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Research paper

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 $CD8\alpha^+$ subset, it is likely that the cells contribute to T cell development and perhaps negative selection of self-reactive lymphocytes.

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 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-13Rα1$^{+/+}$-GFP and IL-13Rα1$^{-/-}$ 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

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

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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

2.3.1. 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-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].

2.3.2. 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.

2.3.3. 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

2.6.1. Ag uptake assay

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

2.6.2. 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.

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 (10 μM 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