Lst1 deficiency has a minor impact on course and outcome of the host response to influenza A H1N1 infections in mice

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Abstract

Background: Previously, we performed a quantitative trait locus (QTL) mapping study in BXD recombinant inbred mice to identify host genetic factors that confer resistance to influenza A virus infection. We found Lst1 (leukocyte specific transcript 1) as one of the most promising candidate genes in the Qivr17-2 locus because it is non-functional in DBA/2 J mice. Several studies have proposed that LST1 plays a role in the immune response to inflammatory diseases in humans and has additional immune-regulatory functions. Here, we evaluated the relevance of LST1 for the host response to influenza A infection in B6-Lst1−/− mutant mice.

Findings: To investigate the role of LST1, we infected B6-Lst1−/− mutant and C57BL/6 N wild-type mice with a low-virulent influenza A virus (PR8M; H1N1). Lst1 deficient mice exhibited significantly increased body weight loss at days 5 and 6 after infection and slightly increased lethality compared to infected wild-type mice. Determination of viral loads, histopathological examination and analysis of immune cell composition in bronchoalveolar lavage of infected lungs did not reveal any obvious differences between KO and wild-type mice.

Conclusions: The absence of Lst1 leads to a slightly more susceptible phenotype. However, deletion of Lst1 in DBA/2 J mice alone does not explain the high susceptibility of this strain to PR8M influenza infections.

Keywords: Influenza A virus, Lst1, KO mouse mutant, Animal model

Findings

Each year, about 500 million people are infected by influenza A virus worldwide, of which about 500,000 die. We and others showed that the genetic background strongly influences the course and outcome of influenza A virus infections in different mouse inbred strains [1–4]. To identify genes that influence resistance or susceptibility to influenza A infections, we performed a genome-wide quantitative mapping study using the BXD recombinant inbred strains derived from C57BL/6 J and DBA/2 J. When infected with a low virulent isolate of PR8 influenza A virus (designated PR8M), DBA/2 J mice are highly susceptible and die within 5–7 days post infection (p.i.), whereas C57BL/6 J mice survive [1, 5]. We found about 30 candidate genes across five QTL regions [2]. One of the most promising candidates in the Qivr17-2 (quantitative trait for influenza virus resistance on chromosome 17) locus was the leukocyte specific transcript 1 (Lst1). The human gene LST1 and its mouse homologue Lst1, formerly described as B144 [6], are located in the MHC class III locus encoding numerous genes involved in the immune response [7, 8]. Transcripts are most abundant in immune cells, especially B cells, T cells, monocytes and dendritic cells [9]. Lst1 expression has been shown to be up-regulated by inflammatory stimuli [8, 10]. DBA/2 J mice exhibit a deletion in the Lst1 which results in a translational frame shift that most likely causes a premature stop codon and thus results in a truncated, non-functional protein [2]. Expression of Lst1 transcripts was up-regulated in C57BL/6 J mice after infection with influenza A virus (PR8M; H1N1). Expression levels increased already at day 2 post infection (p.i.), showing a peak of expression at day 8 p.i.. At later time points Lst1 expression decreased and reached levels that were similar to non-infected mice on day 18 p.i. [2, 11]. Thus, Lst1 expression was found both
during the innate and adaptive phase of the host response to influenza and peaked at the time point of T cell infiltration. Bio-GPS (http://biogps.org) expression studies show that \textit{Lst1} was mainly expressed in immune cells including mast cells, macrophages and dendritic cells.

To further characterize the role of LST1 after influenza A infection, we studied the host response in a \textit{Lst1} mouse knock-out (KO) model. The KO strain C57BL/6 N-\textit{Lst1}\textsuperscript{tm1(KOMP)Vlcg} was created from the ES cell clone 12118A-B1 (obtained from the KOMP Repository; www.komp.org). It harbors a reporter-tagged deletion of the \textit{Lst1} gene. We confirmed insertion of the targeting cassette into the coding region of exon two and three by PCR genotyping (Additional file 1: Figure S1A) and showed absence of transcripts in knock-out mice by reverse transcription PCR (Additional file 1: Figure S1B).

We then infected eight to twelve weeks old female C57BL/6 N-\textit{Lst1}\textsuperscript{tm1(KOMP)Vlcg} as well as wild type control mice (C57BL/6 N) with $2 \times 10^5$ focus forming units (FFU) of the low-virulent mouse-adapted A/PuertoRico/8/34 H1N1 virus (PR8M; [5]) after anesthesia with intra-peritoneal injection with a mixture of 100 mg/ml Ketamine and 20 mg/ml Xylazine. All animal experiments were approved by an external committee and according the guidelines of the animal welfare law in Germany (Permit numbers: 33.942502-04-051/09, 3392 42502-04-13/1234). Changes in body weight and survival rates were monitored for 14 days p.i. (Fig. 1). Mice with a loss of more than 30% of the starting body weight were euthanized and recorded as dead. \textit{Lst1} KO mice exhibited significantly increased body weight loss at days 5 and 6 p.i. All wild-type mice survived the infection with PR8M virus whereas the KO mice showed a lethality of 20%. However, this difference in survival was not statistical significant. No difference in survival and body weight loss was observed between wildtype and \textit{Lst1} KO mice after infection with another influenza A virus subtype (A/Hong Kong/01/68 H3N2, [12], Additional file 1: Figure S2). Furthermore, comparison of viral loads [5] between lungs of PR8M infected \textit{Lst1}\textsuperscript{−/−} and wild-type mice at day 3 and 5 p.i. did not reveal significant differences (Fig. 2). Histopathological analysis of infected lungs showed no obvious alteration in morphology and immune cell
infiltration in KO mice compared to the wild-type controls (Fig. 3). To assess the magnitude and composition of immune cell infiltrates in the airways, we analyzed bronchoalveolar lavage (BAL). We were not able to detect significantly different amounts of immune cells neither in infected nor in mock-infected BAL fluids (Fig. 4).

In conclusion, B6-Lst1−/− KO mice are slightly more susceptible to PR8M influenza A infection which is reflected in an increased body weight loss and slightly reduced survival rate. The susceptibility of KO mice is much less pronounced compared to DBA/2 J mice. Thus, our results show some contribution of Lst1 to susceptibility of DBA/2 J mice. However, its effect is rather small. Therefore, other genes with polymorphisms in Qivr17-2 as well as polymorphic genes in other QTL regions are contributing to the high susceptibility of DBA/2 J mice. Furthermore, it is conceivable that Lst1 has a major contribution by interacting with additional DBA/2 J alleles in Qivr17-2 or other QTLs that were mapped previously. These results emphasize the influence of different gene loci on the host response to influenza A H1N1 infection in mice.

There are numerous reports describing that LST1 plays an important role in the immune response to inflammatory diseases in humans [10, 13–15], in bacterial infections [10] and in signal transduction [16, 17]. It has been demonstrated that LST1 plays a crucial role for transmembrane cell to cell communication [18], which was shown to be important for the intercellular transport of bacteria
and retroviruses [19]. Furthermore, the expression of the Lst1 gene was shown to be up-regulated in response to lipopolysaccharide, interferon-γ gene was shown to be up-regulated in response to Lst1 and retroviruses [19]. Furthermore, the expression of the Lst1 gene was shown to be up-regulated in response to lipopolysaccharide, interferon-γ and bacterial infections [10].

The LST1 gene has been studied extensively at the gene and mRNA level [20], but the biological functions of the protein product are largely unknown. Overexpression of LST1 in several human cell lines leads to the formation of filopodia-like membrane protrusions [21]. Recently, it was proposed that LST1 promotes the formation of tunneling nanotubes [18]. Interestingly, it has been shown that several persistent viruses, like HIV and herpes viruses, are able to use those nanotubes for intercellular transfer [22–24]. This mechanism of viral spread has not been demonstrated for influenza A viruses. In addition, it was shown recently that tunneling nanotubes promote networking of immune cells and can mediate transfer of MHC class I molecules between distant cells [25]. This function might be disturbed in Lst1 KO mice leading to a slightly enhanced susceptibility to PR8M influenza A. We found in other mouse knock-out lines that the low-virulent PR8 virus (PR8M, [5]) is well suited to detect even small differences in susceptibility. However, it is still possible that Lst1 KO mice may exhibit a larger difference in phenotype when infected with another influenza virus subtype or variant.

To our knowledge the Lst1 knock-out mouse model described here is the first in vivo model investigating the role of LST1 during influenza A infections. However, several open questions still remain with respect to the many biological functions of LST1 in other contexts. The mouse model which we generated will help to elucidate also these other functions.

**Additional file**

**Additional file 1: Figure S1.** Targeting and genotyping strategy of the Lst1 KO strain. Mice were genotyped by using different primer pairs leading to PCR products which allowed the identification of wild-type (310 bp), KO (400 bp) and heterozygous animals (310 bp and 400 bp) (A). Primers (blue arrow) used for genotyping: Lst1-fwd: 5′-TGCGTGGCTCAGTCAC TA-3′; Lst1-rev: 5′-AGGCCCAACAATAGTCTAAC-3′; neodfwd: 5′-TCTAGTACTTGTTTG-3′; neodrev: 5′-AGGACCAACAATAATGCTTAC-3′; hubiP: promoter from the human ubiquitin C gene, neor: coding sequence for neomycin phosphotransferase, p(A): polyadenylation signal, black arrow: direction of gene transcription, black boxes: Lst1 coding region. To confirm the knock-out of the (Lst1) gene in C57BL/6 N-Lst1tm1(KOMP)Vlcg mice we performed reverse transcription. Figure S2. No difference in changes of body weight or survival rate between Lst1 KO and C57BL/6 N mice after infection with H3N2 influenza A virus Male C57BL/6 N-Lst1tm1(KOMP)Vlcg (n = 7) and C57BL/6 J mice (n = 10) were infected intranasally with 2x10^5 FFU H3N2 virus (A/HK/01/68) in 20 μl PBS. Body weight (A) and survival (B) were determined for each day p.i. for a period of 14 days. Percent weight change is shown with reference to the starting body weight. Significances were calculated using non-parametric Mann Whitney U test. (p < 0.01 for day 1) and Logrank test for survival rates (not significant). (PDF 230 kb)

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

SRL, HK and KS conceived the experiments and wrote the manuscript. SRL and BH performed the experiments; BH generated the Lst1 mutant mice from cryopreserved sperm. RLOL determined viral load in lung homogenates. All authors read and approved the final manuscript.
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