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Clonal deletion of thymocytes by circulating dendritic cells homing to the thymus

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Dendritic cell (DC) presentation of self antigen to thymocytes is essential to the establishment of central tolerance. We show here that circulating DCs were recruited to the thymic medulla through a three-step adhesion cascade involving P-selectin, interactions of the integrin VLA-4 with its ligand VCAM-1, and pertussis toxin–sensitive chemoattractant signaling. Ovalbumin-specific OT-II thymocytes were selectively deleted after intravenous injection of antigen-loaded exogenous DCs. We documented migration of endogenous DCs to the thymus in parabiotic mice and after painting mouse skin with fluorescein isothiocyanate. Antibody to VLA-4 blocked the accumulation of peripheral tissue–derived DCs in the thymus and also inhibited the deletion of OT-II thymocytes in mice expressing membrane-bound ovalbumin in cardiac myocytes. These findings identify a migratory route by which peripheral DCs may contribute to central tolerance.

The random rearrangement of T cell receptor genes in the thymus continuously generates autoreactive T cells that must be removed from the lymphocyte pool or be rendered innocuous to maintain self-tolerance. Most T cells that encounter their cognate major histocompatibility complex (MHC)–peptide complex before completion of thymic development are eliminated by clonal deletion, a key process leading to central tolerance[1]. The presence of an agonist peptide in the thymus can also result in the differentiation of $CD4^+CD25^+$ regulatory T cells[2] that impose tolerance in the periphery by dampening the response of conventional lymphocytes[3]. Central tolerance is therefore believed to be restricted to those antigens that gain access to the thymic environment and can be effectively displayed on the surface of antigen-presenting cells; that is, proteins that are expressed locally or that circulate in the bloodstream. Although some tissue-specific proteins may be expressed in the thymus[4,5], no active transport of self antigens from the periphery has been described so far.

Dendritic cells (DCs) are very efficient antigen-presenting cells found in all peripheral organs and lymphoid tissues. In the periphery, they are strategically positioned to sample antigens from tissues and carry them to draining lymph nodes for processing and presentation to recirculating T cells[6]. When exposed to appropriate stimuli such as lipopolysaccharide, proinflammatory cytokines or other indicators of cellular distress, DCs undergo maturation, resulting in enhanced antigen presentation and increased expression of costimulatory molecules[6]. The maturing DCs gain access to lymph nodes through afferent lymphatic vessels and induce naive T cells to proliferate and to differentiate into effector cells. Immature DCs are also thought to carry antigen to lymph nodes and to interact with naive T cells, but without a previous maturation stimulus, those interactions result in abortive activation of the T cells, which can be eliminated, rendered unresponsive or induced to differentiate into regulatory T cells[7].

Tissue-resident DCs can reach the circulation and carry antigen to distal organs such as the bone marrow via the blood[8]. Thus, we reasoned that in the steady state, the DC network would provide a suitable mechanism for sampling tissue-specific self antigens, otherwise 'invisible' to the differentiating thymocytes, and carrying them to the thymus via the bloodstream. Here we provide evidence of a multistep adhesion pathway that recruited circulating DCs to the thymus; we also show that peptide-presenting DCs that homed to the thymus induced clonal deletion of developing antigen-specific T cells and, therefore, might make a previously unappreciated contribution to the establishment of central tolerance.

RESULTS

Immature DCs migrate to the thymus

Immature $CD11c^{hi}$ MHC class II–positive DCs constitute a rare but discrete cell population in normal mouse and human blood[9] (**Supplementary Fig. 1** online). This circulating DC population is composed of cells with diverse histories; although some could derive from newly generated bone marrow emigrants, there is also evidence that DCs in other tissues can return to the bloodstream after acquiring antigenic material at peripheral sites[8,9]. To model the trafficking properties of circulating DCs, we did adoptive transfer experiments in which we enriched DC populations obtained from the spleens of donor mice exposed to the ligand for the receptor tyrosine kinase Flt3 (Flt3L), labeled the cells with CFSE (carboxyfluorescein

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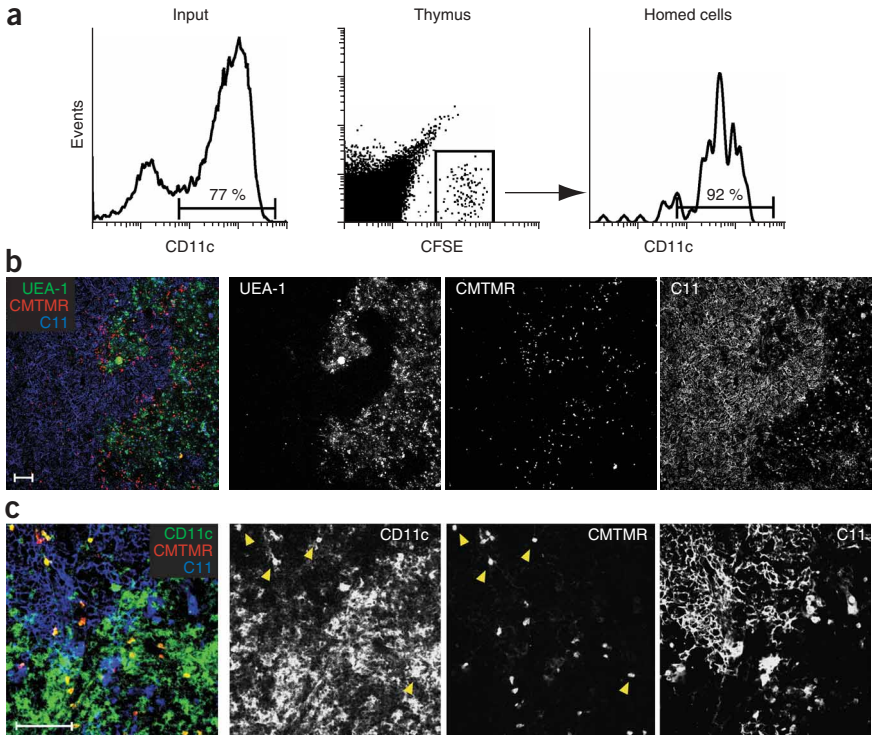

Figure 1 Blood-borne DCs are recruited to the thymus and localize to the medulla. (**a**) Flow cytometry of CD11c expression. After splenic DC populations were expanded by Flt3L, cells were partially purified, labeled with CFSE and injected intravenously into wild-type mice; 18 h later, thymi were collected and single-cell suspensions were analyzed. Left, CD11c expression in the input population; middle, thymocyte suspension showing homed CFSE$^+$ cells; right, CD11c expression on gated CFSE$^+$ cells. Numbers above bracketed lines indicate the frequency of CD11c$^+$ events. Data are representative of more than ten individual experiments. (**b**,**c**) Confocal microscopy of thymus sections (overlay (far left) and single-channel images). Mice were injected intravenously with CMTMR-labeled DCs; 18 h later, thymi were collected and cryosections 20 μm in thickness were fixed and were stained with a lectin delineating the medulla (UEA-1) or with antibodies to markers for the cortex (C-11) or DCs (CD11c). Yellow arrowheads indicate colocalization of CD11c and CMTMR signals. Scale bars, 100 μm. Data are representative of two experiments.

succinimidyl diester) and administered them intravenously to syngeneic recipient mice. Consistent with published observations[8], we recovered abundant CFSE$^+$CD11c$^+$ DCs from spleen, bone marrow, liver and lung 18 h after injection. In addition, we noted that some of the transferred DCs were also recruited to the thymus (**Fig. 1a**). Compared with the transferred input, which contained 20–30% CD11c$^-$ contaminating cells (**Fig. 1a**, left), the population of CFSE$^+$ cells in the thymus was enriched for CD11c$^+$ cells (**Fig. 1a**, right), in contrast to all other organs analyzed.

To determine the localization of the transferred DCs, we analyzed thymic cryosections by immunofluorescence at 18 h after injection. The fluorescence-tagged donor DCs were concentrated in the medulla in proximity to the cortico-medullary junction (**Fig. 1b**), in the same region where endogenous thymic DCs are found[10]. In contrast, we found very few homed cells in the cortex. Costaining with antibody to CD11c (anti-CD11c) confirmed that most homed donor cells were DCs and localized together with the bulk of the endogenous thymic CD11c$^+$ cell population (**Fig. 1c**).

Splenic DCs include at least three distinct subsets: CD8α$^-$CD11b$^+$ 'myeloid' DCs, CD8α$^+$CD11b$^-$ DCs (formerly known as 'lymphoid' DCs) and B220$^+$CD11c^{lo} plasmacytoid DCs[11]. In spleens of mice exposed to Flt3L, we also found a CD8α$^-$CD11b$^-$ population that has been identified before in Peyer's patches and lymph nodes[12,13]. All DCs respond to 'danger signals' (bacterial products, inflammatory cytokines and others) by upregulating costimulatory molecules and antigen-presentation machinery and thus become competent to prime naive T cells[6]. Maturation also influences the expression of chemokine receptors and adhesion molecules and, therefore, the trafficking of DCs[14]. Thus, we tested the influence of subset membership and maturation state on the migration of DCs to the thymus.

Comparison of the subset distribution of DCs in the purified input population to the frequency of each subset after short-term (2-hour) homing showed that all DC populations analyzed had similar access to the thymus (**Fig. 2a**, left). Only CD8α$^+$ DCs were slightly underrepresented in spleen and thymus compared with the input, but this

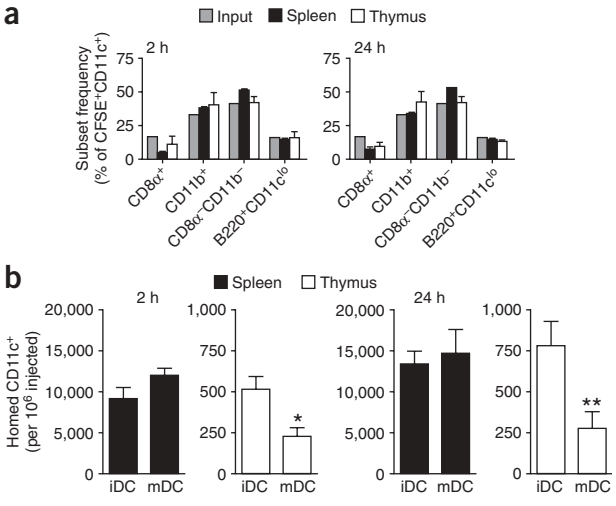

Figure 2 Phenotype of DCs recruited to the thymus. (**a**) Staining of thymi and spleens for B220, CD8α, CD11b and CD11c at 2 h or 24 h after DC populations expanded *in vivo* with Flt3L were labeled with CFSE and administered intravenously to wild-type mice ($n = 2$ mice per group). Data are presented as percent cells belonging to a particular subset in the input (gray bars) or gated on the homed CFSE$^+$CD11c$^+$ population in spleen (filled bars) and thymus (open bars). (**b**) Homing, assessed by flow cytometry for the presence of CD11c$^+$TRITC$^+$ and CD11c$^+$CFSE$^+$ cells. Freshly purified immature DCs (iDC) or DCs stimulated overnight with 1 μg/ml of lipopolysaccharide (mDC) were differentially labeled with CFSE or TRITC and were injected intravenously into wild-type mice ($n = 4$ or more mice). Spleens and thymi were collected at various times (above graphs) and single-cell suspensions were analyzed. Data are presented as total number of homed CD11c$^+$ cells per 1×10^6 cells injected. *, $P < 0.05$, and **, $P < 0.001$, compared with freshly purified DCs. Data are pooled from two (**a**) or three (**b**) experiments (error bars, s.e.m.).

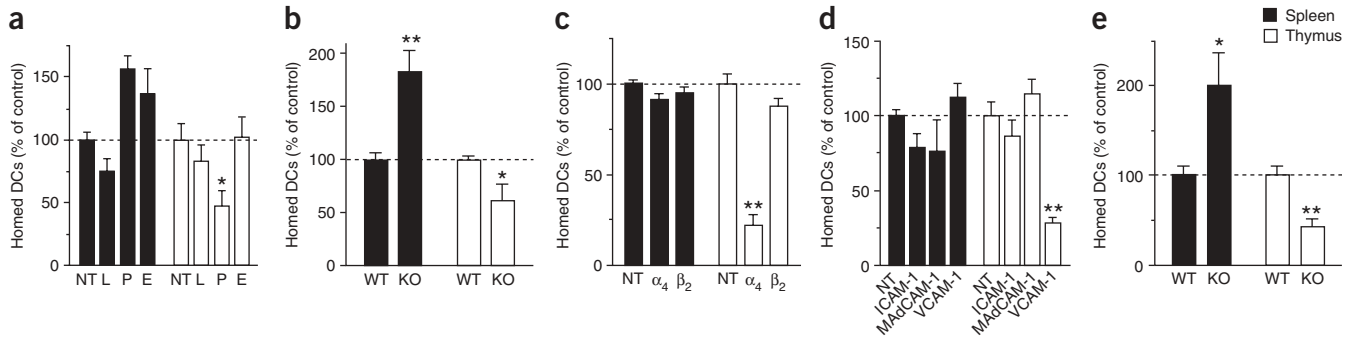

Figure 3 P-selectin and VLA-4–VCAM-1 interactions mediate the recruitment of DCs to the thymus. (**a**) Homing of DCs whose populations were expanded *in vivo* with Flt3L, then were labeled with CFSE and were injected intravenously into wild-type mice ($n = 6$ or more mice per group) either alone (untreated (NT)) or in combination with 100 μg of mAb to L-selectin (L), P-selectin (P) or E-selectin (E); 18 h later, spleens and thymi were collected and single-cell suspensions were analyzed by flow cytometry for the presence of $CD11c^+CFSE^+$ cells. Data are presented as the percentage of homed DCs in untreated recipients. (**b**) Homing of CFSE-labeled DCs injected intravenously into wild-type (WT) or P-selectin-deficient (KO) mice ($n = 6$ mice per group); spleens and thymi were analyzed 18 h later as described in **a**. (**c**) Homing of DCs left untreated (NT) or pretreated with 50 μg/ml of blocking mAb to α_4 or β_2 integrin, then differentially labeled with CFSE or TRITC and injected intravenously into wild-type mice ($n = 6$ mice per group); spleens and thymi were analyzed 2 h later as described in **a**. (**d**) Homing of CFSE-labeled DCs injected intravenously into wild-type mice ($n = 4$ mice per group) either alone (NT) or in combination with 100 μg mAb to ICAM-1, MAdCAM-1 or VCAM-1; spleens and thymi were analyzed 18 h later as described in **a**. (**e**) Homing of CFSE-labeled DCs injected intravenously into wild-type (WT) or VCAM-1-knockout (KO) mice ($n = 5$ mice per group); spleens and thymi were analyzed 18 h later as described in **a**. Dashed horizontal lines indicate the mean homing of untreated DCs in control mice (NT or WT; error bars, s.e.m.). *, $P < 0.05$, compared with untreated (**a**) or wild-type (**b**); **, $P < 0.01$, compared with wild-type (**b**,**e**) or untreated (**c**,**d**). Data are pooled from three (**a**,**b**) or two (**c**–**e**) experiments.

was most likely due to their propensity to become trapped in the lungs after intravenous injection[8]. Subset frequencies remained essentially unaltered after 24 h (**Fig. 2a**, right), indicating that all DCs were retained and survived equally well regardless of their surface phenotype. In contrast, maturation by overnight exposure to lipopolysaccharide from *Escherichia coli* reduced the ability of DCs to home to the thymus in short term (2-hour) and long-term (24-hour) adoptive transfer experiments by 55% and 65%, respectively (**Fig. 2b**). This 'preferential' recruitment of immature DCs was unique to the thymus, as we found no difference in the number of homed immature and mature cells in the spleen (**Fig. 2b**), bone marrow or other lymphoid organs (data not shown). We concluded that all subtypes of blood-borne immature but not mature DCs can efficiently gain access to the thymus, where they localize mainly in the medulla.

Molecular mechanism of DC recruitment

Homing of circulating leukocytes to most tissues is mediated by multistep adhesion cascades in which initial selectin-dependent rolling interactions are converted to firm adhesion by chemokine-induced activation of specific integrins[15]. To delineate the molecular components of the multistep cascade responsible for the homing of DCs to the thymus, we did adoptive transfers in the presence or absence of blocking monoclonal antibodies (mAbs) to a panel of candidate traffic molecules. Of the three selectins typically involved in rolling interactions[15], only the absence of endothelial P-selectin significantly reduced homing, as demonstrated by antibody inhibition (**Fig. 3a**; 47% of untreated control) and by the use of P-selectin-deficient recipients (**Fig. 3b**; 62% of wild-type). However, neither E-selectin nor L-selectin was involved. Combined blockade of P-selectin and E-selectin did not result in additional inhibition compared with the effect of anti-P-selectin alone (data not shown), ruling out the possibility of an auxiliary function for E-selectin in this setting. We recovered more DCs from the spleen (**Fig. 3a**,**b**) and peripheral blood (data not shown) in the absence of P-selectin, indicating that this pathway is required for DC traffic to some but not all organs.

Leukocyte integrins that can mediate intravascular firm arrest belong to either the β_2 or the α_4 subfamily[15]. Adoptively transferred DCs pretreated with neutralizing mAb to β_2 reached the thymus as efficiently as untreated control cells, whereas blockade of α_4 integrins resulted in inhibition of about 80% (**Fig. 3c**). Homing of DCs to the thymus was also reduced by blockade of VCAM-1 (the main endothelial ligand for the integrin VLA-4 ($\alpha_4\beta_1$)), but not by blockade of MAdCAM-1 (a ligand for the integrin $\alpha_4\beta_7$), ICAM-1 (a ligand for the integrin $\alpha_L\beta_2$ (also called LFA-1)) or the integrin $\alpha_M\beta_2$ (also called Mac-1; **Fig. 3d**,**e**). Thus, the only integrin critical for homing of DCs to the thymus is VLA-4. Consistent with that conclusion, DCs purified from LFA-1-deficient or integrin β_7–deficient donors homed to the thymus as efficiently as wild-type DCs (data not shown). By analogy with the molecular adhesion cascade for homing of DCs to the bone marrow identified by intravital microscopy[8], it seems very likely that VLA-4–VCAM-1 interactions are responsible for firm adhesion, although VLA-4 can also mediate rolling *in vitro*[16] and *in vivo*[17].

G protein–coupled receptors commonly provide the signal for integrin activation and consequent arrest of rolling leukocytes[15].

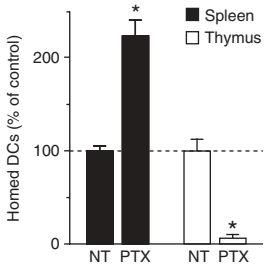

Figure 4 $G\alpha_i$-mediated signaling is required for homing of DCs to the thymus. DC populations were expanded *in vivo* with Flt3L and cells were left untreated (NT) or were incubated for 1 h with 100 ng/ml of PTX, were differentially labeled with CFSE or TRITC and then were injected intravenously into wild-type mice ($n = 4$); 18 h later, spleens and thymi were collected and single-cell suspensions were analyzed by flow cytometry for the presence of $CD11c^+CFSE^+$ and $CD11c^+TRITC^+$ cells. Dashed horizontal lines indicate the mean homing of untreated DCs in control mice (NT; error bars, s.e.m.). Data are presented as percentage of homed untreated DCs. *, $P < 0.001$, compared with untreated. Data are pooled from two experiments.

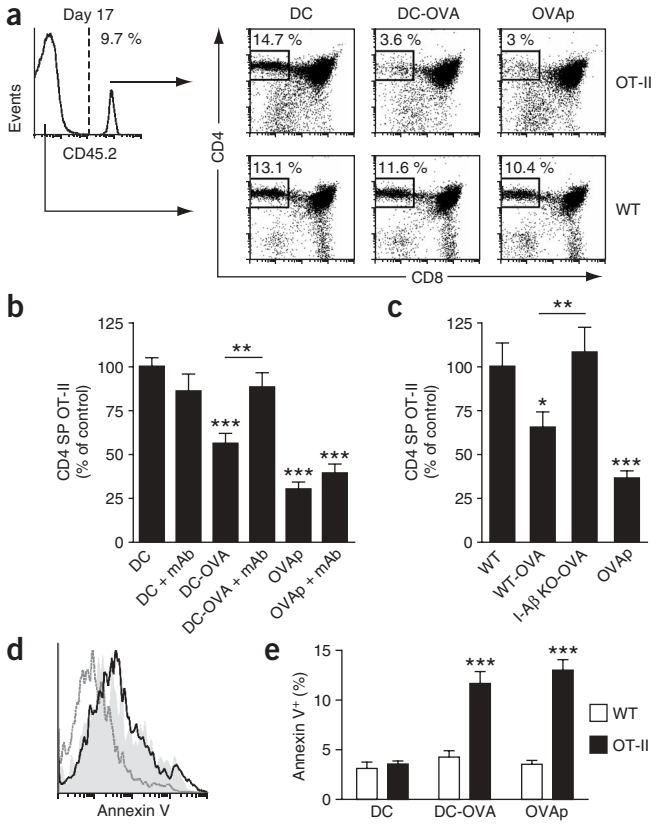

Figure 5 Circulating DCs recruited to the thymus induce apoptosis and clonal deletion of antigen-specific thymocytes. (**a**) Flow cytometry of CD45.2, CD4 and CD8 expression in single-cell suspensions of thymi from bone marrow–chimeric mice (**Supplementary Fig. 2**), analyzed on day 17 after bone marrow transplantation. Numbers above boxed areas indicate percent CD4 SP cells in $CD45.2^+$ (OT-II) and $CD45.2^-$ (WT) gates. DC, untreated DCs; DC-OVA, DCs loaded with 20 μM OVA(323–339); OVAp, soluble OVA(323–339) (50 nmol, intravenously). (**b**) Thymi of bone marrow chimeras ($n = 8$ mice per group) analyzed as described in **a**. +mAb, cells pretreated with 50 μg/ml of nondepleting mAb to integrin α_4 and mice treated with 100 μg mAb to VCAM-1. **, $P < 0.01$, DC-OVA versus DC-OVA+mAb; ***, $P < 0.001$, versus DC. (**c**) Thymi of bone marrow chimeras ($n = 7$ mice per group), analyzed and presented as in **b**. WT-OVA, wild-type DCs loaded with 1 mg/ml of OVA; I-Aβ KO-OVA, I-Aβ-knockout DCs loaded with OVA. *, $P < 0.05$, versus WT; **, $P < 0.01$, WT-OVA versus KO-OVA; ***, $P < 0.001$, versus WT. Data in **b**,**c** are presented as the frequency of CD4 SP cells among all OT-II ($CD45.2^+$) cells in treated mice relative to the average frequency of CD4 SP OT-II cells in thymi of control mice that received only unpulsed DCs. (**d**,**e**) Representative annexin V staining (**d**) of OT-II CD4 SP cells in a bone marrow chimera treated with DCs alone (dotted line), OVA(323–339)-loaded DCs (filled gray histogram) or free OVA(323–339) (black line); and quantification of annexin V staining (**e**) in thymi of bone marrow chimeras ($n = 3$ mice per group) for OT-II CD4 SP cells (OT-II) or wild-type CD4 SP cells (WT). ***, $P < 0.001$, versus OT-II DC. Data are representative of five (**a**) or two (**d**) experiments or are pooled from three (**b**) or two (**c**,**e**) experiments.

These receptors recognize specific chemoattractants (such as chemokines) on the lumenal aspect of the endothelium and signal via members of the $G\alpha_i$ family, which are inactivated by *Bordetella pertussis* toxin (PTX). Indeed, homing of DCs to the thymus but not the spleen was substantially inhibited by pretreatment with PTX (**Fig. 4**), indicating involvement of one or more $G\alpha_i$-dependent chemoattractant receptors in this pathway. PTX treatment did not affect cell viability, as assessed by annexin V staining of PTX-treated DCs after *in vivo* homing or 18 h of culture *in vitro* (data not shown). These results collectively indicated that immature DCs home to the thymus through a multistep adhesion cascade, using P-selectin for rolling, VLA-4–VCAM-1 for firm arrest, and one (or more) as-yet-unidentified chemoattractant(s) signaling through G_i-coupled receptors.

Thymus-tropic DCs induce clonal deletion

Having identified and characterized a mechanism for the recruitment of circulating DCs to the thymus, we addressed the functional consequences of this migratory event. Given that resident thymic DCs contribute to negative selection[7,10] and that immature DCs were 'preferentially' recruited, we hypothesized that DCs could transport to the thymus antigens acquired in a tolerogenic context (in the absence of maturation stimuli) and induce negative selection by presenting them to developing thymocytes. We designed an experimental protocol to test that hypothesis (**Supplementary Fig. 2** online). We reconstituted lethally irradiated $CD45.1^+$ mice with a 1:1 mixture of lymphocyte-depleted bone marrow cell samples from $CD45.1^+$ wild-type mice and $CD45.2^+$ OT-II × *Rag1*$^{-/-}$ (recombination-activating gene 1–deficient) mice. These bone marrow chimeras generated two cohorts of developing T cells: a polyclonal $CD45.1^+$ population, and $CD45.2^+$ thymocytes exclusively expressing the OT-II T cell receptor, which recognizes a peptide of amino acids 323–339 from chicken ovalbumin (OVA(323–339)) in the context of $I\text{-}A^b$ (ref. 18). The time course of our protocol was dictated by the fact that intravenously injected DCs readily access the spleen and other tissues, where they can activate antigen-specific mature T cells, resulting in the production of inflammatory cytokines and steroid hormones that cause nonspecific death of thymocytes[19]. To avoid those confounding side effects, we adoptively transferred peptide-loaded DCs into chimeric recipient mice 2 weeks after reconstitution; at that time, intrathymic T cell development was already well under way (**Fig. 5a**) but the number of mature OT-II cells in the periphery was still negligible (data not shown).

Bone marrow chimeras received intravenous boluses of 1×10^7 DCs untreated or 'loaded' with OVA(323–339) or, as a positive control, 50 nmol of soluble peptide. Then, 2 d after the last injection, we killed the mice and quantified the frequency and differentiation state of the polyclonal and OT-II thymocytes by flow cytometry. Notably, DCs loaded with OVA(323–339) efficiently eliminated $CD4^+$ single-positive (CD4 SP) OT-II thymocytes (**Fig. 5a**). We also found significant clonal deletion of OT-II cells in mice that received 1×10^6 'loaded' DCs per injection ($P < 0.05$; **Supplementary Fig. 3** online).

Although that result was consistent with the hypothesis that homed DCs may directly eliminate antigen-specific T cells in the thymus, our

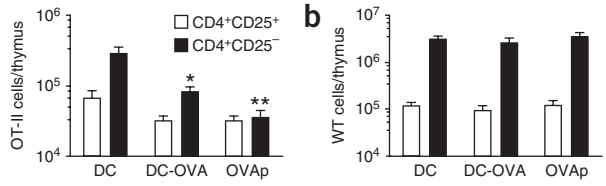

Figure 6 Presentation of agonist peptide by blood-borne DCs does not induce intrathymic differentiation of OT-II regulatory T cells. CD4 SP thymocytes from bone marrow chimera ($n = 8$ mice per group; **Supplementary Fig. 2**) were analyzed for CD25 expression by flow cytometry and absolute numbers of $CD4^+CD25^-$ and $CD4^+CD25^+$ regulatory T cells were calculated for OT-II populations (**a**) and wild-type populations (**b**). *, $P < 0.01$, and **, $P < 0.001$, compared with DCs. Data are pooled from three experiments (error bars, s.e.m.).

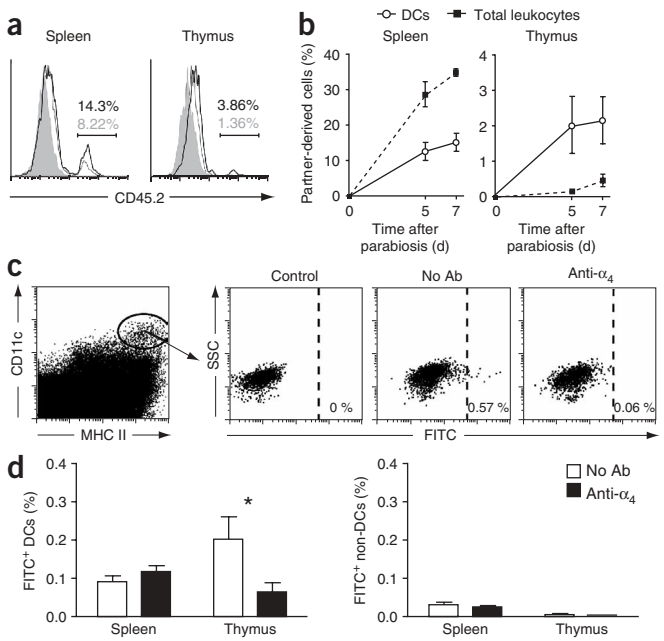

Figure 7 Migration of endogenous DCs to the thymus. (**a**) Representative flow cytometry of DCs in the spleen and thymus of parabiotic pairs at 5 d (gray lines) or 7 d (black lines) after parabiosis. Filled histograms, background staining in control mice. Numbers above bracketed lines indicate percentage of partner-derived DCs at 5 d (gray) and 7 d (black). Histograms are gated on CD11c^{hi}MHC-II^{+} events. Data are representative of two independent experiments. (**b**) Percentage of partner-derived DCs and total leukocytes in parabiotic mice ($n = 4$ mice per time point). (**c**) Detection of DCs in thymi of FITC-painted mice. Far left, gating strategy; remaining plots, FITC staining on DCs from thymi of wild-type mice 48 h after epicutaneous application of FITC. Numbers in bottom right corners indicate percent FITC^{+} cells. Data are representative of four independent experiments. MHC II, MHC class II; SSC, side scatter; Control, mice not painted with FITC; No Ab, PBS alone; Anti-α_4, blocking mAb to α_4. (**d**) Percentage of FITC^{+} DCs (left) or non-DCs (right) in spleen and thymus after administration of FITC and simultaneous injection of 200 μg/mouse of blocking mAb to α_4 or PBS. $n = 11$ mice per group; *, $P < 0.05$. Data in **b**,**d** are pooled from two (**b**) or four (**d**) experiments (error bars, s.e.m.).

findings could also have been explained by the presence of free peptide in the inoculum or by release of peptide or membrane blebs from OVA(323–339)-loaded DCs, which could have been acquired by other antigen-presenting cells in the thymus. To rule out those possibilities, we first sought to determine whether homing of DCs to the thymus was necessary to induce clonal deletion. The simultaneous blockade of α_4 integrins on DCs and of VCAM-1 on the recipients' endothelium not only blocked migration of DCs to the thymus but also abolished the clonal deletion induced by OVA(323–339)-loaded DCs, whereas soluble OVA(323–339) was equally effective in the presence or absence of anti-VCAM-1 (**Fig. 5b**). Next, we sought to determine whether the homed DCs were directly responsible for antigen presentation or acted as antigen carriers. We incubated wild-type or I-Aβ-knockout DCs with whole OVA and transferred them to bone marrow chimeras. Only wild-type DCs and not I-Aβ-knockout DCs induced deletion of OT-II thymocytes (**Fig. 5c**), showing that antigen presentation is directly carried out by the adoptively transferred DCs. Notably, the differentiation of wild-type thymocytes proceeded undisturbed in all mice (data not shown), demonstrating that the deletion of OT-II cells was antigen specific.

The removal of thymocytes in the process of negative selection is typically associated with the induction of apoptosis[1], which can be monitored by the binding of annexin V to the membrane of dying or dead cells. To address whether the disappearance of OT-II cells was due to apoptosis, we stained thymocytes of bone marrow–chimeric recipients of DC or peptide injections with annexin V and compared the staining profiles of CD45.2^{+} (OT-II) and CD45.2^{-} (wild-type) cells. In mice treated with OVA(323–339)-loaded DCs, a significantly larger fraction of CD4 SP OT-II thymocytes was apoptotic than in mice treated with unpulsed DCs ($P < 0.001$), whereas the percentage of annexin V–positive wild-type cells remained the same (**Fig. 5d**,**e**). These data demonstrated that circulating DCs that home to the thymus present peptides to thymocytes, causing their removal by apoptosis-mediated clonal deletion.

CD4^{+}CD25^{+} OT-II thymocytes resist negative selection

CD4^{+}CD25^{+} regulatory T cells are critical in preventing autoimmunity and keeping in check immune responses[3], but the mechanism underlying their generation in the thymus is controversial. Although some experiments have suggested that agonist peptides in the thymus can 'instruct' the differentiation of regulatory T cells[2], other models have been proposed[20]. To address that issue, we analyzed the expression of CD25 on wild-type (CD45.2^{-}) and OT-II (CD45.2^{+}) populations of CD4 SP thymocytes after DC injection.

Injection of OVA(323–339)-loaded DCs or free peptide resulted in massive deletion of CD4^{+}CD25^{-} OT-II thymocytes (by 67% and 90%, respectively; **Fig. 6a**), whereas we detected no increase in the absolute number of CD4^{+}CD25^{+} OT-II regulatory T cells. However, the selective removal of CD25^{-} OT-II cells resulted in a higher frequency of CD25^{+} OT-II cells in the surviving population (from 18% of CD45.2^{+} CD4 SP cells in control bone marrow chimeras to 35% and 50% in mice treated with OVA(323–339)-loaded DCs and free peptide, respectively).

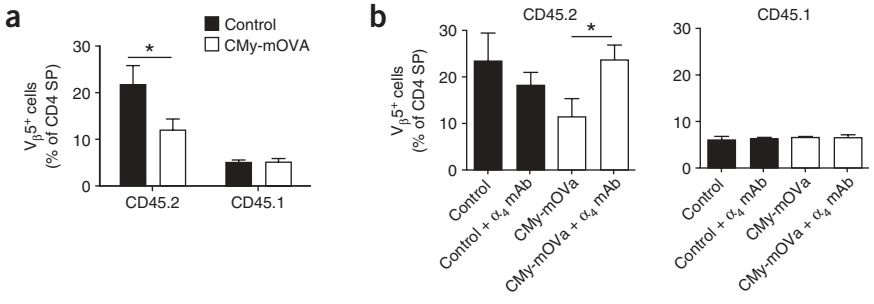

Figure 8 Clonal deletion of OT-II thymocytes in CMy-mOVA mice depends on α_4 integrin function. (**a**) Percent V$_\beta$5^{+} cells in CMy-mOVA mice and littermate control mice (Control) irradiated and reconstituted as described in **Supplementary Fig. 2**; 18 d later, recipient thymi were collected and stained for CD45.2, CD4, CD8α and V$_\beta$5. Results are presented as the percentage of V$_\beta$5^{+} cells in the CD45.2^{+}CD4^{+} CD8α^{-} (CD45.2) or CD45.2^{-}CD4^{+}CD8α^{-} (CD45.1) population; $n = 8$ mice per group; *, $P < 0.05$. (**b**) Percent V$_\beta$5^{+} cells in mice after intraperitoneal administration of 120 μg mAb to integrin α_4 on days 10, 13 and 16 after reconstitution of CMy-mOVA mice and littermate control mice (PBS alone was injected as a control); on day 18 (48 h after the last injection), mice were killed and analyzed as described in **a**. Data are pooled from two experiments (**a**) or were obtained in one experiment (**b**; error bars, s.e.m.; $n = 4$ mice per group).

Emphasizing the antigen specificity of the selection process, the total numbers of $CD4^+CD25^-$ and $CD4^+CD25^+$ wild-type thymocytes remained constant across all experimental groups (**Fig. 6b**).

Endogenous DCs migrate to the thymus

DCs are found not only in the blood but also in thoracic duct lymph[8,9] (**Supplementary Fig. 1**), suggesting that they can reenter the circulation from the periphery. To investigate whether endogenous circulating DCs could also reach the thymus, we surgically joined $CD45.1^+$ and $CD45.2^+$ mice at the flanks, resulting in the establishment of a shared circulation after 3 d (parabiosis). Partner-derived, fully differentiated ($CD11c^{hi}$ MHC class II–positive) DCs migrated to spleen and thymus as early as 5 d after surgery (**Fig. 7a**,**b**). Although the free exchange of DCs in the spleen lagged behind the bulk of recirculating leukocytes (**Fig. 7b**, left), this relationship was inverted in the thymus, where the fraction of partner-derived DCs was eight times larger than the fraction of partner-derived non-DCs (**Fig. 7b**, right). These results demonstrated that endogenous, fully differentiated DCs have the ability to migrate to the thymus via the blood, consistent with published studies of parabiotic mice[21].

We then sought to determine whether DCs could transport antigenic material from the periphery to the thymus. We applied fluorescein isothiocyanate (FITC), a contact sensitizer, to the dorsal skin of wild-type mice. It is known that after such treatment, DCs migrate from the skin to the draining lymph nodes[22], but it is commonly assumed that they do not reach any other organ. However, at 48 h after treatment, a distinct population of $FITC^+$ DCs appeared in the thymus (**Fig. 7c**) and in the spleen (data not shown). Notably, mice treated with mAb to integrin α_4 had significantly fewer $FITC^+$ DCs ($P < 0.05$; **Fig. 7d**, left). This requirement for integrin α_4 function was specific to the thymus, as the number of $FITC^+$ DCs in the spleen did not change. Moreover, the fraction of non-DCs with a bright green fluorescent signal was very small in the thymus and was unaffected by treatment with anti-α_4 (**Fig. 7d**, right), suggesting that skin-resident DCs 'preferentially' acquired the epicutaneously applied FITC before transporting it to the thymus.

Clonal deletion by an extrathymic antigen

To address the physiological relevance of the trafficking of DCs to the thymus, we studied the development of OT-II cells in 'CMy-mOVA' transgenic mice, in which a membrane-bound form of OVA (mOVA) is expressed exclusively in cardiac myocytes (CMy)[23]. We generated mixed bone marrow chimeras by reconstituting irradiated CMy-mOVA mice ($CD45.2^+$) with $CD45.2^+$ bone marrow from OT-II × *Rag1*$^{-/-}$ donors and from congenic $CD45.1^+$ wild-type donors. To detect OT-II thymocytes, we counted $CD4^+CD8^-$ $CD45.2^+V_\beta5^+$ cells 18 d after reconstitution. We used $V_\beta5$ as an additional OT-II T cell receptor–associated marker because despite lethal irradiation, thymi still contained host-derived $CD45.2^+$ thymocytes, which prohibited the use of CD45.2 alone to identify OT-II cells. The fraction of CD4 SP OT-II cells was significantly smaller in the thymi of CMy-mOVA mice than in littermate control mice ($P < 0.05$; **Fig. 8a**). The number of $V_\beta5^+$ cells was fourfold higher among $CD45.2^+$ cells than in the $CD45.1^+$ (non-OT-II) population, but remained unaltered in the latter whether mOVA was expressed in the periphery or not. Thus, OVA-specific thymocytes were selectively deleted in CMy-mOVA hosts.

Next we sought to determine how the deleting antigen came to be presented in the thymus of CMy-mOVA mice. Although transgenic mRNA is not detectable by PCR in the thymi of these mice[23], we could not rule out the possibility that thymic mOVA mRNA was too scarce to be detectable. Thus, it was important to test whether thymus-derived DCs could have acquired the transgenic protein either from an unspecified intrathymic source or by uptake of blood-borne OVA that might have been released from the heart. Uptake of circulating antigen by thymic APCs was most probably instrumental for the deletion of OT-II thymocytes induced by OVA peptide infusion[24] (**Fig. 5a**). Our results (**Fig. 5b**) showed that this well-established mechanism of clonal deletion by thymus-resident antigen-presenting cells did not depend on the VLA-4–VCAM-1 pathway. In contrast, when we treated bone marrow–chimeric mice with mAb to α_4 during the second week after transplantation, the mAb treatment restored the frequency of CD4 SP OT-II cells in CMy-mOVA mice to a frequency equivalent to that in nontransgenic control mice (**Fig. 8b**); the fraction of $V_\beta5^+$ cells among CD4 SP $CD45.1^+$ control cells remained constant in all experimental groups, indicating that VLA-4 inhibition did not interfere with thymocyte selection itself. Thus, the clonal deletion of OT-II thymocytes in CMy-mOVA mice depended on VLA-4-mediated recruitment of cells that probably acquired the deleting antigen from cardiomyocytes and then transported it to the thymus. Given our findings described above, it seems likely that at least some of these peripheral antigen carriers were heart-derived DCs.

DISCUSSION

The fact that DCs set the balance between tolerance and immunity is already well established. DCs that arise in the thymus contribute to central tolerance by inducing clonal deletion of autoreactive thymocytes[7,10]. Conversely, DCs that reside in peripheral tissues migrate to secondary lymphoid organs, where they stimulate T cell responses to pathogens or induce tolerance of autoreactive T cells that have escaped thymic selection[6,7]. Our results here have suggested that DCs with extrathymic origin might also have a role during T cell development. The recruitment pathway identified here allows tissue-resident DCs to collect antigen in the periphery and, after having returned to the bloodstream, to access the thymus. This process may expand the repertoire of selecting antigens presented to differentiating thymocytes, allowing peripheral DCs to participate in the maintenance of central tolerance.

How higher organisms maintain tolerance toward the enormous spectrum of peripheral self and innocuous non-self antigens has been the focus of intense study. One mechanism involves ectopic expression of tissue-specific antigens by medullary thymic epithelial cells[5], which may be directly presented by those cells or may be transferred to thymic DCs for cross-presentation to developing thymocytes[25]. However, the scope of ectopic expression seems to be limited in quantity and quality: tissue-specific antigens have very low expression and several are not expressed at all[5]. Indeed, it seems improbable that a minute population of medullary thymic epithelial cells could faithfully reproduce the overwhelming antigenic diversity created by post-translational modifications, alternative mRNA splicing and differential peptide processing. Moreover, intrathymic gene expression cannot induce tolerance to the broad range of innocuous external antigens derived from commensal flora or food. Thymus-tropic DCs that patrol the periphery and then return to the blood might constitute a mechanism to prevent misguided T cell responses toward at least some of these unpredictable antigens.

Normal blood contains small numbers of circulating differentiated DCs[9], which can capture blood-borne microorganisms[26]. These cells are phenotypically distinct from MHC class II–negative DC precursors[27,28] or $CD11c^{lo}B220^+$ plasmacytoid DCs[29]. Although some of the circulating DCs are probably recent emigrants from the bone marrow, there is mounting evidence that others have entered the blood after

migrating (or originating) in other tissues[8,9]. In fact, DCs are found in thoracic duct lymph, indicating that there is a subtle but continuous flux of tissue-derived DCs that re-enter the blood[8,22]. Increased numbers of circulating DCs may also be encountered in clinical settings; in cancer vaccine trials, autologous DCs modified *ex vivo* have been infused into tumor patients[30,31]. It is likely that the infused DCs exert similar biological activities, presumably at the same anatomical locations as endogenous circulating DCs.

In our experiments, we used splenic DCs whose populations were expanded *in vivo* to obtain a sufficient number of cells that could be tracked after adoptive transfer. Control experiments showed no substantial differences in the migratory activity of splenic versus blood-derived DCs or DC populations expanded with Flt3L versus nonexpanded DC populations (data not shown). Moreover, the fact that all DC subsets injected were recruited equally to the thymus makes it unlikely that our findings reflect the anomalous activity of a nonrepresentative subpopulation of $CD11c^+$ cells in donor spleens. Of note, it has been reported that thymic but not splenic DCs home to the thymus after adoptive transfer[32]. Those findings differ from our observations in which splenic DCs were recruited to the thymus with efficiency similar to or better than that of thymic DCs (data not shown). That discrepancy might be due to the different methods used to purify splenic DCs: extensive manipulation to isolate DCs from the spleen can result in DC maturation, which, as we have shown here, attenuates migration of DCs to the thymus.

Our results have shown that DCs use a classical multistep adhesion cascade to home to the thymus through microvessels in the cortico-medullary junction: P-selectin is required for optimal homing and most likely mediates tethering and rolling interactions; a $G\alpha_i$-mediated, PTX-sensitive chemoattractant signal is absolutely required, as is true for the homing of lymphocytes to most lymphoid organs[15]; and, finally, VLA-4–VCAM-1 interactions are essential to mediate firm arrest and may also contribute to rolling. The adhesion molecules participating in this cascade are the same as those that mediate the recruitment of common lymphoid progenitors to the thymus[33]. However, the chemoattractant signal seems to be distinct from that used by lymphoid progenitors. Those last cells depend on CCR9-CCL25 to home to the thymus[33], whereas DC homing was unaltered in mice treated with anti-CCL25 or several other blocking mAbs to thymic chemokines, including CCL2, CCL5, CCL20 and CXCL12 (data not shown). It also seems unlikely that thymus-expressed CCR7 ligands are involved, as CCR7 is 'preferentially' expressed on mature DCs, which migrated poorly to the thymus.

What is the fate and function of homed DCs in the thymus? Our data have indicated that they can induce antigen-specific clonal deletion. DCs must home to the thymus to exert this effect, and they present the selecting antigen autonomously, without mediation by other antigen-presenting cells. That is consistent with published findings showing that antigen-loaded DCs can eliminate antigen-specific thymocytes *in vitro*[34] and that resident thymic DCs induce negative selection *in vivo*[7,10]. It was initially assumed that the responsible DCs arise exclusively in the thymus, possibly from a common lymphoid progenitor[35]; however, subsequent studies with parabiotic animals[21] have suggested the existence of an immigrating DC population that might participate in negative selection and intrathymic generation of regulatory T cells[36].

Studies of the lifespan and turnover of thymic DCs have shown that there are two kinetically distinct populations of approximately equal size: the first is separated by only 2 d from its last proliferating precursor and therefore is most likely generated locally; the second is composed of DCs that have not divided for at least 1 week[37] and whose entry into the thymus is independent of lymphoid progenitors[21]. Consistent with those observations, we have found that partner-derived DCs in parabiotic mice quickly appeared in the thymus as early as 2 d after a shared circulation was established. That result suggested that fully differentiated DCs access the thymus from the circulation, as blood-borne DC precursors are exceedingly rare and require about 5 d to acquire a full-fledged DC phenotype[28].

At days 5 and 7 after parabiosis, the fraction of partner-derived DCs in the thymus reached about 2%, whereas their frequency among DCs in the blood was about 30% (data not shown). Assuming that the partner-derived DCs faithfully 'report' the trafficking activity of all circulating DCs, we can estimate that at least 7% of thymic DCs were blood derived. Thus, if we assume a total number of about 2×10^5 $CD11c^{hi}$ MHC class II–positive DCs in the thymus of young adult mice[10], at least 1.4×10^4 would be blood-derived immigrants. Intravital microscopy of T cell–DC interactions in lymph nodes has shown that single DCs can contact as many as 5×10^3 T cells per hour[38]. Although such measurements have not yet been done in the thymic medulla, a similar contact frequency in this environment would allow the seemingly small blood-derived fraction of thymic DCs to engage thymocytes in over 1×10^9 interactions per day.

An important issue is the origin of the circulating DCs that entered the thymus. Using FITC painting, we were able to identify in the thymus DCs that had acquired FITC, presumably in the skin. The appearance of $FITC^+$ DCs was dependent on α_4 integrins, suggesting that $FITC^+$ DCs trafficked to the thymus by undergoing specific adhesive interactions. Based on published findings[8] and additional data presented here, we estimate the number of recirculating DCs that pass through the thoracic duct to range from about 2.2×10^4 to 6×10^4 cells per day. Given that the thoracic duct conducts only lymph fluid from below the diaphragm, the total flux of DCs that reenter the blood of a mouse through efferent lymphatic conduits could reach about 1×10^5 cells per day. Our homing experiments showed that the fraction of adoptively transferred immature DCs that could be recovered from the thymus was about 0.08%. That probably underestimates the true homing efficiency of circulating DCs, because many adoptively transferred cells die soon after intravenous injection. Nonetheless, even that conservative estimate suggests a constant migratory stream of at least 80 DCs per day that depart from peripheral tissues and access the thymic medulla in a normal mouse. Given the high efficiency of DCs in making contacts with T cells in lymphoid tissues and the relatively long time spent by thymocytes in the medulla (5 d on average[5]), it is possible that most medullary thymocytes encounter peripheral DCs before leaving the thymus.

Consistent with our hypothesis, autoreactive OT-II thymocytes developing in CMy-mOVA mice were selectively deleted by an α_4 integrin–dependent process. The most plausible interpretation of that observation is that cardiac phagocytic cells, most probably DCs, acquired mOVA from cardiomyocytes and migrated to the thymus through VLA-4–VCAM-1 interactions. Nonetheless, in other experimental settings, tissue-restricted antigens expressed in the pancreas[39] or the central nervous system[40] have failed to induce efficient negative selection in the thymus. However, another study has described bone marrow–dependent deletion of myelin basic protein–specific thymocytes recognizing an epitope of myelin basic protein (amino acids 121–150) not expressed in the thymus[41]. That raises the possibility that the contribution of peripheral DCs to central tolerance might depend on the tissue origin and/or the nature of the selecting antigen. Further studies are needed to explore that hypothesis.

Finally, the finding that mature DCs have a substantially reduced capacity to home to the thymus suggests a mechanism to safeguard

against inadvertent deletion of T cells that respond to pathogen-associated antigens. DCs mature when they encounter 'danger signals', particularly in the context of tissue damage and microbial infections[6]. The loss of thymic tropism should allow mature DCs in the circulation to avoid sequestration in the thymus and thus to accumulate more efficiently at other sites, such as the bone marrow and spleen, where their cargo of pathogen-derived antigens can elicit productive immune responses by fully differentiated T cells[8,9]. In summary, we have shown here that peripheral blood-borne DCs can carry antigens to the thymic medulla, where they are recruited from the circulation through a specific multistep adhesion cascade involving P-selectin and a G protein–coupled stimulus that presumably triggers the high-avidity binding of VLA-4 to VCAM-1. This previously unappreciated migratory pathway can result in antigen-specific clonal deletion of thymocytes and suggests a possible function for peripheral DCs in establishing and maintaining central tolerance.

METHODS

Mice. C57BL/6 (CD45.2 Ly5.2) mice were purchased from The Jackson Laboratory, Taconic or Charles River Laboratories. Congenic CD45.1 Ly5.1 (B6.SJL-*Ptprc*^{a} *Pep3*^{b}/BoyJ) and P-selectin-deficient mice on C57BL/6 background[42] (B6.129S7-*Selp*^{tm1Bay}/J) were obtained from The Jackson Laboratory. I-Aβ-knockout (B6.129-H2-*Ab1*^{tm1Gru} N12) mice were purchased from Taconic. P-selectin-deficient mice on a mixed C57BL/6 × 129 background and control mice were provided by D. Wagner (The CBR Institute for Biomedical Research, Harvard Medical School, Boston, Massachusetts)[43]. Mice with conditional deficiency in VCAM-1 were obtained from P. Koni (Medical College of Georgia, Augusta, Georgia) and were bred as described[44]. T cell receptor–transgenic OT-II × *Rag1*$^{-/-}$ mice (C57BL/6-TgN(OT-II.2a)-*RAG1*^{tm1Mom}) were purchased from Taconic through the National Institute of Allergy and Infectious Diseases exchange program[18,45]. CMy-mOVA mice were obtained from A. Lichtman (Brigham & Women's Hospital, Boston, Massachusetts) and have been described[23]. Mice were housed in an specific pathogen–free, viral antibody–free facility and were used in accordance with guidelines of the animal committees of the CBR Institute and Harvard Medical School (Boston, Massachusetts).

Reagents. Fluorochrome-conjugated mAbs to mouse B220 (RA3-6B2), CD4 (RM4-5), CD8α (53-6.7), CD11b (M1/70), CD11c (HL3), CD25 (PC61), CD45.2 (104) and $V_{\beta}5$ (MR9-4), and purified neutralizing mAbs to mouse integrin β_2 (GAME-46), ICAM-1 (3E2) and P-selectin (RB40.34) were purchased from BD Biosciences. Neutralizing mAbs to mouse integrin α_4 (PS/2), L-selectin (Mel-14), E-selectin (9A9), MAdCAM-1 (MECA-367) and VCAM-1 (MK2.7) were purified from the supernatants of cultured hybridomas obtained from American Type Culture Collection. Chimeric, nondepleting, anti-α_4 (CRL19.11) was provided by R. Palframan (Celltech, London, UK). For immunofluorescence, FITC-conjugated antibody to 'pan cytokeratin' (C-11) was obtained from Sigma-Aldrich; biotinylated *Ulex Europaeus* agglutinin I (UEA-1) was purchased from Vector Laboratories. OVA(323–339) (H_2N-ISQAVHAAHAEINEAGR-OH) was purchased from New England Peptide. Endotoxin-free OVA in crude egg white extract was a gift from M. Boes (Harvard Medical School, Boston, Massachusetts)[46]. PTX was from Calbiochem.

DC isolation and culture. C57BL/6 mice were injected subcutaneously with 2×10^6 to 5×10^6 B16 melanoma cells secreting Flt3 ligand as described[47]. After 10–14 d, mice were killed and DCs were purified by density-gradient centrifugation over Optiprep (Sigma-Aldrich) by collection of the low-density fraction[8]. These preparations routinely contained 75–85% CD11c$^+$ DCs. In some experiments, DC maturation was induced by culture for 18–24 h in complete medium in the presence of 1 μg/ml of lipopolysaccharide (*E. coli* 0.26:B6; Sigma-Aldrich). Mature DC cultures typically resulted in enrichment in CD11c$^+$ cells (90–95%) and in the upregulation of classical maturation markers (CD86 and MHC class II) for all CD11c$^+$ cells.

Homing assay. Immature or mature DCs were labeled for 15 min at 37 °C with 30 μM CFSE, 2 mM TRITC (tetramethylrhodamine-5-(and-6)-isothiocyanate) or 10 mM CMTMR (5-(and-6)-(((4-chloromethyl)benzoyl) amino)tetramethylrhodamine; all from Molecular Probes). Dead cells and excess label were removed by centrifugation over FCS, and 5×10^6 to 20×10^6 labeled DCs were then injected in the tail veins of syngeneic recipient mice. In some experiments, DCs were pretreated with 50 μg/ml of blocking mAb and were washed before injection to avoid the inhibition of adhesion molecules on simultaneously injected control populations; for inhibition of endothelial adhesion molecules, 100 μg mAb was injected along with the labeled DCs. Mice were killed after 18 h in most experiments and after 2 h in experiments in which homing receptors were blocked on the DCs to minimize new expression after mAb treatment. Then, single-cell suspensions were generated from spleens and thymi and cell samples were incubated with anti-CD11c and analyzed on a FACScalibur (BD Biosciences). The total number of homed DCs was calculated by multiplication of the fraction of CFSE$^+$ (or TRITC$^+$) CD11c$^+$ events by the total cellularity of the target organ.

Immunofluorescence. Thymi were embedded in optimum cutting temperature compound and were 'snap-frozen'. Sections 20 μm in thickness were prepared, were air-dried and were fixed for 10 min with ice-cold ethanol. After being blocked with 10% (volume/volume) mouse serum, sections were incubated with various reagents. Cortex and medulla were identified by staining with mAb C-11 to 'pan cytokeratin'[48] and the lectin UEA-1 (ref. 49), respectively. For secondary detection of UEA-1–biotin and CD11c-biotin, sections were incubated with Alexa Fluor 633–conjugated streptavidin (Molecular Probes).

Clonal deletion assay. C57BL/6 congenic (CD45.1$^+$) recipients were lethally irradiated on day 0 by the administration of two doses of 650 rads. Bone marrow cells were isolated from donor OT-II × *Rag1*$^{-/-}$ (CD45.2$^+$) and wild-type (CD45.1$^+$) mice. Mature lymphocytes were removed by incubation with biotinylated antibodies to NK1.1 (PK136), CD3 (145-2C11) and CD19 (1D3), followed by immunomagnetic depletion with streptavidin-conjugated dynabeads (DynalBiotech); 5×10^6 to 10×10^6 of the remaining cells were injected intravenously into each irradiated recipient mouse. Then, 2 weeks later, fresh DCs were isolated from C57BL/6 mice injected with B16 cells secreting Flt3 ligand, were purified as described above, were 'loaded' for 45 min at 37 °C with 20 μM OVA(323–339) with or without 50 μg/ml of nondepleting, α_4-blocking antibody CRL19.11 and were administered intravenously to the bone marrow chimeras. In some experiments, wild-type or I-Aβ-knockout DCs were 'loaded' for 2 h at 37 °C with 1 mg/ml of whole OVA. Mice were treated twice, on days 14 and 15 after irradiation, with 1×10^7 loaded or unloaded DCs or with 50 nmol of soluble peptide and were killed on day 17 unless otherwise indicated. In some experiments, regulatory T cells were counted by gating on CD25^{hi} events (mean fluorescent intensity of more than 500) among CD4 SP cells.

Parabiosis. For parabiosis, 6- to 8-week-old female C57BL/6 mice were anesthetized to full muscle relaxation and were joined as described[50]. The lateral aspects of each mouse were shaved, matching skin incisions were made from the olecranon to the knee joint of each mouse and the subcutaneous fascia was bluntly dissected to expose about 0.5 cm of skin. Olecranons and knees were joined with a single 2-0 silk suture, and the dorsal and ventral skins were approximated by staples or continuous suture.

FITC painting. Mice were anesthetized and hair was removed from their dorsal skin. After skin was stripped three times with transparent tape to weaken the stratum corneum, 500 μl of a 0.5% solution of FITC in acetone and olive oil (4:1, volume/volume) was applied to the skin. The use of dibutyl phthalate was avoided to minimize skin sensitization and ensuing DC maturation. Mice were killed and organs were collected for analysis 48 h later. For detection of the rare FITC$^+$CD11c^{hi} MHC class II–positive cells, 3×10^6 and 5×10^6 total events were collected for spleen and thymus, respectively.

Statistical analysis. Data are presented as mean ± s.e.m. unless otherwise noted. Statistical significance was assessed by the two-tailed unpaired Student's *t*-test for comparison of two groups or by one-way analysis of variance followed by the Student Newman Keuls post-test for more than two groups. Differences with P values of less than 0.05 were considered significant.

Note: Supplementary information is available on the Nature Immunology website.

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AUTHOR CONTRIBUTIONS

R.B. designed and executed all experiments, unless otherwise stated; M.L.S. equally contributed to the design and realization of all adoptive transfers (**Figs. 2–6**); P.S. generated parabiotic mice; N.G. and A.H.L. provided CMy-mOVA-transgenic mice; and R.B. and U.H.v.A. prepared the manuscript with help from M.L.S.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Corrigendum: Surface-bound chemokines capture and prime T cells for synapse formation

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In the version of this article initially published, the label at the far right of the horizontal axis of **Figure 5a** is incorrect. The correct label should be α-β_1. The error has been corrected in the PDF version of the article.

Corrigendum: Clonal deletion of thymocytes by circulating dendritic cells homing to the thymus

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In the version of this article initially published, the third sentence in the legend of Figure 6 is incorrect. The correct sentence should read "*, $P < 0.01$, and **, $P < 0.001$, compared with DCs". In the last sentence of the legend to Figure 8, 'obtainted' should read 'obtained'. On page 1098, in the first sentence of the first full paragraph, 'fused' should read 'used'. These errors have been corrected in the HTML and PDF versions of the article.