Journal of Neuroimmunology 219 (2010) 81–89

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Sphingomyelinase dependent apoptosis of dendritic cells following treatment with amyloid peptides

Nguyen Thi Xuan [a], Ekaterina Shumilina [a], Daniela S. Kempe [a], Erich Gulbins [b], Florian Lang [a,*]

[a] Department of Physiology, University of Tübingen, Gmelinstr. 5, D-72076 Tübingen, Germany
[b] Institute of Molecular Biology, University of Duisburg-Essen, Hufelandstrasse 55, 45122 Essen, Germany

ARTICLE INFO

Article history:
Received 8 July 2009
Received in revised form 9 November 2009
Accepted 2 December 2009

Keywords:
Ceramide
Phosphatidylserine
Cell death
IAPP
Amyloid β-peptide
$A\beta_{1-42}$

ABSTRACT

Amyloid peptides are formed during inflammation and modify the function of immune cells. The present study explored the effect of amyloid β-peptide ($A\beta_{1-42}$) and islet amyloid polypeptide (IAPP) on bone marrow derived dendritic cells (DCs). DCs were treated with $A\beta_{1-42}$ or IAPP with subsequent assessment of ceramide formation, caspase 8 and 3 activity, DNA fragmentation and phosphatidylserine exposure. In addition, TNFα secretion was assessed in lypopolysaccharide (LPS)-stimulated $A\beta_{1-42}$- or IAPP-treated DCs. Within 24 h $A\beta_{1-42}$ and IAPP triggered ceramide formation, caspase 8 and caspase 3 activation, DNA fragmentation and annexin V binding in DCs obtained from wild type mice, whereas in DCs from sphingomyelinase deficient ($asm^{-/-}$) mice and in wild type DCs treated with sphingomyelinase inhibitor amitriptyline all these effects were strongly impaired. Moreover, ceramide formation was also reduced in wild type DCs in which acid sphingomyelinase (Asm) was silenced with Asm-targeted siRNA. Finally, $A\beta_{1-42}$ and IAPP treatment was further followed by a decline of TNFα formation in wild type DCs. In conclusion, amyloid peptides induce DC apoptosis presumably through activation of acid sphingomyelinase resulting in production of ceramide.

1. Introduction

Amyloid fragments may be formed during inflammation, trigger the formation of pro-inflammatory cytokines and thus participate in the orchestration of inflammatory disease (Eikelenboom et al., 2008).

Amyloid precursor peptides, such as $A\beta_{1-42}$ fragment, have previously been shown to stimulate suicidal death in a variety of cells including neurons (Abdul et al., 2006; Huang and May 2006; Malaplate-Armand et al., 2006; Ran et al., 2006; Raynaud and Marcilhac 2006; Yu et al., 2006), endothelial cells (Donnini et al., 2006), neutrophils (Park et al., 2006), erythrocytes (Nicolay et al., 2007) and pancreatic β-cells (Konarkowska et al., 2006; Matveyenko and Butler 2006; Zhang et al., 2009). The amyloid peptides may be effective through the endoplasmatic reticulum (ER) stress pathway (Haataja et al., 2008; Huang et al., 2007), stimulation of Ca^{2+} entry (Park et al., 2006), calpain (Raynaud and Marcilhac 2006), oxidative stress (Abdul et al., 2006; Huang and May 2006; Ran et al., 2006) and ceramide formation (Malaplate-Armand et al., 2006). ER stress has been identified as an important mechanism inducing apoptosis in Alzheimer's disease, Parkinson's disease and type 2 diabetes (Haataja et al., 2008).

Islet amyloid polypeptide IAPP is coexpressed and cosecreted with insulin by pancreatic β-cells (Haataja et al., 2008). It has a direct paracrine effect on β-cells inhibiting insulin secretion (Ohsawa et al., 1989). In common with other amyloidogenic proteins, IAPP has the propensity to form membrane permeant toxic oligomers (Haataja et al., 2008). In type 2 diabetes β-cell loss is associated with abnormal aggregation of IAPP (Haataja et al., 2008). Human IAPP and amyloid β protein share structural properties and the prevalence of Alzheimer's disease is increased in individuals with type 2 diabetes (Janson et al., 2004).

Amyloid β peptides can bind to dendritic cells (DCs) (Schmitt et al., 1997), antigen-presenting cells essential for initiating and directing antigen-specific T-cell responses (Banchereau et al., 2000; Dubsky et al., 2005). DCs play a pivotal role in the control of tolerance needed to prevent the onset of autoimmunity (Adler and Steinbrink 2007; Banchereau et al., 2000; Dubsky et al., 2005). It is increasingly evident that the failure of DCs to maintain tolerance can lead to autoimmune and/or inflammatory diseases, such as type 1 diabetes (Lang et al., 2005; Uno et al., 2007; Yoon and Jun 2005). In type 2 diabetes, the frequency of peripheral DCs is diminished (Seifarth et al., 2008). DCs are involved in various neuroinflammatory disorders including multiple sclerosis, HAM/TSP (HTLV-I-Associated Myelopathy/Tropical Spastic Paraparesis), Alzheimer's disease and prion-associated diseases (Manuel et al., 2007). Selective ablation of bone marrow-

Abbreviations: DC, dendritic cells; IAPP, islet amyloid polypeptide; Asm, acid sphingomyelinase; $asm^{-/-}$, acid sphingomyelinase deficient mice; S1P, sphingosine-1-phosphate; IL, interleukin; $A\beta_{1-42}$, amyloid β-peptide; TNF, tumor necrosis factor.

* Corresponding author. Physiologisches Institut der Universität Tübingen, Gmelinstr. 5, D-72076 Tübingen, Germany. Tel.: +49 7071 29 72194; fax: +49 7071 29 5618.
E-mail address: florian.lang@uni-tuebingen.de (F. Lang).

0165-5728/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2009.12.002

derived DCs was shown to result in increased formation of amyloid plaques in a mouse model of Alzheimer's disease (Butovsky et al., 2007).

Amyloid β peptides induce the pro-inflammatory differentiation of DCs (Ciaramella et al., 2009). In contrast to the well documented proapoptotic effect of amyloid peptides on other cells, a stimulating effect of amyloid peptides on apoptosis of DCs has not been reported. Thus, the present study explored the effect of $A\beta_{1-42}$ and IAPP on DC apoptosis. It is shown that exposure of murine bone marrow derived DCs to amyloid peptides was followed by stimulation of caspase activity, DNA fragmentation, stimulation of phosphatidylserine scrambling of the cell membrane, all events typical for apoptosis (Green and Reed 1998; Gulbins et al., 2000). The events were preceded by ceramide formation, pointing to activation of acid sphingomyelinase (Asm). Thus, additional experiments have been performed in DCs from sphingomyelinase deficient mice.

2. Results

2.1. $A\beta_{1-42}$ or IAPP induce Asm-dependent ceramide formation in DCs

To determine the effect of amyloid peptides on the function of murine bone marrow derived dendritic cells (DCs), the cells were grown in GM-CSF containing media for 8 days and subsequently exposed to the $A\beta_{1-42}$ or IAPP for 24 h. As shown in Fig. 1, the exposure of wild type DCs to amyloid peptides was followed by stimulation of ceramide formation. For statistically significant effects on ceramide production 5 μM IAPP and 200 nM $A\beta_{1-42}$ were required (Fig. 1B, C). Ceramide formation was significantly decreased by treatment with LPS (100 nM, 24 h, Fig. 1B and C). Strongly reduced ceramide formation was observed in amyloid peptide-treated DCs from mice lacking functional acid sphingomyelinase ($asm^{-/-}$) (Fig. 1A, D) or in wild type cells treated with sphingomyelinase inhibitor amitriptyline (Fig. 1D). Moreover, the requirement of Asm in ceramide production was further confirmed in wild type DCs in which Asm expression was silenced by Asm siRNA (Fig. 1D). Efficiency of silencing measured in real-time PCR was about 50%. In these Asm siRNA cells, ceramide formation upon treatment with amyloid peptides was also suppressed (Fig. 1D).

2.2. Amyloid peptides enhance Asm-dependent DNA fragmentation

Further experiments were performed to elucidate the effect of $A\beta_{1-42}$ and IAPP on DNA fragmentation, one of the hallmarks of apoptosis. The exposure to amyloid peptides was followed by an increase of cells in the sub-G1 phase, a marker for fragmented DNA (Fig. 2). The effect reached statistical significance at the concentration of 200 nM $A\beta_{1-42}$ and 1 μM IAPP (Fig. 2B, C). In contrast, DCs derived from bone marrow of $asm^{-/-}$ mice or wild type amitriptyline-treated DCs were more resistant to stimulation of DNA fragmentation by amyloid peptides (Fig. 2A, D). Stimulation of the DCs with LPS significantly decreased DNA fragmentation (Fig. 2B, C).

2.3. Amyloid peptides lead to Asm-dependent caspase activation

The stimulation with $A\beta_{1-42}$ and IAPP was further followed by activation of caspase 8 and 3 as measured by FACS analysis and western blotting (Figs. 3, 4). Amyloid peptides at concentrations ≥200 nM $A\beta_{1-42}$ and ≥1 μM IAPP stimulated the caspases in wild type DCs and to a much less extent in DCs derived from bone marrow of $asm^{-/-}$ mice (Figs. 3, 4) or in amitriptyline-treated wild type DCs (Figs. 3D, 4D).

2.4. Amyloid peptides increase phosphatidylserine exposure at the cell membrane in an Asm-dependent way

As illustrated in Fig. 5, $A\beta_{1-42}$ and IAPP further increased annexin V binding, which reflects the phosphatidylserine exposure at the cell membrane. Accordingly, $A\beta_{1-42}$ and IAPP exposure was followed by scrambling of phospholipids in the cell membrane, a hallmark of apoptosis. Again, DCs derived from bone marrow of $asm^{-/-}$ mice or wild type amitriptyline-treated DCs were more resistant to stimulation of cell membrane scrambling (Fig. 5A, D).

2.5. Amyloid peptides decrease TNFα formation

To determine, whether amyloid peptides could affect the function of DCs, the release of TNFα was determined in LPS-stimulated (100 ng/ml, 4 h) DCs. As shown in Fig. 6, the exposure of wild type DCs to $A\beta_{1-42}$ (200 nM, 4 h) or IAPP (1 μM, 4 h) indeed decreased TNFα formation.

3. Discussion

The present study discloses a novel effect of amyloid peptides $A\beta_{1-42}$ and IAPP, i.e. the triggering of suicidal death of murine bone marrow derived dendritic cells (DCs). Obviously, the effect of amyloid peptides results from stimulation of acid sphingomyelinase (Asm) with subsequent formation of ceramide. Accordingly, genetic knockout of Asm abrogates the proapoptotic effect of $A\beta_{1-42}$ and IAPP. Ceramide has been shown to participate in the stimulation of cell death in a variety of cells including T-lymphocytes (Gulbins et al., 1997), hepatocytes (Lang et al., 2007), erythrocytes (Bentzen et al., 2007; Lang et al., 2006; Nicolay et al., 2006) and pancreatic beta cells (Newsholme et al., 2007). Moreover similar to our study, in pancreatic islet cells amyloid peptides have been recently shown to induce apoptosis via activation of Asm (Zhang et al., 2009). The sequence of events in amyloid peptide-induced apoptosis could be the following: DC stimulation with amyloid leads to translocation of Asm from an intracellular compartment onto the cell surface. This resulted in exposure of the Asm on the extracellular membrane leaflet and subsequent formation of ceramide. Ceramide formation results in caspase 8 autocatalysis that initiates apoptosis induction. Caspase 8 can then directly activate caspase 3. The activation of caspase 3 executes apoptosis by triggering DNA fragmentation and proteolysis of intracellular proteins.

In human DCs it was shown that ceramide can induce cell death in the absence of serum and that pharmacological inhibition of neutral/alkaline ceramidases, which leads to accumulation of ceramide, sensitizes DCs to ceramide-induced cell death (Franchi et al., 2006). Antigen uptake and presentation by DCs could be inhibited by exogenously added or endogenously produced ceramides (Sallusto et al., 1996). Sphingosine-1-phosphate (S1P), which is generated from ceramide by the consecutive actions of ceramidase and sphingosine kinase was found to be a counterplayer of ceramide which can potently induce cell proliferation (Huwiler and Pfeilschifter 2006). S1P was shown to mediate migration of mature murine DCs (Czeloth et al., 2005). Moreover, inhibiting sphingosine kinase suppressed a Th1 polarization via the inhibition of immunostimulatory activity in murine DCs (Jung et al., 2007). Thus, a balance between ceramide and S1P may be decisive for DC responsiveness.

Compelling evidence supports the importance of DC survival in the control of immune responses. Mature DCs are short-lived cells both *in vitro* and *in vivo*. The short life span of these cells could represent an important mechanism controlling the normal immune response and ensuring adequate space for the constant influx of fresh DCs loaded with different antigens (Josien et al., 2000; Seifarth et al., 2008). DCs with an increased lifespan can induce stronger immune responses and even autoimmunity (Josien et al., 2000; Wang et al., 1999). On the other hand, premature apoptosis in DCs could impair the nascent T cell-dependent response and thus weaken the defense against infectious disease. DCs appear to exhibit mechanisms that counterbalance apoptotic stimuli that otherwise efficiently induce apoptosis in macrophages. Thus, mature DCs are relatively resistant to the proapoptotic action of TNFα (Leverkus et al., 2000; Lundqvist et al., 2002) and CD95-mediated apoptosis (Ashany et