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Maturation of function in dendritic cells for tolerance and immunity

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Abstract

The capacity of antigen presenting dendritic cells (DC) to function in both tolerance and immunity is now well documented. The function and characteristics of different DC subsets are reviewed here and their capacity to activate T cells under different conditions of maturation and activation is discussed. The immunogenic potential of exosomes produced by DC is also considered in light of evidence that the capacity of exosomes to activate T cells for tolerance or immunity appears to mirror that of the parent DC. A model is proposed whereby exosomes produced by immature DC can function to maintain peripheral tolerance, while exosomes produced by more mature DC can stimulate effector T cells.

Keywords: dendritic cells • immunity • tolerance • exosomes

Introduction

In 1973, Ralph M. Steinman and Zanvil A. Cohn reported the identification of a novel cell type in the peripheral lymphoid organs of mice [1]. These large plastic-adherent cells were initially identified structurally. Like other mononuclear leukocytes, they

had numerous organelles including abundant mitochondria, endosomes of various structure and density and an irregular eccentric nucleus containing heterochromatin arranged along the nuclear envelope. However, these cells were distinct from other

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cells within lymphoid tissues by their unique cytoplasmic extensions arranged as dendrites of varying length, width, form and number. As a result of their distinct morphology the authors proposed that these cells be termed dendritic cells (DC).

Although initial studies differentiated DC from other leukocytes important in immune responses, like lymphocytes and mononuclear phagocytes, it was not long before their importance in immunity was predicted [1–3]. Within 5 years of their initial characterisation, spleen-derived DC were found to be 100 times more effective than lymphocytes and macrophages in stimulating primary allogeneic mixed leukocyte reactions (MLR) [4]. This finding was extended further by subsequent studies demonstrating that murine DC could cluster with T lymphocytes and initiate a primary syngeneic MLR albeit weaker than the allogeneic MLR response. This property was a function that separated DC from all other spleen cell populations [5]. With these investigations began the characterisation of what is now recognised as the most important antigen presenting cell (APC) in adaptive immunity. Nearly 25 years on, their unique capacity to stimulate naïve lymphocytes, in particular T lymphocytes, is still the most definitive functional characteristic DC can attain [6].

Tissue distribution, subtypes and ontogeny

Early studies on DC indicated they were of bone marrow origin [3]. Developing DC precursors are thought to migrate from bone marrow to blood [7], from where they supply the interstitial DC that can be observed throughout the non-lymphoid peripheral organs of the body [6]. DC have been found in heart, liver, thyroid, pancreas, bladder, kidney, ureter and skin, the latter of which contain the extensively characterised DC termed Langerhans cells (LC) [8, 9]. Fully developed DC have also been observed in the circulatory networks of the body, including blood [10] and afferent lymphatics where they are called veiled cells [11]. These represent DC emigrating from peripheral organs into lymphoid tissues [9]. Within lymphoid tissues, DC can be subdivided into a number of sub-populations based on their expression of cell surface markers.

Currently, the best marker for murine DC in lymphoid tissues is CD11c [12]. However, depending on their location within these organs, and/or their point in development, they can also express combinations of the 'lymphoid markers', CD4 and CD8 α , the 'myeloid markers' CD11b and F4/80, and the markers DEC205 and 33D1 which are relatively restricted to DC populations [7, 12, 13]. DC which have a phenotype: CD4⁺/CD8 α ⁻CD11b⁺F4/80⁺DEC205^{-/low}33D1⁺, are located mainly within the marginal zones of spleen, while CD4⁻CD8 α ⁺CD11b⁻F4/80⁻DEC205⁺33D1⁻DC are located mainly in the T cell-rich paracortical areas of spleen and are termed interdigitating DC [7, 13–15]. CD4⁻CD8 α ⁺CD11b⁻DEC205⁺DC also appear to be the dominant subtype in murine thymus and have also been found in lymph nodes [13]. In addition, lymph nodes contain a CD4⁻CD8 α ⁻CD11b⁺DEC205^{low} subgroup of DC as well as CD4⁻CD8 α ^{low}CD11b⁺DEC205⁺DC which also express Langerin, a characteristic marker of LC. These are thought to be DC immigrants from skin [13].

Murine DC expressing the lymphoid-associated marker, CD8 α , were initially defined as a lymphoid subtype derived from lymphoid precursors based on reports indicating that they could be propagated from CD4^{low} thymic T cell progenitors [16]. DC lacking CD8 α were initially defined as myeloid DC derived from myeloid precursors based on reports demonstrating that they could be propagated efficiently from myeloid progenitors [17]. However, CD8 α ⁺ and CD8 α ⁻ DC have now been derived from both common myeloid and common lymphoid progenitors [18, 19] and it has been shown that CD8 α ⁻ DC can develop into CD8 α ⁺ DC *in vivo* [7]. Furthermore, a common DC precursor has recently been identified in blood, which has a CD11c⁺CD11b⁺B220⁺MHC-II⁻ phenotype and is committed to the production of CD8 α ⁺ and CD8 α ⁻ DC as well as a newly identified DC subset bearing B220 which corresponds to the plasmacytoid DC present in humans [7]. The emerging view of DC development in mice appears to be that bone marrow-derived hemopoietic stem cells can differentiate into many of the DC subsets through either a committed blood CD11c⁺CD11b⁺B220⁺MHC-II⁻ DC precursor, a common myeloid progenitor and/or a common lymphoid progenitor [7].

For those in the field, the study of DC development and the definition of lineage relationships between phenotypically distinct DC subsets has been more difficult than anticipated. In retrospect, the reason for this appears to relate to plasticity in DC development, uncharacteristic of other hematopoietic lineages [20]. Theories on the myeloid or lymphoid lineage relationships between DC subsets have been disputed and corrected over time (see for example ref [21]). The current thinking is that under steady-state or non-inflammatory conditions, there are three main classes of immature DC resident in peripheral lymphoid tissues of mice: the myeloid-like CD11c⁺CD11b⁺CD8 α ⁻DC and the lymphoid-like CD11c⁺CD11b⁻CD8 α ⁺DC making up the 'conventional' DC, and the CD11c^{low}B220⁺ plasmacytoid (p) precursor DC which express CD8 α upon activation [22]. Cells of the p-DC lineage express lymphoid markers including pT α and early D-J rearrangement at the IgH locus [23]. In contrast, monocyte-derived DC develop *in vivo* under inflammatory conditions which drive them from blood into lymph nodes for antigen presentation [24].

In vivo studies have now confirmed that conventional DC and p-DC derive from the Flt3⁺ subset of both common lymphoid and common myeloid progenitors [25, 26]. It is also possible to derive these different DC types by culture of Flt3⁺ bone marrow cells in the presence of different defined cocktails of growth factors including Flt3L [27, 28]. These studies indicate that DC development mediated by Flt3L can occur via multiple pathways from Flt3⁺ bone marrow precursors. However, Flt3L is not specific for DC, and can stimulate expansion of hematopoietic cells of other lineages [29]. *In vivo* evidence in support of this plasticity was obtained after lymphocytic choriomeningitis infection of mice which showed transdifferentiation of p-DC into myeloid-like DC [30]. This plasticity was first detected as an increase in the number of myeloid DC over p-DC but subsequently p-DC derived from infected bone marrow were shown to differentiate into myeloid-like DC after *in vitro* culture with Flt3L [30]. In the least, these two DC subsets must share an immediate common precursor which is responsive to Flt3L. No further committed DC progenitor has been identified other than the Flt3⁺ subsets in bone marrow.

In our hands, splenic stromal cells which support DC development do not express Flt3L transcripts and produce only immature myeloid-like DC [31, 32]. This raises the possibility that a more committed progenitor of myeloid DC is maintained in spleen. Consistent with this hypothesis is evidence that spleen contains a majority of endogenous, immature DC [33] which are thought to be involved in the induction and maintenance of peripheral tolerance [34, 35]. Similarly, the major population of DC in thymus is a CD8 α ⁺ population which arises from an endogenous CD4^{low} lymphoid precursor population [36]. These DC are thought to play a major role in the induction of self tolerance through negative selection.

Functions of dendritic cells and the role of maturation

The paradigm for DC function is to classify DC residing in non-lymphoid peripheral tissues in the immune steady-state as immature. These cells are primarily involved in antigen recognition and uptake. DC that have attained both the capacity to migrate to secondary lymphoid tissues and the capacity to stimulate T cells have been defined as mature. This terminology reflects the functional development of DC.

Immature dendritic cells

The DC located in peripheral tissues in the immune steady-state have characteristics which make them ideally suited to monitor their environment for pathogens and to facilitate their uptake [6]. They are said to be 'immature' and express a large array of receptors that can specifically recognise pathogen-related molecules. These include Toll-like receptor (TLR)-2, TLR-3, TLR-4, TLR-5, TLR-8 and TLR-9 [37], which have specific recognition for a range of molecules including prokaryote-derived lipoproteins, glycolipids, flagellin, CpG DNA and lipopolysaccharides (LPS) [38]. Immature DC, also express several C-type lectins, like the mannose receptor, DEC205 and DC-SIGN, which recognise carbohydrate structures on pathogens [39]. Once in contact with antigen,

immature DC use several pathways to facilitate uptake. These include receptor-mediated endocytosis through C-type lectins and Fc γ II/III [6, 40]. They also have high capacity to non-specifically endocytose particulates and solutes through phagocytosis and macropinocytosis. Although many of these pathways appear to be utilised for uptake of pathogen-related molecules they may also be utilised for uptake of self antigens [41]. Indeed, immature DC also express $\alpha_v\beta_3$ -integrins, $\alpha_v\beta_5$ -integrins and CD36, which help to facilitate continuous uptake of apoptotic material in the immune steady state [42]. These may be important in DC-mediated maintenance of peripheral self tolerance [41].

Once in the endocytic pathway of DC, internalised antigens must be processed before they can be displayed to lymphocytes in association with Major Histocompatibility Complex (MHC) molecules. The endosomes in which this process occurs are present late in the endocytic pathway. They are mildly acidic and contain lysosomal proteins, including lysosome-associated membrane protein (LAMP)-1 and LAMP-2 and the tetraspanins CD63 and CD82 [43]. The acidic nature of these endosomes and an abundance of cysteine proteases (cathepsins B, H, S and L) and aspartic hydrolases (cathepsins D and E) with acidic pH optima, allows them to degrade a variety of exogenous antigens [44–46]. These endosomes also accumulate newly synthesised MHC Class-II (MHC-II) $\alpha\beta$ heterodimers due to their association with the invariant chain, which has endosomal sorting, leucine-rich N-terminal motifs [47]. Due to their high expression of MHC-II, these specialised antigen processing compartments have been termed MHC-II-rich compartments (MIIC) [43]. These appear to be particularly prevalent in immature DC [48].

Recent studies have shown that proteins sequestered into the MIIC of immature DC can be transported directly into the cytosol where they follow the pathway for MHC Class-I (MHC-I) presentation [49]. Normally, newly synthesised MHC-I molecules within the endoplasmic reticulum (ER) associate with peptides derived from cytosol proteins [50]. This process is mediated by the ER resident transporter associated with antigen processing (TAP) which facilitates the trafficking of proteasome-processed peptides from the cytosol into the

ER. MHC-I/peptide complexes are then thought to directly traffic to the plasma membrane (PM) without intersecting the endosomal pathway, allowing presentation of antigen peptides from intracellular pathogens to CD8⁺ T cells [50]. However, several reports have demonstrated that DC can present extracellular antigens *via* MHC-I molecules in a process termed 'cross presentation' [51–53]. This occurs primarily through a TAP-dependent pathway, where proteins from MIIC are transported to the cytosol, and processed by proteasomes before being complexed with MHC-I in the ER [49, 52, 53]. In addition, recent studies have demonstrated that DC can 'cross present' through TAP-independent pathways, where processed antigens within MIIC appear to complex with resident MHC-I molecules present in the MIIC of maturing DC [54–56]. However, before DC can complex processed peptide antigen to MHC molecules and display them at the cell surface, they must first undergo a process of functional maturation.

Mature dendritic cells

One of the first properties attained by 'maturing DC' is the capacity to migrate from non-lymphoid peripheral organs through afferent lymph to the T cell-rich paracortical areas of the proximal secondary lymphoid tissue [57, 58]. This is shown diagrammatically in Fig. 1. This has been well studied in LC where upon maturation there is downregulation of the adhesion molecule, E-cadherin, which acts through homotropic interactions to keep LC within the keratinocytes of the skin [59]. Maturing LC also downregulate the chemokine receptor (CCR)-6, whose ligand CCL20 (MIP3 α) helps localise these cells to dermal tissues [60]. Concomitantly, maturing LC also upregulate CD44 and the integrin $\alpha_v\beta_3$. Both are receptors for osteopontin, a factor important in LC migration to lymph nodes [61]. Maturing LC also upregulate CCR-7 [62]. Ligands for CCR-7 include secondary lymphoid tissue chemokine (SLC: CCL21) which is expressed by lymphatic endothelium and cells in the T cell-rich paracortical areas of secondary lymphoid tissues. Cells in the T cell-rich areas also express CCL19 (MIP3 β) another CCR-7 ligand [60]. Expression of CCR-7 is thought to enable maturing DC to migrate towards lymphatic

endothelium and to concentrate within T cell-rich areas. DC located within these areas amplify this chemotatic signal since they also express CCL21 and CCL19 [60].

The maturing DC is also characterised by tight control over the formation of MHC/peptide complexes and their expression on the cell surface along with costimulatory molecules. Internalised protein antigen can accumulate in MIIC for up to 60hr in immature DC [55]. However, within 3–4hr after induction of maturation of DC, antigen rapidly begins to complex with MHC-II within MIIC. This process involves downregulation of cystatin C, an inhibitor of cathepsin S. Cathepsin S degrades the invariant chain, leaving its MHC-II-associated invariant chain peptide fragment (CLIP) in the peptide binding groove of MHC-II [63]. MHC-II are then released from the endosomal sorting motifs of the invariant chain and CLIP is thought to be replaced with antigen peptide by the action of the resident catalyst H-2M [64, 65]. MHC-II/peptide complexes then separate from the bulk of unprocessed antigen and accumulate in LAMP-H-2M-compartments designated Class-II vesicles (CIIV), which are located in the periphery of the cell [55]. CIIV appear to emanate from MIIC as extensive tubules that separate off and move towards the cell periphery in a microtubule-dependent manner upon DC maturation [66, 67]. Maturation of DC also triggers neo-biosynthesis of MHC-I [53]. MHC-I, along with CD86 molecules, appear to accumulate in CIIV directly from the ER/trans-Golgi network [55, 67]. CIIV can fuse with the PM resulting in expression of MHC-II, CD86 and MHC-I at the cell surface [55, 67]. Concomitantly, maturing DC downregulate their endocytic capacity, thereby preventing reabsorption and degradation of MHC-II/peptide complexes and promoting their stable expression at the cell surface [68].

Functional maturation culminates with DC residing in T cell-rich areas of lymphoid tissues presenting peptide antigens acquired in the periphery in the context of MHC to passing T cells. MHC molecules are expressed 10–100 fold higher on mature DC than on B cells and monocytes [69]. Mature DC also upregulate expression of several costimulatory molecules including CD80, CD86 and CD40 [70] and also begin expression of a novel chemokine, DC-CK1, that preferentially attracts naïve (CD45RA⁺) T cells [71]. They also

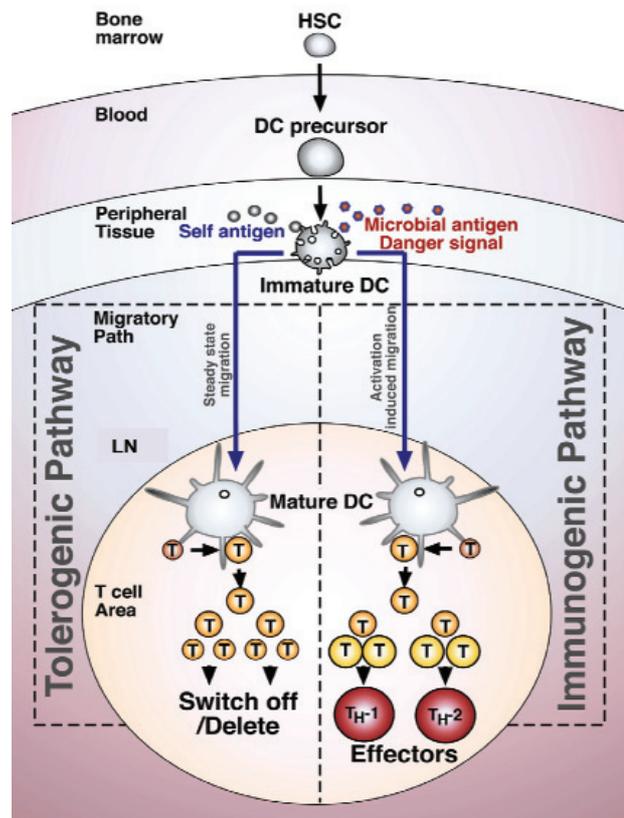


Fig. 1 A dual role for DC in immunity and tolerance. Hemopoietic stem cells (HSC) in bone marrow seed DC precursors in blood, which supply peripheral tissues with immature DC. Immature DC constitutively take up surrounding antigens, both self and foreign. In the immune steady-state, DC would commonly take up self antigens, mature and follow a tolerogenic pathway of development. They migrate with self antigens to T cell areas of lymphoid tissues where they present self antigen in the context of MHC and promote unresponsiveness in T cells specific for self antigens. Upon exposure to 'danger' signals, immature DC undergo activation during their maturation leading acquisition of immunogenic characteristics. They migrate to lymph nodes (LN) and present foreign antigen in the context of MHC and costimulatory molecules to T cells which are driven to differentiate into effector cells. The type of effector T cells generated, either T_H-1 or T_H-2, is influenced by the DC, and is dependent on the activating antigen. It is currently not known what signals lead to maturation of DC for T cell tolerance or how activation with 'danger' signals leads to maturation and T cell immunity.

express several adhesion molecules including CD2, CD11a, CD54 (ICAM-1), CD58 (LFA-3), and the integrins $\beta 1$ and $\beta 2$ [6, 9]. These properties are thought to enable mature DC to attract and cluster with naïve T lymphocytes as has been observed *in vivo* [72]. MHC-II molecules in maturing DC have been observed to traffic in CIIV-like compartments directly toward the PM adjacent to interacting T cells in an antigen-dependent manner [66]. This culminates in MHC/antigen complex expression at the PM and selection of clustered antigen-specific T cells and their subsequent stimulation through costimulatory molecules. CD4⁺ T cells respond by increasing surface expression of CD40L, which can in turn interact with CD40 on mature DC empowering them to directly stimulate naïve CD8⁺ T cells [73, 74]. This bypasses the need for direct spatial interaction of CD8⁺ T cells with T helper (T_H)-1 cells [73, 74].

Outcome of dendritic cell-T cell interactions

Although DC maturation can result in generation of effector T cells which facilitate immunity, functional maturation also appears to be required by DC which switch off T cell reactions. Two different pathways of DC development and functional maturation can lead to production of DC which can function in either tolerance or immunity. These are shown diagrammatically in Fig. 1. The capacity to induce tolerance is important in the maintenance of peripheral self tolerance in the immune steady state [75]. A role for thymic DC in the central tolerisation of T cells to self antigens is well established [76]. More recently, a role for DC in the peripheral tolerisation of CD4⁺ T cells and CD8⁺ T cells *in vivo* has also been demonstrated in murine models and was thought to be mediated by immature DC [77, 78]. However, in the immune steady state, DC are continually taking up self antigens including apoptotic material and acquiring some properties of mature DC [75]. These include the capacity to migrate carrying self antigens to the draining lymph nodes [79–82]. Migration coincides with upregulation of MHC and costimulatory molecules and downregulation of endocytic capacity, classic features associated with DC maturation [83–85]. DC with these characteristics in lymph nodes draining an antigen source

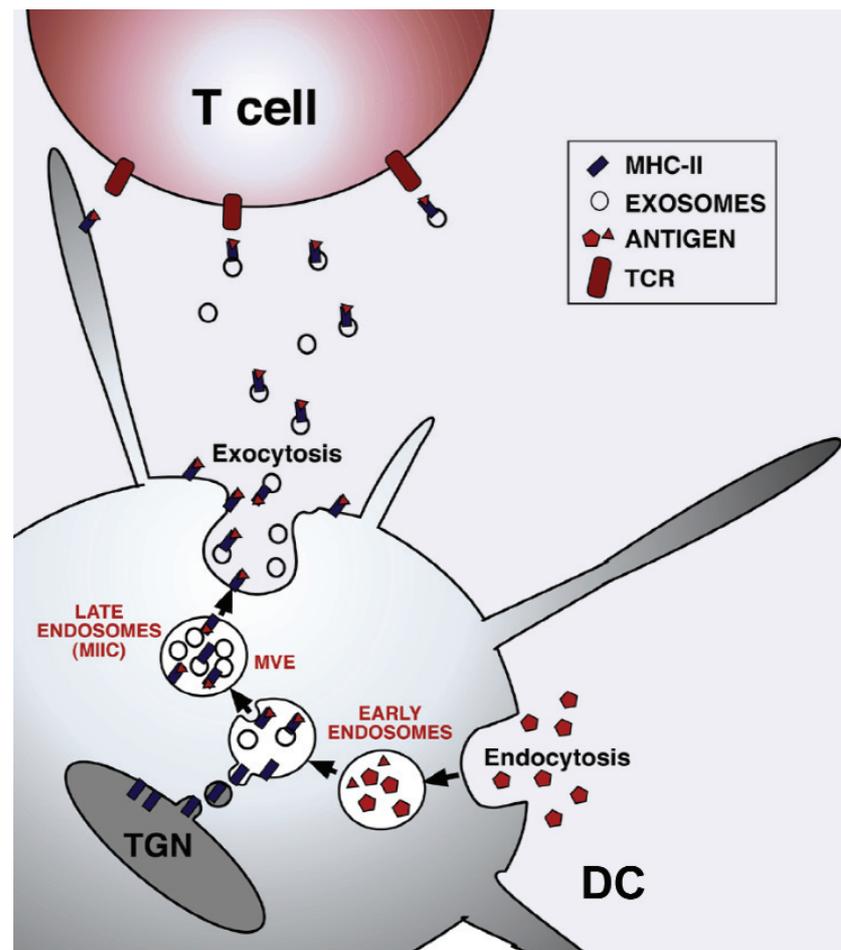
have been found to induce antigen-specific T cell tolerance [83]. Recent studies have also demonstrated that mature DC can induce CD8⁺ T cell tolerance by inducing transient cell proliferation and apoptosis, while immature DC produce CD8⁺ T cell ignorance [86]. Thus, in the immune steady state DC appear able to induce peripheral T cell tolerance that requires some functional maturation of DC.

A variety of 'danger' signals can lead to activation as well as maturation of DC [87]. These include, pathogen components like LPS, CpG DNA and double-stranded viral RNA, cytokines released during inflammation like tumour necrosis factor (TNF)- α , interleukin (IL)-1 and IL-4 and T cell ligands including CD40L and RANKL [6, 62]. However, depending on the type of danger signal, the DC subset receiving them and/or the cytokine profile present during T cell activation, DC can become activated in different ways leading to a diversity in the effector T cell responses generated [88–90]. For instance, CD8⁺ murine DC can produce high levels of IL-12 and prime naïve CD4⁺ T cells to secrete TH1 cytokines, while CD8⁻ murine DC once activated prime naïve CD4⁺ T cells to secrete T_H-0/T_H-2 cytokines [91]. While LPS derived from *E. coli* has been found to stimulate IL-12 production in CD8⁺ DC resulting in T_H-1 effector responses, LPS derived from *P. gingivalis* does not induce IL-12 production by CD8⁺ DC but induces a T_H-2 response [88]. Similarly, the yeast stage of the fungus *C. albicans* stimulates DC to produce IL-12 and a T_H-1 response, while the hyphae stage of *C. albicans* stimulates DC to produce IL-4 and a T_H-2 response [92]. Furthermore, DC in the presence of T_H cells producing IL-4 preferentially induce T_H-2 responses, while DC in the presence of interferon (IFN)- γ and IL-12 producing T_H cells, preferentially induce T_H-1 responses [90]. It is thought that a range of different activation states amongst the DC population allows generation of effector lymphocytes appropriate to deal with the pathogen at hand.

A role for dendritic cell-derived exosomes

Recent studies have shown that another defining property of DC is their capacity to secrete membrane vesicles called exosomes which can induce antigen-specific T cell responses [93–96]. Exosomes are

Fig. 2 Pathway for production of exosomes by DC. Antigens taken up by DC enter the endocytic pathway. Newly synthesised MHC-II molecules are transported from the trans-Golgi-network (TGN) to the endosomal pathway where they intercept with processed peptide antigens and form MHC-II rich compartments (MIIC). The limiting membrane of MHC-II rich compartments invaginate inwardly and bud off to give intraluminal vesicles. The multivesicular endosomes (MVE) formed by this process can fuse with the plasma membrane, thereby transporting their MHC-II/antigen complexes to the cell surface where internal vesicles or exosomes are released. Exosomes expressing MHC-II/antigen complexes can then trigger T cell activation. The mechanism of exosome-mediated T cell activation is not currently well understood. It could involve direct interaction with T cells (as shown), or indirect activation involving uptake of exosomes by functionally mature APC for antigen presentation.



thought to originate from intracellular compartments called multivesicular endosomes (MVE) by the inward invagination and budding from the limiting endosomal membrane. Exosomes isolated from antigen presenting cells like B cells and DC can express MIIC-specific markers like LAMP-1, MHC-II, CD63 and CD82 [56, 96]. When MVE fuse with the PM, exosomes are released into the extracellular environment. The process of production and release of exosomes by DC is shown in Fig. 2.

When B cell-derived exosomes were found to express MHC-II, including MHC-II in peptide-binding conformation [96], it was hypothesised that they could stimulate T cells. The response however was lower than that induced by parent B cells. Similarly, DC produced by *in vitro* culture of murine bone marrow precursors with GM-CSF and IL-4 were shown to produce immunostimulatory exosomes [56]. These DC-derived exosomes expressed MHC-I and CD86, and generated CD8⁺ T cell responses *in vivo* against

tumours [56]. In contrast, exosomes derived from steady state MHC-II^{-low} DC produced in long term stromal cultures express LAMP-1, a marker of MVE, but lack expression of the costimulator CD86 and MHC-II [95]. Isolated exosomes were found to be incapable of stimulating CD4⁺ T cells *in vitro*. They could induce an anti-tumour response *in vivo* probably through exosome uptake by immunostimulatory DC [95]. Exosomes can therefore reflect the functional state and behaviour of antigen presenting cells that release them and not all DC-derived exosomes will be immunogenic. One extension of this hypothesis is that exosomes released by DC maturing in the steady state in the absence of 'danger' and activation signals, may be important mediators of peripheral tolerance. A further extension of the model is that foreign antigen taken up by DC in the steady state will induce tolerance to the antigen, and self antigens presented on DC in the presence of 'danger' signals could lead to an autoreactive response.

Despite poor capacity to stimulate T cell responses *in vitro*, DC-derived exosomes have been shown to have potent immunostimulatory potential *in vivo*, particularly in CD8⁺ cytotoxic T cell responses against established tumours in mice [56, 95]. This finding applies to both MHC-II⁺ DC generated *in vitro* by culture of precursors with cytokines like GM-CSF and IL-4 [56], as well as to non-immunogenic MHC-II^{-low} DC derived in long term stromal cultures in the absence of activating cytokines [95]. The increased immunogenicity of exosomes *in vivo* has been attributed to the uptake and representation of antigens by immunogenic DC in the animal. Exosomes are thought to be involved in the 'spreading' of MHC-II/peptide complexes between DC [97]. Indirect T cell stimulation *in vivo* by exosomes taken up by recipient DC has been well documented for both CD8⁺ T cells [98, 99] and for CD4⁺ T cells [100]. These *in vivo* findings suggest that exosomes may play an important immunoregulatory role in DC function and also in immune response development.

In the steady state in the absence of pathogenic invasion or inflammation, DC exist in an immature form [9]. It is not surprising, therefore, that exosomes produced by immature DC as opposed to more mature or activated DC express no CD86 [95] and may play a role in maintaining peripheral tolerance. In this situation, immature or maturing peripheral DC, would constantly process self antigens, and secrete exosomes bearing MHC/self antigen complexes. These exosomes could then disperse and arrive at draining lymph nodes where resident immature lymphoid DC could then endocytose or re-present exosomes. By displaying exosome-derived MHC/self antigen complexes, resident immature DC could maintain peripheral tolerance and so control autoreactive T cells. This would allow a constant source of peripheral self antigen to be presented to autoreactive T cells at the lymphoid tissues.

Upon pathogenic invasion however, exosomes could play a T cell sensitising role. In this situation, inflammatory mediators like TNF- α and products associated with pathogenic invasion like LPS, would trigger activation and maturation of DC, that had taken up and processed foreign antigen [53]. Activated DC, expressing high levels of MHC/foreign antigen complexes and costimulatory molecules, could migrate to the draining lymph node where they meet and stimulate T cells [9]. Activated DC still actively processing foreign antigen and

expressing costimulatory molecules may secrete exosomes, incorporating costimulatory molecules, as well as MHC/peptide complexes. Exosomes rich in the ICAM-1 adhesion molecule as well as MHC/peptide and costimulatory molecules have recently been shown to be very effective in T cell activation [98]. Exosomes dispersing to the draining lymph node would then be presented on residential lymphoid DC for stimulation of T cells.

Exosomes playing the role of an antigen-bearing vector could allow transfer of large amounts of antigenic material from peripheral tissues to lymph nodes without the need for migration of large numbers of cells. This would also allow immunostimulatory DC which can no longer process antigen to present new foreign material from the periphery. Some evidence supporting a role for a soluble form of MHC/antigen complexes being transferred to the lymph node from peripheral tissues exists in the literature [101, 102].

Conclusions

Originally it was thought that different DC subsets may adopt specific roles in the immune response, however the dominant picture emerging shows less functional distinction between lineages of DC, so that each of the myeloid, lymphoid-like and plasmacytoid DC can function in both tolerance and immunity. The difficulties associated with *ex vivo* isolation of DC subsets, and particularly more immature DC, have impacted on our capacity to clearly delineate the function of DC in different states of maturation. The study of DC maturation of function is now complicated by evidence for plasticity amongst DC subsets. Tolerogenic DC appear to represent cells in a short-lived, immature or maturing state and so the properties of these cells are difficult to capture or immortalise in studies reliant on subset isolation. DC-derived exosomes can express immunostimulatory molecules and effectively mimic the immune potential of the parent DC. They have been shown to be very effective inducers of immunity following adoptive transfer. A role for DC-derived exosomes in tolerance induction is also proposed in light of the need for a migratory vector for efficiently moving antigen from the periphery into lymph nodes for tolerisation of T cells.

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References

1. **Steinman RM, Cohn ZA.** Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med.* 1973;137: 1142–62.
2. **Steinman RM, Cohn ZA.** Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties *in vitro.* *J Exp Med.* 1974; 139: 380–97.
3. **Steinman RM, Lustig DS, Cohn ZA.** Identification of a novel cell type in peripheral lymphoid organs of mice. 3. Functional properties *in vivo.* *J Exp Med.* 1974; 139: 1431–45.
4. **Steinman RM, Witmer MD.** Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. *Proc Natl Acad Sci USA.* 1978; 75: 5132–6.
5. **Nussenzweig MC, Steinman RM.** Contribution of dendritic cells to stimulation of the murine syngeneic mixed leukocyte reaction. *J Exp Med.* 1980; 151: 1196–212.
6. **Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K.** Immunobiology of dendritic cells. *Annu Rev Immunol.* 2000; 18: 767–811.
7. **del Hoyo GM, Martin P, Vargas HH, Ruiz S, Arias CF, Ardavin C.** Characterization of a common precursor population for dendritic cells. *Nature* 2002; 415: 1043–7.
8. **Hart DN, Fabre JW.** Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. *J Exp Med.* 1981; 154: 347–61.
9. **Hart DN.** Dendritic cells: unique leukocyte populations which control the primary immune response. *Blood* 1997; 90: 3245–87.
10. **Freudenthal PS, Steinman RM.** The distinct surface of human blood dendritic cells, as observed after an improved isolation method. *Proc Natl Acad Sci USA.* 1990; 87: 7698–702.
11. **Spry CJ, Pflug AJ, Janossy G, Humphrey JH.** Large mononuclear (veiled) cells like 'Ia-like' membrane antigens in human afferent lymph. *Clin Exp Immunol.* 1980; 39: 750–5.
12. **Steinman RM.** The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol.* 1991; 9: 271–96.
13. **Shortman K, Liu YJ.** Mouse and human dendritic cell subtypes. *Nat Rev Immunol* 2002; 2: 151–61.
14. **Agger R, Crowley MT, Witmer-Pack MD.** The surface of dendritic cells in the mouse as studied with monoclonal antibodies. *Int Rev Immunol* 1990; 6: 89–101.
15. **Witmer MD, Steinman RM.** The anatomy of peripheral lymphoid organs with emphasis on accessory cells: light-microscopic immunocytochemical studies of mouse spleen, lymph node, and Peyer's patch. *Am J Anat.* 1984; 170: 465–81.
16. **Saunders D, Lucas K, Ismaili J, Wu L, Maraskovsky E, Dunn A, Shortman K.** Dendritic cell development in culture from thymic precursor cells in the absence of granulocyte/macrophage colony-stimulating factor. *J Exp Med.* 1996; 184: 2185–96.
17. **Inaba K, Inaba M, Deguchi M, Hagi K, Yasumizu R, Ikehara S, Muramatsu S, Steinman RM.** Granulocytes, macrophages, and dendritic cells arise from a common major histocompatibility complex class II-negative progenitor in mouse bone marrow. *Proc Natl Acad Sci USA.* 1993; 90: 3038–42.
18. **Traver D, Akashi K, Manz M, Merad M, Miyamoto T, Engleman EG, Weissman IL.** Development of CD8 α -positive dendritic cells from a common myeloid progenitor. *Science* 2000; 290: 2152–4.
19. **Manz MG, Traver D, Miyamoto T, Weissman IL, Akashi K.** Dendritic cell potentials of early lymphoid and myeloid progenitors. *Blood* 2001; 97: 3333–41.
20. **O'Garra A, Trinchieri G.** Are dendritic cells afraid of commitment? *Nat Immunol* 2004; 5: 1206–8.
21. **Naik S, Vremec D, Wu L, O'Keeffe M, Shortman K.** CD8 α ⁺ mouse spleen dendritic cells do not originate from the CD8 α dendritic cell subset. *Blood* 2003; 102: 601–4.
22. **O'Keeffe M, Hochrein H, Vremec D, Pooley J, Evans R, Woulfe S, Shortman K.** Effects of administration of progenipoietin 1, Flt-3 ligand, granulocyte colony-stimulating factor, and pegylated granulocyte-macrophage colony-stimulating factor on dendritic cell subsets in mice. *Blood* 2002; 99: 2122–30.
23. **Corcoran L, Ferrero I, Vremec D, Lucas K, Waithman J, O'Keeffe M, Wu L, Wilson A, Shortman K.** The lymphoid past of mouse plasmacytoid cells and thymic dendritic cells. *J Immunol.* 2003; 170: 4926–32.
24. **Randolph GJ, Beaulieu S, Lebecque S, Steinman RM, Muller WA.** Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. *Science* 1998; 282: 480–3.
25. **D'Amico A, Wu L.** The early progenitors of mouse dendritic cells and plasmacytoid predendritic cells are within the bone marrow hemopoietic precursors expressing Flt3. *J Exp Med.* 2003;198: 293–303.
26. **Karsunky H, Merad M, Cozzio A, Weissman IL, Manz MG.** Flt3 ligand regulates dendritic cell development from Flt3⁺ lymphoid and myeloid-committed progenitors to Flt3⁺ dendritic cells *in vivo.* *J Exp Med* 2003; 198: 305–13.
27. **Brasel K, De Smedt T, Smith JL, Maliszewski CR.** Generation of murine dendritic cells from flt3-ligand-supplemented bone marrow cultures. *Blood* 2000; 96: 3029–39.
28. **Karsunky H, Merad M, Mende I, Manz MG, Engleman EG, Weissman IL.** Developmental origin of interferon- α -producing dendritic cells from hematopoietic precursors. *Exp Hematol.* 2005; 33: 173–81.

29. **Brasel K, McKenna HJ, Morrissey PJ, Charrier K, Morris AE, Lee CC, Williams DE, Lyman SD.** Hematologic effects of flt3 ligand *in vivo* in mice. *Blood* 1996; 88: 2004–12.
30. **Zuniga E, McGavern D, Pruneda-Paz J, Teng C, Oldstone MB.** Bone marrow plasmacytoid dendritic cells can differentiate into myeloid dendritic cells upon virus infection. *Nature Immunology* 2004; 5: 1227–34.
31. **Despars G, Ni K, Bouchard A, O'Neill TJ, O'Neill HC.** Molecular definition of an *in vitro* niche for dendritic cell development. *Exp Hematol.* 2004; 32: 1182–93.
32. **O'Neill HC, Wilson HL, Quah B, Abbey JL, Despars G, Ni K.** Dendritic cell development in long-term spleen stromal cultures. *Stem Cells* 2004; 22: 475–86.
33. **Wilson NS, El-Sukkari D, Belz GT, Smith CM, Steptoe RJ, Heath WR, Shortman K, Villadangos JA.** Most lymphoid organ dendritic cell types are phenotypically and functionally immature. *Blood* 2003; 102: 2187–94.
34. **Groux H, Fournier N, Cottrez F.** Role of dendritic cells in the generation of regulatory T cells. *Semin Immunol.* 2004; 16: 99–106.
35. **Tan JKH, O'Neill HC.** Maturation requirements for dendritic cells in T cell stimulation leading to tolerance *versus* immunity. *J Leukoc Biol.* 2005; 78: 319–24.
36. **Wu L, Li CL, Shortman K.** Thymic dendritic cell precursors: relationship to the T lymphocyte lineage and phenotype of the dendritic cell progeny. *J Exp Med.* 1996; 184: 903–11.
37. **Colonna M, Krug A, Cella M.** Interferon-producing cells: on the front line in immune responses against pathogens. *Curr Opin Immunol.* 2002; 14: 373–9.
38. **Akira S, Takeda K, Kaisho T.** Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol.* 2001; 2: 675–80.
39. **Figdor CG, van Kooyk Y, Adema GJ.** C-type lectin receptors on dendritic cells and Langerhans cells. *Nat Rev Immunol.* 2002; 2: 77–84.
40. **Sallusto F, Cella M, Danieli C, Lanzavecchia A.** Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J Exp Med.* 1995; 182: 389–400.
41. **Steinman RM, Turley S, Mellman I, Inaba K.** The induction of tolerance by dendritic cells that have captured apoptotic cells. *J Exp Med.* 2000; 191: 411–6.
42. **Albert ML, Pearce SF, Francisco LM, Sauter B, Roy P, Silverstein RL, Bhardwaj N.** Immature dendritic cells phagocytose apoptotic cells *via* $\alpha_v\alpha_5$ and CD36, and cross-present antigens to cytotoxic T lymphocytes. *J Exp Med.* 1998; 188: 1359–68.
43. **Geuze H.** EJCBLecture. A novel lysosomal compartment engaged in antigen presentation. *Eur J Cell Biol.* 1994; 64: 3–6.
44. **Blum JS, Cresswell P.** Role for intracellular proteases in the processing and transport of class II HLA antigens. *Proc Natl Acad Sci USA* 1988; 85: 3975–9.
45. **Blum JS, Fiani ML, Stahl PD.** Localization of cathepsin D in endosomes: characterization and biological importance. *Adv Exp Med Biol.* 1991; 306: 281–7.
46. **Mizuochi T, Yee ST, Kasai M, Kakiuchi T, Muno D, Kominami E.** Both cathepsin B and cathepsin D are necessary for processing of ovalbumin as well as for degradation of class II MHC invariant chain. *Immunol Lett.* 1994; 43: 189–93.
47. **Pieters J, Bakke O, Dobberstein B.** The MHC class II-associated invariant chain contains two endosomal targeting signals within its cytoplasmic tail. *J Cell Sci.* 1993; 106: 831–46.
48. **Thery C, Regnault A, Garin J, Wolfers J, Zitvogel L, Ricciardi-Castagnoli P, Raposo G, Amigorena S.** Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat shock protein hsc73. *J Cell Biol.* 1999; 147: 599–610.
49. **Rodriguez A, Regnault A, Kleijmeer M, Ricciardi-Castagnoli P, Amigorena S.** Selective transport of internalized antigens to the cytosol for MHC class I presentation in dendritic cells. *Nat Cell Biol.* 1999; 1: 362–8.
50. **Neeffjes JJ, Stollorz V, Peters PJ, Geuze HJ, Ploegh HL.** The biosynthetic pathway of MHC class II but not class I molecules intersects the endocytic route. *Cell* 1990; 61: 171–83.
51. **Svensson M, Wick MJ.** Classical MHC class I peptide presentation of a bacterial fusion protein by bone marrow-derived dendritic cells. *Eur J Immunol.* 1999; 29: 180–8.
52. **Mitchell DA, Nair SK, Gilboa E.** Dendritic cell/macrophage precursors capture exogenous antigen for MHC class I presentation by dendritic cells. *Eur J Immunol.* 1998; 28: 1923–33.
53. **Rescigno M, Citterio S, Thery C, Rittig M, Medaglini D, Pozzi G, Amigorena S, Ricciardi-Castagnoli P.** Bacteria-induced neo-biosynthesis, stabilization, and surface expression of functional class I molecules in mouse dendritic cells. *Proc Natl Acad Sci USA* 1998; 95: 5229–34.
54. **Ruedl C, Storni T, Lechner F, Bachi T, Bachmann MF.** Cross-presentation of virus-like particles by skin-derived CD8(-) dendritic cells: a dispensable role for TAP. *Eur J Immunol.* 2002; 32: 818–25.
55. **Turley SJ, Inaba K, Garrett WS, Ebersold M, Unternaehrer J, Steinman RM, Mellman I.** Transport of peptide-MHC class II complexes in developing dendritic cells. *Science* 2000; 288: 522–7.
56. **Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, Ricciardi-Castagnoli P, Raposo G, Amigorena S.** Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat Med.* 1998; 4: 594–600.
57. **Fossom S.** Lymph-borne dendritic leucocytes do not recirculate, but enter the lymph node paracortex to become interdigitating cells. *Scand J Immunol* 1988; 27: 97–105.
58. **Cumberbatch M, Kimber I.** Tumour necrosis factor- α is required for accumulation of dendritic cells in draining lymph nodes and for optimal contact sensitization. *Immunology* 1995; 84: 31–5.
59. **Schwarzenberger K, Udey MC.** Contact allergens and epidermal proinflammatory cytokines modulate Langerhans cell E-cadherin expression *in situ*. *J Invest Dermatol.* 1996; 106: 553–8.

60. **Dieu MC, Vanbervliet B, Vicari A, Bridon JM, Oldham E, Ait-Yahia S, Briere F, Zlotnik A, Lebecque S, Caux C.** Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J Exp Med.* 1998; 188: 373–86.
61. **Weiss JM, Renkl AC, Maier CS, Kimmig M, Liaw L, Ahrens T, Kon S, Maeda M, Hotta H, Uede T, Simon JC.** Osteopontin is involved in the initiation of cutaneous contact hypersensitivity by inducing Langerhans and dendritic cell migration to lymph nodes. *J Exp Med.* 2001; 194: 1219–29.
62. **Flores-Romo L.** *In vivo* maturation and migration of dendritic cells. *Immunology* 2001; 102: 255–62.
63. **Pierre P, Mellman I.** Developmental regulation of invariant chain proteolysis controls MHC class II trafficking in mouse dendritic cells. *Cell* 1998; 93: 1135–45.
64. **Denzin LK, Cresswell P.** HLA-DM induces CLIP dissociation from MHC class II $\alpha\beta$ dimers and facilitates peptide loading. *Cell* 1995; 82: 155–65.
65. **Martin WD, Hicks GG, Mendiratta SK, Leva HI, Ruley HE, Van Kaer L.** H2-M mutant mice are defective in the peptide loading of class II molecules, antigen presentation, and T cell repertoire selection. *Cell* 1996; 84: 543–50.
66. **Boes M, Cerny J, Massol R, Op den Brouw M, Kirchhausen T, Chen J, Ploegh HL.** T-cell engagement of dendritic cells rapidly rearranges MHC class II transport. *Nature* 2002; 418: 983–8.
67. **Chow A, Toomre D, Garrett W, Mellman I.** Dendritic cell maturation triggers retrograde MHC class II transport from lysosomes to the plasma membrane. *Nature* 2002; 418: 988–94.
68. **Villadangos JA, Cardoso M, Steptoe RJ, van Berkel D, Pooley J, Carbone FR, Shortman K.** MHC class II expression is regulated in dendritic cells independently of invariant chain degradation. *Immunity* 2001; 14: 739–49.
69. **Inaba K, Pack M, Inaba M, Sakuta H, Isdell F, Steinman RM.** High levels of a major histocompatibility complex II-self peptide complex on dendritic cells from the T cell areas of lymph nodes. *J Exp Med.* 1997; 186: 665–72.
70. **Banchereau J, Steinman RM.** Dendritic cells and the control of immunity. *Nature* 1998; 392: 245–52.
71. **Adema GJ, Hartgers F, Verstraten R, de Vries E, Marland G, Menon S, Foster J, Xu Y, Nooyen P, McClanahan T, Bacon KB, Figdor CG.** A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. *Nature* 1997; 387: 713–7.
72. **Ingulli E, Mondino A, Khoruts A, Jenkins MK.** *In vivo* detection of dendritic cell antigen presentation to CD4(+) T cells. *J Exp Med.* 1997; 185: 2133–41.
73. **Bennett SR, Carbone FR, Karamalis F, Flavell RA, Miller JF, Heath WR.** Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* 1998; 393: 478–80.
74. **Ridge JP, Di Rosa F, Matzinger P.** A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature* 1998; 393: 474–8.
75. **Lutz MB, Schuler G.** Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? *Trends Immunol.* 2002; 23: 445–9.
76. **Brocker T.** Survival of mature CD4 T lymphocytes is dependent on major histocompatibility complex class II-expressing dendritic cells. *J Exp Med.* 1997; 186: 1223–32.
77. **Bonifaz L, Bonnyay D, Mahnke K, Rivera M, Nussenzweig MC, Steinman RM.** Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. *J Exp Med.* 2002; 196: 1627–38.
78. **Hawiger D, Inaba K, Dorsett Y, Guo M, Mahnke K, Rivera M, Ravetch JV, Steinman RM, Nussenzweig MC.** Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions *in vivo*. *J Exp Med.* 2001; 194: 769–79.
79. **Albert ML, Sauter B, Bhardwaj N.** Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature* 1998; 392: 86–9.
80. **Huang FP, Platt N, Wykes M, Major JR, Powell TJ, Jenkins CD, MacPherson GG.** A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J Exp Med.* 2000; 191: 435–44.
81. **Hemmi H, Yoshino M, Yamazaki H, Naito M, Iyoda T, Omatsu Y, Shimoyama S, Letterio JJ, Nakabayashi T, Tagaya H, Yamane T, Ogawa M, Nishikawa S, Ryoike K, Inaba K, Hayashi S, Kunisada T.** Skin antigens in the steady state are trafficked to regional lymph nodes by transforming growth factor- β 1-dependent cells. *Int Immunol.* 2001; 13: 695–704.
82. **Parr MB, Kepple L, Parr EL.** Antigen recognition in the female reproductive tract. II. Endocytosis of horseradish peroxidase by Langerhans cells in murine vaginal epithelium. *Biol Reprod.* 1991; 45: 261–5.
83. **Akbari O, DeKruyff RH, Umetsu DT.** Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immunol.* 2001; 2: 725–31.
84. **Brand CU, Hunger RE, Yawalkar N, Gerber HA, Schaffner T, Braathen LR.** Characterization of human skin-derived CD1a-positive lymph cells. *Arch Dermatol Res.* 1999; 291: 65–72.
85. **Ruedl C, Koebel P, Bachmann M, Hess M, Karjalainen K.** Anatomical origin of dendritic cells determines their life span in peripheral lymph nodes. *J Immunol.* 2000; 165: 4910–6.
86. **Albert ML, Jegathesan M, Darnell RB.** Dendritic cell maturation is required for the cross-tolerization of CD8+ T cells. *Nat Immunol.* 2001; 2: 1010–7.
87. **Matzinger P.** Tolerance, danger, and the extended family. *Annu Rev Immunol.* 1994; 12: 991–1045.
88. **Liu YJ, Kanzler H, Soumelis V, Gilliet M.** Dendritic cell lineage, plasticity and cross-regulation. *Nat Immunol.* 2001; 2: 585–9.
89. **Rescigno M.** Dendritic cells and the complexity of microbial infection. *Trends Microbiol.* 2002; 10: 425–61.

90. **Schuhbauer DM, Mitchison NA, Mueller B.** Interaction within clusters of dendritic cells and helper T cells during initial Th1/Th2 commitment. *Eur J Immunol.* 2000; 30: 1255–62.
91. **Pulendran B, Smith JL, Caspary G, Brasel K, Pettit D, Maraskovsky E, Maliszewski CR.** Distinct dendritic cell subsets differentially regulate the class of immune response *in vivo*. *Proc Natl Acad Sci USA.* 1999; 96: 1036–41.
92. **d'Ostiani CF, Del Sero G, Bacci A, Montagnoli C, Spreca A, Mencacci A, Ricciardi-Castagnoli P, Romani L.** Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans*. Implications for initiation of T helper cell immunity *in vitro* and *in vivo*. *J Exp Med.* 2000; 191: 1661–74.
93. **Denzer K, Kleijmeer MJ, Heijnen HF, Stoorvogel W, Geuze HJ.** Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. *J Cell Sci.* 2000; 113: 3365–74.
94. **Thery C, Zitvogel L, Amigorena S.** Exosomes: composition, biogenesis and function. *Nat Rev Immunol.* 2002; 2: 569–79.
95. **Quah B, O'Neill HC.** The immunogenicity of dendritic cell-derived exosomes. *Blood Cells Mol Dis.* 2005; 35: 94–110.
96. **Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, Geuze HJ.** B lymphocytes secrete antigen-presenting vesicles. *J Exp Med.* 1996; 183: 1161–72.
97. **Segura E, Nicco C, Lombard B, Veron P, Raposo G, Batteux F, Amigorena S, Thery C.** ICAM-1 on exosomes from mature dendritic cells is critical for efficient naïve T-cell priming. *Blood* 2005; 106: 216–23.
98. **Thery C, Duban L, Segura E, Veron P, Lantz O, Amigorena S.** Indirect activation of naïve CD4⁺ T cells by dendritic cell-derived exosomes. *Nat Immunol.* 2002; 3: 1156–62.
99. **Vincent-Schneider H, Stumptner-Cuvelette P, Lankar D, Pain S, Raposo G, Benaroch P, Bonnerot C.** Exosomes bearing HLA-DR1 molecules need dendritic cells to efficiently stimulate specific T cells. *Int Immunol.* 2002; 14: 713–22.
100. **Andre F, Chaput N, Scharz N, Flament C, Aubert N, Bernard J, Lemonnier F, Raposo G, Escudier B, Hsu D-H, Tursz T, Amigorena S, Angevin E, Zitvogel L.** Exosomes as potent cell-free peptide-based vaccine. I. Dendritic cell-derived exosomes transfer functional MHC Class I/peptide complexes to dendritic cells. *J Immunol.* 2004; 172: 2126–36.
102. **Carbone FR, Belz GT, Heath WR.** Transfer of antigen between migrating and lymph node-resident DCs in peripheral T-cell tolerance and immunity. *Trends Immunol.* 2004; 25: 655–8.
103. **Smith AL, Fazekas de St Groth B.** Antigen-pulsed CD8 α ⁺ dendritic cells generate an immune response after subcutaneous injection without homing to the draining lymph node. *J Exp Med.* 1999; 189: 593–8.