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Akt-Mediated Proinflammatory Response of Mononuclear Phagocytes Infected with *Burkholderia cenocepacia* Occurs by a Novel GSK3β-Dependent, IκB Kinase-Independent Mechanism

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The environmental bacterium *Burkholderia cenocepacia* causes opportunistic lung infections in immunocompromised individuals, particularly in patients with cystic fibrosis. Infections in these patients are associated with exacerbated inflammation leading to rapid decay of lung function, and in some cases resulting in cepacia syndrome, which is characterized by a fatal acute necrotizing pneumonia and sepsis. *B. cenocepacia* can survive intracellularly in macrophages by altering the maturation of the phagosome, but very little is known on macrophage responses to the intracellular infection. In this study, we have examined the role of the PI3K/Akt signaling pathway in *B. cenocepacia*-infected monocytes and macrophages. We show that PI3K/Akt activity was required for NF-κB activity and the secretion of proinflammatory cytokines during infection with *B. cenocepacia*. In contrast to previous observations in epithelial cells infected with other Gram-negative bacteria, Akt did not enhance IκB kinase or NF-κB p65 phosphorylation, but rather inhibited GSK3β, a negative regulator of NF-κB transcriptional activity. This novel mechanism of modulation of NF-κB activity may provide a unique therapeutic target for controlling excessive inflammation upon *B. cenocepacia* infection. *The Journal of Immunology*, 2011, 187: 000–000.

*Burkholderia cenocepacia* is one of the members of the *Burkholderia cepacia* complex. This is a Gram-negative opportunistic respiratory pathogen that represents a threat to immunocompromised individuals, especially patients with cystic fibrosis (1–3). What is particularly troublesome is that these bacteria are also highly resistant to many antibiotics, making treatment difficult (4, 5). Because of this, it is critical to gain an understanding of host immune responses to this organism, as this may provide alternative means to overcoming the current limitations for treatment.

One major contributing factor to the morbidity and mortality caused by *B. cenocepacia* infection is an exacerbated inflammatory response, which causes collateral tissue damage (6, 7). Accordingly, administration of anti-inflammatory corticosteroids has been associated with favorable patient outcome during *B. cepacia* infection (8). It has also been demonstrated in vivo that TLR/MyD88-driven inflammation is detrimental to the host, as MyD88−/− mice show a survival advantage over wild-type mice after challenge with *B. cenocepacia* (9). Indeed, TLR5 plays a key role in promoting exacerbated inflammation in susceptible individuals (10, 11).

One of the key downstream effects of TLR/MyD88 pathway activation is TNF-α production. This cytokine has been shown to be a major mediator of mortality in an in vivo mouse model of *B. cenocepacia* infection, as TNF-α−/− mice were protected against a challenge lethal to wild-type mice (9). Hence, an understanding of how infection leads to TNF-α production may lead to newer, more effective treatments designed to regulate TNF-α production and other deleterious proinflammatory responses.

Numerous studies have highlighted the importance of macrophages during *B. cenocepacia* infection, as they are a site of bacterial replication much like lung epithelial cells (12, 13). It is interesting to note that CFTR-defective– or CFTR-inhibitor–treated wild-type murine macrophages show delayed phagolysosomal fusion compared with control (14). This helps to explain the persistence of *B. cenocepacia* in individuals with cystic fibrosis, as their macrophages would be less able to control the bacteria. Monocytes/macrophages are also major producers of inflammatory mediators such as TNF-α and IL-8 (7, 15–17), which contribute to the hyperinflammatory state following *B. cenocepacia* infection.

PI3K/Akt signaling is known to regulate various biological functions, including the proinflammatory response to TLR signaling. However, its effect on inflammatory response differs, depending upon several factors that remain to be fully understood (18). In this study, we have investigated the role of PI3K/Akt signaling on IκB kinase (IKK)/NF-κB activation and the ensuing proinflammatory response from mononuclear phagocytes infected with...
B. cenocepacia. We demonstrate that PI3K and Akt promote NF-
κB activity and proinflammatory cytokine production, but not through IKK nor NF-κB p65 phosphorylation. Instead, PI3K/Akt serves to inactivate GSK3β, a downstream repressor of NF-κB. These findings suggest that targeting PI3K/Akt/GSK3β signaling may be of therapeutic value within the context of B. cenocepacia infection.

Materials and Methods

Cells and reagents

RAW 264.7 cells obtained from American Type Culture Collection were cultured in RPMI 1640 (Life Technologies-BRL, Rockville, MD) supplemented with 5% heat-inactivated FBS (HyClone, Logan, UT), 10 mM L-glutamine, penicillin (10,000 U/ml), 3,000 U/ml streptomycin (10,000 µg/ml) (Invitrogen, Carlsbad, CA). The BAY 11-7085 (5 µM) IKK inhibitor was a generous gift from D. Guttridge (The Ohio State University). LY294002 (20 µM) PI3K inhibitor was obtained from Calbiochem (San Diego, CA). SB216763 (2 µM) GSK3β inhibitor was obtained from Sigma-Aldrich (St. Louis, MO). DMEM vehicle control (0.2%) was obtained from Sigma-Aldrich. Akt inhibitor X (10 µM) was obtained from Calbiochem and dissolved in water. Abs against phospho-AktSer173, phospho-IKKα/βSer180/181, phospho-NF-κBp65-Ser536, phospho-GSK3α/βSer9, and GSK3β were purchased from Cell Signaling (Beverly, MA). Abs against Akt and actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Bone marrow-derived macrophages

Wild-type and MyrAkt-expressing mice were sacrificed according to institution-approved animal care and use protocols. Bone marrow cells were collected and differentiated, as previously described, with M-CSF (R&D Systems, Minneapolis, MN) and polymyxin-B (CalBiochem) (19–21).

Peripheral blood monocyte isolation

Human peripheral blood monocytes (PBM) were isolated by centrifugation through a Ficoll gradient, followed by CD14-positive selection by MACS (Miltenyi Biotec, Auburn, CA), according to manufacturer instructions, as previously described (22).

Bacterial infections

All monocyte/macrophage infections were conducted in 5 or 10% heat-inactivated FBS-containing RPMI 1640 without antibiotic. B. cenocepacia K56-2 isolate was grown in Luria Bertani medium (Sigma-Aldrich) for 12–14 h to postlogarithmic phase. OD at 600 nm was taken to assess the density of cultures and calculate the multiplicities of infection. Serial dilutions of cultures and plating on Luria Bertani agar plates. At 24-h postplating, CFUs were counted. Lysed samples were immediately serially diluted 10-fold and plated on Luria Bertani agar plates. At 24–36 h postplating, CFUs were counted. The dual-antibiotic CFU assays were conducted as above, with the exception that parental strain K56-2 was used and extracellular bacteria were killed with gentamicin (500 µg/ml) plus cefazidime (250 µg/ml) for 1 h (12, 23). We found that this killed >98% of K56-2 B. cenocepacia.

Luciferase assays

Transfected macrophages were uninfected or infected for 5 h (time of robust NF-κB–luciferase activity; Supplemental Fig. 1) with B. cenocepacia. At the end of the infection period, cells were washed with PBS and then lysed in 200 µl Luciferase Cell Culture Lysis Reagent (Promega, Madison, WI). Luciferase activity was then measured using Luciferase Assay Reagent (Promega), as previously described (20). Data are expressed as percentage of increase over matched uninfected control samples.

Transfection

A total of 5 µg pcDNA vector with or without wild-type human GSK3β, Addgene plasmid 14753 (24) (Addgene, Cambridge, MA), was used for each transfection with 10 × 10^6 RAW 264.7 cells. Amaxa solution V was used for electroporation using program U-14, as previously described (20). Infections were performed 14 h postransfection. Overexpression was con-
firmed by Western blotting of GSK3β. For NF-κB activity assays, 2 μg NF-κB luciferase reporter plasmid was transfected into RAW 264.7 cells, as described above. Transfection efficiency is routinely tested by flow cytometry analysis of enhanced GFP-transfected cells. We are able to achieve ≥80% transfection efficiency in Raw 264.7 cells with minimal effect on cell viability.

**Results**

**PI3K signaling is required for the proinflammatory response to B. cenocepacia**

PI3K signaling leads to generation of phosphatidylinositol-(3,4)-bisphosphate and phosphatidylinositol-(3,4,5)-trisphosphate, which

**FIGURE 2.** Akt promotes the proinflammatory cytokine production elicited by *B. cenocepacia*. A–C, PBM were pretreated with H2O vehicle control or the Akt inhibitor Akt(X) (10 μM) for 30 min, and then infected with *B. cenocepacia* (Bc) at a MOI of 5 for 8 h. Sandwich ELISAs were done to measure TNF-α (A), IL-6 (B), or IL-8 (C) in cell-free supernatants. Three independent experiments were done. *p < 0.05, Student t test. D, Matched PBM samples run in parallel to that of A were lysed, and pSer-Akt was measured by Western blotting, followed by reprobe for actin as a loading control. E, Bone marrow-derived macrophages from wild-type mice and transgenic mice expressing a macrophage-specific myristoylated Akt were lysed, and pSer-Akt was measured by Western blotting. F–H, Bone marrow-derived macrophages from wild-type or MyrAkt-expressing mice were infected with *B. cenocepacia* (Bc) at a MOI of 5 for 8 h. Sandwich ELISAs were done to measure TNF-α (F), IL-6 (G), and RANTES (H). This represents a triplicate of biological samples. *p < 0.05, Student t test.

**FIGURE 3.** PI3K/Akt does not regulate phagocytosis or intracellular replication of *B. cenocepacia* within macrophages. A, 4 × 10^5^ bone marrow-derived macrophages (BMM) were pretreated with DMSO or LY294002 for 30 min and then infected with *B. cenocepacia* at a MOI of 5 for 1 h. At the end of infection, cells were washed and treated with gentamicin to kill extracellular bacteria for 30 min. Cells were washed, lysed, serially diluted, and plated on Luria Bertani agar. Graphs represent the average and SD of recovered CFUs for three independently infected samples. A Student t test was done to determine significance. *p < 0.05. B, Wild-type or MyrAkt BMM were infected with *B. cenocepacia* (Bc) at a MOI of 5 for 1 h, as done in A. C, 4 × 10^5^ BMM were pretreated with DMSO or LY294002 for 30 min, and then infected with *B. cenocepacia* at a MOI of 5 for 1 h. Samples were lysed at 1 h or cultured in fresh macrophage culture medium for 8 or 24 h and then lysed. Graphs represent the average and SD of recovered CFUs for three independently infected samples. A Student t test was done to determine significance. *p < 0.05. D, 4 × 10^5^ wild-type or MyrAkt BMM were infected and treated, as in C.
result in phosphorylation and activation Akt at the plasma membrane. This drives numerous cellular processes, including the production and release of proinflammatory mediators (18, 25). In this study, we examined PI3K/Akt signaling in primary human monocytes infected with *B. cenocepacia*. PBM were isolated and infected with *B. cenocepacia* at a MOI of 5 for time points of 20, 40, or 60 min. Whole-cell lysates were then probed for phospho-Akt levels by Western blotting, followed by reprobing for total Akt. It is apparent that Akt is robustly activated within 20 min of infection and is sustained through the first hour (Fig. 1A). We also tested the ability of killed *B. cenocepacia* to activate Akt and found that both live and dead bacteria induced this response within 1 h (data not shown). Therefore, this signaling event is not dependent upon bacterial viability.

To then investigate the function of PI3K on host-inflammatory response, PBM were pretreated with vehicle control or the PI3K inhibitor LY294002 (26) and infected with *B. cenocepacia* at a MOI of 5 for 8 h. The effectiveness of the inhibitor under these conditions was verified by Western blotting of phospho-Akt. PBM pretreated with vehicle showed high levels of Akt activation upon infection, whereas PBM given the PI3K inhibitor and infected did not (Fig. 1B).

Next, we examined the production of proinflammatory cytokines in the presence or absence of the PI3K inhibitor in PBM infected with *B. cenocepacia*. Matched supernatant from the samples in Fig. 1B was collected, and proinflammatory cytokine production was assayed by ELISA. Infected PBM showed strong induction of TNF-α, IL-6, and IL-8 compared with uninfected cells. However, PBM pretreated with LY294002 produced significantly less post-infection (Fig. 1C–E). Similar results were obtained with bone marrow-derived murine macrophages (data not shown). Therefore, the inflammatory response to *B. cenocepacia* by monocytes/macrophages requires PI3K signaling.

Akt promotes the proinflammatory response to *B. cenocepacia*

Given that class I PI3K activate Akt (25) and we find that PI3K is required for the monocyte/macrophage proinflammatory response to *B. cenocepacia*, we next examined whether Akt activation could be linked to the proinflammatory response in infected PBM. Therefore, PBM were pretreated with the Akt inhibitor Akt (X) (27) or vehicle control and infected with *B. cenocepacia* for 8 h. Inhibition of Akt led to reduced production of TNF-α, IL-6, and IL-8 compared with cells pretreated with vehicle control (Fig. 2A–C). The reduction in IL-6 did not reach statistical significance, however, and this may be due to residual Akt activation even in the presence of Akt(X) following infection (Fig. 2D).

The above findings suggest that Akt activation is required for cytokine production following *B. cenocepacia* infection. To test whether Akt could drive production of these cytokines, we infected bone marrow-derived macrophages from wild-type mice or from mice expressing a macrophage-specific myristoylated Akt (MyrAkt) (28). We verified the constitutive activation of Akt in unstimulated macrophages by phospho-Akt Western blot (Fig. 2E). Following 8-h infection of wild-type and MyrAkt macrophages with *B. cenocepacia*, we performed ELISAs and found significantly enhanced production of TNF-α, IL-6, and RANTES in the MyrAkt-expressing cells (Fig. 2F–H).

PI3K/Akt do not regulate uptake nor intramacrophage replication of *B. cenocepacia*

PI3K signaling has been reported to influence both phagocytosis (29, 30) and intramacrophage survival (21) of other Gram-negative bacteria. To evaluate its role in macrophages infected with *B. cenocepacia*, we investigated bacterial uptake and replication in PBM after inhibition of PI3K. To overcome the natural antibiotic resistance of *B. cenocepacia* and to facilitate these experiments, we used an aminoglycoside-sensitive strain of *B. cenocepacia* (strain MH1K) that can be killed with a low concentration of gentamicin, but does not differ with the parental isolate in terms of intracellular survival and phagosomal trafficking (13). We pretreated primary murine macrophages with LY294002 and infected with *B. cenocepacia* MH1K. In parallel, we also infected MyrAkt-expressing macrophages. CFU were determined at 1 h of infection to estimate bacterial uptake (Fig. 3A, 3B) and at 8 and 24 h postinfection to estimate intracellular replication (Fig. 3C, 3D). We found no influence of PI3K/Akt activity on internalization or bacterial replication. We also performed these experiments with the parental *B. cenocepacia* strain plus the dual-antibiotic treatment of gentamicin (500 μg/ml) and ceftazidime (250 μg/ml), as done by Sajjan et al. (12), and the results also indicated no effect of PI3K/Akt on phagocytosis or intracellular replication of *B. cenocepacia* in mononuclear phagocytes (data not shown). These findings suggest that PI3K/Akt can be targeted to limit inflammation without altering the intramacrophage life cycle of *B. cenocepacia*.

IKK/NF-κB are activated by *B. cenocepacia* and required for inflammatory responses

We have shown that PI3K/Akt promotes inflammatory responses to *B. cenocepacia* without influencing uptake or replication in

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**FIGURE 4.** The IKK/NF-κB pathway is activated by *B. cenocepacia* and is required for proinflammatory cytokine production. A. PBM were infected with *B. cenocepacia* at a MOI of 5 for 20, 40, or 60 min. Cells were lysed and proteins analyzed by Western blotting for phospho-IKKα/β, followed by anti-actin as a loading control. B, pSer536 of NF-κB p65 was measured in matched samples from Fig. 3A by Western blotting and reprobed with anti-actin as a loading control. C–E, PBM were pretreated with DMSO vehicle control or an IKK/NF-κB inhibitor, BAY7085 (5 μM), for 30 min, and then infected with *B. cenocepacia* (Bc) at a MOI of 5 for 8 h. Sandwich ELISAs were done to measure levels of TNF-α (C), IL-6 (D), or IL-8 (E) in cell-free media samples. Graphs represent three biological samples; *p < 0.05, Student t test. R, resting or uninfected samples. **Downloaded from http://www.jimmunol.org/ by guest on May 13, 2017**
Strikingly, the PI3K inhibitor (LY294002) also strongly impaired infection with B. cenocepacia, and Western blotting was done to measure phosphorylation of IKKα/β (A), phospho-NF-κBp65 (Ser536) (B), and phosphor-serine Akt (C). Actin and total Akt were probed as loading controls. D and E, Bone marrow-derived macrophages from wild-type or myrAkt-expressing mice were infected with B. cenocepacia at a MOI of 5 for 20, 40, or 60 min. Cells were lysed and Western blotting was done to measure phosphorylation of IKKα/β (D) and phospho-NF-κBp65 (Ser536) (E). Actin was used as a loading control. R, resting or uninfected samples.
FIGURE 6. The PI3K/Akt pathway regulates NF-κB activity through GSK3β. A. RAW264.7 cells were transfected with a NF-κB luciferase reporter. Fourteen hours posttransfection, these cells were infected with B. cenocepacia (Bc) at a MOI of 5 for 5 h, the infection time yielding robust NF-κB-luciferase activity (Supplemental Fig. 1). Luciferase activity was measured by a luminometer, and values were converted into percentage of increase over matched resting/uninfected samples. B. PBMs were infected with B. cenocepacia for 20, 40, or 60 min. Western blotting was done on cell lysates to measure phospho-GSK3α/β, and then reprobed with anti-actin as a loading control. C. Matched cell lysates from Fig. 5B were tested by Western blotting for phospho-GSK3β, and then reprobed for total GSK3β as a loading control. D. PBMs were treated with DMSO vehicle control or the LY294002 for 30 min, and then infected with B. cenocepacia for 20, 40, or 60 min. Western blots were done on cell lysates for phospho-GSK3α/β, followed by reprobing for actin as a loading control. E. RAW264.7 cells were transfected with a NF-κB luciferase reporter plasmid. Transfected cells were pretreated with DMSO vehicle control or SB-216763 GSK3 inhibitor for 30 min, and then infected with B. cenocepacia (Bc) at a MOI of 5 for 5 h. Cells were lysed, and luciferase activity was measured by a luminometer. Data are expressed as percentage of increase in activity over matched uninfected control. F. RAW 264.7 cells were pretreated with DMSO vehicle control or SB-216763, and then infected with B. cenocepacia (Bc) at a MOI of 5 for 8 h. Cleared cell lysates were assayed for TNF-α production by ELISA. Data represent the average of three samples, and error bars denote SD. *p < 0.05, Student t test. R, uninfected cells.

37) NF-κB activity. This molecule is normally active until phosphorylation occurs on Ser21 of GSK3α (38) or Ser9 of GSK3β (39). We infected PBMs with B. cenocepacia and examined both GSK3α and GSK3β phosphorylation by Western blotting. As shown in Fig. 6B, infection induces strong phosphorylation of GSK3α/β at Ser21 and Ser9. Specific examination of Ser9 on GSK3β confirmed its phosphorylation following infection (Fig. 6C).

It is reported that Akt controls this inhibitory phosphorylation of GSK3β (40), so we tested whether PI3K activity during B. cenocepacia infection was required for GSK3α/β phosphorylation. PBMs were pretreated with either the PI3K inhibitor LY294002 or vehicle control, followed by infection. Western blotting shows rapid phosphorylation of GSK3α/β with vehicle control. However, PI3K inhibition strongly attenuated this infection-mediated phosphorylation (Fig. 6D). This suggests that inhibition of GSK3β following B. cenocepacia infection is PI3K dependent. To determine whether GSK3β modulated phosphorylation of NF-κB, PBMs were pretreated with either DMSO or the GSK3β inhibitor SB-216763 (41), and then infected for 20, 40, or 60 min. Western blots showed that inhibition of GSK3β did not lead to increases in NF-κB phosphorylation (Supplemental Fig. 2). This suggests that any effect of GSK3β would be at the functional level, downstream of NF-κB phosphorylation.

To test this, we examined the effect of GSK3β on NF-κB and inflammatory response during B. cenocepacia infection, using the NF-κB luciferase reporter, as in Fig. 6A. Reporter-transfected cells were treated with vehicle control or with the GSK3β-specific inhibitor SB-216763. Following this, cells were infected with B. cenocepacia (Bc) for 5 h. Results showed that inhibition of GSK3β led to increased NF-κB reporter activity compared with vehicle control (Fig. 6E). Inhibitor treatment alone led to a modest, yet measurable increase in basal NF-κB activity (Supplemental Fig. 3), which also supports the findings (35–37) that GSK3β can repress NF-κB activity. Collectively, these results suggest that, within the context of B. cenocepacia-infected macrophages, GSK3β represses NF-κB activity, but not NF-κB phosphorylation.

This is also in agreement with the findings that both PI3K and Akt promote NF-κB activity because these signaling molecules inactivate GSK3β. To test the functional outcome of this, we collected supernatants from infected macrophages and measured TNF-α production by ELISA. Results showed a significant increase in TNF-α with the GSK3β inhibitor (Fig. 6F).

Overexpression of GSK3β represses NF-κB and the proinflammatory response

To confirm the function of GSK3β as a repressor of NF-κB and inflammatory response to B. cenocepacia, we cotransfected
RAW264.7 macrophages with a NF-κB luciferase reporter and either vector control or wild-type GSK3β (24). Macrophages overexpressing GSK3β showed reduced NF-κB activity compared with vector control in response to B. cenocepacia infection (Fig. 7A). Consistent with our earlier results showing that GSK3β represses NF-κB activity in infected macrophages, GSK3β overexpression decreased basal NF-κB activity by 50%. We also found reduced production of TNF-α in overexpressing macrophages (Fig. 7B). Verification of GSK3β overexpression was done by Western blotting (Fig. 7C).

In summary, our results show that PI3K/Akt and NF-κB activation are required for proinflammatory responses to B. cenocepacia. However, rather than driving NF-κB activation through IKK phosphorylation, PI3K/Akt signaling serves to inactivate the inhibitory GSK3β.

**Discussion**

We have found that PI3K/Akt signaling inactivates GSK3β to permit enhanced NF-κB activity and production of proinflammatory cytokines following B. cenocepacia infection. Akt has been shown to regulate NF-κB activity through its influence on IKK (42). Akt can directly phosphorylate IKKα to mediate NF-κB nuclear translocation and gene transcription (43) depending on the ratio of IKKα to IKKβ within the cell (44). PI3K can also lead to phosphorylation and activation of NF-κBp65 through Akt, independently of IκB degradation (45). Within the context of B. cenocepacia infection, however, it appears that the inactivation of GSK3β is the major mechanism by which PI3K/Akt modulated NF-κB activity. Of note, the PI3K pathway did not regulate expression of other negative regulators such as IRAK-M (Supplemental Fig. 4).

**FIGURE 7.** GSK3β overexpression inhibits B. cenocepacia-induced NF-κB activation and inflammatory response. A, RAW264.7 macrophages were transfected with a NF-κB luciferase reporter and either vector control or wild-type GSK3β plasmid. Fourteen hours posttransfection, macrophages were infected with B. cenocepacia at a MOI of 5 for 5 h. Cells were lysed, and luciferase activity was measured by a luminometer. Data are expressed as percentage of increase over matched uninfected control. B, RAW264.7 macrophages were transfected with vector control or wild-type GSK3β plasmid. Fourteen hours posttransfection, the macrophages were infected with B. cenocepacia at a MOI of 5 for 24 h. Cleared cell lysates were collected and assayed for TNF-α production by ELISA. Data represent the average of three samples, and error bars denote SD. *p < 0.05, Student t test. C, Western blots to measure GSK3β were done with cell lysates from uninfected macrophages transfected with either vector or GSK3β plasmid, followed by reprobes for actin as loading control. Densitometry quantification of GSK3β overexpression is shown and normalized by actin. D, Model of interactions among IKK, PI3K/Akt, and GSK3β pathways for regulating NF-κB and inflammatory response to B. cenocepacia.
cellular replication of B. cepacia within epithelial cells (23), presumably through an effect on autophagy. The difference between these findings may be reflective of macrophage versus epithelial cell or even LY294002 versus wortmannin pan-PI3K inhibition. However, we also find through genetic means that Akt does not influence macrophage phagocytosis or intramacrophage replication of B. cepacia. Thus, PI3K and Akt both strongly influence the macrophage inflammatory response without affecting B. cepacia uptake or survival.

One critical aspect related to this finding is that B. cepacia BC7, representing the virulent ET12 lineage that is associated with cepacia syndrome-related deaths, directly binds to the TNF receptor (TNFR1) to induce MAPK activation and IL-8 proinflammatory activity of the proinflammatory transcription factor NF-kB (54). This current study was done with the K56-2 isolate, which too represents the highly virulent ET12 lineage (12). This strongly suggests that inhibition of PI3K/Akt may be an ideal therapeutic strategy to combat cepacia syndrome, as it regulates NF-kB at a point downstream of initial IKK activation. As such, it would be effective even against this TNF-driven inflammatory response.

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Disclosures

The authors have no financial conflicts of interest.

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