Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Orai1 is involved in leptin-sensitive cell maturation in mouse dendritic cells

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ARTICLE INFO

Article history:
Received 14 July 2018
Accepted 21 July 2018
Available online xxx

Keywords:
Leptin
Jak2
Orai1
DCs
SOCE

ABSTRACT

Store operated calcium entry(SOCE) is known to play a pivotal role in DCs functions including migration, maturation and antigen-presenting ability. Orai1, the major component of SOCE which mainly pairs with Stim1, is surely involved in the regulation of DCs functions. Leptin is recently found to mature DCs, we aim to evaluate the role of Orai1 in leptin-induced dendritic cells(DCs) maturation process and elucidate the mechanism. To this end, Flow cytometry and ELISA were utilized to detect the costimulatory molecule CD86 expression and IL-12 secretion, respectively. Transwell assay was used to examine DCs migration capacity. To evaluate the activity of SOCE, calcium(Ca^{2+}) imaging was performed. Firstly, we confirmed the positive effects of leptin upon SOCE and Orai1 expression in DCs isolated from mouse bone marrow. Secondly, we showed that the effects of leptin on DCs migration and maturation are Orai1 dependent. Moreover, Janus kinase 2(Jak2) silencing inhibited leptin-induced Orai1 expression and influenced DCs functions including migration and maturation as well as IL-12 secretion. In conclusion, our results imply that leptin regulates Orai1 by activating Jak2 signaling pathway, hence facilitating DCs migration and maturation.

1. Introduction

Dendritic cells(DCs) are powerful antigen-presenting cells and subdivided into conventional DCs of myeloid origin and plasmacytoid DCs of lymphoid origin. Once immature DCs sense danger signals and take up an antigen, they undergo a complete maturation process and transform to mature DCs. This transformation process contains the reduction of endocytic ability, antigen-presenting and costimulatory molecules expression, cytokines release and migration to draining lymph nodes [1]. Furthermore, DCs functions including DC activation, maturation, migration, and formation of immunological synapses with T cells are known to be governed by Ca^{2+} signaling [1–4].

In non-excitable cells, cytosolic Ca^{2+} influx is mainly regulated by Ca^{2+} release from intracellular stores and activation of store-operated Ca^{2+} entry(SOCE) due to subsequent intracellular stores depletion, which is also termed as Ca^{2+}-release activated Ca^{2+} channel(CRAC). SOCE is accomplished by the pore-forming Ca^{2+} channel subunits Orai1, Orai2 and Orai3 [5,6] as well as their regulators Stim1 and Stim2 [7]. Cytosolic Ca^{2+} concentration regulates various DC functions [8] including maturation [9] and induction of oxidative burst [4], SOCE activity is therefore sure subjected to impact on the orchestration of DC functions. All the Orai and Stim isoforms are confirmed in DCs [10]. As the major components of SOCC, Orai1/STIM1 exhibit the most prominent impact on SOCE and is critical for DC maturation [11,12].

Leptin is an adipocyte-derived cytokine that modulates immune responses via leptin receptors(LepRs) [13]. Among several isoforms of LepRs, LepRb has a long cytoplasm domain and definite signaling capacity through the Janus kinase (JAK)/STAT(signal transducer and activator of transcription factor) pathway [14]. Leptin induces the maturation of DCs along with concomitant facilitation to T cell proliferation and Th1 cytokine production [14–17]. But how does leptin promote DCs maturation?

To answer the question, we cultured DCs, examined the role of leptin on DCs migration and ascertained whether Orai1was involved in the process.

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https://doi.org/10.1016/j.bbrc.2018.07.108
0006-291X/

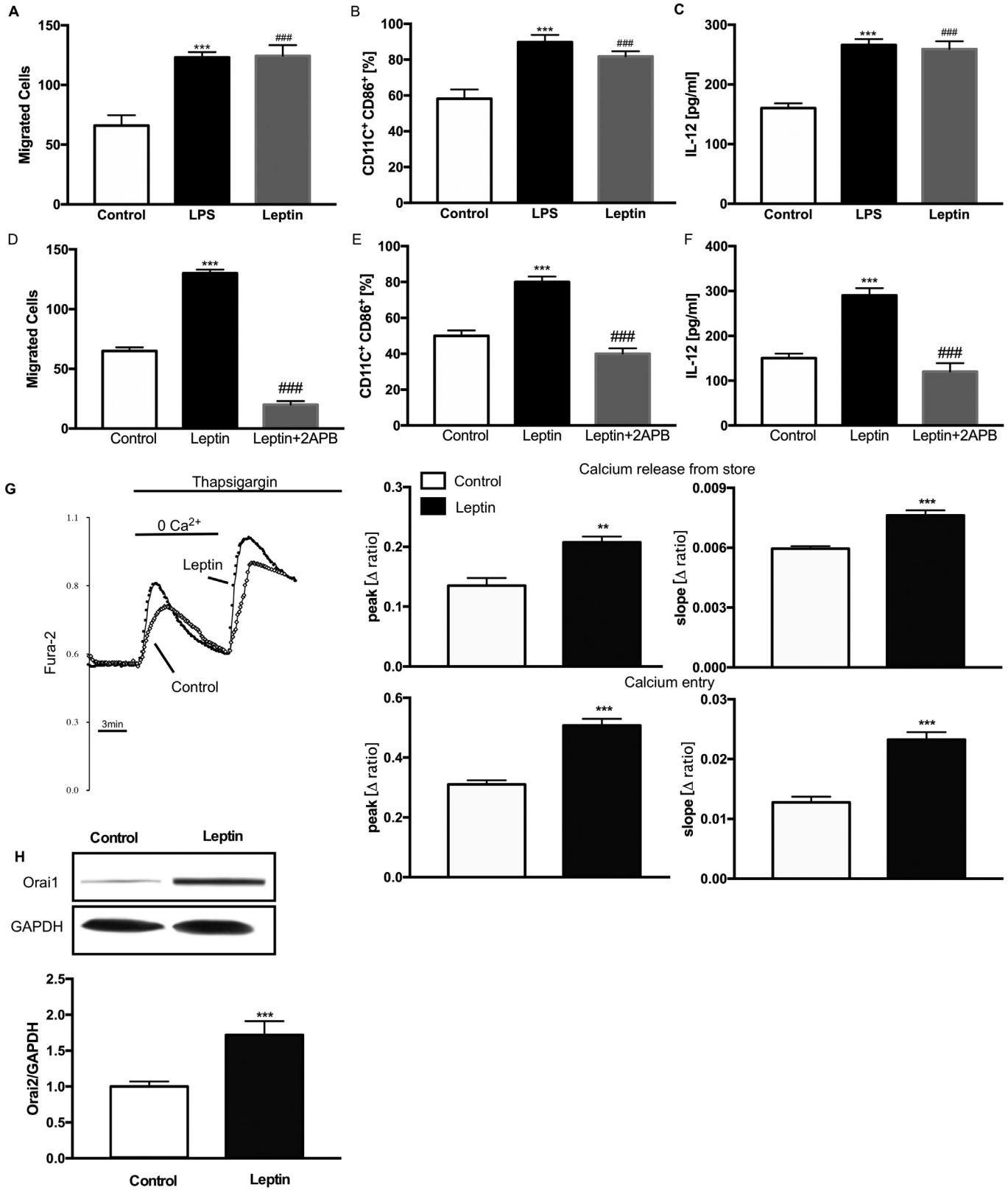

Fig. 1. Increased migration and maturation after administration of leptin. A. Arithmetic means ± SEM(n = 5) of the number of migrated DCs under control and leptin(100 nM)(12h) or LPS(1 μg/ml)(4h) administration. B. Arithmetic means ± SEM(n = 6) of the percentage of $CD11c^+CD86^+$ DCs under control and leptin(12h) or LPS(4h) administration. C. Arithmetic means ± SEM(n = 6) of IL-12 secretion in DCs with leptin(12h) or LPS treatment.*** ($p < 0.001$) indicates significant difference between control and LPS treatment.###($p < 0.001$) indicates significant difference between control and leptin treatment. D. Arithmetic means ± SEM(n = 5) of the number of migrated DCs with leptin(100 nM) treatment in the presence of 2-APB(50 μM)(12h). E. Arithmetic means ± SEM(n = 6) of the percentage of $CD11c^+CD86^+$ DCs with leptin(100 nM) treatment in the presence of 2-APB(50 μM) (12h). F. Arithmetic means ± SEM(n = 6) of IL-12 secretion in DCs with leptin(100 nM) treatment in the presence of 2-APB(50 μM) (12h). G. Representative tracings of fura-2 fluorescence-ratio in fluorescence spectrometry before and following extracellular Ca^{2+} removal and addition of thapsigargin(1 μM), as well as readdition of extracellular Ca^{2+} in DCs with or

Please cite this article in press as: J. Yan, et al., Orai1 is involved in leptin-sensitive cell maturation in mouse dendritic cells, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.07.108