Inhibition of LTA4H expression promotes Staphylococcus aureus elimination by planarians

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The study of host-pathogen interactions in model organisms offers a means to characterize mechanisms and new critical innate immune genes that are conserved in higher eukaryotes. In vertebrates, leukotriene A4 hydrolase (LTA4H) is known to play a role in bacterial growth restriction. Planarian, a non-vertebrate organism, has the extraordinary ability to fight a wide spectrum of bacterial pathogens and have the faculty to regenerate an entire organism from a tissue fragment. However, the antibacterial response of the planarian remains poorly understood. We evaluated the contribution of LTA4H in the antibacterial response of the planarian, and we have observed that the silencing of the Smed-LTA4H gene by RNA interference promotes the S. aureus clearance, suggesting a role of LTA4H in the microbicidal activity of planarians.

Objective
The role of LTA4H in resolving a bacterial infection in a model of resistance to bacterial infection, such as planarians, remains unknown. Therefore, we investigated the role of LTA4H in the planarian species Schmidtea mediterranea, infected with S. aureus.

Introduction
The planarian, a non-vertebrate, is actively used to investigate developmental and regeneration processes (Elliott 2012[1]). In addition, this platyhelminth is a model used to investigate the evolutionary conserved mechanism of antibacterial response because of its resistance to infection (Abnave 2014[2]). Indeed, the planarian species Schmidtea mediterranea and Dugesia japonica are able to eliminate a large spectrum of human pathogens, including Staphylococcus aureus (Abnave 2014[2]) a microbe responsible for nosocomial disease and for causing pneumonia, abscess, sepsis, toxic shock syndrome (Peres 2013[3]). In vertebrates, Leukotriene A4 hydrolase (LTA4H) is a ubiquitously expressed enzyme that catalyzes the final step in the synthesis of leukotriene B4 (LTB4), a potent pro-inflammatory lipid mediator derived from arachidonic acid (Snelgrove 2011[4]). LTA4H controls the balance of pro-inflammatory and anti-inflammatory eicosanoids and determines the expression of tumor necrosis factor (TNF)-α. The expression of LTA4H induces the production of pro-inflammatory cytokines; in contrast, the inhibition of LTA4H reduces the LPS-induced production of pro-inflammatory cytokines, up-regulates the production of the anti-inflammatory cytokine interleukin-10, and enhances bacterial invasion or bacterial susceptibility (Tobin 2010[5]) (Curtis 2011[6]) (Dunstan 2015[7]) (Yang 2014[8]) (Tobin 2012[9]).
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Figure 1. The silencing of Smed-LTA4H enhanced bacterial elimination. (a) Protein Alignment between Hs-LTA4H (NP_000886.1) and predicted Smed-LTA4H. The results are color coded for amino acid conservation and the scoring scheme works from 0, for the least conserved alignment position, to 10, for the most conserved alignment position. (b) Planarians were challenged with S. aureus (1x10^9 bacteria), and Smed-LTA4H expression was evaluated using RTqPCR. S. aureus induced a transient expression of Smed-LTA4H. The results are presented as the mean ± SD (three worms per time point processed individually in triplicate, number of experiments = 3, *p<0.05). (c) The S. mediterranea eGFP (RNAi) and the S. mediterranea LTA4H (RNAi) were fed with S. aureus (1x10^9 bacteria), and the CFUs per worm were counted over time. S. aureus were eliminated in 6 days in control and 4 days in planarians silenced for Smed-LTA4H. The results are expressed as the mean ± SD (ten worms per time point, number of experiments = 3, *p<0.05). (d) The amount of bacteria ingested by the S. mediterranea eGFP (RNAi) and the S. mediterranea LTA4H (RNAi) after feeding (Time 0 day) was determined by counting the CFUs. The S. mediterranea eGFP (RNAi) and the S. mediterranea LTA4H (RNAi) had ingested the same amount of bacteria. The results are expressed as the mean ± SD (ten worms per time point, number of experiments = 3). (e) The efficiency of the Smed-LTA4H silencing by RNAi in the S. mediterranea was determined using RTqPCR. In S. mediterranea LTA4H (RNAi) animals, the level of expression of Smed-LTA4H is diminished of 79% compare to the control (eGFP RNAi). The results are expressed as the mean ± SD (three worms per time point processed individually in triplicate, number of experiments = 3, *p<0.05).
Results & Discussion
First, we searched for a homologue to Homo sapiens (Hs)-LTA4H (NM_000895.2) in Schmidtea mediterranea using the transcriptome database PlanMine (http://planmine.mpi-cbg.de/planmine/begin.do). Our TBLASTN analysis, using the Hs-LTA4H (NP_000886.1) as a query and the planarian transcriptomic data (dd_Smed_v6), identified 10 sequences producing significant alignments with Hs-LTA4H (lcl|dd_Smed_v6_733_0_2, e-value 1e-160; lcl|dd_Smed_v6_733_0_1, e-value 3e-160; lcl|dd_Smed_v6_3027_0_1, e-value 8e-142; lcl|dd_Smed_v6_2224_0_1, e-value 2e-21; lcl|dd_Smed_v6_15623_0_1, e-value 2e-20; lcl|dd_Smed_v6_6470_0_1, e-value 7e-20; lcl|dd_Smed_v6_6938_0_1, e-value 7e-20; lcl|dd_Smed_v6_1249_0_1, e-value 2e-17; lcl|dd_Smed_v6_8086_0_1, e-value 2e-13; lcl|dd_Smed_v6_7405_0_1, e-value 6e-06). Using FGENESH+ (http://www.softberry.com/), we predicted Schmidtea mediterranea (Smed)-LTA4H, as an homologue to Hs-LTA4H. Analysis with BLASTx showed a 44% homology at the protein level (97% of cover, e-value 2e-166) for predicted Smed-LTA4H with Hs-LTA4H (Figure 1a). Second, using quantitative real time PCR, we evaluated the expression level of the Smed-LTA4H in worms challenged by S. aureus. Our results demonstrated that S. aureus induced the mRNA expression of the Smed-LTA4H. We observed the level of mRNA expression of the Smed-LTA4H increase transiently from a relative fold change of 1 to a maximum relative fold change of 4.5, which was reached after 12 hours of challenge with S. aureus (Figure 1b). These data suggest that Smed-LTA4H is induced in response to S. aureus infection. Next, using RNA interference, we inhibited the expression of the Smed-LTA4H in planarians, and the Smed-LTA4H (RNAi) animals were fed S. aureus. We evaluated the clearance of S. aureus at 3, 6, and 9 days post-feeding using a direct measurement of the colony-forming units (CFUs) (Figure 1c). Two days after infection, we observed that the S. aureus CFU count was less important in the Smed-LTA4H (RNAi) worms (1.31x10^3 S. aureus CFU/worm) than in the control Smed-eGFP (RNAi) worms (1.20x10^4 S. aureus CFU/worm). Four days after infection, the Smed-LTA4H (RNAi) worms had fully eliminated the S. aureus, whereas in the control, eGFP (RNAi) worms S. aureus was still detected (1.12x10^0 ± 6.44x10^1 CFU/worm). S. aureus was detected for 2 additional days in the control eGFP (RNAi) worms. The observed difference between the Smed-LTA4H (RNAi) worms and the control eGFP (RNAi) worms in the rate of S. aureus elimination was not due to differences in the level of infection at T0, as there was no significant difference in the number of S. aureus CFU/worm detected at T0 between the Smed-LTA4H (RNAi) worms and the control eGFP (RNAi) worms (1.56x10^6 ± 5.31x10^5 vs 1.35x10^6 ± 6.50x10^5, respectively) (Figure 1d). The inhibition of Smed-LTA4H expression via RNAi increases the rate of elimination of S. aureus in planarians. It is interesting to note that S. aureus induces the expression of Smed-LTA4H, suggesting that a survival strategy of S. aureus is to induce the expression of Smed-LTA4H to decrease the rate of its elimination in planarians. However, this strategy fails, probably because of other antibacterial mechanisms engaged by planarians to fight microbes. The knock down efficiency of Smed-LTA4H was confirmed using real time RT-qPCR. The expression of Smed-LTA4H was reduced by 80% compared to the control Smed-eGFP (RNAi) worms (Figure 1e). Taken together these data shows the silencing of the Smed-LTA4H does not affect the capacity of planarians to ingest bacteria and promotes the capacity of planarians to eliminate S. aureus. The specificity of the RNAi against LTA4H has been controlled. Indeed, for Smed-LTA4H transcript for which the RNAi was designed, the theoretical target accuracy has been calculated. We find a number of theoretical off target equal to 0, thus a target accuracy of 100%, excluding that the observed effect was due to an off target effect. The data from this study demonstrate: (1) S. aureus induces the expression of the Smed-LTA4H and survives up to 6 days in planarians, and (2) the knock down of the Smed-LTA4H significantly increased the antimicrobial activity of planarians, and S. aureus were eliminated by 4 days after infection. To date, there is no published data on the role of the LTA4H in the antibacterial processes of non-vertebrates. Therefore, the only comparisons we can make to the results from our study on non-vertebrate are based on the findings in vertebrates. In vertebrates, the overexpression of the LTA4H is responsible for an increase in TNF-induced cell necrosis leading to bacterial death. In contrast, a reduction in the LTA4H expression leads to an increase in bacterial growth. These data suggest that Smed-LTA4H is induced in response to S. aureus infection. Next, using RNA interference, we inhibited the expression of the Smed-LTA4H in planarians, and the Smed-LTA4H (RNAi) animals were fed S. aureus. We evaluated the clearance of S. aureus at 3, 6, and 9 days post-feeding using a direct measurement of the colony-forming units (CFUs) (Figure 1c). Two days after infection, we observed that the S. aureus CFU count was less important in the Smed-LTA4H (RNAi) worms (1.31x10^3 S. aureus CFU/worm) than in the control Smed-eGFP (RNAi) worms (1.20x10^4 S. aureus CFU/worm). Four days after infection, the Smed-LTA4H (RNAi) worms had fully eliminated the S. aureus, whereas in the control, eGFP (RNAi) worms S. aureus was still detected (1.12x10^0 ± 6.44x10^1 CFU/worm). S. aureus was detected for 2 additional days in the control eGFP (RNAi) worms. 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In this study, we demonstrate that S. aureus survival in the absence of LTA4H is significantly associated with tuberculosis meningitis, lymph node tuberculosis, bone tuberculosis and other extra-pulmonary tuberculosis with the exception of pleural tuberculosis in humans (Curtis 2011(S6) Snelgrove 2011(S4)) Yang 2014(S8)). Therefore in vertebrates, LTA4H deficiency likely leads to bacterial proliferation and a failure to resolve the bacterial infection due to the resultant anti-inflammatory properties, whereas overexpression of the LTA4H is associated with pro-inflammatory properties and thus leads to bacterial death. Here, we observed that LTA4H deficiency in planarians has the opposite effect compared to observations in vertebrates because LTA4H deficiency promotes the clearance of bacteria in planarians. This observed difference might be linked to the extraordinary capacity of planarians to regenerate any part of their body. In fact, it has been suggested that inhibition of the LTA4H expression by maresin-1 (MaR1), induced a faster regeneration of the planarian head (Serhan 2012(S10)) in planarians, the autophagy process is required for tissue regeneration (Gonzalez Estevéez 2007(S11)) and bacterial clearance (Abnave 2014(S9)). The invalidation of the LTA4H gene could result in an increase in autophagy and a disruption of the tissue homeostasis equilibrium (controlled via autophagy), thereby leading to an increase in the elimination of the bacteria.
Conclusions
We can speculate through a comparison with mammalians that $LTA_4H$ expression is associated with anti-inflammatory profile in planarians, but this must be proven by further experiments. However, we can conclude that in this study we have shown that the silencing of the $LTA_4H$ gene in planarians enhances the capability of planarians to kill $S. aureus$. These results suggest that in planarians, $LTA_4H$ expression is taking part to the anti-bacterial mechanisms engaged by planarians to fight microbes.

Limitations
These experiments were conducted using a non-vertebrate model that is highly resistant to infection and has the capacity to continuously regenerate. The particular biological properties of the planarians could explain the unexpected role of the $LTA_4H$ compared to other organisms. In addition, we have specifically worked with $S. aureus$, it could be interesting to analyze $LTA_4H$ deficient planarians challenged with other microorganisms, such as $Mycobacterium tuberculosis$, which are often used to study the function of the $LTA_4H$ in vertebrates.

Conjectures
As suggested above, it would be interesting to evaluate the contribution of autophagy in the microbicidal mechanisms mediated by $LTA_4H$ in planarians. For that, it will be important to analyze the level of the autophagy in the $LTA_4H$ knockdown planarians, the expression level of autophagy markers, such as MORN2 [Abnave 2014], and the planarian homologue of Hs-dap-1 [Gonzalez Estevez 2007] and the level of apoptosis by tunnel assay. It will be also interesting to determine the cells population expressing $LTA_4H$, as well as the co-expression of $LTA_4H$ and autophagy makers in planarians. In addition, whether the regeneration process failed or is affected by the knock down of $LTA_4H$ should be examined to explain the role of $LTA_4H$ in bacterial elimination by planarians. In another hand, we cannot exclude a contribution of the α-toxin produces by $S. aureus$ [Berube 2013] in our observation. It will be interesting to see if others strains of $S. aureus$ which do not produce α-toxin, such as the $S. aureus$ RN4220 strain, have the same effect on Smed-$LTA_4H$ expression, and the effect of the silencing of Smed-$LTA_4H$ on $S. aureus$ RN4220 behaviours.
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Additional Information

Please see https://scienecematters.io/articles/201604000011.

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Ethics Statement

Not Applicable

Citations


