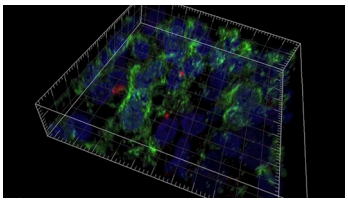
SUPPLEMENTAL MATERIAL

Nothelfer et al., http://www.jem.org/cgi/content/full/jem.20130914/DC1



Video 1. Animation of confocal imaging of 150-µm-thick tissue sections which shows that *S. flexneri* is found intracellularly in B lymphocytes within ILFs. The animation moves through human colonic tissue after ex vivo infection by WT bacteria orthogonally. Bacteria were stained with an antibody specific for *S. flexneri 5a* (red) and B cells with anti-CD20cy (green). DAPI nuclei staining is shown in blue.

Table S1. Regulation of pro- and anti-apoptotic proteins after infection as assessed by a protein-based apoptosis array

Protein name	WT	T3SA⁻ + IpaD
Bad	1.27 ± 0.09[a]	1.37 ± 0.21[b]
Bax	1.38 ± 0.19[b]	1.21 ± 0.11[a]
Bcl-2	2.00 ± 0.44[b]	1.91 ± 0.23[b]
Bcl-x	1.11 ± 0.36[a]	0.61 ± 0.15[c]
Pro-Casp 3	0.94 ± 0.03	0.93 ± 0.01
Cleaved Casp 3	0.90 ± 0.10	1.00 ± 0.04
Catalase	1.03 ± 0.07	1.03 ± 0.10
cIAP-1	0.97 ± 0.16	1.04 ± 0.14
c-IAP 2	0.76 ± 0.07[d]	0.91 ± 0.17
Claspin	0.78 ± 0.09[d]	1.03 ± 0.15
Clusterin	1.29 ± 0.40[a]	1.08 ± 0.17
Cyt c	1.50 ± 0.09[b]	1.57 ± 0.13[b]
TRAIL R1	0.93 ± 0.08	0.82 ± 0.01[d]
TRAIL R2	0.95 ± 0.04	0.78 ± 0.12[d]
FADD	1.27 ± 0.16[b]	1.37 ± 0.22[b]
Fas	0.86 ± 0.26[d]	0.75 ± 0.12[d]
HIF-1a	0.97 ± 0.03	0.91 ± 0.13
HO-1	1.02 ± 0.13	0.75 ± 0.12[d]
HO-2	1.05 ± 0.20	0.91 ± 0.16
HSP27	1.06 ± 0.01	0.99 ± 0.10
HSP60	1.03 ± 0.29	0.96 ± 0.14
HSP70	0.85 ± 0.10[d]	0.93 ± 0.07
HTRA2	1.03 ± 0.09	1.06 ± 0.02
Livin	1.13 ± 0.13[a]	1.05 ± 0.08
PON2	1.02 ± 0.15	1.10 ± 0.06[a]
p21	0.90 ± 0.05	1.28 ± 0.08[a]
p27	0.94 ± 0.10	1.14 ± 0.14[a]
p-p53(S15)	0.89 ± 0.10[d]	0.99 ± 0.08
p-p53 (S46)	0.76 ± 0.05[d]	0.93 ± 0.21
p-p53 (S392)	0.71 ± 0.15[d]	0.80 ± 0.05[d]
p-Rad17 (S635)	1.12 ± 0.32[a]	1.03 ± 0.15
SMAC	1.05 ± 0.07	1.05 ± 0.14
Survivin	0.86 ± 0.16[d]	0.81 ± 0.15[d]
TNF R1	0.92 ± 0.14	0.75 ± 0.15[d]
XIAP	0.92 ± 0.10	1.15 ± 0.13[a]

Pro- and anti-apoptotic protein levels for the entire assay are shown for infection with WT and T3SA⁻ + IpaD as fold change over T3SA⁻ alone. Fold changes are color-coded for the detection of increased (green) or decreased (red) protein amounts. Data are presented as mean ± SEM of three independent experiments.

[a]Fold change over T3SA⁻: >1.1 .

[b]Fold change over T3SA⁻: >1.3.

[c]Fold change over T3SA⁻: <0.7.

[d]Fold change over T3SA⁻: <0.9.

Table S2. List of *S. flexneri* strains used for infections

Name	Description	Reference
WT	M90T, *S. flexneri* 5a WT	Sansonetti et al., 1982
T3SA$^-$	SF401, *mxiD* mutant, unable to assemble T3SA	Allaoui et al., 1993
WT GFP	GFP-expressing M90T	Jaumouillé et al., 2008
T3SA$^-$ GFP	GFP-expressing *mxiD*	Jaumouillé et al., 2008
icsA	SC557, *icsA* mutant, non-motile in cell cytoplasm	Bernardini et al., 1989
mxiE	SF1060, *mxiE* mutant, transcription factor for several virulence effectors	Mavris et al., 2002
spa15	SF1601, *spa15* mutant, chaperone of several virulence effectors	Page et al., 2002
ipaC	SF621, *ipaC* mutant, translocator	Ménard et al., 1993
ipaD	SF622, *ipaD* mutant, needle tip protein	Ménard et al., 1993
ipaD/pIpaD	SF622 (pD1), complemented *ipaD* mutant	Ménard et al., 1993

The name used throughout the text is given with a description of the strain and the reference that first describes it.

Table S3. List of DNA oligonucleotide primers used for gene expression quantification

Gene name	Forward sequence (5′–3′)	Reverse sequence (5′–3′)	Reference
TLR1	CAGTGTCTGGTACACGCATGGT	TTTCAAAAACCGTGTCTGTTAAGAGA	Zarember and Godowski, 2002
TLR2	GGCCAGCAAATTACCTGTGTG	AGGCGGACATCCTGAACCT	Zarember and Godowski, 2002; QIAGEN quantitech; SABiosciences
TLR3	TGGTTGGGCCACCTAGAAGTA	TCTCCATTCCTGGCCTGTG	Zarember and Godowski, 2002
TLR4	CAGAGTTTCCTGCAATGGATCA	GCTTATCTGAAGGTGTTGCACAT	Zarember and Godowski, 2002
TLR5	TGCCTTGAAGCCTTCAGTTATG	CCAACCACCACCATGATGAG	Zarember and Godowski, 2002
TLR6	GAAGAAGAACAACCCTTTAGGATAGC	AGGCAAACAAAATGGAAGCTT	Zarember and Godowski, 2002
TLR7	ATCTTGGCACCTCTCATGCT	AAGAATTTGTCTCTTCAGTGTCCA	In house, QIAGEN quantitech
TLR8	TTATGTGTTCCAGGAACTCAGAGAA	TAATACCCAAGTTGATAGT CGATAAGTTTG	Zarember and Godowski, 2002
TLR9	GGACCTCTGGTACTGCTTCCA	AAGCTCGTTGTACACCCAGTCT	Zarember and Godowski, 2002; QIAGEN quantitech
TLR10	TGTTATGACAGCAGAGGGTGATG	GAGTTGAAAAAGGAGGTTATAGGATAAATC	Zarember and Godowski, 2002

Forward and reverse sequences for each gene are provided alongside the original reference in which it was described. In the case in which multiple primer probes were used to robustly assess expression levels, the commercial provider is also indicated.

Table S4. List of antibodies used for immunoblotting

Antigen	Source	Buffer	Dilution
Actin	Sigma-Aldrich	3% milk in TBST	1:5,000
Bad	Santa Cruz Biotechnology, Inc.	3% milk in TBST	1:200
Bak	Cell Signaling Technology	3% milk in TBST	1:5,000
Bax	Millipore	3% milk in TBST	1:1000
Bcl-2	Santa Cruz Biotechnology, Inc.	3% milk in TBST	1:200
Bcl-xl	Cell Signaling Technology	3% milk in TBST	1:1,000
IκBα	Santa Cruz Biotechnology, Inc.	3% milk in TBST	1:1,000
FADD	BD	TBST	1:250

Antigen specificity, company, and conditions used for probing are given for each antibody.

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