Citrobacter rodentium NleB Protein Inhibits Tumor Necrosis Factor (TNF) Receptor-associated Factor 3 (TRAF3) Ubiquitination to Reduce Host Type I Interferon Production*

Received for publication, May 17, 2016, and in revised form, June 14, 2016. Published, JBC Papers in Press, July 7, 2016, DOI 10.1074/jbc.M116.738278

Xiaofei Gao1, Thanh H. Pham1, Leigh Ann Feuerbacher1, Kangming Chen1, Michael P. Hays1, Gyanendra Singh1, Christian Rueter1,†, Ramon Hurtado-Guerrero2‡, and Philip R. Hardwidge1,§

From the 1Whitehead Institute, Cambridge, Massachusetts 02142, 2Case Western Reserve University, Cleveland, Ohio 44106, the 3College of Veterinary Medicine, Kansas State University, Manhattan, Kansas 66506, the 4National Institute of Occupational Health, Meghani Nagar, Ahmedabad 380016, Gujarat, India, the 5Institute of Infectiology, University of Münster, D-48149 Münster, Germany, and the 6Fundacion ARAID, Edificio CEEI ARAGÓN and Institute of Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza, BIFI-IQFR (CSIC) Joint Unit, Mariano Esquillor s/n, Campus Rio Ebro, Edificio I+D, 50018 Zaragoza, Spain

Interferon signaling plays important roles in both intestinal homeostasis and in the host response to pathogen infection. The extent to which bacterial pathogens inhibit this host pathway is an understudied area of investigation. We characterized Citrobacter rodentium strains bearing deletions in individual type III secretion system effector genes to determine whether this pathogen inhibits the host type I IFN response and which effector is responsible. The NleB effector limited host IFN-β production by inhibiting Lys63-linked ubiquitination of TNF receptor-associated factor 3 (TRAF3). Inhibition was dependent on the glycosyltransferase activity of NleB. GAPDH, a target of NleB during infection, bound to TRAF3 and was required for maximal TRAF3 ubiquitination. NleB glycosyltransferase activity inhibited GAPDH-TRAF3 binding, resulting in reduced TRAF3 ubiquitination. Collectively, our data reveal important interplay between GAPDH and TRAF3 and suggest a mechanism by which the NleB effector inhibits type I IFN signaling.

Many bacterial pathogens utilize a type III secretion system (T3SS)2 to inject virulence proteins (effectors) into host cells to subvert various host functions (1). Characterizing the identity and mechanism of bacterial effectors expressed by attaching-effacing pathogens, which include enterohemorrhagic Escherichia coli (EHEC), enteropathogenic E. coli (EPEC), and Citrobacter rodentium, has been a subject of extensive research in recent years (2). Effector subversion of host pathways regulated by the transcription factor NF-κB has been relatively well studied (3), but less attention has been given to the potential inhibition of host interferon signaling, which also functions as an important early mediator of host defense.

Virally infected cells secrete interferon to protect other cells from subsequent infection (4). Type I IFNs (e.g. IFN-α and IFN-β) bind to a common IFN-α/β receptor (5), initiating a signal transduction cascade that ultimately induces the transcription of genes with promoters containing IFN-stimulated response elements (6). Type I IFNs induce antiviral activity via double-stranded RNA-dependent protein kinase and 2′,5′-oligoadenylate synthetase to promote viral mRNA degradation and inhibit translation, respectively (7).

Type I IFN production is stimulated not only by viral infection but also by bacterial components, including LPS and flagellin (8). LPS binding to Toll-like receptor 4 (TLR4) induces a type I IFN response mediated by the TRIF-related adaptor molecule and TIR-domain-containing adapter-inducing interferon-β adaptor proteins (9). TRIF-related adaptor molecule-TIR-domain-containing adapter-inducing interferon-β signaling results in the activation of TRAF family member-associated NF-κB activator-binding kinase (TBK1) and inducible 1κB kinase (IKKε (10)). Serine phosphorylation of interferon-regulatory factor 3 (IRF3) is mediated by TBK1/IKKε to induce IRF3 dimerization and nuclear translocation (11). Phosphorylated IRF3 (and IRF7) dimers then associate with AP-1, high-mobility group proteins, and NF-κB to form an IFN-β enhanceosome (12).

IFNs are important for maintaining intestinal homeostasis and for responding to pathogen infection (13). Accordingly, pathogens have also evolved strategies to interfere with host type I IFN signaling. For example, the anthrax lethal factor inhibits host IFN production by cleaving MAPKK6 (14) and by inhibiting STAT1 and ISGF3 activation (15). The deubiquitinating protease activity of Yersinia YopJ blocks TLR induction of type I IFN at the level of TAK1 activation by inhibiting TRAF3/6 ubiquitination (16). Vaccinia virus protein K7 prevents IRF3 activation by inhibiting the interaction between DEAD box protein 3 and IKKε (17). The Ebola virus VP35 protein blocks TBK1/IKKε interaction with IRF3/7 (18).

A recent study found that IFN-β is induced by EPEC infection and that the T3SS effector NleD both inhibits IFN-β induction and enhances TNF expression to promote barrier disruption (19). However, whether C. rodentium T3SS effectors may also inhibit...
host IFN-β induction is unclear. Here, we screened C. rodentium strains bearing deletions in individual effector genes to determine the extent to which this pathogen might inhibit the host IFN-β response. We found that the T3SS effector NleB limits host IFN-β production by inhibiting TRAF3 Lys63-linked ubiquitination.

**Results**

**NleB Inhibits the Generation of a Type I IFN Response**—To determine whether C. rodentium T3SS effectors inhibit the host type I IFN response, we monitored the survival of a recombinant vesicular stomatitis virus (VSV-G) expressing GFP (20). HeLa cells were first infected with C. rodentium strains possessing (WT) or lacking (ΔnleB) a functional T3SS for 2 h or were instead treated with LPS for 30 min as a positive control. HeLa cell supernatants were then removed, filtered, and applied to Vero cells for 12 h, which were then infected with VSV-G/GFP (multiplicity of infection = 5) for 30 h. Where indicated, Vero cells were pretreated with a type I IFN receptor blocking antibody (Ab, IFNAR2) before adding culture supernatants. B, representative images from screening multiple C. rodentium effector deletion strains. C, quantification of VSV-G/GFP intensity (arbitrary units) ± IFNAR2 antibody, normalized to DAPI intensity. *, significantly different VSV-GFP intensity compared with WT infection (one-way ANOVA, n = 3).

To test the hypothesis that this phenotype was due to the release of type I IFN from the infected HeLa cells, we used an antibody that blocks the IFN-α/β receptor. Pretreating Vero cells with this antibody blocked the ability of HeLa cell supernatants to inhibit virus replication (Fig. 1C). Thus, C. rodentium induces a host IFN-β response, and the T3SS effector NleB blocks the transduction of this response.

**NleB Inhibits IFN-β and RANTES Gene Transcription**—We then conducted in vitro experiments to determine the impact of NleB on IFN-β signaling pathways. To determine whether NleB inhibits transcription of the IFN-β gene, we performed quantitative RT-PCR analysis of IFNβ1 transcript abundance after infection with WT, ΔescN, or ΔnleB C. rodentium. Although infection with ΔescN stimulated IFNβ1 expression similar to LPS treatment, infection with the WT did not (Fig. 2A). This phenotype was attributable to NleB, as deleting nleB relieved the inhibition of IFNβ1 gene transcription observed with WT C. rodentium. We obtained similar data for the chemokine gene RANTES (CCL5, Fig. 2B).

IRF3 is a transcription factor that binds IFN-β and other innate immune gene promoters (21). Because NleB inhibited IFNβ1 and RANTES expression, which are both direct targets of IRF3, we tested whether IRF nuclear abundance was altered after bacterial infection. By fractionating cell lysates after bacterial infection, we observed that, although WT C. rodentium inhibited the accumulation of nuclear IRF3, infection with ΔescN and ΔnleB did not (Fig. 2C). We reached similar conclusions after using immunofluorescence microscopy and scoring the number of cells with predominantly nuclear IRF3 (Fig. 2D and E).

**NleB Reduces the Nuclear Abundance of STAT1—IFN-β stimulation results in the activation of the JAK-STAT pathway, which has a critical role in regulating the immune response to**
NleB Inhibits Interferon Production

**FIGURE 2. NleB inhibits IFN-β signaling.** A, quantification of IFN-β mRNA abundance after LPS treatment or after 3-h *C. rodentium* infection. *, significantly different (inbl) compared with WT infection (one-way ANOVA, n = 3). B, quantification of RANTES mRNA levels. *, significantly different (ranest) compared with WT infection (one-way ANOVA, n = 3). C, IRF3 immunoblotting of nuclear extracts derived from HeLa cells infected with *C. rodentium* strains versus time. D, immunofluorescence microscopy analysis of IRF3 (green) and cell nuclei (blue) after LPS stimulation or *C. rodentium* infection. E, relative abundance of nuclear IRF3 (3 h post-infection) normalized to poly-(ADP-ribose) polymerase. *, significantly different nuclear [IRF3] compared with WT infection (one-way ANOVA, n = 3). F, immunoblot analysis of cytoplasmic (C) and nuclear (N) fractions of HeLa cells infected with *C. rodentium* WT or ΔnleB or mock-infected with PBS in the presence or absence of IFN-β (5000 units/ml) stimulation for 30 min. Protein mobility differences between cytoplasmic and nuclear fractions are due to the differing [NaCl] in buffers used for cellular fractionation. G, quantification of the fold change in nuclear p-STAT1 in the absence or presence of IFN-β stimulation followed by *C. rodentium* infection. p-STAT1 signal intensity was normalized to tubulin (cytoplasmic) and poly(ADP-ribose) polymerase (nuclear). *, significantly different nuclear p-STAT1 (one-way ANOVA, n = 3).

NleB selectively inhibits the Lys63-linked TRAF3 ubiquitination (Fig. 3). Quanti-fication of these data is shown in Fig. 3, C and D.

Performing these studies with cells that were subsequently infected with *C. rodentium* strains possessing or lacking NleB revealed the selective impact of NleB on Lys63-linked TRAF3 ubiquitination. Although Lys63-linked TRAF3 ubiquitination did not differ among infection conditions, Lys63-linked TRAF3 ubiquitination was significantly inhibited in cells infected with either WT or ΔnleB/pnleB *C. rodentium* in contrast to its ubiquitination after infection with ΔnleB *C. rodentium* (Fig. 3, B–D). We corroborated these data by transfecting NleB in the absence of bacterial infection. Transfecting NleB into RAW264.7 cells infected with ΔnleB-linked TRAF3 ubiquitination (Fig. 3E). Overall, these data suggest that NleB selectively inhibits Lys63-linked TRAF3 ubiquitination.

Bacterial infection. After cytokine stimulation and activation of JAK, STATs, including STAT1, become phosphorylated and translocate to the nucleus, where they can activate the expression of immune response genes (22). To determine whether NleB inhibits p-STAT1 nuclear translocation, we evaluated the relative abundance p-STAT1 in HeLa cells infected with *C. rodentium* strains with or without IFN-β stimulation. We fractionated the cells to separate nuclear from cytoplasmic components. As expected, p-STAT1 nuclear abundance significantly increased after stimulation with IFN-β (Fig. 2, F and G). p-STAT1 nuclear abundance was reduced in samples infected with WT *C. rodentium* even after stimulation with IFN-β (Fig. 2, F and G). By contrast, ΔnleB *C. rodentium* failed to inhibit p-STAT1 nuclear translocation, indicating that NleB inhibits IFN-β-induced p-STAT1 nuclear translocation (Fig. 2, F and G).
**NleB Targets GAPDH to Regulate Lys^{63} Ubiquitination of TRAF3**—We showed previously that NleB functions as a translocated glycosyltransferase enzyme that covalently modifies host proteins with GlcNAc to subvert their normal functions (25). Specifically, we found that NleB disrupts TRAF2 signaling, leading to inhibition of the pro-inflammatory NF-κB pathway (25). We also revealed that the glycosylation enzyme GAPDH functions as a co-activator of TRAF2 activity. The modification of GAPDH with GlcNAc by NleB prevents GAPDH from binding to and activating TRAF2, leading to a reduced NF-κB response to infection (25).

To determine the extent to which the phenotypes we observed with TRAF3 were also dependent upon GAPDH, we first determined whether GAPDH interacts with TRAF3. By transfecting TRAF3-FLAG, we observed that GAPDH immunoprecipitated with TRAF3, similar to its ability to immunoprecipitate with TRAF2 (Fig. 4A). Co-incubation of GAPDH with NleB almost abolished the binding of GAPDH to TRAF3, at low concentrations of TRAF3, as shown in pulldown assays and in ELISAs (Fig. 4, B and C), similar to our previous observations with GAPDH binding to TRAF2 (25). Although a ~20% complex formation in the presence of NleB was achieved, this is contrast to ~100% complex formation in the absence of NleB (Fig. 4C).

Then we investigated whether the GAPDH-TRAF3 interaction is important for TRAF3 activity in response to LPS. We transfected GAPDH siRNA targeting the GAPDH 3′ UTR and complemented these knockdown cells with two GAPDH-Myc plasmids, either WT or a C150S GAPDH mutant (which does not interact with TRAFs (25)). After LPS treatment, Lys^{63}-Ub levels on TRAF3 were significantly elevated, as expected (Fig. 4D). Complementing GAPDH knockdown cells with WT, but not with C150S GAPDH, supported TRAF3 ubiquitination. Co-transfecting WT NleB, but not the glycosyltransferase-deficient mutant NleB(AAA) (25), significantly reduced Lys^{63}-linked TRAF3 ubiquitination and inhibited the association of GAPDH with TRAF3 (Fig. 4D).

**Discussion**

NleB is a translocated glycosyltransferase enzyme that was originally studied for its ability to suppress NF-κB activation (26, 27). We found previously that NleB disrupts TRAF2 signaling by modifying GAPDH with GlcNAc and preventing GAPDH from binding to and activating TRAF2 (25). The EPEC NleB1 effector modifies arginine residues of host proteins (N-GlcNAcylation) rather than the more typical targeting of serine/threonine residues (O-GlcNAcylation) (28, 29). In particular, it was found that EPEC NleB1 glycosylates the death domains of TRADD, FADD, RIPK1, and TNFR1, blocking the assembly of the TNFR1 complex. This N-GlcNAcylation disrupts TNF signaling in EPEC-infected cells, thus impacting NF-κB activation, apoptosis, and necroptosis (28, 29). A recent paper defined numerous amino acids that are important to the and immunoprecipitated using anti-FLAG antibody. Ubiquitination was assessed by immunoblotting for HA to detect ubiquitin (Ub) conjugates. C, quantification of Lys^{63}-ubiquitin from the experiments depicted in B, D, quantification of Lys^{63}-ubiquitin from the experiments depicted in B, *, significantly different ubiquitination levels compared with WT infection (one-way ANOVA, n = 3). E, Lys^{63} and Lys^{63} ubiquitination of TRAF3 as a function of NleB transfection. IP, immunoprecipitation; WB, Western blot.

---

**FIGURE 3. NleB inhibits Lys^{63}-ubiquitination of TRAF3.** A, cells were stimulated with 1.0 μg/ml poly(IC) for 12 h, *, significantly different luciferase activity compared with HA control transfection (one-way ANOVA, n = 3). B, Raw 264.7 cells were transfected with TRAF3-FLAG and either treated with LPS (1.0 μg/ml, 30 min) or infected with C. rodentium strains for 3 h. Cells were lysed.
NleB Inhibits Interferon Production

FIGURE 4. Role of GAPDH in TRAF3 ubiquitination. A, HEK293T cells were co-transfected with Myc-GAPDH and FLAG-TRAF2 or FLAG-TRAF3. After 48 h, cell lysates were immunoprecipitated (IP) with anti-FLAG antibody (Sigma), followed by immunoblotting with anti-GAPDH or anti-FLAG antibody. WB, Western blot. B, His-GAPDH immobilized to nickel-nitrioltriacetic acid-agarose beads were incubated with GST, GST-TRAF3, FLAG-NleB, or GST-TRAF3 + FLAG-NleB. Protein interactions were analyzed using immunoblotting. C, Immunolun-2 plates were coated with 1 µg of His-GAPDH and overlaid with GST-TRAF3 in the presence or absence of FLAG-NleB. Protein binding was detected using anti-GST antibody and 1-Step Ultra TMB-ELISA solution. Absorbance at 450 nm was measured. D, HEK-Blue hTLR4 cells were transfected with the indicated plasmids and GAPDH siRNAs. After 72 h, cells were treated with 200 ng/ml LPS for 40 min. Cell lysates were immunoprecipitated using FLAG antibody and immunoblotted for FLAG-TRAF3, HA-K63-ubiquitin, and GAPDH-Myc.
NleB inhibited the TRAF3 and GAPDH interaction by modifying GAPDH with GlcNAc. Other studies have shown that EPEC NleB1 glycosylates the death domains of TRADD, FADD, and other death domain-containing proteins (28, 29). Although these results are able to explain NleB function in the TNF/NF-κB signaling pathway, they fail to explain the function of NleB in the TLR4/type I IFN pathway we report here. The role of GAPDH in T cell activation has been described recently (32). GAPDH functions as a “switcher” of T cell activation by binding to AU-rich elements within the 3’ UTR of IFN-γ mRNA to suppress the translation of IFN-γ (32). Therefore, selective targeting of GAPDH might provide an interesting framework with which to understand the multiple effects of NleB on immune signaling pathways. Overall, our data suggest that C. rodentium NleB affects both NF-κB and interferon signaling.

Experimental Procedures

VSV-GFP assay—HeLa cells (ATCC) were grown in 6-well plates and infected with C. rodentium strains for 3 h. After infection, the cell supernatants were collected and filtered (0.25 μm). Vero cells (ATCC) were grown in 24-well plates on coverslips and treated with 50 μl of cell-free HeLa supernatants for 12 h and then infected with VSV-G/GFP (multiplicity of infection = 5) for 30 h. Vero cells were fixed with 3.7% paraformaldehyde before processing for immunofluorescence microscopy. Where indicated, Vero cells were treated with IFNAR2 antibody (1.0 μg/ml) before adding HeLa supernatants.

IRF3 Nuclear Translocation—HeLa cells were grown in 6-well plates and infected with C. rodentium strains where 70% confluent for 2–7 h. LPS (100 ng/ml, 1 h) was used as a positive control to induce IRF nuclear translocation. Nuclear extraction was performed as described previously (33), and nuclear proteins were resolved using SDS-PAGE and immunoblotted for IRF3 (Cell Signaling Technology).

STAT1 Assays—Overnight cultures of C. rodentium were diluted 1:10 in DMEM and grown for 3 h without shaking to an A600 of 0.9–1.0. HeLa cells were treated with IFN-β (5000 units/ml, PBL Assay Science) for 30 min to promote STAT1 phosphorylation and nuclear translocation (34). Cells were then inoculated with 100 μl of C. rodentium cultures for 3 h. Cells were subsequently washed with PBS, and cell fractionation was conducted as described (35). The concentration of p-STAT1 in the nuclear and cytoplasmic subcellular fractions was normalized to poly(ADP-ribose) polymerase and tubulin abundance, respectively.

Immunoprecipitation and Immunoblotting—Raw 264.7 cells (ATCC) were transfected, treated, and infected as indicated. Cells were then harvested into PBS, pooled, and centrifuged at 16,200 × g for 5 min. Supernatants were removed, and cells pellets were lysed in 20 mM Tris HCl (pH 8.0), 2 mM EDTA, 137 mM NaCl, 1% (w/v) Nonidet P-40, and 10% (v/v) glycerol. Samples were incubated on ice for 30 min, and cell lysates were collected by centrifugation at 7800 × g for 10 min at 4 °C. Protein G Dynabeads (Invitrogen) were used with appropriate antibodies for immunoprecipitation. Western blots were imaged using an Odyssey infrared imaging system (LI-COR).

Luciferase Assays—HEK 293T cells (ATCC) were co-transfected at a ratio of 10:1 (1.0 μg of total DNA) with a firefly luciferase construct driven by a consensus IFN-β promoter together with a Renilla luciferase plasmid and with NleB-HA or an HA control plasmid. After 24 h, cells were stimulated with 1.0 μg/ml poly(I:C) for 12 h. Cells were then lysed with passive lysis buffer, and lysates were analyzed by using the Dual-Luciferase kit (Promega) with firefly fluorescence units normalized to Renilla fluorescence units. Luciferase assays were performed in triplicate with at least three independently transfected cell populations.

RT-PCR—cDNA was prepared from 1 μg of RNA by using the Superscript First Strand System (Invitrogen) with oligo(dt) primer. Real-time PCR was performed in triplicate using SYBR Green PCR Master Mix (Ambion) in a Fast 7500 sequence detection system (Applied Biosystems). Relative transcription levels were calculated by using the ΔΔCt method.

Ubiquitination Assays—Anti-FLAG M2 affinity resin was washed twice with cold TBS buffer (50 mM Tris HCl (pH 7.4) and 150 mM NaCl) and centrifuged at 8000 × g for 30 s at 4 °C. HEK-Blue hTLR4 cells (InvivoGen) were transfected and treated with LPS. Cells were harvested and washed with cold PBS. Cell pellets were lysed in lysis buffer (50 mM Tris HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, and 1% Triton X-100) on ice for 30 min and then mixed with anti-FLAG M2 affinity resin and rotated at 4 °C overnight. Resins were centrifuged at 8000 × g for 30 s at 4 °C and then washed three times with cold TBS buffer. Eluates were immunoblotted with FLAG-TRAF3, GAPDH-Myc, and HA-Lys63-ubiquitin.

ELISAs—Immulon-2 96-well plates (Dynatech) were coated with 1.0 μg of GAPDH and incubated at 37 °C for 1 h. Plates were washed with 0.05% PBS-Tween and blocked in 5% (w/v) nonfat milk in PBS-Tween. After washing, the plates were overlaid with different amounts of GST-TRAF3 or with GST-Traf3 that had been labeled with GlcNAc by NleB. After 1-h incubation at 37 °C, primary and secondary antibodies were added. Plates were developed with 1-Step Ultra TMB-ELISA solution (Thermo Scientific) and then quenched with 2 M H2SO4. Absorbance at 450 nm was measured.

Pulldown Assays—GAPDH was immobilized on nickel-nitrilotriacetic acid-agarose beads (Qiagen) and then incubated with 1.0 μg of purified GST or GST-Traf3 in the presence or absence of FLAG-NleB for 4 h at 4 °C. Beads were centrifuged and washed three times with 20 mM HEPES (pH 7.9), 150 mM KCl, 0.2 mM EDTA, 0.1% Nonidet P-40, 10% glycerol, and 1.0 mM DTT. Samples were analyzed using immunoblotting.

Statistical Analyses—Immunoblotting, luciferase, and RT-PCR assays were analyzed statistically using one-way ANOVA and Dunn’s multiple comparisons test. Data shown are the mean ± S.E. of at least three replicates. p < 0.05 was considered significant.

Author Contributions—P. R. H. conceived and coordinated the study and wrote the paper. X. G., T. H. P., L. A. F., K. C., M. P. H., and G. S. designed, performed, and analyzed the experiments. C. R. and R. H. G. assisted with analysis and interpretation of data. All authors reviewed the results and approved the final version of the manuscript.

References
1. Cornelis, G. R. (2010) The type III secretion injectisome, a complex nanomachine for intracellular ‘toxin’ delivery. Biol. Chem. 391, 745–751
2. Sanchez-Villamil, J., and Navarro-Garcia, F. (2015) Role of virulence factors on host inflammatory response induced by diarrheagenic Escherichia coli pathotypes. Future Microbiol. 10, 1009–1033

3. Rahman, M. M., and McFadden, G. (2011) Modulation of NF-κB signaling by microbial pathogens. Nat. Rev. Microbiol. 9, 291–306

4. Isaacs, A., and Lindemann, J. (1957) Virus interference: I. the interferon. Proc. R. Soc. Lond. B. Biol. Sci. 147, 258–267

5. Mogensen, K. E., Lewerenz, M., Reboul, J., Lutfalla, G., and Uzé, G. (1999) The type I interferon receptor: structure, function, and evolution of a family business. J. Interferon Cytokine Res. 19, 1069–1098

6. Levy, D. E., Marié, L., and Prakash, A. (2003) Ringing the interferon alarm: differential regulation of gene expression at the interface between innate and adaptive immunity. Curr. Opin. Immunol. 15, 52–58

7. van den Broek, M. F., Müller, U., Huang, S., Zinkernagel, R. M., and Aguet, M. (1995) Immune defence in mice lacking type I/II type II interferon receptors. Immunol. Rev. 148, 5–18

8. Belardelli, F. (1995) Role of interferons and other cytokines in the regulation of the immune response. APAMS 103, 161–179

9. Karaghiosoff, M., Hovanessian, A. G., Levy, D. E., and Marié, I. J. (2005) Regulation of interferon and other cytokines gene expression by deacetylation. J. Immunol. 172, 747–751

10. Gold, J. A., Hoshino, Y., Hoshino, S., Jones, M. B., Nolan, A., and Weiden, M. D. (2004) Exogenous γ and α/β interferon rescue human macrophages from cell death induced by Bacillus anthracis. Infect. Immun. 72, 1291–1297

11. Sweet, C. R., Conlon, J., Golenbock, D. T., Goguen, J., and Silverman, N. (2007) Yop1 targets TRAF proteins to inhibit TLR-mediated NF-κB, MAPK and IFN signaling pathway. Cell. Microbiol. 9, 2700–2715

12. Chang, C. H., Curtis, J. D., Maggi, L. B., Jr., Faubert, B., Villarino, A. V., O’Sullivan, D., Huang, S. C., van der Windt, G. J., Blagih, J., Qiu, J., Weber, J. D., Pearce, E. J., Jones, R. G., and Pearce, E. L. (2013) Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell 153, 1239–1251

13. Gao, X., Fan, W., Mateo, K., Callegari, E., Wang, D., Deng, W., Puente, J., Li, F., Chasseau, M. S., Finlay, B. B., Lenardo, M. J., and Hardwidge, P. R. (2009) STAT-phosphorylation-independent induction of interferon regulatory factor-9 by interferon-β. J. Interferon Cyto- kine Res. 30, 163–170