REVIEW
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# Thymic dendritic cells

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*T cells bearing receptors with high affinity for self-antigens are responsible for the generation of autoimmune diseases. Therefore, potentially autoreactive thymocytes must be eliminated or inactivated in normal individuals. Induction of tolerance in thymocytes occurs by a process of negative selection controlled by the thymic stroma, and in particular by thymic dendritic cells (DCs). Here, Carlos Ardavín reviews current knowledge on thymic DCs and their role in negative selection.*

Dendritic cells (DCs) are key elements in the establishment of T-cell immune responses and, as such, have extraordinary antigen-presenting capacity. Their potential as antigen-presenting cells (APCs) relies on their ability to endocytose, process and retain a wide variety of antigens on their surface and to present these to specific T cells. DCs also have a highly efficient costimulatory potential based on their constitutive expression of B7-1 (CD80), B7-2 (CD86) and CD40 molecules. Moreover, DCs are strategically located in most potential sites of antigen entry, either in lymphocyte-deprived epithelial microenvironments or in a variety of lymphoid organs. Furthermore, they display a remarkable migratory capacity upon contact with an antigen, which allows the transport and presentation of antigenic peptides to specific T cells (and therefore the development of an effector response) in locations that may be very distant from the original site of antigen entry.

Within the thymus, however, DCs do not behave as classical APCs. Here, effective interaction between thymic DCs and thymocytes does not induce the series of T-cell activation events occurring in the periphery but determines the negative selection of autoreactive thymocytes, thereby leading to the generation of central T-cell tolerance. Despite extensive knowledge concerning peripheral DCs, the biology of thymic DCs is not fully understood. This review summarizes the current data on thymic DCs and their role in negative selection, and discusses the relationships between DCs and lymphoid lineages. Box 1 introduces some key features of thymic DCs.

**Box 1. Characteristics of thymic dendritic cells**

- Irregular-shaped cells (20–30 μm) with long, interdigitating, cytoplasmic processes
- Excentrically located nucleus with scant heterochromatin
- Few organelles, concentrated in the juxtanuclear area
- Characteristic Birbeck granules, also found in peripheral dendritic cells and Langerhans cells
- Located mainly in the cortico-medullary border and medulla
- Represent approximately 0.1% of bone-marrow-derived thymic cells
- Detected at day 14 of gestation in the mouse
- Short intrathymic lifespan: turnover rate of 2–3 weeks

## Phenotype of thymic DCs

Several studies dealing with the phenotype of thymic DCs have been published[1–7]. Although these reports have provided a precise definition of thymic DC markers, they have also generated some controversy concerning the expression by thymic DCs of certain cell-surface molecules. Analysis of the phenotypic variations seen during thymic DC development *in vitro*[8], in experimental models involving thymus reconstitution after irradiation by transfer of early CD4$^{lo}$ precursors[9], and in thymic DCs transferred to culture[3,7,10] suggests that discrepancies between individual studies probably reflect the strategy used for DC isolation.

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**Table 1. Phenotype of mouse thymic DCs**

| Molecule | Alternative designation | Expression[a] | Notes | Refs |
|---|---|---|---|---|
| **Lymphoid precursor markers** | | | | |
| Sca-1 | Ly-6A/E, MTS23 | + | Expressed by HSCs; signal transduction potential | 12[c], 13, 15 |
| Sca-2 | | + | Expressed by $CD4^{lo}$ precursors, but not by HSCs | 9, 14 |
| c-kit | CD117 | + | Receptor for SCF | 9 |
| Thy-1 | CD90 | + | Transferred from thymocytes, as demonstrated in thymic chimeras | 9, 17 |
| BP-1 | | + (30–50%) | Expressed by early B-cell precursors, but not by HSCs; thymic DCs express BP-1 mRNA | 9, 16 |
| B220 | CD45R | – | Expressed by B-cell precursors and mature B cells, but not by HSCs | 4, 16 |
| CD45 | | + | Differentially expressed by MHC class $II^{lo}$ and MHC class $II^{hi}$ thymic DC subpopulations | 4, 5 |
| **T-cell markers** | | | | |
| CD2 | | + | Involved in CD2–CD48-mediated APC–T-cell interaction | 9, 19 |
| CD3 | | – | | 4 |
| CD4 | | Low | Expressed at high levels by human thymic DCs | 4, 18, 33 |
| CD25 | IL-2Rα | + (10–15%) | Upregulated upon culture (>50%) | 9 |
| CD8α | | + | Thymic DCs express CD8α mRNA; expressed by a subpopulation of splenic DCs | 4, 9, 22 |
| CD8β | | + | Downregulated upon culture; probably transferred from thymocytes; not expressed by splenic DCs | 4, 9 |
| **DC markers** | | | | |
| N418 | CD11c | + | Within the thymus, only DCs express N418 at high levels | 9, 24 |
| DEC-205 | NLDC-145 | + | Endocytic receptor involved in antigen processing; expressed by thymic epithelial cells | 9, 25 |
| M342 | | + | Intracellular perinuclear antigen | 27[d] |
| **Costimulatory molecules** | | | | |
| B7-1 | CD80 | ?[b] | Constitutively expressed by peripheral DCs; controversial role in negative selection | 22, 29, 32 |
| B7-2 | | + | Constitutively expressed by peripheral DCs; controversial role in negative selection | 22, 29, 30[d] |
| CD40 | | + | Involved in negative selection, as demonstrated by blocking the CD40–CD40L interaction | 31[d], 87 |
| HSA | CD24 | + | Differentially expressed by MHC class $II^{lo}$ and MHC class $II^{hi}$ thymic DC subpopulations | 4, 5 |
| **MHC molecules** | | | | |
| MHC class I | | + | Involved in T-cell selection | 4, 94 |
| MHC class II | | + | Involved in T-cell selection; defines MHC class $II^{lo}$ and MHC class $II^{hi}$ thymic DC subpopulations | 4, 5, 94 |
| **Adhesion molecules** | | | | |
| LFA-1 | CD11a/CD18 | + | Involved in DC–thymocyte interactions in *in vitro* T-cell deletion assays | 4, 82 |
| ICAM-1 | CD54 | + | Involved in DC–thymocyte interactions in *in vitro* T-cell deletion assays | 4, 82 |
| MAC-1 | CD11b/CD18 | – /low | | 4 |
| CD44 | | + | Differentially expressed by MHC class $II^{lo}$ and MHC class $II^{hi}$ thymic DC subpopulations | 4, 5 |
| **Myeloid markers** | | | | |
| FcγRII | CD32 | – /low | Expressed by Langerhans cells | 4, 95 |
| F4/80 | | – | Macrophage marker | 4 |
| Gr-1 | | – | Myeloid differentiation antigen | 4, 15 |

Abbreviations: APC, antigen-presenting cell; CD40L, CD40 ligand; DC, dendritic cell; HSA, heat-stable antigen; HSC, hematopoietic stem cell; ICAM-1, intercellular cell adhesion molecule 1; IL-2R, interleukin 2 receptor; LFA-1, leukocyte function-associated molecule 1; MHC, major histocompatibility complex; Sca-1, stem cell antigen 1; SCF, stem cell factor.

[a]–, not expressed; + expressed by all, or the majority of, thymic DCs (except when the percentage of positive cells is indicated); low, expressed at low levels.

[b]In this report, B7 expression was detected using a CTLA-4 fusion protein binding to B7-1 and B7-2.

[c]DCs isolated by an adherence-based method.

[d]Results obtained by *in situ* immunohistochemical staining.

**Table 2. Comparison of the phenotypes of murine HSCs, CD4$^{lo}$ precursors, pro-T cells and thymic DCs**

| | Expression | | | |
|---|---|---|---|---|
| Molecule | HSCs | CD4$^{lo}$ precursors | Pro-T cells[a] | Thymic DCs |
| Sca-1 | + | + | + | + |
| Sca-2 | – | + | + | + |
| c-kit | + | + | + | + |
| Thy-1 | Low | Low | Intermediate | Intermediate[b] |
| HSA | Low | Intermediate | Intermediate | High |
| CD44 | High | High | High | High |
| CD25 | – | – | + | +[c] |
| CD2 | – | – | – | + |
| CD4 | Low | – | – | Low |
| CD8 | – | – | – | + |
| MHC class II | – | – | – | + |
| BP-1 | – | – | – | + |

Abbreviations: TCR, T-cell receptor; for other abbreviations, see Table 1 footnotes.
[a]c-kit$^+$CD44$^+$CD25$^+$ T-cell-lineage-committed precursor, with non-rearranged TCR genes.
[b]Thy-1 expression by thymic DCs has been demonstrated to be due, at least in part, to transfer from thymocytes[17].
[c]10–15% of 4°C-isolated DCs express CD25, which is upregulated on thymic DCs upon culture[9].

The 'classical' isolation methods based on differential DC adherence, which led to the discovery of DCs by Steinman and Cohn[11], involve the culture of DCs for 12–14 h at 37°C in medium supplemented with fetal calf serum. Important phenotypic differences are seen between thymic DCs isolated by adherence-based methods and freshly isolated thymic DCs purified by means of alternative techniques involving low-density gradients, immunomagnetic bead depletion and/or fluorescence-activated cell sorting (FACS)[4]. These isolation methods are performed entirely at 4°C and avoid DC culture *in vitro*, thereby allowing the analysis of DCs expected to retain the characteristics of those found *in vivo*. Therefore, unless specifically stated, data on the phenotype of thymic DCs presented here derive from FACS analysis performed on DCs purified by methods that do not use differential adherence or culture (here termed 4°C-isolated DCs) or, when indicated, from *in situ* immunohistochemical staining of thymus sections. Table 1 summarizes the phenotype of thymic DCs in the mouse system (where most of the studies have been carried out), although the most relevant information dealing with murine as well as human thymic DC phenotype is also considered below.

## Mouse thymic DCs

### *Lymphoid precursor markers*

Mouse thymic DCs express a series of cell-surface antigens, such as Sca-1 (stem cell antigen 1, Ly-6A/E), Sca-2, c-kit (stem cell factor receptor), Thy-1 and CD45 (Refs 4, 9, 12, 13), found in the earliest CD4$^{lo}$ intrathymic T-cell precursor population[14]. Sca-1, c-kit and Thy-1 are also expressed by bone marrow hematopoietic stem cells (HSCs) (Ref. 15). Interestingly, BP-1, a marker of early B-cell precursors but not of bone marrow HSCs (Ref. 16), is expressed by thymic DCs (Ref. 9). Thy-1 expression on DCs has been shown to be due, at least in part, to passive acquisition from thymocytes[17], and is downregulated following *in vitro* culture.

### *T-cell markers*

Strikingly, murine thymic DCs express T-cell antigens such as CD2, CD8 and CD25 (Refs 4, 9), as well as low levels of CD4 (Ref. 18). The significance of CD2 for DCs is unknown: CD2 plays an important role in peripheral APC–T-cell interactions[19], but negative selection is not significantly affected in CD2-deficient mice[20]. It is not known whether CD2 is produced by DCs themselves or is acquired from surrounding thymocytes. However, mouse thymic DCs express CD8α at both the mRNA and protein levels[4]. Since CD8α expression is stable after DC culture, whereas CD8β is downregulated[9], it has been proposed that thymic DCs express a CD8αα homodimer, as do extrathymically derived T cells, rather than the CD8αβ heterodimer expressed by thymus-derived T cells: CD8β would thus be present on DCs only if transferred from thymocytes. As with CD2, the role of CD8 at the thymic DC surface remains to be elucidated, although it has been proposed that CD8 might be involved in triggering thymocyte apoptosis by interacting with the α3 domain of thymocyte major histocompatibility complex (MHC) class I molecules[21]. Interestingly, a recent study reported that CD8$^+$ but not CD8$^-$ splenic DCs expressed Fas ligand (FasL), and induced Fas-mediated apoptosis of CD4$^+$ T cells[22]. CD25 (interleukin 2 receptor α chain) expression by a proportion of DCs may be indicative of an activated state of some thymic DCs. CD25 is upregulated on thymic DCs upon culture[9], and this upregulation is augmented by adding granulocyte–macrophage colony-stimulating factor (GM-CSF) to the culture medium. Moreover, human DC activation through CD40 crosslinking is accompanied by upregulation of CD25 (Ref. 23).

As summarized in Table 2, the data on lymphoid precursor markers and T-cell markers indicate that thymic DCs, HSCs and lymphoid precursors share some relevant cell-surface antigens. This suggests a relationship between DCs and lymphoid lineages, as discussed below.

### *DC markers*

Despite extensive efforts by different research groups during the past decade, a molecule expressed exclusively by DCs has not been

discovered. However, some well-characterized cell-surface and intracellular antigens represent extremely useful tools in the study of both thymic and peripheral DCs. The N418 marker, corresponding to the leukocyte integrin CD11c, remains the best thymic and peripheral DC marker. Within the thymus, only thymic DCs express this antigen at high levels. Although low expression of N418 by peripheral monocytes and macrophages has been reported[24], under controlled experimental conditions, N418 can be used as a DC-specific marker in the mouse. The dendritic epithelial cell (DEC)-205 antigen, detected by the monoclonal antibody (mAb) NLDC-145, is an endocytic receptor involved in antigen processing[25], and is expressed at high levels by thymic DCs as well as by thymic cortical epithelial cells: comparative B-cell expression is only 2–10% of this level[26]. Finally, the mAb M342 detects an intracellular antigen on DCs, and allows specific *in situ* detection of thymic medullary DCs, although peritoneal B cells and stimulated splenic B cells are also M342$^+$ (Ref. 27).

### *Costimulatory molecules*

Costimulatory interactions such as B7–CD28 and CD40–CD40 ligand (CD40L) have been demonstrated to play an essential role in T-cell activation and T-cell-dependent humoral and inflammatory responses; in addition, CD40–CD40L interactions can regulate APC function through upregulation of B7-1 and B7-2 (Refs 28, 29). However, despite several reports dealing with the involvement of these molecules in negative selection of T cells (see below), little information is available concerning their expression by thymic DCs, although CD40 and B7-2 have been demonstrated to be constitutively expressed by splenic DCs (Refs 22, 29, 30). B7-2 and CD40 have been detected *in situ* in the thymic medulla by immunohistochemical staining[30,31], but no precise characterization of the cells expressing these molecules was made. By using a CTLA-4 fusion protein as a ligand, B7 expression by thymic DCs (isolated by an adherence-based method) has been reported[32]. In addition, recent results using 4°C-isolated DCs have demonstrated the constitutive expression of B7-2 and CD40 by mouse thymic DCs (C. Ardavín, unpublished).

## Human thymic DCs

Despite the numerous studies performed on human peripheral DCs, few reports deal with thymic DCs in humans. However, analysis of cell-surface markers of freshly isolated human thymic DCs by Lafontaine *et al.*[3] and Shortman and colleagues[18,33] has allowed their phenotypic characterization.

Human thymic DCs express high levels of MHC class I and class II molecules, CD45 and CD11c (Refs 3, 18, 33). In addition, they express the adhesion molecules leukocyte function-associated molecule 1 (LFA-1), intercellular adhesion molecule 1 (ICAM-1) and CD44 (Refs 3, 33), but lack some lymphoid precursor markers such as CD34 and CD7 (Ref. 33), as well as B-cell, natural killer (NK)-cell and myeloid markers such as CD14, CD16, CD19 and CD56 (Refs 3, 33). Human thymic DCs express neither CD2 (which is expressed by mouse thymic DCs) nor CD1, although CD1 is strongly upregulated upon culture[3]. Interestingly, while mouse thymic DCs express high levels of CD8 but low levels of CD4, human thymic DCs express low levels of CD8 but high levels of CD4 (Ref. 33). The functional significance of this differential expression of CD4 and CD8 by murine versus human DCs is not known. However, CD4 expression by thymic and peripheral human DCs is of particular relevance since it mediates susceptibility to infection by human immunodeficiency virus (HIV)[34], and thus has important implications for AIDS pathogenesis.

## APC potential of thymic DCs

As discussed above, thymic DCs express a series of molecules known to have a crucial role in T-cell costimulation, as well as the endocytic receptor DEC-205. More importantly, thymic DCs process and present a variety of endogenous and exogenous self- and nonself-antigens, including MHC molecules[35,36], minor histocompatibility antigens[37], viral proteins[38,39], and viral[40] and bacterial[41] superantigens (SAgs). Moreover, the ability of thymic DCs to activate T cells has been demonstrated in different experimental systems[35,36,38,42,43].

These studies have demonstrated that thymic DCs are as efficient APCs as their splenic counterparts and may even be superior in certain experimental conditions[36,43]; furthermore, DCs are the most efficient APCs for both endogenous and exogenous antigens, and viral SAgs (Refs 36, 42, 43). Interestingly, in addition to their strong APC potential, thymic DCs have by far the highest antigen retention capacity compared with other APCs, including cortical epithelial cells and splenic DCs, as demonstrated by estimating the *in vivo* half-life of antigen–MHC class II complexes[44]. The functional relevance of the APC capacity of thymic DCs in relation to the induction of T-cell tolerance will be discussed below.

## Thymic DC precursors

DCs have been demonstrated to derive ultimately from HSCs during fetal and adult life[45–49], but characterization of the DC-lineage-committed precursors has not yet been achieved. Definition of the progenitors of thymic DCs is of particular relevance in our understanding of the mechanisms controlling the generation of T-cell tolerance. An extrathymic or intrathymic origin of thymic DCs will determine important differences in the availability of peptides of self- or nonself-antigens that could be presented intrathymically by DCs to autoreactive T-cell clones. Evidence of an intrathymic development of thymic DCs has been reported by Steinman and colleagues, who described the formation of DCs *in vitro* when thymic cells lacking CD4, CD8, surface immunoglobulin and MHC class II were cultured in the presence of interleukin 1 (IL-1)[50]. Recently, it was demonstrated that the earliest intrathymic CD4$^{lo}$ T-cell precursor population in the mouse produces T cells and thymic DCs after intrathymic transfer into sub-lethally irradiated congenic mice[49]. Interestingly, the T-cell:DC ratio of donor origin obtained after reconstitution was equivalent to that reported in the adult mouse thymus[2]. Additionally, when injected intravenously, CD4$^{lo}$ T-cell precursors generated T cells, B cells, and thymic and splenic DCs

**Table 3. Experiments supporting the existence of a common multipotent progenitor population for T-cell, B-cell, DC and NK-cell lineages**

| Cell population assayed | Origin | Experimental model | Progenitor cells obtained | Ref. |
|---|---|---|---|---|
| HSCs | Mouse bone marrow | Intrathymic transfer after irradiation | Thymus: T cells, DCs<br>Periphery: T cells | 49 |
| $CD4^{lo}$ precursors | Mouse thymus | Intrathymic transfer after irradiation | Thymus: T cells, DCs<br>Periphery: T cells | 49 |
| $CD4^{lo}$ precursors | Mouse thymus | Intravenous transfer after irradiation | Thymus: T cells, DCs<br>Periphery: T cells, DCs, B cells | 49 |
| $CD4^{lo}$ precursors | Mouse thymus | FTOC | T cells, NK cells | 62 |
| $CD4^-CD8^-Fc\gamma RII/III^+$ precursors | Mouse fetal thymus | Intrathymic transfer after irradiation | Thymus: T cells<br>Periphery: T cells | 60 |
| $CD4^-CD8^-Fc\gamma RII/III^+$ precursors | Mouse fetal thymus | Intravenous transfer after irradiation | Thymus: not analyzed<br>Periphery: NK cells | 60 |
| $c\text{-}kit^+CD25^-CD44^+$ pro-T cells | Mouse fetal thymus | Intrathymic transfer after irradiation | Thymus: T cells<br>Periphery: T cells | 61 |
| $c\text{-}kit^+CD25^-CD44^+$ pro-T cells | Mouse fetal thymus | Intravenous transfer after irradiation | Thymus: none[b]<br>Periphery: B cells, $NK1.1^+$ cells | 61 |
| $CD34^+CD10^+Lin^-$ precursors[a] | Human bone marrow | SCID-hu thymus assay | T cells | 57 |
| $CD34^+CD10^+Lin^-$ precursors | Human bone marrow | Liquid cultures in the presence of IL-1, IL-3, IL-6, IL-7, SCF, GM-CSF, TNF, FL, EPO | DCs | 57 |
| $CD34^+CD10^+Lin^-$ precursors | Human bone marrow | Stroma-supported cultures in limiting dilution in the presence of IL-3, IL-6, LIF | DCs, B cells | 57 |
| $CD34^+CD10^+Lin^-$ precursors | Human bone marrow | Stroma-supported cultures in limiting dilution in the presence of IL-3, IL-6, LIF, IL-2, IL-7, IGF-I | DCs, B cells, NK cells | 57 |
| $CD34^+CD44^{int}$ precursors | Human postnatal thymus | Culture in the presence of IL-7 | T cells ($CD34^-CD44^-$), DCs and monocytic cells ($CD34^-CD44^+$) | 56 |
| $CD34^+CD38^{dim}$ precursors | Human postnatal thymus | Hybrid human/mouse FTOC | T cells | 59 |
| $CD34^+CD38^{dim}$ precursors | Human postnatal thymus | Culture in the presence of SCF, GM-CSF, TNF-$\alpha$ | DCs | 59 |
| $CD34^+CD38^{dim}$ precursors | Human postnatal thymus | Stroma-supported cultures in limiting dilution in the presence of SCF, IL-7, IL-2 | NK cells | 59 |

Abbreviations: DC, dentritic cell; EPO, erythropoietin; FL, FLT3/FLK2 ligand; FTOC, fetal thymic organ culture; GM-CSF, granulocyte–macrophage colony-stimulating factor; IGF-I, insulin-like growth factor I; IL, interleukin; LIF, leukemia inhibitory factor; NK, natural killer; SCF, stem cell factor; SCID-hu, severe combined immunodeficiency mice grafted with fetal human tissue; TNF, tumor necrosis factor.
[a]$Lin^-$: cells devoid of lineage-specific cell-surface markers.
[b]Fetal progenitors may lose their capacity to home back to the thymus[61].

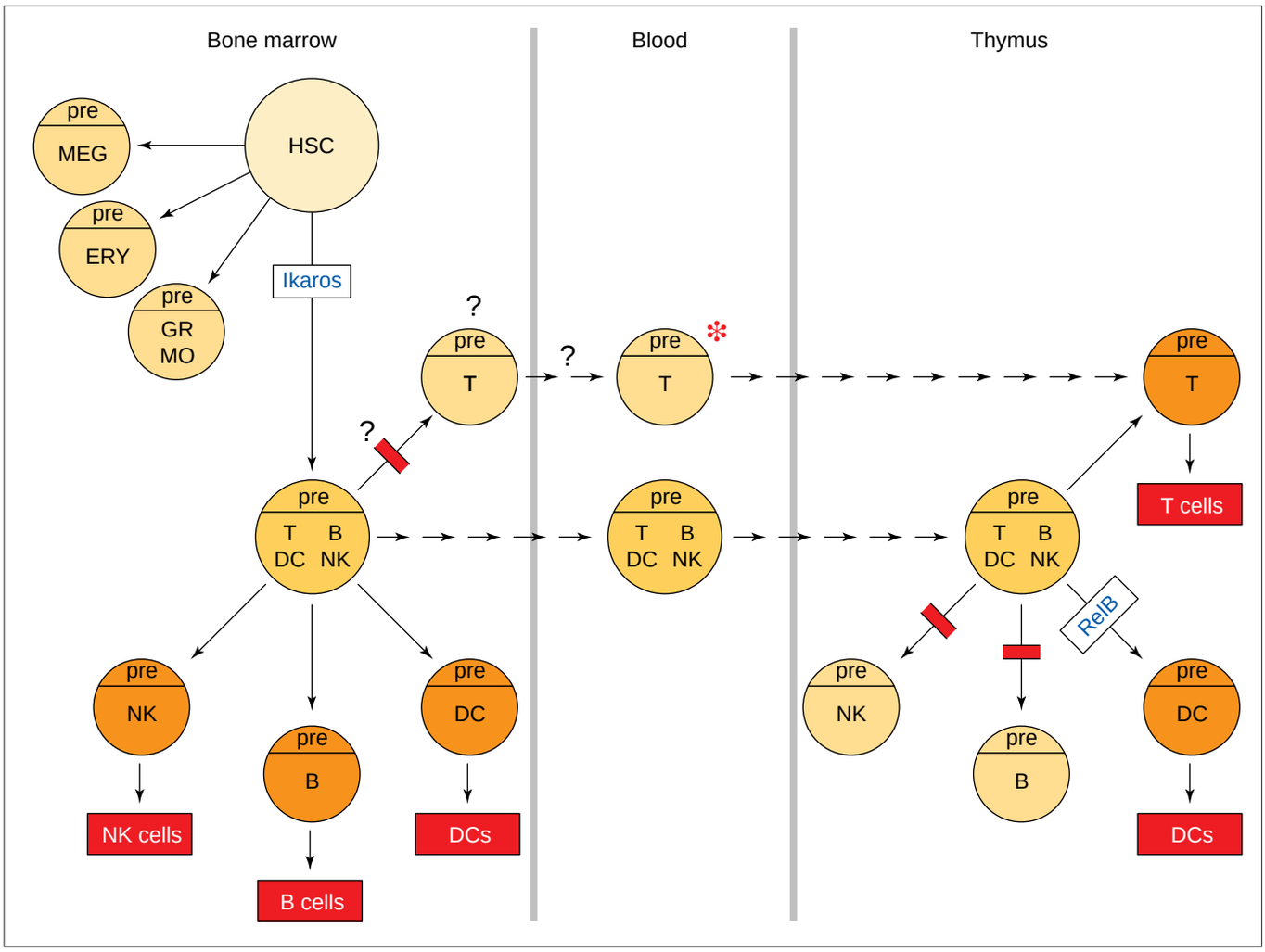


**Fig. 1.** *Model of differentiation and commitment of cell lineages. Continuous arrows indicate differentiation; small, discontinuous arrows indicate migration. Red interruptions in continuous arrows indicate blockages to the corresponding differentiation pathways. Ikaros and RelB boxes indicate the differentiation blockages occurring in mice deficient for these transcription factors (see text for details). The asterisk indicates that circulating T-cell-lineage-committed precursors have been isolated from mouse fetal blood, but have not been described in adults. Abbreviations: B, B cell; DC, dendritic cell; ERY, erythrocyte; GR, granulocyte; HSC, hematopoietic stem cell; MEG, megakaryocyte; MO, monocyte; NK, natural killer cell; T, T cell.*

(Ref. 49), but were devoid of myeloid or erythroid differentiation potential[51].

These results demonstrate the existence of functional intrathymic DC precursors and provide the first clear evidence supporting a relationship between the DC and lymphoid lineages – a relationship already suggested by the fact that these lineages share some cell-surface markers (Table 2). Therefore, the earliest T-cell precursors in adult mice (recently termed thymic lymphoid progenitors[52]) have a multipotential differentiative capacity, and are not yet committed to the T-cell lineage. Interestingly, a c-kit$^{lo}$Thy-1$^{+}$ T-cell-lineage-committed precursor has been isolated from day 15.5 mouse fetal blood[53]. These precursors generated T cells after intrathymic or intravenous transfer, but had no B-cell, myeloid or erythroid potential, indicating that T-cell lineage commitment can precede thymus colonization in the mouse fetus.

Evidence from different experimental systems over the past four years supports the hypothesis that T cells, DCs, B cells and NK cells can originate from a common multipotent progenitor (summarized in Table 3). Generation of B cells *in vitro* from intrathymic fetal and adult T-cell precursors has been reported[54,55]. More importantly, DC precursors have also been identified within the human CD34$^{+}$CD44$^{int}$ subset of postnatal thymic precursors[56], and a CD34$^{+}$CD10$^{+}$Lin$^{-}$ common progenitor population for T cells, DCs, B cells and NK cells has recently been described in human bone marrow[57]. Interestingly, in the latter report, Galy and colleagues demonstrated the T-cell reconstitution potential of CD34$^{+}$CD10$^{+}$Lin$^{-}$ precursors in the SCID-hu thymus assay (injection of precursor cells into allogeneic fetal thymic grafts depleted of their endogenous thymocytes and implanted under the kidney capsule of severe combined immunodeficiency mice)[57]. Furthermore, the differentiation of this progenitor population *in vitro* into defined cell lineages can be driven by different cytokines. Recent data also indicate the existence of a common intrathymic precursor for human T cells, DCs and NK cells: CD34$^{bright}$CD1$^{-}$ human fetal thymus progenitors generated T cells in fetal thymic organ culture (FTOC), and generated NK cells when cultured in the presence of IL-2, IL-7 and stem cell factor (SCF, c-kit ligand)[58]. More interestingly, it was recently reported that CD34$^{+}$CD38$^{dim}$ human thymic precursors differentiated

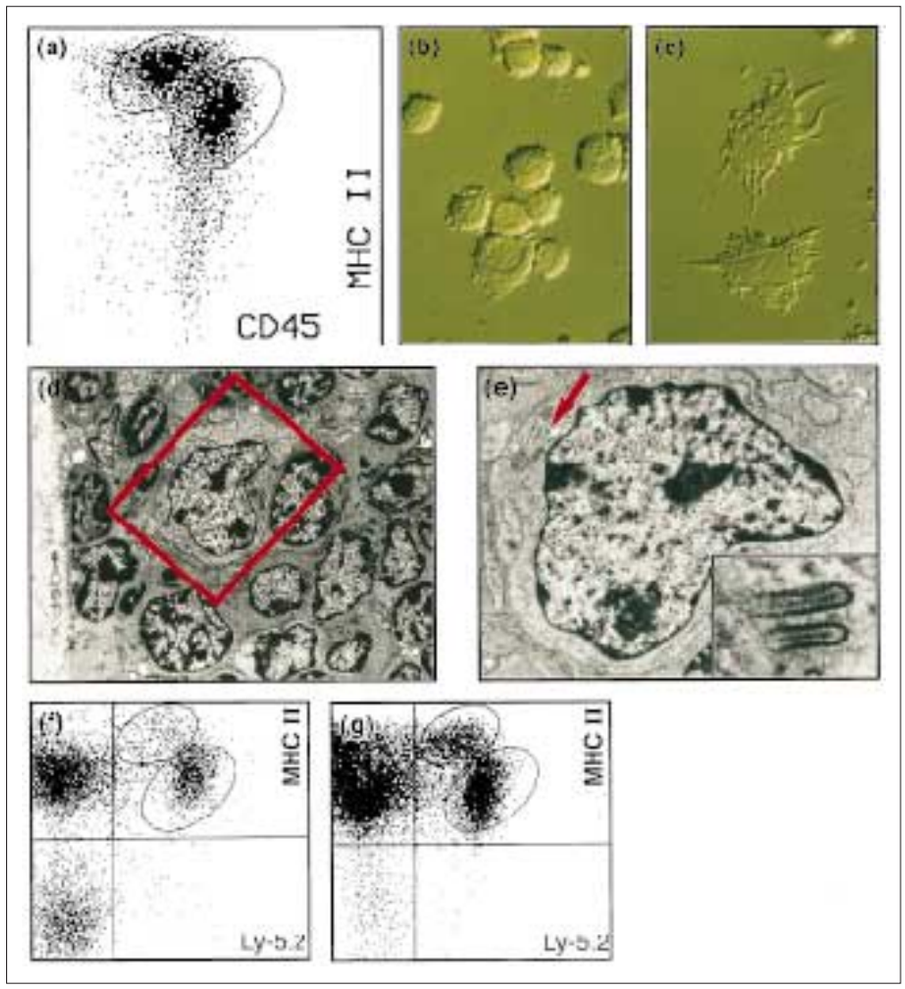


**Fig. 2.** *Thymic DC subpopulations. (a) MHC class II versus CD45 (Ly-5) profile of mouse thymic DCs. Differential expression of these markers allows the definition of MHC class II$^{lo}$ (CD45$^{hi}$) and MHC class II$^{hi}$ (CD45$^{lo}$) DC subpopulations; these subpopulations are highlighted with lower and higher circles, respectively. (b, c) Nomarski differential interference microscopy micrographs of MHC class II$^{lo}$ and MHC class II$^{hi}$ subpopulations separated by FACS (×400). (d) Electron microscopy micrograph of the subcapsular area of the mouse thymus (×1500). (e) Enlargement of the area indicated in (d) showing a lymphoblast-like cell possessing Birbeck granules (arrow) in its cytoplasm; this cell probably corresponds to an MHC class II$^{lo}$ immature DC (×6200). The inset shows a high-magnification micrograph of Birbeck granules indicated by the arrow (×91 200). (f, g) MHC class II versus donor-type CD45 (Ly-5.2) expression of DC preparations 7 and 11 days, respectively, after thymus reconstitution by intrathymic transfer of CD4$^{lo}$ precursor cells, showing the sequential appearance of MHC class II$^{lo}$ (lower circle) and MHC class II$^{hi}$ (higher circle) DCs. Abbreviations: DC, dendritic cell; FACS, fluorescence-activated cell sorting; MHC, major histocompatibility complex.*

into T cells in a hybrid human–mouse FTOC system, and generated DCs when cultured in the presence of SCF, GM-CSF and tumor necrosis factor α (TNF-α) or NK cells in the presence of SCF, IL-7 and IL-2 (Ref. 59). In the mouse, a fetal thymocyte population that lacks the T-cell receptor (TCR), CD4 and CD8, but expresses FcγRII/III, has been shown to generate T cells when transferred intrathymically and to generate NK cells after intravenous transfer[60]. In addition, c-kit$^+$CD25$^-$ precursors isolated from day 14–15 mouse fetal thymus (claimed to be functionally analogous to CD4$^{lo}$ precursors in the adult thymus) can generate B cells and NK1.1$^+$ cells after intravenous injection[61]. Finally, CD4$^{lo}$ precursors can differentiate into T cells and NK1.1$^+$TCR$^-$ NK cells in FTOC (Ref. 62).

Collectively, these data suggest a strong link between lymphoid, DC and NK-cell lineages, and support the existence in the bone marrow and thymus of a multipotent precursor population capable of differentiating into T cells, DCs, B cells or NK cells. This concept has also been reinforced by the fact that a mutation in the DNA-binding domain of the *Ikaros* gene product, a transcription factor controlling the expression of lymphoid-restricted genes, blocked the differentiation of T cells, B cells and NK cells[63], whereas the erythroid and myeloid lineages were not affected. DC differentiation was not specifically addressed in this report but it has recently been shown that mice lacking the C-terminus of the *Ikaros* gene product had deficient lymphoid and DC development[64].

A model of the differentiation and commitment of DC and lymphoid lineages is presented in Fig. 1. Multipotent T/DC/NK/B precursors will generate, under the control of bone marrow or thymic microenvironments, either peripheral DCs, B cells or NK cells, or, alternatively, T cells and thymic DCs. This model considers a direct commitment of the multipotent T/DC/B/NK precursors into monospecific T, DC, NK and B precursors. However, as previously proposed[57], experimental evidence suggests that commitment to the different lymphoid lineages could occur by the gradual restriction of the differentiation potential of the multipotent T/DC/NK/B precursor [e.g. pre-T/DC/NK/B → pre-T/DC/NK (and pre-B) → pre-T/DC (and pre-NK) → pre-T and pre-DC]. In this sense, it has been demonstrated that CD4$^-$CD8$^-$CD44$^+$CD25$^+$c-kit$^+$ pro-T cells, downstream from CD4$^{lo}$ precursors, lack B-cell differentiation potential but retain the capacity to generate DCs and NK cells[62,65]. In the mouse, IL-1α and TNF-α appear to have a crucial role in the commitment of thymic lymphoid progenitors to the T-cell lineage[61]. The signals controlling DC lineage commitment remain largely unknown in the murine system, but differentiation of human thymic DCs has been reported to require IL-7 (Ref. 56). Interestingly, analysis of *rel*B mutant mice indicates that differentiation of thymic DCs may be controlled by the relB subunit of the NF-κB transcription factor complex[66]; DC formation is severely impaired in these mice, while lymphoid and myeloid differentiation is unaffected. Further studies are necessary to establish definitively the mechanisms controlling commitment to the lymphoid and DC lineages from the thymic lymphoid progenitor.

## Thymic versus peripheral DCs

Experimental evidence presented above supports the notion that DCs develop intrathymically from a lymphoid precursor. This concept then poses the question of whether thymic and peripheral DCs represent different cell lineages. If the model presented in Fig. 1 is correct, thymic and peripheral DCs originate from the same precursor population, and therefore would represent a unique cell lineage. Differential microenvironmental conditions would determine the differentiation of DCs in a thymic or extrathymic location. In support of this, it has been demonstrated that intravenous transfer of $CD4^{lo}$ thymic precursors results in the development both of thymic and of splenic donor-type DCs (Ref. 49). In addition, as mentioned previously, functional studies using highly purified thymic and splenic DCs have not revealed significant differences between them concerning antigen internalization, processing and presentation capacity, as well as T-cell costimulation and activation potential, although they appear to have a differential capacity to induce the viral SAg-mediated proliferation of $CD4^+$ TCR-transgenic thymocytes[67]. However, phenotypic differences between thymic and splenic DCs have been reported in the mouse. Thymic, but not splenic, DCs express the B-cell precursor antigen BP-1 (Ref. 17). Furthermore, whereas both DC populations are $CD11c^+$, all thymic DCs express DEC-205 and M342, whereas splenic DCs appear to express these markers heterogeneously. It has been proposed that less-mature $DEC\text{-}205^-M342^-$ DCs located at the periphery of the white pulp generate $DEC\text{-}205^+M342^+$ DCs located in the central zone of the periarteriolar lymphatic sheath[27].

CD8 expression also differs between thymic DCs and their splenic counterparts. Thymic DCs are $CD8\alpha^+\beta^+$, while splenic DCs are $CD8\alpha^+\beta^-$ (Ref. 4). However, as mentioned above, loss of CD8β but not of CD8α after short-term culture[9] may indicate that CD8β is transferred to thymic DCs from surrounding thymocytes and, therefore, that DCs, regardless of their origin, express the CD8αα homodimer. More interestingly, whereas all thymic DCs express CD8α, this marker allows the definition of $CD8^-$ and $CD8^+$ splenic DC subpopulations[4]. In addition, it has been reported that $CD8^-FasL^-$ DCs induced a strong proliferative T-cell response, but $CD8^+FasL^+$ DCs induced a weaker response associated with Fas-mediated T-cell apoptosis[22]. Although the functional significance of these results is not well understood, it has been proposed that $CD8^-$ and $CD8^+$ splenic DCs represent two different DC lineages, with $CD8^+$ splenic DCs related to $CD8^+$ thymic DCs[22]. This is supported by recent data showing that, after intravenous transfer, HSCs generated both $CD8^-$ and $CD8^+$ splenic DCs, whereas only $CD8^+$ splenic DCs were obtained with $CD4^{lo}$ thymic precursors[65]. An alternative explanation is that $CD8^-$ and $CD8^+$ splenic DCs constitute two maturation and/or functional stages, as suggested previously on the basis of the expression of DEC-205 and M342. In this sense, two subsets of DCs have been identified in human blood on the basis of their CD11c expression[7]: the $CD11c^-$ subset is functionally immature, whereas $CD11c^+$ mature DCs display a strong T-cell-stimulatory capacity. Interestingly, as discussed below, MHC class II expression defines two major DC subpopulations in the mouse thymus.

## Thymic DC subpopulations

Murine thymic DCs can be subdivided in two discrete subpopulations on the basis of their MHC class II expression[5]: MHC class $II^{lo}$ DCs and MHC class $II^{hi}$ DCs. Both subsets express the DC markers DEC-205 and CD8α. MHC class $II^{hi}$ DCs express lower levels of heat stable antigen (HSA), CD44 and CD45 than do MHC class $II^{lo}$ DCs. MHC class II versus CD45 expression allows a clear definition of thymic DC subpopulations (Fig. 2a), and therefore their separation by FACS. FACS-sorted MHC class $II^{lo}$ and MHC class $II^{hi}$ thymic DCs display distinctive morphological characteristics: MHC class $II^{lo}$ DCs are rounded cells with an irregular surface (Fig. 2b), whereas MHC class $II^{hi}$ DCs are irregular-shaped cells with long cell surface processes characteristic of mature DCs (Fig. 2c). Thymic reconstitution by intrathymic transfer of $CD4^{lo}$ precursors[49] demonstrated that MHC class $II^{lo}$ and MHC class $II^{hi}$ DC subpopulations appear sequentially. A population of MHC class $II^{lo}$ DCs appeared seven days after transfer, whereas very few MHC class $II^{hi}$ DCs could be detected at this stage (Fig. 2f). After 11 days, however, both DC subpopulations were found (Fig. 2g). As shown in Fig. 3a, these results suggest that MHC class $II^{lo}$ DCs represent an immature stage of thymic DC differentiation originated from $CD4^{lo}$ precursors in the outer cortex. Migration of MHC class $II^{lo}$ immature DCs to the cortico-medullary junction and medulla would be paralleled by final DC differentiation resulting in mature MHC class $II^{hi}$ cells. In support of this hypothesis, electron microscopy on normal mouse thymus revealed the existence of blast-like cells in the subcapsular cortex (Fig. 2d, e), possessing Birbeck granules characteristic of DC and Langerhans cells, and these cells most likely correspond to immature MHC class $II^{lo}$ DCs. The significance of MHC class $II^{lo}$ and MHC class $II^{hi}$ DCs in T-cell negative selection is currently under investigation.

## Role of thymic DCs in negative selection

Negative selection is the process by which T-cell tolerance is induced intrathymically, and involves the deletion or functional inactivation of autoreactive T-cell clones whose TCRs determine high-affinity TCR–peptide–MHC interactions (reviewed in Ref. 68). Naturally occurring peptides that mediate negative selection may derive from self- or nonself-antigens of intra- or extracellular origin, such as MHC molecules[69], minor histocompatibility antigens[70], circulating proteins[71], viral proteins[72], as well as viral or bacterial SAg (Refs 41, 72). Although thymic epithelial cells can induce T-cell clonal deletion in certain experimental systems[72–74], extensive evidence supports the concept that DCs have a crucial role in T-cell negative selection[68]. As discussed below, the negative selection potential of DCs relies on their antigen internalization, processing and presenting capacity[36,42], their location within the thymus, and their extended antigen-retention capacity[44]. By contrast, the thymic distribution of cortical epithelial cells, as well as their relative inefficiency in internalizing exogenous proteins or activating T-cell clones and TCR-transgenic T cells in the presence of specific antigen[35,38,42,75] (which is probably due to their inability to deliver costimulatory signals[35,42]), place considerable constraints on their capacity to induce tolerance.

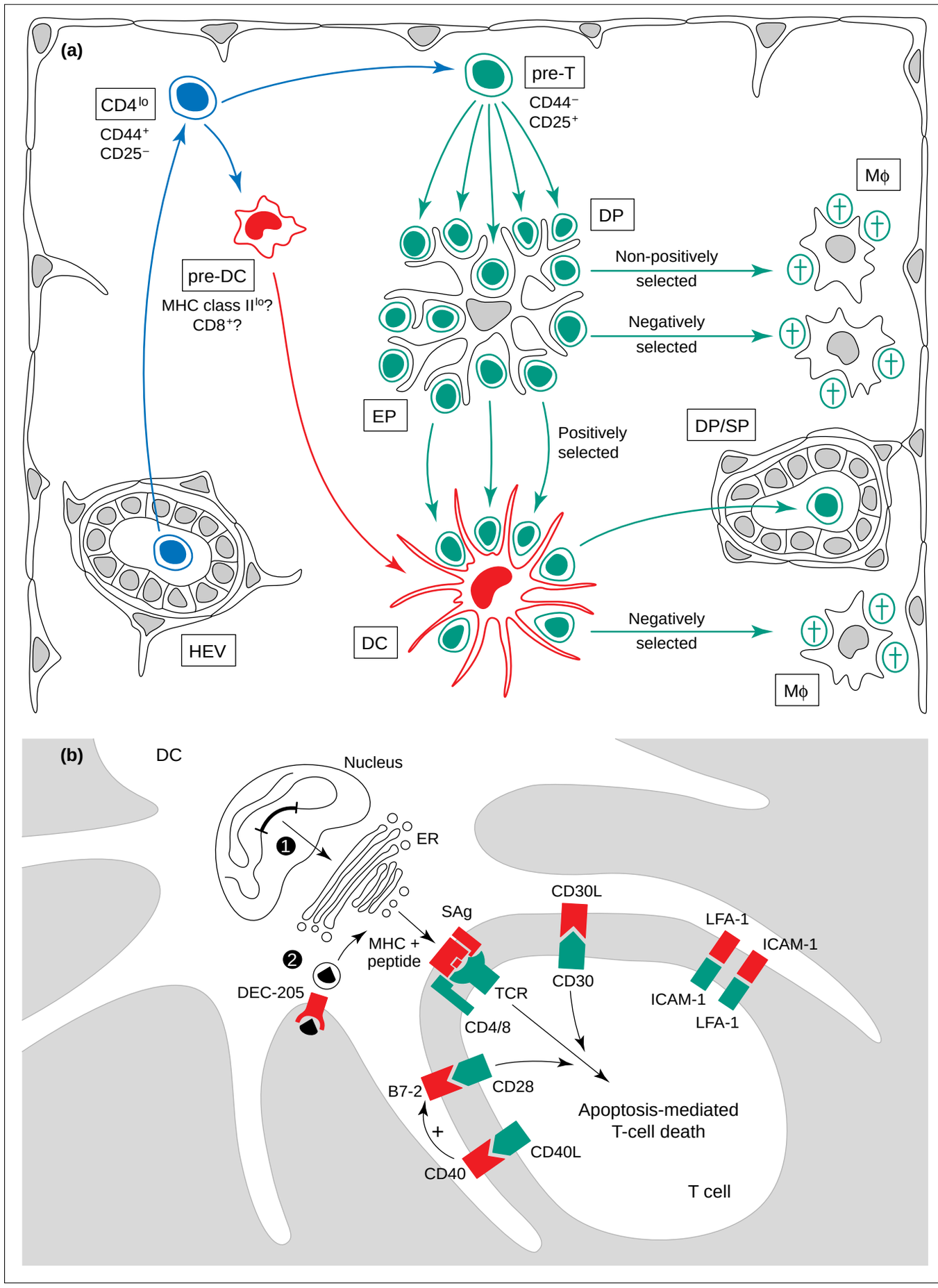
(a)
CD4lo
CD44+
CD25−
pre-T
CD44−
CD25+
pre-DC
MHC class IIlo?
CD8+?
DP
Mϕ
Non-positively
selected
Negatively
selected
EP
Positively
selected
DP/SP
HEV
DC
Negatively
selected
Mϕ
(b)
DC
Nucleus
ER
1
SAg
MHC +
peptide
2
DEC-205
CD30L
CD30
TCR
CD4/8
LFA-1
ICAM-1
ICAM-1
LFA-1
B7-2
CD28
+
CD40
CD40L
Apoptosis-mediated
T-cell death
T cell

**Fig. 3.** *Role of thymic DCs in negative selection. (a) Schematic representation of the differentiation and function of DCs in the mouse thymus, integrating the differential role of thymic epithelial cells and DCs in T-cell selection. Multipotent CD4$^{lo}$ precursors migrate to the thymus via HEVs, and differentiate into DC precursors and T-cell precursors in the subcapsular cortex. DC maturation is accompanied by migration to the cortico-medullary border and medulla. Immature DP thymocytes with functional, self-MHC-restricted TCRs are positively selected by cortical epithelial cells, and migrate to the cortico-medullary border and medulla. Non-positively selected DP thymocytes, and negatively selected autoreactive DP thymocytes bearing TCRs with high affinity for self-antigens, undergo apoptosis and are eliminated by cortical Mϕs. Positively selected, non-autoreactive, SP thymocytes exit the thymus via HEVs. Autoreactive DP/SP thymocytes bearing TCRs with high affinity for antigenic peptides presented by thymic DCs are negatively selected by apoptosis-mediated clonal elimination, and are removed by medullary Mϕs. (b) Scheme of the molecular interactions involved in DC-mediated T-cell negative selection. Antigenic peptides presented by thymic DCs include (1) those originated by cytosolic degradation of endogenous self- and nonself-proteins encoded by the DC genome, via the endoplasmic reticulum (ER), and (2) those derived from exogenous self- and nonself-proteins taken up by receptor molecules, such as DEC-205, involved in the endocytic pathway. High-affinity MHC–peptide–TCR interactions ultimately lead to apoptosis-mediated T-cell death. Candidate molecules and their ligands that could be involved in negative selection signaling are shown (see text for details). Abbreviations: CD40L, CD40 ligand; DC, dendritic cell; DP, double positive; EP, epithelial cell; HEV, high endothelial venule; ICAM-1, intercellular cell adhesion molecule 1; LFA-1, leukocyte function-associated molecule 1; MHC, major histocompatibility complex; Mϕ, macrophage; SAg, superantigen; SP, single-positive; TCR, T-cell receptor.*

Tolerance towards a defined antigen is generated depending on its availability and concentration within the thymic microenvironment. Thus, the nature and concentration of the antigen may have important consequences on the stage of differentiation of the T-cell being deleted, and on the internalization, processing and costimulatory requirements of the deleting APC. Limiting amounts of antigen may provoke the need for highly efficient processing and/or costimulatory signals, whereas a high concentration of antigen may override these requirements. Interestingly, soluble circulating antigens have easier access to the medulla than to the cortex[42,68,76]. This suggests that negative selection of MHC class II-restricted T-cell clones specific for exogenous antigens that require internalization and endosomal degradation occurs mainly in the medulla and cortico-medullary junction, as demonstrated in TCR-transgenic mice with specificity for the C5 component of complement[71]. Concerning the T-cell developmental stage at which intrathymic tolerance is achieved *in vivo*, a variety of experimental models have illustrated that for MHC class I- as well as class II-restricted thymocytes, double-positive (DP)[72,77,78], single-positive (SP)[71,72,78] and intermediate differentiation stages[69,79] can be clonally deleted.

Despite a large literature on T-cell negative selection, some crucial questions remain unanswered concerning the naturally occurring negatively selecting antigens, the timing of clonal deletion, the differential role of the separate thymic APC populations, and the molecular mechanisms involved. Figure 3a shows an integrated model of the role of epithelial cells and DCs in T-cell selection, based on the data presented above. Potentially autoreactive cortical DP thymocytes bearing TCRs with high affinity for peptide–MHC complexes are clonally eliminated when the corresponding antigen is available in the cortex and can be efficiently processed and presented by cortical epithelial cells. Due to their functional APC restrictions, it has been proposed[68] that epithelial cells preferentially induce the clonal deletion of MHC class I-restricted thymocytes that recognize endogenously synthesized peptides. This is supported by experiments analyzing the clonal deletion of TCR-transgenic thymocytes in irradiated bone marrow chimeras that express the negative selection restriction element on host tissue[72]. Eventually, MHC class II-restricted thymocytes specific for exogenous antigens can be negatively selected at the cortical DP stage, when those antigens are present in the cortex at high concentration[77]. Positively selected DP/SP thymocytes that have avoided initial deletion would be negatively selected at the later checkpoint when their MHC class I- or II-restricted TCRs recognize with high affinity their specific antigenic peptides presented by thymic DCs. Both *in vivo* and *in vitro* experimental approaches have provided evidence for the capacity of thymic DCs to induce the negative selection of T cells with specificities for MHC molecules[69], minor histocompatibility antigens[37], circulating proteins[71], viral antigens[38,72], viral SAgs (Ref. 67) and bacterial SAgs (Ref. 41). The antigen internalization and processing potential, as well as the costimulatory capacity, of thymic DCs may allow the negative selection of T-cell clones specific for antigens against which T-cell tolerance cannot be induced by cortical epithelial cells[71,80].

Little is known about the molecular mechanisms controlling the process of negative selection. Molecules expressed by thymic DCs, and known to play an essential role in peripheral T-cell activation, costimulation and apoptosis, remain poorly understood in the context of tolerance induction. DC–thymocyte molecular interactions that could be involved in negative selection are represented in Fig. 3b and discussed below. LFA-1–ICAM-1 interactions are implicated by *in vitro* experiments in which deletion of lymphocytic choriomeningitis virus (LCMV)- or H-Y-specific TCR-transgenic DP thymocytes was blocked by anti-LFA-1 antibodies[81,82]. However, contradictory results have been reported on the role of the Fas–FasL interaction in negative selection: whereas blocking of FasL does not impair *in vitro* deletion of TCR-transgenic DP thymocytes[83], both *in vivo* and *in vitro* thymocyte apoptosis has been induced with anti-Fas antibodies[84].

Some controversy also exists concerning costimulation during negative selection. Deletion of SAg-reactive[32] and H-Y-specific TCR-transgenic thymocytes[85] is not blocked by *in vivo* or *in vitro* treatment with CTLA-4–Ig, nor is the negative selection of SAg-reactive and H-2L$^{d}$-specific TCR thymocytes impaired in CD28-deficient mice[86]. Although these experimental approaches suggest that the B7–CD28 interaction is not required in T-cell clonal deletion, its involvement cannot be ruled out when antigen is present only in limiting amounts and may therefore require highly efficient

presentation in order to determine a high-avidity TCR–peptide–MHC interaction. This is supported by a recent report analyzing the involvement of the CD40–CD40L interaction in the negative selection of SAg-reactive and cytochrome *c*-specific TCR-transgenic thymocytes by anti-CD40L antibody treatment and in CD40L-deficient mice[87]. The results demonstrated that the CD40–CD40L interaction plays an essential role in negative selection when antigen or SAg were produced endogenously, but not when administered exogenously at supraphysiological concentration. It was proposed that the CD40–CD40L interaction participates in T-cell clonal deletion by mediating APC upregulation of costimulatory molecules that are required for thymocyte signaling during negative selection when antigen is produced under physiological conditions. Supraphysiological antigen concentration may induce thymocyte deletion in the absence of costimulation. Finally, recent evidence suggests that CD30, a member of the TNF receptor superfamily, participates in negative selection, as clonal deletion of H-Y-TCR-transgenic thymocytes is defective in CD30-deficient mice[88].

On the basis of the existing data, a model integrating the molecular interactions controlling intrathymic T-cell deletion cannot as yet be defined. Moreover, signal-transducing molecules such as $p56^{lck}$, $p21^{ras}$, mitogen-activated protein kinase (MAPK) or calcineurin that are known to play an essential role in T-cell signaling during peripheral activation or positive selection do not appear to participate in negative selection[89–92]. Interestingly, a recent report describing ZAP-70-deficient mice[93] has demonstrated the involvement of the ZAP-70 protein tyrosine kinase in anti-CD3-induced apoptosis, and in the clonal deletion of ovalbumin-specific TCR-transgenic thymocytes.

## Concluding remarks

Recent studies analyzing the differentiation potential of both human and mouse lymphoid precursors support a close developmental relationship between DC and lymphoid lineages. Within the thymus, the existence of a common functional precursor restricted to T cells and $CD8^+$ DCs suggests that thymic DCs may represent a separate DC lineage, and this has important consequences for the origin and availability of self-antigens governing the clonal elimination or inactivation of autoreactive T cells. Future studies on thymic DC biology, based on experimental approaches approximating physiological conditions, should provide new insights on the underlying mechanisms of induction of T-cell immunological tolerance.

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