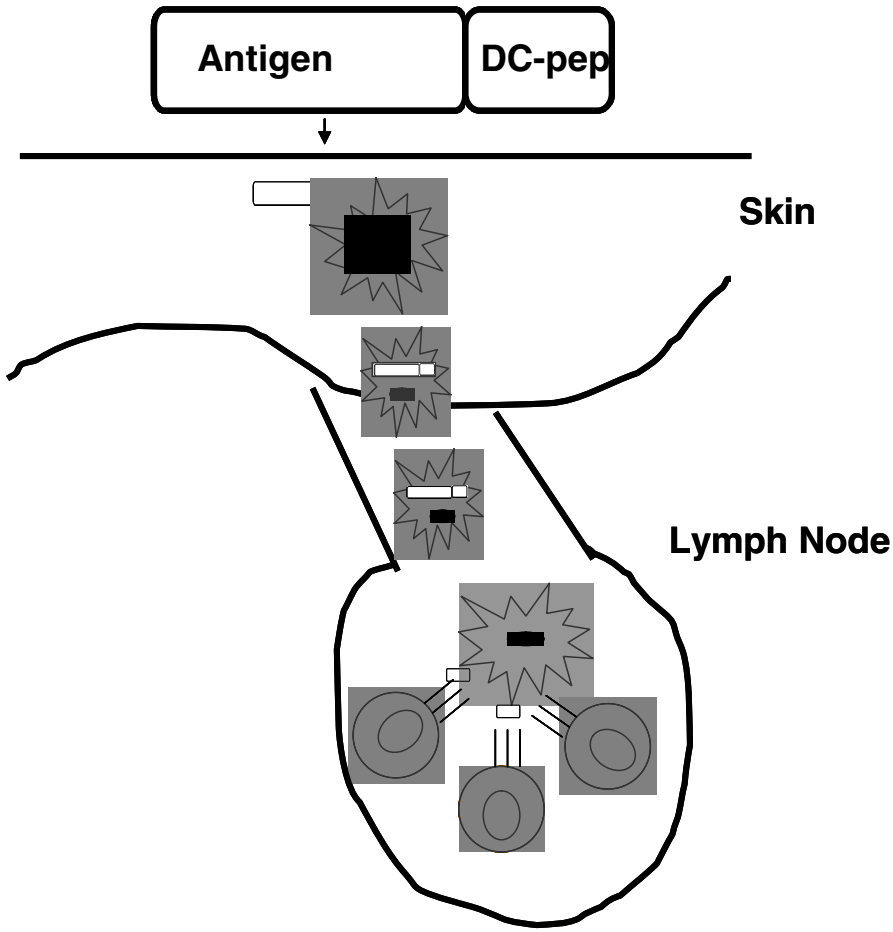

Figure 5
Transdermal delivery of immunogenic fusion protein by cutaneous DCs. Genetically engineered immunogenic fusion protein can be transdermaly administrated into the skin whereby cutaneous DC subsets can capture it via DC-peptide motifs fused to immunogen subunits. Loaded cutaneous DC subsets can be activated, leave the skin and enter the lymph nodes where they can present processed antigen as immunogenic peptides to T cells eliciting specific T cell immune responses.

enables them to regulate specific T- and B-cell responses *in vivo* [63-65]. Other, studies also clearly demonstrated that Gram-positive bacteria such as *Lactobacilli* can successfully be used in order to deliver vaccine peptides to immune component cells [66-68].

More specifically, in order to target any vaccine to DCs, recently, a novel strategy was proposed. Mohamadzadeh et al. fused a subunit of hepatitis C virus with a DC-binding peptide. Studies are ongoing to express such immunogenic fusion proteins by a strain of Lactobacilli [69]. Such a *Lactobacillus* strain will express and secretes the immunogenic protein in the intestinal region. DCs will be able to capture such immunogen via the motifs of DC-binding peptides. Such binding of an immunogen to DCs will facilitate rapid internalization of the immunogen into DCs. DCs will then process and present it to T cells residing in the gut. These cells will be activated and will circulate through the body in order to elicit specific T cell immune responses against the pathogen of interest.

A transdermal delivery system also offers an interesting route to approach DC subsets in order to enhance immunity against cancer or pathogens. Accordingly, the immune system of the skin harbors two very potent antigen-presenting DC subsets which induce primary antigen specific T cell immune responses [70]. Furthermore, careful experimentation of various vaccine delivery routes has shed light on the skin and its immune mechanisms. It has previously been shown that cutaneous DC subsets can be

targeted and activated in situ in order to achieve specific T cell mediated immune responses [70-74]. Thus, the feasibility of using immunogenic DC-peptide fusion proteins should be tested to determine whether administration of such immunogenic fusion proteins will induce the activation of cutaneous DC subsets that in turn prime antigen-specific T cells *in situ*.

DCs play a crucial role in host-pathogen interactions. A recent example [75] involves the report in human papilloma virus 16 which is strongly associated with the development of cervical cancer, that in infected cells the E6 oncogenic protein limits the numbers of LC in infected epidermis. This appears to decrease the host's ability to mount an effective immunological response to HPV 16. We anticipate that future studies will be focused on enhancing functional aspects of DCs to prevent such events and establish novel vaccine strategies to efficiently target immunogenic antigens or inhibitory agents to DCs in order to elicit or suppress specific immune responses *in vivo*.

Conclusions

1. Dendritic cells play a significant role in immunopathogenesis.

2. The functions of dendritic cells involve cancer, infectious diseases and tolerance.

3. Novel approaches in vaccine design can occur by targeting dendritic cells.

Competing interests

None declared.

Author's contributions

Dr. M. Mohamadzadeh is the corresponding author and designed the draft of the manuscript. Dr. R. Luftig contributed to the viral-related segments and overview of the manuscript. Both authors read and approved the final manuscript.

Abbreviations

TNF: Tumor Necrosis Factors

GM-CSF: Granulocyte macrophage colony stimulating Factor

CD: Cluster Density

IL-1: Interleukin-1

TGF: Transforming growth factor

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