Lymph node stromal cells acquire peptide–MHCII complexes from dendritic cells and induce antigen-specific CD4+ T cell tolerance

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Dendritic cells (DCs), and more recently lymph node stromal cells (LNSCs), have been described to tolerize self-reactive CD8+ T cells in LNs. Although LNSCs express MHCII, it is unknown whether they can also impact CD4+ T cell functions. We show that the promoter IV (pIV) of class II transactivator (CIITA), the master regulator of MHCII expression, controls endogenous MHCII expression by LNSCs. Unexpectedly, LNSCs also acquire peptide–MHCII complexes from DCs and induce CD4+ T cell dysfunction by presenting transferred complexes to naive CD4+ T cells and preventing their proliferation and survival. Our data reveals a novel, alternative mechanism where LN-resident stromal cells tolerize CD4+ T cells through the presentation of self-antigens via transferred peptide–MHCII complexes of DC origin.

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Abbreviations used: BEC, blood endothelial cell; CH, contact hypersensitivity; CIITA, class II transactivator; DN, double negative; FRC, fibroblastic reticular cell; LEC, lymphatic endothelial cell; LNSC, LN stromal cell.

Self-reactive T cells that escape thymic negative selection are kept in check by peripheral tolerance mechanisms that include T cell anergy and deletion. Research into how self-reactive T cells are tolerized in LNs has focused largely on DCs. Depending on their functional status, antigen presentation by DCs can indeed lead to different forms of T cell tolerance (Steinman et al., 2003; Helft et al., 2010). Recently, however, LN-resident radio-resistant cells, the LN stromal cells (LNSCs), have been suggested to contribute to peripheral T cell tolerance. These cells can be discriminated based on their lack of CD45 expression and the differential expression of podoplanin (gp38) and PECAM (CD31). Fibroblastic reticular cells (FRCs, gp38$^+$CD31$^-$) produce chemokines such as CCL19 and CCL21, thereby providing a scaffold on which the CC-chemokine receptor 7 (CCR7)$^+$ T cells and DCs can migrate and establish contact (Turley et al., 2010). In LNs, blood endothelial cells (BECs, gp38$^-$CD31$^+$) lining the high endothelial venules are crucial for lymphocyte entry (Mueller and Germain, 2009). Afferent lymphatic endothelial cells (LECs, gp38$^+$CD31$^+$) promote DC entry (Johnson et al., 2006; Acton et al., 2012), as well as antigen delivery (Sixt et al., 2005; Roozendaal et al., 2009), into LNs, whereas efferent LECs regulate T cell egress from LNs (Cyster and Schwab, 2012). The function of so-called double-negative (DN) stromal cells (gp38$^-$CD31$^-$) remains unknown. For many years, LNSCs were thought to only play an architectural role in LN construction and homeostasis. More recently, however, studies have identified LNSCs as active players in modulating adaptive immune responses (Swartz and Lund, 2012). In vitro, DC adhesion to LECs leads to decreased levels of co-stimulatory

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The Rockefeller University Press $30.00
J. Exp. Med. 2014 Vol. 211 No. 6 1153-1166
www.jem.org/cgi/doi/10.1084/jem.20132000

The Journal of Experimental Medicine

molecules by DCs (Podgrabinska et al., 2009). Furthermore, FRCs inhibit the proliferation of newly activated T cells through a NOS2-dependent mechanism, but also indirectly affect T cell proliferation by suppressing DC functions (Khan et al., 2011; Lukacs-Kornek et al., 2011; Siegert et al., 2011). In addition, FRCs can suppress acute T cell proliferation both in vitro and in vivo (Siegert et al., 2011). Other studies have convincingly demonstrated a role for LNSCs in maintaining peripheral $CD8^+$ T cell tolerance via direct presentation of self-antigens to self-reactive $CD8^+$ T cells. Unlike DCs, which acquire antigens and subsequently cross-present self-peptides to $CD8^+$ T cells in the draining LNs, LNSCs ectopically express and present PTAs (peripheral tissue antigens) to $CD8^+$ T cells, and consequently induce clonal deletion of self-reactive $CD8^+$ T cells (Lee et al., 2007; Nichols et al., 2007; Gardner et al., 2008; Magnusson et al., 2008; Yip et al., 2009; Cohen et al., 2010; Fletcher et al., 2010). In addition, we have recently shown that tumor-associated LECs can scavenge tumor antigens and cross-present them to cognate $CD8^+$ T cells, driving their dysfunctional activation (Lund et al., 2012). The lack of expression of co-stimulatory molecules such as CD80/86, and high PD-L1 expression levels at the surface of LECs (Fletcher et al., 2010; Tewalt et al., 2012), were proposed as the major mechanisms by which these cells induce deletional $CD8^+$ T cell tolerance.

While accumulating evidence suggests that direct antigen presentation by LNSCs promotes $CD8^+$ T cell deletion, it is unknown whether LNSCs can similarly contribute to $CD4^+$ T cell tolerance. As previously described, FRCs, BECs, and LECs express MHCII under virally induced inflammatory conditions or IFN-γ treatment (Malhotra et al., 2012; Ng et al., 2012). However, little is known about the regulation of MHCII expression by LNSCs.

Here, we show that endogenous MHCII expression by LNSCs is controlled by the IFN-γ–inducible promoter IV (pIV) of class II transactivator (CIITA). Due to basal pIV activity, LNSCs express low levels of MHCII upon steady state and up-regulate these molecules when exposed to IFN-γ. Unexpectedly, in addition to low endogenous basal expression, the majority of MHCII molecules detected at LEC, BEC, and FRC surface were acquired from DCs. Furthermore, antigen-presenting DCs transfer antigenic peptide–MHCII (pMHCII) complexes to LNSCs, in a process dependent on both cell–cell contact and DC-derived exosomes. Importantly, acquired pMHCII complexes were presented by LECs, BECs, and FRCs to $CD4^+$ T cells and promoted cognate $CD4^+$ T cell dysfunction by impairing their survival and response to further restimulation. These data suggest that LNSCs serve more diverse roles than previously thought in regulating $CD4^+$ T cell immunity.

RESULTS

CIITA pIV drives IFN-γ–mediated MHCII up-regulation, but not basal MHCII expression, by LECs, BECs, and FRCs

We first characterized steady-state MHCII expression by primary murine LNSCs. As previously described (Malhotra et al., 2012), LECs, BECs, and FRCs, but not DN cells (Fig. S1), expressed low basal levels of MHCII molecules (Fig. 1 A). MHCII expression is almost exclusively controlled by a single master regulatory factor, CIITA (Reith et al., 2005). Expression of CIITA is regulated mainly at the transcriptional level by a large regulatory region that contains three distinct promoters in mice, pI, pIII, and pIV (Fig. S2; Reith et al., 2005). We quantified pI, pIII, and pIV mRNA in FACS-sorted FRCs, BECs, and LECs from $CD45^{neg}$-enriched LN fractions. Although pI and pIII mRNAs were undetectable, all three LNSC subpopulations expressed pIV mRNA (Fig. 1 B). Because pIV is induced by IFN-γ, we injected WT and pIV knockout ($pIV^{-/-}$) mice with IFN-γ and observed that LECs, BECs, and FRCs isolated from WT mice strongly up-regulated MHCII compared with untreated WT mice (Fig. 1 C). In contrast, LECs, BECs and FRCs isolated from $pIV^{-/-}$ mice did not increase MHCII after IFN-γ treatment (Fig. 1 C), demonstrating that IFN-γ–mediated MHCII up-regulation by LNSC is pIV dependent. Surprisingly, although only pIV mRNA was detected in LNSC (Fig. 1 B), basal MHCII expression was slightly reduced but not abrogated in cells isolated from untreated $pIV^{-/-}$ mice. Similarly, this reduced but clear expression of MHCII was detectable in IFN-$\gamma R^{-/-}$ LNSCs (Fig. 1 D), indicating that although steady-state IFN-γ is partially responsible for pIV-dependent basal MHCII expression, other mechanisms driving MHCII expression must also exist.

CIITA-independent MHCII expression is an event that only exists rarely (Arancibia-Cárcamo et al., 2004; Zinzow-Kramer et al., 2012). LNSCs isolated from mice deficient for the full CIITA gene ($CIITA^{-/-}$ mice) were, however, found negative for MHCII expression (Fig. 1 E), ruling out a CIITA-independent MHCII expression by LNSCs. Even though no other CIITA promoter activity was detected except for pIV (Fig. 1 B), we nevertheless checked for MHCII expression by LNSCs isolated from mice deficient for pI ($pI^{-/-}$), and double deficient for pIII and pIV ($pIII+IV^{-/-}$). Whereas $pIII+IV^{-/-}$ LNSCs express similar levels of MHCII compared with $pIV^{-/-}$ LNSCs, MHCII expression was dramatically and significantly reduced in LECs, BECs, and FRCs from $pI^{-/-}$ mice (Fig. 1 E). This result was unexpected because pI has been shown to control CIITA expression only in conventional DCs, macrophages, and microglial cells (Reith et al., 2005).

LNSCs acquire MHCII from hematopoietic cells

Even though pI mRNA was undetectable in WT LNSCs, $pI^{-/-}$ LNSCs exhibited a dramatic decrease in MHCII expression. We hypothesized that although LNSCs endogenously up-regulated MHCII molecules in an IFN-γ–inducible pIV-dependent manner (Fig. 1 C), they may also acquire MHCII from hematopoietic cells under steady state. Accordingly, MHCII expression by macrophages and DCs was not totally abrogated in $pI^{-/-}$ mice (Fig. 2 A) and may explain the remaining low MHCII expression by $pI^{-/-}$ LNSCs (Fig. 1 E). An alternative explanation is that $pI^{-/-}$ mice are pIV sufficient,

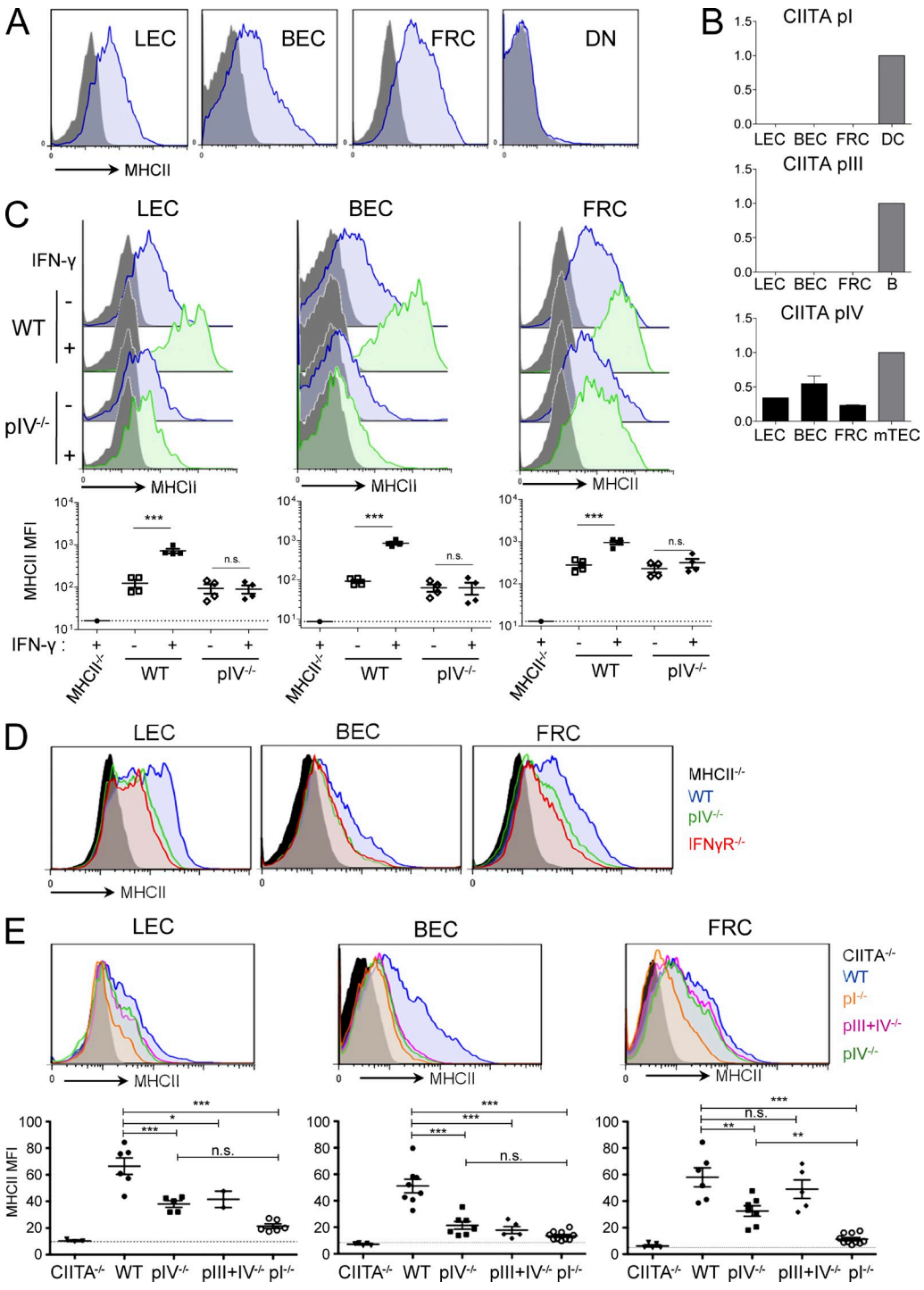

Figure 1. CIITA pIV controls IFN-γ–mediated up-regulation, but not steady-state expression of MHCII by LNSCs. (A) LNSCs were defined by FACS as follows: LEC, CD45$^-$ CD31$^+$ gp38$^+$; BEC, CD45$^-$ CD31$^+$ gp38$^-$; FRC, CD45$^-$ CD31$^-$ gp38$^+$; DN, CD45$^-$ CD31$^-$ gp38$^-$. Histograms show MHCII expression levels by each LNSC population (gray histograms, isotype control). Data are representative of 4 independent experiments with at least 3 mice each. (B) Ciita promoters pI, pIII, and pIV mRNA levels were quantified by qPCR from FACS-sorted LEC, BEC, or FRC. Indicated control cells (gray bars) were used as reference. Data are representative of 3 independent experiments, with a pool of 10 mice each. (C) Mice were injected subcutaneously (green) or not (blue) with IFN-γ and draining LNs were collected 24 h later. Histograms show MHCII expression levels on LEC, BEC, and FRC. Graphs depict MHCII MFI where each symbol represents individual mouse. Data are representative of 3 independent experiments, with 4 mice per group. ***, $P < 0.001$, n.s. = not significant. Error bars depict mean ± SEM. (D and E) Histograms show MHCII expression levels on LEC, BEC, and FRC from indicated mice. Histograms are representative of at least 2 independent experiments with 2–3 mice per group. (E) Graphs depict MHCII MFI where each symbol represents individual mouse. Data are pooled from 3 independent experiments with 2–3 mice per group. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s. = not significant. Error bars depict mean ± SEM.

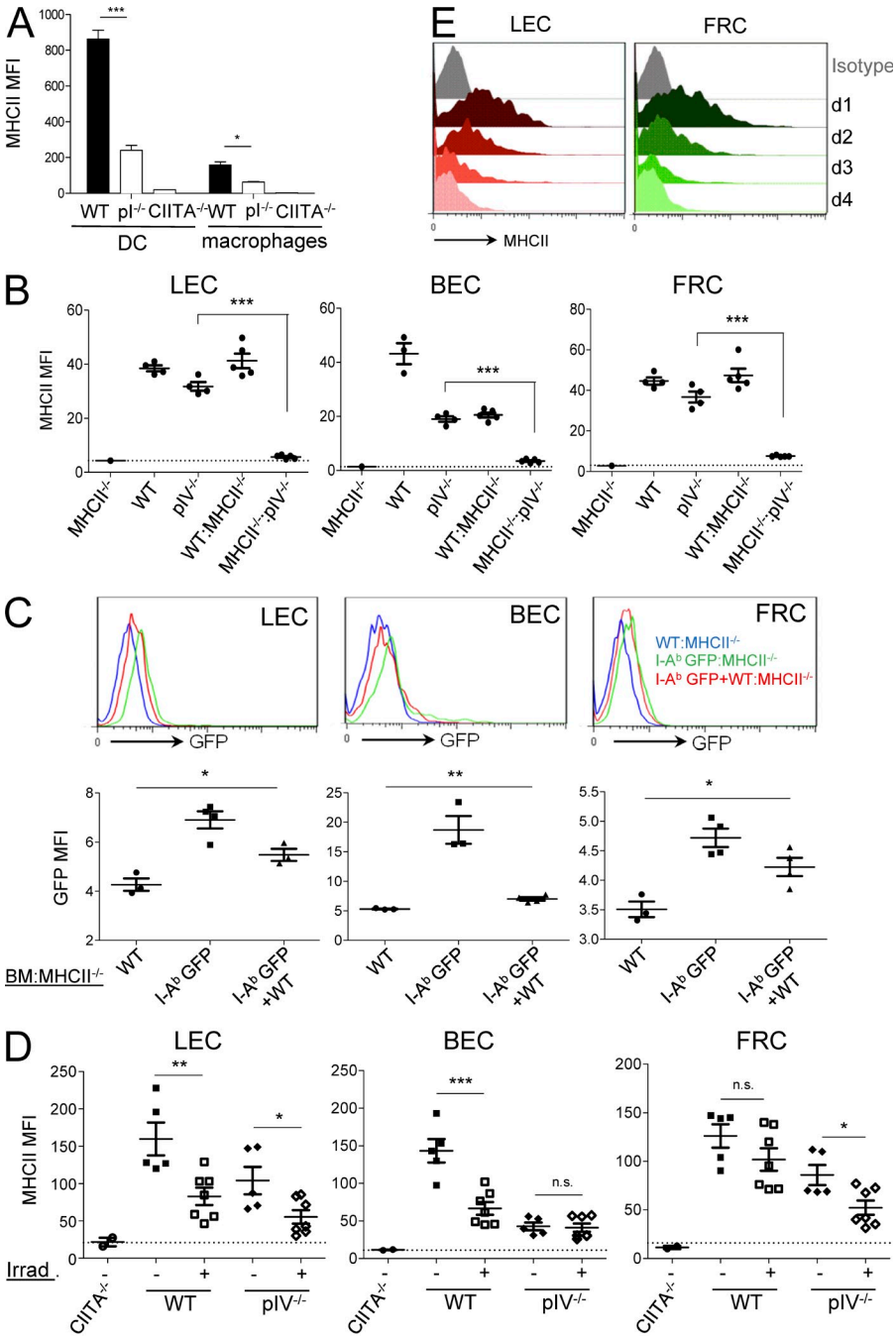

Figure 2. LNSCs acquire MHCII molecules from hematopoietic cells. (A) MHCII MFI on splenic macrophages and BMDCs from WT, pI$^{-/-}$, and CIITA$^{-/-}$ mice. Error bars indicate mean + SEM. Data are representative of 4 independent experiments, with 2–3 mice per group. *, $P < 0.05$; ***, $P < 0.001$. (B) MHCII MFI on LEC, BEC, and FRC from indicated control mice and WT:MHCII$^{-/-}$ or MHCII$^{-/-}$: pIV$^{-/-}$ BM chimeric mice which were generated as described in Materials and methods. Graphs are representative of 3 independent experiments with 5 mice per group. ***, $P < 0.001$. Error bars depict mean ± SEM. (C) Histograms show GFP expression gated on MHCII^{+} LEC, BEC, and FRC from indicated BM chimeric mice generated in MHCII$^{-/-}$ recipients. Graphs are representative of 2 independent experiments, with 3–4 mice per group. *, $P < 0.05$; **, $P < 0.01$. Error bars depict mean ± SEM. (D) Mice were either sublethally irradiated or untreated, and LNs harvested 3 d later. Data show MHCII MFI on LEC, BEC, and FRC and are representative of 2 independent experiments, with 7 mice per group. Each symbol represents an individual mouse. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Error bars depict mean ± SEM. (E) LN cells were cultured and nonadherent hematopoietic cells were removed daily. LEC and FRC were analyzed at indicated times for MHCII expression. Data are representative of at least 3 independent experiments with minimum 3 mice per group.

and as mentioned previously, low levels of IFN-γ in vivo promote slight endogenous MHCII expression by LNSCs (Fig. 1 D). The hypothesis of MHCII acquisition by LNSCs from hematopoietic cells was tested by generating BM chimeric mice in which only the hematopoietic cell compartment or, conversely, the radioresistant stromal cells originate from WT. As mentioned above (Fig. 1), control pIV$^{-/-}$ LNSCs exhibited a reduced but significant MHCII expression compared with WT LNSCs (Fig. 2 B). Because there is no endogenous MHCII expression in pIV$^{-/-}$ LNSCs, we considered that this expression reflects only acquired MHCII molecules. Consistently, MHCII expression by LNSCs from WT BM: MHCII$^{-/-}$ chimeric mice was restored to similar levels compared with pIV$^{-/-}$ LNSCs, demonstrating that steady-state MHCII at LNSC surfaces were acquired from hematopoietic cells (Fig. 2 B). An almost total lack of MHCII expression by LNSCs from MHCII$^{-/-}$ BM: pIV$^{-/-}$ chimeric mice was observed (Fig. 2 B), suggesting that LNSCs do acquire MHCII from hematopoietic cells. In addition, we reconstituted irradiated MHCII$^{-/-}$ recipient mice with a mixture (1:1) of WT and I-A^{b} GFP^{+} BM cells. Levels of GFP expression by MHCII^{+} LECs, BECs, and FRCs were intermediate in mixed

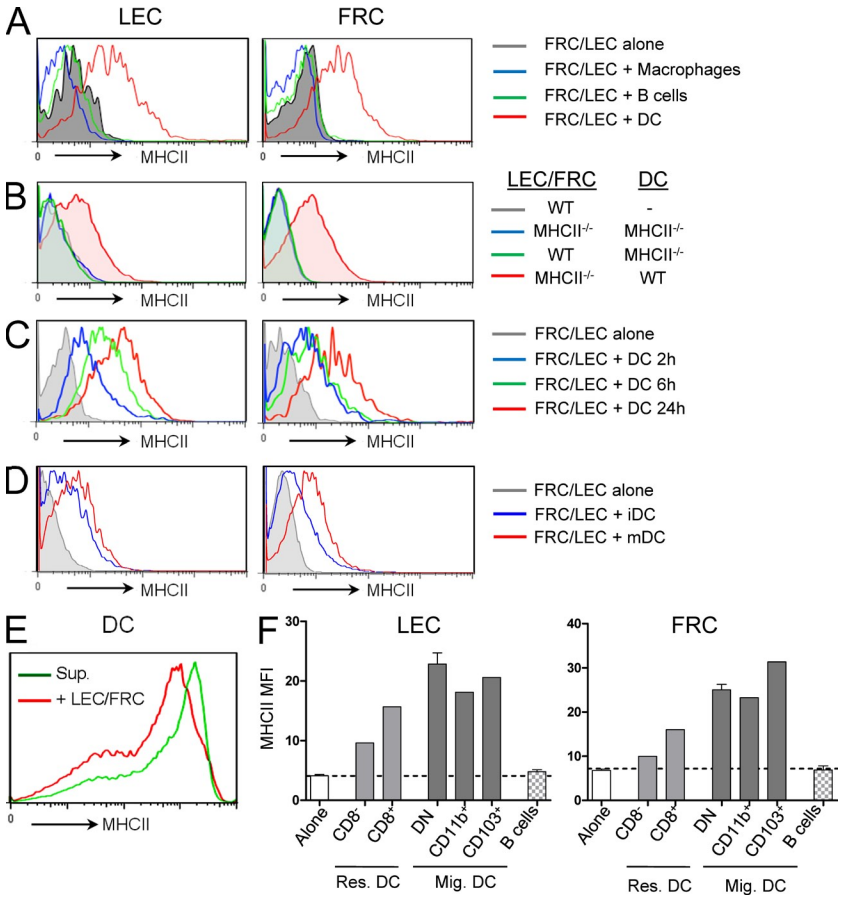

Figure 3. DCs transfer MHCII molecules to LNSCs in vitro. (A) LECs/FRCs from WT mice were co-cultured alone (gray) or in the presence of macrophages (blue), B cells (green), or DCs (red) for 24 h. (B) WT or MHCII$^{-/-}$ LECs/FRCs were co-cultured for 24 h in the presence or absence of DCs from either WT or MHCII$^{-/-}$ mice, as indicated. (C) LECs/FRCs from WT mice were co-cultured alone (gray) or in the presence of DCs for 2 h (blue), 6 h (green), or 24 h (red). (D) LECs/FRCs from WT mice were co-cultured alone (gray) or in the presence of DCs pretreated (red) or not (blue) with LPS for 24 h. (A–D) Histograms show MHCII levels on LEC (left) and FRC (right). Data are representative of at least 2 independent experiments with minimum 3 mice per group. (E) MHCII expression by LPS-treated DCs cultured with either LEC/FRC (red) or in the presence of LEC/FRC culture supernatant (Sup., green) for 24 h. Data representative of 3 independent experiments with 3 mice per group. (F) LECs/FRCs from WT mice were co-cultured alone or with indicated sorted LN DC subsets for 24 h. Graphs show MHCII MFI on LEC and FRC and are representative of 2 independent experiments. Each experiment is a pool of 10 mice. Error bars depict mean ± SEM.

BM chimeras compared with control MHCII$^{-/-}$ recipient mice reconstituted with exclusively WT or I-A^b GFP$^+$ BM cells (Fig. 2 C). Thus, LNSCs from mixed BM chimeric mice express both nonfluorescent (from WT BM) and GFP$^+$ (from I-A^b GFP$^+$ BM) MHCII molecules, reinforcing the idea that nonhematopoietic radioresistant LECs, BECs, and FRCs acquire MHCII from hematopoietic cells. To further test our hypothesis, we sublethally irradiated WT mice to eliminate hematopoietic cells. pIV$^{-/-}$ mice were included to avoid any possible endogenous MHCII up-regulation after irradiation-induced inflammation. 3 d after irradiation, the number of hematopoietic cells in LN was dramatically reduced (not depicted) and correlated with a significant decrease in MHCII expression by both WT and pIV$^{-/-}$ LNSCs (Fig. 2 D). These results indicated that LNSCs transiently acquire MHCII molecules from hematopoietic cells in vivo. To analyze this phenomenon in vitro, we generated LEC/FRC cultures (Lukacs-Kornek et al., 2011). In brief, LN cells were cultured for 5–7 d and washed daily to remove nonadherent hematopoietic cells. Consistent with our hypothesis that LNSCs acquire most MHCII from hematopoietic cells in steady state, removal of hematopoietic cells correlated with the disappearance of MHCII expression levels on LECs and FRCs over time (Fig. 2 E). This also reinforced our in vivo results showing that transferred MHCII are not stable at the surface of LNSC (Fig. 2 D).

DCs transfer MHCII to LNSCs in vitro

We next investigated which cell types are involved in the transfer of MHCII molecules to LNSCs. MHCII-negative LECs and FRCs (Fig. 3 A) were co-cultured for 24 h with MHCII$^+$ cells, namely macrophages, B cells, or LPS-treated DCs. We observed that only DCs were capable of transferring MHCII to LNSCs (Fig. 3 A). In vivo experiments further suggested that this transfer is exclusively mediated by DCs. First, MHCII expression by pIII+IV$^{-/-}$ LNSCs was comparable to pIV$^{-/-}$ LNSCs (Fig. 1 E), demonstrating that B cells, which rely on pIII, are not involved in this process. Second, MHCII expression by LNSCs was not reduced after depletion of macrophages using clodronate liposomes (unpublished data). Only WT and not MHCII$^{-/-}$ DCs transferred MHCII to both WT and MHCII$^{-/-}$ LNSC, ruling out the possibility that DC-derived soluble inflammatory mediators induce endogenous MHCII up-regulation by LNSCs (Fig. 3 B). Furthermore, MHCII transfer from DCs to LECs and FRCs was detectable within 2 h of co-culture and increased over time (Fig. 3 C). Finally, we investigated whether MHCII transfer to LECs and FRCs was different depending on DC activation state. Both untreated DCs (immature DCs [iDCs]) and LPS-treated DCs (mature DCs [mDCs]) transferred MHCII to LNSCs. LNSCs co-cultured with mDC acquired more MHCII compared with those incubated with iDC (Fig. 3 D). Interestingly, DCs cultured for 24 h with LEC/FRC cultures

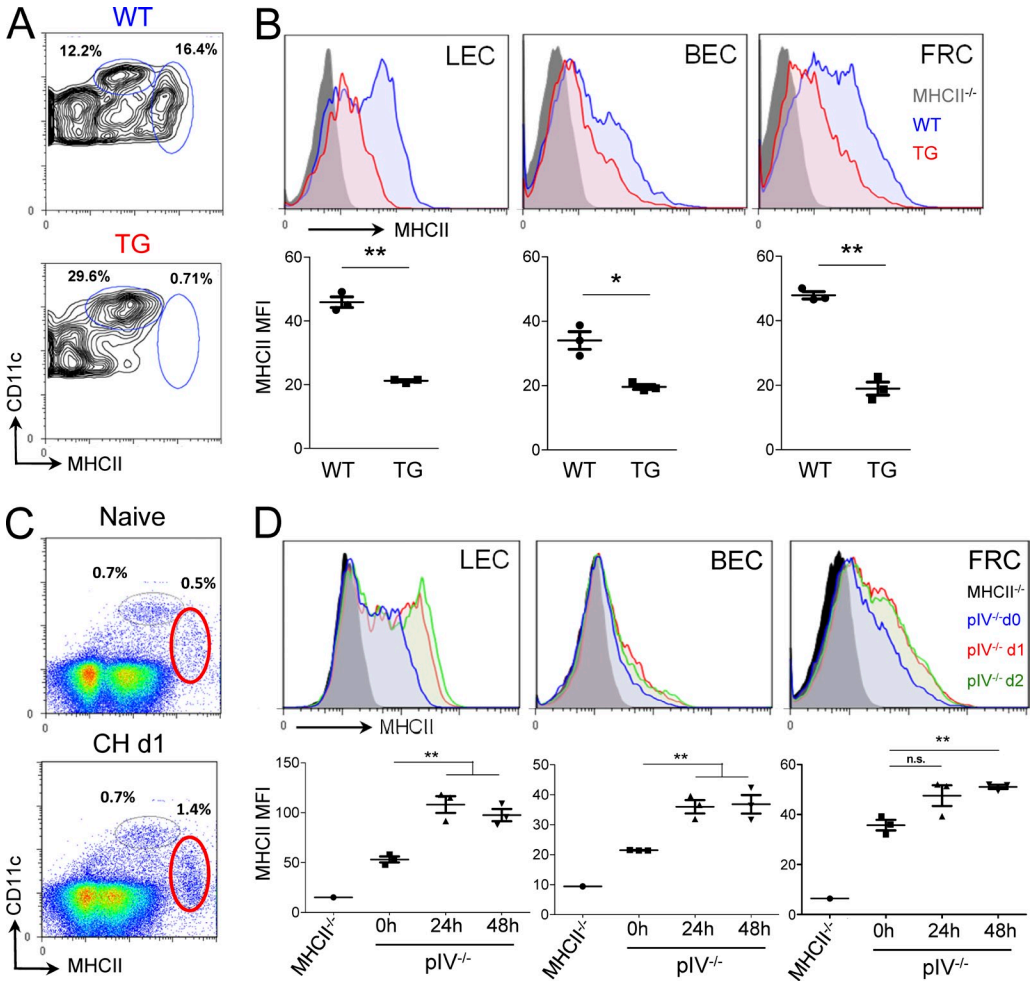

Figure 4. DCs transfer of MHCII molecules to LNSCs in vivo. (A and B) Frequencies of resident (CD11c^{hi}MHCII^{int}) and migratory (CD11^{int}MHCII^{hi}) DC populations (A), and MHCII expression on LNSCs (B), in LN from WT and K14-VEGFR3-Ig (TG) mice. Graphs show MHCII MFI on LEC, BEC, and FRC from WT and TG mice. Each symbol represents an individual mouse. (C and D) A 1:1 acetone-butyl phthalate mixture was applied or not in the skin of pIV$^{-/-}$ mice. Frequencies of resident and migratory DC populations in the draining LNs 1 d after CH (C). MHC-II levels in LEC, BEC, and FRC 0, 24, and 48 h after CH (D). Graphs show MHCII MFI on LEC, BEC, and FRC. (A–D) Data are representative of at least 2 independent experiments with minimum 3 mice per group. *, $P < 0.05$; **, $P < 0.01$. Error bars depict mean ± SEM.

expressed lower MHCII compared with DCs cultured alone (Fig. 3 E), consistent with MHCII transfer from DCs to LNSCs. Importantly, we observed that different ex vivo purified DC subsets from steady-state LN (Fig. S3) transferred MHCII to MHCII-deficient LECs and FRCs (Fig. 3 F), with an efficacy that correlated with the DCs' own MHCII expression (Fig. S3).

DCs transfer MHCII to LNSCs in vivo

To definitively demonstrate that LNSCs acquire MHCII molecules from DCs in vivo, we manipulated the numbers of DCs in LNs and analyzed the impact on MHCII expression by LNSCs. First, K14-VEGFR3-Ig transgenic mice (Mäkinen et al., 2001) that are deficient in skin-afferent lymphatics, resulting in an abrogation of skin DC homing to LN (Fig. 4 A), exhibited reduced MHCII expression in LECs, BECs, and FRCs compared with WT cells (Fig. 4 B). Next, we used a model of contact hypersensitivity (CH) to enhance migration of skin DCs to draining LNs (Fig. 4 C; Thomas et al., 2012). To avoid endogenous MHCII up-regulation by LNSCs due to skin inflammation and thereby quantify only DC transfer–mediated MHCII expression, CH was induced in pIV$^{-/-}$ mice. Expression of MHCII was significantly increased at 24 and 48 h in LEC and BEC populations and at 48 h in FRCs after CH (Fig. 4 D). Altogether, our data demonstrated that LNSCs acquire MHCII molecules from DCs in vivo and that the intensity of MHCII expression by LNSCs proportionally correlates with the numbers of DCs in LNs.

Mechanisms of MHCII transfer from DCs to LNSCs

To investigate whether MHCII transfer from DCs to LNSCs relies on active processes, isolated LN LEC/FRC cultures were co-cultured with paraformaldehyde (PFA)-pretreated DCs. These paralyzed DCs entirely lost their ability to transfer

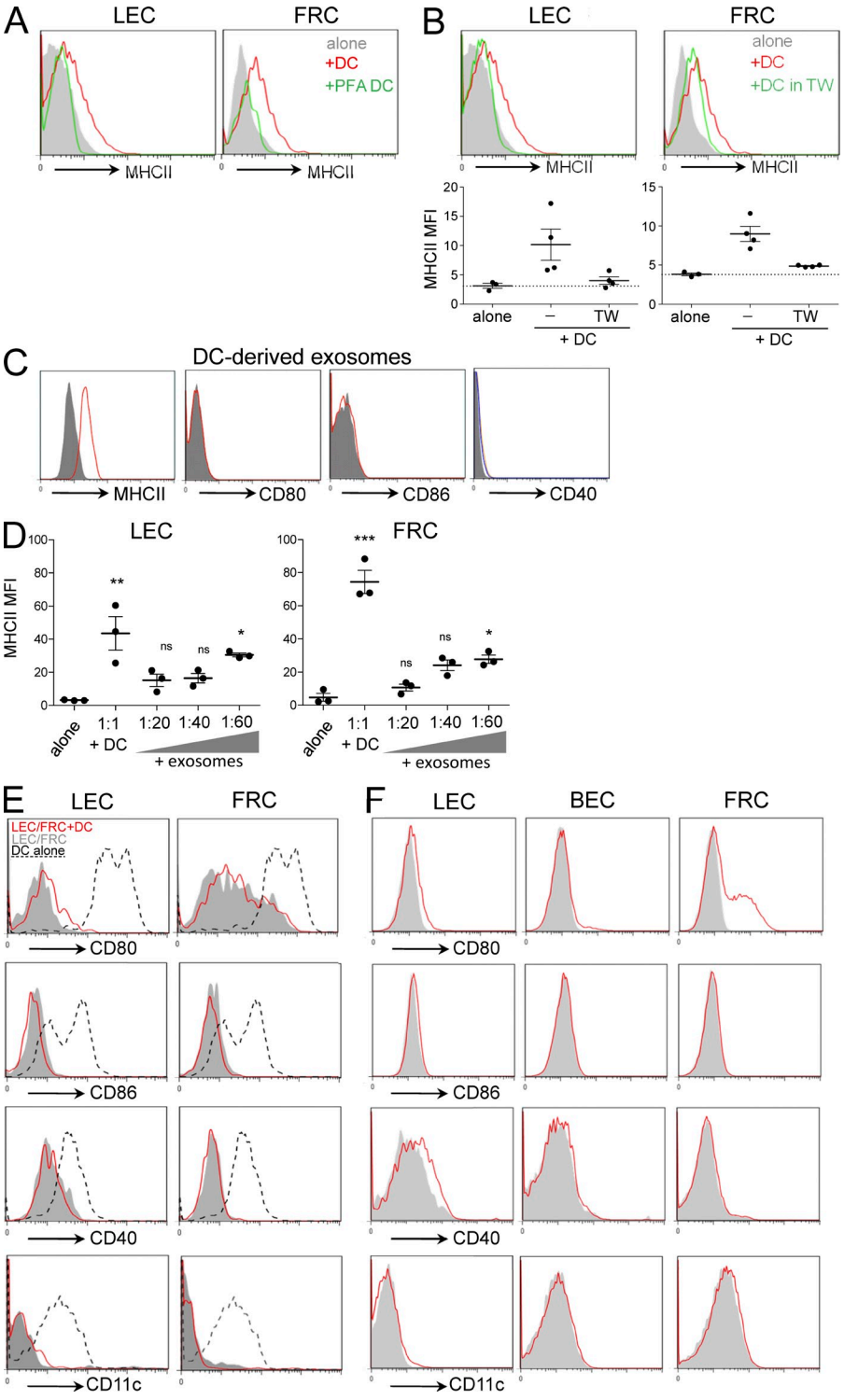

Figure 5. Mechanisms of MHCII transfer. (A) LECs/FRCs were co-cultured overnight with BMDCs previously treated (DC) or not with PFA (PFA DC). Histograms show MHCII levels on LEC and FRC. Data are representative of 3 independent experiments. (B) LECs/FRCs were co-cultured overnight with BMDCs in the same compartment or in two compartments separated by a transwell (TW) membrane. Histograms show MHCII levels on LEC and FRC. Graphs show MHCII MFI on LEC (left) and FRC (right). Data are representative of 3 independent experiments. Error bars depict mean ± SEM. Each symbol represents experimental replicates. (C) Expression of MHCII, CD80, CD86, and CD40 by DC-derived exosomes. Histograms are representative of 2 independent experiments. (D) 5×10^4 LECs/FRCs were co-cultured overnight with 5×10^4 (1:1) LPS-treated BMDCs or exosomes purified from 10^6 (1:20), 2×10^6 (1:40), or 3×10^6 (1:60) LPS-treated BMDCs. Data show MHCII MFI on LEC and FRC and are representative of 2 independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s. = not significant. Error bars depict mean ± SEM. Each symbol represents experimental replicates. (E) Levels of CD80, CD86, CD40, and CD11c expression by LEC and FRC cultured alone or co-cultured overnight with LPS-treated BMDCs. Red histograms represent expression of indicated molecules by LEC and FRC compared with isotype (gray histograms). Dotted histograms represent expression of the molecules by BMDCs. Data are representative of 3 independent experiments. (F) Expression of CD80, CD86, CD40, and CD11c by LEC, BEC, and FRC isolated from LN (red histograms) compared with isotype (gray histograms). Histograms are representative of 2 independent experiments with 2–4 mice per group.

MHCII to LECs and FRCs (Fig. 5 A), suggesting an active transfer of MHCII from donor to recipient cells. To investigate whether this process was cell–cell contact dependent, we co-cultured DCs and LEC/FRCs in different compartments separated by a culture insert membrane, and observed a dramatic reduction of MHCII expression on LECs and FRCs (Fig. 5 B). However, small amounts of MHCII were detectable on these cells (Fig. 5 B), suggesting that DC-derived vesicles might also be involved in transferring MHCII molecules in vitro. Indeed, DC-derived exosomes have been previously described to mediate intercellular transfer of surface proteins (Davis, 2007). To determine whether exosomes were involved in the process of MHCII transfer from DCs to LNSCs, we isolated and purified exosomes from cultured DCs as previously

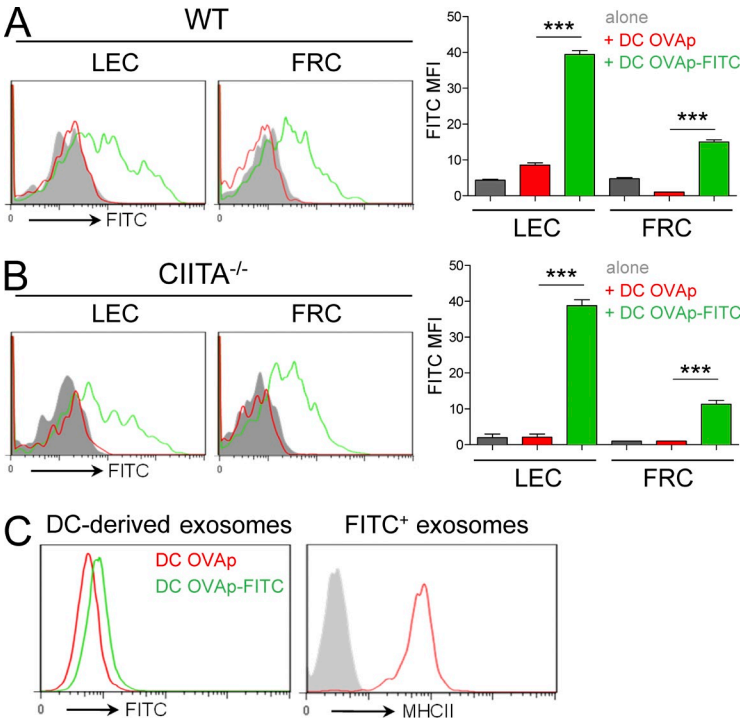

Figure 6. DCs transfer pMHCII complexes to LEC and FRC in vitro. (A and B) WT (A) and CIITA$^{-/-}$ (B) LEC/FRC were co-cultured with BM-DCs preloaded with OVA$_{323-339}$ (red histograms) or FITC-labeled OVA$_{323-339}$ (green histograms). After 24 h, FITC signals was analyzed by FACS in LECs and FRCs and compared with isotype control (gray histogram). Graphs show FITC MFI and are representative of 3 independent experiments. Error bars depict mean ± SEM. ***, $P < 0.001$. (C) Exosomes were purified from supernatant of 5×10^6 BMDCs loaded with FITC-labeled (green) or unlabeled (red) OVA peptide (1 µM). DC-derived exosomes were coupled with latex beads and analyzed by FACS for FITC fluorescence (left) and for MHCII expression (compared with isotype control in gray) after gating on FITC$^+$ exosomes (right). Data representative of 3 independent experiments.

described (Théry et al., 2002). We found that DC-derived exosomes were positive for MHCII expression (Fig. 5 C). When DC-derived exosomes were added to LEC/FRC cultures, MHCII expression increased in a dose-dependent manner on both LECs and FRCs (Fig. 5 D), but to a lesser extent compared with DCs (Fig. 5 D).

Strikingly, in vitro cultured LECs and FRCs did not express co-stimulatory molecules, nor did they acquire them when co-cultured with DCs (Fig. 5 E). Accordingly, DC-derived exosomes were negative for co-stimulatory molecules (Fig. 5 C). Furthermore, ex vivo LNSCs were negative for the CD11c marker and expressed low levels of co-stimulatory molecules (Fig. 5 F). Altogether, these results suggest that the process of transfer from DCs to LNSCs is restricted to MHCII molecules and that DCs transfer MHCII to LNSCs in a mainly cell contact–dependent manner, although other mechanisms, including DC-derived exosomes, might also play a role.

LNSCs present pMHCII acquired from DCs and induce CD4$^+$ T cell tolerance

Next, we asked whether peptide-loaded MHCII molecules (pMHCII) could also be transferred from DCs to LNSCs, and if so, whether these transferred complexes could be functionally presented to CD4$^+$ T cells. To this end, we first loaded DCs with FITC-labeled OVA$_{323-339}$ peptide and, after thorough washing, co-cultured them with LEC/FRCs. We found efficient transfer of FITC fluorescence to both LECs and FRCs (Fig. 6 A), suggesting that DCs can transfer pMHCII complexes to LNSCs in vitro. Importantly, similar results were obtained using CIITA$^{-/-}$ LEC/FRC cells, ruling out the possibility that endogenous MHCII molecules expressed by LECs and FRCs were loaded with free FITC$^+$ OVA peptide (Fig. 6 B). Consistent with a possible role for exosomes in the transfer of pMHCII to LNSCs, exosomes derived from FITC-labeled OVA$_{323-339}$-loaded DCs were FITC$^+$ (Fig. 6 C). Importantly, all FITC$^+$ exosomes were MHCII positive, reinforcing the idea that exosomes might transfer pMHCII complexes rather than free peptide (Fig. 6 C).

To study the impact of pMHCII transfer from DCs to LNSCs on CD4$^+$ T cells in vivo, we took advantage of the mouse model CD11cDOG (CD11cDTR-OVA-eGFP) in which OVA protein is exclusively expressed by DCs (Hochweller et al., 2008; Fig. 7 A). In contrast to DCs, LNSCs purified from CD11cDOG mice did not express OVA mRNA (Fig. 7 B), excluding the possibility that in this model, LNSCs endogenously express and may directly present OVA to T cells. Next, LNSCs and DCs were sorted from either WT or CD11cDOG mice and incubated for 3 d with CFSE-labeled OT-II CD4$^+$ T cells. As expected, T cells proliferated well after culture with CD11cDOG but not WT DCs (Fig. 7 C). Although we did not observe any significant proliferation when OT-II cells were incubated with WT or CD11cDOG LECs, BECs, or FRCs, Annexin V staining revealed that more T cells became apoptotic when co-cultured with CD11cDOG LECs compared with WT LECs (Fig. 7 C). Thus, consistent with published studies showing that LECs can induce T cell deletion (Lund et al., 2012; Tewalt et al., 2012) and correlating with the higher expression of PD-L1 by LECs compared with other LNSC populations observed (Fig. 7 D), our data suggest that under steady state, LECs acquire pMHCII complexes from DCs and promote CD4$^+$ T cell apoptosis in an antigen-specific manner.

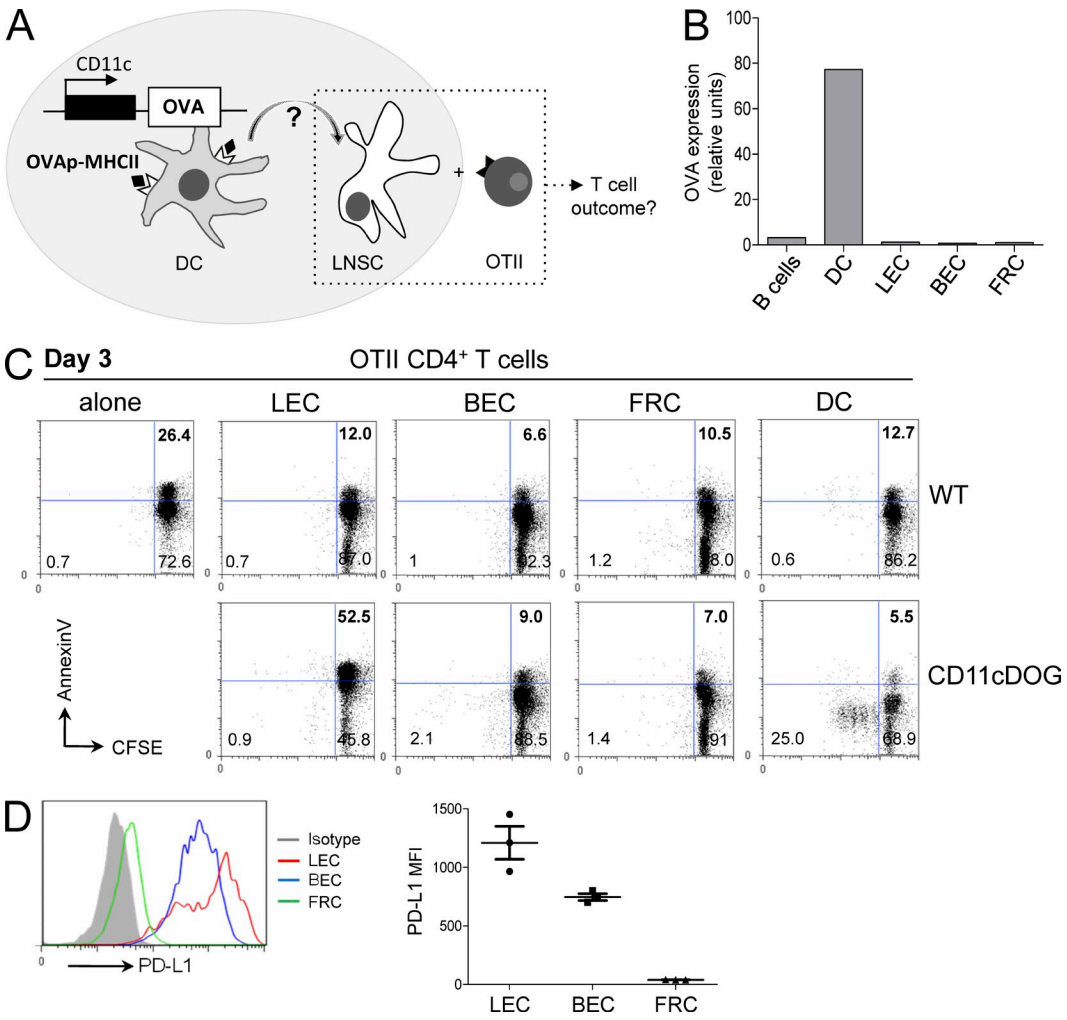

Figure 7. LECs presenting pMHCII acquired from DCs promote T cell apoptosis. (A) A schematic view of the protocol used to test the transfer of pMHCII (OVAp-MHCII) from DCs to LNSCs in CD11cDOG mice and its impact on OVA-specific CD4+ T cell (OTII) outcome. LNSCs were purified from CD11cDOG mice (in which OVA is selectively expressed in DCs) and co-cultured with OTII cells. (B) OVA mRNA levels were quantified by qPCR from FACS-sorted B cells, DCs, LECs, BECs, or FRCs of CD11cDOG mice. Data are representative of 2 independent experiments with a pool of 7 to 12 mice each. (C) LNSCs and DCs were purified from CD11cDOG or WT mice, and ex-vivo cultured with TCR transgenic CD45.1 CD4+ T cells (OTII). (C) CFSE and Annexin V profiles of OTII cells, gated on CD4+ and CD45.1+ cells after 3 d of culture with indicated cells. Dot plots are representative of 3 independent experiments with 16 mice per group. (D) Histograms show PD-L1 expression by LECs, BECs, and FRCs compared with isotype control (gray). Graphs represent PDL-1 MFI for each cell subset and are representative of 3 independent experiments with 3 mice. Error bars depict mean ± SEM.

To further investigate the inhibitory capacity of LNSCs, T cells were harvested and restimulated using anti-CD3/CD28 antibodies after 3 d of culture. OT-II cells that were not exposed to any other cells noticeably proliferated upon CD3/CD28 stimulation (Fig. 8 A), as observed for T cells exposed for 3 d to WT DCs. However, OT-II cells exposed to WT LECs responded less efficiently to restimulation compared with unexposed T cells, confirming that LNSCs suppress T cell proliferation independently of the presentation of pMHC complexes (Khan et al., 2011; Lukacs-Kornek et al., 2011; Siegert et al., 2011). Ex vivo, LECs appeared to be more efficient compared with BECs and FRCs at inhibiting T cell proliferation independently of antigen presentation (Fig. 8 A). Importantly, the proliferation of T cells preexposed to CD11cDOG LECs, BECs, and FRCs was further reduced compared with T cells preexposed to WT LECs, BECs, and FRCs (Fig. 8 A). We did not observe any differences in T reg cell frequency, whether T cells were precultured alone or with LNSNs derived from WT or CD11cDOG mice (unpublished data). To firmly demonstrate that pMHCII complexes are transferred from DCs to LNSC in vivo, and to rule out that free OVA released from CD11cDOG DCs is captured and presented via endogenous MHCII expressed at the surface of LNSCs, experiments were repeated using BM chimeric mice in which LNSCs do not express endogenous MHCII. CD11cDOG BM cells were used to reconstitute irradiated CIITA$^{-/-}$ recipient mice. Using this setting, we confirmed that LECs, BECs, and FRCs isolated from CD11cDOG:

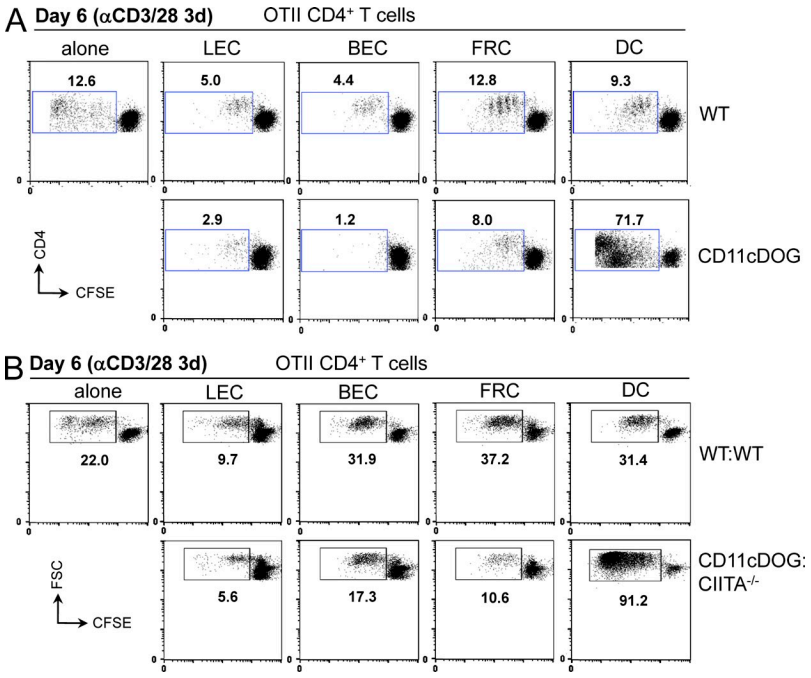

Figure 8. T cells preexposed to LNSCs presenting acquired pMHCII failed to proliferate upon restimulation. (A) OTII+ cells were harvested after 3 d of exposure to indicated cells from either CD11cDOG or WT mice (as in Fig. 7 C) and restimulated with anti-CD3/anti-CD28 for 3 additional d. Proliferation (percentage) of gated CD45.1+ CD4+ T cells measured by CFSE dilution. Dot plots are representative of 3 independent experiments with 16 mice per group. (B) LNSCs and DCs were purified from CD11cDOG:WT or WT:WT chimeric mice, and ex vivo–cultured with TCR transgenic CD45.1 CD4+ T cells (OTII) for 3 d. OTII+ cells were harvested from and restimulated with anti-CD3/anti-CD28 for 3 additional d. Proliferation (percentage) of gated CD45.1+ CD4+ T cells measured by CFSE dilution. Dot plots are representative of 2 individual experiments with a pool of 20 mice/group.

CIITA$^{-/-}$ mice inhibit T cell proliferation in an antigen-specific manner compared with LNSCs isolated from WT:WT mice (Fig. 8 B). Altogether, our data demonstrated that LNSCs acquired $OVA_{323-339}$–MHCII complexes from DCs in vivo, and induced an antigen-specific inhibition of OT-II survival and proliferation.

DISCUSSION

Emerging evidence suggests that stromal cells in LNs play an important role in the regulation of peripheral immune responses (Bajénoff et al., 2006; Mueller and Germain, 2009). Many current studies have focused on dissecting the complex mechanisms responsible for LNSC immunoregulation. Particular LNSC subsets, mainly LECs and FRCs, suppress activated CD4+ and CD8+ T cell proliferation independently on the presentation of pMHC complexes (Khan et al., 2011; Lukacs-Kornek et al., 2011; Siegert et al., 2011). LNSCs also express various PTAs and contribute to the maintenance of deletional peripheral CD8+ T cell tolerance (Lee et al., 2007; Nichols et al., 2007; Magnusson et al., 2008; Cohen et al., 2010; Fletcher et al., 2010; Tewalt et al., 2012). However, whether the different LNSC subpopulations influence CD4+ T cell tolerance is still unclear. LECs, BECs, and FRCs have been shown to express low surface MHCII levels in steady-state and to increase expression upon inflammation (Malhotra et al., 2012). Our study clarifies the mechanisms accounting for the regulation of MHCII expression by LNSCs. We show that LECs, BECs, and FRCs, but not DN cells, express MHCII in steady state and up-regulate surface MHCII upon exposure to IFN-γ. We further demonstrated that LNSCs exclusively express pIV of CIITA, and that IFN-γ–mediated MHCII expression is pIV-dependent.

Our data provide ample evidence that low endogenous MHCII expression by LNSCs is driven by basal pIV activity, whereas the majority of MHCII molecules are acquired from DCs. First, LECs/FRCs cultured in vitro do not express MHCII but acquire these molecules after co-culture with DCs, but not B cells or macrophages. Furthermore, changes in DC numbers in LN in vivo proportionally modulate MHCII expression levels by LNSCs. First, a decrease in DC numbers caused by sublethal mouse irradiation significantly reduces MHCII expression by LNSCs. This process mainly eliminates resident DCs, and not some of the radioresistant migratory DC subsets such as Langerhans cells and dermal DCs, suggesting that LN-resident DCs contribute to MHCII transfer to LNSCs. In contrast, preventing skin-resident DC trafficking to LNs dramatically decreases surface MHCII expression levels on LNSCs. Interestingly, although significantly reduced, MHCII molecules can still be detected at the surface of LNSC, suggesting that both migrating and LN-resident DCs transfer MHCII molecules to LNSCs. Conversely, promoting skin DC migration to LN using the CH model increases MHCII expression by LNSCs. This result was obtained in pIV$^{-/-}$ mice, in which LNSCs cannot endogenously express MHCII molecules, demonstrating that increased DC numbers enhance the process of MHCII transfer to LNSCs. Interestingly, both steady-state and activated DCs, as well as different DC populations purified from LN, can transfer MHCII to LNSC, showing that this process is not restricted to specific DC activation states or subsets. Rather, the amount of MHCII transferred seems to be dependent on the level of expression of those molecules by DCs.

Evidence that DCs can transfer pMHCII complexes at the surface of LNSCs was provided in vitro using a fluorescent peptide and also in vivo using mice in which only DCs

express the specific antigen. The transfer of pMHC complexes from one cell to another has already been demonstrated with pMHCI transfer between DCs (Wakim and Bevan, 2011) and pMHCII from DCs to NK cells (Nakayama et al., 2011). Different mechanisms for intercellular protein transfer between immune cells have been described, including the uprooting of proteins from cell membranes, the transfer of protein ectodomains after proteolytic cleavage, the transfer of enclosed membrane bodies or vesicles, small membrane bridges which occur after intercellular membrane fusion, and finally membrane nanotubules which may derive from membrane fusion or bridges (Davis, 2007). In vitro, transfer of MHCII to LNSCs is dramatically dampened when DCs and LNSCs were cultured in separated compartments, suggesting that this process is mostly cell–cell contact–dependent. However, our data suggest that DC-derived exosomes might also play a role. Mechanisms by which cells take up DC-derived exosomes are not yet well defined. Exosomes can either be adsorbed to the cell surface of the recipient cell nonspecifically or through active phagocyte or receptor-mediated endocytosis. In this regard, differential expression of adhesion molecules has been involved in their target and uptake by DCs (Segura et al., 2007). Therefore, differential adhesion molecule expression on LECs, BECs, and FRCs (Malhotra et al., 2012) might lead to different levels of exosome uptake. Whether exosomes actually play a role in pMHCII complex transfer from DCs to LNSCs in vivo remains to be determined. Indeed, MHCII transfer was considerably less efficient using exosomes compared with BM-DCs, suggesting that cell–cell contact dependent mechanisms are mainly involved. This is a sound hypothesis, given the location of LNSCs along the DC migratory pathway, and requires further study.

Whether cultured alone or in the presence of DCs, LECs and FRCs remained CD11c negative and expressed the same low levels of co-stimulatory molecules, suggesting that the process of transfer from DCs to LNSCs is restricted to MHCII molecules, as already proposed for NK cells (Nakayama et al., 2011). An alternative possibility is that DCs also transfer other molecules to LNSCs, which are rather actively internalized from the cell surface and MHCII molecules would be recycled to the cell membrane.

LNSCs exhibit many features needed to induce T cell tolerance, including their localization in LNs allowing permanent interactions with T cells, the absence of co-stimulatory molecules, and the presence of co-inhibitory molecules. A recent study demonstrated that one particular subset of $CD45^{lo}CD11c^{int}gp38^{neg}CD31^{neg}$ cells in secondary lymphoid organs, the eTACs, induces cognate $CD4^+$ T cell inactivation (Gardner et al., 2013). eTACs were described to originate from both radioresistant and radiosensitive origins, and to express low levels of CD11c and CD45. Here we provide convincing evidence that radioresistant $CD45^{neg}$ LECs, BECs, and FRCs also impact $CD4^+$ T cell responses. First, we confirmed published observations that proliferation of $CD4^+$ T cells exposed to LECs, BECs, and FRCs was inhibited independently of antigen presentation by LNSCs. Second, OVA-specific $CD4^+$ T cell proliferation and survival were significantly impaired when T cells were cultured with LNSCs from CD11cDOG mice in which OVA is restricted to DCs. Interestingly, we observed an antigen-specific increase of T cell apoptosis which seems to be restricted to LECs and correlates with higher PD-L1 expression by LECs compared with FRCs and BECs. However, further investigations would be necessary to dissect LEC ability to induce T cell deletion. Altogether, our data demonstrated that LNSCs acquire pMHCII complexes from DCs and consequently impair self-antigen $CD4^+$ T cell responses under steady-state conditions.

Our findings describe a novel mechanism, in which LNSCs maintain $CD4^+$ T cell peripheral tolerance. Besides conventional direct presentation of self-antigens to T cells, DCs can also transfer self-pMHCII complexes to LNSCs that in turn present these complexes to T cells and consequently induce dysfunctional self-antigen $CD4^+$ T cell responses. Further studies are needed to decipher the relative contribution of DCs and LNSCs in maintaining T cell tolerance, and to determine whether endogenous expression of MHCII molecules by LNSCs similarly affects $CD4^+$ T cell responses either upon steady-state or inflammatory conditions.

MATERIALS AND METHODS

Mice and treatments. C57BL/6 WT mice were either purchased from Charles River or bred in our specific pathogen-free facility. $MHCII^{-/-}$ (*H2-Aa*$^{-/-}$; Köntgen et al., 1993), $pI^{-/-}$, $pIV^{-/-}$ (Waldburger et al., 2003), $pIII+IV^{-/-}$ (LeibundGut-Landmann et al., 2004), $Ciita^{-/-}$ (Laufer et al., 1996), CD11cDOG (Hochweller et al., 2008), OT-II $CD45.1^+$ (Barnden et al., 1998), K14-VEGFR3-Ig (provided by K. Alitalo, Institute of Biomedicine, University of Helsinki, Helsinki, Finland; Mäkinen et al., 2001), $I\text{-}A^b$ GFP (Boes et al., 2002; provided by A.M. Lennon-Dumenil, INSERM U932, Institut Curie, Paris, France), and *Ifngr1*$^{-/-}$ (The Jackson Laboratory) mice have been previously described. These mouse strains are on a C57BL/6 background and were housed and maintained under SPF conditions. All animal husbandry and experiments were approved by and performed in accordance with guidelines from the Animal Research Committee of the University of Geneva. When indicated, IFN-γ (1 µg, both flanks) was injected subcutaneously. For some experiments, mice were irradiated with two consecutive doses of 300 cGy (Gammacell 40 Exactor).

LNSC isolation. LNSCs were obtained as previously described (Link et al., 2007). In brief, total skin or skin-draining LNs from individual or 10–15 pooled mice were cut into small pieces and digested in RPMI containing 1 mg/ml Collagenase IV (Worthington Biochemical Corporation), 40 µg/ml DNase I (Roche), and 2% FBS. Undigested cells were further digested with 1 mg/ml Collagenase D, and 40 µg/ml DNase I (Roche). The reaction was stopped by addition of 5 mM EDTA and 10% BSA. Samples were further disaggregated through a 70-µm cell strainer and blocked with anti-CD16/32 antibody. Single cell suspensions were negatively selected using CD45 microbeads and magnetic bead column separation (Miltenyi Biotec).

Antibodies, flow cytometry, and cell sorting. Anti-gp38 (clone 8.1.1), anti-CD31 (clone 390), anti-CD11b (clone M1-70), anti-CD11c (clone N418), anti-CD45.1 (A20), anti-IAb (AF6.120.1), anti-CD80 (clone 16-10A1), anti-CD86 (clone GL1), anti-PD-L1 (clone 10F.9G2), anti-CD63 (clone NVG-2), and anti-F4/80 (clone BM8) mAbs were from BioLegend. Anti-CD45 (clone 30F11), anti-CD103 (clone M290), anti-CD16/32 FcγRIII (clone 2.4G2), anti-$I\text{-}A^d$/$I\text{-}E^d$ (clone 2G9), anti–AnnexinV mAbs were from BD. Anti-CD19 (1D3), anti-CD8 (clone 53–6.7), anti-CD40 (clone HM40-3), and anti-CD4 (GK1.5) were from eBioscience. Anti-CD3 and anti-CD28 were from Bio X Cell. For flow cytometry, enriched $CD45^{neg}$ cells

were stained with mAbs against CD45, gp38, and CD31. In certain experiments, cells were further stained with mAbs against MHCII or isotype control as indicated. Cells were either acquired on a FACSCalibur (BD) or sorted using a MoFlowAstrios (Beckman Coulter), and analyzed using FlowJo software (Tree Star).

Generation of BM chimeric mice. BM was recovered from tibia and femurs by flushing with PBS-EDTA, dissociated by repeated passages through a 20-gauge needle. 5–7 × 10^6 cells were injected intravenously into irradiated (two consecutive doses of 450 cGy) recipient mice. Reconstitution was assessed by analyzing blood cells by flow cytometry after 6–8 wk.

Generation of BM–derived DCs, macrophages, and B cell isolation. DCs were generated by culturing BM cells for 7–10 d in the presence of 20 ng/ml GMCSF in RPMI supplemented with 10% heat-inactivated fetal bovine serum, 50 mM 2-mercaptoethanol, 100 mM sodium pyruvate, and 100 µM penicillin/streptomycin at 37°C, 5% CO_2. For some experiments, DCs were treated with 1 µg/ml LPS (Enzo Life Sciences) for the last 16 h of culture. For macrophage differentiation, BM cells were cultured for 7 d in DMEM containing 4.5 glucose, supplemented with 20% FBS, 30% L929 supernatant, and 1% penicillin/streptomycin. B cells were purified from the spleen of naive WT mice using the CD19 depletion kit and magnetic bead column separation (Miltenyi Biotec).

LEC/FRC in vitro culture. A mixture of FRC/LEC culture was performed as previously described (Fletcher et al., 2011). In brief, LNs from 1–3 mice were dissected and digested with a freshly made enzymatic solution comprised of RPMI-1640 containing 0.8 mg/ml Dispase, 0.2 mg/ml Collagenase P, and 0.1 mg/ml DNase I (Roche). Tubes were incubated at 37°C in a water bath and gently inverted at 5-min intervals to ensure the contents were mixed. After 20 min, LNs were very gently mixed using a 1-ml pipette. Large fragments were allowed to settle before replacing the supernatant for fresh digestion mix. Supernatant containing the digested cells was added into 10 ml of cold FACS buffer (2% FCS and 5 mM EDTA in PBS). These steps were repeated every 10 min until all LNs were completely digested. Cells were washed and filtered through a 70-µm cell strainer, quantified using a hemocytometer, and plated in 6-well plates at a concentration of 1 × 10^7 cells/well. Cell culture media was αMEM supplemented with 10% batch-tested, low Ig FCS, and 1% penicillin/streptomycin. Plates were washed and culture media was renewed every day to remove nonadherent cells. After 5 d, cultures primarily contained LECs and FRCs. When indicated, BM-derived DCs, macrophages, splenic B cells, or DC-derived exosomes were added to the cultures for 2–24 h depending on the experiment. For transwell assays, BMDCs were added to the upper chamber of a 6-well plate and separated from LEC/FRC cultures by a 0.4-µm-pore semipermeable membrane (Corning Costar). When indicated, BMDCs were pretreated for 1 h with paraformaldehyde (PFA, 4%; Wako) or incubated with either 1 µM OVA (GenScript) or OVA-FITC (Eurogentec) peptides at 37°C and extensively washed with PBS before co-culture.

Exosome purification. BMDCs were activated with LPS, loaded with either 1 µM OVA peptide (GenScript) or 1 µM OVA peptide-FITC (Eurogentec), and cultured in exosome-depleted medium for 24 h. Exosomes were then purified using serial centrifugations: 300 *g* for 10 min, 2,000 *g* for 10 min, 10,000 *g* for 30 min, and 100,000 *g* for 70 min (twice). Pellet was only kept for the last two ultracentrifugations and resuspended in small volumes of PBS. For FACS analysis, exosomes were coupled to Aldehyde-Sulfate Latex beads 4% wt/vol 9 µm (Life Technologies; 10:1 ratio), and identified as $CD63^+$. In indicated experiments, exosomes were incubated for 12 h with LNSCs on a plate shaker at 37°C.

Ex vivo antigen presentation assay. LNSCs or DCs were sorted using a MoFlowAstrios (Beckman Coulter) from pooled skin draining LN of CD11cDOG or nontransgenic WT control mice, as described in the previous sections. The purity of LNSCs and DCs routinely exceeded 97%. $CD4^+$ T cells were purified from OT-II TCR transgenic mice from spleen and LN using $CD4^+$ T cell depletion kit and the MACS system (Miltenyi Biotec). Naive CFSE-labeled OT-II cells (5 × 10^4) were co-cultured with sorted 5 × 10^4 LNSC or DC for 3 d. After 3 d, OT-II cells were harvested and culture in an anti-CD3/anti-CD28 (1 µg/ml) precoated plates for an additional 3 d. Cells were stained for surface markers and proliferation was measured by CFSE dilution in a FACSCalibur (BD).

CH model. A 1:1 mixture of acetone and dibutyl phthalate was applied epicutaneously in the back skin of mice (Vigl et al., 2011). Draining LNs were excised and analyzed after 24 or 48 h.

RNA isolation and quantitative RT-PCR. Total RNA was prepared with TRIZOL Reagent (Invitrogen) from sorted LNSCs. cDNA was synthesized with random hexamers and Superscript II reverse transcription (Invitrogen). PCRs for CIITA promoters were performed with the iCycler iQ Real-Time PCR detection System and iQ SYBR green super mix (Bio-Rad Laboratories). Results were quantified with a standard curve generated with serial dilutions of a reference cDNA preparation. Primer sequences: CIITA pI forward, 5′-CAGGGACCATGGAGACCATAGT-3′ and reverse, 5′-CAGGTAGCTGCCCTCTGGAG-3′; CIITA pIII forward, 5′-GGTTCCTGGCCCTTCTGG-3′ and reverse, 5′-ATCCATGGTGGCACACAGACT-3′; and CIITA pIV forward, 5′-CAGCACTCAGAAGCACGGG-3′ and reverse, 5′-ATCCATGGTGGCACACAGACT-3′. Results were normalized with GAPDH mRNA expression and quantified with a standard curve generated with serial dilutions of a reference thymic cDNA preparation. OVA mRNA levels were measured by TaqMan PCR in an ABI 7700 PCR machine (Azco Biotech). Primer sequences: OVA forward, 5′-CCATTTGCCAGTGGGACAAT-3′, reverse, 5′-TCAAGCTGCTCAAGGCCTG-3′, and Tg probe, 5′-(FAM)CATGTTGGTGCTGTTGCCTGATGAAGTC(TAMRA)-3′; and internal control forward, 5′-CACGTGGGCTCCAGCATT-3′, reverse, 5′-TCACCAGTCATTTCTGCCTTTG-3′, and probe, 5′-(JOE NHS Ester)CCAATGGTCGGGCACTGCTCAA(BHQ1)-3′.

Statistical analysis. Statistical significance was assessed by the two-tailed unpaired Student's *t* test and ANOVA using Prism 5.0 software (GraphPad Software).

Online supplemental material. Fig. S1 shows flow cytometry dot plots describing LNSC subpopulations. Fig. S2 shows differential regulation of the gene encoding the MHC CIITA in indicated cell subtypes. Fig. S3 depicts the gating strategy used for the FACS sorting of different DC subsets in LN for Fig. 3 F. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20132000/DC1.

We thank Jean-Pierre Aubry-Lachainaye for flow cytometry cell sorting, Boris Lee and Amanda Proudfoot for critical reading of the manuscript, and Hans Acha-Orbea, Sebastian Amigorena, Sachiko Hirosue, Efthymia Vokali, and Amanda Lund for helpful discussion. We are grateful to Kari Alitalo for kindly providing the K14-VEGFR3-Ig transgenic mice.

T. Suter is supported by the Clinical Research Priority Program (CRPP) Multiple Sclerosis of the University of Zurich, Zurich, Switzerland. This work was supported by the Swiss National Science Foundation (310030-127042 to S. Hugues and 31-135756 to M.A. Swartz), by the European Research Council (281365 to S. Hugues and 323053 to M.A. Swartz), and by the Leenaards Foundation (to S. Hugues and M.A. Swartz).

The authors declare no competing financial interests.

Submitted: 19 September 2013
Accepted: 8 April 2014

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