Macrophages play a pivotal role in the pathogenesis of a variety of diseases. These studies were performed to characterize the mechanisms by which Toll-like receptor 4 (TLR4)-mediated NF-κB activation promotes resistance to cell death in macrophages. When NF-κB activation was inhibited by a super-repressor, 1eBα, the TLR4 ligand lipopolysaccharide induced the activation of caspase 8, the loss of mitochondrial transmembrane potential (ΔΨm), and apoptotic cell death in macrophages. The inhibition of caspase 8 activation suppressed DNA fragmentation but failed to protect macrophages against the loss of ΔΨm and resulted in necrotic cell death. In contrast, the reduction of receptor-interacting protein 1 suppressed the loss of ΔΨm and inhibited apoptotic cell death. Further, when caspase 8 activation was suppressed, the knock down of receptor-interacting protein inhibited the loss of ΔΨm and necrotic cell death. These observations demonstrate that following TLR4 ligation by lipopolysaccharide, NF-κB is a critical determinant of macrophage life or death, whereas caspase 8 determines the pathway employed.

Macrophages play a pivotal role in infection, atherosclerosis, and chronic inflammation such as observed in rheumatoid arthritis. The ligation of Toll-like receptors (TLRs) activates intracellular signal transduction pathways, including NF-κB (1). Expressed on macrophages and monocytes, TLR4 is critical for the recognition of lipopolysaccharide (LPS) from Gram-negative bacteria. Recent studies have demonstrated that endogenous TLR4 ligands are highly expressed at sites of chronic inflammation, characterized by the accumulation of macrophages, such as the joints of patients with rheumatoid arthritis (2–8). Resistance to apoptosis may contribute to the persistence of chronic inflammation (9). Therefore, microbial and endogenous TLR4 ligands may promote resistance to apoptosis through the activation of NF-κB.

Apoptosis may be initiated by two different pathways, the death receptor-mediated pathway and the mitochondria-dependent pathway.

EXPERIMENTAL PROCEDURES

Materials—LPS and polymyxin B sulfate were obtained from Sigma. RPMI, FBS, PBS, l-glutamine, penicillin, and streptomycin were obtained from Invitrogen. Propidium iodide (PI) was from Roche Applied Science and rhodamine 123 (Rh123) was from Molecular Probes (Eugene, OR). Anti-TNFRI antibody was from R&D Systems (Minneapolis, MN). Caspase 8 (Ac-IETD-afc) synthetic fluorogenic substrate was purchased from Enzyme Systems Products (Livermore, CA). Geldanamycin was obtained from Calbiochem.

Cell Isolation and Culture—Buffy coats (Lifesource, Glenview, IL) were obtained from healthy donors. Mononuclear cells, isolated by Histopaque (Sigma) gradient centrifugation, were separated by countercur-
LPS-induced Macrophage Cell Death

current centrifugal elution (JE-6B; Beckman Coulter, Palo Alto, CA) in the presence of 10 μg/ml polymyxin B sulfate as previously described (23–26). Isolated monocytes were ≥90% pure as determined by morphology, nonspecific esterase staining, and CD14 expression examined by flow cytometry (data not shown). Monocytes were adhered to plates for 1 h in RPMI and 1 μg/ml polymyxin B sulfate. Following adherence, monocytes were differentiated in vitro for 7 days in RPMI containing 20% heat-inactivated FBS, 1 μg/ml polymyxin B sulfate, 0.35 mg/ml l-glutamine, 120 units/ml penicillin and streptomycin (20% FBS/RPMI) (23–27).

Adenovirus Infection of Primary Macrophages—Primary macrophages were infected at a concentration of 50 m.o.i. (multiplicity of infection) with adenoviral vectors expressing a super-repressor IκBα (Ad IκBα) or a control vector (β-galactosidase or CMV-blank, Ad Control) for 2 h as previously described (23, 25, 26, 28). Where indicated, macrophages were co-infected with Ad DN FADD (5 m.o.i.), Ad Bcl-xL (6 m.o.i.), AdCrmA (25 m.o.i.) or Ad control (5, 6, or 25 m.o.i., respectively). Within each experiment the total concentration of adenoviral vector was held constant. After infection, 20% FBS/RPMI was added, and the cells were incubated overnight. The macrophages were then washed and incubated in 20% FBS/RPMI for an additional 12 h (total of 32 h from the initiation of the infection). The infected cells were treated with LPS (10 ng/ml) or control medium for 12 or 24 h. The cells were then harvested and analyzed as described.

Cell Transfection—In vitro differentiated macrophages were transfected with either nonspecific or RIP siRNA (final, 100 nM; Dharmacon, Inc., Lafayette, CO) employing Lipofectamine 2000 according to the manufacturer’s directions (Invitrogen). The cells were then incubated for 72 h prior to analysis by immunoblot assay or adenoviral infection.

Electron Microscopy—In vitro differentiated macrophages cultured in the presence or absence of Ad IκBα, LPS, and IETD were harvested, and cell pellets were fixed overnight at 4 °C in a 0.2 M sodium cacodylate buffer (pH 7.4) containing 2% glutaraldehyde. Samples were post-fixed in cacodylate-buffered 1% osmium tetroxide, dehydrated, and embedded in Epon 812 (Nacalai Tesque, Osaka, Japan) for ultrathin sectioning. The samples were stained with uranyl acetate and lead citrate and examined with a JEOL 1220 electron microscope.

Analysis of Mitochondrial Transmembrane Potential and Cell Membrane Integrity—Mitochondrial dysfunction was assessed utilizing the cationic lipophilic green fluorescent Rh123 as previously described (23, 25, 26, 28, 29). Disruption of ΔΨm is associated with a lack of Rh123 retention and a decrease in fluorescence. Cultures were incubated with Rh123 (0.1 μg/ml) for 30 min, harvested, and washed by PBS. To assess cell membrane integrity simultaneously, PI (3.3 μg/ml) was added to the cells prior to analysis by flow cytometry. For histogram analysis, objects with minimal light scatter representing debris were gated out.

Determination of Subdiploid DNA Content—At the indicated time points, cells were harvested, fixed in 70% ethanol, and stained with PI (50 μg/ml) as previously described (30). The apoptotic profile was determined by flow cytometry utilizing a Beckman-Coulter EpicsXL flow cytometer and system 2 software. The subdiploid DNA peak (<2N DNA) immediately adjacent to the G0/G1 peak (2N DNA) represented apoptotic cells and was quantitated by histogram analyses. Objects with minimal light scatter representing debris were excluded as previously described (31), so that quantitation of the subdiploid population would not be inappropriately skewed (23, 25, 26, 28).

Immunoblot Analysis—Whole-cell extracts were prepared from 7-day differentiated macrophages that were treated as indicated. Extracts were electrophoresed on SDS-PAGE 12.5% polyacrylamide gels and transferred to Immobilon-P (Millipore, Bedford, MA) by semi-
phages infected with Ad IκBα. These results demonstrate that LPS-induced apoptosis in macrophages was not mediated by TNFR1.

Caspase 8 Is Necessary for LPS-induced DNA Fragmentation but Not Mitochondrial Dysfunction or Cell Death—Because previous studies demonstrated that caspase 8 was activated by LPS when NF-κB activation was suppressed (16), the role of caspase 8 was examined. Macrophages were infected with the control adenoviral vector or Ad IκBα and then treated with LPS for 12 h. Cells were harvested, and the lysates were analyzed for caspase 8 activation employing a substrate-specific fluorometric assay. Compared with the control-infected macrophages, LPS induced a 10–15-fold activation of caspase 8-like activity when NF-κB activation was inhibited (Fig. 3A). Because the caspase 8 inhibitor IETD-fmk suppressed >90% of the LPS-induced caspase 8-like activity (Fig. 3A), this inhibitor was employed to define the effects of caspase 8 inhibition on apoptotic cell death. The inhibition of caspase 8-like activity with IETD-fmk markedly suppressed LPS-induced DNA fragmentation following NF-κB inhibition (Fig. 3B). In contrast, IETD-fmk did not protect macrophages from the loss of ΔΨm (Fig. 3C) or cell death (Fig. 3D), which actually increased. To define the mode of cell death, electron microscopy was performed. Macrophages infected with Ad IκBα and treated with IETD-fmk plus LPS demonstrated characteristics of necrotic cell death, including vacuolization of the cytoplasm, ruptured cell membrane, and lack of DNA fragmentation (Fig. 3E).

Bcl-xL Protects against Apoptosis, Not Necrosis—To further define the role of the mitochondria in LPS-induced apoptosis, Bcl-xL was ectopically expressed, employing an adenoviral vector (Ad Bcl-xL) in macrophages co-infected with Ad IκBα or Ad control. In macrophages infected with Ad IκBα, ectopically expressed Bcl-xL (Fig. 3B) significantly (p < 0.001) suppressed the LPS-induced DNA fragmentation, loss of ΔΨm (Fig. 3C), and cell death (Fig. 3D). Macrophages were also examined to define the effects of Bcl-xL when caspase 8 activity was also blocked. The combination of IETD-fmk plus Bcl-xL completely prevented DNA fragmentation (Fig. 3B). In contrast, the protection provided by Bcl-xL against the loss of ΔΨm and the induction of cell death was lost when caspase 8 activity was suppressed (Fig. 3, C and D). Therefore, Bcl-xL protected against apoptotic, but not against non-apoptotic or necrotic, cell death. These observations suggest that caspase 8 activation regulates the mode of LPS-induced macrophage cell death when NF-κB activation is repressed.

**FADD Is Upstream of Caspase 8**—An earlier study suggested that TLR4-induced apoptosis was mediated through FADD (12). Therefore, studies were performed to determine the effect of a dominant negative (DN) FADD on caspase 8-like activity and on apoptosis. The adenoviral-mediated expression of a DN FADD suppressed caspase 8-like activity (Fig. 4A), suggesting that FADD was upstream of caspase 8 in LPS-induced apoptosis. Consistent with its effects on caspase 8, the expression of the DN FADD also suppressed LPS-induced DNA fragmentation (Fig. 4B). In contrast, the DN FADD failed to prevent either cell death or the loss of ΔΨm (data not shown), consistent with the results observed with IETD-fmk. These observations suggest that FADD is upstream of caspase 8, which contributes to the apoptotic phenotype.

**RIP Contributes to LPS-induced Macrophage Apoptotic Cell Death**—Because RIP may contribute to TNFα-induced non-apoptotic cell death (35) and because activated caspase 8 may cleave RIP, the effect of LPS-
induced caspase 8 activation on RIP expression was examined. LPS-induced caspase 8-like activity induced by LPS. Macrophages were infected with Ad lxBa or control adenoviral vector at 50 m.o.i. The infected macrophages were then pretreated with 20 μM IETD-fmk or Me2SO alone for 1 h, followed by 10 ng/ml LPS for 12 h. Caspase 8-like activity was examined as described under “Experimental Procedures.” B, macrophages were infected with Ad BCi-xL, control adenoviral vector, and the cell lysates were analyzed for BCi-xL protein expression by immunoblot analysis (insert). Tubulin was the loading control. B–D, macrophages were co-infected with Ad IBxL (50 m.o.i.) and Ad BCi-xL (6 m.o.i.) or control adenoviral vector (CV, 56 m.o.i.). The infected macrophages were pretreated with 20 μM IETD-fmk or control medium (control) for 1 h before the addition of LPS. The macrophages were infected and treated as described above and were then harvested and analyzed for DNA fragmentation (B), the loss of 2N DNA (C), and the loss of membrane integrity (D). The results in panels B–D represent the mean ± 1 S.E. of one experiment performed in triplicate that is representative of five independent experiments. **, p < 0.001 between Ad IBxL-infected, LPS-treated cells incubated with control medium with Me2SO and/or control vector (Ad Control) versus those treated with IETD-fmk and/or infected with Ad BCi-xL. E, electron microscopy was performed on untreated macrophages (top panel) and those infected with Ad lxBa and treated with LPS plus IETD-fmk (bottom panel). The scale bars are 2 μm.

induced caspase 8 activation on RIP expression was examined. LPS-induced caspase 8-like activity induced by LPS. Macrophages were infected with Ad lxBa or control adenoviral vector at 50 m.o.i. The infected macrophages were then pretreated with 20 μM IETD-fmk or Me2SO alone for 1 h, followed by 10 ng/ml LPS for 12 h. Caspase 8-like activity was examined as described under “Experimental Procedures.” B, macrophages were infected with Ad BCi-xL, control adenoviral vector, and the cell lysates were analyzed for BCi-xL protein expression by immunoblot analysis (insert). Tubulin was the loading control. B–D, macrophages were co-infected with Ad IBxL (50 m.o.i.) and Ad BCi-xL (6 m.o.i.) or control adenoviral vector (CV, 56 m.o.i.). The infected macrophages were pretreated with 20 μM IETD-fmk or control medium (control) for 1 h before the addition of LPS. The macrophages were infected and treated as described above and were then harvested and analyzed for DNA fragmentation (B), the loss of 2N DNA (C), and the loss of membrane integrity (D). The results in panels B–D represent the mean ± 1 S.E. of one experiment performed in triplicate that is representative of five independent experiments. **, p < 0.001 between Ad IBxL-infected, LPS-treated cells incubated with control medium with Me2SO and/or control vector (Ad Control) versus those treated with IETD-fmk and/or infected with Ad BCi-xL. E, electron microscopy was performed on untreated macrophages (top panel) and those infected with Ad lxBa and treated with LPS plus IETD-fmk (bottom panel). The scale bars are 2 μm.

the expression of RIP was suppressed employing siRNA (Fig. 5B). Compared with nonspecific control siRNA, the reduction of RIP resulted in protection against LPS-induced cell death (Fig. 5B). Further, the suppression of RIP also protected against DNA fragmentation (Fig. 5C) and
the loss of $\Delta \Psi_m$ (Fig. 5D). These observations demonstrate that even though LPS-induced caspase 8 activation was capable of cleaving RIP, RIP contributed to TLR4-mediated apoptotic cell death.

**RIP Contributes to Caspase 8 Activation**— Because earlier studies suggested that RIP may be upstream of caspase 8 in TLR4 signaling (19), we examined the effects of the reduction of RIP on caspase activation. Macrophages were incubated with geldanamycin, which dissociates RIP from HSP90, resulting in the reduction of RIP by proteasomal degradation (36, 37). Geldanamycin pretreatment for 12 h resulted in a marked reduction of RIP (Fig. 6A). When RIP was reduced employing geldanamycin, caspase 8-like activity was suppressed (Fig. 6B). When examined by Western blot, the reduction of RIP was also associated with a marked decrease of activated p18 caspase 8 (Fig. 6C). In contrast, partially processed p41/43 caspase 8 was equivalent in the presence or absence of geldanamycin (Fig. 6C). Consistent with the effects on caspase 8 activation, the processing of Bid, which is downstream of caspase 8, was suppressed in the presence of geldanamycin (Fig. 6C). These observations suggest that RIP mediates TLR4-induced caspase 8 activation in macrophages.

**RIP Mediates Non-apoptotic Death in Macrophages**— Studies were performed to determine the effects of RIP on non-apoptotic cell death. The reduction of RIP by geldanamycin, as with the RIP siRNA, resulted in suppression ($p < 0.05$) not only of the apoptotic phenotype (Fig. 7A) but also the loss of $\Delta \Psi_m$ (Fig. 7B) and cell death (Fig. 7C). To suppress caspase 8, an adenoviral vector expressing CrmA was employed to co-inflect macrophages with the Ad IxBo. Inhibition of caspase 8 with CrmA protected against DNA fragmentation (Fig. 7A) but failed to protect against the loss of $\Delta \Psi_m$ (Fig. 7B) or the loss of membrane integrity (Fig. 7C) mediated by LPS, similar to the results observed with IETD-fmk or the DN FADD. In contrast, when RIP also was suppressed, CrmA prevented apoptotic cell death (Fig. 7, A and B) and the loss of $\Delta \Psi_m$ was further diminished (Fig. 7B). To confirm the effects of RIP on non-apoptotic cell death, the expression of RIP was suppressed employing siRNA and then macrophages were infected with a control vector or AdIxB. When these macrophages were incubated with LPS plus IEDT-fmk to inhibit caspase 8, the knock down of RIP suppressed the loss of cell membrane integrity (Fig. 7D) and the loss of $\Delta \Psi_m$ (Fig. 7E). These observations demonstrate that the non-apoptotic cell death that becomes apparent when caspase 8 activity is inhibited was suppressed by the reduction of RIP.

**DISCUSSION**

Macrophages play a critical role in a variety of diseases including rheumatoid arthritis, atherosclerosis, infection, and inflammatory bowel disease. NF-kB activation is critical not only for the induction of inflammation but also for macrophage survival, because NF-kB regulates the expression of both pro-inflammatory and anti-apoptotic proteins (23, 38, 39). Here we have documented that NF-kB activation is essential for macrophage survival following TLR4 ligation with LPS. When NF-kB activation is prevented, LPS-induced macrophage apo-
Apoptotic cell death proceeds through a RIP- and caspase 8-dependent mechanism, and ectopically expressed Bcl-xL is effective at preventing apoptotic cell death (Fig. 8). When caspase 8 activation is also suppressed, the death-inducing pathway is converted to one that is RIP dependent, non-apoptotic, which involves the loss of ΔΨm, and the macrophages are no longer protected by Bcl-xL (Fig. 8). The current

FIGURE 6. The reduction of RIP inhibits caspase 8 activation. The reduction of RIP is associated with the decreased activation of caspase 8. Macrophages were infected with Ad IxBα or control adenoviral vector (Ad Control) at 50 m.o.i. and then treated with 0.5 μM geldanamycin (GA) or control medium for 12 h. Some cultures were harvested and examined for RIP by immunoblot analysis (A), and the remaining cultures were incubated with LPS (10 ng/ml) or control medium (CM) for an additional 12 h. These cells were harvested and analyzed for caspase 8-like activity (B) and by immunoblot analysis using a monoclonal antibody to caspase 8 that detects the cleaved p18 fragment (C). The results in panel A represent the mean ± S.E. of three independent experiments; those in panel B are representative of two independent experiments.

FIGURE 7. Depletion of RIP protects against non-apoptotic cell death. A–C, human macrophages were infected with Ad IxBα (50 m.o.i.) or the control adenoviral vector (CV, 50 m.o.i.) alone or plus AdCm α or additional control vector (25 m.o.i.) for 24 h prior to the addition of Me2SO or geldanamycin (0.5 μM) for an additional 12 h. Subsequently, LPS (10 ng/ml) was added, and the macrophages were cultured for an additional 17 h and then examined for apoptosis (% apoptotic cells) and the loss of mitochondrial integrity (B, Rh123 retention), and the loss of cell viability (C, PI+ cells). D and E, in vitro differentiated human macrophages were transfected with nonspecific (NS) or RIP siRNA (RIPi) for 72 h and then infected with Ad IxBα (50 m.o.i.) or a control adenovirus for 24 h prior to addition of LPS (10 ng/ml) and IETD-fmk (20 μM). The cells were harvested after an additional 17 h and examined for loss of membrane integrity (% PI+ cells) or loss of ΔΨm (E, Rh123). The results in panels A–C represent the mean ± S.E. of three experiments and in panels D and E two experiments, each performed in duplicate. *, p < 0.05 or **, p < 0.01 versus control treatment.
LPS-induced Macrophage Cell Death

Following the inhibition of NF-κB, caspase 8 was critical for the induction of LPS-induced apoptosis. The DN FADD, IETD-fmk, CrmA, and z-VAD-fmk (data not shown) each inhibited caspase 8 activation and markedly suppressed LPS-induced DNA fragmentation. These observations are consistent with those employing a macrophage cell line and the proteasome inhibitor MG132 to inhibit NF-κB activation (12, 13, 16). When the activation of caspase 8 was not suppressed, Bcl-xL was effective at preventing DNA fragmentation, the loss of mitochondrial integrity, and cell death. The LPS-induced apoptosis may be mediated through the caspase 8-mediated activation of Bid (Fig. 6A) and caspase 3, which was not examined in this study. Our data suggest further that RIP contributes to apoptosis, because the knock down of RIP protected not only against LPS-induced caspase 8 activation, Bid cleavage, and DNA fragmentation but also against the loss of ΔΨm and the induction of cell death. These observations suggest that RIP is upstream of caspase 8. Supporting this interpretation, cell death induced by the overexpression of RIP was suppressed by the inhibition of caspase 8 or the expression of DN FADD, indicating that the action of RIP to promote apoptosis was upstream of FADD and caspase 8 (19, 45). Recent observations employing DN FADD and macrophages deficient in Toll/interleukin-1R domain-containing adapter-inducing IFN-β (TRIF) or myeloid differentiation factor 88 demonstrated that TRIF and FADD, but not myeloid differentiation factor 88, were upstream of caspase 8 activation in TLR4-mediated apoptosis (12, 13, 16) (Fig. 8). Although FADD did not interact directly with TRIF (19), it was capable of interacting with RIP through their death domains (44). Together, these observations suggest that following TLR4 ligation, TRIF may interact with RIP, promoting apoptosis by recruiting FADD and the activation of caspase 8 (Fig. 8).

Another potential explanation for the apoptotic effects of RIP may be that the carboxy-terminal fragment of caspase 8-cleaved RIP (RIPc), which contains the death domain, may continue to promote apoptosis. The overexpression of either RIPc or wild type RIP induced apoptotic cell death (35, 46, 47). In contrast, expression of the amino-terminal fragment of caspase 8-cleaved RIP, which possesses the kinase domain, failed to induce cell death (35, 46). Therefore, caspase 8-cleaved RIP does not prevent, but may promote, the pro-apoptotic effects of RIP.

However, our data suggest that caspase 8-mediated RIP cleavage is associated with the loss of its ability to induce non-apoptotic cell death, suggesting that intact death and kinase domains are required. When caspase 8 activation was suppressed, although the apoptotic phenotype was reduced there was no protection against loss of ΔΨm or cell death. The pattern of death observed was consistent with necrosis (20, 37, 48). When caspase 8 activation was suppressed, RIP was not cleaved, suggesting that full-length RIP may be necessary for this mode of cell death. The importance of RIP in non-apoptotic cell death was demonstrated by the protection provided by the reduction of RIP induced by geldanamycin or siRNA. The observation that Bcl-xL provided no protection to the mitochondria when the activation of caspase 8 was suppressed is consistent with an alternate, non-caspase-mediated mechanism of macrophage cell death. This interpretation is supported by recent studies showing that full-length, but not caspase 8-cleaved, RIP was responsible for the induction of TNFα plus z-VAD-fmk-induced necrosis (37). Our observations are also consistent with the report (20) that RIP-mediated non-apoptotic cell death induced through the Fas, TNFR1, and TNF-related apoptosis-inducing ligand (TRAIL) pathways was dependent upon RIP kinase activity. Prior studies have demonstrated that overexpressing RIP or RIPc results in apoptosis and the kinase domain is dispensable, similar to the role of RIP in TNFR1-induced NF-κB activation (49). In contrast, the induction of RIP-mediated non-apoptotic cell death requires an additional activation signal and the kinase domain is required (20, 37). When NF-κB activation was not suppressed, caspase 8 was not activated, RIP was not cleaved, and no cell death was observed. Therefore, these observations suggest that LPS-induced NF-κB activation protects not only against the activation of caspase 8 but also against the induction of the ability of RIP to induce necrosis (Fig. 8).

In conclusion, our data suggest that in macrophages when NF-κB activation is suppressed the inhibition of caspase 8 converts cell death from apoptotic to non-apoptotic cell death, which is mediated through RIP. These two forms of cell death may have different physiological

---

*H. Liu, L. Pagliari, and R. M. Pope, unpublished observations.*

---

**FIGURE 8. Schematic representation of the mechanisms contributing to LPS-induced macrophage cell death following the inhibition of NF-κB activation.** Upon ligation by LPS, TLR4 recruits adaptor molecules myeloid differentiation factor (MyD88) and TRIF, which leads to the activation of NF-κB. Following the inhibition of NF-κB activation, LPS induces macrophage apoptosis through two pathways, one of which involves caspase 8 activation, which may be mediated through effects on the mitochondria, and a direct effect on apoptosis. Caspase 8 activation leads to the cleavage of RIP, and the inhibition of caspase 8 activity promotes RIP-mediated non-apoptotic cell death.
LPS-induced Macrophage Cell Death

consequences (50, 51) that will be important to consider for therapeutic interventions involving inhibition of NF-κB activation when TLR4 signaling will result either from microbial sources or endogenous TLR ligands that exist locally in chronic inflammatory conditions such as rheumatoid arthritis.

Acknowledgments—We thank Mary Paniagua and Jeffery Nelson from the Flow Cytometry Core Facility in the Robert H. Lurie Comprehensive Cancer Center at Northwestern University Feinberg School of Medicine for assistance in fluorescence-activated cell sorter analysis.

REFERENCES

1. Medzhitov, R. (2001) Nat. Rev. Immunol. 1, 135–145
2. Ohashi, K., Burkart, V., Flohe, S., and Kolb, H. (2000) J. Immunol. 164, 558–561
3. Okamura, Y., Watiari, M., Jerud, E. S., Young, D. W., Ishizaka, S. T., Rose, J., Chow, J. C. and Schrottner, P. (2002) Nat. Immunol. 171, 99–111
4. Jobin, C., Panja, A., Helebrand, C., Tempe, N. S., and McLeod, J. D. (2003) Oncogene 22, 1575–1582
5. Darzynkiewicz, Z., Yuan, G., Li, X., Gorczyca, W., Murakami, T., and Traganos, F. (1997) Cytometry 27, 1–20
6. Schenck, P., Schneider, S., Miehlke, R., and Prehm, P. (1995) J. Immunol. 160, 410–418
7. Ashkenazi, A., and Dixit, V. M. (1998) Science 281, 1305–1308
8. Ohashi, K., Burkart, V., Flohe, S., and Kolb, H. (2000) J. Immunol. 160, 410–418
9. Mancini, M., Anderson, B. O., Caldwell, E., Sedgbinassah, M., Paty, P., and Heesenko, D. M. (1997) J. Cell Biol. 138, 449–469
10. Kiener, P. A., Davis, P. M., Starling, G. C., Mehnlin, C., Klebanoff, S. J., Ledbetter, J. A., and Liles, W. C. (1997) J. Exp. Med. 185, 1511–1516
11. Liu, H., Li, X., Su, X., Xu, L. G., Bin, L. H., Zhang, J., and Shu, H. B. (2004) Oncogene 23, 5415–5425
12. Pagliara, L. J., Perlman, H., Liu, H., and Pope, R. M. (2000) Mol. Cell Biol. 20, 8855–8865
13. Holler, N., Pagliara, L. J., Georgaropas, C., Mano, T., Walsh, K., and Pope, R. M. (1999) J. Exp. Med. 190, 1679–1688
14. Liu, H., Ma, Y., Pagliara, L. J., Perlman, H., Yu, C., Lin, A., and Pope, R. M. (2004) J. Immunol. 172, 1907–1915
15. Kucharczak, J., Simmons, M. J., Fan, Y., and Gelinas, C. (2003) Oncogene 22, 8961–8982
16. Xaus, J., Comalada, M., Villedor, A. F., Lloberas, A., Argiles, J. M., and Strauss, J. F., III (2001) J. Immunol. 167, 4423–4429
17. Mimizuku, M., Janossy, M., Sartor, R. B. (1998) Hepatology 28, 1357–1366
18. Tschopp, J. (2004) Nat. Rev. Immunol. 4, 4491–4499
19. Kim, J., Choi, E. J., and Joe, C. O. (2000) Oncogene 19, 4491–4499
20. Barcia, R. N., Valle, N. S., and McLeod, J. D. (2003) J. Biol. Chem. 278, 7925–7933
21. Xaus, J., Comalada, M., Valledor, A. F., Lloberas, J., Lopez-Soriano, F., Argiles, J. M., and Strauss, J. F., III (2001) J. Immunol. 167, 4423–4429
22. Medzhitov, R., Bermouli, K. B., Auderset, K., Joseph, J. M., and Gross, N. (2004) Oncogene 23, 5415–5425
23. Liu, H., Perlman, H., Liu, H., and Pope, R. M. (2000) Mol. Cell Biol. 20, 8855–8865
24. Ruckdeschel, K., Mannel, O., and Schrottner, P. (2002) Nat. Immunol. 1, 2514–2526
25. Ohashi, K., Burkart, V., Flohe, S., and Kolb, H. (2000) J. Immunol. 160, 410–418
26. Pagliara, L. J., Perlman, H., Liu, H., and Pope, R. M. (2001) J. Exp. Med. 194, 113–126
27. Chann, F. K., Shisler, J., Bisby, J. G., Kelce, M., Zheng, L., Appel, M., Orenstein, J., Moss, R., and Lenardo, M. J. (2003) J. Biol. Chem. 278, 51613–51621
28. Liu, H., Perlman, H., Pagliara, L. J., and Pope, R. M. (2001) J. Exp. Med. 194, 113–126
29. Liu, H., Perlman, H., Pagliara, L. J., and Pope, R. M. (2001) J. Exp. Med. 194, 113–126
30. Mancini, M., Anderson, B. O., Caldwell, E., Sedghiabassah, M., Paty, P., and Heesenko, D. M. (1997) J. Cell Biol. 138, 449–469
31. Gallo, N., and Liu, H., Tu-Rapp, H., Hisen, H. J., Ibrahim, S. M., Cole, S. M., and Pope, R. M. (2004) Nat. Immunol. 5, 380–387
32. Liu, H., Perlman, H., Pagliara, L. J., and Pope, R. M. (2001) J. Exp. Med. 194, 113–126
33. Kim, J., Choi, E. J., and Joe, C. O. (2000) Oncogene 19, 4491–4499
34. Kucharczak, J., Simmons, M. J., Fan, Y., and Gelinas, C. (2003) Oncogene 22, 8961–8982
35. Ashkenazi, A., and Dixit, V. M. (1998) Science 281, 1305–1308
36. Barcia, R. N., Valle, N. S., and McLeod, J. D. (2003) J. Biol. Chem. 278, 7925–7933
37. Chan, F. K., Shisler, J., Bisby, J. G., Kelce, M., Zheng, L., Appel, M., Orenstein, J., Moss, R., and Lenardo, M. J. (2003) J. Biol. Chem. 278, 51613–51621
38. Liu, H., Perlman, H., Pagliara, L. J., and Pope, R. M. (2001) J. Exp. Med. 194, 113–126
39. Mancini, M., Anderson, B. O., Caldwell, E., Sedghiabassah, M., Paty, P., and Heesenko, D. M. (1997) J. Cell Biol. 138, 449–469
40. Perlman, H., Sata, M., Le Roux, A., Sedlak, T., Branellec, D., and Walsh, K. (1998) EMBO J. 17, 3576–3586
41. Ruckdeschel, K., Mannel, O., and Schrottner, P. (2002) Nat. Immunol. 171, 99–111
42. Hsu, L. C., Mo Park, J., Zhang, K., Luo, J. L., Kaufman, R. J., Eckmann, L., Moss, B., and Lenardo, M. J. (2003) J. Biol. Chem. 278, 51613–51621
43. Darzynkiewicz, Z., Yuan, G., Li, X., Gorczyca, W., Murakami, T., and Traganos, F. (1997) Cytometry 27, 1–20
44. Jobin, C., Panja, A., Helebrand, C., Tempe, N. S., and McLeod, J. D., and Sartor, R. R. (1998) J. Immunol. 160, 410–418
45. Ashkenazi, A., and Dixit, V. M. (1998) Science 281, 1305–1308
46. Mancini, M., Anderson, B. O., Caldwell, E., Sedghiabassah, M., Paty, P., and Heesenko, D. M. (1997) J. Cell Biol. 138, 449–469
47. Mancini, M., Anderson, B. O., Caldwell, E., Sedghiabassah, M., Paty, P., and Heesenko, D. M. (1997) J. Cell Biol. 138, 449–469
48. Kucharczak, J., Simmons, M. J., Fan, Y., and Gelinas, C. (2003) Oncogene 22, 8961–8982
49. Kucharczak, J., Simmons, M. J., Fan, Y., and Gelinas, C. (2003) Oncogene 22, 8961–8982
50. Kucharczak, J., Simmons, M. J., Fan, Y., and Gelinas, C. (2003) Oncogene 22, 8961–8982