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Disruption of *Serinc1*, which facilitates serine-derived lipid synthesis, fails to alter macrophage function, lymphocyte proliferation or autoimmune disease susceptibility

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ABSTRACT

During immune cell activation, serine-derived lipids such as phosphatidylserine and sphingolipids contribute to the formation of protein signaling complexes within the plasma membrane. Altering lipid composition in the cell membrane can subsequently affect immune cell function and the development of autoimmune disease. Serine incorporator 1 (SERINC1) is a putative carrier protein that facilitates synthesis of serine-derived lipids. To determine if SERINC1 has a role in immune cell function and the development of autoimmunity, we characterized a mouse strain in which a retroviral insertion abolishes expression of the *Serinc1* transcript. Expression analyses indicated that the *Serinc1* transcript is readily detectable and expressed at relatively high levels in wildtype macrophages and lymphocytes. The ablation of *Serinc1* expression in these immune cells, however, did not significantly alter serine-derived lipid composition or affect macrophage function and lymphocyte proliferation. Analyses of *Serinc1*-deficient mice also indicated that systemic ablation of *Serinc1* expression did not affect viability, fertility or autoimmune disease susceptibility. These results suggest that *Serinc1* is dispensable for certain immune cell functions and does not contribute to previously reported links between lipid composition in immune cells and autoimmunity.

1. Introduction

The mobilization of protein receptors and adaptors into appropriate signaling complexes within plasma membranes is critical for activating and regulating different cellular processes (Lingwood and Simons, 2009; Simons and Sampaio, 2011; Sonnino and Prinetti, 2013). Lipids also contribute to the formation of these signaling complexes (Chiantia and London, 2013; Ramstedt and Slotte, 2006; Róg and Vattulainen, 2014). In particular, lipids and protein signaling complexes can form ordered membrane domains, which have been shown to be important for stabilizing signaling events that mediate immune cell activation and function (Dykstra et al., 2003; Szöor et al., 2010; Varshney et al., 2016). In macrophages, recognition of lipopolysaccharide (LPS) by toll-like receptor (TLR) 4 can induce clustering of TLR4 to ordered membrane domains where

Abbreviations: *Serinc*/SERINC/*SERINC*, serine incorporator; TDE2, tumor differentially expressed 2; BM-MΦs, bone marrow derived macrophages; gt, gene-trap; ANA, anti-nuclear antibodies; LPS, lipopolysaccharide.

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it interacts with CD14 to recruit downstream signaling molecules (Fessler and Parks, 2011; Płóciennikowska et al., 2015; Triantafilou et al., 2002; Zhu et al., 2010). In lymphocytes, ordered membrane domains and lipids have been shown to stabilize or enhance T-cell receptor (TCR) or B-cell receptor (BCR) signaling that promotes cell responses (Dinic et al., 2015; Gupta and DeFranco, 2007; Miguel et al., 2011; Rentero et al., 2008; Wu et al., 2015; Zech et al., 2009). Indeed, immune cells isolated from patients with autoimmune diseases, such as systemic lupus erythematosus (SLE), have been shown to have altered activation thresholds associated with altered localization of signaling molecules within the plasma membrane (Flores-Borja et al., 2007; Jury et al., 2004; Kabouridis and Jury, 2008; Krishnan et al., 2004; Michel and Bakovic, 2007). Although the underlying mechanisms are still debated, one important component of such ordered membrane domains is serine-derived lipids (Chiantia and London, 2013; Ramstedt and Slotte, 2006; Róg and Vattulainen, 2014). In particular, serine incorporator 1 (SERINC1) was reported to localize to the plasma membrane and facilitate the synthesis of serine-derived lipids including phosphatidylserine and sphingolipids (Grossman et al., 2000; Inuzuka et al., 2005). We therefore postulated that the disruption of *Serinc1* gene expression would alter the levels of serine-derived lipids in the plasma membrane, leading to abnormal immune cell function that affects the development of autoimmune disease in mice.

SERINC1 (also known as TMS-2 and TDE2) belongs to a family of putative carrier proteins, which consists of five members that are highly conserved among eukaryotes, with mammalian SERINC1-5 sharing approximately 31–58% amino acid homology (Bossolasco et al., 2006; Grossman et al., 2000; Inuzuka et al., 2005; Krueger et al., 1997; Ren et al., 2014). The five members are predicted to have 11 transmembrane domains with a conserved myc-type helix-loop-helix dimerization domain (Inuzuka et al., 2005). Functionally, SERINC proteins are proposed to facilitate the synthesis of phosphatidylserine and sphingolipids, which are essential membrane lipids that require the amino acid serine (Inuzuka et al., 2005; Merrill, 2011; van Meer and Lisman, 2002; Voelker, 1990). Serine, however, is synthesized in the cytosol by 3-phosphoglycerate dehydrogenase. Due to their unique 11 transmembrane domains, SERINC proteins are predicted to act as a carrier for serine, a polar and hydrophilic amino acid, into lipid bilayer membranes (Inuzuka et al., 2005). In addition, SERINC proteins are predicted to act as scaffold proteins for the two enzymes, phosphatidylserine synthase and palmitoyltransferase, that synthesize phosphatidylserine and sphingolipids, respectively (Inuzuka et al., 2005). In this way, SERINC proteins are proposed to bring together both the substrate and the lipid synthetic enzymes within the lipid membrane bilayer. While direct evidence for this complex mechanism is currently lacking, overexpression of either rat *Serinc1*, *Serinc2* or *Serinc5* in mammalian COS cells can enhance synthesis of phosphatidylserine and sphingolipids indicating that SERINC proteins are able to affect serine-derived lipid synthesis (Inuzuka et al., 2005). However, a role for SERINC1 in serine-derived lipid synthesis has yet to be demonstrated in immune cells.

To investigate *Serinc1* in immune cells, we took advantage of a previously established mouse strain deficient for *Serinc1* expression on a mixed genetic background derived from the C57BL/6 and 129S5 mouse strains (B6;129S5-*Serinc1*$^{Gt(OST454985)Lex}$, henceforth termed B6;129S5-*Serinc1*$^{gt/gt}$ (Tang et al., 2010)). This *Serinc1*-deficient mouse strain was initially generated as part of an effort to establish and characterize a mouse knockout library of secreted and transmembrane proteins (Tang et al., 2010). Specifically, Tang et al. subjected >400 knockout mouse strains to a systematic screen designed to test different phenotypes, including some immune system abnormalities. Their screen suggested that B6;129S5-*Serinc1*$^{gt/gt}$ mice had no gross immunological defects (Tang et al., 2010). These initial studies, however, did not investigate membrane lipid composition and were limited out of necessity to a few immunological parameters using peripheral blood and small cohort sizes, which may have missed specific immune cell effects and immune-related disease pathology.

In this study, we confirmed that the *Serinc1* transcript is expressed in immune cells and conducted additional analyses of the B6;129S5-*Serinc1*$^{gt/gt}$ mouse strain. Following confirmation that these mutant mice lack expression of *Serinc1*, we measured different immune cell phenotypes to determine if *Serinc1* deficiency affects immune cell numbers or function. Interestingly, the loss of *Serinc1* expression did not significantly change the membrane lipid composition in macrophages, T cells or B cells, nor did these *Serinc1*-deficient immune cells demonstrate altered functional responses when activated. Moreover, *Serinc1*-deficient mice did not exhibit altered susceptibility to developing autoimmune disease. Other SERINC family members have been shown to facilitate synthesis of serine-derived lipids (Inuzuka et al., 2005) and may compensate for the loss of *Serinc1* in this mutant mouse strain. Our study represents the first report for which a member of this gene family is directly investigated in mouse immune cells and demonstrates that disruption of *Serinc1* expression alone is unlikely to grossly affect immune cell function or the development of autoimmune disease.

2. Materials and methods

2.1. Animals

The B6;129S5-*Serinc1*$^{gt/gt}$ mouse strain was obtained from the Mouse Knockout Consortium. This mouse strain was generated by a gene trap strategy (*via* retroviral insertion) on a mixed B6;129S5 genetic background (Tang et al., 2010). Briefly, 129S5 ES cell clones containing a gene trap mutation in *Serinc1* were microinjected into C57BL/6J-*Tyrc-Brd* blastocysts. Resulting chimeric offspring were backcrossed with C57BL/6J-*Tyrc-Brd* females to produce F_1 heterozygous males. Sperm obtained from male F_1 heterozygous were frozen and archived. Recovery of F_1 heterozygotes was achieved using C57BL/6 females. Wildtype and mutant *Serinc1* mice were generated from the same B6;129S5-*Serinc1* colony. Experiments used either mice that were littermates or mice generated from a number of breeding pairs within our B6;129S5-*Serinc1* colony in order to establish larger cohorts. Mice were bred and maintained in specific pathogen-free conditions at St Vincent's Institute and experiments approved by the institutional animal ethics committee.

2.2. Genotyping of the B6;129S5-Serinc1 mouse strain

Genomic DNA was extracted from tail biopsies by standard methods. PCR-based genotyping was performed using the following oligos: Serinc1Gt F (5′- AAGCTTCATTGCTTTGGGTGAGGT), Serinc1Gt R (5′-TCTCAATGGAATTGCTGTGTGCCC), Neo F (5′-ATGCCCGACGGCGATGATCT) and Neo R (5′-CGGGAGCGGCGATACCGTAA). Amplicon products are visualized *via* standard agarose gel electrophoresis methods.

2.3. Gene expression analyses

Total RNA was isolated from tissues using TRIzol reagent (Invitrogen) and from immune cell subsets using RNeasy Mini Kit (Qiagen). Total RNA was treated with DNase (Qiagen) and cDNA was synthesized using Superscript III reverse transcriptase (Invitrogen). qPCR analysis was used to quantitate *Serinc* family gene expression. Ct values were normalized using two reference genes (*Hprt* and *Hmbs*). Standard curves were generated for all primer sets to ensure exponential increase of targeted transcripts during amplification (efficiency = $10(-1/\text{slope}) = \sim 2$). ΔCt for each tissue was