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Phosphatidylinositol 3-Kinase Confers Resistance to Encephalomyocarditis and Herpes Simplex Virus-Induced Cell Death Through the Activation of Distinct Downstream Effectors

Christine Prejean,* Tulika Sarma,* Anna Usacheva,* Brian Hemmings,† Lewis Cantley,§ David A. Fruman,§ Lynda A. Morrison,¶ R. Mark Buller,¶ and Oscar R. Colamonici‡*

The Janus kinase/STAT pathway has emerged as the paradigm of IFN-induced protection from viral infections. However, the possible participation of other signaling proteins in this protection is not clearly understood. In this report, we demonstrate that activation of phosphatidylinositol 3-kinase (PI3K) by either serum factors or IFNs blocks cell death induced by encephalomyocarditis virus (EMCV) and HSV. This increased resistance to virus-induced cell death does not involve the activation of the STAT pathway and occurs in the presence of normal viral replication. Interestingly, the cell uses two different PI3K regulated pathways to block EMCV- and HSV-induced cell death. The increased sensitivity of p85α−/− embryonic fibroblasts to EMCV-induced cell death is specifically corrected by overexpression of an activated allele of Akt/protein kinase B, but not activated mitogen-activated protein kinase extracellular kinase. Conversely, the augmented sensitivity of p85α−/− cells to HSV-induced cell death was compensated for by expression of an activated form of mitogen-activated protein kinase extracellular kinase, but not by activated Akt/protein kinase B. We conclude from these data that PI3K-activated pathways function in parallel with the Janus kinase/STAT pathway to protect cells from the lethal effects of viruses. The Journal of Immunology, 2001, 167: 4553–4559.

Type I and type II IFNs constitute the first line of defense against viral infections and also play a role in the control of cell proliferation (1). Type I IFNs include a family of proteins represented by IFN-α, IFN-β, and IFN-ω, while IFN-γ is the only known type II IFN (1, 2). Binding of type I and type II IFNs to their distinct heterodimeric receptors (also referred to as IFN-ωR and IFN-γR) results in the activation of the Janus kinase (Jak)/STAT pathway. In IFN-α and IFN-γ pathways, tyrosine kinases (Jak1/Tyk2 and Jak1/Jak2, respectively) become activated and phosphorylate receptor chains. The phosphorylated tyrosines act as docking sites for STAT factors via conserved SH2 domains. STATs 1 and 2 are activated by the type I IFN receptor and STAT1 is activated by the type II IFN receptor. Jak5 presumably phosphorylate the STATs, which allows two STATs to homodimerize or heterodimerize by reciprocal phosphotyrosine-SH2 domain interactions. Thus, type I IFNs induce dimerization of STAT1 and STAT2, which, along with the p48 protein, form the IFN-stimulated gene factor 3 complex, while IFN-γ induces the γ-activated factor complex composed of STAT1 dimers. These complexes translocate to the nucleus, bind to the IFN-stimulated response element (ISRE) and IFN-γ activated sequence (GAS) motifs in specific genes, and induce transcription of genes required to produce an antiviral state (for reviews see Refs. 2–4) (Fig. 1A).

The importance of the Jak/STAT pathway has been demonstrated by the generation of knockout mice with targeted disruption of genes encoding the IFN-γ chains or associated Jak and STAT proteins. These mice lack the ability to mount an efficient antiviral response and die shortly after viral challenge (5–9). Interestingly, it has been shown that other signaling proteins are activated by IFNs, including mitogen-activated protein kinases (MAPKs), SHP1 and 2, CrkL/Crk II, p95^onc, insulin receptor substrate-1, and phosphatidylinositol (PtdIns) 3-kinase (PI3K) (10–20). However, the role of these signaling proteins in the antiviral response has not been elucidated.

PI3K is probably the most intriguing due to its multiple functions. PI3K is a dimer composed of adaptor and catalytic subunits and plays a critical role in many cellular events such as cell survival, proliferation, and adhesion (21–24). Cytokines, IFNs, and growth factors activate class I PI3K, which contains one of three forms of the adaptor subunit that are encoded by three different genes: p85α, p85β, and p55γ. The α gene encodes adaptor subunits with molecular masses of 85, 55, and 50 kDa (p85α, p55α,
Several signaling pathways diverge downstream of activated PI3K, including two critical in promoting cell survival, the Ser/Thr kinase Akt/protein kinase B (PKB) (reviewed in Refs. 24 and 27), and extracellular regulated kinase (ERK) (28). ERK is part of the p21<sup>ras</sup> oncprotein (Ras)-RAF-MEK-MAPK signaling pathway and mediates both cell growth and survival. Akt/PKB is a Ser/Thr kinase with important antiapoptotic functions. There are three isoforms of Akt/PKB that are encoded by three different genes: Akt1/PKB<sub>a</sub>, Akt2/PKB<sub>b</sub>, and Akt3/PKB<sub>y</sub>. Akt is composed of an amino-terminal pleckstrin homology domain, a catalytic domain, and a 70-aa regulatory domain at the C terminus. Akt/PKB is regulated by phosphorylation of conserved threonine and serine residues. In Akt1/PKB<sub>a</sub>, these residues are found in the catalytic domain, T308, and regulatory domain, S473. Akt/PKB is activated by the phospholipids produced by PI3K activity. These lipids bind the pleckstrin homology domain of Akt/PKB and target the protein to the cell membrane where 3'-phosphoinositide kinases 1 and 2 phosphorylate residues T308 and S473, thereby activating the kinase domain. Activated Akt/PKB promotes cell survival in both the cytosol and the nucleus in several ways. The proapoptotic protein BAD and the Forkhead family of transcription factors are both targets of Akt. Phosphorylation of these proteins by Akt impairs their apoptotic functions. Akt also promotes cell survival through phosphorylation of I<sub>κB</sub> kinases and caspase-9, and acts to stabilize the mitochondria (reviewed in Ref. 27).

In this paper, we investigated the possible involvement of PI3K in the cell survival activity induced by IFNs and serum against cell death induced by viruses. Our data show that mouse embryonal fibroblast (MEF) clones lacking the p85<sub>a</sub> adaptor subunit are more susceptible to EMVC- and HSV-induced cell death. This effect is independent of the activation of the STAT pathway, implying that PI3K and STAT pathways function in parallel. Interestingly, overexpression of an activated form of Akt (Akt<sub>m</sub>) in p85<sub>a</sub>−/− cells was able to rescue the impaired response to the RNA virus EMCV, but not to HSV type 1 (HSV-1), a DNA virus. Conversely, expression of an activated form of MEK (MEK<sup>+</sup>) conferred resistance to HSV-1 but not to EMCV. These data suggest that HSV and EMCV very likely induce cell death through different mechanisms that are inhibited by distinct pathways downstream of PI3K. We propose that PI3K plays a central role in the regulation of virus-induced cell death through different downstream pathways.

**Materials and Methods**

**Reagents**

The pan-anti-p85 Ab was previously described (29). The anti-Akt Ab was purchased from Upstate Biotechnology (Lake Placid, NY). Anti-hemagglutinating Ag (HA) and anti-p85 Ab was previously described (29). The anti-Akt Ab was purchased from Cell Signaling Technology (Danvers, MA). Recombinant murine (mu) IFN-α, recombinant mouse IFN-γ, recombinant mouse Flg, and recombinant mouse IFN-a3, b1, and b2 were kindly provided by M. Roussel, St. Jude Children’s Research Hospital, Memphis, TN (31). Clones were selected in 500 μg/ml hygromycin and screened by Western blotting with the appropriate anti-tag Ab.

**Stable transfectants**

MEFp85α−/−<sup>53.9</sup> and MEFp85α−/−<sup>92.1</sup> cells were cotransfected with pCEP4 vector, which carries the hygromycin resistance gene, and the HA-tagged activated Akt/PKB (Akt<sub>m</sub>/p), under the control of the CMV promoter (30), or with myc-tagged MEK<sup>+</sup> under the control of an inducible promoter (kindly provided by M. Roussel, St. Jude Children’s Research Hospital, Memphis, TN) (31). Clones were selected in 500 μg/ml hygromycin and screened by Western blotting with the appropriate anti-Ab.

**Susceptibility to virus-induced cell death and protection by IFNs**

Cells were seeded in 96-well plates at 15,000 cells/well in DMEM supplemented with 10% FBS in 96-well plates (15,000 cells/well) and replacing the medium with the indicated concentration of FBS after the cells were attached to the plates. Two-fold dilutions were performed starting at 1/1,000 (multiplicity of infection (MOI) 0.5) for EMVC in a final volume of 100 μl. For experiments using HSV-1, cells were infected at the indicated MOIs in DMEM containing 10% FBS. Cell viability was determined using MTT after 24 h for EMVC or 3 and 6 days for HSV-1.

The induction of an antiviral response by IFNs was determined using a standard antiviral assay (32). MEFp85α−/−<sup>92.1</sup> and MEFp85α−/−<sup>92.3</sup> (p85<sub>a</sub>−/− and p85α−/−<sup>−3</sup>) and p85α−/−<sup>53.9</sup> (p85α−/−<sup>−</sup>) were incubated with the indicated amounts of murine type 1 or II IFNs (muIFN) for 18 h in DMEM containing 0.5% FBS. The medium was removed and replaced with the lowest EMCV dilution that killed 100% of the cells in 24 h (MOIs of 0.05 and 0.005) for p85<sup>−−/−</sup> and p85<sup>−/−</sup> cells, respectively. Cell viability was also determined using MTT assay. Similar results were observed with different muIFN preparations (natural muIFN-αβ, recombinant muIFN-α4, and IFN-a-ad), although these preparations have different specific activities.

**Viral replication**

Cells were seeded in 24-well plates and infected at the indicated MOIs. The initial time point was 1 h to allow virus adsorption to the cells. Virus titer was determined by a standard plaque assay with Vero cells.

**Immunoprecipitation, Western blots, and in vitro kinase assays**

Cells were treated with 250 U/ml the indicated IFNs for 20 min. For experiments with LY294002 (LY), cells were preincubated with 25 μM LY for 1 h before IFN treatment. Cells were lysed and subjected to immunoprecipitation and Western blotting following procedures described previously (33). For in vitro kinase assays, immunoprecipitates were incubated with 2 μg of substrate and 2 μCi of [γ<sup>32</sup>P]ATP in a final volume of 30 μl of kinase buffer (25 mM MgCl<sub>2</sub>, 25 mM HEPES (pH 7.5), 2 mM DTT, 25 mM β-glycerophosphate, and 0.1 mM sodium orthovanadate). Myelin basic protein (MBP) and histone 2-B (H2B) were used as substrates for ERK and Akt, respectively. After 20 min, reactions were stopped by adding 4× SDS-loading buffer and boiling.

**Northern blotting**

Cells were treated with the indicated IFNs for 20 h in the presence or absence of 25 μM of LY. Total RNA was extracted using the Trizol kit, (Life Technologies, Gaithersburg, MD), and 20 μg were used for Northern blotting. [γ<sup>32</sup>P]CTP-labeled STAT1, oligoadenylate synthetase gene (OAS), and actin were sequentially used for hybridization. The same preparation and concentration of IFNs used in these experiments failed to produce an IC<sub>50</sub> in a standard antiviral assay.

**Results**

MEFp85α−/−<sup>−</sup> cells have increased susceptibility to EMVC

Two pathways critical in promoting cell survival diverge downstream of activated PI3K, the Ser/Thr kinases Akt/PKB (reviewed in Refs. 24 and 27) and ERK (28). This potentially places PI3K in a central position with regard to IFN-mediated inhibition of virus-induced cell death (Fig. 1A). If this premise is correct, then PI3K-deficient cells should be more susceptible to virus-induced cell death. It was not possible to develop MEF cell lines with a null mutation of the PI3K-p110<sub>α</sub> catalytic subunit because p110<sub>α</sub>−/− mice died very early during gestation (34). However, MEFs have been generated from mice carrying a deletion of the α forms of the adaptor subunit (29). Thus, MEFp85α−/−<sup>−</sup> cells were used to test the hypothesis that activation of PI3K by IFNs could contribute to the antiviral effect by prolonging survival of virus-infected cells.

We first determined the sensitivity of MEFp85α−/−<sup>−</sup> and MEFp85α−/−<sup>+</sup> cells to the cytotoxic effect of an RNA virus, EMVC. Both cell lines were plated in 96-well plates in 2% serum.
and treated with virus in 2-fold dilutions. Interestingly, the MOI required to kill the majority of p85α−/− cells was ~100-fold lower than that of p85α+/+ cells (Fig. 1B), suggesting that the activation of PI3K by growth factors present in serum plays a role in resistance against EMCV-induced cell death.

We next determined the capacity of these cells to induce an antiviral state in response to IFN-α or IFN-γ under low-serum conditions (0.5% FBS) to minimize PI3K activation by serum factors. Fig. 1 (C and D) shows that p85α−/− cells require more muIFN-αβ or muIFN-γ than wild type cells for protection against EMCV. This defect in the antiviral response was always more pronounced for IFN-γ. These results indicate that activation of PI3K by IFN-α or IFN-γ contributes to the antiviral effect.

Because the attenuated response to IFNs could correlate with low expression of p85β in MEFp85α−/− cell lines, cell lysates were subjected to Western blotting with a pan-p85 Ab that recognizes both p85α and β isoforms (Fig. 1C, inset, and Ref. 29). We found that the p85β isoform, if present, is able to complement the p85α−/− mutation, resulting in a milder defect or no alteration at all in the antiviral response (e.g., p85α−/−.8 and data not shown). These data strongly suggest that activation of PI3K plays a role in determining sensitivity to EMCV-induced cell death and the induction of an antiviral state by IFN-α and IFN-γ.

The Jak/STAT pathway is not impaired in p85α−/− cells

We next tested whether increased sensitivity of p85α−/− cells to EMCV corresponded to a defect in the activation of the Jak/STAT pathway. This was conducted under IFN concentrations that prevented viral-induced cell death in the p85α+/+ cells, but not in p85α−/− cell lines. (The same preparation and concentration of IFNs used in these experiments failed to produce an IC50 in p85α−/− cells in a standard antiviral assay.) We initially assessed the activation of the Jak/STAT pathway by studying the induction of tyrosine phosphorylation of Jak1, STAT1, and STAT2 after treatment with IFN-α or IFN-γ for 15 min. Fig. 2 shows that muIFN-α and muIFN-γ induced comparable levels of tyrosine phosphorylation of Jak1, STAT1, and STAT2 in both p85α−/− and p85α+/+ cells at IFN doses that showed marked impairment in the induction of the antiviral response of p85α−/− cells (Fig. 2, A–C, and data not shown). These results indicate that the defect observed in p85α−/− cells is not due to a defect in the activation of the Jak/STAT pathway.

To further characterize the activation of the IFN system, we performed Northern blots to study the IFN-driven induction of specific genes in MEFp85α−/− and MEFp85α+/+ cells. These experiments were also conducted in cells pretreated with the PI3K inhibitor LY to determine whether PI3K plays a role in the induction of specific genes by IFN-α and/or IFN-γ. Fig. 2D shows that IFN-stimulated gene factor 3-driven induction of the OAS by IFN-α and induction of transcription of the ST1 gene through the GAS element by IFN-α and IFN-γ were identical in p85α−/− and p85α+/+ cells. Additionally, the data show that preincubation with LY does not inhibit IFN-driven transcription. This eliminates the possibility that PI3K-induced activation of Akt or MEK influences Jak/STAT signaling. These results demonstrate that PI3K contributes to the antiviral activity of IFN-α and IFN-γ independently of the Jak/STAT pathway, and works as a parallel, STAT-independent mechanism.
Akt and ERK are activated by IFNs through PI3K

Because PI3K signaling diverges into several pathways, we next wanted to determine which pathways mediated an increased resistance to virus-induced cell death. The primary targets for investigation were Akt/PKB and ERK, because activation of these survival pathways could increase resistance to virus-induced cell death. A previous report indicated that the ERK pathway may be activated by IFN-α and IFN-γ (12), possibly through PI3K (35).

To determine whether Akt/PKB and ERK were activated by IFNs through PI3K, MEFP85α+/+ and MEFP85α−/− cells were treated with IFNs α and γ at 250 U/ml, and then in vitro kinase assays were performed after immunoprecipitation with anti-Akt (Fig. 3A) or anti-ERK (Fig. 3B) Abs. Treatment with IFN-α or IFN-γ induced an increase in Akt/PKB activity, as seen by H2B phosphorylation, in p85α+/+ cells, but not in MEFP85α−/− cells (Fig. 3A). Similarly, IFNs α and γ produced an increase in MBP phosphorylation, which is indicative of ERK activity, in p85α+/+ cells but not in p85α−/− cells (Fig. 3B). These results confirm that activation of Akt/PKB and ERK by IFNs requires functional PI3K (35, 36).

**Activated Akt increases resistance to EMCV-induced cell death**

To determine which PI3K downstream effector was responsible for the increase in cell survival, we stably transfected constitutively active forms of Akt/PKB (Akt/PKB m/p-membrane targeted) or MEK (MEK*) into MEFP85α+/+ and MEFP85α−/− cells to bypass the requirement of PI3K for activation. Fig. 3C shows the expression of HA-Aktm/p- and myc-MEK*-tagged constructs in different clones. Immunoblotting with an anti-phospho-Akt shows Akt activity in stably transfected cells, but not in cells transfected with empty vector. Similarly, good levels of expression of MEK* were detected in p85α−/− and p85α+/+ cells.

We next tested the susceptibility of these cells to EMCV infection. Surprisingly, overexpression of activated Akt/PKB not only overcame the sensitivity to EMCV-induced cell death observed in p85α−/− cells, but also made them highly resistant to this virus even under conditions in which activation of PI3K by growth factors present in the serum was minimal (Fig. 4A, left panel). A similar increase in resistance to EMCV was observed in p85α−/− cells transfected with activated MEK/PKB. This increase was not observed in cells transfected with control vector (Fig. 4A, left panel) or activated MEK. Activation of PI3K by factors present in a higher concentration of serum (Fig. 4A, right panel) increased the resistance to EMCV-induced cell death of control MEFP85α+/+ cells, and, to a lesser extent, p85α−/− cells transfected with the control vector. This effect was also detected in nontransfected cells (Fig. 1B). These results suggest that activation of the PI3K-Akt/PKB pathway is responsible for the resistance to virus-induced cell death.

**Aktm/p does not increase survival by inhibiting viral replication**

The increased survival of cells expressing a constitutively active form of Akt/PKB could be explained by a decrease in viral replication through activation of the Jak/STAT pathway. We determined the titer of EMCV in cultures 24 h after infection using two different replicates. Although the titer of EMCV in cultures transfected with Aktm/p was lower than in control nontransfected cultures, this decrease was not statistically significant. These results suggest that activation of the PI3K-Akt/PKB pathway is responsible for the resistance to virus-induced cell death.
Akt.1 and serum for 6 h and then treated with 250 U/ml muIFN-α or muIFN-γ or were left untreated (CT). Lyastes were immunoprecipitated with an anti-Akt Ab and subjected to an in vitro kinase assay using H2B as a substrate. Western blotting with anti-Akt Ab was used as a control for loading. B, Cells were stimulated with IFNs as in A and immunoprecipitated with an anti-ERK polyclonal Ab. An in vitro kinase assay was subsequently performed using MBP as a substrate. Immunoblotting was performed with the same anti-ERK Ab used for immunoprecipitation (lower panel). C, p85α+/+53.9 and p85α−/−92.3 cells were stably transfected with control vector (pCMV5), the membrane-targeted form of Akt/PKB (pCMV-HA-Aktm/p) (30), or myc-tagged, inducibly activated MEK (pMAM-MEK*) as previously described (31). MEK* was induced by incubating the cells in ZnSO₄ for at least 16 h. Expression of Aktm/p and MEK* was detected by immunoprecipitation with Abs against the tags (HA and myc, respectively) followed by Western blotting with the same Abs used for immunoprecipitation.

FIGURE 3. IFNs activate ERK and Akt/PKB through PI3K. A, MEFp85α+/+53.9 and p85α−/−92.3 cells were incubated in the absence of serum for 6 h and then treated with 250 U/ml muIFN-α or muIFN-γ or were left untreated (CT). Lyastes were immunoprecipitated with an anti-Akt Ab and subjected to an in vitro kinase assay using H2B as a substrate. Western blotting with anti-Akt Ab was used as a control for loading. B, Cells were stimulated with IFNs as in A and immunoprecipitated with an anti-ERK polyclonal Ab. An in vitro kinase assay was subsequently performed using MBP as a substrate. Immunoblotting was performed with the same anti-ERK Ab used for immunoprecipitation (lower panel). C, p85α+/+53.9 and p85α−/−92.1 cells were stably transfected with control vector (pCMV5), the membrane-targeted form of Akt/PKB (pCMV-HA-Aktm/p) (30), or myc-tagged, inducibly activated MEK (pMAM-MEK*) as previously described (31). MEK* was induced by incubating the cells in ZnSO₄ for at least 16 h. Expression of Aktm/p and MEK* was detected by immunoprecipitation with Abs against the tags (HA and myc, respectively) followed by Western blotting with the same Abs used for immunoprecipitation.

Constitutively active MEK increases resistance to HSV-1

We also studied the sensitivity of wild type and p85α−/− cells to HSV-1 to test whether resistance to virus-induced cell death is a characteristic specific to EMCV or a general mechanism that may also apply to other viruses. The p85α+/+ cells were less susceptible to HSV-1-induced cell death than were p85α−/− cells transfected with empty vector (Fig. 4C, −/−CMV.2). Surprisingly, the defect in p85α−/− cells was not corrected by activated Akt (−/−Akt.1 and −/−Akt.4), but rather by transfection of activated MEK (−/−MEK.9^*). There were no significant differences in HSV-1 replication between p85α−/− cells expressing activated MEK or control vector (data not shown). The finding that a specific antiapoptotic pathway is required for a distinct virus, i.e., Akt protects against EMCV but not HSV-1, indicates that these mechanisms are specific and not due to a general resistance to apoptosis due to expression of an activated kinase. These data indicate that HSV-1 and EMCV induce cell death by activation of different apoptotic pathways that are inhibited by different survival pathways regulated by PI3K.

Discussion

It is well known that IFNs activate PI3K; however, the role of this pathway in any biological effect induced by IFNs has not been established. This report demonstrates that activation of PI3K is important in the response to viral agents at two different levels. First, activation of PI3K by serum factors induces resistance to virus-induced cell death. This is demonstrated by the finding that cells lacking p85α are more susceptible to virus-induced cell death. Second, during the antiviral response, activation of PI3K by IFN-α or IFN-γ increases protection against viral agents. This is
particularly evident at lower doses of IFNs in cells lacking p85α (Fig. 1, C and D). Interestingly, the impaired antiviral response in MEFp85α/−/− cells occurs in the presence of normal STAT activation and gene induction (Fig. 2) and is not inhibited by PI3K inhibitors. This strongly suggests that PI3K functions in parallel to the STAT pathway, possibly by activating specific survival pathways (see below). Therefore, we conclude that there are two different components involved in the IFN-induced antiviral state: activation of the STAT pathway that controls viral replication and activation of PI3K that “helps” the antiviral response by making cells more resistant to virus-induced cell death. In this respect, the activation of PI3K by IFNs has the same role as the activation by serum factors.

The STAT-independent contribution of PI3K is further demonstrated by the finding that expression of activated downstream effectors of PI3K, i.e., Akt and MEK, rescue the defect observed in p85α/−/− cells by promoting cell survival without affecting viral replication (Fig. 4B and data not shown). Moreover, the survival role of PI3K in viral infections is further underscored by two findings: 1) cells lacking p85α are more susceptible to EMCV (Fig. 1B); and 2) conditions that decrease the activation of PI3K, such as serum starvation, increase susceptibility to EMV-induced cell death (Fig. 4A). Thus, activation of PI3K by IFNs during the antiviral response or factors present in serum during viral infection play a role in fending off virus-induced cell death.

The role of PI3K in antagonizing virus-induced cell death is not unique to a single virus class, as it can protect cells infected with RNA (EMCV) and DNA (HSV) viruses. However, the downstream effectors are different: ERK for HSV and Akt/PKB for EMCV. This suggests that HSV and EMCV probably induce cell death by activating different apoptotic pathways. This would explain why Akt increases cellular resistance to EMV and MEK increases resistance to HSV-1. For example, it is possible that EMV up-regulates BAD or FKHRs. The antiapoptotic effect of Akt/PKB could be through inactivation of these proapoptotic proteins (37). Similarly, the antiapoptotic effect of ERK could be mediated by phosphorylation of BAD by ribosomal S6 protein kinase as recently reported for neurons (38). Thus, PI3K is a central player in the control of virus-induced cell death through distinct downstream effectors.

These findings raise the following question: what is the role of PI3K in the normal antiviral response? It is possible that the PI3K pathway is important as a rapid mechanism against virus-induced cell death, giving the cell time to activate transcription of IFN-stimulated genes (ISGs) responsible for the antiviral effect of IFNs. The role of PI3K could be different in the cells initially infected by the virus and any neighboring cells. For instance, the activation of the PI3K pathway by growth factors could delay virus-induced cell death and maintain survival. This would give cells time to express and secrete IFNs to protect neighboring cells. In this scenario, paracrine IFN will induce transcription of ISGs, specific antiviral genes that will stop viral replication. In the infected cells, the survival mechanisms induced by IFN-driven activation of Akt/ PKB and ERK could be important in fending off apoptotic pathways activated by the virus, even after induction of a full antiviral response. For example, the initially infected cells secrete IFNs that will activate the STAT pathway in noninfected neighboring cells, with the final result of stopping viral replication. However, if a given virus encodes or contains a protein that induces cell death, the activation of the STAT pathway in the healthy neighboring cells may not be able to stop this effect, particularly if high numbers of viral particles are released by the infected cells. It should also be considered that PI3K activity could help to restrict the spread of certain viruses that require cell death for release, because it has been postulated that viruses may induce apoptosis as a way of leaving the cell without eliciting an immune response.

Finally, it should be noted that previous reports indicated that activation of ERK by IFN was required for induction of ISGs (12). Contrary to this, the finding that neither cells treated with the PI3K inhibitor LY nor MEFp85α/−/− cells have a defect in the induction of ISGs through the ISRE or GAS elements strongly suggests that ERK activity is not necessary for full activation of the STAT pathway or ISG induction. It is also worth noting that these results confirm previous reports (35) that indicated ERK is activated downstream of PI3K. The exact mechanism of MAPK activation is not understood. It may occur through a Ras-dependent or Ras-independent mechanism (36).

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