Shigella-induced Apoptosis Is Dependent on Caspase-1 Which Binds to IpAB*

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We report here that the Shigella invasion plasmid antigen (Ipa)B, which is sufficient to induce apoptosis in macrophages, binds to caspase (Casp)-1, but not to Casp-2 or Casp-3. Casp-1 is activated and its specific substrate interleukin-1β is cleaved shortly after Shigella infection. Macrophages isolated from Casp-1 knock-out mice are not susceptible to Shigella-induced apoptosis, although they respond normally to other apoptotic stimuli. Shigella kills macrophages from casp-3, casp-11, and p53 knock-out mice as well as macrophages overexpressing Bel-2. We propose that Shigella induces apoptosis by directly activating Casp-1 through IpAB, bypassing signal transduction events and caspases upstream of Casp-1. Taken together these data indicate that Shigella-induced apoptosis is distinct from other forms of apoptosis and seems uniquely dependent on Casp-1.

Shigellae are the etiological agents of bacillary dysentery, an acute form of diarrhea accompanied by blood and mucus. Dysentery is an epidemiologically important disease which is often fatal in children. Shigellae are invasive bacteria that penetrate the colonic tissue and initiate an acute inflammation which is fatal in children. Shigellae are invasive bacteria that penetrate the colonic tissue and initiate an acute inflammation which is fatal in children. Shigellae are invasive bacteria that penetrate the colonic tissue and initiate an acute inflammation which is fatal in children.
however, to some apoptotic stimuli. Casp-3 is therefore required in many, but not all pathways that lead to programmed cell death. Casp-2 is alternatively spliced and generates a pro- and an anti-apoptotic molecule (26). In casp-2 /−− mice apoptosis mediated by perforin and granzyme B was defective but developmental cell death of motor neurons was accelerated (27). The position of Casp-2 in a “caspase cascade” is still unclear (28, 29).

Here, we show that IpaB binds to Casp-1, but not Casp-2 or Casp-3. As expected, Casp-1 is activated and IL-1β is cleaved during a Shigella infection. Using macrophages elicited from mice with different targeted deletions, we demonstrate that Shigella requires Casp-1, but not Casp-3 or Casp-11 to induce apoptosis. Shigella-induced apoptosis proceeds in the absence of the apoptosis promoter p53 and in the presence of the anti-apoptotic protein Bel-2. We propose a model where Shigella bypasses upstream apoptotic regulators to directly activate Casp-1.

EXPERIMENTAL PROCEDURES

Ligand Blot of His-tagged Caspase Fusion Proteins—His-tagged caspases were constructed by releasing the cDNA from pJ.348 (17) by a BamHI, SalI digestion, cloning into pGSTag (30), and subsequently subcloning into pRSETA (Invitrogen, Carlsbad, CA). casp-2 (31) and casp-3 (22) were amplified by polymerase chain reaction from pMSN2.4 and pSK-CPP32a, respectively (kindly provided by Dr. S. Kumar; Hanson Cancer Research, Adelaide, Australia, and Dr. E. S. Alnemri; Jefferson Cancer Institute, Philadelphia, PA). The polymerase chain reaction products were cloned into the BamHI and NotI sites of pRSETA and sequenced.

The constructs (His-casp-1, -2, and -3) were transformed into Escherichia coli BL21(DE3)/pLySs and induced with 0.5 mM isopropyl-1-thio-β-d-galactopyranoside at an OD600 of 0.8–1.0 (1 h at 37 °C). Bacterial pellets were resuspended in binding buffer containing 1 mM phenylmethylsulfonyl fluoride, lysed by sonication, and the His-tagged caspases were purified by nickel chelate chromatography according to the manufacturer’s instructions (Novagen, Madison, WI). His-casp-3 was soluble and stable under the above conditions, while His-casp-2 had to be solubilized from inclusion bodies by addition of 1.5% methyl-β-cyclodextrin. His-casp-1 and -2 were stabilized by addition of either 100 μg/ml acetyl-Tyr-Val-Ala-Asp-CHO (VYD-CHO) or 100 μg/ml acetyl-Asp-Glu-Val-Asp-CHO (DEVD-CHO), respectively. The purified His-tagged caspases were quantified by Western blot using a monoclonal anti-T7-TAG antibody (Novagen).

The probes for the ligand blot experiment, GSTag-IpaB and GSTag, were purified and labeled with protein kinase A/32P-ATP as described (9). Nitrocellulose membranes containing similar amounts of purified caspases were purified and labeled with protein kinase A/32P[γ-]ATP as described (9). Nitrocellulose membranes containing similar amounts of protein were loaded into each lane (Fig. 1). We verified the amount of protein loaded into each lane (Fig. 1) using a goat anti-IL-1β antiserum (Boehringer Mannheim, Indianapolis, IN). For IL-1β experiments, 106 cells were treated with serum-free RPMI 1640, and, when indicated, the cells were incubated for 1 h with 50 μM YVAD-CMK. After infection at a multiplicity of infection of 50 the macrophages were lysed in situ at given time points as described previously (35). Samples containing equal protein amounts were separated on 15% SDS-polyacrylamide gels, transferred to nitrocellulose, and analyzed by Western blot using either a rabbit anti-Casp-1 (kindly provided by Dr. D. Miller; Merck, Rahway, NJ), or a goat anti-IL-1β (R&D Systems, Minneapolis, MN) antibody.

RESULTS

IpaB Binds to Casp-1, But Not to Casp-2 or Casp-3—The S. flexneri strain IpaB was shown to bind to a caspase in macrophage lysates both in vitro and in vivo (9). The IpaB-binding caspase was tentatively identified as Casp-1 using a polyclonal antiserum (9). To confirm that IpaB binds Casp-1, and test whether other caspases also bind this bacterial protein, we performed a ligand blot assay. We chose to test Casp-1, Casp-2, and Casp-3 as representative members of the three caspase subfamilies (11).

Purified His-tagged Casp-1, Casp-2, and Casp-3 were resolved by SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose. An anti-T7 monoclonal antibody which recognizes an epitope within the His-tag was used to detect IL-1β. Lysates were made at the indicated time points and analyzed by immunoblots for the maturation of Casp-1 and casp-2. However, we were not able to detect IL-1β in these lysates. The results are shown in Fig. 1. IpaB binds to Casp-1, but not to Casp-2 or Casp-3, as confirmed by ligand blot analysis.

In vivo studies showed that IpaB binds to Casp-1, but not to Casp-2 or Casp-3, when Shigella flexneri was injected into mice. The results are shown in Fig. 1. IpaB binds to Casp-1, but not to Casp-2 or Casp-3, as confirmed by ligand blot analysis.
IL-1β. We detected Casp-1 maturation from the 45-kDa precursor to the 20-kDa subunit as early as 20 min after infection (Fig. 2A). This anti-Casp-1 antibody recognizes the 20 kDa, but not the 10-kDa subunit of the mature enzyme. As expected, expression of Casp-1 was not up-regulated after activation with LPS and Casp-1 did not mature in macrophages infected with the non-virulent strain BS176. We also tested whether the caspase inhibitor YVAD could block caspase maturation. YVAD inhibited Casp-1 maturation (Fig. 2B), as well as Shigella-induced apoptosis (9).

IL-1β, a substrate of Casp-1, was cleaved with similar kinetics to Casp-1. Not surprisingly, expression of IL-1β was only detected in LPS-activated macrophages. IL-1β was not cleaved in macrophages infected with BS176 (Fig. 2C). IL-1β maturation was significantly retarded in macrophages preincubated with YVAD (Fig. 2D). These data show that Casp-1 is activated in Shigella-infected macrophages.

Casp-1 −/− Macrophages Are Resistant to Shigella-induced Apoptosis in Vitro—To further investigate the role of Casp-1 in Shigella-induced apoptosis, we infected peritoneal macrophages from wild type mice with virulent (M90T) or avirulent (BS176) S. flexneri. Lysates collected at the indicated post-infection time points were analyzed by Western blot. A, Western probed with an anti-Casp-1 antibody. Casp-1 is expressed at comparable levels in naive and LPS-activated macrophages and matures shortly after infection with M90T as indicated by the appearance of the 20-kDa band. B, Western probed with an anti-Casp-1 antibody of macrophages treated with the Casp-1 inhibitor YVAD-cmk before infection. Casp-1 maturation is blocked by YVAD-cmk. C, Western probed with an anti-IL-1β antibody. IL-1β is expressed only in LPS-activated macrophages and matures after infection with M90T as indicated by the appearance of the 17-kDa band. D, Western probed with an anti-IL-1β antibody of macrophages treated with the Casp-1 inhibitor YVAD-cmk before infection. As expected, IL-1β maturation is blocked by YVAD-cmk.

![Fig. 2. Casp-1 matures during S. flexneri infection. Naive or LPS-activated peritoneal macrophages from wild type mice were infected with virulent (M90T) or avirulent (BS176) S. flexneri. Lysates collected at the indicated post-infection time points were analyzed by Western blot. A, Western probed with an anti-Casp-1 antibody. Casp-1 is expressed at comparable levels in naive and LPS-activated macrophages and matures shortly after infection with M90T as indicated by the appearance of the 20-kDa band. B, Western probed with an anti-Casp-1 antibody of macrophages treated with the Casp-1 inhibitor YVAD-cmk before infection. Casp-1 maturation is blocked by YVAD-cmk. C, Western probed with an anti-IL-1β antibody. IL-1β is expressed only in LPS-activated macrophages and matures after infection with M90T as indicated by the appearance of the 17-kDa band. D, Western probed with an anti-IL-1β antibody of macrophages treated with the Casp-1 inhibitor YVAD-cmk before infection. As expected, IL-1β maturation is blocked by YVAD-cmk.](image)

by other stimuli (18), Shigella-mediated macrophage death requires casp-1.

Casp-3 and Casp-11 Are Not Necessary for Shigella-induced Apoptosis—We tested whether macrophages from casp-3 knock-out mice (25) were susceptible to Shigella-induced cytotoxicity. Peritoneal macrophages isolated from wild type and casp-3 −/− mice were killed with equal efficiency by Shigella (Fig. 4A). As expected, BS176 was not cytotoxic.

Similar results were obtained with macrophages from casp-11 knock-out mice (21). Casp-11 appears to be an upstream regulator of Casp-1 activation (21). As shown in Fig. 4B, virulent Shigella killed macrophages isolated from control or casp 11 −/− mice to the same extent. BS176 was not cytotoxic to macrophages from either mice lineage. Since Casp-11 is induced after LPS activation, we also tested LPS-activated macrophages from control and casp 11 −/− mice. M90T induced cell death with equal efficiency in both types of activated macrophages (data not shown).

Shigella-induced Apoptosis Is Independent of p53 and Cannot be Inhibited by Bcl-2—To further investigate the nature of the apoptotic cascade engaged in macrophages infected by Shigella, we tested the role of the tumor suppressor, p53, as well as the apoptosis inhibitor oncogene bcl-2. p53 is necessary for apoptosis induced by numerous stimuli (36). Conversely, apoptosis is often inhibited by overexpression of Bcl-2 (37, 38). Shigella killed p53 −/− as efficiently as wild type macrophages (Fig. 5A), demonstrating that Shigella-induced apoptosis is independent of p53. BS176 was unable to kill either wild type or p53 −/− macrophages.

In order to test whether overexpression of Bcl-2 affects Shigella-induced apoptosis, the macrophage-like cell line RAW stably transfected with either the vector alone, or the vector expressing Bcl-2 (34) was infected with M90T or BS176. As shown in Fig. 5B, overexpression of Bcl-2 did not protect macrophages from Shigella killing. BS176 is not cytotoxic to either bcl-2 or vector-transfected cells. Similar results were obtained with differentiated U937 cells overexpressing Bcl-xL, another anti-apoptotic protein of the Bcl-2 family (Ref. 39, data not shown).

Expression of Bcl-2 in the transfected macrophages was confirmed by Western blot (data not shown). Furthermore, in order to determine whether the Bcl-2 overexpressed in RAW cells was functional, we treated these macrophages with the topoisomerase inhibitor Etoposide, an inducer of apoptosis that is known to be inhibited by Bcl-2. Twenty-four hours after treatment almost 50% of the vector control cells yet only 5% of the Bcl-2 expressing cells died (Fig. 5C), indicating that Bcl-2 expressed in RAW cells is biologically active.

![Fig. 3. Macrophages from casp-1 −/− mice are not susceptible to S. flexneri induced apoptosis. Peritoneal macrophages were isolated from wild type or casp-1 −/− mice and infected with either M90T or a deletion mutant in ipaB (AIPA). At the indicated time points, cytotoxicity was determined by measuring the release of cytoplasmic lactate dehydrogenase (LDH). Only macrophages from wild type mice are susceptible to Shigella-induced apoptosis.](image)
DISCUSSION

Among the caspases, Casp-1 appears to be unique since it induces apoptosis and activates two potent proinflammatory cytokines, IL-1\(\beta\) and IL-18 (15, 16). Interestingly, these cytokines do not encode a signal sequence and it is still unknown how they reach the extracellular space after cleavage by Casp-1 (40, 41). The link between apoptosis and inflammation by Casp-1 is surprising, since apoptosis is classically considered to be an immunologically silent cell death (42). Indeed, during development and homeostasis, numerous apoptotic events occur without eliciting inflammation. The role of macrophage cell death in the release of IL-1\(\beta\) and IL-18 is still not understood.

Although Casp-1 induces apoptosis when overexpressed in tissue culture cells (17) the role of this enzyme in apoptosis has remained enigmatic. casp-1\(^{-/-}\) mice develop normally and macrophages from these mice undergo apoptosis induced by several stimuli (18, 19). These results suggest either that Casp-1 function is redundant or is not required in the pathways leading to apoptosis in development or in response to certain stimuli.

The Shigella invasin IpaB binds specifically to Casp-1 but not Casp-2 or Casp-3 (Fig. 1). Based on sequence homology, Casp-1, Casp-2, and Casp-3 are representatives of the three caspase subfamilies (11). It seems unlikely that IpaB binds to caspases closely related to Casp-1, since Shigella kills macrophages derived from casp-11\(^{-/-}\) mice. Casp-11 is closely related to casp-1 and is classified within the same caspase family (Fig. 4). IpaB bound both the zymogen of Casp-1 as well as a protein of 30 kDa that could be an intermediate maturation product.

Casp-1 is processed shortly after Shigella infection suggesting that IpaB can promote this process (Fig. 2A). Cleavage of pro-IL-1\(\beta\) indicates that the mature Casp-1 is active during infection (Fig. 2C). The irreversible inhibitor YVAD-cmk has higher affinity for Casp-1 than for other caspases. This inhibitor blocks Shigella-induced apoptosis (9) and also Casp-1 activity as shown by its inhibition of IL-1\(\beta\) cleavage (Fig. 2D). Surprisingly, YVAD also inhibits Casp-1 maturation (Fig. 2B). Although in cell lysates, YVAD has a significantly higher affinity for mature Casp-1 than for its zymogen (43), the affinity of the Casp-1 zymogen for YVAD in vivo is not known. Our results could indicate that in Shigella infections: 1) YVAD inhibits the autocatalytic activity of the zymogen, 2) that mature Casp-1 activity is necessary to activate the Casp-1 precursor, or 3) that a different caspase with high affinity for YVAD is necessary for Casp-1 activation.

Casp-1 is necessary for Shigella-induced apoptosis since these bacteria do not kill macrophages from mice with a targeted deletion in this protease (Fig. 3). These results are surprising, as macrophages isolated from casp-1\(^{-/-}\) are susceptible to other apoptotic stimuli (18). Although Casp-3 is implicated in many different apoptotic processes and is thought to be a downstream effector protease in a caspase cascade (11), it is not necessary for Shigella killing (Fig. 4).

**FIG. 4. S. flexneri is cytotoxic to macrophages from both casp-3 and casp-11 knock-out mice.** A, peritoneal macrophages were isolated from wild-type (○) or casp-3 \(^{-/-}\) (■) mice or B, from casp-11 \(^{-/-}\) (□) mice. Macrophages were infected either with M90T or BS176. Cytotoxicity was determined by measuring the release of LDH after 3 h. Shigella is cytotoxic to macrophages from both casp-3 \(^{-/-}\) and casp-11 \(^{-/-}\) mice.

**FIG. 5. S. flexneri is cytotoxic to macrophages from p53 knock-out mice and to RAW cells overexpressing Bcl-2.** A, peritoneal macrophages isolated from wild-type (○) or p53 \(^{-/-}\) (□) mice and B, RAW cells transfected with the vector control (■) or with the vector encoding bcl-2 (□) were infected with either M90T or BS176. C, RAW cells transfected with the vector control (■) or with the vector encoding bcl-2 (□) were treated with Etoposide for 24 h. Cytotoxicity was determined by measuring the release of LDH. M90T is cytotoxic to macrophages from both p53 knock-out mice and to RAW cells overexpressing Bcl-2. However, Bcl-2 could protect RAW cells from Etoposide-induced apoptosis.
Recently, Casp-11 was shown to regulate Casp-1 activation (21). Data presented here suggest that Shigella directly activates apoptosis through Casp-1, since macrophages from casp-11−/− mice are insensitive to Shigella cytotoxicity (Fig. 4). The independence of Shigella-induced apoptosis of Casp-11 is not surprising since both naive and activated wild type or casp-11 macrophages are susceptible to Shigella-induced cell death (Figs. 2 and 4, Ref. 33), although Casp-11 is up-regulated only in activated macrophages. Thus, Shigella induces a unique form of apoptosis which is dependent on Casp-1, does not require Casp-3, and bypasses the requirement for Casp-11 activation. The pro-apoptotic function of Casp-1 in Shigella-induced apoptosis appears to be independent of mature IL-1β. Pretreatment of macrophages with IL-1 receptor antagonist, a natural competitor of IL-1, does not prevent Shigella-induced apoptosis.2 Furthermore, filtered, IL-1 rich, supernatants of macrophages infected with Shigella are not cytotoxic to naïve macrophages.3 These results strongly suggest that Casp-1-induced apoptosis does not function through IL-1α.

Consistent with the model that Shigella induces apoptosis by directly activating Casp-1 is the observation that this process does not require p53 (Fig. 5). p53 is a transcriptional activator that is necessary for apoptosis initiated by diverse stimuli after G1 arrest in the cell cycle (36). The susceptibility of p53−/− macrophages to Shigella is not surprising, since S. flexneri codes a protein homologous to IpaB, the Salmonella invasion protein (Sip)B which also binds to Casp-1.4 We propose the following model for apoptosis during Shigella infections remains to be determined. Salomonna, however, encodes a protein homologous to IpaB, the Salmonella invasion protein (Sip)B which also binds to Casp-1.4 Macrophages infected with Shigella release large amounts of IL-1 (33) and treatment of animals with the IL-1 receptor antagonist, a natural inhibitor of macrophages with IL-1 receptor antagonist, a natural competitor of IL-1, does not prevent Shigella-induced apoptosis.2 Furthermore, filtered, IL-1 rich, supernatant of macrophages infected with Shigella are not cytotoxic to naïve macrophages.3 These results strongly suggest that Casp-1-induced apoptosis does not function through IL-1α.

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REFERENCES

1. Zychlinsky, A., and Sansonetti, P. J. (1997) Trends Microbiol. 5, 201–204
2. Lederberg, J., and Pai, T. J. (1997) Vaccine 15, 168–179
3. Ménard, R., Déhio, C., and Sansonetti, P. J. (1996) Trends Microbiol. 4, 220–226
4. Ménard, R., Sansonetti, P. J., and Parsot, C. (1993) J. Bacteriol. 175, 5899–5906
5. Ménard, R., Prevost, M. C., Gounou, P., Sansonetti, P. J., and Déhio, C. (1996) Proc. Natl Acad. Sci. U. S. A. 93, 1254–1258
6. Zychlinsky, A., Prevost, M. C., and Sansonetti, P. J. (1992) Nature 358, 167–168
7. Zychlinsky, A., Thirumalai, K., Arondel, J., Cantey, J. R., Aliprantis, A., and Sansonetti, P. J. (1996) Infect. Immunol. 64, 6575–6588
8. Islam, D., Veress, B., Bardhan, P. K., Lindberg, A. A., and Christenson, B. (1997) Infect. Immunol. 65, 739–749
9. Chen, Y., Smith, M. R., Thirumalai, K., and Zychlinsky, A. (1998) EMBO J. 17, 2353–2360
10. Thirumalai, K., Kim, K., and Zychlinsky, A. (1997) Infect. Immunol. 65, 787–793
11. Thornberry, N. A. (1996) Br. Med. Bull. 53, 478–490
12. Almeirini, E. S., Livingston, D. J., Nicholson, D. W., Salvesen, G., Thornberry, N. A., Wong, W. W., and Yuan, J. (1996) Cell 87, 171
13. Cerretti, D. P., Kozlowsky, C. J., Mosley, B., Nelson, N., Van Ness, K., Greenstreet, T. A., Marsh, C. J., Krennich, S. R., Druck, T., Cannizzaro, L. A., Huebner, K., and Black, R. A. (1992) Science 256, 97–100
14. Thornberry, N. A., Bull, H. G., Calaycay, J. R., Chapman, K. T., Howard, A. D., Costoura, M. J., Miller, D. K., Molinaeux, S. M., Weidner, J. R., Annans, J., Elliston, K. O., Ayala, J. M., Casano, F. J., Chou, D., Jing-J. F., Rigger, L. A., Gaffney, E. P., Limjucuo, G., Palyha, O. C., Raju, S. M., Rolando, A. M., Sailey, J. P., Yamin, T. T., Lee, T. D., Shively, J. E., MacCoss, M., McLeod, R. A., Schmidt, M., and Toetz, M. (1998) Science 281, 177–180
15. Ghayur, T., Banerjee, S., Hugunin, M., Butler, D., Herzog, L., Carter, A., Quintal, L., Sekut, L., Talanian, R., Paskind, M., Wang, W., Kamen, R., Tracey, D., and Allen, H. (1997) Nature 386, 619–623
16. Gu, Y., Kuida, K., Teuteu, H. K., Ku, G., Fleming, M. A., Hayashi, N., Higashimo, K., Okamura, H., Nakakuni, K., Kurimoto, M., Tanimoto, T., Flavel, R. A., Sato, V., Harding, M. W., Livingston, D. J., and Su, M. S. (1997) Science 275, 296–299
17. Miyara, M., Zhu, H., Rotello, R., Hartwig, E. A., and Yuan, J. (1993) Cell 75, 653–660
18. Li, X., Allen, H., Banerjee, S., Franklin, S., Herzog, L., Johnston, C., McDowell, J., Paskind, M., Rodman, L., Safied, J., Tewne, E., Tracey, D., Wardwell, S., Wei, F.-Y., Wong, W., Kamen, R., and Seshadri, T. (1997) Cell 80, 401–411
19. Kuida, K., Lippe, J. A., Ku, G., Harding, M. W., Livingston, D. J., Su, M. S.-S., and Flavel, R. A. (1995) Science 267, 206–209
20. Wang, S., Miura, M., Zhu, Y., Liao, S., O’Hara, K., Hanson, S. M., and Lin, L.-N. (1995) J. Biol. Chem. 271, 20580–20587
21. Wang, S., Miura, M., Jung, Y.-k., Zhu, H., Kawasaki, H., Greenberg, A.-h., and Yuan, J. (1996) J. Biol. Chem. 271, 768–774
22. Fernandez-Almeirini, T., Litwack, G., and Almeirini, E. S. (1994) J. Biol. Chem. 269, 39761–39764
23. Tewari, M., Quan, I. T., O’four, K., Desnoyers, S., Zeng, Z., Beidler, D. R., Poirier, G. G., Salvesen, G. S., and Dixit, V. M. (1995) Cell 81, 801–809
24. Nicholson, D. W., Ali, A., Thornberry, N. A., Vaillancourt, J. P., Ding, C. K., Gallant, M., Gareau, Y., Griffis, P. R., Lavelle, M., Lazebnik, Y., Munday, N. A., Raju, S. M., Smulson, E. M., Yamin, T. T., Yu, V. L., and Miller, D. K. (1995) Nature 376, 37–43
25. Kuida, K., Zheng, T. S., Na, S., Kuzan, C., Yang, D., Karasuyama, H., Rakis, P., and Flavel, R. A. (1996) Nature 384, 368–372
26. Jiang, Z. H., Zhang, W. J., Rao, Y., and Wu, J. Y. (1998) Proc. Natl Acad. Sci. U. S. A. 95, 9155–9160
27. Bergeron, L., Perez, O. I., MacDonald, G., Shi, L., Sun, Y., Jurissova, A., Varmuza, S., Latham, K. E., Flaws, J. A., Salter, J. C., Harra, H., Moskowitz, M. A., Li, E., Greenberg, A., Tilly, J. L., and Yuan, J. (1998) Genes Dev. 12, 1304–1314

2 H. Hilbi, J. E. Mos, D. Hersh, Y. Chen, J. Arondel, S. Banerjee, R. A. Flavel, J. Yuan, P. J. Sansonetti, and A. Zychlinsky, unpublished results.
3 H. Hilbi, J. E. Mos, D. Hersh, Y. Chen, J. Arondel, S. Banerjee, R. A. Flavel, J. Yuan, P. J. Sansonetti, and A. Zychlinsky, unpublished observation.
4 D. Hersh, D. M. Monack, M. R. Smith, N. Ghor, S. Fulkow, and A. Zychlinsky, manuscript in preparation.
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28. Harvey, N. L., Butt, A. J., and Kumar, S. (1997) *J. Biol. Chem.* 272, 13134–13139
29. Li, H., Bergeron, L., Cryns, V., Pasternack, M. S., Zhu, H., Shi, L., Greenberg, A., and Yuan, J. (1997) *J. Biol. Chem.* 272, 21010–21017
30. Ron, D., and Dressler, H. (1992) *BioTechniques* 13, 866–869
31. Kumar, S., Kinoshita, M., Noda, M., Copeland, N. G., and Jenkins, N. A. (1994) *Genes Dev.* 8, 1615–1620
32. Sansonetti, P. J., Kopecko, D. J., and Formal, S. B. (1981) *Infect. Immun* 34, 75–83
33. Zychlinsky, A., Fitting, C., Cavaillon, J. M., and Sansonetti, P. J. (1994) *J. Clin. Invest.* 94, 1328–1332
34. Messmer, U. K., Reed, U. K., and Brune, B. (1996) *J. Biol. Chem.* 271, 20192-20197
35. Hilbi, H., Chen, Y., Thirumalai, K., and Zychlinsky, A. (1997) *Infect. Immunol.* 65, 5165–5170
36. Levine, A. J. (1997) *Cell* 88, 323–331
37. Yang, E., and Korsmeyer, S. J. (1996) *Blood* 88, 386–401
38. Reed, J. (1997) *Nature* 387, 773–776
39. Boise, L. H., Gonzales-Garcia, M., Postema, C. E., Ding, L., Lindsten, T., Turka, L. A., Mao, X., Nunez, G., and Thompson, C. B. (1993) *Cell* 74, 597–608
40. Dinarello, C. A. (1996) *Blood* 87, 2095–2147
41. Gillespie, M. T., and Horwood, N. J. (1998) *Cytok. Growth Fact. Rev.* 9, 109–116
42. Savill, J., Fudok, V., Henson, P., and Haslett, C. (1993) *Immunol. Today* 14, 131–136
43. Yamin, T.-T., Ayala, J. M., and Miller, D. K. (1996) *J. Biol. Chem.* 271, 13273–13282
44. Sansonetti, P. J., Arondel, J., Cavaillon, J.-M., and Huerre, M. (1995) *J. Clin. Invest.* 96, 884–892
45. Friedlander, R. M., Gagliardini, V., Hara, H., Fink, K. B., Li, W., MacDonald, G., Fishman, M. C., Greenberg, A. H., Moskowitz, M. A., and Yuan, J. (1997) *J. Exp. Med.* 185, 933–940
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