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OCILRP2 signaling synergizes with LPS to induce the maturation and differentiation of murine dendritic cells

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ABSTRACT

Osteoclast Inhibitory Lectin-related Protein 2 (OCILRP2) is a typical type II transmembrane protein and belongs to C-type lectin-related protein family. It is preferentially expressed in dendritic cells (DC), B lymphocytes, and activated T lymphocytes. Upon binding to its ligand, OCILRP2 can promote CD28-mediated co-stimulation and enhance T cell activation. However, the role of OCILRP2 in DC development and activation is unclear. In this report, we present evidence that recombinant protein OCILRP2-Fc inhibits the generation and LPS-induced maturation of murine bone marrow-derived dendritic cells (BMDCs) by downregulating the expression of CD11c, MHC-II, and co-stimulators CD80 and CD86. OCILRP2-Fc also reduces the capacity of BMDCs to take up antigens, activates T cells, and secret inflammatory cytokines such as IL-6, IL-12, and TNF-α. Additionally, we show that OCILRP2-Fc may cause the aforementioned effects through inhibiting NF-κB activation. Therefore, OCILRP2 is a new regulator of DC maturation and differentiation following TLR4 activation.

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1. Introduction

Dendritic cells (DC) as the main antigen-presenting cells (APC) are unique cells that can fully activate naïve T lymphocytes by providing three types of signals: (1) antigen presentation (signal 1); (2) co-stimulatory signal (signal 2); (3) polarizing (signal 3) [1–3]. Signal 1 is mediated by major histocompatibility complex (MHC) coupled with antigenic peptides that are presented to CD8 (through MHC I) or CD4 (through MHC II) T cells. Signal 2 is dependent on co-stimulatory molecules such as B7 family. Signal 3 is provided by cytokines or growth factors secreted by DC. However, the capacity of DC to activate T cells is solely dependent on the status of DC maturation. The immature DC is primarily responsive of antigen uptake and process. The matured DC are responsive of antigen presentation and provoking the expression of co-stimulatory molecules [4,5]. DC maturation is a complicated process, to which many factors contribute in different ways. For instance, DC maturation can be triggered by pathogen associated molecular patterns (PAMP) (e.g., lipopolysaccharide, LPS) through engaging the pattern recognition receptors (PRR) such as toll-like receptors (TLR) [6,7]. Other factors such as CD4-NK cells can also mediate DC maturation via contact-dependent and independent pathways [8]. The accumulating evidence demonstrated that the transcription factor NF-κB participates in driving DC maturation processes [9–12].

Osteoclast Inhibitory Lectin-related Protein 2 (OCILRP2) belongs to C-type lectin-related (Clr) protein family. It is a typical type II transmembrane protein with a short cytoplasmic domain, for which no known signaling motifs are found [13–15]. This protein has been documented to be selectively expressed in immune tissues, with the highest expression in DC, B lymphocytes, and activated T lymphocytes. Consistently, its ligand, NK-cell receptor protein 1 member f (NKRP1f) is also expressed in DC and activated lymphocytes. OCILRP2 interacting with its ligand provides an additional positive signal to enhance B7.1/CD28-mediated T cell proliferation as well as IL-2 production [16]. Silencing OCILRP2 leads to intrinsic impairment in T cell response to anti-CD3 and anti-CD28 stimulation as well as APC presenting antigens. OCILRP2-silenced T cells are incapable of proliferation, and decrease the production of IL-2 upon antigen priming. Moreover, NF-κB activation is also impaired as the result of OCILRP2 silencing [17]. Although OCILRP2 is

Abbreviations: APC, antigen-presenting cells; BMDCs, bone marrow-derived dendritic cells; Clr, C-type lectin-related; GM-CSF, granulocyte-macrophage colony stimulating factor; NKRP1f, NK-cell receptor protein 1 member f; OCILRP2, Osteoclast Inhibitory Lectin Related Protein 2; PAMP, pathogen associated molecular patterns; PRR, pattern recognition receptors; TLR, toll like-receptors.

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dominantly expressed in DC, its role in DC maturation and activation is still unclear.

In this paper, we report that recombinant OCILRP2-Fc protein impaired the mouse bone marrow-derived dendritic cells (BMDCs) development and activation in vitro. This suppression was attributed to restrained transcription factor NF-κB activation as a result of blockade of OCILRP2 signaling. These findings suggest that OCILRP2 signaling plays an important role in DC maturation and functional differentiation.

2. Materials and methods

2.1. Reagents and mice

Recombinant OCILRP2-Fc which fused with OCILRP2 extracellular domain and human IgG1 Fc fragment was produced by cultured CHO cells, and purified by using protein-A column. Cytokine detecting ELISA kits, recombinant murine granulocyte-macrophage colony stimulating factor (rmGM-CSF) and interleukin-4 (rmIL-4) were purchased from R&D system. FITC-conjugated anti-mouse CD11c, CD80, MHC-II, and PE-conjugated anti-mouse CD86 antibodies were bought from eBioscience. Polyclonal anti-IκB antibody was bought from Cell Signaling Technology. LPS and FITC-labeled dextran (M.W. = 40,000) were purchased from Sigma–Aldrich. 6–8 weeks old BALB/c mice were purchased from Beijing Laboratory Animal Research Centre. All experiments were approved by the Animal Ethics Board of Henan University and performed in accordance with EU Directive 2010/63/EU.

2.2. Dendritic cells generation and culture

BMDCs were prepared as described previously [18] with a little modifications. Briefly, bone marrow cells were collected from tibias and femurs of 6–8 weeks old BALB/c mice, and passed through a nylon mesh to remove small pieces of bone and debris, then red blood cells were lysed within ACK lysis buffer (0.15 M NH_4Cl, 1.0 M $KHCO_3$, 0.1 mM Na_2EDTA). After washing, cells were resuspended in RPMI 1640 complete medium supplemented with 10% FBS, rmGM-CSF (20 ng/ml), rmIL-4 (20 ng/ml), 2-mercaptoethanol (50 mM) and with recombinant OCILRP2-Fc protein (10 μg/ml) or control human IgG. Cells were cultured in 24-well plates at 1×10^6 cells/ml, 1 ml in each well. Non-adherent cells were discarded after having been cultured for 24 h, and medium was replaced every other day. On day 6, cells were cultured for additional 24 h in the presence of LPS (500 ng/ml) to induce BMDCs maturation. Non-adherent and loose adherent cells consisting of mostly BMDCs were harvested and purified by MACS using anti-CD11c antibody–coupled magnetic beads (Miltenyi Biotec).

2.3. Cell surface markers expression analysis

Bone marrow cells were cultured with OCILRP2-Fc or control human IgG for 5 days, then cells were harvested or stimulated by LPS for another day. 1×10^6 cells were incubated respectively with fluorescence-conjugated anti-mouse CD11c, CD80, MHC-II or CD86 monoclonal antibody for 30 min on ice, the appropriate conjugated isotype-matched IgGs were used as control. After washing 3 times, samples were acquired on FACS Calibur with CellQuest software and analyzed with FlowJo software.

2.4. Fluorescein isothiocyanate (FITC)-dextran uptake

2×10^6 Immature BMDCs or LPS-induced mature BMDCs with different treatment were incubated with 1 mg/ml of FITC-dextran for 2 h at 37 °C. Parallel cultured BMDCs were pre-cooled and incubated with FITC-dextran at 4 °C as negative control. Subsequently, cells were harvested and washed 3 times with cold PBS, then acquired on FACS Calibur.

2.5. Allogeneic mixed leukocyte reaction

Graded numbers of BMDCs were cultured with 2×10^5 $CD4^+$ T cells in triplicate in round-bottom 96-well plates, $CD4^+$ T cells were purified from allogeneic mouse spleen by MACS (Miltenyi Biotec). After 3 days of culturing, each well was pulsed with 1 μCi [^{3}H]thymidine and incubated for additional 18 h. And then the incorporation of radionuclide into DNA was measured by liquid scintillation counter.

2.6. Electrophoretic mobility shift assay (EMSA)

EMSA was performed as described previously [17]. In brief, BMDCs with different treatment were harvested, cells were lysed within 100 μl of Buffer C (20 mM Hepes, 25% glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM PMSA, 1 mM DTT, leupeptin and aprotinin) plus 0.1% NP40 followed by sonication. Soluble fractions were separated from sonicated cell lysate by centrifugation (14,000 rpm). 5 μg of cell lysate were incubated with ^{32}P-labeled IgκB probe at room temperature for 20 min and then resolved on 6% native polyacrylamide gel.

2.7. Western blotting

Whole cell lysate was prepared by using lysis buffer containing 50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% sodium deoxycholate, 0.1% SDS, 1% Triton X-100, 1 mM PMSF and 1 × protease inhibitor cocktail (Roche). Protein concentrations were determined by BCA assay. Equal quantities of samples were run on 10% SDS–PAGE gels and transferred onto nitrocellulose membrane. The membrane was probed with polyclonal anti-IκB antibody (1:500 dilution) and followed by horseradish peroxidase-conjugated secondary antibody, specific bands were visualized via the ECL kit according to the manufacturer's instruction.

2.8. Cytokine ELISA

The same number of immature BMDCs with different treatment were seeded in each well of 24-well plate followed by LPS (500 ng/ml) stimulating. Cell culture supernatants were collected at different time points (0, 6, 12, 24, and 48 h post-stimulation with LPS). Cytokine concentrations were determined by ELISA kits according to the manufacturer's instructions.

2.9. Statistical analyses

SPSS13.0 software was used for the statistic analysis. Data were presented as mean ± standard deviations (SE), and analyzed using two-way ANOVA and paired student's *t*-test. Differences were considered significant when $p < 0.05$.

3. Results

3.1. OCILRP2-Fc suppresses BMDC generation and maturation

In order to investigate whether OCILRP2 signaling affects DC generation and maturation, we surveyed cell surface marker expression in both immature BMDCs and LPS-induced mature BMDCs with OCILRP2-Fc treatment. 74% of immature BMDCs and 85% of mature BMDCs were CD11c positive, before purification. The expression level of CD11c in OCILRP2-Fc treated cells was

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