A Requirement for the p85 PI3K Adapter Protein BCAP in the Protection of Macrophages from Apoptosis Induced by Endoplasmic Reticulum Stress

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A Requirement for the p85 PI3K Adapter Protein BCAP in the Protection of Macrophages from Apoptosis Induced by Endoplasmic Reticulum Stress

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Macrophages are innate immune cells that play key roles in regulation of the immune response and in tissue injury and repair. Although macrophages are known for their capacity to mediate the efficient clearance of apoptotic and damaged cells by phagocytosis, they themselves undergo apoptosis in response to a variety of stimuli in health and disease. For example, in atherosclerosis (reviewed in Ref. 1) or infection with Gram-negative bacteria (2), macrophages face the dual challenge of endoplasmic reticulum (ER) stress and exposure to innate immune ligands. Whether macrophages survive or die under these conditions may have a direct impact on the overall host response to these pathological stimuli. Many specific factors that regulate apoptosis in macrophages exposed to these challenges are unknown.

Like other cells, macrophages use the PI3K/Akt pathway for cell survival (3–5). The class I PI3K, composed of heterodimeric subunits, is recruited to the plasma membrane by various Src homology 2 (SH2) domain-binding adapter proteins and transmembrane receptors. In some cases, the identity of the relevant adapter proteins is known. For example, macrophages use Gab2 to recruit p85 PI3K during phagocytosis (6). However, the requirement for specific adapter proteins in many other functions in these cells, such as protection from apoptosis, is unknown.

B cell adapter protein (BCAP) was originally isolated as a binding partner for the SH2 domain of PI3K in chicken DT40 B cells (7). Disruption of the BCAP gene in DT40 cells led to impaired recruitment of PI3K, inhibition of phosphatidylinositol-3,4,5-trisphosphate generation, and defective activation of Akt in response to ligation of the BCR (7). Surprisingly, deletion of the BCAP gene in mice did not lead to altered PI3K activity in B cells; rather, B cells from these mice demonstrated defective activation of NF-κB due to decreased expression of the NF-κB subunits, c-Rel and RelA. In addition, BCAP-deficient cells demonstrated defects in survival that were attributed to insufficient expression of c-Rel (8). In contrast to these results, BCAP-null NK cells appeared to be more resistant to apoptosis despite defects in ITAM-mediated Akt activation (9). Although differences in the role of BCAP in survival in B cells and NK cells are hard to reconcile, it was noted that BCAP-null NK cells were more mature than their wild-type (WT) counterparts, whereas BCAP-null B cells are less mature (9). Thus, it is difficult to distinguish a role for BCAP in survival and development in these cells.

The other major cell type known to express BCAP is the macrophage (10). A functional role for BCAP in macrophages has not been reported. In this study, we identified BCAP as an impor-
tient prosurvival protein in macrophages. We tested the hypothesis that BCAP is required for macrophage survival during conditions likely to be encountered in specific disease states characterized by ER stress and exposure to the innate immune ligand, LPS.

Materials and Methods

Reagents

EnzChek Caspase-3 Assay Kit, Lipofectamine 2000, and rabbit IgG against ERK1/2 were from Invitrogen (Carlsbad, CA). Antibodies against BCAP was as described (7). Abs against Cbl-b (G-1), c-Cbl (C-15), Syk (LR and N-19), Vav (C-14), LAT (FL-233), SLPI-76 (H-300), RelA (C-20), c-Rel (N), and Actin (C-11) were from Santa Cruz Biotechnology (Santa Cruz, CA). Abs against Gab1 and Gab2 were from Millipore (Billerica, MA). Abs against Akt, phospho-Akt (Ser473), phospho-IKK (N), and Actin (C-11) were from Santa Cruz Biotechnology (Santa Cruz, CA). Abs against Gab1 and Gab2 were from Millipore (Billerica, MA). Abs against Akt, phospho-Akt (Ser473), phospho-IKK (N), and Actin (C-11) were from Santa Cruz Biotechnology (Santa Cruz, CA).

Cells

BCAP−/− mice back-crossed onto a C57BL/6 background were previously described (8) and maintained in specific pathogen-free conditions at the Institute of Comparative Medicine (Columbia University). All experiments were approved by the Columbia University Institutional Animal Care and Use Committee. Bone marrow-derived macrophages (BMDMs) were generated by harvesting stem cells from bone marrow obtained by aspiration of mouse femurs. Cells were subjected to hypotonic lysis to remove erythrocytes and plated at a density of 3 × 105 cells in 10 cm² Petri dishes. Cells were grown in complete medium (RPMI 1640, 10% (v/v) FCS, 20% (v/v) L929-cell conditioned medium (LCM) as a source of CSF-1 (11), 100 U/ml penicillin G, and 100 μg/ml streptomycin) in a tissue culture incubator maintained at 5% CO2 and 37°C. Cells were passaged every 3 d and used for experiments after 5–6 d of culture. Some experiments were done with BMDMs that were frozen on day 5 in DMSO Freeze Medium (BioVeris, Gaithersburg, MD) and later rethawed for further experimentation.

FIGURE 1. Induction of BCAP by LPS. A, BMDMs (8 × 105) were incubated in medium containing 1% FCS and in the presence or absence of 100 ng/ml LPS for the indicated times at 37°C, subjected to detergent lysis, SDS-PAGE, and immunoblotting using an antisera against BCAP or IgG against actin. Arrowheads refer to the four major isosforms of BCAP. B, Quantitation of BCAP protein expression as described in Materials and Methods. Data represent mean ± SEM, n = 5. *p < 0.05, ** p < 0.01. C, Adherent BMDMs (8 × 105) were incubated in medium containing 1% FCS and in the presence or absence of 100 ng/ml LPS for the indicated times at 37°C. RNA was isolated and subjected to quantitative RT-PCR. Relative BCAP mRNA was normalized to peptidylprolyl isomerase A control mRNA. Data represent mean ± SEM, n = 6. *p < 0.001, ** p < 0.0001. D, BMDMs (8 × 105) were incubated in medium containing 1% FCS and in the presence or absence of 100 ng/ml LPS for 24 h at 37°C, subjected to detergent lysis, SDS-PAGE, immunoblotting using Abs against the indicated proteins, and densitometry. Immunoblotting for Gab1 and Gab2 in BMDMs demonstrated two predominant bands, denoted “upper” and “lower,” which corresponded with their predicted, and slightly less than predicted, MWs, respectively. Data represent mean ± SEM, n = 4–7. *p < 0.05, ** p < 0.01, ***p < 0.001. E, Expression of PI3K SH2 adapter proteins in macrophages from BCAP−/− mice. Adherent BMDMs (8 × 105) were subjected to detergent lysis, SDS-PAGE, immunoblotting using Abs against the indicated proteins, and densitometry. Data represent mean ± SEM, n = 4–7. *p < 0.05, ** p < 0.01.

Cell stimulation

At the beginning of each experiment, 8 × 105 BMDMs were replated onto 13-mm-diameter round glass coverslips in 24-well plates or in 24-well plates without coverslips. Cells were maintained in medium containing 1% FCS and in the presence or absence of 100 ng/ml LPS for 24 h at 37°C, followed by further incubation in the presence or absence of 1 μM thapsigargin for an additional 24 h at 37°C.

Quantification of apoptotic nuclei

Quantification of apoptotic nuclei was performed as described in Ref. 12. In brief, cells were fixed with 3.7% formaldehyde for 5 min, stained with 1 μM 4′,6-diamidino-2-phenylindole in the presence of 0.2% Triton X-100 for an additional 2 min, and subjected to fluorescence microscopy to visualize nuclear morphology. Nuclei were scored as apoptotic if they were obviously pyknotic and cells had a shrunken or retracted appearance. At least 200 cells in five separate fields per coverslip were scored for evidence of apoptotic nuclei; fields were selected at random, and all cells in the field were scored for viability in a blinded fashion and verified by two independent reviewers. Assays were performed in triplicate.

Caspase-3 activity assay

Adherent BMDMs (1 × 105) were maintained in medium containing 1% FCS in the presence or absence of 100 ng/ml LPS for 24 h at 37°C, followed by incubation in the presence or absence of 1 μM thapsigargin for an additional 6 h at 37°C. After 24 h, the medium was removed and cells were frozen at −80°C for 20′, thawed. Cellular debris was removed by centrifugation, and supernatants were added to microplate wells. Fluorometric caspase-3 activity assays, performed according to the manufacturer’s instructions, were performed in triplicate and corrected for substrate background fluorescence.

Quantitative RT-PCR

Total RNA was harvested from 2 × 105 adherent BMDMs using the RNeasy Mini Kit (Qiagen, Germantown, MD) according to the manufacturer’s instructions. RNA was primed with random hexamers and reverse transcribed into cDNA using the Superscript III First-Strand
mRNA measurements were performed in triplicate for BCAP was normalized to that of PPIA (Quantitect; Qiagen). All mRNA measurements were performed in triplicate.

Materials and Methods

After stimulation, cells were centrifuged at 7000 × g for 10 s and lysed in RIPA buffer (150 mM sodium chloride, 0.5% IPEGAL, 0.5% sodium deoxycholate, 0.05% SDS, 1 mM EDTA, 1 mM sodium orthovanadate, 50 mM sodium fluoride, 1 mM phenylmethylsulfonylfluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 50 mM Tris-HCl, pH 7.4) for 10 min at 4˚C, followed by sonication using a tip-probe sonicator and centrifugation at 13,000 × g for 10 min to remove insoluble debris. The supernatant was preclared with nonimmune rabbit serum prebound to protein A/G agarose preincubated with rabbit serum against BCAP, or nonimmune rabbit serum control, for 90 min. Beads were washed extensively and boiled in Laemmli buffer for 5 min. Proteins were resolved by SDS-PAGE, transferred onto nitrocellulose, and immunoblotted using the indicated Abs. Immunoblots were developed using ECL. Where indicated, band intensities were quantitated by densitometry using ImageJ software (version 1.38; http://rsb.info.nih.gov/ij/).

Retroviral transduction of BMDMs

WT and a quadruple yxM mutant (targeting Y264, Y420, Y445, and Y460) were cloned into pMX-ires-GFP (http://www.lablife.org/p?a=vdb_view&id=g2.tT07GqFTNuTUD3dWf6ud.c1pQEk-), as described (13). Plasmids were transfected with a packaging vector, pCL-Eco, into HEK293T cells using Lipofectamine 2000. Supernatants were stored at −80˚C until use. Bone marrow aspirate cultures were plated at 90–100% confluence in 6-well plates and incubated with viral supernatant in the presence of 4 μg/ml polybrene. Cells were centrifuged at 600 rpm for 2 h at room temperature (“spinfection”) followed by incubation overnight at 37°C in a 5% CO2 incubator. The retroviral infection protocol was repeated the following day. Cells were maintained for an additional 3–7 d at 37°C in a 5% CO2 incubator prior to performing experiments.

Statistical analysis

Data analysis was performed using two-tailed one-sample t tests or repeated measures ANOVA for matched observations followed by Bonferroni post hoc analysis using GraphPad Prism software version 4.0c (http://www.graphpad.com). Data were log-normal transformed unless otherwise specified.

Results

Addition of LPS leads to upregulation of BCAP but not several other PI3K adapter proteins

BCAP is a member of a family of phylogenetically ancient proteins whose other members are vertebrate BANK and Dof, an adapter protein necessary for signaling of the FGF receptor homologue Heartless, in Drosophila (14). As the Dof/BCAP/BANK motif is conserved throughout evolution, we hypothesized that BCAP might be important in innate immune signaling in macrophages. We first determined whether addition of LPS affected expression of BCAP in BMDMs. LPS induced a time-dependent increase in the expression of three of four of the major isoforms of BCAP expressed in these cells. LPS induced an increase in both BCAP protein (Fig. 1A, 1B) and mRNA (Fig. 1C). The increase in BCAP3 protein was more than 10-fold. Similar results were obtained with Pam3Cys, an innate immune ligand of TLR2 (not shown). To determine whether induction of BCAP by LPS was a general feature of PI3K adapter proteins, we determined the relative amounts of eight other p85 PI3K adapter proteins expressed in macrophages. Of these, only Gab1 demonstrated

FIGURE 2. BMDMs from BCAP−/− mice are more susceptible to ER stress-associated cell death in the presence of LPS. Adherent BMDMs (1 × 105) were maintained in medium containing 1% FCS in the presence or absence of 100 ng/ml LPS for 24 h at 37°C, followed by incubation in the presence or absence of 1 μM thapsigargin for an additional 24 h at 37°C. Cells were fixed and stained with DAPI to visualize nuclei. A, Fluorescence micrograph of macrophages maintained in 1% FCS in the absence (Control) or presence (LPS/thaps) of LPS and thapsigargin at 37°C for 48 h. Note pyknotic nuclei after addition of LPS and thapsigargin, typical of apoptotic cells. Representative of 10 such micrographs. Scale bar, 10 μm. B, Adherent BMDMs were incubated in the absence or presence of LPS and thapsigargin as described above except maintained in serum-free medium. Viability was determined by nuclear morphology, as described in Materials and Methods. Data represent mean ± SEM, n = 4–7. C, Adherent BMDMs were incubated in the absence or presence of LPS and thapsigargin as described above and maintained in 1% FCS at 37°C for 48 h. Viability was determined by nuclear morphology, as described in Materials and Methods. Data represent mean ± SEM, n = 4–7. D, Adherent BMDMs were incubated in the absence or presence of LPS for 24 h and thapsigargin for an additional 6 h as described above and maintained in 1% FCS at 37°C. Caspase-3 activity, expressed as percentage increase compared with BMDMs not treated with LPS or thapsigargin, was determined as described in Materials and Methods. Black bars, WT macrophages; gray bars, BCAP−/− macrophages. Data represent mean ± SEM, n = 3. *p < 0.05.
a significant increase and Gab2 demonstrated a trend in increased expression, in response to LPS, although both to a much lower extent than BCAP (Fig. 1D). Notably, expression of both Gab1 and Gab2 were increased in unstimulated macrophages from BCAP-deficient mice (Fig. 1E). Collectively, these data indicate that BCAP is among the few PI3K adapter proteins whose expression level increased after the addition of the TLR4 ligand, LPS.

BCAP is required for maximal protection against apoptosis in response to ER stress in BMDMs

As BCAP is a PI3K adapter protein and the PI3K/Akt pathway is required for protection against apoptosis in multiple cell types, including macrophages (15–20), we determined whether BMDMs deficient in BCAP were susceptible to apoptosis. We attempted to induce apoptosis using various activators of the extrinsic pathway of apoptosis (e.g., TNF-α, FasL, TRAIL), but we found that these agents did not induce apoptosis in BMDMs, even when cultured in the absence or presence of serum and LCM (not shown). These results are consistent with earlier studies that report significant resistance to apoptosis in mature macrophages subjected to growth factor withdrawal or addition of activators of the extrinsic pathway of cell death (21–23). Because macrophages are known to be susceptible to apoptosis induced by ER stress (24–27), we determined whether expression of BCAP influenced survival after addition of thapsigargin, a sarcoplasmic/endoplasmic reticulum calcium ATPase inhibitor that activates ER stress and the intrinsic pathway of apoptosis (28). Macrophages that lacked BCAP were more sensitive to thapsigargin-induced cell death when cultured in the absence of LCM, a source of GM-CSF (Fig. 2A, 2B). When cultured in the presence of LCM, BMDMs from WT mice were substantially resistant to the apoptosis-inducing effect of thapsigargin (Fig. 2C). As LPS is known to sensitize mouse peritoneal macrophages to apoptosis induced by ER stress (29), we determined whether this was also true for mouse BMDMs. Indeed, BMDMs pretreated overnight in LPS were rendered more sensitive to thapsigargin-induced apoptosis (Fig. 2C). BCAP-deficient macrophages were 27% more susceptible to apoptosis under these conditions (Fig. 2C). The increased apoptosis in BCAP-deficient cells in the presence of LPS and thapsigargin was also reflected in increased caspase-3 activity (Fig. 2D). Curiously, BCAP-deficient BMDMs incubated with LPS alone also demonstrated increased caspase-3 activation (Fig. 2D) even in the absence of overt signs of apoptosis (Fig. 2C). This may have been due to the possibility that LPS-treated BCAP-deficient BMDMs were in relatively early stages of apoptosis in which overt changes in nuclear morphology might not have been apparent. Thus, under conditions in which BCAP is induced >10-fold in WT cells by LPS, BMDMs are susceptible to ER stress-induced apoptosis, an effect that is more apparent in the absence of BCAP expression.

BCAP is required for maximal activation of Akt in response to addition of LPS and thapsigargin in BMDMs

As BCAP is a PI3K adapter protein, and PI3K is required for protection against apoptosis in macrophages (30–35), we tested whether decreased cell survival in macrophages from BCAP−/− mice was correlated with decreased activation of Akt. The

![FIGURE 3](image_url)

**FIGURE 3.** Defective activation of Akt in BMDMs from BCAP−/− mice in response to LPS and thapsigargin. Adherent BMDMs (8 × 10^5) were maintained in medium containing 1% FCS in the presence or absence of 100 ng/ml LPS at 37°C for 24 h, followed by incubation in the presence or absence of 1 μM thapsigargin for an additional 24 h at 37°C. Cells were subjected to detergent lysis, SDS-PAGE, immunoblotting using Abs against the indicated proteins (A), and densitometry (B, C). Note inability of BCAP-deficient macrophages to manifest an increase in phospho-Akt in the presence of LPS, thapsigargin, or LPS and thapsigargin. Black bars, WT macrophages; gray bars, BCAP−/− macrophages. Data represent mean ± SEM, n = 6. *p < 0.05; **p < 0.01.

![FIGURE 4](image_url)

**FIGURE 4.** Activity of ERK in BMDMs from WT and BCAP−/− mice in response to LPS and thapsigargin is equivalent. Adherent BMDMs (8 × 10^5) were maintained in medium containing 1% FCS in the presence or absence of 100 ng/ml LPS at 37°C for 24 h, followed by incubation in the presence or absence of 1 μM thapsigargin for an additional 24 h at 37°C, subjected to detergent lysis, SDS-PAGE, immunoblotting using Abs against the indicated proteins (A), and densitometry (B). Data represent mean ± SEM, n = 6.
phospho-Ser473 Akt content of macrophages was increased over baseline in macrophages from WT mice (Fig. 3). However, macrophages from BCAP<sup>−/−</sup> mice failed to demonstrate increased phospho-Ser473 Akt content in response to LPS or thapsigargin; indeed, the phospho-Ser473 Akt content of BCAP-deficient macrophages declined in response to these stimuli (Fig. 3). There was also decreased basal Akt activity in BCAP-deficient macrophages and decreased and/or delayed activation of Akt at early times after addition of LPS (Supplemental Fig. 1). Therefore, BCAP expression was necessary for maximal Akt activity after addition of LPS or thapsigargin, and its absence sensitized macrophages to thapsigargin-induced decreases in Akt activity.

BCAP is not required for maximal activation of ERK as well as expression of the NF-κB subunits, c-Rel and RelA

In addition to the PI3K/Akt pathway, other signal transduction pathways are important in macrophage survival. For example, ERK has been reported to be an important macrophage survival factor in some (35–40) but not all (30, 41, 42) studies, and we detected decreased ERK phosphorylation in response to LPS or thapsigargin in BMDMs from both WT and BCAP-deficient mice, as well as a trend for enhanced ERK phosphorylation in unchallenged BMDMs from BCAP-deficient mice (Fig. 4). Regardless of the magnitude of this effect, there were no significant differences between ERK activation in these cells challenged with either LPS, thapsigargin, or both, suggesting that the survival defect in BCAP-deficient cells was not due to decreased ERK activity. There was also no change in the phosphorylation status of ERK at early time points after addition of LPS (Supplemental Fig. 2).

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Regardless of the magnitude of this effect, there were no significant differences between ERK activation in these cells challenged with either LPS, thapsigargin, or both, suggesting that the survival defect in BCAP-deficient cells was not due to decreased ERK activity. There was also no change in the phosphorylation status of ERK at early time points after addition of LPS (Supplemental Fig. 2). This was previously shown that B cells from BCAP<sup>−/−</sup> mice are susceptible to apoptosis due to decreased expression of the NF-κB subunits, c-Rel and RelA (8). However, the level of expression of these proteins in BCAP-deficient BMDMs was not significantly different than in WT mice (Fig. 5A, 5B). This was further supported by the finding that in unstimulated BMDMs, addition of LPS induced a rapid increase in the phosphorylation in the active loop of IKKα/β, which was unimpaired in BCAP-deficient macrophages after early time points (Supplemental Fig. 3) and after 24 h (mean IKKα/β-normalized staining density, 0.486 versus 0.679, WT versus BCAP<sup>−/−</sup>, n = 3; not significant). These data indicate that there are fundamental differences in the phenotype of BCAP-deficient macrophages and B cells.

The importance of the four YxxM consensus binding sites for p85 PI3K in protein against apoptosis by BCAP

BCAP was originally isolated as a p85 SH2 domain-binding protein from chicken DT40 B cells. It contains four consensus binding sites for the SH2 domain of p85 PI3K. We found that the phosphotyrosyl

FIGURE 5. Expression of c-Rel and RelA in BMDMs from WT and BCAP<sup>−/−</sup> mice. Adherent BMDMs (8 × 10<sup>5</sup>) were maintained in medium containing 1% FCS in the presence or absence of 100 ng/ml LPS at 37°C for 24 h, followed by incubation in the presence or absence of 1 μM thapsigargin for an additional 24 h at 37°C. Cells were subjected to detergent lysis, SDS-PAGE, immunoblotting using Abs against the indicated proteins (A), and densitometry (B, C). Data represent mean ± SEM, n = 6.
content of BCAP1 and BCAP3 was low, but detectable, in LPS-treated BMDMs (Fig. 6A). As we found that a fraction of BCAP in these cells was present in Triton X-100–insoluble “lipid rafts” (not shown), confirming earlier observations in DT40 cells (7), we suspect that the apparently low stoichiometry of phosphorylation reflects this small but functionally important fraction. We found that expression of WT BCAP, but not a non-phosphorylatable 4YF mutant allele lacking the four consensus PI3K binding sites, could rescue the defect observed in BCAP-deficient BMDMs (Fig. 6B).

Discussion
In this study we demonstrated that BCAP was highly induced by the innate immune ligand, LPS, in BMDMs. BCAP expression was required for maximal survival in the presence of LPS and ER stress, two stresses likely to be present during specific innate immune challenges. BCAP was required for Akt activation, but neither for ERK activation nor expression of the NF-κB subunits, c-Rel and RelA. Finally, the antiapoptotic activity of BCAP required the presence of tyrosine residues in the four YxxM consensus binding sites for the p85 subunit of PI3K.

Specific signaling intermediates required for macrophage survival have been identified only recently. For example, IRF-2 is required for maximal survival of macrophages, most likely as a transcriptional repressor of caspase-1 (43). The Tec family members, Tec and Btk, are required for survival pathways downstream of the M-CSF receptor (44). Macrophages from phospholipase C β3-deficient mice were defective in protection against apoptosis by ligands of various GPCRs, consistent with a role for phospholipase C β3 in signal transduction by GPCRs (45). The importance of the PI3K/Akt pathway in survival of macrophages and other cells is now well established (15–20), although the precise downstream targets of PI3K and Akt that are required for survival of macrophage subpopulations are known in only a few cases. For example, mTOR/S6K is required for survival of osteoclasts, most likely to allow for ongoing protein synthesis (46). In another study, Akt activity was required for maximal expression of the antiapoptotic protein Mcl-1 but not other Bcl-2 family members (19). It is likely that more relevant antiapoptotic targets of PI3K will be revealed in the future.

In contrast to the insights provided by these studies, much less is known about the precise mechanisms by which potentially antiapoptotic signaling modules are activated or recruited to the membrane. Indeed, there are many potential adapter proteins capable of interacting with the p85 regulatory subunit of PI3K, and redundancy can be expected. Nevertheless, we were successful in identifying one nonredundant PI3K adapter protein that was highly induced by an innate immune ligand. Although these studies have not defined precisely which receptors use BCAP to couple to PI3K, preliminary experiments measuring Akt activation indicate that these include c-fms, the receptor for CSF-1, a known survival factor for macrophages, and FcγRI (data not shown). Notably, although we found BCAP-deficient macrophages somewhat defective in FcγRI-mediated Akt activation, we found no defects in FcγRII-mediated, or FcγRIII-mediated phagocytosis (data not shown). FcγR-mediated phagocytosis is a known PI3K-dependent process (reviewed in Refs. 47, 48), and Gab2, an adapter protein that interacts with the SH2 domain of p85 PI3K, is required for FcγR-mediated phagocytosis (6). It is notable that of the eight potential PI3K adapter proteins we examined, only Gab1/Gab2 were up-regulated in BCAP-deficient cells. This suggests the possibility that, in macrophages, BCAP and Gab proteins are functionally redundant, although structurally dissimilar. This would also account for the rather modest decrease in survival we observed in BCAP-deficient macrophages. We would predict that macrophages deficient in both BCAP and Gab1 and/or Gab2, if viable, would demonstrate profound defects in survival.

What is the immunological significance of the findings from this study? Macrophages are sentinels of the innate immune system and are among the first cells to respond to pathogenic microbes. Upon encountering pathogenic bacteria and fungi, they are exposed to a harsh extracellular environment composed of virulence factors supplied by the pathogens and by oxidative and other potentially damaging agents supplied by the host. Under these conditions, macrophages demonstrate evidence of ER stress, as indicated by expression of C/EBP homologous protein and other markers of ER stress (1, 2). With the possible exception of infections with pathogenic mycobacteria, in which apoptosis is required for optimal mycobacterial killing (49, 50), continued macrophage viability is crucial for survival of the host. We suggest that as macrophages engage their pathogenic targets, a phyleogenetically conserved program of survival is triggered, which allows macrophages to withstand ongoing cellular insults. Among the critical components of this program is the PI3K/Akt signaling module, and among the means by which PI3K is recruited to the membrane, and therefore activated, is by binding BCAP. Further experiments using mice in which BCAP is absent only from the macrophage compartment will be necessary to test the validity of this model.

Disclosures
The authors have no financial conflicts of interest.

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