Original Paper

SGLT1 Deficiency Turns Listeria Infection into a Lethal Disease in Mice

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Key Words

Glucose uptake • Na⁺-coupled glucose transport • Listeria infection • TNF-α and IL-12a • Survival, •Liver • Spleen • Kidney • Lung • Bacterial clearance

Abstract

Background: Cellular glucose uptake may involve either non-concentrative glucose carriers of the GLUT family or Na⁺-coupled glucose-carrier SGLT1, which accumulates glucose against glucose gradients and may thus accomplish cellular glucose uptake even at dramatically decreased extracellular glucose concentrations. SGLT1 is not only expressed in epithelia but as well in tumour cells and immune cells. Immune cell functions strongly depend on their metabolism, therefore we hypothesized that deficiency of SGLT1 modulates the defence against bacterial infection. To test this hypothesis, we infected wild type mice and gene targeted mice lacking functional SGLT1 with *Listeria monocytogenes*. **Methods:** SGLT1 deficient mice and wild type littermates were infected with 1x10⁴ CFU *Listeria monocytogenes* intravenously. Bacterial titers were determined by colony forming assay, SGLT1, TNF-α, IL-6 and IL-12a transcript levels were determined by qRT-PCR, as well as SGLT1 protein abundance and localization by immunohistochemistry. **Results:** Genetic knockout of SGLT1 (*Slc5a1*^{-/-} mice) significantly compromised bacterial clearance following *Listeria monocytogenes* infection with significantly enhanced bacterial load in liver, spleen, kidney and lung, and significantly augmented hepatic expression of TNF-α and IL-12a. While all wild type mice survived, all SGLT1 deficient mice died from the infection. **Conclusions:** SGLT1 is required for bacterial clearance and host survival following murine Listeria infection.

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Introduction

Bacterial growth depends on availability of nutrients such as glucose and is augmented by hyperglycemia [1-8]. Extracellular glucose concentration could be lowered by cellular uptake and utilization of glucose. Glucose uptake could be accomplished by Na⁺-coupled glucose carriers SGLT1 and SGLT2 [9]. Unlike the facilitative glucose carriers of the GLUT family [10-12], the Na⁺-coupled glucose carriers can transport glucose against a chemical gradient and accomplish cellular glucose accumulation even at low extracellular glucose concentrations [9]. SGLT1 is expressed in intestinal epithelial cells and SGLT1 as well as SGLT2 in renal epithelial cells thus accomplishing the concentrative cellular uptake of glucose from the intestinal or renal tubular lumen across the apical cell membrane [9, 13]. The low luminal glucose concentrations provide some protection against luminal bacterial growth and genetic or pharmacological inhibition of the carriers may favor the development of urinary tract infection [14, 15] and diarrhea [16, 17]. SGLT1 expression is, however, not restricted to renal and intestinal epithelia [18], but has also been observed in heart [18, 19], lung [18], liver [18], tumor cells [20-27] and lymphocytes [28]. The expression of SGLT1 in lymphocytes prompted us to speculate that SGLT1 expression could modulate immune function during bacterial infection.

In the present study, we explored whether SGLT1 participates in the orchestration of anti-bacterial defense. To this end, SGLT1 deficient mice and their wild type littermates were intravenously infected with 1×10⁴ CFU *Listeria monocytogenes*, and bacterial load, inflammatory cytokines, and survival determined.

Materials and Methods

Mice

The study was performed in age matched male SGLT1 deficient (*Slc5a1*^{-/-}) and wild type (*Slc5a1*^{+/+}) mice on C57BL/6J background. All animals were housed in single-ventilated cages in a controlled facility. Animal experiments were authorized by the Nordrhein Westfalen Landesamt für Natur, Umwelt und Verbraucherschutz (Recklinghausen, Germany), and in accordance with the German law for animal protection or according to institutional guidelines at the Ontario Cancer Institute of the University Health Network and at McGill University.

Immunostaining and microscopy

Immunohistochemistry in mouse tissue cryosections of spleen, small intestine and liver was performed using previously described protocols [16, 29, 30]. In short, tissue samples were fixed with 4% *p*-formaldehyde, rinsed, and cut in 4 μm thick cryosections which underwent antigen unmasking steps that included a microwave heating in 10 mM citrate buffer (pH 6.0), followed by incubation with antibodies. The noncommercial rabbit-raised polyclonal mSgt1 antibody [16] was used diluted 1:50 at 4°C overnight, followed by a commercial secondary antibody (Cy3-labeled goat anti-rabbit IgG, Jackson ImmunoResearch Laboratories, USA; dilution 1:800). The stained cryosections were covered with Vectashield (Vector Laboratories, USA), and inspected under the fluorescence microscope OPTON III RS (Opton Feinttechnik, Germany). The images were taken with the attached SPOT RT digital camera and software (Diagnostic Instruments, USA), imported and processed in Adobe Photoshop. Additional immunofluorescent staining and histological analysis was performed on snap-frozen tissue as previously described [31]. In brief, sections were fixed with acetone for 10 min, and non-specific antigens were blocked in phosphate-buffered saline (PBS) containing 2% FCS for 10 min, followed by various staining's for 45 min. Images were acquired with a fluorescence microscope (KEYENCE BZ II analyzer).

Isolation of total RNA, reverse transcription and qRT-PCR

Total RNA from tissue samples was isolated by using TRIzol (Ambion, Thermo Fisher Scientific), reverse transcribed into cDNA using QuantiTect Reverse Transcription Kit (Qiagen). Quantitative real-time PCR was done by using SYBR green master mix (Applied Biosystems, Darmstadt, Germany) and analyzed by Roche light-cycler 480. For expression analysis of target genes, expression levels were normalized to GAPDH

mRNA and calculated using the ΔC_t method. The following SYBR green primers were used purchased from Qiagen:

SGLT1:	QT00112679
GAPDH:	QT01658692
TNF α :	QT00104006
IL-6:	QT00098875
IL-12a:	QT01048334

Listeria infection

SGLT1 deficient mice and littermate controls were infected intravenously by 1×10^4 CFU *Listeria monocytogenes* and followed during the course of infection. Mice were sacrificed 48 hours later and organs were frozen in tissue-tek for immunohistological analysis. Liver sections were cut at 8 μ m in cryotome and stained using anti-*Listeria* (clone LZA2, Santa Cruz) followed by secondary anti-mouse FITC-labeled IgG (Jackson ImmunoRes.) and Ly-6G and F480 for granulocytes and macrophages, respectively. Images were taken at fluorescent digital microscope (Keyence BZ 9000).

For detection of bacterial load, colony forming assay was done in 24-well plates on brain-heart infusion agar. Organs were harvested 4 days post-infection, homogenized, diluted and plated on the agar plates. The plates were inoculated at 37°C for bacterial plaques, which were counted manually.

Statistical analysis

Data are provided as means $\pm$ SD, n represents the number of mice investigated. All experiments were done with at least 3 mice per group. Data were tested for significance using parametric unpaired Student t-test unless specified. Results with $p < 0.05$ were considered statistically significant.

Results

SGLT1 expression in liver, spleen and intestine

Quantitative RT-PCR was employed in order to define SGLT1 transcript levels in murine liver, spleen and small intestine (jejunum). In naïve mice, the SGLT1 transcript levels were in liver and spleen orders of magnitude smaller than those in intestine (Fig. 1A). Expression of SGLT1 was confirmed by immunohistochemistry (Fig. 1B). As expected, no appreciable staining was observed in tissues from SGLT1 deficient mice.

SGLT1 sensitive bacterial clearance following *Listeria monocytogenes* infection

As illustrated in Fig. 2A-D, the bacterial titers in liver, spleen, kidney and lung were significantly higher in SGLT1 deficient mice (*Slc5a1*^{-/-}) than in wild type mice (*Slc5a1*^{+/+}). The observations were again confirmed by immunohistochemistry illustrating an enhanced abundance of *Listeria monocytogenes* in *Slc5a1*^{-/-} mice as compared to *Slc5a1*^{+/+} mice (Fig. 2E). While foci of replicating *Listeria* were highly infiltrated by granulocytes in *Slc5a1*^{+/+}

Fig. 1. SGLT1 expression in liver, spleen and small intestine. A: Arithmetic means $\pm$ SD (n = 3/group) of SGLT1 transcript levels in naïve mice showing its maximum expression in small intestine (SI) as compared to spleen and liver. B: Immunohistochemistry (n = 3/group) of SGLT1 protein in spleen, jejunum (Small intestine) and liver of wild type (WT) and SGLT1 deficient (SGLT1^{-/-}) mice, 48 hrs post-infection with 1×10^4 CFU *Listeria monocytogenes* intravenously. In WT mice, the protein was immune-localized to the brush-border membrane of jejunal enterocytes and luminal membrane of bile ducts, but not in spleen. The staining was absent in SGLT1^{-/-} mice. Bar, 20 μ m (for all images).

Fig. 2. Impairment of bacterial clearance in SGLT1 deficient mice. A-D: Bacterial titers in (A) liver, (B) spleen, (C) kidney and (D) lung of wild type mice (closed boxes) (n=4) and SGLT1 deficient mice (open boxes) (n=5), as measured by colony forming assay, 96 hrs after 1×10^4 CFU *Listeria monocytogenes* infection. E: Immunohistochemical staining of monocytes forming granulomas in liver sections of control and SGLT1 deficient mice, 48 hrs (n=3/group) and 96 hrs (control n = 4, SGLT1 deficient n=6) post-infection with *Listeria monocytogenes* representing less granulomas and thus bacterial clearance in control mice. F: Arithmetic means $\pm$ SD (n = 3/group) of granulomas quantified by ImageJ analysis 48 hrs post-infection with *Listeria monocytogenes* in WT and SGLT1 deficient mice (F480 = F480 positive macrophages, Ly-6G = Ly-6G positive granulocytes). *(p<0.01), ***(p<0.0001) indicate significant difference between SGLT1 deficient mice and wild type mice.

mice, *Slc5a1*^{-/-} mice showed only limited granulocyte infiltration in relation to *Listeria* growth (Fig. 2E). Therefore *Slc5a1*^{-/-} mice showed enhanced numbers of granuloma, which contain massive *Listeria* but little granulocytes (Fig. 2F).

Anti-bacterial response in wild type and SGLT1 deficient mice

Following infection with *Listeria monocytogenes* the hepatic TNF- α and IL-12 α transcript levels increased to significantly higher values in *Slc5a1*^{-/-} mice than in *Slc5a1*^{+/+} mice. The transcript levels of IL-6 similarly tended to increase to higher values in *Slc5a1*^{-/-} mice than in *Slc5a1*^{+/+} mice, a difference, however, not reaching statistical significance. In the spleen, TNF- α transcript levels increased again to significantly higher values in *Slc5a1*^{-/-} mice than in *Slc5a1*^{+/+} mice, whereas the splenic IL-6 and IL-12 α transcript levels were similar in *Slc5a1*^{-/-} mice and in *Slc5a1*^{+/+} mice (Fig. 3).

Survival of wild type and SGLT1 deficient mice following a *Listeria monocytogenes* infection

As illustrated in Fig. 4A, all *Slc5a1*^{+/+} mice survived the *Listeria monocytogenes* infection, whereas all *Slc5a1*^{-/-} mice died by

Fig 3. Anti-bacterial response in wild type and SGLT1 deficient mice. A-C: Arithmetic means $\pm$ SD of transcript levels of (A) TNF α , (B) IL-6, and (C) IL-12 α in liver (left panel) and spleen (right panel) in WT (black bars) and SGLT1 deficient mice (white bars), 48 hrs post-infection with 1×10^4 CFU *listeria monocytogenes* intravenously (n=3/group). *(p<0.01), ***(p<0.001) indicate significant difference between SGLT1 deficient mice and wild type mice.

Fig. 4. Lethal course of *listeria monocytogenes* infection in SGLT1 deficient mice. A: Survival analysis of wild type mice and SGLT1 deficient mice after intravenous infection with 1×10^4 CFU *Listeria monocytogenes*. While 100% of SGLT1 deficient mice died from the infection, all wild type mice survived (n = 7/group). B: Survival analysis of WT (*Slc5a1*^{+/+}) mice maintained on normal or low-glucose diet after 1×10^4 CFU *listeria monocytogenes* intravenous infection (n = 4/group). **($p < 0.001$) indicate significant difference between SGLT1 deficient mice and wild type mice (Mantel-cox test).

day five from the infection. As *Slc5a1*^{-/-} mice lack SGLT1, they were maintained on low-glucose diet. To test whether the diet has any effect on outcome of *listeria* infection, we maintained *Slc5a1*^{+/+} mice on either control diet or low-glucose diet for 3 weeks and then subjected the animals to 1×10^4 CFU *Listeria monocytogenes* infection. As a result, the infection outcome in *Slc5a1*^{+/+} mice was not influenced by the diet, i.e. all *Slc5a1*^{+/+} mice on regular diet (n = 4) and low-glucose diet (n = 4) survived the infection (Fig. 4B). Thus, the different diets did not account for the impaired anti-bacterial response following *Listeria* infection in *Slc5a1*^{-/-} mice.

Discussion

The present study demonstrates for the first time that the high affinity Na⁺ coupled glucose transporter SGLT1 participates in the orchestration of the host defense against a *Listeria monocytogenes* infection. Following systemic infection with *Listeria monocytogenes* the pathogen enters the liver, the main target organ of *Listeria* [32]. The pathogen is similarly found in the spleen and, to a lesser extent, in kidney and lung. More importantly, lack of SGLT1 leads to excessive bacterial growth, and enhanced formation of granulomas. SGLT1 deficiency thus has a striking influence on animal survival following *Listeria monocytogenes* infection. While all wild type mice survived, all SGLT1 deficient mice died from the infection.

The present observations raise the question, how the host cell SGLT1 expression and function is critically important for the limitation of bacterial load. SGLT1 may be critically important for the function and survival of the major anti-bacterial innate immune cells, granulocytes and macrophages. Obviously, these innate immune cells require glucose degradation by glycolysis for ATP generation [33, 34]. ATP is mainly generated from glycolysis under aerobic and anaerobic conditions [35, 36]. Lack of SGLT1 may thus compromise glucose uptake, function and survival of granulocytes entering infected tissue with low extracellular glucose concentration. In addition, metabolism can regulate immune functions [37, 38]. Therefore lack of SGLT1 might induce an oxidative metabolism in granulocytes which could limit their function and survival [39]. At least in theory, SGLT1 expression in host cells may further influence the growth of bacteria by lowering the extracellular glucose concentration in areas of bacterial growth. SGLT1 accomplishes uphill transport of glucose, driven by the steep electrochemical gradient of Na⁺ across the cell membrane [9]. Low extracellular glucose concentrations were expected to compromise bacterial growth and survival. Thus, the present observations could reflect SGLT1-dependent clearance of glucose from extracellular fluid curtailing nutrient supply of the pathogen. Further possibilities cannot be ruled out. In any case, the present observations reveal that SGLT1 is critically important for the host defense against *Listeria monocytogenes*.

Cellular glucose uptake by Na⁺-coupled requires subsequent ATP-consuming extrusion of the co-transported Na⁺ by the Na⁺/K⁺ ATPase. Energy depletion impairs extrusion of the co-

transported Na⁺ leading to cellular K⁺ loss, depolarization, Cl⁻ entry, cellular accumulation of NaCl with osmotically obliged water and thus cell swelling [40]. As Na⁺/K⁺ ATPase extrudes 3 Na⁺ ions for one ATP, the energy required for extrusion of co-transported Na⁺ is, however, only a fraction of the energy gained by glucose degradation, even if glucose is utilized for ATP generation by glycolysis without oxidative metabolism.

The present observations raise a potential caveat in the use of SGLT1 inhibitors for the treatment of diabetes and cancer [17, 41]. The inhibitors may, similar to genetic knockout of SGLT1, compromise the host defense against bacteria and thus lead to lethal infections. Whether humans are similarly sensitive to lack of SGLT1 as mice, however, remains to be shown.

In conclusion, during *Listeria monocytogenes* infection SGLT1 apparently plays a decisive role in the host defense against this pathogen. Accordingly, SGLT1 deficiency leads to excessive bacterial load in several organs and turns *Listeria monocytogenes* infection into a lethal disease.

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Disclosure Statement

None.

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