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Death or survival: Membrane ceramide controls the fate and activation of antigen-specific T-cells depending on signal strength and duration

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Abstract

Sphingomyelinase (SMase)-mediated release of ceramide in the plasma membrane of T-lymphocytes induced by different stimuli such as ligation of Fas/CD95, irradiation, stress, inflammation or anticancer drugs primarily involves mitochondrial apoptosis signaling, but under specific conditions non-apoptotic Fas-signaling was also reported. Here we investigated, using a quantitative simulation model with exogenous C2-ceramide (and SMase), the dependence of activation and fate of T-cells on the strength and duration of ceramide accumulation. A murine, influenza virus hemagglutinin-specific T-helper cell (IP12-7) alone or together with interacting antigen presenting B-cells (APC) was used. C2-ceramide induced apoptosis of T_H cells above a 'threshold' stimulus (>25 μM in 'strength' or >30 min in duration), while below the threshold C2-ceramide was non-apoptotic, as confirmed by early and late apoptotic markers (PS-translocation, mitochondrial depolarization, caspase-3 activation, DNA-fragmentation). The modest ceramide stimuli strongly suppressed the calcium response and inhibited several downstream signal events (e.g. ERK1/2-, JNK-phosphorylation, CD69 expression or IL-2 production) in T_H cells during both anti-CD3 induced and APC-triggered activation. Ceramide moderately affected the Ca^{2+}-release from internal stores upon antigen-specific engagement of TCR in immunological synapses, while the influx phase was remarkably reduced in both amplitude and rate, suggesting that the major target(s) of ceramide-effects are membrane-proximal. Ceramide inhibited Kv1.3 potassium channels, store operated Ca^{2+}-entry (SOC) and depolarized the plasma membrane to which contribution of spontaneously formed ceramide channels is possible. The impaired function of these transporters may be coupled to the quantitative, membrane raft-remodeling effect of ceramide and responsible, in a concerted action, for the suppressed activation. Our results suggest that non-apoptotic Fas stimuli, received from previously activated, FasL+ interacting lymphocytes in the lymph nodes, may negatively regulate subsequent antigen-specific T-cell activation and thus modulate the antigen-specific T-cell response.
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Keywords: Antigen specific T-cell activation; AICD; Cell death; Phospholipid death pathway; Ion channels; Rafts; Survival

1. Introduction

Ceramides are essential lipid components of all mammalian cell membranes, including T-lymphocytes [1–4], where they are often released dynamically from sphingomyelin upon activation of sphingomyelinases (SMase: neutral or acidic forms) by various external stimuli [5,6]. Among others, stress or receptor-mediated cell-death stimuli activate such local ceramide accumulation in T-lymphocytes. Ceramides were reported as mediators of apoptotic signaling [5–7] through the sphingolipid/mitochondrial death pathway, either as membrane-restructuring molecules [4,8,9] or second messengers [2–4]. Several other functions (e.g. regulation of cell proliferation or T-cell differentiation) were also devoted to ceramides, although many of them are still

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strongly debated [2]. Recent studies revealed some of their possible molecular targets in T-cell membranes during execution of the death signals, such as voltage-gated potassium channels [10,11] or the ion channels involved in calcium-release activated calcium current (I_{CRAC}) [12].

Fas/CD95 death receptor engagement by FasL can also generate ceramide release in the plasma membrane of activated T-cells. This interaction (either *cis* or *trans*) may result in apoptotic cell death eliminating the functionally unnecessary T-cells from the repertoire. The T-cell repertoire is also fine-tuned by the so-called activation-induced cell death (AICD) undergoing through Fas-dependent or Fas-independent pathways [13]. Fas-dependent AICD strongly depends on Fas- and FasL-expressions, as well as on the subsequent ceramide accumulation in the plasma membrane.

Recent 'in vivo imaging' data on intact lymph nodes [14–16] envisioned T-cells as highly motile cells very frequently forming short or long lived (antigen-dependent or antigen-independent) cell–cell contacts with many kinds of cellular constituents, such as dendritic cells, B-cells, other T-cells, etc. This allows us to speculate that T-cells may receive Fas-stimuli varying in strength and duration during these serial contacts made with APCs or other lymphocytes within lymph nodes. Similarly to the APC-mediated fine tuning of TCR-signaling [17], the response of T-cells to Fas/ceramide and their further fate may also be very sensitive to the stimulus strength and duration. Non-apoptotic [18] or costimulatory [19] signaling upon Fas engagement were reported recently under specific conditions. These data raise the questions how *Fas/ceramide signals* are integrated with *TCR-mediated activation signals* when received in costimulation and how the response of T-cells is influenced by the sequence of the two signals.

Therefore, in the present study we aimed to investigate how the strength and duration of Fas/ceramide stimulus influences the cellular response of mouse helper T-cells, using an influenza virus $HA_{317-329}$ peptide specific mouse T_H-hybridoma cell line (IP12-7) [20]. Since Fas-ligation was reported earlier to suppress TCR-mediated activation of Jurkat T-cells evoked by crosslinking with anti-CD3 mAb [21], we studied the effect of ceramide mediated signals on mouse helper T-cell activation in an immunological synapse (IS) model, where B-lymphoma antigen presenting cells (APC) with different costimulatory capacity [17] were compared. Finally, we also investigated how the AICD of mouse T_H cells is influenced by membrane ceramide under non-apoptotic conditions.

Our data revealed a double-faced effect of ceramide accumulation in the plasma membrane on the fate of T-cells. Depending on the strength and duration of the stimulus (exogenous C2-ceramide or SMase treatment) the ceramide mediated signals were either non-apoptotic or generated apoptosis. The non-apoptotic ceramide stimuli remodeled plasma membrane rafts accompanied with inhibition of several ion transport activities that in turn down-modulated TCR-mediated signaling and the T-cell response. This novel regulatory effect of ceramide on T-cell activation may have in vivo significance when the Fas and antigen signals are received in costimulation, in the lymph nodes.

2. Materials and methods

2.1. Cells

IP12-7 T-cell hybridoma of helper phenotype was developed from BALB/c mice pre-immunized with the $HA_{317-341}$ peptide and subsequently infected with the A/PR/8/34 human influenza A virus [20]. Two murine B-lymphoma cells 2PK3 (ATCC TIB203, IEd/Ed-Ad) and A20 (ATCC TIB208, I-Ad/Ed) were used as APCs. All cell lines were cultured in RPMI-1640 supplemented with 2 mM L-glutamine, 1 mM Na-pyruvate, 50 μM 2-mercaptoethanol, antibiotics and 5% FCS. The human Jurkat (ATCC TIB152) T-leukemia cell line was cultured in RPMI-1640 supplemented with 2 mM L-glutamine, 1 mM Na-pyruvate, antibiotics and 10% FCS.

2.2. Synthetic antigenic peptide, monoclonal antibodies and other reagents

The synthetic peptide $HA_{317-329}$, comprising the C-terminal region of the HA1 subunit of the human influenza virus A/PR/34/8 (H1N1 subtype) hemagglutinin (HA) was synthesized by the classical solid phase tBoc method. The peptide was purified by HPLC (peptide purity >96%) and characterized by amino acid analysis and mass spectroscopy.

The following monoclonal antibodies (mAb) were used in the study: murine anti-CD3 (145-2C11, hamster IgG, a generous gift of G. Laszlo), anti-Fas (Jo2, hamster IgG) and anti-CD69 (H1.2F3, hamster IgG) mAbs from Pharmingen (San Diego, CA). Phospho-ERK1/2 rabbit polyclonal antibody specific for pThr202/pTyr204 and pThr185/pTyr187 residues of ERK1 and ERK2, respectively, and protein A-peroxidase conjugate were purchased from Sigma-Aldrich (St. Louis, MO). The anti-pan-ERK mouse IgG2a monoclonal antibody was from Transduction Laboratories (UK), while the affinity-purified rabbit polyclonal antibody against Kv1.3 voltage-gated potassium channels was obtained from Alomone Labs (Israel). Polyclonal antibody specific for both the full length and the cleaved form of poly-(ADP-ribose) polymerase (PARP) was from Biomol Research Laboratories (Plymouth Meeting, PA). Phospho-SAPK/JNK (Thr183/Tyr185) and SAPK/JNK rabbit polyclonal antibodies were from New England Biolabs (Beverly, MA). Anti-ceramide antibody (MID-15B4) was obtained from Alexis (Lausen, Switzerland), while anti-CD48 (MEM102) and FITC-anti-IgM were generous gifts of V. Horejsi (Academy of Sciences of Czeh Republic) and G. Laszlo (Eötvös Lorand University, Budapest, Hungary), respectively.

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