ERK Activation Is Required for Double-stranded RNA- and Virus-induced Interleukin-1 Expression by Macrophages*

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Double-stranded (ds) RNA, which accumulates during viral replication, activates the antiviral response of infected cells. In this study, we have identified a requirement for extracellular signal-regulated kinase (ERK) in the regulation of interleukin 1 (IL-1) expression by macrophages in response to dsRNA and viral infection. Treatment of RAW 264.7 cells or mouse macrophages with dsRNA stimulates ERK phosphorylation that is first apparent following a 15-min incubation and persists for up to 60 min, the accumulation of iNOS and IL-1 mRNA following a 6-h incubation, and the expression of iNOS and IL-1 at the protein level following a 24-h incubation. Inhibitors of ERK activation prevent dsRNA-induced ERK phosphorylation and IL-1 expression by macrophages. The regulation of macrophage activation by ERK appears to be selective for IL-1, as ERK inhibition does not attenuate dsRNA-induced iNOS expression by macrophages. dsRNA stimulates both ERK activation and IL-1 expression by macrophages isolated from dsRNA-dependent protein kinase (PKR)-deficient mice, indicating that PKR does not participate in this antiviral response. These findings support a novel PKR-independent role for ERK in the regulation of the antiviral response of IL-1 expression and release by macrophages.
duced MAPK kinase activation is less clear. It was first shown that dsRNA stimulates c-Jun N-terminal kinase (JNK) and p38 activation in MEF. In these studies, dsRNA-induced p38 activation was attenuated in PKR¯/¯ MEF while JNK appeared to be activated by a PKR-independent mechanism (24). More recently, dsRNA and viral infection have been shown to stimulate p38 and JNK activation in MEF isolated from PKR¯/¯ mice. While JNK activation appears to require a PKR-dependent inhibition of protein synthesis (25), p38 activation by dsRNA is not dependent on PKR or PKR-mediated inhibition of protein synthesis (25). While these findings indicate that MAPK are activated by dsRNA, the role of PKR and the mechanisms by which PKR may mediate MAPK activation have yet to be fully elucidated.

In this study, the role of the MAPK pathway, specifically ERK, in dsRNA-induced macrophage activation has been examined. We show that the MAP/ERK kinase (MEK) selective inhibitors U0126 and PD98059 prevent dsRNA- and virus-induced ERK phosphorylation, IL-1 expression, IL-1β reporter activity, and IL-1 release by macrophages. The role of ERK in the regulation of inflammatory gene expression in response to virus infection appears to be selective for the IL-1 pathway, as ERK inhibition does not affect the ability of dsRNA to stimulate iNOS expression or nitrite production by macrophages. Using peritoneal macrophages isolated from PKR¯/¯ and PKR±/± mice we show that the genetic absence of PKR does not modulate the ability of dsRNA to induce IL-1 expression and release or to stimulate ERK phosphorylation. These findings support a role for ERK activation in the PKR-independent regulation of the antiviral response of IL-1 expression and release by macrophages.

EXPERIMENTAL PROCEDURES

Materials and Animals—Defined fetal bovine serum was purchased from Hyclone (Logan, UT). Poly IC, myelin basic protein, and protein A-Sepharose were purchased from Sigma Chemical Co. Poly IC was prepared as previously described (4). U0126 was from CalBiochem (San Diego, CA), and PD98059 was purchased from Alexis Biochemicals (San Diego, CA). The human IL-1β promoter luciferase reporter plasmid (XL-Luc) has been previously described (32) and was provided by Dr. Matthew Fenton (Boston University, Boston, MA). Rabbit anti-phospho-ERK was purchased from Promega (Madison, WI). Rabbit anti-ERK1, -ERK2, and -JNK were gifts from Dr. Peter Howley (Harvard Medical School, Boston, MA) and Dr. Charles Rodi (Amersham Biosciences). 3ZD monoclonal mouse anti-IL-1β was from Biological Resources Branch, DCTD at the NCI. PKR−/− mice (C57BL/6/J×SV129 background) were the generous gift of Dr. Randal Kaufman (University of Michigan, Ann Arbor, MI) and have been previously described (33–35). C57BL/6/J×SV129 (PKR±/±) mice were obtained from Jackson Laboratories (Bar Harbor, ME). iNOS and cyclophilin cDNA were the generous gift of Dr. Charles Rodi (Amersham Biosciences) and Dr. Steve Carroll (University of Alabama-Birmingham, Birmingham, AL), respectively. IL-1α and IL-1β cDNAs were gifts from Dr. Cliff Bellone (Saint Louis University, St. Louis, MO) and have been previously described (36). The wild-type ERK1 plasmid was the gift of Dr. Joseph Baldassare (Saint Louis University). The B cells of encephalomyocarditis virus (EMCV) were the generous gift of Dr. J. W. Yoon (University of Calgary, Calgary, Alberta, Canada). All other reagents were obtained from commercially available sources.

Cell Culture, Peritoneal Macrophage Isolation, and EMCV Infection—RAW 264.7 and RINm5F cells were removed from growth flask by treatment with 0.05% trypsin, 0.02% EDTA for 5 min at 37°C. Cells were then washed with media and plated at the indicated concentrations. The cells were allowed to adhere for 2 h under an atmosphere of 5% CO₂, 95% air before the initiation of experiments. Peritoneal exudate cells (PEC) were isolated from PKR−/− and PKR+/− mice by lavage as previously described (37). After isolation, 4×10⁶ cells/condition were incubated in 400 µl of complete CMRL-1066 (CMRL-1066 containing 10% heat-inactivated fetal bovine serum, 1% l-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin) for 2 h under an atmosphere of 5% CO₂, 95% air. The cells were washed three times with 400 µl of complete CMRL-1066 to remove non-adherent cells before initiation of experiments. RAW 264.7 cells (4×10⁷/400 µl of DME) were infected with the indicated amounts of EMCV virus in 100 µl of DME for 30 min, 300 µl of DME was added, and the cells were incubated for 24 additional hours. EMCV was propagated as previously described (15).

Nitrite and IL-1 Determinations—Nitrite production was determined by the Greiss assay, and IL-1 release was measured using the RINm5F cell bioassay as previously described (38, 39). The RINm5F cell IL-1 bioassay assay measures cumulative biological activity of both IL-1α and IL-1β.

Western Blot Analysis—Total cellular protein was separated by SDS-PAGE and transferred to High Bond ECL nitrocellulose membranes (Amersham Biosciences) under semidry conditions as previously described (9). Antibody dilutions were: rabbit anti-mouse iNOS, 1:2,000; mouse anti-pre-IL-1β, 1:2,000; rabbit anti-phospho-ERK, 1:1,000; rabbit anti-phospho-p38, 1:1,000; rabbit anti-phospho-JNK, 1:1,000; rabbit anti-ERK1, 1:1,000; rabbit anti-JNK, 1:1,000; horseradish peroxidase-conjugated donkey anti-mouse, 1:5,000; and horseradish peroxidase-conjugated donkey anti-rabbit, 1:7,000. Antigen detection was by ECL (Amersham Biosciences) according to the manufacturer’s specifications.

Northern Blot and RT-PCR Analysis—Total RNA was isolated from RAW 264.7 cells using the RNaseasy kit (Qiagen, Santa Clarita, CA) according to the manufacturer’s instructions. Northern blot analysis and RT-PCR were performed using iNOS, IL-1α, IL-1β, cyclophilin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes as previously described (9, 40). Cyclophilin was used as an internal RNA loading control for Northern blots, and GAPDH used as a control for the RT reaction.

Transient Transfections and IL-1β Reporter Assays—RAW 264.7 cells were transiently transfected with 1 µg of the human IL-1β luciferase reporter (positions −3757 to +11; XL-LUC) and 1 µg of the pCMV-SPORT-β-galactosidase (Invitrogen) control plasmid using the Qiagen Superfect reagent according to the manufacturer’s instructions. After an 8-h incubation at 37°C experiments were initiated by addition
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RESULTS

Effects of MEK Inhibition on dsRNA-induced iNOS Expression, Nitrite Production, and IL-1 Expression and Release by RAW 264.7 Macrophages—Macrophage activation in response to dsRNA includes the expression of iNOS and production of nitric oxide (9, 14). Also, dsRNA and viral infection have been shown to stimulate ERK activation in macrophages (15, 24, 27), and LPS-induced iNOS expression is sensitive to ERK inhibition (43). Therefore, the potential role of ERK in dsRNA-induced macrophage activation was examined using selective inhibitors (U0126 and PD98059) of MEK, the upstream kinase of p38 and JNK.

Figure 3. Effects of PD98059 on dsRNA-induced IL-1 expression by RAW 264.7 macrophages. RAW 264.7 cells (5 × 10⁶/2 ml DME) were pretreated with PD98059 for 30 min, poly(IC) was added, and the cells were cultured for 6 additional hours at 37°C. Total RNA was isolated and used to determine IL-1α and IL-1β mRNA accumulation by Northern blot analysis (A). Cyclophilin mRNA accumulation is shown as a control for RNA loading. RAW 264.7 cells (5 × 10⁶/ml DME) were pretreated with the indicated concentrations of PD98059 for 30 min, poly(IC) (50 μg/ml) was added, and the cells were cultured for 24 additional hours. The cells were then isolated and pro-IL-1β protein expression was determined by Western blot analysis (B). The concentration-dependent effects of PD98059 on dsRNA-induced p38 and JNK phosphorylation following a 30-min incubation were examined by Western blot analysis using phosphospecific (p38-P, JNK-P) antisera (C). Total JNK is shown as a loading control. The results IL-1 mRNA accumulation, pro-IL-1β protein expression, and p38 and JNK phosphorylation are representative of three independent experiments.

Figure 2. MEK inhibition prevents dsRNA-induced IL-1 expression and release by RAW 264.7 macrophages. RAW 264.7 cells (4 × 10⁷/400 μl of DME) were pretreated with the indicated concentrations of U0126 for 30 min, poly(IC) (50 μg/ml) was added, and the cells were cultured for 24 additional hours. IL-1 released into the culture supernatant was determined using the RINm5F cell bioassay (A). RAW 264.7 cells (5 × 10⁶/2 ml DME) were pretreated with U0126 or MeSO (vehicle control, DMSO) for 30 min, poly(IC) was added, and the cells were incubated for 6 additional hours at 37°C. Total RNA was isolated and used to determine IL-1α and IL-1β mRNA accumulation by Northern blot analysis (B). Cyclophilin mRNA accumulation is shown as a control for RNA loading. RAW 264.7 cells (4 × 10⁶/ml DME) were pretreated with the indicated concentrations of PD98059 for 30 min, poly(IC) (50 μg/ml) was added, and the cells were cultured for 24 additional hours. The cells were then isolated and pro-IL-1β protein expression was determined by Western blot analysis (C). The concentration-dependent effects of PD98059 on dsRNA-induced p38 and JNK phosphorylation following a 30-min incubation were examined by Western blot analysis using phosphospecific (p38-P, JNK-P) antisera (C). Total JNK is shown as a loading control. The results IL-1 mRNA accumulation, pro-IL-1β protein expression, and p38 and JNK phosphorylation are representative of three independent experiments.

Densitometry and Image Analysis—Autoradiograms were scanned into NIH Image version 1.59 using a COHU high performance CCD camera (Brookfield, WI) and densities determined using NIH Image version 1.59 software.

Immunoassay—The K71R mutation in human ERK1 was made using the Stratagene (La Jolla, CA) QuickChange PCR-plasmid mutagenesis kit using 5'-TGCGCATCAGAAAGATACGC-3' and 5'-GCTGATTTTCTGATGCCA-3' (mutated bases in bold) primers according to the manufacturer's specifications. This mutation, which was confirmed by sequence analysis, has previously been shown to inhibit ERK kinase activity (42). RAW 264.7 cells were transiently transfected with 2 μg of wild-type ERK1 or ERK1(K71R) using Superfect (Qiagen) according to the manufacturer's instructions. After a 48-h incubation at 37°C, poly(IC) was added, and the cells were cultured for 30 additional min. The cells were then harvested, ERK was immunoprecipitated, and the ability of immunoprecipitated ERK to phosphorylate myelin basic protein was examined as previously described (42). ERK kinase activity was quantitated by densitometry.

Effects of MEK Inhibition on dsRNA-induced iNOS Expression, Nitrite Production, and IL-1 Expression and Release by RAW 264.7 Macrophages—Macrophage activation in response to dsRNA includes the expression of iNOS and production of nitric oxide (9, 14). Also, dsRNA and viral infection have been shown to stimulate ERK activation in macrophages (15, 24, 27), and LPS-induced iNOS expression is sensitive to ERK inhibition (43). Therefore, the potential role of ERK in dsRNA-induced macrophage activation was examined using selective inhibitors (U0126 and PD98059) of MEK, the upstream kinase

Densitometry and Image Analysis— Autoradiograms were scanned into NIH Image version 1.59 using a COHU high performance CCD camera (Brookfield, WI) and densities determined using NIH Image version 1.59 software.
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that is responsible for the activation of ERK. Treatment of RAW 264.7 macrophages with 50 μg/ml poly(IC) results in the accumulation of iNOS mRNA following a 6-h incubation, the expression of iNOS at the protein level and a 5-fold increase in nitrite production following a 24-h incubation (Fig. 1, A–C). The ERK selective inhibitor U0126, at concentrations of 0.01–5 μM, does not attenuate iNOS mRNA accumulation, iNOS protein expression, or nitrite production by RAW 264.7 cells. Similar results for nitrite production and iNOS expression were obtained with the second MEK inhibitor, PD98059 at concentrations of 0.1–10 μM (data not shown). MEK inhibition slightly increases the levels of nitrite produced by RAW 264.7 cells treated with poly(IC); however, this minor increase did not achieve statistical significance.

A second antiviral response activated by dsRNA in macrophages is the expression and release of the proinflammatory cytokine, IL-1 (4, 9). A 24-h incubation of RAW 264.7 cells with poly(IC) (50 μg/ml) results in a ~50-fold increase in IL-1 release and the accumulation of pro-IL-1β protein (Fig. 2, A and B, respectively). Consistent with IL-1 release, treatment of RAW 264.7 cells with poly(IC) stimulates the accumulation of both IL-1α and IL-1β mRNA as determined by Northern blot analysis following a 6-h incubation, and IL-1β luciferase reporter activity following a 24-h incubation (Fig. 2, C and D, respectively). The MEK selective inhibitor U0126 attenuates poly(IC)-induced IL-1α and IL-1β mRNA accumulation, IL-1β promoter activity, pro-IL-1β protein expression, and IL-1 release by RAW 264.7 cells, with maximal inhibition at 5 μM.

To confirm these findings the effects of a second MEK selective inhibitor, PD98059 on IL-1α and IL-1β mRNA, and protein expression in response to poly(IC) treatment was examined. PD98059 at 10 μM inhibits poly(IC)-induced IL-1α and IL-1β mRNA accumulation and pro-IL-1β protein expression (Fig. 3, A and B). To confirm the selectivity of the ERK inhibitors, the effects of PD98059 and U0126 on dsRNA-induced p38 and JNK phosphorylation were examined. At concentrations that prevent dsRNA-induced IL-1α and IL-1β expression, PD98059 (10

Fig. 4. Effects of MEK inhibition on EMCV-induced pro-IL-1β expression by RAW 264.7 cells. A, RAW 264.7 cells (4 × 10⁶/400 μl DME) were infected with the indicated multiplicity of infection (MOI) of EMCV for 24 h, the cells were isolated and pro-IL-1β expression was determined by Western blot analysis. B, RAW 264.7 cells, pretreated with U0126 for 30 min, were infected for 24 h with EMCV at an MOI of 0.25. The cells were then isolated, and pro-IL-1β expression was determined by Western blot analysis. Results are representative of three independent experiments.

Fig. 5. dsRNA stimulates ERK activation and dnERK inhibits dsRNA-induced IL-1 expression by macrophages. A, RAW 264.7 cells (4 × 10⁶/400 μl DME) were pretreated with 5 μM U0126 for 30 min, poly(IC) (50 μg/ml) was added, and the cells were cultured for an additional 30 min. The cells were isolated and phosphorylated ERK (p44-P and p42-P) and p44 ERK (loading control) were determined by immunocomplex kinase assay (B). The expression levels of ERK and dnERK were determined by Western blot analysis and are shown in the inset (B). RAW 264.7 cells (4 × 10⁶/2 ml of DME), transiently transfected with ERK or dnERK, were treated with 50 μg/ml poly(IC) for 30 min, and ERK activity was determined by immunocomplex kinase assay (B). The expression levels of ERK and dnERK were determined by Western blot analysis and are shown in the inset (B).

Fig. 6. MEK inhibition does not affect dsRNA + IFN-γ-induced iNOS expression or nitrite production by peritoneal macrophages. Peritoneal macrophages isolated from PKR(−/−) and PKR(+/+) mice (4 × 10⁶ cells/400 μl of complete CMRL-1066) were preincubated for 30 min with U0126, poly(IC) was added, and the cells were cultured for 24 additional hours. Nitrite production was determined on the culture supernatants (A), and iNOS protein expression determined by Western blot analysis of the isolated cells (B). The results for nitrite production are the average ± S.E. of three independent experiments, and iNOS protein expression is representative of three independent experiments.
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To confirm that poly(IC) recapitulates host responses to a viral infection, the effects of encephalomyocarditis virus (EMCV) infection on the expression of IL-1β by RAW 264.7 cells were evaluated. As shown in Fig. 4A, infection of RAW 264.7 cells with EMCV stimulates the accumulation of IL-1β following a 24-h incubation. Importantly, EMCV-induced IL-1β expression is attenuated by U0126 (5 μM, Fig. 4B) at a concentration that prevents poly(IC)-induced IL-1β expression by RAW 264.7 cells (Fig. 2). These findings indicate that EMCV and poly(IC) elicit similar antiviral responses in RAW 264.7 cells and provide additional evidence to support a role for ERK in the regulation of IL-1β expression by macrophages in response to a viral infection.

dsRNA stimulates ERK activation and dnERK inhibits dsRNA-induced IL-1 expression by macrophages—To confirm that U0126 prevents ERK activation, the effects of this MEK kinase inhibitor on ERK phosphorylation were examined by Western blot analysis. Treatment of RAW 264.7 cells for 30 min with poly(IC) (50 μg/ml) results in the phosphorylation of ERK, an effect that is prevented by U0126 at concentrations (5 μM) that inhibit dsRNA-induced IL-1 expression and release (Fig. 5A). In a similar manner PD98059 (10 μM) prevents poly(IC)-stimulated ERK phosphorylation (data not shown). Consistent with the stimulatory actions of poly(IC) on ERK phosphorylation, dsRNA activates ERK as determined by immunocomplex kinase assays. For these experiments, RAW 264.7 cells, transiently transfected with either wild type or dnERK (ERK1(K71R)), were incubated with or without poly(IC) for 30 min, and then the phosphorylation of myelin basic protein by immunoprecipitated ERK was examined. A 30-min incubation with poly(IC) stimulates an 4.5-fold increase in ERK activity (myelin basic protein phosphorylation) in RAW 264.7 cells expressing wild-type ERK; however, poly(IC) fails to stimulate myelin basic protein phosphorylation in RAW 264.7 cells expressing dnERK (Fig. 5B). In this immunocomplex kinase assay wild type and dnERK are expressed at levels 2–3-fold higher than the levels of endogenous ERK (Fig. 5B, inset). RT-PCR was used to examine the role of ERK in the regulation of IL-1α and IL-1β mRNA accumulation by macrophages in response to dsRNA and EMCV infection. A 6-h incubation with poly(IC) results in the accumulation of both IL-1α and IL-1β mRNA in RAW 264.7 cells transfected with wild-type ERK (Fig. 5C). In contrast, IL-1α and IL-1β mRNA accumulation is attenuated in RAW 264.7 cells transfected with dnERK. In a similar fashion, EMCV-induced IL-1α and IL-1β mRNA accumulation is attenuated in RAW 264.7 cells transfected with dnERK (Fig. 5D). The transfection efficiency in these experiments was greater than 70% (as determined by β-galactosidase staining, and the levels of wild type and dnERK expression were comparable to those shown in Fig. 5B, inset). These findings indicated that dsRNA stimulates ERK activation and provide further evidence to support a role for ERK in the regulation of dsRNA- and EMCV-induced IL-1 expression by macrophages.

MEK inhibition does not affect dsRNA-induced iNOS expression and nitrite production but prevents IL-1 expression and release by peritoneal macrophages—Consistent with the lack of a role for ERK in dsRNA-induced iNOS expression by RAW 264.7 cells, U0126 (5 μM) does not modulate poly(IC) +
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IFN-γ-induced nitrite production (Fig. 6A) or iNOS expression (Fig. 6B) by PEC isolated from PKR−/− and PKR−/+ mice. In contrast to RAW 264.7 cells, naive mouse peritoneal macrophages require two proinflammatory signals for iNOS expression and nitrite production (44), and we have previously shown that neither poly(IC) nor IFN-γ alone stimulate iNOS expression or nitric oxide production by PEC (44). Also, we have shown that PKR is not required for poly(IC) + IFN-γ-induced iNOS expression and nitric oxide production by mouse PEC (4).

While ERK does not appear to participate in the regulation of iNOS expression, it is required for poly(IC) and poly(IC) + IFN-γ-induced IL-1 release by mouse macrophages. Treatment of PEC isolated from PKR−/− and PKR−/+ mice for 24 h with either poly(IC) or poly(IC) + IFN-γ results in the release of IL-1 to similar levels, and this IL-1 release is prevented by U0126 (Fig. 7A). U0126 also prevents poly(IC) (data not shown) and poly(IC) + IFN-γ-induced IL-1β protein accumulation in PEC isolated from PKR−/− and PKR−/+ mice (Fig. 7B). These findings, which are consistent with the inhibitory actions of U0126 on IL-1 expression and release by RAW 264.7 cells, support a PKR-independent role for ERK in dsRNA-induced IL-1 expression and release by primary mouse macrophages.

To confirm that dsRNA stimulates ERK activation, and to determine if PKR is required for this activation, the effects of poly(IC) on ERK phosphorylation by PEC isolated from PKR−/− and PKR−/+ mice were examined. As shown in Fig. 7C, poly(IC) stimulates the time-dependent phosphorylation of ERK that is first apparent following a 15-min incubation and that persists for up to 60 min. PKR does not appear to participate in ERK activation, as poly(IC) stimulates ERK phosphorylation to similar levels in PEC isolated from PKR−/− and PKR−/+ mice. In addition, the stimulatory actions of a 30-min incubation with poly(IC) on ERK phosphorylation are prevented by U0126 (5 μM, Fig. 7D). These findings indicate that the presence of PKR is not required for the activation of ERK by dsRNA in PEC.

DISCUSSION

Recently, Magun and co-workers (25) have proposed that dsRNA triggers two separate antiviral programs, cell suicide (apoptosis), and a survival pathway associated with proinflammatory cytokine production. At the core of this proposal is the ability of PKR to regulate each of these antiviral responses. The first antiviral pathway is that of apoptosis, a process of self-elimination that removes virally infected cells. This pathway of programmed cell death is activated by dsRNA, is dependent on PKR (45, 46), and appears to be a widely used antiviral response as evidenced by the multiple strategies used by viruses to evade apoptosis (Ref. 8 and references therein). The second antiviral pathway activated by dsRNA or viral infection is the expression and release of proinflammatory cytokines such as interferons and interleukins. The role of PKR in the regulation of cytokine expression has not been clearly defined, in part because of results that support or refute a role for PKR in the regulation of NF-κB and MAPK activation in response to dsRNA. PKR was originally believed to be required for dsRNA-induced NF-κB activation and p38 phosphorylation based on studies in MEF isolated from PKR−/− mice (24, 35). More recently, dsRNA and EMCV infection have been shown to stimulate NF-κB and p38 activation in MEF isolated from PKR−/− mice to levels similar to MEF isolated from wild-type mice (25), suggesting that PKR-independent pathways also participate in the antiviral response.

In this study, we present evidence to further support the presence of a PKR-independent antiviral response pathway that is activated by viral infection and dsRNA and that regulates the expression and release of the proinflammatory cytokine IL-1. We show that dsRNA treatment or EMCV infection stimulates IL-1 expression and release in an ERK-dependent fashion. Selective inhibition of MEK, the upstream ERK kinase, prevents dsRNA and EMCV-induced IL-1 expression and release. The role of ERK in regulating the antiviral response appears to be selective for IL-1 expression as MEK inhibition does not modulate dsRNA-induced iNOS expression or nitric oxide production by macrophages. PKR does not appear to be required for this response, as dsRNA stimulates ERK activation and ERK-dependent IL-1 expression by macrophages isolated from PKR−/− mice. These findings suggest that PKR is not required for the activation of ERK or the expression and release of IL-1 by macrophages in response to dsRNA or viral infection. However, this conclusion must be tempered by the possibility that other “PKR-like” molecules may compensate for the absence of PKR (in PKR-deficient macrophages), and the recent evidence that the PKR−/− mice used in this study may be incomplete knockouts (47).

In summary, these studies have identified a novel mechanism by which viral infection and dsRNA stimulate the expression and release of IL-1 by macrophages. dsRNA or EMCV infection stimulates ERK activation and ERK participates in the transcriptional regulation of IL-1 expression by macrophages. PKR does not appear to be required for dsRNA-induced ERK activation or the transcriptional activation of IL-1 expression. The downstream targets of ERK that regulate IL-1 expression in response to dsRNA and virus infection have yet to be defined. One likely candidate is PU.1, a member of the ets family of transcription factors, and a transcription factor known to participate in the activation of IL-1 expression in response to LPS. In support of a role for PU.1 in the regulation of virus-induced IL-1 expression, we have recently shown by gel shift analysis that poly(IC) stimulates the DNA binding activity of PU.1 in macrophages in an U0126-sensitive manner.2 Additional transcription factors also participate in the regulation of IL-1 expression in response to dsRNA. Inhibitors of NF-κB have been shown to prevent dsRNA-induced IL-1 expression and release by mouse macrophages (9). In addition, interferon regulatory factor-4 in combination with PU.1 has been shown to synergistically activate the human IL-1 reporter by interacting at the distal PU.1 consensus binding element (32, 48). Importantly, this transcriptional regulation of antiviral response genes following viral infection or treatment with dsRNA appears to be selective. We show that dsRNA-stimulated iNOS expression is not sensitive to inhibitors of ERK, while ERK inhibition prevents virus and dsRNA-stimulated IL-1 expression and release. Recently, we have identified a role for the calcium-independent phospholipase A2 (iPLA2) in the regulation of dsRNA and EMCV-induced iNOS expression, and the role of iPLA2 in this antiviral response appears to be selective for iNOS as inhibitors of this phospholipase fail to modulate IL-1 expression in response to dsRNA (49). In this study we have not examined the upstream molecules that activate ERK; however, it is likely that the recently described receptor for dsRNA, TLR3 mediates ERK activation (50). These findings indicate that activation of the antiviral responses is cell type selective and that the pathways that regulate specific antiviral responses may be selective for individual target genes or cellular responses.

Acknowledgments—We thank Colleen Bratcher for expert technical assistance and Dr. Joseph Baldassare for advice concerning the immunocomplex kinase assays. We also thank Dr. Matthew Fenton for the human IL-1β luciferase reporter construct and Dr. J. W. Yoon for providing EMC virus.

L. B. Maggi and J. A. Corbett, unpublished observations.
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J. Biol. Chem. 2003, 278:16683-16689.
doi: 10.1074/jbc.M211744200 originally published online February 27, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M211744200

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