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Elma Lima Leite[1,2], Arthur Gautron[3], Aurelie Nicolas[1], Jordane Ossemond[1], Fillipe L. R. do Carmo[1 2], David Gilot[3], Vasco Azevedo[2], Friedrich Goetz[4], Yves Le Loir[1], Michael Otto[5], Nadia Berkova[1]

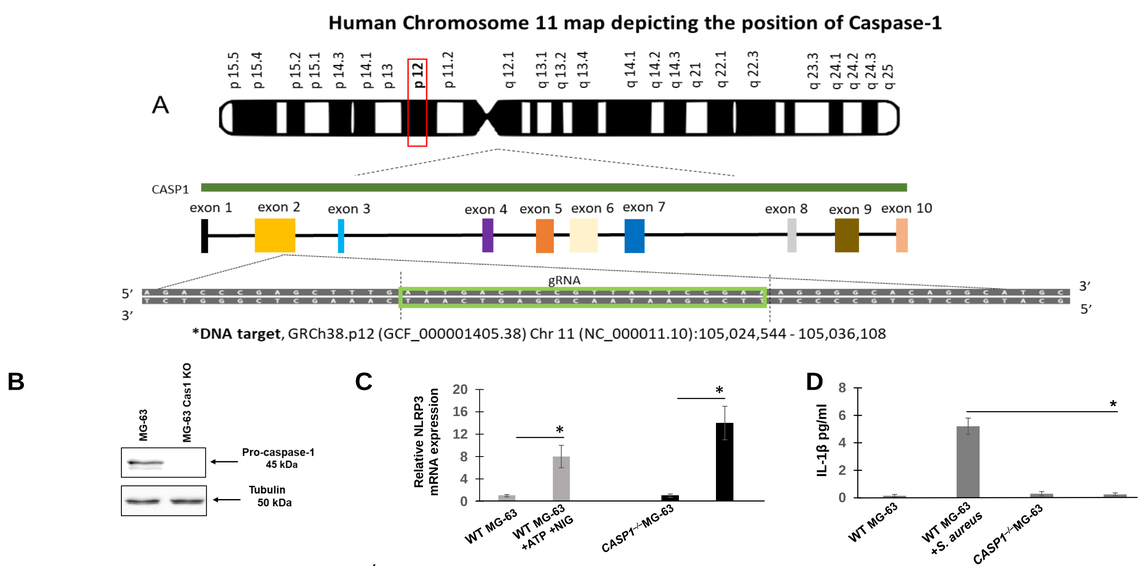
[1] Institut National de la Recherche Agronomique, Unité Mixte de Recherche 1253 STLO, Rennes, France; Agrocampus Ouest, Unité Mixtes de Recherche 1253 STLO, Rennes, France.
[2] Instituto de Ciências Biológicas - Universidade Federal de Minas Gerais, Belo Horizonte- Minas Gerais, Brazil.
[3] Univ Rennes, CNRS, IGDR [(Institut de génétique et développement de Rennes)]-UMR 6290, F-35000, Rennes, France.
[4] Paul-Ehrlich-Institute, Federal Regulatory Agency for Vaccines and Biomedicines, Langen 63225, Germany
[5] Mikrobielle Genetik, Universität Tübingen, D-72076 Tübingen, Germany
[6] Laboratory of Human Bacterial Pathogenesis, US National Institutes of Health, Bethesda, Maryland, 20892, USA.

CONTEXT & AIM

- The inflammasome is a multi-protein signaling platform that assembles after recognition of danger signals and/or pathogens. Once assembled, inflammasomes initiate signaling by activation of downstream proteases, most notably Caspase-1 and Caspase-11, which then proteolytically mature pro-IL-1β, pro-IL-18, and pro-IL-33.
- *Staphylococcus aureus* is a gram-positive bacterium that can cause several fatal infections and is also the predominant cause of bone infections worldwide. The quorum-sensing system in *S. aureus* known as the accessory gene regulator (Agr). Agr regulates the expression of many virulence factors including the expression of PSMs encoding genes.
- In this study, we investigated the involvement of inflammasomes in the model of persistent infection of human osteoblast-like cells with the help of the *CASP1*$^{-/-}$MG-63 cell line that was established using the CRISPR-Cas9 gene editing system.

RESULTS

Generation of CASP1$^{-/-}$ MG-63 cells using the CRISPR-Cas9 gene editing system



A. Generation of CASP1$^{-/-}$MG-63 cells targeting exon 2 of the CASP1 gene

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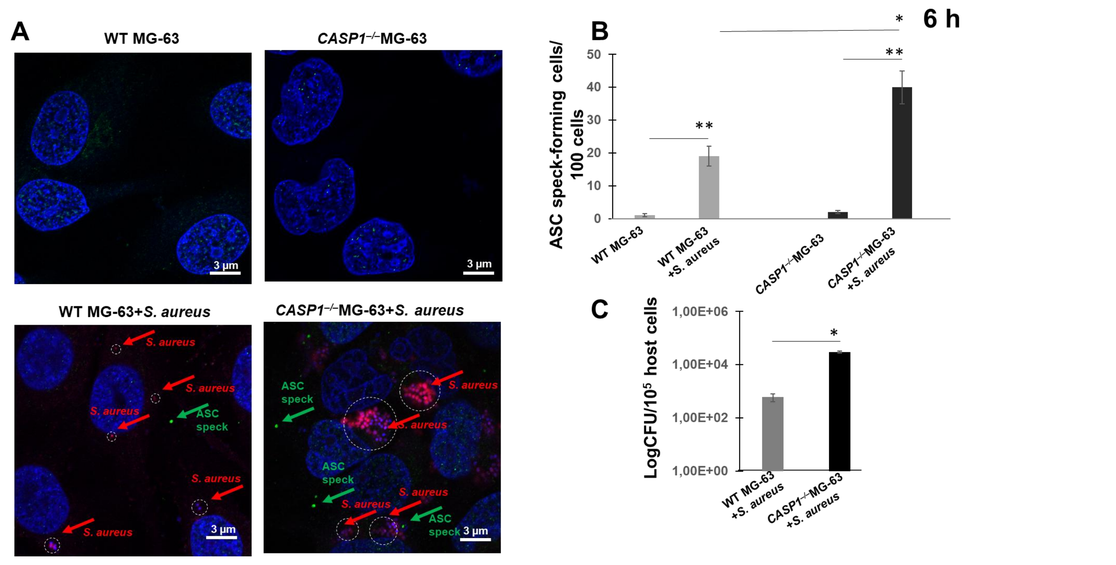
B. Detection of 45-kDa pro-caspase-1 in WT MG-63 vs CASP1 $^{-/-}$ MG-63 cells by Western blot analysis using anti-caspase-1 antibody.
C. Analysis of mRNA levels of NLRP3 in WT MG-63, CASP1$^{-/-}$ MG-63 cells by RT-qPCR.
D. The level of IL-1β in cell supernatants was determined by ELISA.

Higher number of ASC specks in CASP1$^{-/-}$ MG-63 cells compared to WT MG-63 cells

MOI 50:1



A. WT MG-63 or CASP1$^{-/-}$ MG-63 cells were exposed to *S. aureus* SA113 (red fluorescence, red arrows). Six hours post-infection cells were immunostained with anti PYCARD antibody.

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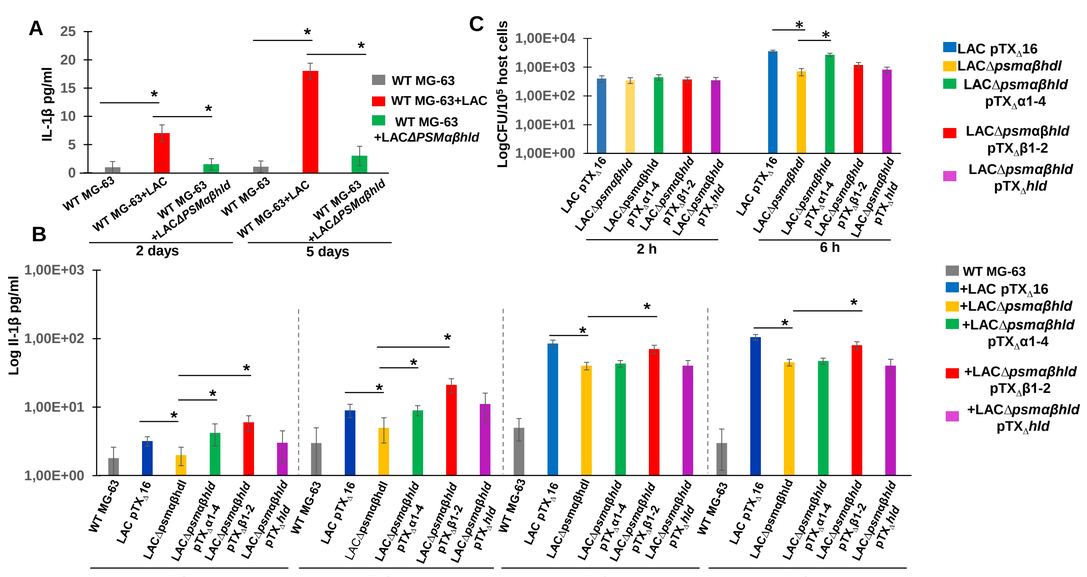
B. Quantification of the amount of ASC-speck forming cells/100 cells.
C. Cell lysates were plated on BHI agar, and CFU were determined. CFU values of cells exposed to *S. aureus* were normalized to 10^5 host cells.

S. aureus phenol-soluble modulins stimulate IL-1β release from infected osteoblast-like MG-63 cells



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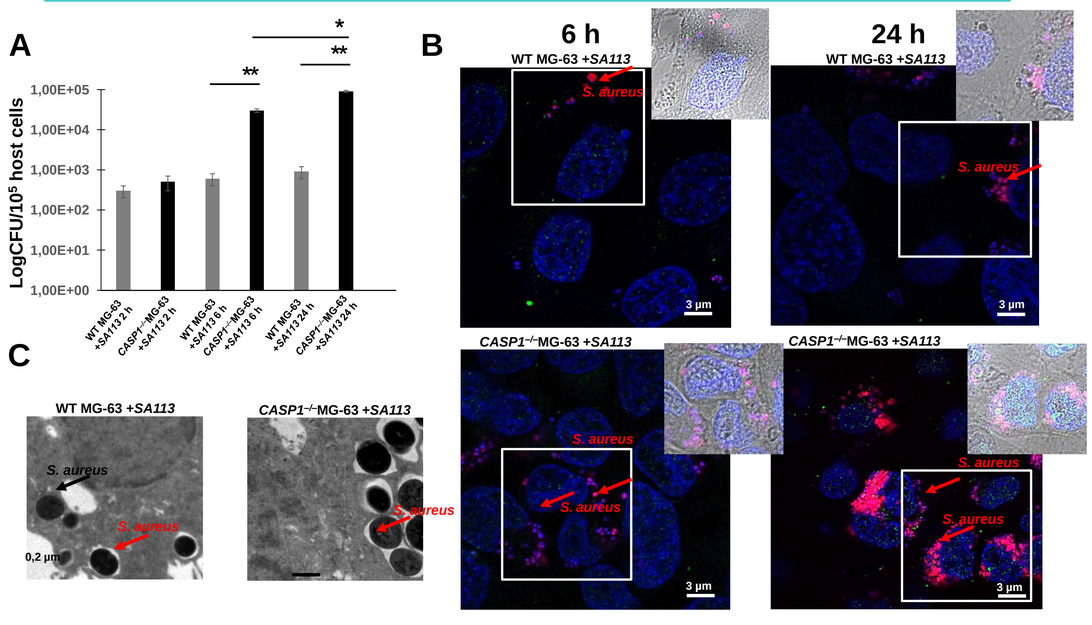
A. WT MG-63 cells were exposed to wild type LAC (USA300) and its isogenic mutant LAC *Δpsmαβhld*. The level of IL-1β in cell supernatants was determined by ELISA.
B. MG-63 cells were exposed to USA300 LAC (pTXΔ16), which carries the control plasmid, the deletion mutant LACΔ*psmαβhld* (pTXΔ16) and the complemented strains.
C. WT MG-63 or CASP1$^{-/-}$ MG-63 cells were exposed to USA300 LAC, the deletion mutant LAC*Δpsmαβhld* (pTXΔ16) and the complemented strains. Then, cell lysates were plated on BHI agar, and CFU were determined. CFU values were normalized to 10^5 host cells.

Involvement of caspase-1 in bacterial clearance



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A. WT MG-63 or CASP1$^{-/-}$ MG-63 cells were exposed to a fluorescent *S. aureus* SA113 strains. CFU values were normalized to 10^5 host cells.
B. WT MG-63 or CASP1 $^{-/-}$ MG-63 cells exposed to *S. aureus* SA113. Six hours and 24 hr post-infection cells were stained with anti-PYCARD antibody (green staining). Nuclear DNA was labeled with DAPI (blue staining). The number of viable bacteria recovered from infected cells was significantly higher in CASP1$^{-/-}$MG-63 than in WT MG-63 cells.
C. Transmission electron micrographs of WT MG-63 or CASP1$^{-/-}$ MG-63 cells infected with SA113 strain.

CONCLUSION

- **Osteoblast-like cells induce an immune response against *S. aureus* through inflammasomes activation and processing of IL-1β.**
- **The active caspase-1 restrict intracellular replication of *S. aureus* in non-professional phagocytes in addition to professional phagocytes, suggesting the pivotal role of caspase-1 in *S. aureus* clearance independently from the type of cells.**
- **Inflammasomes related IL-1β production by infected osteoblast-like cells appears to be particularly dependent on PSMβ among PSMs.**

Centre
Bretagne - Normandie

65 rue de Saint Brieuc
35 042 Rennes Cedex
nadejda.berkova@inrae.fr

PHC Germaine de Stael, France–Switzerland
Intramural Research Program, NIH, USA
INRAE, Metaprogram GISA, LONGhealth–MPP10573
Deutsche Forschungsgemeinschaft
CAPES, Brazil