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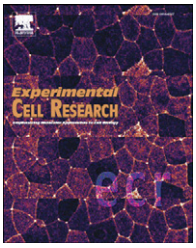

Research Article

Mast cell death induced by 24(S),25-epoxycholesterol

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ABSTRACT

Mast cell is one of the central effectors in inflammatory responses. Recent studies suggest that a promising therapeutic approach for various inflammatory immune diseases, including rheumatoid arthritis, multiple sclerosis, and type I allergies, is to inhibit mast cell growth and/or survival. Studies also indicate that a balanced lipid metabolism is crucial for regulating the life span of cells. Oxysterol is a well-known regulator of lipid metabolism and has diverse functions, such as inhibition of the mevalonate isoprenoid pathway, efflux of free cholesterols, and synthesis of cholesterol esters. Here, we show that 24(S),25-epoxycholesterol, a representative endogenous oxysterol, induces apoptosis in bone marrow-derived murine mast cells. Furthermore, we have revealed, for the first time, that the accumulation of neutral lipids catalyzed by acyl-CoA: cholesterol acyltransferase in the cells was involved in induction of mast cell apoptosis. Our present findings confer new insights into the roles of lipid metabolism during oxysterol-mediated mast cell apoptosis.

Introduction

Mast cell activation through Fc receptors is responsible for the onset of a variety of immune diseases, such as rheumatoid arthritis, multiple sclerosis, and type I allergies [1,2]. Previous studies employing mast cell-deficient mice suggest that reducing mast cell number in inflammation sites is a promising therapeutic strategy for chronic inflammatory disorders [3–6]. Indeed, mast cell apoptosis, triggered by Leflunomide or Imatinib, contributes to the anti-inflammatory actions of these medicines on rheumatoid arthritis [7,8]. Therefore, the discovery of natural substances that can reduce mast cell survival is important.

Homeostasis of lipid metabolism is essential for cell survival of leukocytes, including T cells and granulocytes. For example, statins, which are synthetic inhibitors of HMG CoA reductase, induce apoptosis in $CD4^+$ T cells, eosinophils, and neutrophils by suppressing the mevalonate isoprenoid pathway [9–12]. In addition, treatment of a rat mast cell line with Lovastatin results in apoptotic cell death [13]. Based on these findings, we hypothesized that primary mast cells as well as other leukocytes may die by the

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Abbreviations: BMMCs, bone marrow-derived mast cells; LXR, liver X nuclear receptor; 25-OHC, 25-hydroxycholesterol; 22(R)-OHC, 22(R)-hydroxycholesterol; 24(S),25-EC, 24(S),25-epoxycholesterol; ACAT, acyl-CoA:cholesterol acyltransferase; FITC, fluorescein isothiocyanate; PI, propidium iodide

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dysregulation of lipid metabolism. To address this hypothesis, we employed natural oxysterol cholesterol derivatives, 24(S),25-epoxycholesterol (24(S),25-EC), 22(R)-hydroxycholesterol (22(R)-OHC), and 25-hydroxycholesterol (25-OHC), because they are endogenous HMG CoA reductase inhibitors [14,15], and 25-hydroxycholesterol causes apoptotic cell death in immature T cells and leukemia cells [16,17].

In addition to inhibiting HMG CoA reductase, oxysterols have additional functions in lipid metabolism. Oxysterols act as potent activators of the synthesis of cholesterol esters [18,19] and the liver X nuclear receptor (LXR) [20,21]. Recently, Thewke et al. also reported that the 7-ketocholesterol-induced accumulation of cholesterol esters elicited apoptotic cell death in macrophages in an acyl-CoA:cholesterol acyltransferase (ACAT)-dependent fashion [22,23]. In contrast, the biological functions of LXR activation in cell death by oxysterol are largely unknown. However, the activation of LXR with synthetic ligands has both pro-apoptotic and pro-survival effects under different experimental conditions [24,25]. These findings suggest that LXR activation may affect oxysterol-induced cell death.

Here, we report that 24(S),25-EC loading induces apoptotic cell death in bone marrow-derived murine mast cells (BMMCs). Furthermore, we investigated how 24(S),25-EC-induced dysregulation of lipid metabolism reduced mast cell survival.

Materials and methods

Reagents

Oxysterols 24(S),25-EC and 22(R)-OHC were purchased, respectively, from Biomol (Plymouth Meeting, PA , USA) and Cayman (Ann Arbor, Michigan, USA). Nile red, 25-OHC, Oil red O, Sandoz58-035, and mevalonate were purchased from Sigma (St Louis, MO, USA). Recombinant murine Interleukin (IL)-3 was purchased from PeproTech (Rocky Hill, NJ, USA). FR179254 [26] and z-DEVD-fmk were purchased from Calbiochem (San Diego, CA, USA). Hoechst33342 was purchased from Dojindo (Kumamoto, Japan). Lovastatin and GERI-BP002-A [27] were from Biomol. A synthetic LXR antagonist, 5CPPSS-50 [28,29], was a kind gift from Dr. Y. Hashimoto (The University of Tokyo, Tokyo, Japan). Mevalonate, Lovastatin, and oxysterols were stored as 20 mM stock solutions in 99.5% ethanol at −30 °C. z-DEVD-fmk, 5CPPSS-50, and ACAT inhibitors (Sandoz58-035, FR179254, GERI-BP002-A) were stored as 20 mM stock solutions in DMSO at −30 °C.

Preparation of BMMCs and primary human mast cells

C57BL/6J mice were purchased from the Charles River Laboratories in Japan (Kanagawa, Japan). Following the animal care committee approval, all experiments were performed in accordance with the Nihon University guidelines for the care and use of laboratory animals. BMMCs were prepared from the femurs of 4- to 6-week-old C57BL/6J mice. Briefly, bone marrow cells were cultured in RPMI1640 (Sigma) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS; SAFC Biosciences, Tokyo, Japan), and 4 ng ml^{-1} recombinant IL-3 (PeproTech). BMMCs were used for experiments after they were cultured for 6 to 8 weeks. We obtained approval of the ethical review boards to prepare primary human mast cells from cord blood-derived $CD34^+$ mononuclear cells (RIKEN BioResource center, Tsukuba, Japan). The $CD34^+$ cells were cultured in serum-free Iscove methylcellulose medium (Stem Cell Technologies Inc., Vancouver, BC, Canada) and Iscove modified Dulbecco medium containing SCF at 200 ng ml^{-1}, IL-6 at 50 ng ml^{-1} and IL-3 at 1 ng ml^{-1} as previously described [30]. On day 42 of culture, methylcellulose was dissolved in phosphate-buffered saline (PBS) and the cells were resuspended and cultured in Iscove modified Dulbecco medium containing SCF at 100 ng ml^{-1} and IL-6 at 50 ng ml^{-1} with 2% FBS.

Treatment of BMMCs with oxysterols

BMMCs (5×10^5 ml^{-1}) were incubated in culture medium containing oxysterols (10 μM, 20 μM, or 40 μM) for 3 h to 24 h. In some experiments, cells were treated with pharmacological inhibitors or with their vehicles for 1 h prior to oxysterol loading.

Evaluation of cell death

Apoptotic cell death and overall cell death were evaluated by double staining with fluorescein isothiocyanate (FITC)-conjugated annexin V and propidium iodide (PI) as previously described [31]. BMMCs (5×10^5 ml^{-1}) treated with or without oxysterols for 24 h were labeled with annexin V and PI and then analyzed with a FACSCalibur (Becton Dickinson Biosciences, San Jose, CA, USA). Subsets of cells were determined as follows: annexin V^+/PI^- (early apoptotic cells), annexin V^+/PI^+ (late apoptotic cells), and annexin V^-/PI^- (living cells).

Flow cytometric analysis of caspase 3/7 activation and mitochondrial membrane potential

BMMCs (5×10^5 ml^{-1}) were treated with or without oxysterols for 18 h. Caspase 3/7 activation was determined with reagents of the Dual Sensor: MitoCasp (Cell Technology Inc. Mountain View, CA, USA) according to the manufacturer's protocol. Mitochondrial membrane potential was detected with Mito Flow (Cell Technology Inc.) according to the manufacturer's protocol. Stained cells were evaluated with a FACSCalibur.

DNA fragmentation assay

After treatment with oxysterol for 18 h, the BMMCs were harvested and washed twice with PBS. DNA was prepared from the cells with an Apoptosis Ladder Detection kit (Wako Chemical, Tokyo, Japan) or a Quick Apoptotic DNA Ladder Detection kit (Bio Vision, Mountain View, CA, USA). DNA fragmentation was resolved on a 1% agarose gel and visualized with ethidium bromide.

Staining of nucleus

BMMCs (5×10^5 ml^{-1}) were treated with or without oxysterols for 15 h to 18 h. The nuclei were stained with Hoechst33342 for 20 min at room temperature and then analyzed with an Olympus IX71 inverted microscopy (Olympus, Tokyo, Japan).

Detection of intracellular lipids

Neutral lipids were detected by staining with oil red O or Nile red. BMMCs (5×10^5 ml^{-1}) were treated with or without oxysterols for