Science Immunology

immunology.sciencemag.org/cgi/content/full/1/2/eaah3539/DC1

Supplementary Materials for

IL-1β is an innate immune sensor of microbial proteolysis

Christopher N. LaRock,* Jordan Todd, Doris L. LaRock, Joshua Olson, Anthony J. O'Donoghue, Avril A. B. Robertson, Matthew A. Cooper, Hal M. Hoffman, Victor Nizet*

*Corresponding author. Email: clarock@ucsd.edu (C.N.L.); vnizet@ucsd.edu (V.N.)

Published 19 August 2016, *Sci. Immunol.* **1**, eaah3539 (2016)
DOI: 10.1126/sciimmunol.aah3539

The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at immunology.sciencemag.org/cgi/content/full/1/2/eaah3539/DC1)

Supplemental Methods

Adverse Event Reporting

The FDA Adverse Event Reporting System (FAERS) database was accessed at its web portal: www.fda.gov/Drugs/InformationOnDrugs/ucm135151. Numbers of infections by individual bacterial species were counted by the queries: (Escherichia) or (Staphylococcus) or (Tuberculosis) or (Legionella) and all adverse events identified where consistent with disease caused by the indicated pathogenic species. The query (Streptococcal) revealed infections due to other pathogenic streptococci including group B *Streptococcus*, group C *Streptococcus*, viridans streptococci, and *Streptococcus pneumonia* (pneumococcus). To differentiate group A *Streptococcus* (GAS, aka *Streptococcus pyogenes*) infections from other streptococcal infections, the query was: ("Necrotising fasciitis" OR "Beta haemolytic" OR pharyngitis OR "toxic shock" AND streptococcal ANDNOT ("Alpha haemolytic" OR Pneumonia OR Peptostreptococcus)). This query scheme provided values for total adverse events for each condition. To calculate events associated with IL-1 inhibition, the generic and trade names of approved IL-1 inhibitors were added to the query (anakinra kineret rilonacept arcalyst canakinumab ilaris). Total adverse events associated with these drugs were calculated by querying these drug names without a concurrent search for adverse events, and the total number records quantified by null query.

Specific GAS infection types were identified by queries for (Streptococcal OR "beta haemolytic) and sequentially, each of the associated diseases: "necrotising fasciitis" or sepsis or erysipelas or "toxic shock" or pharyngitis or glomerulorephritis. Events associated with IL-1 inhibition were added to the query with the addition of (anakinra kineret rilonacept arcalyst canakinumab ilaris).

Drug classes associated with streptococcocal necrotising fasciitis where queried by (streptococcal "necrotising fasciitis") and the generic and trade names of drugs belonging to that class, including inhibitors of IL-1 (anakinra kineret rilonacept arcalyst canakinumab ilaris), TNF (etanercept enbrel adalimumab humira infliximab remicade certolizumab cimzia golimumab simponi lenalidomide revlimid), IL-6 (tocilizumab atlizumab actemra), CD20 (rituximab rituxan), CD80/86 (orencia abatacept belatacept nulojix), DMARDs (trexall methotrexate leflunomide arava sulfasalazine), and NSAIDs (voltaren diclofenac ibuprophen motrin advil di-antalvic movicox meloxicam naproxen asprin). These classes encompassed the anti-inflammatory drugs associated with necrotising fasciitis, and all trade and brand names reported as associated with the infections. Total usage of these drugs was queried by drug names without the accompanied search for adverse events. Positive records were downloaded and manually examined in their entirety. Duplicate records were identified due to redundancy in multiple fields, including similar date of reporting, patient age, list of full medications taken, outcomes, and complications. Duplicate records identified in the present analysis are outlined in table S2. Proportional reporting risk (PRR)[1,2] was calculated as: (adverse incidents with suspect drug / total reports with suspect drug) / (total adverse incidents without suspect drug / total reports without suspect drug).

1. S. Evans, P. C. Waller, S. Davis, Use of proportional reporting ratios (PRRs) for signal generation from spontaneous adverse drug reaction reports. *Pharmacoepidemiology and drug safety* **10**, 483-486 (2001).

2. K. E. Kip, J. M. Swoger, L. M. Grandinetti, A. M. I. Barrie, J. B. Greer, M. D. Regueiro, Tumor Necrosis Factor α Antagonist–associated Psoriasis in Inflammatory Diseases: An Analysis of the FDA Adverse Event Reporting System. *Inflammatory Bowel Diseases* **19**, 1164 (2013).

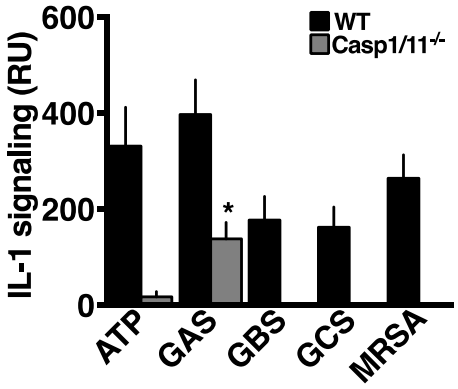

Fig. S1. Examinaion of inflammasome dependence of IL-1β activation.

Total IL-1 signaling induced by macrophages treated with ATP after overnight priming with LPS, or infected with GAS, GBS (group B *Streptococcus*), GCS (group C *Streptococcus*) or MRSA (methicillin-resistant *Staphylococcus aureus*) over a 2 h period.

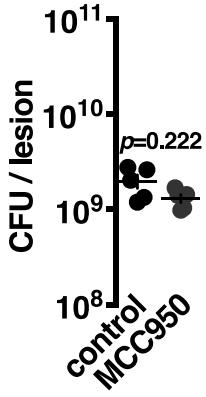

Fig. S2. Potentiation of GAS infection by NLRP3 inhibition.
Quantification of GAS CFU within lesions of MCC950-treated or control mice 72 h post-intradermal infection.

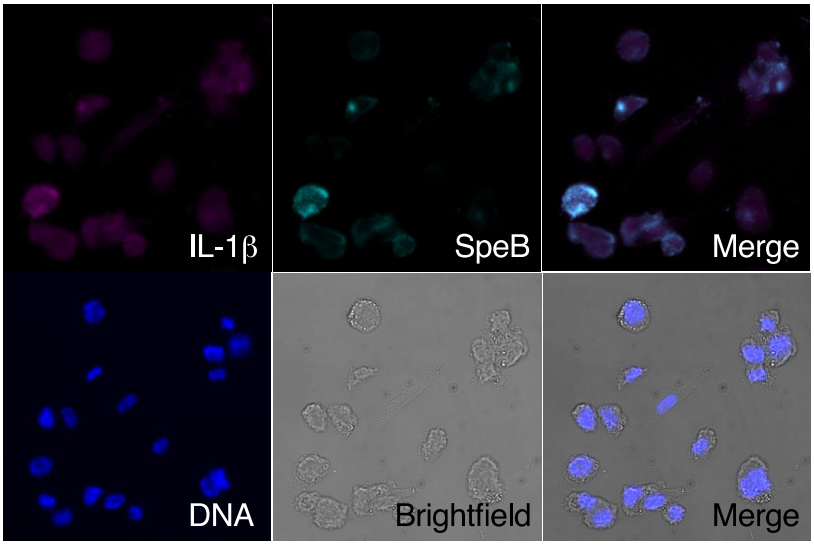

Fig. S3. SpeB colocalization with IL-1β.

Immunofluorescent microscopy analysis examining colocalization of SpeB with IL-1β 4 h post-infection of BMM with GAS.

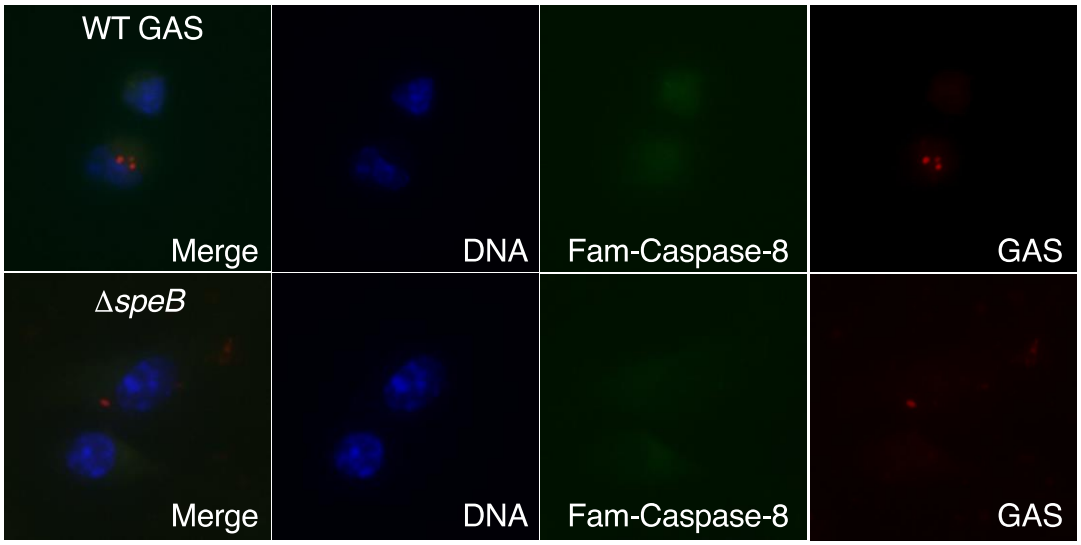

Fig. S4. SpeB does not activate caspase-8 or caspase-8–like proteolysis.
Immunofluorescent microscopy analysis examining examination of activation of caspase-8 using the Fam-Caspase-8 activity based assay, 4 h post-infection of BMM with GAS.

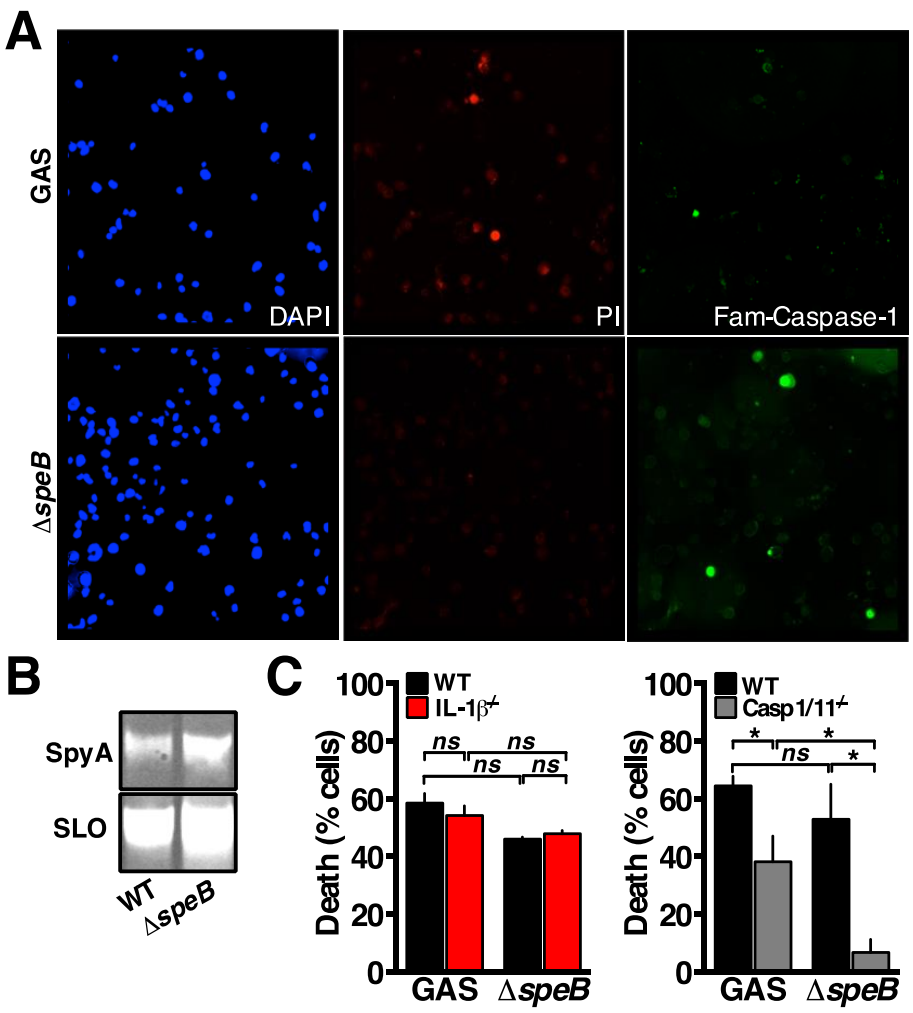

Fig. S5. SpeB induces cell death and antagonizes canonical inflammasome activation.
(**A**) SpeB induces significant death as measured by uptake of propidium iodide (PI) without the involvement of caspases or the inflammasome since active caspase-1 (measured by FLICA) is absent in these cells. Greater caspase activation is observed by Δ*speB* GAS, suggesting SpeB partially antagonizes canonical inflammasome activation. (**B**) SpeB degrades the inflammasome-activating PAMPs SpyA (*38*) and SLO (*49*). (**C**) Cell death, measured by release of cytosolic lactate dehydrogenase, is fully independent of IL-1β, and independent of caspase-1 in the presence of SpeB.

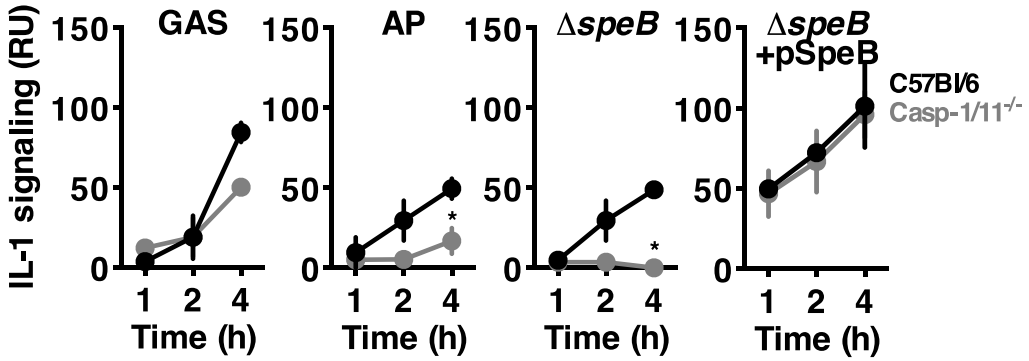

Fig. S6. Kinetics of activation by caspase-1 or SpeB during infection.

BMM from wild-type or caspase-1/-11$^{-/-}$ C57Bl/6 mice were infected with wild-type, animal-passaged (AP), Δ*speB* GAS, or strains trans-complemented (Δ*speB*+pSpeB; (*28*)) for SpeB expression, were monitored for release of bioactive IL-1 with IL-1R reporter cells at the indicated time intervals.