Immunology Letters 191 (2017) 40–46

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

In vivo blockade of T cell development reveals alternative pathways for generation of intraepithelial lymphocytes in mice

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ARTICLE INFO

Keywords:
Thymus
Intestinal lymphocytes
Cell differentiation
Transcription factors
T cells

ABSTRACT

Intraepithelial lymphocytes (IELs) are resident cells localized within the intestinal epithelia and play an important role in regulating gut inflammations and host defense against pathogens. CD8α$^+$ TCRαβ$^+$ IELs are heterogeneous populations that are generated from T cell precursors including CD4$^-$ CD8α$^-$ double-negative (DN) cells and CD4$^+$ CD8α$^+$ double-positive (DP) cells. However, developmental pathways of TCRαβ$^+$ IELs remained unclear. To gain insight into the mechanisms, we generated mice (Bcl11b$^{\Delta DN2}$ mice) that lack thymic precursors (DN CD5$^+$ TCRβ$^+$ cells) for CD4$^-$ CD8αα$^+$ TCRαβ$^+$ IELs. Unexpectedly, we found that, in the absence of the precursors in thymi of Bcl11b$^{\Delta DN2}$ mice, CD4$^-$ CD8αα$^+$ TCRαβ$^+$ IELs were still present in the intestine though the number was reduced. Adoptive transfer experiment showed that their precursors were highly enriched in CD8α$^+$ TCRβ$^-$ thymocytes. The CD4$^-$ CD8αα$^+$ TCRαβ$^+$ IELs in Bcl11b$^{\Delta DN2}$ mice are distinguished by Thy1.2 expression and are indeed present in WT mice. Taken together, our study reveal a novel developmental pathway for CD8αα$^+$ TCRαβ$^+$ IELs.

1. Introduction

Intestine in mammals is one of the mucosal tissues that are continuously exposed to challenges by pathogenic organisms in ingested food and water. Intraepithelial lymphocytes are localized within the epithelium of the intestine, and play an important roles in maintaining homeostasis by fighting against pathogens and regulating excessive inflammations [1–3]. Indeed, these populations are highly conserved in vertebrates including fish and mammals [4]. Therefore, elucidation of full pictures of developmental pathways of IELs provides us novel insights into T cell biology involved in mucosal immunity.

Bcl11b is a transcription factor essential for T cell development [5–7]. T cell development is initiated after migration of haematopoietic stem cells to the thymus. Most immature T cell precursors are identified by the lack of CD4 and CD8α expressions, known as double-negative (DN) cells. DN cells are further classified into 5 populations, DN1, DN2a, DN2b, DN3 and DN4 cells, as they mature. T cell identity is fixed at the DN2b stage at which Bcl11b has an indispensable role. Indeed, in Bcl11b-deficient mice, T cell development is completely blocked at the DN2a stage. DN4 cells become DP cells at which TCRα rearrangement occurs and then give rise to conventional αβ T cells and unconventional αβ T cells including invariant natural killer T (iNKT) cells and CD4$^-$ CD8αα$^+$ TCRαβ$^+$ IELs [8–11]. During the intrathymic development of CD4$^-$ CD8αα$^+$ TCRαβ$^+$ IELs, DN CD5$^+$ TCRβ$^+$ cells were identified as their precursors in the thymus after the DP stage [9]. In contrast to αβ T cell development through the DP stage, iNKT cell subsets bypassing DP stage have been identified [12]. Thus, Bcl11b-deficient mice could be a novel tool for analysis of T cells derived from DN stages.

To gain insights into developmental pathways of TCRαβ$^+$ IELs, we generated mice in which T cell development was blocked before the DP stage by inducing T cell-specific disruption of Bcl11b (designated as Bcl11b$^{\Delta DN2}$ mice hereafter), because Bcl11b-deficient mice were shown to be fatal around perinatal period [13,14]. Here we found that DN CD5$^+$ TCRβ$^+$ thymocytes were absent in Bcl11b$^{\Delta DN2}$ mice. Unexpectedly, however, CD4$^-$ CD8αα$^+$ TCRαβ$^+$ IELs were still present in the small intestine of Bcl11b$^{\Delta DN2}$ mice. Thy1.2 expression was higher in CD4$^-$ CD8αα$^+$ TCRαβ$^+$ IELs from Bcl11b$^{\Delta DN2}$ mice than those from WT mice. Adoptive transfer experiment suggested that precursors for Thy1.2$^+$ CD4$^-$ CD8αα$^+$ TCRαβ$^+$ IELs were enriched in CD8α$^+$ TCRβ$^-$ thymocytes. Thus, we identify a novel developmental pathway for CD8αα$^+$ TCRαβ$^+$ IEL subset.

Abbreviations: IELs, intraepithelial lymphocytes; DN, double negative; DP, double-positive; MHC, major histocompatibility complex

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http://dx.doi.org/10.1016/j.imlet.2017.09.008
Received 8 July 2017; Received in revised form 21 September 2017; Accepted 23 September 2017
Available online 27 September 2017

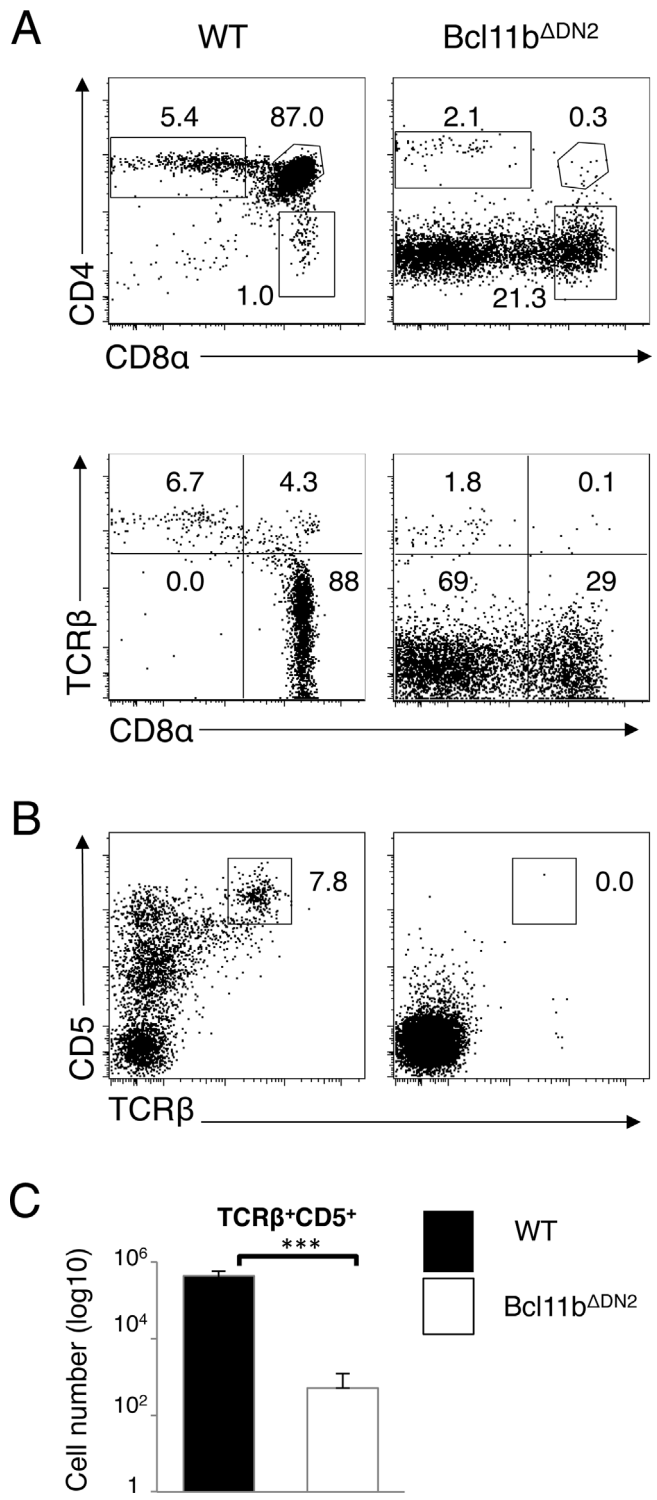

Fig. 1. Precursors for CD4$^-$ CD8αα$^+$ TCRαβ$^+$ IELs are completely absent in Bcl11b$^{\Delta DN2}$ mice.
Thymocytes in WT and Bcl11b$^{\Delta DN2}$ mice were analyzed after gating on total lymphocytes (A) or DN cells (B). (C) Bar graph shows absolute numbers of TCRβ$^+$ IEL precursors (TCRβ$^+$CD5$^+$) in thymus from WT (n = 8) and Bcl11b$^{\Delta DN2}$ (n = 7) mice. Asterisks indicate statistical significance as determined by Student's *t*-test (***, $p < 0.001$). Data are representative of more than five independent experiments.

2. Materials and methods

2.1. Mice

Bcl11b$^{flox/flox}$ mice were provided by R. Kominami (Niigata University, Niigata, Japan). *Rag1-Cre* mice were provided by T.Rabbits (Leeds Institute of Molecular Medicine, Leeds, United Kingdom). *CD3ε$^{\Delta 5/\Delta 5}$* mice were kindly provided by Bernard Malissen (Centre d'Immunologie de Marseille- Luminy, France). Bcl11b$^{\Delta MatT}$ mice were generated by crossing *Bcl11b$^{flox/flox}$* mice with *MaT-Cre* mice which were kindly provided by S Yamasaki (Kyushu University, Fukuoka, Japan) [15]. 8–12 week-old mice were used throughout the experiments. Experiments were carried out in accordance with Guidelines for Animal Experiments. This study was approved by Committee of Ethics on Animal Experiments in the Faculty of Medicine, Kyushu University.

2.2. Cell preparation from various tissues

Small intestine tissues were dissected and Peyer's Patches were carefully removed. After flushing of fecal contents with ice-cold HBSS, small intestines were opened longitudinally, gently rinsed several times of the same ice cold buffer and cut laterally into small pieces, and placed in 50 ml plastic tubes, incubated in 199 media buffer for 30 min at 37 °C with constant shaking in water bath. After centrifugation at 440*g* for 5 min, pellets were resuspended in RPMI 1640 containing 10% FCS. Dissociated cells were filtered through the gauze mesh and cells were further purified using 40/70% Percoll gradient centrifugation for 20 min at 2200 rpm. Single cell suspensions of thymocytes were prepared by grinding the organs by ends of two glass slides.

2.3. Antibodies and flow cytometric analysis

The following antibodies were purchased from Biolegend (San Diego, CA): anti-TCRβ (H57-597), anti-CD8α (53–6.7), anti-CD8β (YTS156.7.7), anti-CD5 (53–7.3), Anti-CD4 (RM 4–5), anti-CD103 (2E7), anti-B220 (RA3-6B2) and anti-Thy1.2 (53–2.1). Anti-I-A/I-E (M5/114.15.2) mAb was obtained from eBioscience (San Diego, CA). FITC-conjugated anti-Bcl11b (Clone 25B6) mAb was obtained from Abcam. Dead cells were excluded adding propidium iodide. Intracellular staining was performed according to the manufacturer's instructions (BD Biosciences). Stained cells were acquired on FACS Verse flow cytometer (BD Biosciences). The data were analyzed using FlowJo software version 9.9.4 (Tree Star).

2.4. Adoptive transfer experiment

After fluorescence-activated cell sorting by BD FACS Aria (BD Biosciences), cells (1×10^5 cells/mouse) were transferred into gender-matched *CD3e$^{\Delta 5/\Delta 5}$* mice. At 7–8 weeks after transfer, mice were sacrificed for the analysis.

2.5. Statistical analysis

Statistical analysis was calculated by the Student *t*-test using Prism software (GraphPad Software, San Diego, CA). Differences with values of $p < 0.05$ were considered to be statistically significant.