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A distinct dendritic cell population arises in the thymus of IL-13Rα1-sufficient but not IL-13Rα1-deficient mice

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Abstract

IL-13 receptor alpha 1 (IL-13Rα1) associates with IL-4Rα to form a functional IL-4Rα/IL-13Rα1 heteroreceptor (HR) through which both IL-4 and IL-13 signal. Recently, HR expression was associated with the development of M2 type macrophages which function as antigen presenting cells (APCs). Herein, we show that a subset of thymic resident dendritic cells (DCs) expressing high CD11b (CD11b^{hi}) and intermediate CD11c (CD11c^{int}) arise in HR-sufficient but not HR-deficient mice. These DCs, which originate from the bone marrow are able to take up Ag from the peritoneum, traffic through the spleen and the lymph nodes and carry it to the thymus. In addition, since the DCs are able to present Ag to T cells, express high levels of the costimulatory molecule CD24, and comprise a CD8α$^+$ subset, it is likely that the cells contribute to T cell development and perhaps negative selection of self-reactive lymphocytes.

Keywords

Dendritic cell; CD11b; CD11c; thymus; antigen presentation; IL-13Rα1

1. Introduction

IL-4 signals through the conventional IL-4R (IL-4Rα/common γ) and the IL-4Rα/IL-13Rα1 heteroreceptor (HR) while IL-13 signals only through the HR as IL-13Rα2 serves as a decoy receptor [1, 2]. Mice in which the IL-13Rα1 is made non-functional by gene ablation do not express the HR and offer a useful HR$^{-/-}$ mouse model to define the role the

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Author Contributions

S.B. and M.M.M. designed and conducted the experiments, analyzed and interpreted the data, and drafted the manuscript. A.N.C-R. and T.K.U. advised on experimental design and edited the manuscript. H.Z. conceived the project, advised on experimental design, and wrote the manuscript.

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IL-4Rα/IL-13Rα1 HR plays in the development of myeloid cells [3, 4]. For instance, we have previously demonstrated that the HR plays a role in the development of M2 type macrophages [4]; an observation that bodes well with reports indicating that IL-4Rα expression is subset specific and shapes the function of macrophages [5, 6]. Also, BM-derived HR-positive (HR^{+}) stem cells that settle in the thymus, which are known as early thymic progenitors (ETPs), give rise to myeloid cells that are able to function as APCs [3] while their HR-negative counterparts give rise to T cells [7]. The HR^{+}-ETP-derived, as well as non-ETP-derived, myeloid cells may contribute to central tolerance of self-reactive T cells to prevent the development of autoimmunity. In fact, $HR^{-/-}$ mice are more susceptible to induction of experimental allergic encephalomyelitis (EAE), an autoimmune disease mediated by myelin reactive T cells [8]. As DCs represent perhaps the most prominent APCs that support central T cell tolerance [9, 10], one would envision the HR to play a role in the generation of DCs able to contribute to the process of thymic T cell selection. To this end, we conducted a comparative fine analysis of DC subsets in different organs of $HR^{+/+}$ relative to $HR^{-/-}$ mice. The findings indicate that a distinct subset of DCs characterized by expression of high levels of CD11b ($CD11b^{hi}$) and intermediate levels of CD11c ($CD11c^{int}$) is observed in the thymus of $HR^{+/+}$ but not $HR^{-/-}$ mice. These cells originate from the bone marrow, reside in the thymus, and include $CD8\alpha^{+}$ and $CD8\alpha^{-}$ DCs, both of which express the thymus homing molecule PSGL-1. Also, while both subsets take up Ag from the periphery and travel through lymphoid organs, the $CD8\alpha^{+}$ subset seems to be more efficient at Ag transfer to the thymus. Given that the subsets reside in the thymus and are able to present Ag to T cells, it is logical to envision a role for these APCs in T cell selection.

2. Materials and methods

2.1. Mice

All animal experiments were done according to protocols approved by the University of Missouri Animal Care and Use Committee. C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). IL-13Rα$1^{+/+}$-GFP and IL-13Rα$1^{-/-}$ C57BL/6 mice were previously described [3, 7]. Ovalbumin (OVA)-specific OT-II-TCR transgenic mice were previously described [11]. Only female mice were used throughout the study. Animals were 6–8 weeks old at the time experiments were performed. All animals were maintained under specific pathogen–free conditions in individually ventilated cages and kept on a 12 h light-dark cycle with access to food and water ad libitum.

2.2. Antigens

OVA peptide (OVAp) which encompasses aa residues 323–339 (ISQAVHAAHAEINEAGR) of OVA is recognized by OT-II-TCR transgenic T cells in the context of H-2^{b} MHC haplotype and was purchased from EZBiolab (Carmel, IN). OVA-Alexa 488 was purchased from Molecular Probes (Eugene, OR) and was used to track Ag uptake and DC-trafficking to the thymus.

2.3. Flow Cytometry

Antibodies—anti-CD4 (RM4-5), anti-CD8 (53-6.7), anti-CD25 (7D4), anti-CD44 (IM7), anti-CD45.1 (A20), anti-CD45.2 (104), anti-CD11b (M1/70), anti-CD11c (HL3), anti-

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PDCA (927), anti-CCR2 (SA203G11), anti-CCR5 (7A4), anti-CCR7 (4B12), anti-CCR9 (CW-1.2), anti-CD69 (H1.2F3), anti-CD80 (16-10A1), anti-CD86 (P03.1), anti-MHCII (NIMR-4), anti-SIRPα (P84), and PSGL-1 (2PH1) antibodies were purchased from BD Biosciences (San Jose, CA). Anti-IL-13Rα1 monoclonal antibody (1G3-A7) was produced in our laboratory [4] and recognizes cell surface IL-13Rα1 dimerized with IL-4Rα1[7].

Fluorochromes—These include antibodies conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), PE-Cy5, PE-Cy5.5, peridinin-chlorophyll-protein complex (PerCP)-Cy5.5, PE-Cy7, allophycocyanin (APC), APC-Cy7 (or APCeFluor780), or biotin. Biotinylated antibodies were revealed with Streptavidin PE.

Sample Analysis—The samples were read on a Beckman Coulter CyAn (Brea, CA) and analyzed with FlowJo software version 10 (Tree Star). Dead cells were excluded using 7-aminoactinomycin D (7-AAD; EMD Biosciences).

2.4. Bone Marrow Chimeras

CD45.2 $HR^{-/-}$ C57BL/6 mice were lethally irradiated (900 rads) and given 10×10^6 BM cells from CD45.2 $HR^{-/-}$ C57BL/6 mice with or without the addition of 1×10^6 BM LSK ($Lin^- sca1^+cKit^+$) cells from CD45.1 $HR^{+/+}$ C57BL/6 mice. Thymic cells were harvested and analyzed 3 weeks after BM reconstitution.

2.5. ELISA

Cytokine production was measured by ELISA according to the standard BD biosciences protocol (San Jose, CA) using anti-cytokine antibodies for IFN-γ (capture R4-6A2, biotinylated XMG1.2) and IL-5 (capture TRFK5, biotinylated TRFK4). The OD450 was read on a SpectraMax 190 counter (Molecular Devices, Sunnyvale, CA) and analyzed with SoftMAX Pro software v3.1.1. Cytokine concentrations were extrapolated from the linear portion of a standard curve generated by graded amounts of recombinant IFN-γ and IL-5 (Peprotech, Rocky Hill, NJ).

2.6. Ag Uptake and DC trafficking to the thymus

Ag uptake assay—Sorted $CD11b^{hi}CD11c^{int}$ cells were incubated with 10 μg Alexa-488-OVA for 30min at 37°C. Cells were then washed 3 times in PBS, and analyzed for OVA uptake by measuring Alexa-488 fluorescence.

DC trafficking—100 μg Alexa-488-OVA or DMSO control were injected i.p. into $HR^{+/+}$ C57BL/6 mice. Seven days later, thymic, spleen and lymph node cells were labelled with antibodies against CD11b, CD11c, and CD8α and the APC subsets were assessed for Alexa-488 fluorescence.

2.7. Cell Sorting

Thymic cells were stained with anti-CD11b and anti-CD11c antibodies and the $CD11b^{hi}CD11c^{int}$ population was sorted on a Beckman Coulter MoFlo XDP (Brea, CA). Cell purity was routinely checked and only sorts with a purity of >95% were used in this study.

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2.8. T Cell Proliferation

APC function was assessed by measuring the ability of sorted $CD11b^{hi}CD11c^{int}$ DCs to induce proliferation of T cells. Accordingly, naïve OT-II cells (10×10^6 cells/ml) isolated from the spleen with MACS $CD4^+$ T cell isolation kit (Miltenyi) were labelled with 5 μM CFSE for 10 min at 37°C. T cell proliferation was measured by CFSE dilution upon incubation with OVAp-loaded (10μM per 2×10^6 cells) $CD8\alpha^+$ or $CD8\alpha^-$ $CD11b^{hi}CD11c^{int}$ DCs.

2.9. Statistical Analysis

Data were analyzed using either an unpaired, two-tailed Students t-test, or one-way ANOVA as indicated. All statistical analyses were performed using Prism software version 4.0c (GraphPad).

3. Results

3.1. A distinct dendritic cell population emerges in the thymus of $HR^{+/+}$ but not $HR^{-/-}$ mice

We have previously demonstrated that the IL-4Rα/IL-13Rα1 heteroreceptor (HR) contributes to the development and function of APCs including macrophages [4, 12] and neonatal DCs as well as basophils[13]. Herein, comparative analysis was performed in $HR^{+/+}$ and $HR^{-/-}$ mice to determine whether the HR plays a role in populating the thymus with functional APCs. The results show that the thymus, but not other organs, of $HR^{+/+}$ mice display a unique DC population expressing high levels of CD11b and intermediate levels of CD11c ($CD11b^{hi}CD11c^{int}$) that is not apparent in $HR^{-/-}$ mice (Fig. 1A). Results from several experiments indicate that the number of these $CD11b^{hi}CD11c^{int}$ DCs in the thymus of $HR^{+/+}$ mice is significantly higher than $HR^{-/-}$ mice (Fig. 1B) perhaps suggesting that the HR is required for their development. This statement is supported by data showing that most of the cells express intermediate levels of the HR (Fig. 1C). Furthermore, about two-thirds of these thymic $CD11b^{hi}CD11c^{int}$ cells do not express CD4 or CD8α subset markers while about one-third express CD8α but not CD4 (Fig. 1D). Moreover, 86% of these cells do not express PDCA1 marker indicating that the cells are not plasmacytoid DCs (Fig. 1E). As plasmacytoid DCs do not express CD11b [14], the PDCA1 expression observed with 14% of the cells may represent background levels. Overall, a distinct population of $CD11b^{hi}CD11c^{int}$ DCs that reside in thymus is observed in $HR^{+/+}$ but not $HR^{-/-}$ mice.

3.2. Thymic $CD11b^{hi}CD11c^{int}$ DCs originate from bone marrow progenitors

To determine whether the $CD11b^{hi}CD11c^{int}$ thymic DCs originate from the bone barrow (BM), CD45.2 $HR^{-/-}$ mice were lethally irradiated, reconstituted with 10×10^6 $HR^{-/-}$ CD45.2 BM cells alongside 1×10^6 CD45.1 $HR^{+/+}$ or $HR^{-/-}$ $Lin^-Sca1^+c\text{-}Kit^+$ (LSK) bone marrow stem cells. Three weeks later host thymic cells were harvested and analyzed for presence of CD45.1 $CD11b^{hi}CD11c^{int}$ DCs. The results show that mice recipient of $HR^{+/+}$, but not those given $HR^{-/-}$, LSK BM cells had $CD11b^{hi}CD11c^{int}$ DCs (Fig. 2). These findings indicate that $CD11b^{hi}CD11c^{int}$ DCs originate from BM precursors.

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3.3. CD11b^{hi}CD11c^{int} cells can be distinguished from other thymic APCs by higher expression of CD24 marker

Four groups of thymic APCs can be identified in HR$^{+/+}$ mice on the basis of CD11b and CD11c expression (Fig. 3A). These include previously described CD11b^{hi}CD11c^{-} macrophages and CD11b^{-}CD11c^{hi} and CD11b^{int}CD11c^{hi} (cDCs) [15] as well as our newly defined CD11b^{hi}CD11c^{int} populations (Fig. 3A). As far as chemokine receptors, the CD11b^{hi}CD11c^{int} DCs had similar CCR2, CCR5 and CCR7 expression patterns as macrophages and CD11b^{-} cDCs while the CD11b^{int}CD11c^{hi} cDCs had much higher expression than the other APC subsets (Fig. 3B). CCR9 expression was similar in all cell types. The macrophages displayed a usual expression of costimulatory and MHCII molecules while the CD11b^{hi}CD11c^{int} DCs had expression patterns similar to both type of cDCs (Fig.3C). As far as SIRPα migratory marker, all cells except the CD11b^{-} cDCs had high levels of expression perhaps indicating that the CD11b^{hi}CD11c^{int} DCs migrate to the thymus from the periphery. Interestingly, the CD11b^{hi}CD11c^{int} DCs express high levels of CD24 relative to all other APCs. In all, the CD11b^{hi}CD11c^{int} DCs display a phenotype characteristic of other thymic DCs except for CD24 which is usually associated with APCs involved in thymic T cell selection [16].

3.4. CD8α^{+} CD11b^{hi}CD11c^{int} DCs carry antigen from the periphery to the thymus

The CD11b^{hi}CD11c^{int} DCs express both CCR9 and SIRPα and therefore can migrate from one organ to another. Furthermore, since the cells also express high levels of CD24 one would envision that they will be able to carry Ag from the periphery to the thymus where they might contribute to T cell selection. Given that this unique DC population comprise CD8α^{+} and CD8α^{-} subsets, we sought to determine whether the cells manifest discrepancies in migratory marker expression and Ag transfer from the periphery to the thymus. Indeed, the CD8α^{+} subset shows higher expression of CCR7 and CCR9 and lower expression of SIRPα than the CD8α^{-} subset (Fig. 4A). Both populations express similar levels of the thymus homing PSGL-1 molecules (Fig. 4A). These results reflect discrepancies among CD8α^{+} and CD8α^{-} CD11b^{hi}CD11c^{int} DCs perhaps suggesting a differential pattern of migration and Ag transfer from the periphery to the thymus. To test this premise, we began by assessing the subsets for Ag uptake by *in vitro* incubation with ovalbumin (OVA) conjugated to the fluorescent molecule Alexa-488 (Alexa-488-OVA). The results show that both DC subsets are capable of taking up OVA Ag (Fig. 4B). Subsequently, HR$^{+/+}$ mice were injected i.p. with Alexa-488-OVA and 7 days later their thymic, splenic and lymph node APCs were tested for Alexa-488 fluorescence. The findings show that CD8α^{+} CD11b^{hi}CD11c^{int} DCs were more efficient than the CD8α^{-} counterparts or the CD11b^{-} CD11c^{hi} cDCs at Ag transfer to the thymus as over 7% of this cell population was Alexa-488 positive (Fig. 4C). Furthermore, the CD8α^{+} CD11b^{hi}CD11c^{int} DCs were also more efficient than the CD8α^{-} counterpart in transferring Ag through the spleen (Fig. 4D) but this function was equivalent among the two populations in the lymph nodes (Fig. 4E). The cDCs, whether CD8α^{+} or CD8α^{-}, were able to transfer Ag through the spleen and lymph nodes but at lower efficacy (Fig. 4D and E).

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Overall, these findings suggest that the CD8α$^+$ CD11b^{hi}CD11c^{int} DC population serves an important role in carrying peripheral Ag to the thymus and may participate in thymic selection of T cells.

3.5. CD11b^{hi}CD11c^{int} DCs are functionally active in vitro and present Ag to T cells

As the CD8α$^+$ CD11b^{hi}CD11c^{int} DCs were able to carry Ag through different lymphoid organs we sought to determine whether the cells are able to present such an Ag to T cells. The findings show that when the subsets were incubated with CFSE-labeled naïve OVA-specific OT-II T cells and OVAp as Ag, both the CD8α$^+$ and CD8α$^-$ CD11b^{hi}CD11c^{int} DCs induced similar proliferation of the T cells as dilution of CFSE was much more prominent relative to unstimulated T cells (Fig. 5A). Furthermore, both subsets induced IFNγ and IL-5 production by the T cells to a similar extent indicating that these DCs are able to present Ag to naïve T cells and support their differentiation (Fig. 5B). In all, CD11b^{hi}CD11c^{int} DCs can take up and present Ag to T cells.

4. Discussion

This report describes a population of CD11b^{hi}CD11c^{int} DCs in the thymus of HR$^{+/+}$ mice that was not found in HR$^{-/-}$ mice. Although other organs had CD11b^{hi}CD11c^{int} DCs, these were not as distinctive as the thymic population and were similar in both strains. Furthermore, the thymic CD11b^{hi}CD11c^{int} DCs originate from the BM and express the HR. These observations, alongside the fact that CD11b^{hi}CD11c^{int} DCs do not arise in the thymus of HR$^{-/-}$ mice, suggest that the HR is required for their development and/or migration to the thymus especially that expression of the migratory marker SIRPα is up-regulated on these cells [17, 18]. Moreover, the CD11b^{hi}CD11c^{int} DCs are able to capture Ag from the peritoneum and navigate with it through peripheral organs such as the spleen and the lymph node like conventional DCs. Interestingly, the CD11b^{hi}CD11c^{int} DCs were more efficient than cDCs in reaching the thymus while carrying the Ag, a function that is associated mostly with the CD8α$^+$ population. This is perhaps related to the fact that the CD11b^{hi}CD11c^{int} CD8α$^+$ subset had much higher expression of CCR9, a chemokine receptor defined to facilitate hematopoietic progenitor trafficking to the thymus [19]. Since the subsets reside in the thymus and were able to present Ag to naïve CD4$^+$ T cells and induce their differentiation, it is possible that these DCs present Ag in the thymus and perhaps contribute to the process of T cell selection [20, 21]. This idea is more conceivable for the CD11b^{hi}CD11c^{int} CD8α$^+$ subset as circulating CD8α$^+$ cDC have been shown to be critical for thymic T cell development and negative selection of self-reactive T cells [9, 10, 22]. The other interesting finding uncovered in this report is that CD11b^{hi}CD11c^{int} DCs express very high levels of the co-stimulatory molecule CD24, also known as heat stable antigen (HSA) [23]. Since high levels of CD24 on CD8α$^+$ splenic DCs are required for optimal T cell stimulation [24], the thymic CD11b^{hi}CD11c^{int} DCs may utilize CD24/ligand interactions for increased sensitivity in detection and elimination of self-reactive T cells during thymic development.

Overall, a distinct subset of bone marrow derived CD11b^{hi}CD11c^{int} DCs develop in HR$^{+/+}$, but not HR$^{-/-}$, mice. These cells are able to take up Ag and traffic through different

lymphoid organs where they can present the Ag to T cells. As the DCs express high levels of the costimulatory molecule CD24 and reside in the thymus, one would envision the cells to play a role in T cell development and negative selection of self-reactive T cells.

5. Conclusions

This report defines a previously unrecognized population of thymic DCs that are $CD11b^{hi}CD11c^{int}$. These cells arise in $HR^{+/+}$, but not in $HR^{-/-}$, mice, come from BM progenitors, and express the HR. Phenotypic profiling of this new DC subset shows that they express thymus-homing markers and co-stimulatory molecules, especially CD24. As the cells are able to pick up Ag, present it to T cells, and home to the thymus, it may be that they play a role in T cell development and negative selection of self-reactive T cells.

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Abbreviations

BM	bone marrow
ETP	early thymic progenitor
HR	IL-13Rα1/IL-4Rα heteroreceptor
OVA	ovalbumin

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Highlights

- Unique CD11b^{hi} CD11c^{int} DC population arises in murine thymus
- CD11b^{hi} CD11c^{int} DCs rely on IL-13Rα1 for their development
- CD11b^{hi} CD11c^{int} DCs carry peripheral antigen to thymus
- CD11b^{hi} CD11c^{int} thymic DCs present antigen to T cells

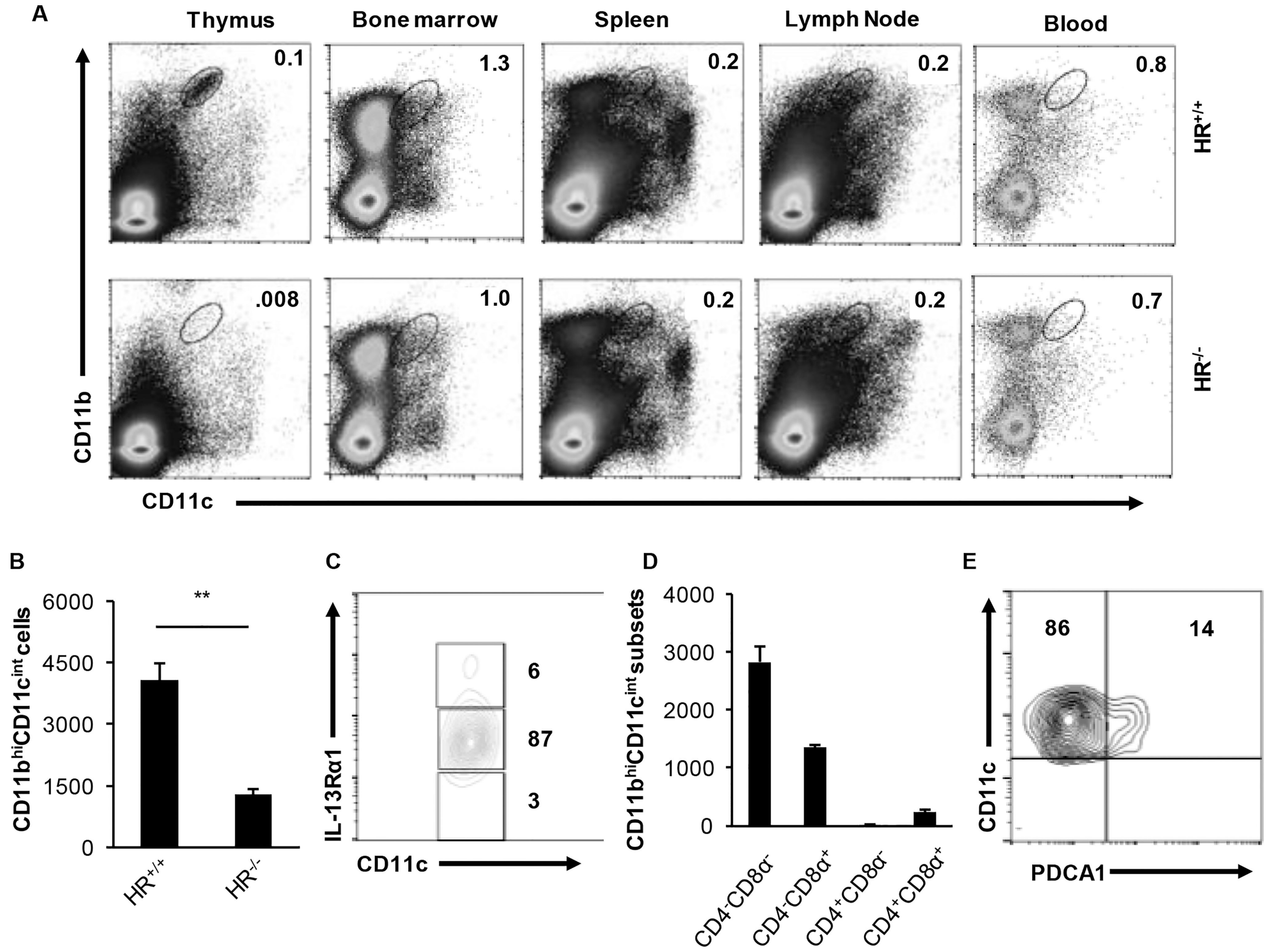

Figure 1. A subset of DCs arise in the thymus of $HR^{+/+}$ but not $HR^{-/-}$ mice

Cells isolated from various organs of $HR^{+/+}$ and $HR^{-/-}$ mice were stained with anti-CD11b and anti-CD11c antibodies and marker expression was analyzed by flow cytometry. (A) Shows dot plot analysis of CD11b and CD11c expression. The numbers indicate the percentages of $CD11b^{hi}CD11c^{int}$ cells within the elliptical gate. (B) Shows the numbers of $CD11b^{hi}CD11c^{int}$ cells. The bars represent the mean ± SD number of cells per 5×10^6 thymic cells. **$p<0.01$ as analyzed by two-tailed, unpaired Student's t-test. (C) shows IL-13Rα1 expression on $CD11b^{hi}CD11c^{int}$ thymic cells as detected by anti-IL-13Rα antibody. (D) Thymic cells from $HR^{+/+}$ mice were stained with antibodies against CD8α, and CD4 and marker expression was analyzed on $CD11b^{hi}CD11c^{int}$ cells. The bars represent the mean ± SD number of cells per 2×10^6 thymic cells. (E) Thymi were harvested from $HR^{+/+}$ mice and analyzed for PDCA1 expression within the $CD11b^{hi}CD11c^{int}$ myeloid DC subset.

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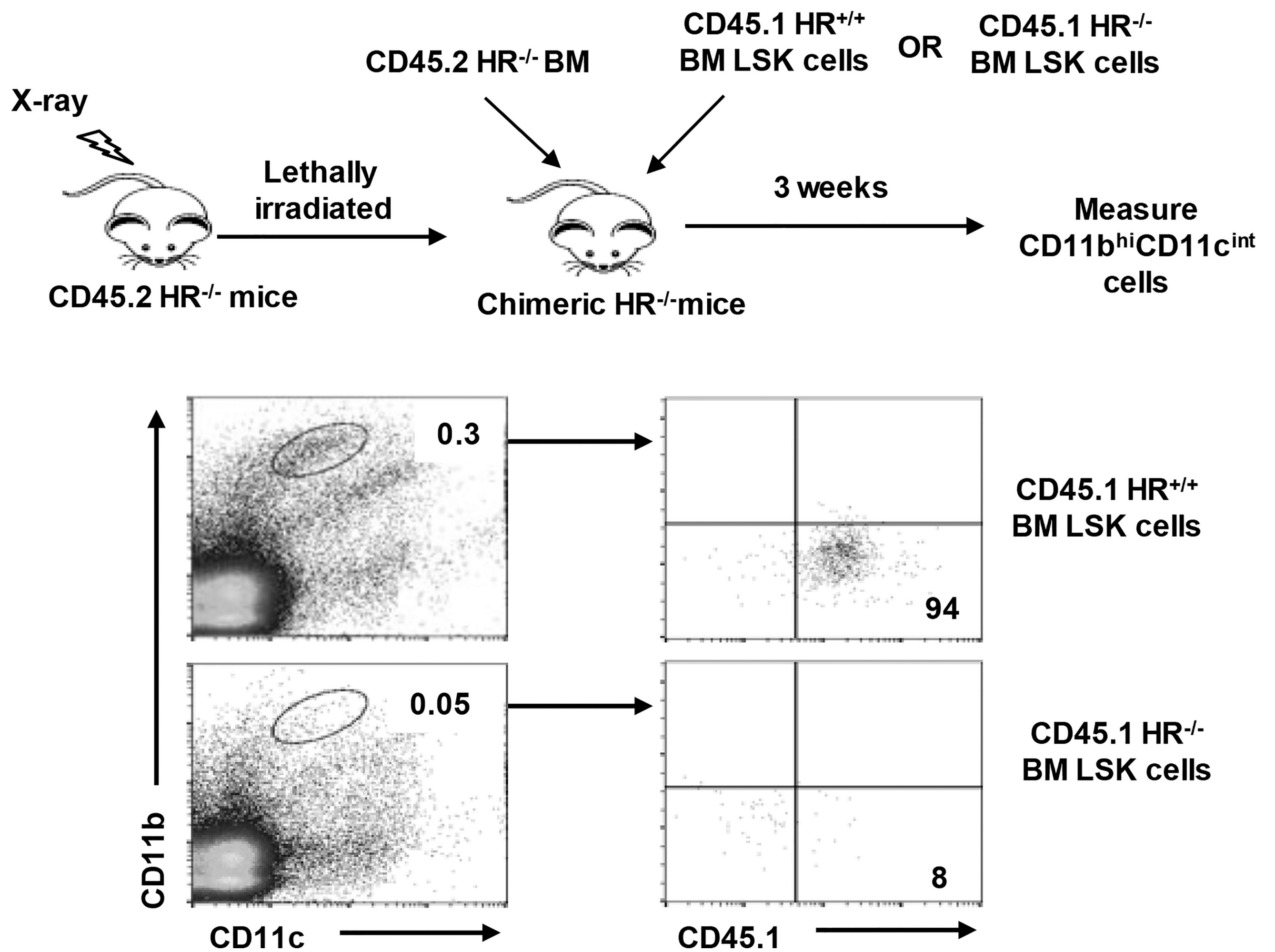

Figure 2. HR$^{+/+}$ but not HR$^{-/-}$ bone marrow stem cells give rise to thymic CD11b^{hi}CD11c^{int} DCs

CD45.2 HR$^{-/-}$ mice were lethally irradiated and then reconstituted with 10×10^6 BM cells from CD45.2 HR$^{-/-}$ mice combined with 1×10^6 lineage$^-$ (Lin$^-$), Sca1$^+$, c-kit$^+$ BM (LSK) cells from either HR$^{+/+}$ or HR$^{-/-}$ mice. Three weeks later, thymic cells were harvested and the CD11b^{hi}CD11c^{int} cells were analyzed for CD45.1 expression.

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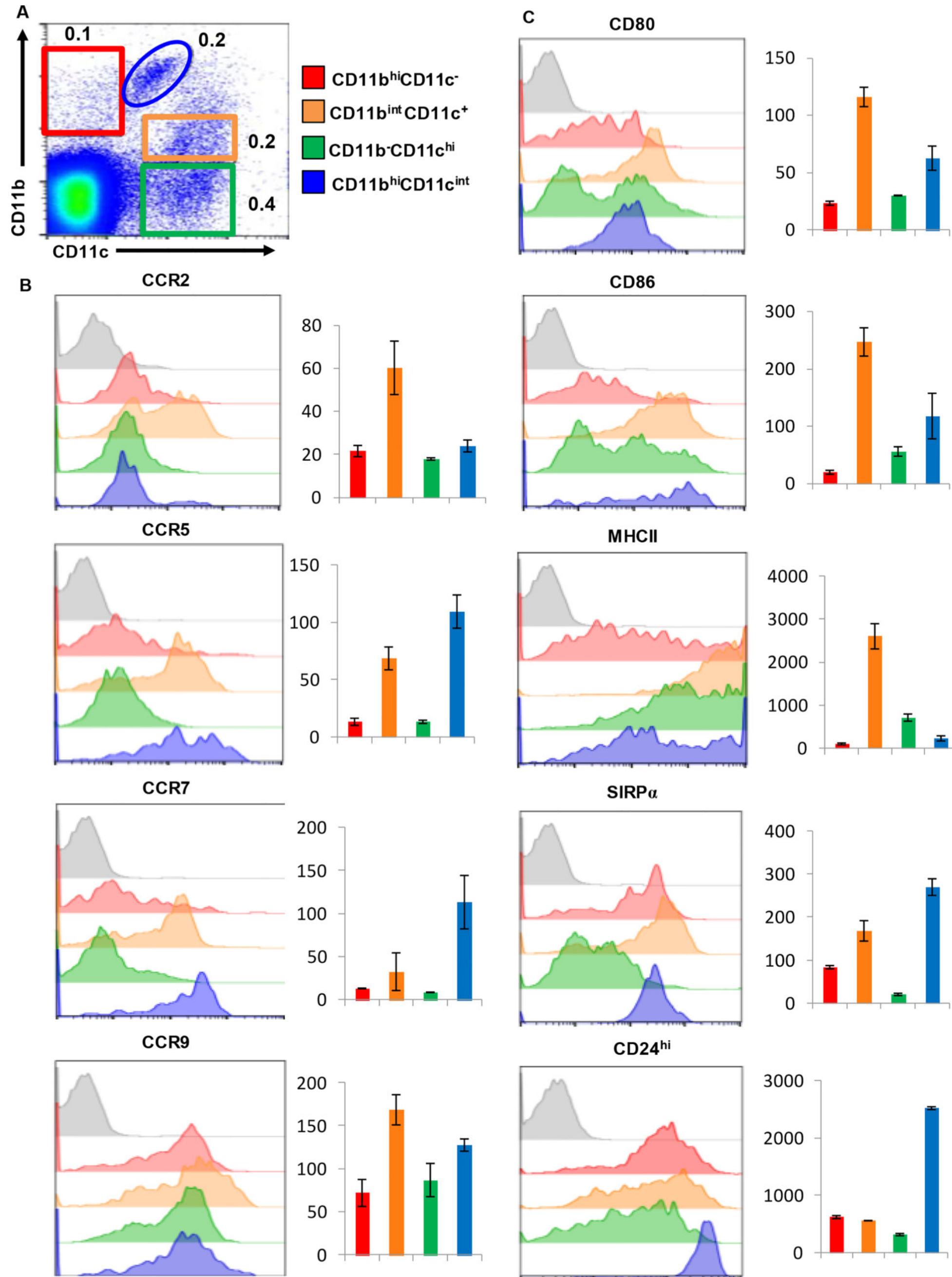

Figure 3. Thymic CD11b^{hi}CD11c^{int} DCs express markers characteristic of antigen presenting cells

Thymic cells from HR$^{+/+}$ mice were stained with antibodies to CD11b, CD11c and analyzed for expression of other markers associated with APCs. (A) Shows four distinct APC populations based on expression of CD11b and CD11c which include CD11b^{hi}CD11c^{-} (red), CD11b^{int}CD11c^{hi} (orange), CD11b^{-}CD11c^{hi} (green) and CD11b^{hi}CD11c^{int} (blue). (B, C) Shows expression of chemokine (B) and other (C) markers associated with APC function relative to isotype control (gray). The histograms illustrate representative experiments while the bar graphs represent the mean cell percentage ± SEM from three independent experiments.

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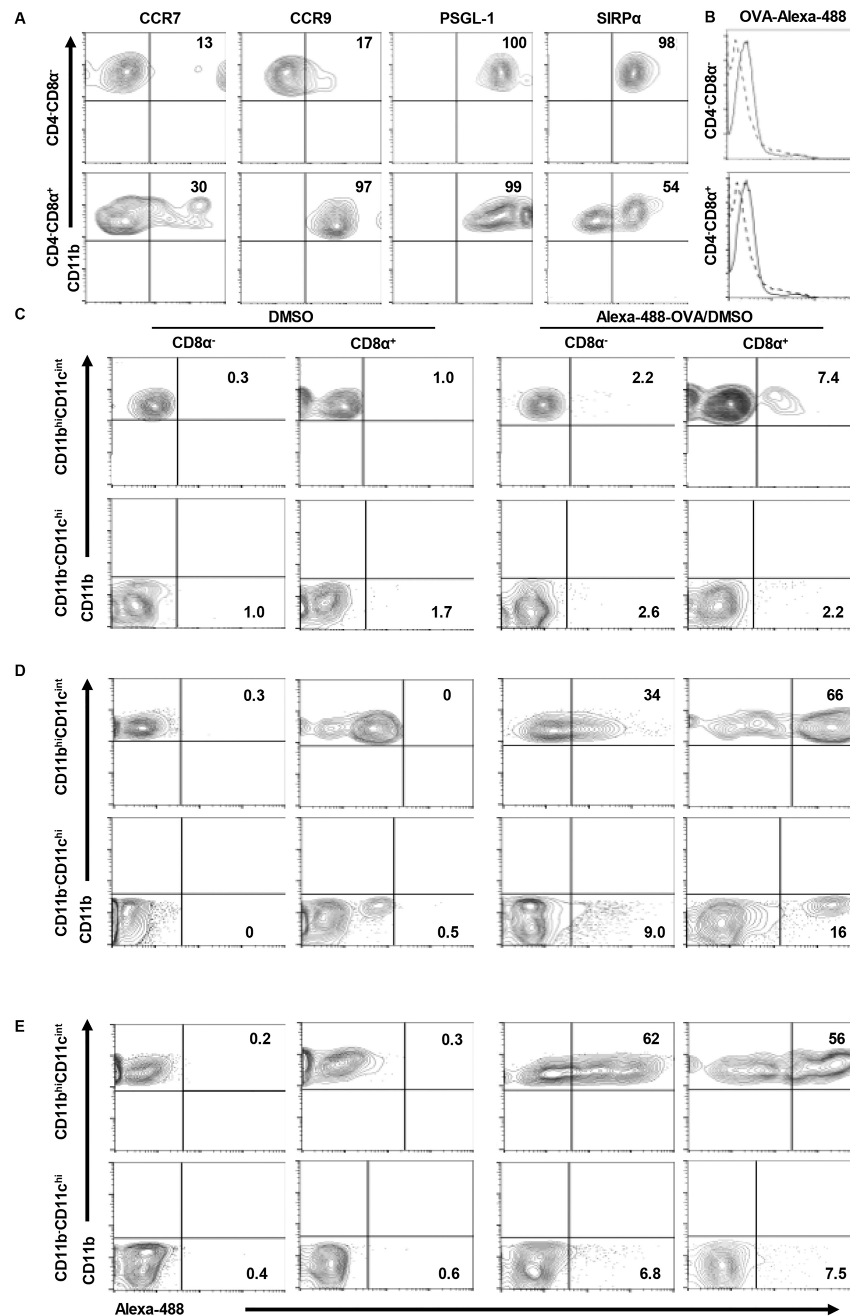

Figure 4. CD11b^{hi}CD11c^{int} cells comprise a population that express the CD8α^{+} DC marker and migrate to the thymus

(A) Thymic cells from HR$^{+/+}$ mice were stained with antibodies to CD11b, CD11c, CD8α, and CD4 along with antibodies to CCR7, CCR9, PSGL-1 or SIRPα. The cells were gated on CD11b^{hi}CD11c^{int} CD4^{-}CD8α^{+} or CD11b^{hi}CD11c^{int} CD4^{-}CD8α^{-} population and analyzed for surface expression of each of the markers. The numbers represent the percentages of cells expressing the indicated marker. (B) Sorted CD8α^{-} and CD8α^{+} CD11b^{hi}CD11c^{int} thymic cells were incubated with OVA-conjugated Alexa-488 (solid lines) or Nil (dashed lines: background auto fluorescence) and analyzed for OVA uptake by flow cytometry. (C–E) HR$^{+/+}$ mice were given i.p. DMSO diluent or 100 μg Alexa-488 conjugated OVA in

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DMSO. Seven days later $CD8\alpha^{+}$ and $CD8\alpha^{-}$ subsets of $CD11b^{hi}CD11c^{int}$ (new DC population) and $CD11b^{-}CD11c^{hi}$ (cDCs) cells from the thymus (C), the spleen (D) and lymph nodes (E) were analyzed for Alexa-488 fluorescence. The numbers represent the percentage of cells stained with Alexa-488 fluorescence.

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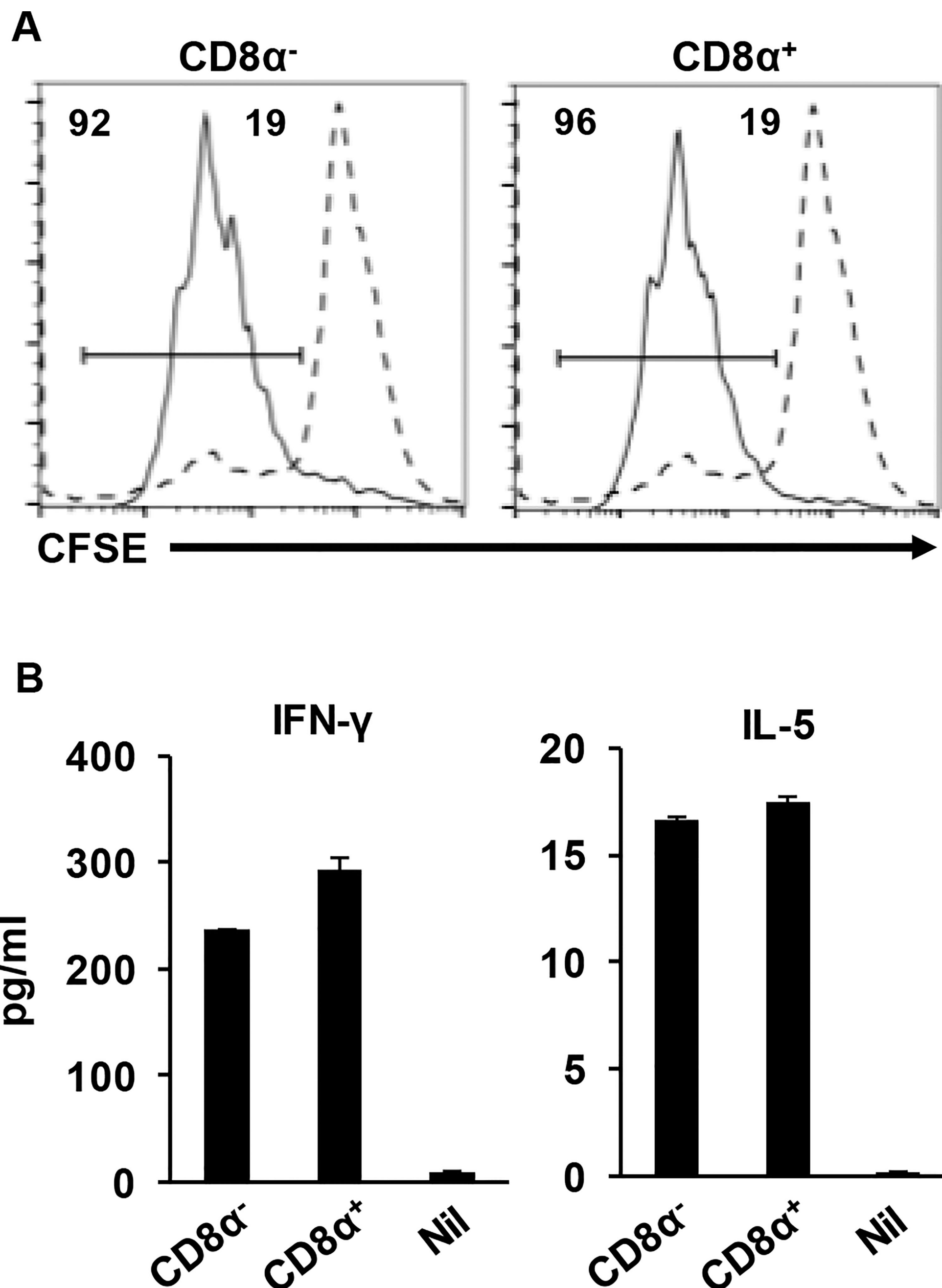

Figure 5. CD11b^{hi}CD11c^{int} thymic DCs present Ag to T cells

(A) OT-II TCR-OVA transgenic CD4^{+} T cells were labelled with CFSE and incubated with sorted thymic CD8α^{-} or CD8α^{+} CD11b^{hi}CD11c^{int} cells in the presence of OVAp or diluent. Proliferation was measured by CFSE dilution induced by OVAp stimulation (solid lines) relative to diluent (dashed lines). The numbers represent the percentage of proliferative cells. (B) OT-II TCR-OVA transgenic CD4^{+} T cells were incubated without (Nil) or with sorted thymic CD8α^{-} or CD8α^{+} CD11b^{hi}CD11c^{int} cells in the presence of OVAp and cytokine

production was measured by ELISA. The bars show IFNγ (left panel) and IL-5 (right panel) secretion. Each bar represents the mean ± SD pg/ml cytokine.

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