Immunology Letters 181 (2017) 51–57

Contents lists available at ScienceDirect

Immunology Letters

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

Lipid mediators foster the differentiation of T follicular helper cells

Tomonori Nagaya[a,b,1], Koji Kawata[a,1], Ryuta Kamekura[a,b], Sumito Jitsukawa[a,b], Terufumi Kubo[c], Motonari Kamei[a], Noriko Ogasawara[b], Ken-ichi Takano[b], Tetsuo Himi[b], Shingo Ichimiya[a,*]

[a] Department of Human Immunology, Research Institute for Frontier Medicine, Sapporo Medical University School of Medicine, Sapporo 060-8556, Japan
[b] Department of Otolaryngology, Sapporo Medical University School of Medicine, Sapporo 060-8556, Japan
[c] Department of Pathology, Sapporo Medical University School of Medicine, Sapporo 060-8556, Japan

ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form 23 October 2016
Accepted 8 November 2016
Available online 10 November 2016

Keywords:
Lipid mediators
T follicular helper cells

ABSTRACT

Lipid mediators such as leukotrienes and lipoxines broadly regulate innate and acquired immunity, and their dysfunction causes various immune-mediated disorders. We previously reported a salient feature of arachidonate 5-lipoxygenase (Alox5), which is responsible for the production of such lipid mediators, in the regulation of high affinity antibodies in vivo. The aim of this study was to determine the functional significance of Alox5-related lipid mediators during the processes of acquired humoral responses. The results of in vitro experiments using lymphocytes in tonsils and blood specimens showed that lipoxin A4 (LXA4) and leukotriene B4 (LTB4) have the capacity to differentiate naïve CD4$^+$ T cells into T follicular helper (Tfh) cells, which activate naïve B cells to form germinal centers. Such a function of LXA4 was further supported by results of in vitro studies using BML-111 and BOC-2, which are an agonist and an antagonist, respectively, of FPR2 of an LXA4-specific cell-surface receptor. The results suggest that such lipid mediators have a potential role in the development of lymphoid follicles through the regulation of Tfh cell differentiation.

© 2016 European Federation of Immunological Societies. Published by Elsevier B.V. All rights reserved.

1. Introduction

Lymphoid organs such as lymph nodes, tonsils, and the spleen control immune responses to efficiently inactivate and eliminate pathogens [1]. In addition to a unique capacity to concentrate foreign antigens and antigen-presenting cells (APCs), such lymphoid organs characteristically contain a representative site of lymphoid follicles surrounded by interfollicular regions and T cell zones harboring variable numbers of B cells and CD4$^+$ T cells. T follicular helper (Tfh) cells actively move around lymphoid follicles and encourage cognate interaction with B cells, which are eventually activated to form germinal centers to produce high-affinity antibodies and memory B cells [2–4]. This elaborated process is linked to the initiation of antigen-specific immune responses and generation of long-lived protective immunity. The structures of lymphoid organs are often functionally altered by infection, aging and various immune-related disorders [5]. Thus, an understanding of lymphoid follicles providing cardinal foci of immune cells is important to recognize physiological and pathological immune conditions.

Our previous study focusing on lymphoid tissues showed that arachidonate 5-lipoxygenase (Alox5) expressed in mantle zone B cells around germinal centers has a unique role in the establishment of antigen-specific antibody responses [6]. Alox5 is an enzyme responsible for the production of lipid mediators, including leukotrienes and lipoxins, and is present in large amounts in resting B cells and APCs such as dendritic cells and macrophages [6,7]. Experimental evidence obtained by using Alox5-deficient mice suggests that Alox5-related lipid mediators support T follicular helper (Tfh) cells in the spleen [6]. Furthermore, the number of follicular B cells in Alox5-deficient mice is significantly decreased compared to that in wild-type mice, indicating that Alox5-related lipid mediators assist T-cell-mediated B-cell responses [6]. Leukotrienes and lipoxins of Alox5-related lipid mediators widely operate innate and acquired immune responses [7,8]. However, little is known about the functional significance of Alox5-related lipid mediators in immune cells of lymphoid tissues.

In this study, we examined the role of leukotrienes and lipoxins associated with Alox5 in the regulation of Tfh cell

Abbreviations: Tfh cells, T follicular helper cells; LXA4, lipoxin A4; LTB4, leukotriene B4; FPR2, formyl peptide receptor 2.

* Corresponding author at: Department of Human Immunology, Research Institute for Frontier Medicine, Sapporo Medical University School of Medicine, South-1, West-17, Chuo-ku, Sapporo 060-8556, Japan.
E-mail address: ichimiya@sapmed.ac.jp (S. Ichimiya).

[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.imlet.2016.11.006
0165-2478/© 2016 European Federation of Immunological Societies. Published by Elsevier B.V. All rights reserved.

function. When receptors specific to lipid mediators in $CD4^+$ T cells were investigated, it was found that naïve $CD4^+$ T cells ($CD3^+CD4^+CD45RA^+CD45RO^-CCR7^+$) preferentially expressed FPR2 (also named ALXR) of a lipoxin A4 (LXA4) receptor at the mRNA level and, to a lesser extent, BLT1 and BLT2 of a leukotriene B4 (LTB4) receptor [9,10]. Experiments using in vitro differentiation models of Tfh cells further revealed that LXA4 as well as LTB4 intensified the differentiation of naïve $CD4^+$ T cells to Tfh cells ($CD3^+CD4^+PD\text{-}1^+CXCR5^+$) but not to Th1 and Th2 cells. The results indicate that Alox5-related lipid mediators affect the development of Tfh cells. Further investigation focusing on lipid mediators would lead to a better understanding of the regulation of Tfh cell differentiation and the pathogenesis of autoimmunity and allergies associated with specific antibody production.

2. Materials and methods

2.1. Tissues and blood samples

Surgically resected specimens of tonsils were obtained from patients admitted to Sapporo Medical University Hospital. Some of the tissues were stored in OCT compound (Sakura, Tokyo, Japan) at −80 °C for frozen tissue sections. Heparinized blood was obtained from healthy volunteers. All tissues were obtained after receiving informed consent and with the approval of the institutional review boards of Sapporo Medical University.

2.2. Reagents

Anti-human mAbs including APC-anti-CD3 (UCHT1), PE-anti-CD3 (UCHT1), APC-Cy7-anti-CD4 (RPA-T4), FITC-anti-CD4 (RPA-T4), PE-anti-PD-1 (EH12.1), PE-anti-Bcl6 (K112-91), PE-Cy7-anti-PD-1 (EH12.1), PE-Cy7-anti-CCR7 (3D12), PerCP-Cy5.5-anti-CXCR5 (RF8B2), PerCP-Cy5.5-anti-CD24 (ML5), FITC-anti-CD27 (M-T271), FITC-anti-CD45RA (HI100), APC-anti-CD45RO (UCHL1), APC-Cy7-anti-CD19 (SJ25C1), BV421-anti-IFN-γ (4S.B3), BV421-anti-IL-4 (8D4-8), BV421-anti-IL-17A (N49-653) (BD Biosciences), BV421-anti-CXCR5 (J252D4; Biolegend) and APC-anti-FPR2 (304455; R&D SYSTEMS) were used for flow cytometry. Lipid mediators (LTB4, LTC4, LTD4, LTE4 and LXA4), FPR2/ALX, an agonist of BML-111 (5[S]-6[R]-7-trihydroxyheptanoic- acid-methyl-ester) (Cayman Chemicals), and FPR2/ALX, an antagonist of BOC-2 (Bachem), were used for in vitro culture experiments.

2.3. Flow cytometry and cell sorting

Human tonsil tissues were mechanically disrupted, and lymphocytes in single cell suspensions were prepared by density gradient centrifugation with Lympholyte (CEDARLANE). Heparinized PBMCs from fresh blood specimens were also isolated with Lympholyte. After standard staining with specific surface markers, cells were analyzed using FACSCanto II or FACSAria II for careful cell sorting (BD Biosciences). In each experiment, specimens were analyzed for singlet events with doublet discrimination, and the purity of FACS-sorted cells reached 95% after validation by reanalysis. To analyze the expression of a Bcl6 transcription factor, IFN-γ, IL-4, IL-17 and FPR2, intracellular staining was performed according to the protocol of the Transcription Factor Buffer Set (BD Biosciences), and then cells were counted by FACSCanto II. Data were examined by using FACSDiVA software (BD Biosciences).

2.4. Cell culture

For naïve $CD4^+$ T cell culture, FACS-sorted human naive $CD4^+$ T cells (1×10^5 cells) from human peripheral blood were stimulated under a Tfh polarization condition supplemented with anti-CD3 mAb (OKT3; 10 μg/ml), anti-CD28 mAb (15E8; 10 μg/ml), IL-6 (25 ng/ml), IL-12 (1 ng/ml), IL-21 (25 ng/ml), TGF-β (5 ng/ml), anti-IL-4 mAb (10 μg/ml), and anti-IFN-γ mAb (10 μg/ml) as described previously [12] in the presence of lipid mediators as described above. After 5 days, polarized Tfh cells were detected by FACS analyses for PD-1 and CXCR5. All of the cytokines used in this study were obtained from Peprotech, and anti-IL-4 and anti-IFN-γ antibodies were obtained from Milteny Biotech. Serum-free AIM-V medium (Invitrogen) containing 50 μg/ml streptomycin and 100 U/ml penicillin was used in all experiments, and all experiments were performed at 37 °C in a humidified atmosphere with 5% carbon dioxide.

2.5. Gene analysis

For quantitative PCR analysis, total RNA extracted by TRIZOL reagent was reverse-transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative PCR was performed with a Step One Real-Time PCR System of Assay-on-Demand probes according to the instructions of the manufacturer (Applied Biosystems). The levels of expression of target genes were calculated using ΔΔCT and comparative methods after normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression.

2.6. Immunohistochemistry

Immunohistochemistry was conducted as described previously [6]. In brief, tissue sections of tonsils were stained with primary Abs in a moisture box at 4 °C overnight and reacted with secondary goat pAbs conjugated to Alexa Fluor dyes (Invitrogen). After staining with DAPI, tissues slides were analyzed using an immunofluorescence microscope (IX71; Olympus) or a confocal laser scanning microscope with image examiner software (LMS510META; Carl Zeiss).

2.7. Cell viability assay

Tfh cell viability was assessed by staining with annexin V-FITC (ImmunoChemistry Technologies) and 7-AAD (BD Biosciences) according to the manufacturer's protocol.

2.8. Statistical analysis

Results are expressed as means and SD. The unpaired Student's *t*-test was used to compare experimental groups with $P < 0.05$ considered significant.

3. Results

3.1. Alox5-releted lipid mediators regulate the differentiation of Tfh cells

We initially focused on analysis of various types of Alox5-releted lipid mediators responsible for Tfh cell differentiation from naïve $CD4^+$ T cells. When the mRNA expression profiles of receptors specific to lipid mediators on $CD4^+$ T cells of tonsils were investigated, it was found that naïve $CD4^+$ T cells ($CD3^+CD4^+CD45RA^+CD45RO^-CCR7^+$) highly expressed FPR2, which is a receptor for LXA4, in comparison to the levels of expression on Tfh cells ($CD3^+CD4^+PD\text{-}1^+CXCR5^+$) and non-Tfh cells ($CD3^+CD4^+PD\text{-}1^-CXCR5^-$) (Fig. 1A). To a lesser extent, naïve $CD4^+$ T cells expressed BLT1 and BLT2, receptors for LTB4, which were also expressed by Tfh cells as well as non-Tfh cells at levels similar to those in naïve $CD4^+$ T cells. CysLTR1 and CysLTR2, which are receptors for cysteinyl leukotrienes including LTC4, LTD4, and

Download English Version:

https://daneshyari.com/en/article/5666611

Download Persian Version:

https://daneshyari.com/article/5666611

Daneshyari.com