Austin Journal of Clinical Immunology

Austin Publishing Group

Open Access Full Text Article

Editorial

Pathogen Hijacking of Crk Adaptor Proteins and Crk-Regulated Signal Transduction Pathways

Neta Sal-Man and Noah Isakov*

Department of Microbiology, Immunology and Genetics, Ben Gurion University, Israel

***Corresponding author:** Noah Isakov, The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben Gurion University of the Negev, P.O.B. 653, Beer Sheva 84105, Israel, Tel: 97286477267; Fax: 97286477626; Email: noah@bgu.ac.il

Received: March 03 2014; **Accepted:** March 05 2014; **Published:** March 07 2014

Microbial pathogens have evolved a variety of strategies to promote their own survival and utilize different tactics to divert host immune responses. Many types ofpathogenic bacteria employ unique secretion systems through which they deliver novel 'weapons', or virulence factorsinto eukaryotic cells, which promote bacteriainvasion and/orperturb diverse host cell functionsto facilitate bacterial colonization.

Among the cellular host proteins that are targeted by invading pathogens, Crk adaptor proteins, which linkdifferent surface receptors to their correspondingsignaling pathways, appear to be preferred targets. Crk was discovered in the late 1980s as a chicken retroviral oncogene product that consists of SH2 and SH3 domains [1,2]. It was later found to have three mammalian homologs: CrkI and CrkII, which are alternative spliced forms of a single gene, and CrkL, which is encoded by a separate gene [3,4]. The Crk proteins are involved in the regulation of many different cellular activities, including gene expression, cell adhesion, migration, proliferation and differentiation, as well as cell transformation and apoptosis [5]. Furthermore, Crk proteins integrate signalsthat are critical for immune cell functions [6,7], and are therefore potential drug targets in maladies caused by cancer, autoimmunity and infection diseases [8,9].

Recent studies suggested that Crk adaptor proteins contribute to bacterial and perhaps also viral pathogenesis by promoting pathogen entry into cells and byserving as targets forvirulence factors that subvert the cellular machinery to create a microenvironment which is beneficial for the pathogen.

Involvement of Crk adaptor proteins in bacterial entry into mammalian cells was first noted in *Yersinia pseudotuberculosis* infection of human epithelial cells [10,11]. *Yersinia* is a Gram-negative bacterium with a type-III secretion system (T3SS), and its intracellular invasion involves an interaction between the bacterial protein, invasin, and the host cell β1 integrin receptor, which triggers the cellular machinery that supports *Yersinia*uptake. Invasin binding to the β1 integrin initiates a host response leading to activation of FAK (focal adhesion kinase)- and/or Src protein tyrosine kinases [12,13]. and phosphorylation of p130Cas (Crk-associated substrate, 130kDa). A subsequent interaction between phospho-p130Cas and Crk is required for further activation of the GTP-binding protein, Rac1, which promotes actin rearrangement and bacterial internalization. Cell transfection with CrkII point mutants in the SH2 (R38V) or SH3N(W169L) domains, which are unable to interact with p130Cas or DOCK180(dedicator of cytokinesis, 180kDa; an upstream regulator of Rac1), respectively, resulted in decreased bacterial uptake, demonstrating the critical role of CrkII and its SH2- and SH3N-domain-binding partners in the bacterial internalization process.

Crk proteins are also required forcell infection by *Shigella flexneri,* another intracellular Gram-negative bacterium with aT3SS [14]. Entry of *S. flexneri* into mammalian cells is made possibleby binding of theIpaA and IpaB bacterial proteinstothe host cell integrin α5β1 and CD44 surface receptors, respectively, which establishthe initial contact [15,16]. A key event in the early phase of the infection is the induction of actin polymerization and cytoskeletal reorganization at the bacteria-host cell contact area, which promotes bacterial internalization. This step involves Abl/Arg-mediated tyrosine phosphorylation of CrkII, which in turn activates the Rho family GTPases, Cdc42 and Rac, leading to actin polymerization and rearrangement of the cytoskeleton [17]. Phosphorylation of CrkII at tyrosine 221 (Y221) is an essential event during cell infection by *S. flexneri,* and is essential for bacterial invasion. Overexpression of aphosphorylation-deficient mutant of CrkII, in which tyrosine 221 is replaced by phenylalanine (Crk Y221F), inhibits bacterial entry into the cells [14].

Additional Crk-regulated host cell proteins that are involved in non-phagocytic cell invasion by *S. flexneri* are cortactin and Unc119 [18,19]. Cortactin is involved in*S. flexneri* entry into epithelial cellsthrough itsbinding to and cooperation with the Crk protein, thuspromoting actin polymerization and cytoskeletal rearrangement [18]. Unc119 acts asan upstream negative regulator of Abl, thereforeinhibits Abl-mediated CrkII phosphorylation at tyrosine 221, and consequently, reduces *S. flexneri*intakebycells. In agreement, knockdownof Unc119 enhanced bacterial invasion, while cell treatment with a cell permeable Unc119 protein lead to a partial inhibition of bacterial internalization [19].

While *Pseudomonas aeruginosa* [20,21]. and *Salmonella enteric* [22]. possess distinct T3SSsand utilize differentarray of virulence factors topromotecell invasion, they subvert common host signaling pathwaysto supporttheiruptake. This twopathogensgain entry into nonphagocytic cells by utilizing the Abl-dependent Crk-mediated signaling pathways that manipulate the host cell actin assembly and promote cytoskeleton rearrangement. Furthermore, the *P. aeruginosa*

Isakov et al. © All rights are reserved

Citation: Sal-Man N, Isakov N. Pathogen Hijacking of Crk Adaptor Proteins and Crk-Regulated Signal Transduction Pathways. Austin J Clin Immunol. 2014;1(3): 1012.

virulence factor, ExoT, can disrupt host cell signaling pathways by ADP-ribosylating CrkI and CrkII at Arg20 within the SH2 domain, thereby interfering with Crk binding to p130Cas and modulating p130Cas-dependent signaling events.

A somewhat different mechanism for cell invasion has been proposed forthe intracellular Gram-positive bacteria, *Listeria monocytogenes*, [23,24]. This bacterium possesses a surface protein,termed INIB, which interacts with a host cell surface receptor tyrosine kinase, termed Met.Binding of INIBactivates the Met catalytic domain, which stimulates a CrkII and Gab1 adaptor protein-regulated signal transduction pathways.Consequently, phosphoinositides 3-kinase (PI-3K) undergoes activation and promotesadditional events that support bacterial entry into the cells. Recently it was shown that the SH3C domain of CrkII is required for activation of PI-3K. This activation promotes changes in actin polymerization necessary for bacterial entry. The finding that the Crk-SH3C domain is essential for bacterial uptake is surprising, since in contrast to the Crk-SH2 and SH3N domain, Crk-SH3C has no known binding partners, and is assumed to function as an integral regulatory region.

A different and novel strategyofinteraction with host cells has been adopted by the Gram-negative bacteria *Helicobacter pylori* [25,26], which colonize the gastric epithelia. *H. pylori*mediates persistent infection by inhibiting cell apoptosis, thereby preventing the rapid epithelial cell turnover that facilitates bacterial clearance. This mechanism is made possible by the *H. pylori* virulence protein, CagA, which is delivered into the host cellswhere itupregulates survival mechanisms and induces anti-apoptotic pathways. The molecular basis of this process involves binding of CagA to CrkI, CrkII and CrkL adaptor proteins, leading to induction of signaling events that activate pro-survival effector molecules, including the MEK/ERK cascade and the anti-apoptotic protein MCL1 (myeloid cell leukemia sequence 1).

It is interesting to note that the Gram-negative bacteria, *Escherichia coli*, also utilize aT3SS to deliver virulence factors that ultimately modify the Crk signaling pathway. While CrkII was found to be selectively recruited to the pedestal of the *enteropathogenicE. coli* (EPEC) and not to that of the *enterohemorrhagicE. Coli* (EHEC) [27], recent studies revealed that EHEC infection coincide with intracellular delivery of a virulence protein NleH1, which physically interacts with CrkL [28]. Binding of CrkL to IKKβandinteractionwith NleH1promotes NleH1 association with the ribosomal protein S3 (RPS3), which leads to modulation ofthe RPS3/NFκB signaling pathway? While the exact effect of NleH1 in the host cell is not fully clear, it is assumed to promote bacterial survival by inhibiting innate immune responses.

A recent study demonstrated that *Chlamydia trachomatis*, a Gram-negative obligate intracellular bacteria and the causative agent of trachoma and sexually transmitted diseases, also engage CrkI and CrkII to promotebacterial recruitment to nascent inclusions,and thereby alteringinnate anti-*Chlamydia* immune mechanisms [29]. This activity is carried out by TepP (translocated early phosphoprotein), which is translocated into the host cells during the early phase of cell entry. Tep Pundergoes pho sphorylation by a host cell kinase andacts as a bacterial linker that associates with host cell CrkI/CrkII to alter the regulation of innate immune response genes.

Viruses also utilize a variety of strategies to evade host cells and/or neutralize anti-viral responses. The 1918 Spanish influenza virus and the avian influenza. A viruseswere found to utilize Crk-regulated signaling pathways to promote intracellular viral replication [30-32]. These viruses utilize their NS1 (nonstructural protein 1) virulence factor, which possesses a proline-rich SH3-binding motif, to bindCrkI/II and CrkL proteins with high affinity,in order todownregulate JNK-ATF2 signaling. The JNK-ATF2 pathway suppresses apoptosis, which is detrimental to viral proliferation, and is therefore inhibited by the virus. Knock-down of the host cell CrkI/II and CrkL proteins have shown to significantly impair viral propagation, indicating that NS1-Crk interaction is critical for viral replication [30].

Altogether, the studiesdescribed here demonstrate that Crk adaptor proteins are essential for cell infection and propagation of a variety of pathogens. Exogenous manipulation of Crk protein expression or function might therefore serve as potential strategies for inhibition of pathogen replication and survival. Furthermore,better understanding of the mechanisms by which different virulence factors hijack cellular effector molecules and signaling pathways may provide crucial information for the design of drugs that ban pathogen propagation by divertingdifferent cellular machineries.

References

1. Mayer BJ, Hamaguchi M, Hanafusa H . A novel viral oncogene with structural similarity to phospholipase C. Nature. 1988; 332: 272-275.
2. Reichman CT, Mayer BJ, Keshav S, Hanafusa H. The product of the cellular crk gene consists primarily of SH2 and SH3 regions. Cell Growth Differ. 1992; 7: 451-460.
3. Matsuda M, Tanaka S, Nagata S, Kojima A, Kurata T . Two species of human CRK cDNA encode proteins with distinct biological activities. Mol Cell Biol. 1992; 12: 3482-3489.
4. ten Hoeve J, Morris C, Heisterkamp N, Groffen J . Isolation and chromosomal localization of CRKL, a human crk-like gene. Oncogene. 1993; 8: 2469-2474.
5. Birge RB, Kalodimos C, Inagaki F, Tanaka S . Crk and CrkL adaptor proteins: networks for physiological and pathological signaling. Cell Commun Signal. 2009; 7: 13.
6. Gelkop S, Babichev Y, and Isakov, N.T cell activation induces direct binding of the Crk adapter protein to the regulatory subunit of phosphatidylinositol 3-kinase (p85) via a complex mechanism involving the Cbl protein. The Journal of biological chemistry. 2001; 276: 36174-36182.
7. Gelkop S, Isakov N. T cell activation stimulates the association of enzymatically active tyrosine-phosphorylated ZAP-70 with the Crk adapter proteins. J Biol Chem. 1999; 274: 21519-21527.
8. Gelkop S, Babichev Y, Kalifa R, Tamir A, Isakov N . Involvement of crk adapter proteins in regulation of lymphoid cell functions. Immunol Res. 2003; 28: 79-91.
9. Feller SM, Lewitzky M . Potential disease targets for drugs that disrupt protein-- protein interactions of Grb2 and Crk family adaptors. Curr Pharm Des. 2006; 12: 529-548.
10. Weidow CL, Black DS, Bliska JB, Bouton AH . CAS/Crk signalling mediates uptake of Yersinia into human epithelial cells. Cell Microbiol. 2000; 2: 549-560.
11. Bruce-Staska PJ, Weidow CL, Gibson JJ, Bouton AH . Cas, Fak and Pyk2 function in diverse signaling cascades to promote Yersinia uptake. J Cell Sci. 2002; 115: 2689-2700.
12. Alrutz MA, Isberg RR . Involvement of focal adhesion kinase in invasin-mediated uptake. Proc Natl Acad Sci U S A. 1998; 95: 13658-13663.

13. Reynolds AB, Kanner SB, Wang HC, Parsons JT . Stable association of activated pp60src with two tyrosine-phosphorylated cellular proteins. Mol Cell Biol. 1989; 9: 3951-3958.

14. Burton EA, Plattner R, Pendergast AM . Abl tyrosine kinases are required for infection by Shigella flexneri. EMBO J. 2003; 22: 5471-5479.

15. Watarai M, Funato S, Sasakawa C . Interaction of Ipa proteins of Shigella flexneri with alpha5beta1 integrin promotes entry of the bacteria into mammalian cells. J Exp Med. 1996; 183: 991-999.

16. Skoudy A, Mounier J, Aruffo A, Ohayon H, Gounon P. CD44 binds to the Shigella IpaB protein and participates in bacterial invasion of epithelial cells. Cell Microbiol. 2000; 2: 19-33.

17. Lamarche N, Tapon N, Stowers L, Burbelo PD, Aspenström P . Rac and Cdc42 induce actin polymerization and G1 cell cycle progression independently of p65PAK and the JNK/SAPK MAP kinase cascade. Cell. 1996; 87: 519-529.

18. Bougnères L, Girardin SE, Weed SA, Karginov AV, Olivo-Marin JC, Parsons JT, et al. Cortactin and Crk cooperate to trigger actin polymerization during Shigella invasion of epithelial cells. J Cell Biol. 2004; 166: 225-235.

19. Vepachedu R, Karim Z, Patel O, Goplen N, Alam R . Unc119 protects from Shigella infection by inhibiting the Abl family kinases. PLoS One. 2009; 4: e5211.

20. Pielage JF, Powell KR, Kalman D, Engel JN . RNAi screen reveals an Abl kinase-dependent host cell pathway involved in Pseudomonas aeruginosa internalization. PLoS Pathog. 2008; 4: e1000031.

21. Sun J, Barbieri JT . Pseudomonas aeruginosa ExoT ADP-ribosylates CT10 regulator of kinase (Crk) proteins. J Biol Chem. 2003; 278: 32794-32800.

22. Ly KT, Casanova JE . Abelson tyrosine kinase facilitates Salmonella enterica serovar Typhimurium entry into epithelial cells. Infect Immun. 2009; 77: 60-69.

23. Dokainish H, Gavicherla B, Shen Y, Ireton K. The carboxyl-terminal SH3 domain of the mammalian adaptor CrkII promotes internalization of Listeria monocytogenes through activation of host phosphoinositide 3-kinase. Cell Microbiol. 2007; 9: 2497-2516.

24. Sun H, Shen Y, Dokainish H, Holgado-Madruga M, Wong A. Host adaptor proteins Gab1 and CrkII promote InlB-dependent entry of Listeria monocytogenes. Cell Microbiol. 2005; 7: 443-457.

25. Suzuki M, Mimuro H, Suzuki T, Park M, Yamamoto T. Interaction of CagA with Crk plays an important role in Helicobacter pylori-induced loss of gastric epithelial cell adhesion. J Exp Med. 2005; 202: 1235-1247.

26. Mimuro H, Suzuki T, Nagai S, Rieder G, Suzuki M . Helicobacter pylori dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach. Cell Host Microbe. 2007; 2: 250-263.

27. Goosney DL, DeVinney R, Finlay BB. Recruitment of cytoskeletal and signaling proteins to enteropathogenic and enterohemorrhagic Escherichia coli pedestals. Infect Immun. 2001; 69: 3315-3322.

28. Pham TH, Gao X, Singh G, Hardwidge PR . Escherichia coli virulence protein NleH1 interaction with the v-Crk sarcoma virus CT10 oncogene-like protein (CRKL) governs NleH1 inhibition of the ribosomal protein S3 (RPS3)/nuclear factor Î°B (NF-Î°B) pathway. J Biol Chem. 2013; 288: 34567-34574.

29. Chen YS, Bastidas RJ, Saka HA, Carpenter VK, Richards KL, et al. The Chlamydia trachomatis Type III Secretion Chaperone Slc1 Engages Multiple Early Effectors, Including TepP, a Tyrosine-phosphorylated Protein Required for the Recruitment of CrkI-II to Nascent Inclusions and Innate Immune Signaling. PLoS pathog. 2014; 10: e1003954.

30. Hrincius ER, Wixler V, Wolff T, Wagner R, Ludwig S, Ehrhardt C. CRK adaptor protein expression is required for efficient replication of avian influenza A viruses and controls JNK-mediated apoptotic responses. Cell Microbiol. 2010; 12: 831-843.

31. Heikkinen LS, Kazlauskas A, Melén K, Wagner R, Ziegler T . Avian and 1918 Spanish influenza a virus NS1 proteins bind to Crk/CrkL Src homology 3 domains to activate host cell signaling. J Biol Chem. 2008; 283: 5719-5727.

32. Miyazaki M, Nishihara H, Hasegawa H, Tashiro M, Wang L . NS1-binding protein abrogates the elevation of cell viability by the influenza A virus NS1 protein in association with CRKL. Biochem Biophys Res Commun. 2013; 441: 953-957.

Austin J Clin Immunol - Volume 1 Issue 3 - 2014
ISSN : 2381-9138 | www.austinpublishinggroup.com
Isakov et al. © All rights are reserved

Citation: Sal-Man N, Isakov N. Pathogen Hijacking of Crk Adaptor Proteins and Crk-Regulated Signal Transduction Pathways. Austin J Clin Immunol. 2014;1(3): 1012.