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Jmjd3-mediated epigenetic regulation of inflammatory cytokine gene expression in serum amyloid A-stimulated macrophages

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ABSTRACT

Serum amyloid A (SAA), a major acute-phase protein, has potent cytokine-like activities in isolated phagocytes and synovial fibroblasts. SAA-induced proinflammatory cytokine gene expression requires transcription factors such as NF-κB; however, the associated epigenetic regulatory mechanism remains unclear. Here we report that Jmjd3, a histone H3 lysine 27 (H3K27) demethylase, is highly inducible in SAA-stimulated macrophages and plays an important role in the induction of inflammatory cytokine genes. SAA-induced Jmjd3 expression leads to reduced H3K27 trimethylation. Silencing of Jmjd3 expression significantly inhibited SAA-induced expression of proinflammatory cytokines including IL-23p19, G-CSF and TREM-1, along with up-regulation of H3K27 trimethylation levels on their promoters. Depletion of Jmjd3 expression also attenuated the release of proinflammatory cytokine genes in a peritonitis model and ameliorated neutrophilia in SAA-stimulated mice. Finally, we observed that Jmjd3 is essential for SAA-enhanced macrophage foam cell formation by oxidized LDL. Taken together, these results illustrate a Jmjd3-dependent epigenetic regulatory mechanism for proinflammatory cytokine gene expression in SAA-stimulate macrophages. This mechanism may be subject to therapeutic intervention for sterile inflammation and atherosclerosis.

1. Introduction

Serum amyloid A (SAA), a major acute-phase protein, is produced by hepatocytes during acute-phase response and released to the blood circulation [1,2]. SAA is also produced in inflammatory tissues in response to microbial infection, tissue injury, neoplastic growth and immunological disorders [3,4]. Elevation in plasma SAA concentration is a clinical indication for inflammatory disorders and is associated with the pathogenesis of chronic diseases such as secondary amyloidosis [5], atherosclerosis [6], obesity [7], diabetes [8] and rheumatoid arthritis [4]. Accumulating evidence supports the notion that SAA also actively participates in the inflammatory process due to its cytokine-like activity. Innate immune cells such as macrophages respond to SAA with changes in their gene expression profile, including the up-regulation of a large number of proinflammatory cytokines such as IL-1β, matrix metalloprotease 9 [9], granulocyte colony stimulated factor (G-CSF) [10], IL-8 [11], IL-12 and IL-23 [12]. Recent studies have shown that the cytokine-like activities of SAA are mediated by cell surface receptors including formyl peptide receptor 2 (FPR2), Toll-like receptors 2 (TLR-2) and TLR-4 [11,13,14]. These receptors activate the transcription factor NF-κB, leading to the induced expression of inflammatory cytokines [11,12]. However, the epigenetic pathways involved in the modulation of SAA-induced inflammatory cytokine gene expression have not been characterized.

Epigenetic modification in response to environmental stimuli plays a fundamental role in inflammatory gene expression [15]. LPS, for example, regulates the transcription of inflammatory cytokine genes in macrophages by altering histone deacetylase (HDAC) expression [16]. HDACs, along with histone acetyltransferases (HATs), are essential for viral infection [17,18]. In eukaryotic cells, chromatin is organized in nucleosomes with DNA wrapped around histone octamers, each assembled with two copies of the H2A, H2B, H3 and H4 subunits [19]. Transcriptional activators typically recruit enzymes that modify the tails of histone through phosphorylation, acetylation, ubiquitination, SUMOylation, methylation, and ADP-ribosylation [20]. These modifications produce different results

Abbreviations: SAA, serum amyloid A; H3K27me3, trimethylation of histone H3 at lysine 27; HDACs, histone deacetylases; FPR2, formyl peptide receptor 2; G-CSF, granulocyte colony-stimulating factor; IL-23p19, interleukin-23 alpha subunit p19; IL-12p40, interleukin-12 p40; IL-12p35, interleukin-12 p35; TREM-1/2, triggering receptor expressed on myeloid cell-1/2; TGF-β, transforming growth factor beta; CXCL1, chemokine (C-X-C motif) ligand 1; IL-1β, interleukin-1 beta; IL-8, interleukin-8; TNF-α, tumor necrosis factor alpha; PMs, peritoneal macrophages; BMDMs, bone marrow-derived macrophages; PI3K, phosphatidylinositide 3-kinases; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; HATs, histone acetyltransferases; oxLDL, oxidized low density lipoproteins; DAMPs, damage associated molecular patterns; PAMPs, pathogen associated molecular patterns; HMGB1, high mobility group box-1.

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in transcriptional regulation. While lysine acetylation of histone by histone acetyltransferases usually increases transcriptional activity, lysine methylation of histone H3 regulates nuclear processes dedicated to the maintenance of active or silent states of gene expression depending on the levels and sites of methylation [21,22].

The broad and potent effect of SAA on the induction of inflammatory cytokine genes suggests the presence of epigenetic regulatory mechanisms. Using PCR-array expression profiling, we found that Jmjd3 (Kdm6b) was markedly induced in SAA-stimulated macrophages. Jmjd3 contains a C-terminal Jumonji C (JmjC) domain and is a demethylase that catalyzes site-specific demethylation of the trimethylated lysine 27 in histone H3 (H3K27me3), resulting in di- and mono-methylated histone H3 (H3K27me2, H3K27me1) [23]. Since its discovery, Jmjd3 has been found to play important roles in regulating polycomb-mediated gene silencing during embryonic development and cell reprogramming [24,25]. A link between Jmjd3 and inflammation has been suggested based on the function of Jmjd3 in controlling macrophage differentiation and cell identity [26] and on the induction of Jmjd3 in macrophages exposed to bacterial products [27]. Since Jmjd3 expression is markedly up-regulated by SAA, we speculated that it might play a role in SAA-induced inflammatory gene transcription. In this report, we show that Jmjd3 reduces the level of H3K27me3, thus promoting the transcription of a variety of inflammatory cytokine genes in SAA-stimulated macrophages.

2. Materials and methods

2.1. Mice

C57BL/6 mice were purchased from Shanghai Laboratory Animal Center (SLAC, Shanghai, China). *Tlr2*$^{-/-}$ mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Age- and sex-matched littermates were used in the experiments. The procedures involving mice were carried out using protocols approved by the Institutional Animal Care and Use Committee at the Shanghai Jiao Tong University. Mice that underwent bone marrow transplantation were housed in sterile filter-top cages and supplied with SulfaTrim water for at least 14 days before and up to 42 days after irradiation.

2.2. Reagents

The Epigenetic Chromatin Modification Enzymes PCR Array Kit was purchased from QIAGEN (PAMM-085A; SABiosciences, Venlo, The Netherlands). Recombinant human SAA was obtained from PeproTech (Rocky Hill, NJ). The content of bacterial endotoxin is less than 0.1 ng/μg protein. LPS from *Escherichia coli* 0111:B4 was purchased from Sigma-Aldrich (St Louis, MO). The inhibitors for protein kinases MEK (U0126) and PI3K (LY294002) were purchased from Calbiochem (San Diego, CA). The anti-Jmjd3 antibody was obtained from Abcam (Cambridge, MA). Antibodies for H3K27me3 and Jmjd3 (C-terminus) were obtained from Millipore (Billerica, MA). Antibodies for HDAC1, β-actin, the anti-rabbit and anti-mouse IgG HRP linked antibodies were obtained from Cell Signaling Technology (Danvers, MA).

2.3. Cells preparation and culture

Mouse macrophages (BMDMs and PMs) were prepared from WT or knockout C57BL/6 mice as described [45]. Human monocytic THP-1 cells (TIB-202), mouse RAW264.7 macrophages (TIB-71), the viral packaging cell line BOSC23 (CRL-11270) were all obtained from ATCC (Manassas, VA). The cells were maintained in RPMI 1640 supplemented with 2 mM of L-glutamine, 10% of FBS (GIBCO), 25 mM HEPES, 100 U/ml penicillin, and 100 mg/ml streptomycin. All cell cultures were kept in a humidified atmosphere with 5% CO_2 at 37 °C.

2.4. siRNA interference

Mouse peritoneal macrophages were transfected with specific siRNA using Silencer® siRNA Transfection II Kit (Ambion) according to the manufacturer's instructions. The cells were then recovered for 48 h before stimulation. The siRNA oligonucleotide were designed and synthesized by Shanghai RIBOBIO Co., LTD (Guangzhou, China). Sequence of MyD88-specific siRNA1 and 'Nonsense' siRNA were shown in Supplementary Table 1.

2.5. Plasmid constructs

Mouse cDNA coding for Jmjd3, JmjC (a.a. 1141–1614) and JmjC carrying an Ala mutation at His-1388 (Mut. JmjC) were a gift from Prof. Gioacchino Natoli (European Institute of Oncology, Milan, Italy) as described in [24]. The cDNAs were subcloned into the multi-cloning sites of a retrovirus-based expression plasmid, MigR1, which also contains an internal ribosome entry site (IRES) for GFP expression (Addgene, Cambridge, MA). Oligonucleotides targeting mouse Jmjd3 were annealed and ligated into the RNAi-Ready pSIREN-RetroQ ZsGreen vector (Clontech, Mountain View, CA). All sequences for Jmjd3 cloning and shRNA were shown in Supplementary Table 1.

2.6. Retrovirus-mediated gene transfer

BOSC23 cells were co-transfected with 6 μg of the constructed plasmid plus 1.5 μg of the pVSV-G plasmid (Clontech, Mountain View, CA) using HG TransGene Reagent (Health & Gene, China). After 6 h, the medium was removed and replaced with fresh medium. Viral supernatants were collected, passed through a filter and concentrated. For infection, cells were incubated with serially diluted retroviral supernatants in the presence of 8 μg/ml Polybrene (Sigma, St. Louis, MO), centrifuged at 2000 rpm for 90 min at 30 °C, followed by incubation at 37 °C for an additional 6 h. The media was replaced with fresh RPMI 1640 supplemented containing 10% FBS. After 48 h, the cells were treated with SAA for the indicated times, and then harvested for different assays.

2.7. Immunofluorescence

RAW264.7 cells were grown on microscope cover glass (ThermoFisher) and fixed with 4% paraformaldehyde at 4 °C. After washing and permeabilization, cells were inversed on the dilution of an anti-H3K27me3 antibody (10 μg/ml) for overnight at 4 °C. The cells were then repeatedly washed with PBS and incubated with 20 μg/ml of Alexa Fluor® 568 Goat Anti-Rabbit IgG (H + L) Antibody (Life technologies, Carlsbad, CA) for 60 min. Nuclei were stained with DAPI (10 μg/ml) for 5 min. The cover glass was washed with PBS and examined under a Leica TCS SP UV confocal laser scanning microscope (Leica, Wetzlar, Germany).

2.8. Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) was performed using a ChIP assay kit (Millipore) according to the manufacturer's with minor modifications. Briefly, after SAA stimulation, RAW264.7 cells were cross-linked and then washed and resuspended in SDS Lysis Buffer. Nuclei were fragmented by sonication. Chromatin fractions were cleared with protein A-agarose beads followed by immnoprecipitation overnight with an anti-H3K27me3 antibody (Millipore) or with control IgG. Cross-linking was reversed, followed by proteinase K digestion. The primer sequences were shown in Supplementary Table 1. Data are presented as the amount of DNA recovered relative to the input control.

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