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B-1 B cell progenitors transiently and partially express keratin 5 during differentiation in bone marrow

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ABSTRACT

Background: Keratin 5 (K5) is a cytoskeletal tissue-specific protein expressed in the epithelial cells of skin and esophagus and ectopic K5 expression in lymphocytes has never been reported.
Objective: Here we demonstrate an ectopic epidermal self-protein expression in B-1 B cell by fate mapping of K5-expressing cells.
Methods: K5-Cre × CAG-CAT-loxP-EGFP double Tg (K5 × GFP) mice that express enhanced GFP under the control of the K5 promoter were employed.
Results: Unexpectedly, $B220^{+}GFP^{+}$ cells were found in LN, spleen, peripheral blood and peritoneal cavity. These cells were $IgM^{+}IgD^{low}CD23^{-}CD43^{+}CD19^{+}CD93^{-}$, indicating that they were B-1 B cells. The number of $B220^{+}GFP^{+}$ cells was significantly larger in spleen than in the other tissues tested. Although GFP^{+} B-1 cells did not express K5 in the periphery, $Lin^{-}CD93^{+}B220^{low-neg}CD19^{+}$ B-1 B cell progenitors expressed GFP and $B220^{+}CD93^{+}$ progenitor cells expressed K5 and MHC-class II in BM, indicating that GFP^{+} B-1 cells transiently expressed K5 and the progenitor cells were potential APC. GFP^{+} B-1 cells in the periphery continued expressing MHC class II and had exogenous antigen-presenting capacity comparable to non-follicular B cells. GFP^{+} B-1 cells spontaneously secreted more IgM than GFP^{-} B-1 cells in vitro.
Conclusion: These results indicate that B-1 B cells transiently and partially express K5 in BM and are potent for both natural antibody production and antigen presentation.

1. Introduction

Keratin 5 (K5) is a cytoskeletal protein in the epithelial cells of skin and esophagus, and is a predominant keratin of the basal and suprabasal cell layers of the epidermis by forming heterodimers with keratin 14. Although K5 is a good marker of epithelial cells, such as keratinocytes, it disappears during differentiation, indicating that K5 expression is transient during the lifespan of epithelial cells [1]. Tg neo-self-antigen driven by the K5 promoter, such as membrane-bound OVA (mOVA) in K5-mOVA Tg mice, has been used for studying the presentation of epidermal self-antigen or the transport of epidermal self-protein by migratory dendritic cells (mDC) [2–4]. K5 is also expressed in lymphoid organs such as thymus, and K5-driven antigen plays a critical role in the depletion

Abbreviations: Aire, autoimmune regulator; CLP, common lymphoid precursor; dTg, double transgenic; EGFP, enhanced green fluorescent protein; K5, keratin 5; LPS, lipopolysaccharide; mDC, migratory dendritic cell; MLR, mixed lymphocyte reaction; mOVA, membrane-bound ovalbumin; mTEC, medullary thymic epithelial cell; skin LN, skin-draining lymph node; SSC, side scatter; TEC, thymic epithelial cell.

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of autoreactive T cells during T-cell maturation [5]. Because K5 is expressed in medullary thymic epithelial cells (mTEC) but not in thymocytes, ectopic K5 expression in lymphocytes has never been reported.

Mouse B-1 B cells are a relatively small population of B cells that spontaneously secrete IgM [6] and constitute most of the B cells present in mouse peritoneal and pleural cavities. B-1 B cells consist of two subsets: $CD5^+$ B-1a B cells secrete natural antibodies recognizing high molecular-weight polymeric antibodies, and $CD5^-$ B-1b B cells secrete antibodies induced after antigenic exposure [7].

Fate mapping analysis using either the Cre-loxP-enhanced GFP (EGFP) or yellow fluorescence protein system has been used for detecting target molecule-expressing cells [8,9]. In this study, K5 expression was re-clarified by fate mapping analysis in which K5-Cre Tg mice expressing Cre recombinase under the K5 promoter were crossed with a GFP reporter strain of CAG-CAT-loxP-EGFP Tg mice. K5 fate mapping analysis revealed that B-1 B cell progenitors transiently express K5 in BM and that B-1 B cells in the periphery are potent, natural antibody producers and APC.

2. Materials and methods

2.1. Ethics statement

All mice were maintained under specific pathogen-free conditions and had free access to chow and water. The mice were sacrificed under deep anesthesia for experiments. All animal care and experimentation were performed in accordance with the guidelines of the Institute of Experimental Animal Sciences Osaka University Medical School for minimizing distress in experimental animals (Approval No. 19-063-0).

2.2. Mice

All mice had a C57BL/6 background. K5 × GFP dTg mice were generated by crossing K5-Cre Tg mice with CAG-CAT-loxP-EGFP Tg mice. K5-Cre Tg mice expressing Cre recombinase under the control of the K5 promoter and CAG-CAT-loxP-EGFP reporter Tg mice expressing EGFP upon Cre-mediated recombination were generated, respectively, as described previously [10,11]. K5-mOVA Tg mice expressed mOVA under the control of the human K5 promoter [2]. OT-I/PL Tg mice were MHC class I ($H\text{-}2K^b$)—restricted OVA-specific TCR Tg mice expressing the monoclonal Vα2 and Vβ5TCR chains on CD90.1 Thy1 congenic mice [12]. OT-II RAG-$2^{-/-}$ Tg mice were MHC class II ($I\text{-}A^b$)-restricted OVA-specific TCR Tg mice (OT-II) on RAG$2^{-/-}$ mice [13]. OT-I/PL and OT-II RAG-$2^{-/-}$ Tg mice were kind gifts from Dr. Masaaki Murakami (Developmental Immunology, Osaka University Immunology Frontier Research Center). Unless otherwise indicated, one mouse was used for each experiment and all results are representative of more than three independent experiments.

2.3. Cell preparation

Cell suspensions of LN or spleen were prepared as follows. Mixtures of cervical, axillary, brachial, and inguinal LN were prepared as skin LN. For splenic cells, 500 μl collagenase D solution (2 mg/ml, Roche, Basel, Switzerland) was injected, and tissues of skin LN, MLN, and spleens were cut into smaller pieces. Each sample was incubated in collagenase D solution for 45 min at 37 °C. Collagenase D-released cells and remaining tissue fragments were passed through a 70-μm cell strainer (BD Biosciences, Franklin Lakes, NJ, USA) and washed, and the cell pellet was resuspended in an appropriate amount of buffer. Peritoneal cells were collected from the abdominal cavities of mice by repeated lavage with PBS. For CD43 staining, collagenase-D preparation was not used.

2.4. Cell sorting

Pooled cell suspensions were prepared from skin LN, MLN, and spleens as described above. For MLR, IgD^{low} cells were then further purified by negative selection using rat-IgG purified anti-mouse IgD (eBioscience, San Diego, CA, USA) as the primary antibody and magnetic Dynal beads conjugated to sheep anti-rat IgG (Invitrogen, Carlsbad, CA, USA) as the second antibody. Then, $B220^+IgD^{low}$ cells were purified from IgD^{low} cells by positive selection using magnetic beads coated with anti-CD45R (B220) antibodies (Miltenyi Biotec, Bergisch Gladbach, Germany). From enriched $IgD^{low}B220^+$ cells, $B220^+GFP^+$ cells and non-follicular B cells, were sorted as GFP^+mPDCA-1^- and GFP^- mPDCA-1^-, respectively, using FACS Aria II (Becton Dickinson, Franklin Lakes, NJ, USA).

For ELISPOT assay, $B220^+$ cells were purified by positive selection using magnetic beads coated with anti-CD45R (B220) antibodies. From these $B220^+$ cells, GFP^+ B-1 B cells, follicular B cells, and GFP^- B-1 B cells were sorted as GFP^+B220^+, $GFP^-CD23^+CD43^-B220^+$, and $GFP^-CD23^-CD43^+B220^+$ cells, respectively, using FACS Aria II.

2.5. MLR

OVA-specific $CD8^+$ cells were prepared from skin LN and MLN of OT-I mice and were purified by negative selection using magnetic beads (Thermo Fisher Scientific). OVA-specific $CD4^+$ cells were also purified from skin LN, MLN, and spleens of OT-II/RAG-$2^{-/-}$ Tg mice by negative selection using magnetic beads (Thermo Fisher Scientific). CFSE-labeled responder cells (5×10^4 OT-I cells or OT-II cells) were co-cultured for 72 h with 1×10^4 sorted stimulator cells. $B220^+GFP^+$ cells, non-follicular B cells, or mDCs were used as stimulator cells. mDCs were sorted as $CD11c^{inter}$ and $CD40^{high}$ from skin LN after enrichment with CD11c Microbeads (Miltenyi Biotec). Responder cells were incubated with OVA protein (1000 μg/ml, Sigma), or MHC class I- or II- restricted OVA peptides (1 μM SIINFEKL or 10 μM, OVA327-339, Sigma-Aldrich St. Louis, MO, USA). T cell proliferation was assessed by CFSE dilution with FACS Canto II (Becton Dickinson).

2.6. FACS analysis

Cells prepared from skin LN, MLN, spleens, BM, and peripheral blood were stained with the following antibodies and reagents: PE-conjugated B220 (BD Biosciences), CD5 (BD biosciences), CD19 (BD Biosciences), $I\text{-}A^b$ (BD Biosciences), IgD (Biolegend), mPDCA-1 (eBioscience), CD45 (BD Biosciences), cytokeratin 5, (Santa Cruz Biotechnology, Santa Cruz, CA, USA), CD40 (BD Biosciences), and CD43 (BD Biosciences); Allophycocyanin-conjugated Lineage Antibody Cocktail (BD Biosciences), B220 (BD Biosciences), and CD21 (BioLegend); PE-Cy7-conjugated CD93 (eBioscience) CD23 (Biolegend); biotin-conjugated IgM (eBioscience); Per-CP-conjugated streptavidin (BD biosciences), CD90.1 (BD biosciences); and CD4 (BD biosciences). Dead cells were excluded by staining with 7-AAD (BD Biosciences). All antibodies and reagents were used with isotype-matched negative controls. Cells were analyzed with a FACS Canto II (Becton Dickinson).

2.7. In vivo LPS injection

Mice were inoculated intraperitoneally (i.p.) with 10 μg LPS (*Escherichia coli* serotype O26:B6, 90–99% pure; Sigma–Aldrich, St. Louis, MO, USA) in 200 μl (0.05 mg/ml) PBS solution or with PBS alone, and were killed 3 days later for flow cytometric analysis.

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