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Dynamin related protein 1-dependent mitochondrial fission regulates oxidative signalling in T cells

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ABSTRACT

In T cells mitochondria-derived reactive oxygen species (ROS) are indispensible for activation of the transcription factor NF-κB, expression of cytokines and the CD95 ligand (CD95L/FasL). Here we show that activation-induced ROS generation is dependent on mitochondrial fission. Inhibition of dynamin related protein 1 (Drp1) results in reduced ROS levels and transcriptional activity of NF-κB leading to diminished proliferation and CD95L-dependent activation-induced cell death (AICD). Upon stimulation Drp1 is S-nitrosylated, which is required for oxidative signalling, AICD and cytokine production. In conclusion, we describe a novel signalling pathway that links TCR-induced nitric oxide release to mitochondrial fission and oxidative signalling.

1. Introduction

T cell receptor (TCR) triggering induces a signalling cascade that leads to T cell activation and activation-induced cell death (AICD) [1]. Upon TCR engagement phospholipase Cγ1 produces inositol-3,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 raises intracellular calcium levels and leads to translocation of NF-AT into the nucleus. DAG triggers the Ras-MAP kinase cascade inducing activation of AP-1 and, in parallel, activation of protein kinase Cθ and subsequently the NF-κB translocation into the nucleus [2]. In parallel, PKC induce a shift in cellular metabolism and finally leads to the release of reactive oxygen species (ROS) [3]. ROS are essential for complete activation of NF-κB and AP-1 [4]. Several sources of ROS in TCR signalling have been described [5,6]. However, accumulating reports suggest that the mitochondrial respiratory chain is the source for early ROS production in T cells [7–10]. ROS signalling and subsequent transcription factor activation are indispensable for expression of interleukin (IL)-2 and the death ligand CD95L (FasL), which induces CD95 (Fas)-dependent apoptosis in pre-activated T cells (AICD) [6,11–13].

Mitochondrial function has been linked to mitochondrial morphology before [14]. In several cell types ROS production was correlated with increased fission [15–17]. In these settings oxidative stress was causative for mitochondrial fragmentation. Therefore, fission might be a way to cope oxidative stress. However, Yu et al. reported that under hyperglycemic conditions mitochondria undergo dynamin related protein 1 (Drp1)-dependent fission, which resulted in increased ROS release, demonstrating that fission also supports ROS formation [18].

In conjunction with T cell activation several functions of Drp1 have been described. Triggering of the CXCL-12 receptor resulted in increased fission and cell motility [19], whereas Drp1 was reported to be essential for TCR recycling at the immunological synapse [20]. These functions of mitochondrial dynamics were linked to local provision of energy-consuming processes like actin remodelling with ATP. However, the function of mitochondrial fission during T cell activation in respect to ROS production has not been investigated.

Abbreviations: AICD, activation induced cell death; CFSE, carboxyfluorescein succinimidyl ester; DAG, diacylglycerol; Drp1, dynamin related protein 1; H_2DCF-DA, 2′,7′-dichlorodihydrofluorescein diacetate; IP_3, inositol 3,4,5-triphosphate; NAME, N^G-Nitroarginine methyl ester; NMMA, N^G-monomethyl arginine; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; ROS, reactive oxygen species; TCR, T cell receptor

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Here, we show a novel signalling pathway, which links TCR signalling to mitochondrial fission, ROS release, and T cell activation.

2. Material and methods

Material and methods are given in the Supplementary data.

3. Results

3.1. Mitochondria undergo fission upon T cell stimulation

Mitochondrial fission was described to be a prerequisite for ROS production [18]. Therefore, we asked whether TCR stimulation results in Drp1-dependent mitochondrial fission. Jurkat cells were transfected with mitochondria-targeted YFP. Upon stimulation with agonistic CD3-engaging antibodies mitochondria showed a fragmented phenotype (Fig. 1A). Microscopic analysis of resting T cells was not possible due to the low numbers of clustered mitochondria (data not shown). Therefore, T cells were activated with PHA in the presence of IL-2. After TCR re-stimulation activated T cells showed increased mitochondrial fission (Fig. 1B and C). Determination of the form factor and aspect ratio of mitochondria further demonstrated mitochondrial fission upon stimulation (Fig. 1D and E).

Drp1 is the crucial factor for mitochondrial fission, which translocates from the cytosol to the outer mitochondrial membrane. Upon T cell stimulation with agonistic anti-CD3 antibodies an accumulation of Drp1 at mitochondria was observed indicating that elevated mitochondrial fragmentation occurs due to an increased fission rate rather than due to a decreased fusion rate (Fig. 1F).

3.2. Drp1 function is required for activation-induced ROS production and NF-κB activation

To investigate whether mitochondrial fission is required for activation-induced ROS generation in T cells we inhibited Drp1 by overexpression of dominant negative Drp1 (dnDrp1) [21] or by siRNA-mediated downregulation in Jurkat cells. ROS production was assessed using H_2DCF-DA. Expression of dnDrp1 or siRNA application reduced activation-induced ROS production (Fig. 2A and B). In addition, reduced ROS levels were observed, when primary T cells were stimulated with anti-CD3 antibodies in the presence of the Drp1 inhibitor Mdivi-1 (Fig. 2C).

ROS are essential for activation of NF-κB [4]. In line with reduced ROS production, overexpression of dnDrp1 (Fig. 2D), downregulation of Drp1 (Fig. 2E) or application of Mdivi-1 (Fig. 2F) led to diminished NF-κB activation as assessed with luciferase-reporter

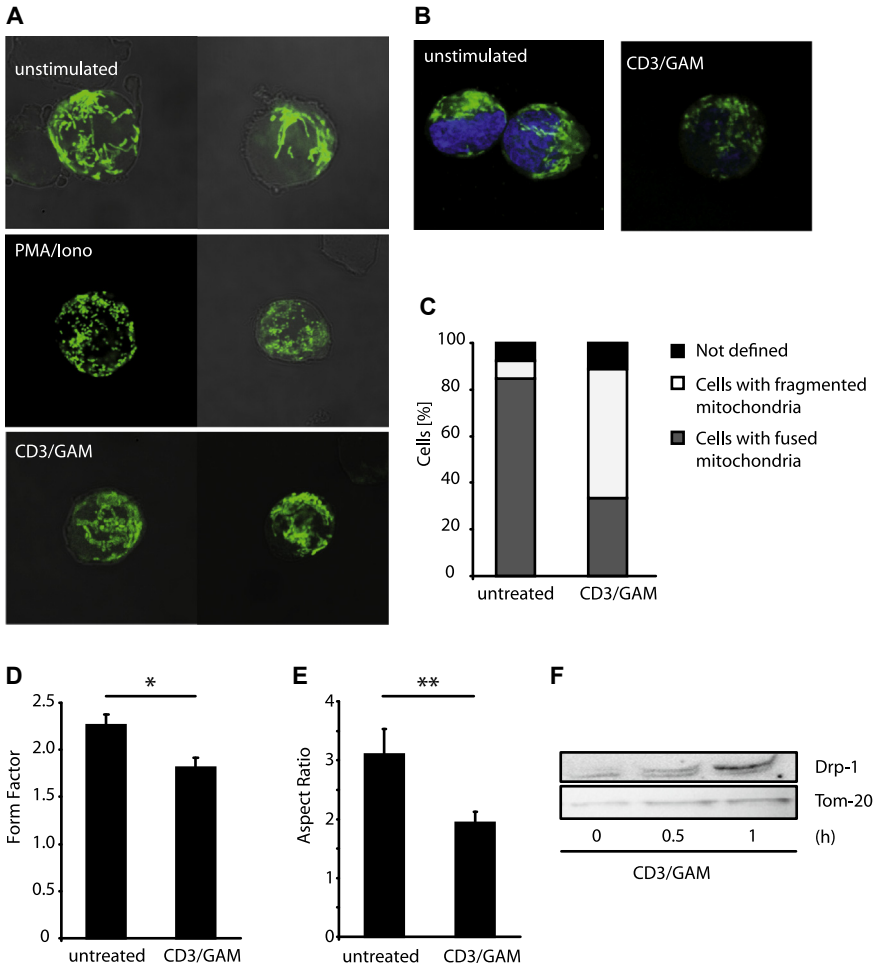

Fig. 1. Mitochondria undergo fission upon T cell stimulation. (A) Representative images of Jurkat cells transfected with mtYFP (upper panel) and stimulated for 45 min either with PMA (10 ng/ml) and iono (1 μM) (middle panel) or anti-CD3 and GAM (1 μg each, lower panel). (B–E) Pre-activated human T cells were stained with Mitotracker and DAPI before activation with anti-CD3 and GAM. (B) Representative image of unstimulated and stimulated pre-activated T cells. Cells with fragmented or fused mitochondria were counted in a blinded fashion (C) or analysed with ImageJ and form factor and aspect ratio were calculated (D,E). (F) T cells were stimulated with anti-CD3 and GAM (1 μg/ml each) for the indicated periods. Mitochondria were isolated by sorting with anti-Tom20 antibodies coupled to magnetic beads. Error bars represent S.E.M. ($^*P < 0.05$; $^{**}P < 0.001$, Student's *t*-test).