

# Dendritic cells and the control of immunity

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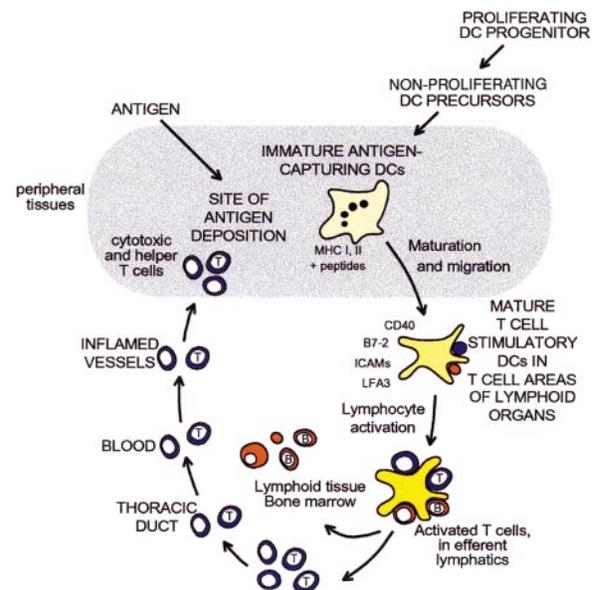
**B and T lymphocytes are the mediators of immunity, but their function is under the control of dendritic cells. Dendritic cells in the periphery capture and process antigens, express lymphocyte co-stimulatory molecules, migrate to lymphoid organs and secrete cytokines to initiate immune responses. They not only activate lymphocytes, they also tolerize T cells to antigens that are innate to the body (self-antigens), thereby minimizing autoimmune reactions. Once a neglected cell type, dendritic cells can now be readily obtained in sufficient quantities to allow molecular and cell biological analysis. With knowledge comes the realization that these cells are a powerful tool for manipulating the immune system.**

Immunology has long been focused on antigens and lymphocytes, but the mere presence of these two parties does not always lead to immunity. A third party, the dendritic cell (DC) system of antigen-presenting cells (APCs), is the initiator and modulator of the immune response. First visualized as Langerhans cells (LCs) in the skin in 1868, the characterization of DCs began only 25 years ago. It was known that 'accessory' cells were necessary to generate a primary antibody response in culture, but it was only once DCs were identified and purified from contaminating lymphocytes and macrophages that their distinct function as APCs became apparent (Box 1).

DCs are efficient stimulators of B and T lymphocytes<sup>1</sup>. B cells, the precursors of antibody-secreting cells, can directly recognize native antigen through their B-cell receptors. T lymphocytes, however, need the antigen to be processed and presented to them by an APC. The T-cell antigen receptors (TCRs) recognize fragments of antigens bound to molecules of the major histocompatibility complex (MHC) on the surface of an APC. The peptide-binding proteins are of two types, MHC class I and MHC class II, which stimulate cytotoxic T cells (CTLs) and helper T cells respectively. Intracellular antigens, cut into peptides in the cytosol of the APC, bind to MHC class I molecules and are recognized by CTLs, which, once activated, can directly kill a target cell. Extracellular antigens that have entered the endocytic pathway of the APC are processed there and generally presented by MHC class II molecules to T-helper cells, which, when turned on, have profound immune-regulatory effects.

Some *raison d'être* for a specialized DC system are now clear (Fig. 1). The initiation of T-cell immunity is rather demanding. Initially, peptides from infected cells located anywhere in the body must be found and recognized by T cells that circulate in the blood stream. The amounts of specific antigen–MHC complexes on tumours and infected cells are typically small (one hundred or less per cell), and must be recognized by rare T-cell clones (usually at

a frequency of 1/100,000 or less) through a TCR that has a low affinity (1  $\mu$ M or less). Moreover, infected cells and tumours frequently lack the co-stimulatory molecules that drive clonal expansion of the T cell, the production of cytokines, and development into killer cells. DCs provide a means of solving these challenges. Located in most tissues, DCs capture and process antigens, and display large amounts of MHC–peptide complexes at their surface (Fig. 1). They upregulate their co-stimulatory molecules and migrate to lymphoid organs, the spleen and the



**Figure 1** Afferent and efferent limbs of immunity that resolve several demands of antigen presentation *in vivo* (see text). Antigens are captured by DCs in peripheral tissues and processed to form MHC–peptide complexes. These immature DCs derive successively from proliferating progenitors and non-proliferating precursors, the latter not being fully committed to form DCs. As a consequence of antigen deposition and inflammation, DCs begin to mature, expressing molecules that will lead to binding and stimulation of T cells in the T-cell areas of lymphoid tissues. If the antigen has also been bound by B cells, then both B and T cells can cluster with DCs, as shown. After activation, T (blue) and B (orange) blasts leave the T-cell area. B blasts move to the lining of the intestine, the bone marrow, and other parts of the lymphoid tissue, such as the medulla of lymph node, with some becoming antibody-secreting plasma cells. T blasts leave the blood at the original site of antigen deposition, recognizing changes in the inflamed blood vessels and responding vigorously to cells that are presenting antigen. This limits the T-cell response to the site of microbial infection.

## Box 1 Dendritic cells and the control of immunity

- Sentinels *in vivo*: *in situ* distribution to optimize antigen capture; migration into lymphoid organs to optimize clonal selection of rare, CD4<sup>+</sup> and CD8<sup>+</sup> T cells
- Initiators of immune responses: stimulation of quiescent, naive and memory, B and T lymphocytes
- Potency in stimulating T cells: capacity of small numbers of DCs and low levels of antigen to induce strong T-cell responses
- Inducers of tolerance: deletion of self-reactive thymocytes, and anergy of mature T cells

lymph nodes, where they liaise with and activate antigen-specific T cells (Fig. 1, right). All of these DC activities can be induced by infectious agents and inflammatory products, so that DCs are mobile sentinels that bring antigens to T cells and express co-stimulators for the induction of immunity.

Not only should the immune system attack that which is foreign or aberrant, it should also leave alone that which is neither to avoid autoimmunity. And again, DCs play a vital part. Normally, mature T cells do not respond to self-peptides that are presented to them: during their development only those T cells that have no, or low, affinity towards the peptide antigens present in the thymus are allowed to mature and to enter the circulation. But not all self-antigens are represented in the thymus. What prevents T cells from being activated by for example brain- or pancreas-derived self-peptides, an event that could ultimately result in the development of multiple sclerosis or diabetes? We shall discuss some evidence that DCs can tolerize the T-cell repertoire to self-antigens, both in the thymus and in the periphery.

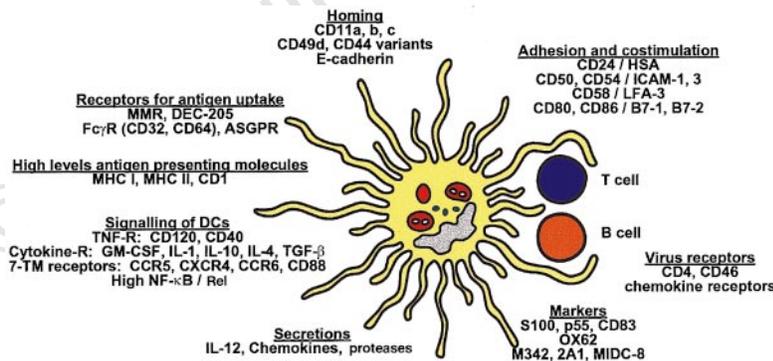
Until recently, the paucity of DCs and the lack of specific markers had impeded research. Both obstacles are being overcome, and sufficient DCs can now readily be prepared from progenitors<sup>2-10</sup>: bone-marrow cultures stimulated with cytokines, and mouse cell lines cultured in GM-CSF (granulocyte-macrophage colony stimulating factor) and interleukin (IL)-4, are both tricks of the trade used to obtain reasonable numbers of DCs. DCs derived from human blood monocytes that have been nurtured in GM-CSF and IL-4, followed by maturation in a monocyte-conditioned medium, are the most potent APCs known<sup>8,9</sup>. These DCs have many features

of primary DCs, including the expression of molecules that enhance antigen capture and selective receptors that guide DCs to and from several sites in the body (Fig. 2). New DC products are being rapidly identified by screening of complementary DNAs<sup>11-13</sup> that will provide more markers for identifying, characterizing and targeting DCs. We shall first review the features of mature DCs and their capacity to stimulate T cells, then discuss the immature antigen-capturing DCs, focusing on their migration to the lymphoid tissues and their development from progenitors; we shall consider their role in B-cell responses and the induction of tolerance and will finish by discussing their role in clinical immunology and pointing out the next challenges.

**Features of mature dendritic cells**

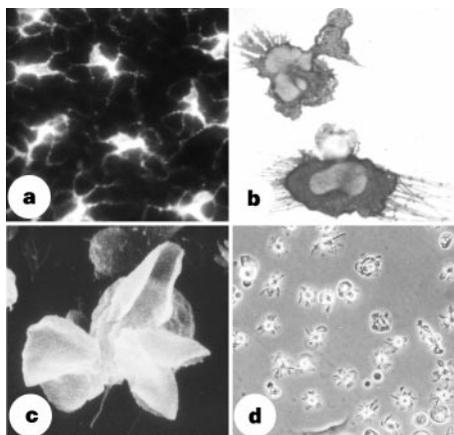
No other blood cell exhibits the shape and motility that give rise to the term ‘dendritic’ cell. *In situ*, as in the skin, airways and lymphoid organs, DCs are stellate (Fig. 3a). When isolated and spun onto slides, DCs display many fine dendrites (Fig. 3b). When looked at with an electron microscope, the processes are long (>10 μm) and thin, either spiny or sheet-like (Fig. 3c). When alive and viewed by phase-contrast microscopy, DCs extend large, delicate processes or veils in many directions from the cell body (Fig. 3d). These bend, retract, and re-extend in a non-polarized fashion for a day or more. Actin cables are scarce<sup>14</sup>. The shape and motility of DCs fit their functions, which are to capture antigens and select antigen-specific T cells.

Terminally differentiated or mature DCs can readily prime T cells (Box 1). Once activated by DCs, these T cells can complete the



**Figure 2** Some features of DCs, including DCs expanded *ex vivo* from precursors. Many monoclonals to antigens that are not rigorously DC-specific, nor well understood, are still useful in identifying DCs. These are CD83, an immunoglobulin-superfamily member; p55, a presumptive actin-bundling protein; S100b,

a calcium-binding cytosolic protein; DEC-205, a multilectin in DCs and many epithelia; antibodies called M342, 2A1 and MIDC-8 which recognize antigens in intracellular granules of mouse DCs; and OX62 on rat DCs and some T cells.



**Figure 3** The unusual shapes of DCs. **a**, DCs in a sheet of epidermis (MHC II stain); **b**, in cytopins stained for surface MHC II; **c**, by scanning electron microscopy; **d**, in the live state by phase-contrast microscopy.

immune response by interacting with other cells, such as B cells for antibody formation, macrophages for cytokine release, and targets for lysis. Immature DCs, on the other hand, are less potent initiators of immunity but specialize in capturing and processing antigens to form MHC peptide complexes. Thus, two key functions of DCs segregate in time: they first handle antigens and then, as mature DCs a day or more later, stimulate T cells.

*In vitro* or *in vivo*, only few DCs are necessary to provoke a strong T-cell response. *In vitro*, DCs can induce a so-called mixed leukocyte reaction (MLR), a model for graft rejection. Leukocytes from one individual, the potential transplant donor, are mixed with T cells from the responder or graft recipient. If donor and recipient are mismatched at the MHC, the T cells begin to proliferate, release cytokines and become CTLs. Normally, the MLR is carried out with equal numbers of stimulators and responders, but only one DC is necessary to turn on 100–3,000 T cells. These early observations introduced the notion that there are cells, DCs, specialized to initiate immunity. Now it is clear that DCs prime T cells not only to mismatched MHC, but to a range of foreign proteins, from superantigens, the microbial proteins that bind directly to MHC molecules without prior processing<sup>15</sup>, to the larger world of more standard proteins that do require processing, including those from infectious agents<sup>16,17</sup> and tumours<sup>18–21</sup>. *In vivo*, immunity develops in lymphoid organs, where the DC–T-cell interaction can be seen for all major classes of T-cell ligands<sup>22–24</sup>. DCs form clusters with antigen-specific T cells, creating a microenvironment in which immunity can develop<sup>22–24</sup>.

So far, no one has been able to identify a single specific molecule to explain the efficacy of DCs in T-cell binding and activation, and the special effects of DCs seem solely to relate to quantitative aspects and their regulation. For example, MHC–peptide complexes<sup>25</sup> are 10–100 times higher on DCs than on other APCs like B cells and monocytes. Mature DCs resist the suppressive effects of IL-10, but synthesize high levels of IL-12 (refs 26–28) that enhance both innate (natural killer cells) and acquired (B and T cells) immunity. DCs also express many accessory molecules that interact with receptors on T cells<sup>29,30</sup> to enhance adhesion and signalling (co-stimulation), for example LFA-3/CD58, ICAM-1/CD54, B7-2/CD86. All these properties (MHC expression, secretion of IL-12 and the expression of co-stimulatory molecules) are upregulated within a day of exposure to many stresses and dangers, including microbial products.

Depending on the conditions, DCs can stimulate the outgrowth and activation of a variety of T cells, which affect the immune

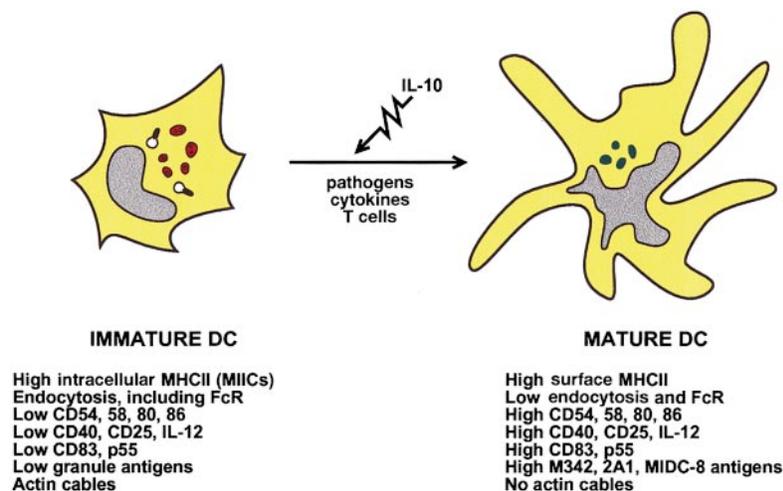
response differently. They can persuade CTLs, which express the accessory molecule CD8 and hence interact with MHC class I bearing cells, to proliferate vigorously, which is unusual for CD8<sup>+</sup> T cells<sup>31,32</sup>. CD4-expressing T-helper cells, on the other hand, scrutinize cells that express MHC class II molecules. In the presence of mature DCs and of the IL-12 they produce<sup>26–28</sup>, these T cells turn into interferon- $\gamma$  (IFN- $\gamma$ )-producing Th1 cells. IFN- $\gamma$  activates the antimicrobial activities of macrophages and, together with IL-12, it promotes the differentiation of T cells into killer cells. So the capacity of DCs to produce IL-12 and Th1 cells will lead to microbial resistance. With IL-4, however, DCs induce T cells to differentiate into Th2 cells which secrete IL-5 and IL-4. These cytokines activate eosinophils and help B cells to make the appropriate antibodies, respectively.

The communication between DCs and T cells seems to be a dialogue rather than a monologue in which the DCs respond to T cells as well. CD40 (ref. 33) and the newly described TRANCE/RANK receptor<sup>34,35</sup> on DCs are ligated by the TNF (tumour-necrosis factor) family of proteins expressed on activated and memory T cells: this leads to increased DC survival<sup>33,34</sup> and, in the case of CD40, upregulation of CD80 and CD86 (ref. 33), secretion of IL-12 (refs 26, 27) and release of chemokines such as IL-8, MIP-1 $\alpha$  and  $\beta$ <sup>33</sup>.

### Immature antigen-capturing dendritic cells

In most tissues, DCs are present in a so-called ‘immature’ state, unable to stimulate T cells. Although these DCs lack the requisite accessory signals for T-cell activation, such as CD40, CD54 and CD86, they are extremely well equipped to capture antigens and—a key event in the induction of immunity—antigens are able to induce full maturation and mobilization of DCs.

The sentinel position of immature DCs stand out when the skin surface, or epidermis, is labelled for DC molecules (Fig. 3a). Humans have about 10<sup>9</sup> epidermal LCs, the immature dendritic cells of the skin that are located above the basal layer of proliferating keratinocytes. Freshly isolated LCs are weak T-cell stimulators, have few MHC- and accessory- molecules, but many antigen-capturing Fc $\gamma$  and Fc $\epsilon$  receptors. This phenotype changes dramatically within a day of culture (Fig. 4): the cells undergo extensive transformation, antigen-capturing devices disappear, and T-cell stimulatory functions increase. When a skin patch is explanted and the LCs are challenged with an antigen, the migration of mature DCs into the culture medium can be observed<sup>36</sup>. The situation is similar *in vivo*. When they encounter a powerful immunological stimulus, for example a contact allergen or a transplant, most of the LCs from



**Figure 4** Features that change during DC maturation. Immature DCs take up antigen in several ways: that is, phagocytosis, macropinocytosis or adsorptive pinocytosis. An example of a pathogenic molecule that will induce maturation is

lipopolysaccharide (LPS); TNF $\alpha$ , GM-CSF are examples of cytokines, and CD40L is an example of a T-cell ligand that binds CD40 on DCs. IL-10 can inhibit maturation.

the epidermis mature and move into dermal lymphatics in search of antigen-specific T cells. Small numbers of antigen-capturing DCs can also be isolated from blood, lung, spleen, heart, kidney, and the B- and T-cell areas of tonsils; these cells lack LC-specific markers (E-cadherin, Birbeck granules, Lag-1), but they also acquire accessory molecules within 1–2 days of culture, before any encounter with T cells. In summary, a pool of cells in our bodies seems to be committed to mature into potent antigen-presenting cells.

What is it that makes a DC such a good APC? Immature DCs have several features that allow them to capture antigen. First, they can take up particles and microbes by phagocytosis<sup>16,17,37,38</sup>. Second, they can form large pinocytotic vesicles in which extracellular fluid and solutes are sampled, a process called macropinocytosis<sup>7</sup>. And third, they express receptors that mediate adsorptive endocytosis, including C-type lectin receptors like the macrophage mannose receptor<sup>7</sup> and DEC-205 (ref. 39), as well as Fcγ and Fcε receptors<sup>6</sup>. Macropinocytosis and receptor-mediated antigen uptake make antigen presentation so efficient that picomolar and nanomolar concentrations of antigen suffice<sup>7</sup>, much less than the micromolar levels typically employed by other APCs. However, once the DC has captured an antigen, which also can provide a signal to mature, its skills to capture antigens rapidly decline, and the time has come to assemble antigen–MHC class II complexes.

The antigen enters the endocytic pathway of the cell. In macrophages most of the protein substrate is directed to the lysosomes, an organelle with only few MHC class II molecules, where the antigen is fully digested into amino acids. Not in DCs; the DC is able to produce large amounts of MHC class II–peptide complexes at a single brief stage of its life. Much of this success may be due to specialized, MHC class II-rich compartments (MIICs) that are abundant in immature DCs<sup>6,14,40,41</sup>. MIICs are late-endosomal structures that contain the HLA-DM or H-2M products, which enhance and edit peptide binding to MHC class II molecules. During maturation of DCs, MIICs convert to non-lysosomal vesicles that discharge their MHC–peptide complexes to the surface<sup>41,42</sup> (Fig. 5). Immature DCs have been compared to idling motors in neutral gear, constantly degrading MHC class II molecules in their MIICs. As soon as an antigen instructs the DCs to move into gear, fragments of antigen are loaded onto class II molecules and these complexes are sent to the cell surface, where they remain stable for days<sup>41,42</sup>.

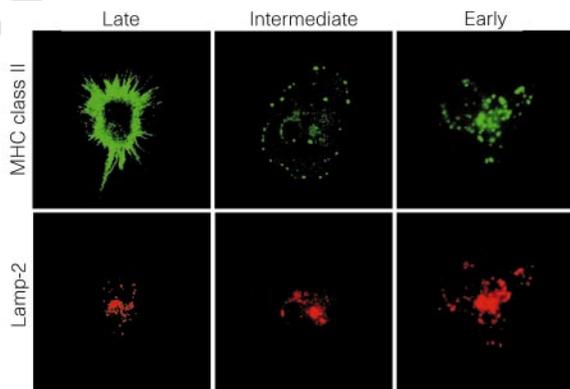
To generate cytotoxic killer cells, which have the capacity to eliminate infected cells and attack transplants and tumour cells, DCs

have to present antigenic peptides complexed to MHC class I molecules to CD8-expressing T cells. This is relatively straightforward if the DC is infected itself, with, for instance, influenza virus. The virus uses the cell's machinery to synthesize viral proteins, which are, like cellular proteins, degraded into peptides by the proteasome. A dedicated peptide transporter then translocates these peptides from the cytosol to the endoplasmic reticulum, where they bind to class I molecules. The peptide-loaded MHC class I complexes travel to the cell surface where they are displayed for scrutiny by T cells. It is less clear, however, how DCs could process and present antigens that have no access to the cytosol in an MHC class I-restricted manner (for instance transplant- and tumour-derived antigens, or antigens from viruses that cannot infect DCs). Nevertheless, they can probably present peptides from non-replicating microbes<sup>32</sup> and dying infected cells<sup>43</sup> by means of MHC class I as efficiently as if they were infected themselves. By processing dying cells, DCs may be able to 'cross-prime' or 'cross-tolerize' T cells to another cell's antigens or self-proteins, which could have major clinical implications, as we will discuss.

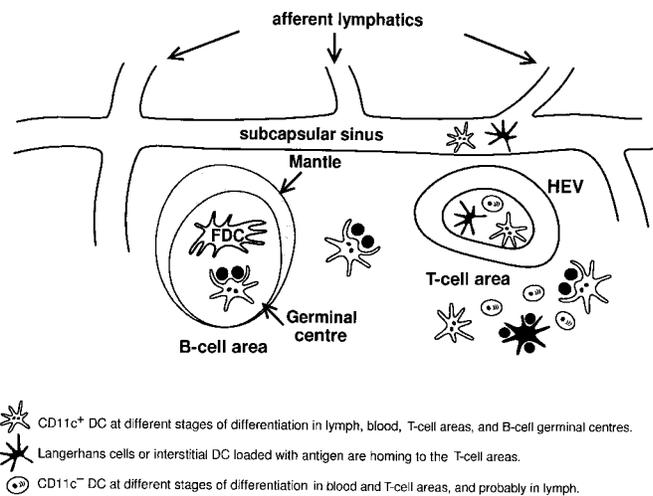
It is clear that maturation of DCs is crucial for the initiation of immunity. It can be influenced by a variety of factors, notably microbial and inflammatory products. Whole bacteria<sup>14</sup>, the microbial cell-wall component LPS<sup>7</sup>, and cytokines like IL-1, GM-CSF and TNF-α, all stimulate DC maturation, whereas IL-10 blocks it<sup>44</sup>. Ceramide, which is induced by maturation signals, can shut down antigen capture by the DC<sup>45</sup>. Mature DCs express high levels of the NF-κB family of transcriptional control proteins (Rel A/p65, Rel B, Rel C, p50, p52)<sup>46</sup> which regulate the expression of many genes encoding immune and inflammatory proteins. Signalling through the TNF-receptor family, for example TNF-R (CD120a/b), CD40 and TRANCE/RANK, results in activation of NF-κB. Therefore, to induce the immune response through activation of DCs, a pathogen or antigen may have to engage the signal transduction pathways of the TNF-R family and TNF-R-associated factors (TRAFs).

**Migration of dendritic cells *in vivo***

Upon activation, DCs travel to the lymphoid tissues such as the spleen and lymph nodes. There, DCs may complete their



**Figure 5** Intracellular MHC II-bearing compartments in immature, maturing and mature DCs. MHC II is shown in green and lysosomal membrane glycoprotein (Lamp-2) (or H-2M) in red. Early or immature DCs have numerous MHC II-rich lysosomes (right); maturing DCs contain MHC II-positive, non-lysosomal vacuoles (green, middle panels) that probably bring large amounts of MHC II to the surface of late or mature DCs (left). Micrographs courtesy of S. Turley and I. Mellman.



**Figure 6** Distinct subsets of DCs in lymphoid organs, possibly derived from separate pathways of development (see text). DCs arrive through afferent lymphatics and HEVs and include cells that are on patrol, or have left peripheral tissues in response to a stimulus, or act as precursors to lymphoid DCs. All DCs may initially enter the T-cell regions to mature into what are called 'interdigitating' cells by electron microscopy. The activated B-cell follicles, with germinal centres, have two cell types: FDCs and CD11c<sup>+</sup> DCs, which present respectively native antigens to B cells and processed antigens to T-helper cells. HEV, high endothelial venule.

maturation<sup>47</sup>, attract T and B cells by releasing chemokines<sup>11</sup> and maintain the viability of recirculating T lymphocytes<sup>48</sup>.

Even in the absence of invading pathogens, a fraction of the DC population seems to move around. These cells are always present in afferent lymph that access the T-cell areas (Fig. 6), but not in efferent lymph, indicating that most of the migrating DCs die after their arrival in lymphoid tissues. In blood, there are two subsets of precursor DCs, one expressing and one lacking CD11c (ref. 49). Both subsets can enter lymphoid organs at high endothelial venules by virtue of CD49d  $\beta$ 1 integrins<sup>50</sup> possibly with different final destinations such as the B-cell (CD11c<sup>+</sup>) and T-cell (CD11c<sup>-</sup>) areas (Fig. 6). DCs along liver sinusoids move in hepatic lymphatics to coeliac lymph nodes<sup>24,51</sup>, and DCs from intestine migrate to mesenteric lymph nodes. The DCs that migrate in the steady state may replenish immature populations or may be on patrol to identify invaders, like other white blood cells.

Not every pathogen or antigen induces a strong T-cell response, but those that do can induce the mobilization and maturation of DCs. Inhaled viruses and bacteria mobilize DCs into airway epithelium, and this influx proceeds as rapidly as that of neutrophils<sup>52</sup>. An injection of LPS causes a massive egress of DCs from skin, heart, kidney and intestine<sup>53,54</sup>, as well as the maturation and movement of spleen DCs from the marginal zone into the T-cell areas<sup>55</sup>. Application of a fluorescent contact allergen to the skin induces DC migration, and a day later fluorescent DCs can be found in the draining nodes. Following transplantation of heart and skin, DCs crawl out of the graft and make their way to lymphoid tissues. When access is prevented by disconnecting the afferent lymphatics, no immune response to skin transplants and contact allergens develops.

How dendritic cells know where to go is largely unknown. LPS<sup>53</sup> stimulates a variety of cells to produce cytokines and chemokines, for example GM-CSF, TNF- $\alpha$ , IL-1, MIP-1 $\alpha$  and  $\beta$ . These products are known to modulate DC movement and maturation. Damage to cytokine-rich cells, such as keratinocytes or mast cells, induced by an antigen could result in the release of preformed mediators like GM-CSF, TNF- $\alpha$  and IL-4. The current focus, however, is on seven-transmembrane-spanning G-protein-coupled receptors, an ever-growing family of proteins that includes receptors for calcitonin-gene-related peptide (which is found in nerve endings that abut on skin LCs<sup>56</sup>), C5a and chemokines<sup>13,57</sup>. These peptides and their receptors may mediate many of the steps required for the migration and targeting of DCs: egress from a tissue, directed movement or chemotaxis, and maintenance of viability.

### Dendritic cell development

There seem to be several pathways to generate DCs. We mentioned earlier that blood monocytes give rise to DCs when cultured with the appropriate cytokines<sup>6-9</sup>. The DC progenitors are also present in bone marrow: a small CD34<sup>+</sup> subset of haematopoietic progenitors gives rise to all blood cells and DCs. The DC progeny is likely to colonize most tissues *in vivo* as immature non-dividing cells. Several cytokines contribute to their growth and differentiation. Both c-Kit ligand and Flt-3 ligand are transmembrane proteins on stromal cells that bind to tyrosine-kinase receptors and sustain DC progenitors<sup>58</sup>; administration of Flt-3 ligand *in vivo* stimulates the outgrowth of functional DCs<sup>10</sup>. GM-CSF and IL-3 (refs 2, 59), products of activated T cells and other cells, also enhance DC differentiation, whereas macrophage (M)-CSF favours differentiation of the precursors into macrophages. TNF and CD40L<sup>3,33,60</sup> block the granulocyte-differentiation pathway and stimulate the final maturation of DCs.

Cells that express the marker CD34 contain progenitors for two discrete DC populations: the epidermal LCs and dermal or interstitial type DCs<sup>61,62</sup>. One functional difference is that only interstitial-type DCs directly stimulate naive B cells to make antibodies<sup>63</sup>. The LC progenitor expresses CLA (a ligand for E-selectin and a skin-homing molecule) and lacks CD14 (a marker that is abundant on

monocytes) and cannot form macrophages. In contrast, the dermal DC progenitors lack CLA, give rise to CD14-positive cells that resemble monocytes, and can form either macrophages in response to M-CSF or DCs in response to GM-CSF and TNF- $\alpha$ <sup>61,64</sup>. Mice deficient in TGF- $\beta$  mice have no epidermal LCs but maintain DCs in other sites<sup>65</sup>, which underscores the peculiarity of LCs.

A tentative but intriguing subset of DCs may be specialized in the induction of immune tolerance<sup>66</sup>. It is proposed that these DCs arise from a progenitor that also yields T cells<sup>67</sup>. IL-3, not GM-CSF, drives the development of these lymphoid DCs, which lack myeloid markers (markers shared by macrophages and granulocytes such as CD11b, CD13 and CD33)<sup>59</sup>. In humans, a distinct lymphoid DC precursor may have been identified; it expresses CD4, lacks CD11c and looks like an antibody-forming plasma cell but develops into a DC in the presence of IL-3 and CD40L<sup>68</sup>.

It is noteworthy that although mouse DC lines can be generated with the help of transforming viruses, there are no examples of spontaneous DC malignancy. In human Langerhans cell granulomatosis, originally called histiocytosis X, typical LCs and eosinophils accumulate. The cause of these granulomas is unknown, but they appear to be clonal in origin and create symptoms as a result of their position in critical sites rather than by overt malignant transformation<sup>69</sup>.

### Dendritic cells and B lymphocytes

DCs, famous for their T-cell-stimulatory properties, are now known to have major effects on B-cell growth and immunoglobulin secretion. B cells and DCs are both APCs and both are essential for antibody responses but for entirely different reasons (reviewed in ref. 1; Box 2); DCs activate and expand T-helper cells, which in turn induce B-cell growth and antibody production. But during this *ménage à trois*, there is more direct DC–B-cell dialogue as well (Fig. 1).

Naive B cells respond uniquely to the interstitial, non-LC type of DC<sup>63,70</sup>, and by secretion of soluble factors<sup>70</sup>, including IL-12, DCs stimulate the production of antibodies directly and the proliferation of B cells that have been stimulated by CD40L on activated T cells. DCs also orchestrate immunoglobulin class-switching of T-cell-activated B cells: IL-10 and TGF- $\beta$  can induce secretion of IgA<sub>1</sub>, but expression of IgA<sub>2</sub> appears to be strictly dependent on a direct interaction between the B cell and the DC<sup>71</sup>. This indicates that DCs are in control of mucosal immunity, and, in fact, DCs can be found in mucosal lymphoid tissues beneath antigen-transporting M cells<sup>72-74</sup> and in T-cell areas.

Follicular dendritic cells, or FDCs, directly sustain the viability, growth and differentiation of activated B cells (Fig. 6). They also organize the primary B-cell follicles, as shown by the absence of FDCs and follicles in TNF- $\alpha$ -knockout mice. FDCs differ from ordinary DCs: they are not bone-marrow-derived<sup>75</sup>, they lack the leukocyte marker CD45, and they display a unique set of molecules at their surface (reviewed in ref. 76), including all known complement receptors (CD11b, a long isoform of CD21 (ref. 77) and CD35). With their receptors for complement and Fc, FDCs capture antibody–antigen complexes and display whole complexes, rather than processed antigens, at their surface for long periods. FDCs are abundantly present within antigen-stimulated B-cell areas, or germinal centres. There, proliferating B cells (centroblasts) undergo

#### Box 2 Differences between dendritic cells and B cells as APCs

- DCs have higher levels of MHC II and accessory molecules
- DCs can make large amounts of IL-12
- DCs can internalize antigens by means of Fc and multilectin receptors; B cells have antigen-specific immunoglobulin receptors and inhibitory Fc $\gamma$ R

somatic mutation, after which they stop dividing (centrocytes) and wait to be triggered by an immune complex on FDCs. B cells that recognize an immune complex with high affinity process the antigen and present it as peptide–MHC complexes to antigen-specific T cells. The T–B-cell interaction ensures the survival of these high-affinity B cells, while the non-stimulated low-affinity B cells apoptose and are phagocytosed by tingible body macrophages.

It is now known that germinal centres contain a second type of dendritic cell, the CD11c<sup>+</sup> DC (Fig. 6). It also carries immune complexes, and is a much more powerful stimulator of T cells than the germinal centre B cells<sup>78</sup>. This may be the DC that brings the antigens to the germinal centre and displays processed antigens to memory T cells.

## Dendritic cells and T-cell tolerance

Most studies focus on the power of DCs to activate T cells, but before T cells encounter foreign antigens, the T-cell repertoire should be tolerized to self-antigens. This occurs in the thymus (central tolerance) by deletion of developing T cells, and in lymphoid organs (peripheral tolerance) probably by the induction of anergy or deletion of mature T cells. In both cases, the DC system that initiates immunity to foreign antigens also appears to tolerize T cells to self-antigens.

In the thymic medulla, DCs present self-antigens in the context of MHC molecules. Thymocytes that have too high an affinity for self-antigens are deleted (negative selection). If antigen-bearing DCs are directly injected into the developing thymus or fetal thymic organ cultures, reactive T cells are deleted. In the thymic cortex, macrophages digest large numbers of dying thymocytes that have failed to undergo positive selection. As these macrophages handle large amount of self-antigens, they seem ideally suited to delete auto-reactive T cells, yet they do not seem to do so: if MHC class II molecules are solely present on DCs in the medulla, negative selection ensues<sup>79</sup>. If, on the other hand, MHC class II molecules are only expressed by cortical epithelium, and not by DCs in medulla, the propensity to autoimmunity increases<sup>80</sup>, indicating that DCs in the medulla are responsible for the deletion of auto-reactive T cells. Also, *in vitro* it is the DC, and not the macrophage, that efficiently deletes auto-aggressive thymocytes<sup>81</sup>.

Recent studies point to an important role for DCs in the induction of peripheral tolerance as well. DCs can capture and present self-antigens that are exclusive to specialized tissues. For example, bone-marrow-derived APCs present peptides, which are derived from insulin-producing  $\beta$ -cells of the pancreas, to T cells in the draining lymph nodes<sup>82–84</sup>; tolerance ensues, probably as a result of T-cell anergy or deletion<sup>83,84</sup>. It has recently been shown that DCs present peptides from apoptotic cells<sup>43</sup>. Accordingly, DCs may be able to present many self-antigens, derived from the normal turnover of somatic cells, to T cells and thus induce tolerance to self-proteins that have no access to the thymus.

What determines whether a DC turns the immune system on or off? Lymphoid DCs are long-lived cells and express very high levels of MHC–self peptide complexes<sup>25</sup>, and maybe T cells become anergic or die in response to abundant and persistent antigens. Maybe distinct DCs are responsible for the different tasks: more resident lymphoid DCs induce tolerance to self, whereas migratory myeloid DCs, including LCs, are activated by foreign antigens in the periphery and move to lymphoid organs to initiate an immune response. Another possibility is that tolerance-inducing DCs are qualitatively different, perhaps expressing a death molecule like Fas ligand<sup>66</sup>.

## Dendritic cells in clinical immunology

Given their central role in controlling immunity, DCs are logical targets for many clinical situations that involve T cells: transplantation, allergy, autoimmune disease, resistance to infection and to tumours, immunodeficiency, and vaccines. In autoimmune diseases

such as psoriasis and rheumatoid arthritis, increased numbers and activation of DCs have been noted. DCs are important APCs in the lung, possibly contributing to allergy and asthma. In transplantation and contact allergy, DCs have been implicated in the induction of both immunity and tolerance. Here we concentrate on three areas where direct data on the role of DCs is available: infection with immunodeficiency viruses, resistance to tumours, and new approaches to vaccination.

Recent results paint a paradoxical picture in which DCs, instead of inducing host resistance, provide a safe haven for several viruses. Cells of the DC system may be hosting latent cytomegalovirus<sup>85</sup>, and Kaposi's virus (KSHV) may likewise be sheltered in patients with multiple myeloma<sup>86</sup>. For HIV-1 and measles, the consequences of DC infection are more overt: especially upon interacting with memory T cells and activated T cells, they sustain the production of many HIV-1, SIV and measles<sup>87–93</sup> particles. Measles turn DCs into multinucleated cells, or syncytia, and suppresses dendritic-cell and T-cell function<sup>91,93</sup>. HIV-1 and SIV also vigorously replicate in DC-derived syncytia *in vitro*<sup>90,94</sup>, but immunosuppression is not apparent at this time. Syncytia are not just a sign of viral toxicity, as is often assumed, but are also true virus factories<sup>90–94</sup>. *In vivo*, infected syncytia have been noted on the surfaces of mucosa-associated lymphoid tissue<sup>72,73</sup>. These so-called lymphoepithelia contain numerous memory B and T cells, as well as DCs that are chronically exposed to maturation stimuli from the environment. But, rather than battling with the infection, mature DCs assist in its spreading by transmitting HIV-1 and SIV to T cells<sup>87,88,90,94</sup>. FDCs in B-cell areas (Fig. 6) appear not to be infected with immunodeficiency virus, but they may play a dual role. By displaying virions complexed with antibody, they nevertheless can elicit resistance, especially B-cell memory, but at the same time they act as long-lived extracellular reservoirs of potentially infectious virus.

Many tumour components do not elicit an antigen-specific T-cell response in patients, which may be due to the absence of functional DCs in tumours. DCs that infiltrate colon and basal-cell skin cancers can lack CD80 and CD86 (ref. 95) and therefore have reduced T-cell stimulatory activity. Likewise, tumours may secrete factors, such as IL-10, TGF- $\beta$  and vascular endothelial growth factor, that reduce DC development and function. Be that as it may, the more DCs infiltrate the tumour, the better the prognosis.

The immune repertoire carries tumour-reactive T cells, especially CTLs, but there is little evidence that these T cells are being activated *in vivo*. However, when tumour antigens are applied to DCs *ex vivo* and these DCs are then reinfused, specific immunity ensues. In animals this strategy can lead to protection against tumours and even a reduction in the size of established tumours<sup>96–98</sup>, and at present similar studies are carried out in patients. Many vehicles for the delivery of tumour antigens to DCs are being considered: viral vectors, naked and plasmid DNA, RNA, liposomes with nucleic acid or protein, and tumour lysates, apoptotic cells and peptides. It is interesting that DCs appear to have a direct lytic potential on certain tumour targets as well<sup>99</sup>.

Vaccine design has yet to target the DC system. DCs can readily elicit helper and killer T cells, antibodies and IL-12, and can operate at mucosal surfaces where protection is needed early during many infections. In contrast, many existing vaccines and adjuvants are weak stimulators of CD8<sup>+</sup> T cells and Th1-type T cells. As some DCs appear to tone down the immune response, vaccines that target these DCs could also be used to induce tolerance, for example to allergens.

The classical approach to vaccination exploits attenuated forms of pathogens to elicit an immune response, and such attenuation is now more feasible using genetic manipulation and new vectors like avipox viruses. *In vitro*, DCs are the only cells that efficiently present inactivated virus<sup>32</sup>, and therefore the efficacy of the new generation of attenuated vaccines could be improved by specific targeting to DCs. The immune response can also be boosted by immunization

with DNA vaccines; even though the DNA is primarily expressed in weak APCs, like dermal and muscle cells, DNA vaccines can activate both CD4- and CD8-bearing cells. DCs isolated from vaccinated animals both express the vaccine DNA<sup>100,101</sup> and present the corresponding peptides to specific T cells<sup>101</sup>. The frequency of transfected DCs is low and greater efficiency should prove valuable.

### Upcoming challenges

We have only just started to appreciate the extraordinary life of dendritic cells and many questions remain unanswered. At the molecular level, there is a sizeable effort to screen expressed sequence tags from DCs to characterize their products. Regulatory molecules of the killer-inhibitor family<sup>102</sup>, of chemokines and chemokine receptors<sup>11,13,103</sup>, and of proteinases<sup>12</sup> have now been identified. This ongoing search should uncover new lymphocyte-binding and antigen-processing molecules, secretory products, and transcription factors that may help to explain the specialized features of DCs.

At the physiological level, many features of DCs need to be understood in more detail to allow successful manipulation of the immune system. What signals lead to maturation and migration of DCs, how do they know where to go, and how do they reach the lymphatics, T-cell areas, and sometimes B-cell areas? What are the roles of the different DC subsets, including their effects on B cells and tolerance? Have DCs a larger repertoire of secretory products than currently appreciated, and are some of these, like IL-12, immune modulators? And, what determines whether a DC turns the immune system on or off?

At the clinical level, the efficacy of DNA vaccines and vectors may crucially depend upon their capacity to transduce DCs. Vaccines in general should improve if they elicit mobilization and maturation of DCs. Emerging evidence points towards a role for DCs in tolerance, and it will therefore be imperative to pursue DCs more vigorously in allergy, transplantation and autoimmunity. On the other hand, many microbes such as HIV-1 and measles may take advantage of the special features of DCs. Widely used immunomodulators like steroids may block DC maturation<sup>47</sup>, and perhaps new drugs can be designed to modulate their functions. The results are awaited of clinical trials in which DCs are being used to elicit immunity to viral infections and tumours.

Although studies of pathogenesis have long focused on the core of immunology by identifying antigens and lymphocytes, all the recent evidence places a new emphasis on the role of DCs in the control of immunity. □

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