EBioMedicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Batf3-dependent CD8α$^+$ Dendritic Cells Aggravates Atherosclerosis via Th1 Cell Induction and Enhanced CCL5 Expression in Plaque Macrophages

Yalin Li [a], Xueyan Liu [a], Wei Duan [a], Hua Tian [b], Guangming Zhu [a], Hao He [a], Shutong Yao [b], Shuying Yi [a], Wengang Song [a,*], Hua Tang [a,*,1]

[a] Institute of Immunology, Taishan Medical University, Taian, Shandong, China
[b] Key Laboratory of Atherosclerosis in Universities of Shandong and Institute of Atherosclerosis, Taishan Medical University, Shandong, China

ARTICLE INFO

Article history:
Received 1 October 2016
Received in revised form 24 March 2017
Accepted 4 April 2017
Available online xxxx

Keywords:
Batf3
CD8α$^+$ DCs
Th1 cells
CCL5
Atherosclerosis

ABSTRACT

Dendritic cells (DCs) play an important role in controlling T cell-mediated adaptive immunity in atherogenesis. However, the role of the basic leucine zipper transcription factor, ATF-like 3 (Batf3)-dependent CD8α$^+$ DC subset in atherogenesis remains unclear. Here we show that *Batf3*$^{-/-}$*Apoe*$^{-/-}$ mice, lacking CD8α$^+$ DCs, exhibited a significant reduction in atherogenesis and T help 1 (Th1) cells compared with *Apoe*$^{-/-}$ controls. Then, we found that CD8α$^+$ DCs preferentially induce Th1 cells via secreting interleukin-12 (IL-12), and that the expression of interferon-gamma (IFN-γ)or chemokine (C-C motif) ligand 5 (CCL5) in aorta were significantly decreased in *Batf3*$^{-/-}$*Apoe*$^{-/-}$ mice. We further demonstrated that macrophages were the major CCL5-expressing cells in the plaque, which was significantly reduced in *Batf3*$^{-/-}$*Apoe*$^{-/-}$ mice. Furthermore, we found CCL5 expression in macrophages was promoted by IFN-γ. Finally, we showed that *Batf3*$^{-/-}$*Apoe*$^{-/-}$ mice displayed decreased infiltration of leukocytes in the plaque. Thus, CD8α$^+$ DCs aggravated atherosclerosis, likely by inducing Th1 cell response, which promoted CCL5 expression in macrophages and increased infiltration of leukocytes and lesion inflammation.

1. Introduction

Atherosclerosis is a chronic, systemic inflammatory disease characterized by immune cells accumulation in the arterial wall and formation of atherosclerotic plaques (Chaudhari et al., 2015). Among these cells, macrophages have been well-characterized for their ability to phagocytose oxidized low density lipoprotein (OxLDL) and become foamy cells (Moore and Tabas, 2011). It has also been suggested that macrophages can secrete inflammatory cytokines and chemokines that are likely related to the promotion of lipoprotein retention and the accumulation of immune cells in the arterial wall (Moore and Tabas, 2011). One in vitro study demonstrated that in response to OxLDL stimuli, macrophages up-regulated mRNA encoding CXC chemokine ligand (CXCL) 1, CXCL2, and chemokine (C-C motif) ligand 5 (CCL5 or Rantes) (Stewart et al., 2010). The majority of these chemokines promote atherogenesis via the accumulation of inflammatory cells (Rolin and Maghazachi, 2014). In particular, treatment with a CCL5 antagonist was found to inhibit the recruitment of T cells and macrophages into the plaque area, and alleviate atherosclerosis (Veillard et al., 2004). However, which cells or molecules promote macrophages secreting CCL5 to enhance leukocyte infiltrating into atherosclerotic plaque in vivo, remains unknown.

A T cell-mediated adaptive immune response also plays an important role in atherosclerosis, as pro and anti-atherogenic CD4$^+$ T cell subsets, as well as their signature cytokines have been defined (Lahoute et al., 2011; Smith et al., 2010; Subramanian et al., 2013). In particular, evidence shows that CD4$^+$ T helper 1 (Th1) cells and their key cytokine, interferon gamma (IFN-γ) are pro-atherosclerotic (Benagiano et al., 2003; Buono et al., 2005). Apolipoprotein-E deficient mice (*Apoe*$^{-/-}$) that lack T-bet (a Th1 cell-differentiating transcription factor) or the IFN-γ receptor have demonstrated a reduction in atherosclerosis development (Buono et al., 2005; Gupta et al., 1997). Moreover, mice that receive exogenous IFN-γ have a larger atherosclerotic lesion area than the control mice (Whitman et al., 2000). However, during atherosclerotic progress, it is unclear how the Th1 cell response is generated, and whether IFN-γ regulates macrophage activation involved in atherosclerotic plaque formation.

DCs have long been recognized as the most potent antigen-presenting cells and are composed of distinct subsets, of which development is specifically regulated by distinct transcriptional factors. For example, interferon regulatory factor 4 (IRF4) and Notch2 are critical for CD11b$^+$ DCs generation, whereas IRF8 and Batf3 are essential for CD8a$^+$ DCs development (Mildner and Jung, 2014). It has been suggested that DCs

* Corresponding authors.
E-mail addresses: wgsong@tsmc.edu.cn (W. Song), htang@tsmc.edu.cn (H. Tang).
[1] Lead Contact.

http://dx.doi.org/10.1016/j.ebiom.2017.04.008

Please cite this article as: Li, Y., et al., Batf3-dependent CD8α$^+$ Dendritic Cells Aggravates Atherosclerosis via Th1 Cell Induction and Enhanced CCL5 Expression in Plaque Macrophages, EBioMedicine (2017), http://dx.doi.org/10.1016/j.ebiom.2017.04.008

play an important role in controlling the T cell-mediated adaptive immune response to regulate atherogenesis (Choi et al., 2011; Gautier et al., 2009; Koltsova et al., 2012; Subramanian et al., 2013; Weber et al., 2011). Moreover, different DC subsets have distinct function in modulating different types of adaptive immunity (Choi et al., 2011; Sage et al., 2014; Weber et al., 2011). For instance, a CCL17-expressing $CD11b^+$ DCs subset promotes atherosclerosis through the inhibition of regulatory T cell (Treg) expansion in lymphoid tissues (Weber et al., 2011). In addition, in mouse models of genetic and high-fat/cholesterol diet-induced dyslipidemia, $CD8\alpha^-$ DCs preferentially induced a Th2 response (Shamshiev et al., 2007). Although the role of $CD8\alpha^+$ DCs in atherosclerosis has been suggested, one study suggested the protective role of $CD8\alpha^+$ DCs but another showed no functions of $CD8\alpha^+$ DCs in atherosclerosis (Choi et al., 2011; Legein et al., 2015). These contradicting results on their role in controlling T cell-mediated atherosclerosis were assiocated with experimental mice that had different gene difficiency. Thus, the function of $CD8a^+$ DCs in atherosclerosis remains to be elucidated.

In the current study, to clarify the role of $CD8a^+$ DCs in atherosclerosis, a double knockout $Batf3^{-/-}Apoe^{-/-}$ mouse was generated and fed with a Western diet. Our findings indicate an important role of Batf3-dependent $CD8\alpha^+$ DCs in controlling Th1 cell cytokine production, and IFN-γ-dependent chemokine CCL5 expression by macrophages during atherosclerotic progression.

2. Materials and Methods

2.1. Mice

$Apoe^{-/-}$ mice(RRID:IMSR_JAX:002052), OT-II mice(RRID:IMSR_JAX:004194)on a C57BL/6 background, and *Batf3* deficient mice $Batf3^{-/-}$ (RRID:IMSR_JAX:013755)were obtained from the Jackson Laboratory (Bar Harbor, ME). $Batf3^{-/-}$ mice were crossed with $Apoe^{-/-}$ mice to generate double knockout mice $Batf3^{-/-}Apoe^{-/-}$. $Batf3^{-/-}Apoe^{-/-}$ mice were genotyped by Shanghai Biowing Applied Biotechnology Ltd., using multiplex PCR with next generation sequencing (Chen et al., 2016). All identified SNP were compared with the Mouse Genome Informatics (MGI) database, and were found to be identical with genotype of C57BL/6. The detailed SNP test results were in Table S1 and Table S2 in the supplementary data.

Female $Batf3^{-/-}Apoe^{-/-}$ mice or the $Apoe^{-/-}$ mice were kept on a chow diet (CD) or fed a Western diet (WD) (21% fat and 0.15% cholesterol) (Beijing keaoxieli company, China) for 12 weeks (wks). These mice were 6–8 wks in age, weighed 21–25 g, and were housed at a constant temperature (24 °C) in a 12-hour (h) dark/12-h light-cycle room in the Taishan Medical University Animal Care Facility, according to institutional guidelines. All animal studies were approved by the Animal Care and Utilization Committee of Taishan Medical University.

2.2. Measurements of Atherosclerotic Lesions

For atherosclerotic lesion measurements, the $Apoe^{-/-}$ mice (n = 8) and the $Batf3^{-/-}Apoe^{-/-}$ mice (n = 8) were fed a Western diet for 12 wks, anesthetized using isoflurane, blood was drawn, and the mice were perfused with 2 mmol/L Ethylene Diamine Tetraacetic Acid (EDTA) (Sigma-Aldrich) in Phosphate Buffered Saline (PBS) via cardiac puncture to remove blood contamination from vascular tissue. The aortas were dissected, and the exposed aortas were stained for lipid depositions with Oil Red O (Sigma-Aldrich), and an en face assay was performed (Iqbal et al., 2012). The heart was embedded in OCT compound, and the aortic roots were sectioned into 5 μm slices, generating ~30–40 sections that spanned the entirety of the aortic root, and then stained with Oil Red O (Sigma-Aldrich), hematoxylin and eosin (H&E) or masson-trichrome (Solarbio, Beijing, China). For comparisons of lesion size between the groups, the mean lesion area was quantified from 10 digitally captured sections per mice (Cipriani et al., 2013). For immunohistochemistry detection, cryosections of the aortic root were stained for the presence of leukocytes (CD45), macrophages (Mac3), DCs (CD11c) and T cells (CD3) using specific antibodies to Mac-3 (M3/84; BD Biosciences Cat# 550292, RRID:AB_393587), as well as eBioscience antibodies to CD45.2 (104; eBioscience Cat# 13-0454-85, RRID:AB_466457), CD11c (N418; eBioscience Cat# 13-0114-82, RRID:AB_466363), and CD3 (145-2C11; eBioscience Cat# 13-0031-85, RRID:AB_466320) using standard immunohistochemistry techniques (Subramanian et al., 2013). Images were viewed and captured with a Nikon Labophot 2 microscope equipped with a Spot RT3 colour video camera attached to a computerized imaging system (Nikon corporation, Tokyo, Japan). Quantitative analysis of plaque area was performed by 2 blinded observers using Image-Pro Plus software 6.0 (Media Cybernetics, MD, USA, RRID:SCR_007369). For the immunohistofluorescence analysis, the cryosections were stained with an antibody against CD45 (104; eBioscience Cat# 47-0451-82, RRID:AB_1548781), Mac3 (M3/84; BD Biosciences Cat# 550292, RRID:AB_393587), CCL5 (Bioss Inc. Cat# bs-1324R-Biotin, RRID:AB_11099534). Streptavidin APC-eFluor 780 (eBioscience Cat# 47-4317-82, RRID:AB_10366688) and Goat Rabbit IgG Secondary antibody (Bioss Inc. Cat# bs-0295G-Biotin,RRID:AB_10894308). Images were viewed and captured with a Laser Scanning Confocal Microscope (ANDOR E2V, Leica, Germany).

2.3. Flow Cytometry Analysis

Splenic single-cell suspensions and aortic single-cell suspensions were prepared as described in Supplemental information. Cell surface molecule staining was performed using combinations of specific antibodies to CD45.2 (104; eBioscience Cat# 45-0454-82, RRID:AB_953590), CD11c (N418; eBioscience Cat# 17-0114-82, RRID:AB_469346), IA/IE (M5/114.15.2; BioLegend Cat# 107630, RRID:AB_2069376), CD8a (53-6.7; eBioscience Cat# 95-0081-42, RRID:AB_1603266), CD11b (M1/70; eBioscience Cat# 12-0112-81, RRID:AB_465546), B7-DC (122; eBioscience Cat# 12-9972-82, RRID:AB_466285), B7-H2 (HK5.3; eBioscience Cat# 12-5985-82, RRID:AB_466094), CD40 (1C10; eBioscience Cat# 12-0401-82, RRID:AB_465649), CD80 (16-10A1; eBioscience Cat# 12-0801-82, RRID:AB_465752), CD86 (GL1; eBioscience Cat# 12-0862-83, RRID:AB_465769), CD62L (MEL-14; eBioscience Cat# 12-0621-83, RRID:AB_465722), and CD103 (M290; BD Biosciences Cat# 557493, RRID:AB_396730). For intracellular cytokine staining, splenocytes were prepared as previously described, and were then stimulated with an RPMI-1640 medium containing 10% fetal calf serum (FCS), 20 ng/mL phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich), 1 μg/mL ionomycin (Sigma-Aldrich), and 5 μg/mL brefeldin A (selleckchem) for 4 h at 37 °C and 5% CO_2. The cells were washed with washing buffer and then stained with an Anti-Mouse CD4-FITC antibody (GK1.5; eBioscience Cat# 11-0042-86, RRID:AB_46489) or an Anti-Mouse CD8-BV650 antibody (53-6.7; eBioscience Cat# 95-0081-41, RRID:AB_1603267). Then the cells were fixed and permeabilized with a Fixation/Permeabilization Solution (BD Biosciences), and stained intracellularly with Anti-Mouse/Rat IL-17A PerCP-Cyanine5.5 (eBio17B7; eBioscience Cat# 45-7177-80, RRID:AB_925754) and Anti-Mouse IFN-γ APC (XMG1.2; eBioscience Cat# 17-7311-82, RRID:AB_469504) antibodies. Intracellular staining for Foxp3 (FJK-16S; eBioscience Cat# 12-5773-82, RRID:AB_465936) was performed using the Transcription factor staining buffer kit (eBioscience). Flow cytometry analysis was performed using a FACS ARIA II (BD Biosciences) equipped with a 405 nm, 488 nm and a 633 nm laser, and the results were analyzed by FlowJo 7.6 software (Tree Star, OR, USA, RRID:SCR_008520).

2.4. Antigen-specific T Cell Proliferation and Polarization

Naïve $CD4^+CD62L^+$ T cells were sorted from the spleens of OT-II mice by fluorescence-activated cell sorting (FACS) Aria II (BD Biosciences) as shown in Fig. S2a, and labeled with 5 μM cell proliferation

Please cite this article as: Li, Y., et al., Batf3-dependent $CD8\alpha^+$ Dendritic Cells Aggravates Atherosclerosis via Th1 Cell Induction and Enhanced CCL5 Expression in Plaque Macrophages, EBioMedicine (2017), http://dx.doi.org/10.1016/j.ebiom.2017.04.008