Pseudomonas aeruginosa-Induced Human Mast Cell Apoptosis Is Associated with Up-Regulation of Endogenous Bcl-xS and Down-Regulation of Bcl-xL

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Pseudomonas aeruginosa-Induced Human Mast Cell Apoptosis Is Associated with Up-Regulation of Endogenous Bcl-xS and Down-Regulation of Bcl-xL

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Mast cells play a critical role in the host defense against bacterial infection. Recently, apoptosis has been demonstrated to be essential in the regulation of host response to Pseudomonas aeruginosa. In this study we show that human mast cell line HMC-1 and human cord blood-derived mast cells undergo apoptosis as determined by the ssDNA formation after infection with P. aeruginosa. P. aeruginosa induced activation of caspase-3 in mast cells as evidenced by the cleavage of D4-GDI, an endogenous caspase-3 substrate and the generation of an active form of caspase-3. Interestingly, P. aeruginosa treatment induced up-regulation of Bcl-xS and down-regulation of Bcl-xL. Bcl-xS and Bcl-xL are alternative variants produced from the same Bcl-x pre-mRNA. The former is proapoptotic and the latter is antiapoptotic likely through regulating mitochondrial membrane integrity. Treatment of mast cells with P. aeruginosa induced release of cytochrome c from mitochondria and loss of mitochondrial membrane potentials. Moreover, P. aeruginosa treatment reduced levels of Fas-associated death domain protein-like IL-1β-converting enzyme-inhibitory proteins (FLIPs) that are endogenous apoptosis inhibitors through interaction with caspase-8. Thus, human mast cells undergo apoptosis after encountering P. aeruginosa through a mechanism that likely involves both the Bcl family protein mitochondrial-dependent and the FLIP-associated caspase-8 pathways. The Journal of Immunology, 2006, 177: 8000–8007.
Bcl-xS and down-regulation of Bcl-xL, a shift from Bcl-xL to Bcl-xS expression in mast cells. In addition, P. aeruginosa infection induced up-regulation of FLIPs levels in mast cells. To our knowledge, this demonstration is the first to show that a shift from Bcl-xL to Bcl-xS is associated with bacteria-induced apoptosis in mammalian cells. Our results suggest that P. aeruginosa-induced human mast cell apoptosis likely involves Bcl family protein mitochondrial-dependent and FLIP-associated death receptor pathways.

FIGURE 1. Induction of human mast cell apoptosis by P. aeruginosa. a and b, HMC-1 cells were treated with increasing concentrations of P. aeruginosa strain 8821 for 24 h before flow cytometric analysis for ssDNA. For the live P. aeruginosa experiment, mast cells were treated with live P. aeruginosa for 3 h at various MOI = 25, 50, or 100, and then a mixture of antibiotics was added to kill P. aeruginosa (200 μg/ml gentamicin, 1% penicillin/l streptomycin (v/v) each 50 U/ml, 100 μg/ml ceftazidime, and 100 μg/ml piperacillin). Live P. aeruginosa induced concentration-dependent generation of ssDNA in HMC-1 5C6 cells. Results are expressed as mean percentage of ssDNA positive cells ± SEM from five independent experiments. *, p < 0.01 compared with HMC-1 cells treated with medium alone. Cells without P. aeruginosa treatment served as controls (NT). Mast cells treated with killed P. aeruginosa (50 killed, MOI = 50; 100 killed, MOI = 100) did not undergo apoptosis. c and d, Human CBMC were treated with medium, live or killed P. aeruginosa 8821 (MOI = 100), for 24 h, then fixed and stained for ssDNA. Similar to HMC-1 cells, CBMC undergo apoptosis after P. aeruginosa treatment. Results are expressed as mean ± SEM. *, p < 0.01 compared with cells treated with medium alone (n = 3).

Alternative splicing of the Bcl-x pre-mRNA gives rise to two transcripts, coding for either a long-form (Bcl-xL) or a short-form (Bcl-xS) of the protein (19). Bcl-xL inhibits apoptosis through heterodimerization with proapoptotic proteins (20). In contrast, Bcl-xS is proapoptotic through antagonizing survival proteins such as Bcl-xL or Bcl-2 (20). Due to the antagonistic functions of these two splice variants, a shift of the Bcl-xL to Bcl-xS ratio regulates the apoptotic process. The shift in splicing from Bcl-xL to Bcl-xS has been achieved by artificial antisense oligonucleotides and rendered cells to apoptosis (21, 22). Although the importance of the balance between Bcl-xL and Bcl-xS in control of apoptosis has been well recognized, it is not known whether this mechanism of alternative splicing is involved in pathogen-host interaction.

In this study, we demonstrated that human mast cells underwent apoptosis as determined by ssDNA formation in response to live, but not killed P. aeruginosa infection. Similarly, live but not killed P. aeruginosa induced caspase-3 activation in human mast cells. Interestingly, P. aeruginosa infection induced up-regulation of Bcl-xL and down-regulation of Bcl-xS, a shift from Bcl-xL to Bcl-xS expression in mast cells. In addition, P. aeruginosa reduced FLIPs levels in mast cells. To our knowledge, this demonstration is the first to show that a shift from Bcl-xL to Bcl-xS is associated with bacteria-induced apoptosis in mammalian cells. Our results suggest that P. aeruginosa-induced human mast cell apoptosis likely involves Bcl family protein mitochondrial-dependent and FLIP-associated death receptor pathways.

Materials and Methods
Reagents
Mouse anti-ssDNA mAb (IgM), biotinylated mouse anti-Bcl-xS/Bcl-xL, rabbit anti-FLIP short, and rabbit anti-FLIP long Abs were purchased from Chemicon International. Mouse anti-rat neutrophil mAb (RP-3, IgM) isotype control was a gift from F. Sendo (Yamagata University, Yamagata, Japan). Rabbit anti-active caspase-3 IgG and rabbit anti-human cytochrome c were purchased from Cell Signaling Technology. Rabbit FITC-conjugated anti-active caspase-3 IgG was purchased from BD Biosciences. Mouse anti-D4-GDI (specific for the 23-kDa form) mAb was purchased from Imgenex. Mouse anti-human Bcl-2 (IgG1) was purchased from Upstate Biotechnology. Goat anti-actin IgG, donkey anti-goat IgG HRP, donkey anti-rabbit IgG HRP, and donkey anti-mouse IgG HRP Ab conjugates were purchased from Santa Cruz Biotechnology. Goat PE-conjugated IgG to mouse IgM was purchased from Immunex. Mouse anti-human Bcl-2 (IgG1) was purchased from Upstate Biotechnology. Goat anti-actin IgG, donkey anti-goat IgG HRP, donkey anti-rabbit IgG HRP, and donkey anti-mouse IgG HRP Ab conjugates were purchased from Santa Cruz Biotechnology. Goat PE-conjugated IgG to mouse IgM was purchased from Immunex. Mouse anti-human Bcl-2 (IgG1) was purchased from Upstate Biotechnology. Goat anti-actin IgG, donkey anti-goat IgG HRP, donkey anti-rabbit IgG HRP, and donkey anti-mouse IgG HRP Ab conjugates were purchased from Santa Cruz Biotechnology. Goat PE-conjugated IgG to mouse IgM was purchased from Immunex. Mouse anti-human Bcl-2 (IgG1) was purchased from Upstate Biotechnology. Goat anti-actin IgG, donkey anti-goat IgG HRP, donkey anti-rabbit IgG HRP, and donkey anti-mouse IgG HRP Ab conjugates were purchased from Santa Cruz Biotechnology.

Mast cells and culture conditions
Human mast cells HMC-1 5C6 were maintained in IMDM in a 5% CO2-humidified atmosphere at 37°C. Culture medium was supplemented with 10% FBS and 50 U/ml each of penicillin and streptomycin.

Highly purified cord blood-derived mast cells (CBMC) (>95% purity) were obtained by long term culture of cord blood progenitor cells as previously described (23). The percentage of mast cells in the cultures was determined by toluidine blue staining (pH 1.0) of cytocentrifuged samples. Mature mast cells after more than 8 wk in culture were identified by their morphological features and the presence of metachromatic granules, at which time they were used for this study.
Murine primary cultured bone marrow-derived mast cells (BMMC) were harvested from the femurs and tibias of C57-black mice from Charles River Breeding Laboratories and maintained as previously described (24). Following 5 wk of culture, mast cell purity of ≥98% was achieved as assessed by toluidine blue staining. Mature mast cells were identified by their morphological features and granule prevalence.

Bacterial preparation and treatment with mast cells

*P. aeruginosa* strain 8821, a gift from Dr. A. Chakrabarty, University of Illinois (Chicago, IL), is a mucoid strain isolated from a cystic fibrosis patient (25). *P. aeruginosa* was cultured in Luria-Bertani broth and harvested when the culture reached an OD at 640 nm of 2 OD units (early stationary phase). Bacteria were washed in PBS and density adjusted to 1 OD unit before use. For killed *P. aeruginosa* experiments, bacteria were treated with gentamicin (100 μg/ml) for 2 h and exposed directly to UV light illumination for 20 min before experimental use. For live *P. aeruginosa* experiment, mast cells were treated with live *P. aeruginosa* for 3 h and throughout the rest of the incubation period. Sample lysates were subjected to SDS-PAGE and analyzed by Western blotting with mAbs specific for active caspase-3 or actin. Representative histograms show that live *P. aeruginosa*-treated HMC-1 SC6, but not medium treated (NT) or killed *P. aeruginosa* (killed 50, MOI = 50; killed 100, MOI = 100) treated cells were stained positive for active caspase-3 (b). Results are expressed as mean percentage of positive staining cells ± SEM of five independent experiments. *, p < 0.05 in c, d and e. Similar to HMC-1 cells, human CBMC after treatment with live, but not killed *P. aeruginosa* 8821 (MOI = 100) for 24 h were stained positive for active caspase-3. Results are expressed as mean ± SEM. *, p < 0.05 (n = 3).

Detection of ssDNA by flow cytometry

*P. aeruginosa*-treated or sham-treated mast cells were fixed, permeabilized, and stained with a mAb specific for segments of ssDNA as previously described (26). Briefly, mast cells were fixed for 1–3 days in methanol at −20°C and subsequently heated in formamide at 70°C for 10 min. Non-specific binding was blocked with 1% nonfat dry milk (w/v) in PBS. Cells were stained with anti-ssDNA or IgM isotype control, followed by washing and incubation with a PE-conjugated anti-mouse IgM Ab. After washing, cells were analyzed with a FACSCalibur flow cytometer (BD Biosciences).

Preparation of total cell lysate

Treated cells (0.25 × 10^5–2.5 × 10^6) were homogenized in ice-cold radioimmunoprecipitation assay buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 50 mM NaHPO_4, 0.25% sodium

**FIGURE 2.** *P. aeruginosa* induces activation of caspase-3 in mast cells. a, HMC-1 cells were treated with medium (NT) or increasing concentrations of live or killed *P. aeruginosa* (MOI = 25, 50 or 100) for 24 h, then lysed in radioimmunoprecipitation assay buffer. When live *P. aeruginosa* bacteria were used, antibiotics were added, the sample lysates were subjected to SDS-PAGE and analyzed by Western blotting with mAbs specific for active caspase-3 or actin. b and c, HMC-1 cells were treated with medium or *P. aeruginosa* for 24 h (MOI = 25, 50 or 100), then fixed and permeabilized for staining with FITC-conjugated anti-active caspase-3 Ab for flow cytometric analysis. Representative histograms show that live *P. aeruginosa*-treated HMC-1 SC6, but not medium treated (NT) or killed *P. aeruginosa* (killed 50, MOI = 50; killed 100, MOI = 100) treated cells were stained positive for active caspase-3 (b). Results are expressed as mean percentage of positive staining cells ± SEM of five independent experiments. *, p < 0.05 in c, d and e. Similar to HMC-1 cells, human CBMC after treatment with live, but not killed *P. aeruginosa* 8821 (MOI = 100) for 24 h were stained positive for active caspase-3. Results are expressed as mean ± SEM. *, p < 0.05 (n = 3).

**FIGURE 3.** *P. aeruginosa*-induced D4-GDI cleavage. HMC-1 cells were treated with live *P. aeruginosa* (strain 8821, MOI = 25, 50, or 100) for 3 h. Subsequently, antibiotics were added to kill the bacteria and further incubated for 21 h (total 24 h incubation). Cells without *P. aeruginosa* treatment served as controls (NT). Cell lysates were subjected to SDS-PAGE and Western blotting for the analysis of a 23-kDa cleavage fragment (specifically generated by active caspase-3) of the endogenous caspase-3 substrate D4-GDI. Blots that were probed for actin served as loading control.
deoxycholate (w/v), 0.1% Nonidet P-40 (v/v), 1 mM Na3VO4, and 1 mM NaF) containing freshly added protease and phosphatase inhibitors (2 mM PMSF, 1 ug/ml aprotinin, 1 ug/ml leupeptin, 1 ug/ml pepstatin A, 5 mM EDTA, 5 mM EGTA, and 2 mM iodoacetamide). Lysates were typically incubated on ice for at least 20 min before centrifugation at 15,000 g to remove cellular debris. Protein was quantified using a protein quantification reagent according to the manufacturer (Bio-Rad).

Western blotting for active caspase-3, D4-GDI, Bcl-xL/Bcl-xS, and FLIPs

Sample lysates containing 75 ug of protein (for caspase-3), 15 ug (for D4-GDI), 5 ug (for FLIPshort), and 30 ug (for FLIPlong, Bcl-xL/Bcl-xS, Bcl-2, or cytochrome c) were boiled for 5 min and subjected to SDS-10% PAGE. Gels were transferred to polyvinylidene difluoride membrane, and nonspecific binding was blocked using 10% nonfat dry milk. Membranes were then incubated overnight at 4°C with Abs to active caspase-3, D4-GDI, FLIP short, FLIP long, Bcl-xL/Bcl-xS, Bcl-2, or cytochrome c and detected by ECL detection reagent (Amersham Biosciences). Membranes were subsequently stripped (62.5 mM Tris-HCl (pH 6.8), 20% SDS (w/v), 100 mM 2-ME) and reprobed for actin.

Detection of active caspase-3 by flow cytometry

Treated mast cells (0.5–1 x 10^6) were fixed in 4% paraformaldehyde and subsequently stored in 10% DMSO in PBS at −80°C until staining. Cells were thawed and permeabilized with 0.1% saponin in PBS for 1 h followed by incubation in 3% BSA/PBS for 1 h to block nonspecific binding. Cells were then stained with FITC-conjugated rabbit mAb to active caspase-3, washed, and analyzed by flow cytometry.

Detection of mitochondrial membrane potential using DioC6

Changes of mitochondrial membrane potential were measured using DioC6 as described elsewhere with minor modifications (27). Mast cells were suspended in 40 nM DioC6 in medium for 30 min at 37°C. Cells were then transferred to flow cytometry polystyrene tubes and kept on ice. Cells were then analyzed by flow cytometry for green fluorescence.

Statistical analysis

Data were analyzed by one way ANOVA followed by Tukey’s posttest, using Instat GraphPad software (version 3.0) to determine the statistical difference between individual treatments. Statistical significance was defined as p < 0.05.

Results

P. aeruginosa induces mast cell apoptosis

To determine whether P. aeruginosa induces mast cell apoptosis, an Ab specific for ssDNA was used because the generation of ssDNA is a specific indicator of apoptosis (26). HMC-1 cells were treated with various concentrations of live or killed P. aeruginosa strain 8821. Treatment of HMC-1 cells with live P. aeruginosa induced ssDNA formation in a concentration-dependent manner (Fig. 1, a and b). Interestingly, no ssDNA formation was detectable when HMC-1 cells were treated with killed P. aeruginosa (Fig. 1, a and b).

P. aeruginosa induces caspase-3 activation in mast cells

Because caspase-3 plays a central role in the execution of apoptosis, several approaches were taken to examine the activation of caspase-3...
Bcl-xS expression in human mast cells

exotoxin A or camptothecin induce
were used as controls.
actin. Cells without treatment (NT)
by Western blotting and ssDNA for-
caspase-3 and Bcl-xL to Bcl-xS levels
induced decrease of the antiapoptotic Bcl family member
Bcl-2 was unaffected by
we determined the ratio of Bcl-xS to Bcl-xL. Treatment of mast
induced a consistent increase in the
P. aeruginosa
induced significant D4-GDI cleavage leading to the generation of
a 23-kDa product, which is specific to caspase-3 activity (Fig. 3).
It is noteworthy that only live P. aeruginosa, but not killed P. aeruginosa, induced enhanced D4-GDI cleavage in mast cells, a pattern consistent with that of ssDNA formation.

P. aeruginosa increases Bcl-xS and decreases Bcl-xL levels in mast cells and decreases mitochondrial membrane potential

Mitochondria play an essential role in the initiation of the apoptotic process by release of proapoptotic substances into the cytosol to activate caspase-3. The balance between the antiapoptotic Bcl family members such as Bcl-xL and Bcl-2 and the proapoptotic Bcl family members such as Bcl-xS controls apoptosis through several mechanisms including maintaining the integrity of the mitochondrial membrane by preventing the release of proapoptotic substances from the mitochondria (29). To examine whether P. aeruginosa regulates the levels of Bcl family members in mast cells, HMC-1 cells were treated with live P. aeruginosa with various MOI for 24 h. Cell lysates were used to examine the levels of Bcl-xS, Bcl-xL, and Bcl-2. Treatment of mast cells with P. aeruginosa induced decrease of the antiapoptotic Bcl family member Bcl-xL and increased the proapoptotic member Bcl-xS (Fig. 4).

Interestingly, the level of Bcl-2 was unaffected by P. aeruginosa treatment (Fig. 4). Because Bcl-xL and Bcl-xS are splice variants, we determined the ratio of Bcl-xS to Bcl-xL. Treatment of mast cells with P. aeruginosa induced a consistent increase in the Bcl-xS to Bcl-xL ratio.

To examine whether Bcl-xS expression is a specific effect induced by P. aeruginosa treatment, we examined the effect of two apoptosis-inducing agents (camptothecin and exotoxin A) on Bcl-xS expression in HMC-1 cells. Camptothecin is an anticancer chemical that induces cell apoptosis by inhibiting topoisomerase I (30). Treatment of HMC-1 with camptothecin (1 μM) induced
However, camptothecin at all concentrations tested (0.1, 1, and 5 μM) served as controls. Cells were then used to stain with DioC6 and analyzed by flow cytometry. Representative flow cytometry histogram showed that P. aeruginosa treatment induces loss of mitochondrial potential in mast cells (a). Results are expressed as mean percentage of positive staining cells ± SEM of three independent experiments. *, p < 0.01 compared with no treatment group (b). c. HMC-1 cells were treated with live P. aeruginosa (Psa Live), killed P. aeruginosa (Psa Killed) (MOI = 100), or camptothecin (Camp, 1 μM) for 24 h. Cells were then subjected to mitochondria/cytosol fractionation to obtain mitochondria-free cytosol fraction. Cytosol proteins were probed for cytochrome c, D4-GDI, or actin by Western blotting. Cells without P. aeruginosa treatment (NT) were used as a control.

To determine whether P. aeruginosa treatment affects mast cell mitochondrial membrane permeability, DioC6 was used. HMC-1 cells were treated with live P. aeruginosa with various MOI for 24 h or treated with killed P. aeruginosa (MOI = 100) for 24 h. HMC-1 cells were also treated with camptothecin (1 μM) for 24 h as a control. After treatment, cells were stained with DioC6. Treatment with camptothecin or live, but not killed P. aeruginosa induced loss of mast cell mitochondrial membrane potential (Fig. 6, a and b), suggesting an increase of mitochondrial membrane permeability.

To further determine a role of mitochondrial pathway in P. aeruginosa-induced mast cell apoptosis, we examined whether P. aeruginosa treatment induces cytochrome c release into cytosol compartment. HMC-1 cells were treated with camptothecin (1 μM), live or killed P. aeruginosa (MOI = 100) for 24 h. Cells were then subjected to mitochondria-cytosol separation using a mitochondria-cytosol fractionation kit (BioVision). The release of cytochrome c in the cytosol fraction was examined by Western blotting. Treatment of mast cells with live P. aeruginosa induced release of cytochrome c into cytosol (Fig. 6c). In contrast, killed P. aeruginosa only induced a low level of cytochrome c release. Treatment with camptothecin also induced a significant release of cytochrome c. Similarly, camptothecin or live but not killed P. aeruginosa induced D4-GDI cleavage when cytosol fractions were probed for cleaved D4-GDI product by Western blotting (Fig. 6c).

P. aeruginosa down-regulates FLIP_short and FLIP_long in mast cells

FLIPs are endogenous proteins that regulate caspase-3 activation through interaction with caspase-8 (14, 31). To determine whether FLIPs are involved in live P. aeruginosa-induced mast cell apoptosis, HMC-1 cells were treated with live or killed P. aeruginosa...
We determined whether P. aeruginosa (3–48 h) did not affect the surface Fas levels as determined by flow cytometry. An active form of caspase-3 in P. aeruginosa infection combined with detection of denatured DNA with a mAb developed technique based on formamide-induced DNA denaturation (1–3). Recently, we demonstrated an active mast cell-pathogen interaction, which leads to secretion of biologically active mast cell mediators such as IL-1β/H9251, IL-8/H9262, and the mechanisms involved in mast cell infection (1–3). To date, this important role of mast cells has been recognized. Depending on a specific cell type, two major pathways have been well described in the initiation of caspase-3 activation, the FLIPs-associated death receptor-caspase-8 pathway and the Bcl family-regulated mitochondrial pathway. Both pathways exist in mast cells (12, 18). Bcl family members consist of proapoptotic proteins (Bcl-xS, Bax, Bad, and others) and the antiapoptotic proteins (Bcl-xL, Bcl-2, and Bcl-w). The balance between proapoptotic and antiapoptotic members determines the fate of many types of cells. Bcl-xL and Bcl-xS are splice variants produced by alternative splicing of Bcl-x pre-mRNA. The antagonistic functions of Bcl-xL (antiapoptotic) and Bcl-xS (proapoptotic) have prompted several studies in an attempt to shift the alternative splicing in an effort to control the apoptotic process (21, 22). We found that a shift from Bcl-xL to Bcl-xS in human mast cells was induced by treatment with P. aeruginosa. To our knowledge, this demonstration represents the first that shows that alternative splicing of Bcl-xL and Bcl-xS is involved in bacterial pathogen-induced apoptosis in mammalian cells. One major advantage of using the mechanism of splicing shift producing variants with opposing effect is that one molecule of antiapoptotic Bcl-xL is replaced with one molecule of proapoptotic Bcl-xS, leading to an increased net effect in the control of apoptotic process.

The production of specific Bcl isoforms with opposite effects on the apoptotic response is likely controlled by the promoter usage (33). Several bcl-x gene promoters have been identified (33, 34). The usage of different promoters leads to the generation of different bcl gene products, with promoter 1 primarily producing Bcl-xL and promoter 2 producing Bcl-xS. Bcl-xL and Bcl-xS (33). Accordingly, it is possible that upon P. aeruginosa infection human mast cells change the usage of bcl gene promoter leading to the decrease of Bcl-xL and increase of Bcl-xS. However, the promoter usage appears to be tissue specific (33). Thus, it remains to be determined whether P. aeruginosa-induced Bcl-x isoform shift also occurs in other cell types or other pathogen-induced apoptosis because different cell types possess distinct mechanisms in response to different bacterial pathogen. In addition, because mechanisms involved in live P. aeruginosa-induced mast cell apoptosis are likely multifactorial, the causative relationship between Bcl-xL/Bcl-xS levels and mast cell apoptosis requires further study.

It has been suggested that members of the Bcl-x family generally control mitochondrial membrane permeability during apoptosis by regulating the electrical and osmotic homeostasis of mitochondria (29, 35). Using DioC6 as a probe, we observed a loss of mitochondrial potential in mast cells treated with P. aeruginosa. In addition, P. aeruginosa treatment induced release of cytochrome c from mitochondria. These results support a role of mitochondrial pathway in P. aeruginosa-induced human mast cell apoptosis.

An additional mechanism in the initiation of caspase-3 activation is the FLIPs-associated death receptor-caspase-8 pathway. Cellular FLIPs structurally resemble caspase-8 except that they lack proteolytic activity (14). Thus, FLIPs function as intrinsic inhibitors of caspase-8 pathway activation. Treatment of mast cells with live P. aeruginosa reduced the protein levels of both FLIP_s and FLIP_l, suggesting a potential role of death receptor-caspase-8 pathway in P. aeruginosa-induced mast cell apoptosis. We also attempted to determine whether levels of cell surface Fas (CD95) on mast cells were altered by P. aeruginosa treatment because Fas was up-regulated by P. aeruginosa on epithelial cells (32). Little changes of surface Fas were observed when human mast cells were treated with P. aeruginosa for various times.
(3–48 h) (data not shown), suggesting that mast cells and epithelial cells likely respond differently to P. aeruginosa.

Interestingly, both ssDNA formation and activation of caspase-3 were induced by live, but not killed P. aeruginosa. Further studies are needed to determine the specific bacterial components that are responsible for inducing mast cell apoptosis. It is possible that live P. aeruginosa releases bacterial toxins that induce mast cell apoptosis. Our recent study showed that P. aeruginosa exotoxin A induces human mast cell apoptosis that is associated with reduced levels of FLIPs, a feature similar to that induced by live P. aeruginosa (10). However, we noticed that unlike live P. aeruginosa, exotoxin A did not induce increased expression of Bcl-xS. In addition, we have demonstrated that mitochondrial pathway is activated by live P. aeruginosa. This result is in contrast to exotoxin A, which induces mast cell apoptosis through a mitochondrial-independent pathway (10). Thus, exotoxin A may not be a major factor responsible for P. aeruginosa-induced mast cell apoptosis. P. aeruginosa-derived azurin and cytchrome c have been implicated in macrophage and mast cell apoptosis (36). Alternatively, P. aeruginosa invasion into mast cells may initiate an intracellular event that directly targets the upstream of initiator caspases.

In summary, we reported for the first time that human mast cells undergo apoptosis after encountering live bacteria, P. aeruginosa, through a mechanism that is associated with a shift of alternative splicing from Bcl-xL to Bcl-xS. P. aeruginosa-induced caspase-3 activation in mast cell is accompanied by the loss of mitochondrial potential and reduced FLIPs levels, suggesting a potential role for the Bcl mitochondrial pathway and FLIPs-caspase-8 pathway in P. aeruginosa-induced mast cell apoptosis.

Disclosures

The authors have no financial conflict of interest.

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