Aspergillus fumigatus-induced Interleukin-8 Synthesis by Respiratory Epithelial Cells Is Controlled by the Phosphatidylinositol 3-Kinase, p38 MAPK, and ERK1/2 Pathways and Not by the Toll-like Receptor-MyD88 Pathway*

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Previous studies have established that phagocytes are key cells of the pulmonary innate immune defense against A. fumigatus, an opportunistic fungus responsible of invasive pulmonary aspergillosis. Macrophages detect A. fumigatus via Toll-like receptors 2 and 4 (TLR2 and -4) and respond by the MyD88-NF-κB-dependent synthesis of inflammatory mediators. In the present study, we demonstrate that respiratory epithelial cells also sense A. fumigatus and participate in the host defense. Thus, the interaction of respiratory epithelial cells with germinating but not resting conidia of A. fumigatus results in interleukin (IL)-8 synthesis that is controlled by phosphatidylinositol 3-kinase, p38 MAPK, and ERK1/2. Using MyD88-dominant negative transfected cells, we also show that IL-8 production is not dependent on the TLR-MyD88 pathway, although the MyD88 pathway is activated by A. fumigatus and leads to NF-κB activation. Thus, our results provide evidence for the existence of two independent signaling pathways activated in respiratory epithelial cells by A. fumigatus, one that is MyD88-dependent and another that is My88-independent and involved in IL-8 synthesis.

Aspergillus fumigatus is an opportunistic fungus responsible for invasive pulmonary aspergillosis (IPA), a life-threatening disease that usually occurs in immunocompromised patients as inhalation of spores by those hosts results in fungal growth inside the lung and hematogenous dissemination (1, 2).

The mechanisms of host resistance to IPA are not completely understood. It is generally accepted that macrophages and neutrophils represent the first two lines of innate host defense against A. fumigatus (3, 4). Specifically, alveolar macrophages phagocyte and kill conidia, whereas neutrophils lyse hyphae of germinated spores (5). However, it is now recognized that epithelial cells also play an important role in the innate defense (6–8). Rather than just representing a physical barrier to prevent pathogen gaining unwanted access to essential organs, the epithelial layer provides a surface where the host can interact with pathogens. The airway epithelium senses microbial products through pattern recognition receptors that bind conserved molecular patterns expressed by micro-organisms. Toll-like receptors (TLRs) have been identified as a major class of pattern recognition receptors (9–11). Recognition of pathogen-associated molecular patterns by TLRs triggers a cascade of cellular signals that culminates in the activation of NF-κB, which leads to inflammatory gene expression and ultimately to the clearance of the infectious agents (11–14). There is accumulating evidence, based on in vitro and in vivo studies, that supports a role for TLRs in A. fumigatus sensing. Findings from various studies demonstrated that A. fumigatus recognition by macrophages, neutrophils, and dendritic cells, whether of mouse (15) or human (16) origin, depends on the presence of TLR2 and TLR4. The importance of TLR2 and TLR4 in IPA has been confirmed by the increased susceptibility of TLR2 and TLR4 knock-out mice to A. fumigatus infections (17–19). However, very little knowledge exists concerning how the pulmonary epithelium responds to A. fumigatus infection and which receptors and signaling pathways might be involved. Most studies have investigated physical interactions between A. fumigatus and the epithelium and found that A. fumigatus conidia can be internalized by epithelial cells (20, 21). Once internalized, some conidia traffic to late endosomes/lysosomes, where they can germinate. Those germinals are able to escape the phagosome and form extracellular hyphae without lysis of the host cells (20). A. fumigatus conidia have been also found to suppress apoptosis of epithelial cells induced by either tumor necrosis factor α (TNF-α) or staurosporine (22). These observations led to hypothesize that A. fumigatus uses epithelial cells...
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as reservoirs for immune evasion and eventually a starting point for dissemination throughout the host (21, 23).

As far as synthesis of cytokines and chemokines are concerned, it has been shown that A. fumigatus hyphae and proteases can activate cultured epithelial cells to produce interleukin (IL)-8 (24–26), a CXC chemokine that is a potent chemoattractant for neutrophils. In support, CXC chemokines, such as KC and MIP-2, have been shown to be components of neutrophil-mediated host defense in a mouse model of IPA (27, 28). However, to date, there are no reports on the regulation of IL-8 gene expression by airway epithelial cells in response to A. fumigatus stimulation. This is of particular interest in the context of the involvement of TLRs in IPA, since respiratory epithelial cells express all of the different types of TLRs, and activation of these TLRs has been shown to induce the production of cytokines, chemokines, and antimicrobial peptides (29–32).

In this study, we investigated the signaling pathways that promote the generation of IL-8 by a human bronchial epithelial cell line stimulated with live A. fumigatus.

**MATERIALS AND METHODS**

**Reagents**—F-12K nutrient mixture (Kaighn’s modification), penicillin and streptomycin, glutamine, and trypsin–EDTA were from Invitrogen (Paisley, UK). The p38 MAPK inhibitor (SB203580) was obtained from Calbiochem, and the PI3K inhibitor (LY294002) and ERK1/2 (PD98059) inhibitors were from Cell Signaling Technology (Danvers, MA). MALP-2 was purchased from Alexis Biochemicals (San Diego, CA). The p38 MAPK inhibitor, the Akt, the p44/42 MAP kinase, the phospho-p38 MAPK (Thr180/Tyr182), the phospho-Akt (Ser473), and the phospho-p44/42 MAPK (Thr202/Tyr204) antibodies were from Cell Signaling Technology (Danvers, MA) and Sigma. Leupeptin, aprotinin, soybean trypsin inhibitor, phenylmethylsulfonyl fluoride, and benzamidine were from Kanazawa University, Japan. After 24 h, cells were stimulated with A. fumigatus (3 × 105 conidia), TNF-α (0.05 ng/ml), or MALP-2 (30 or 100 ng/ml). Following 15 h with the indicated treatments, cells were lysed in a lysis buffer (25 mM Tris (pH 7.4), 8 mM MgCl2, 1 mM dithiothreitol, 1% (v/v) Triton X-100, and 15% (v/v) glycerol), and luciferase activity was measured in the cell lysates using an ECL+ immunoblotting detection system (GE Healthcare) according to the manufacturer’s instructions. Molecular masses were estimated from calibration standards included in each gel.

**Transient and Stable Transfections of BEAS-2B Cells and Reporter Gene Studies**—BEAS-2B cells were seeded at 105 cells/well on 24-well plates 72 h before transient transfection using FuGENE 6 transfection reagent (Roche Applied Sciences), according to the manufacturer’s instructions. Each sample contained either 200 ng of NF-κB-luciferase (kindly provided by Dr. A. Israel, Pasteur Institute, Paris, France) or 200 ng of a luciferase reporter gene driven either by a long IL-8 promoter construct (−1481 bp relative to the transcription start site) or by a short IL-8 promoter construct (−133 bp relative to the transcription start site) containing or not site-specific mutations for the binding of NF-κB, AP-1 (activating protein-1), or NF-κB (nuclear factor-κB) (gifts from Dr. N. Mukaida, Kanazawa University, Japan). After 24 h, cells were stimulated with A. fumigatus (3 × 105 conidia), TNF-α (0.05 ng/ml), or MALP-2 (30 or 100 ng/ml). Following 15 h with the indicated treatments, cells were lysed in a lysis buffer (25 mM Tris (pH 7.4), 8 mM MgCl2, 1 mM dithiothreitol, 1% (v/v) Triton X-100, and 15% (v/v) glycerol), and luciferase activity was measured in the cell lysates using an ECL+ Berthold luminometer. Results are expressed as relative luciferase units.

**Preparation of A. fumigatus Conidia**—A clinical isolate of A. fumigatus (Green strain CBS 144.89) was maintained on 2% malt extract agar slants at 22 °C. Conidia were recovered from cultures grown for 7 days by washing the slant culture with a phosphate-buffered saline, 0.1% Tween 20 solution and gently shaken. Conidia were then washed by centrifugation (5 min at 10,000 × g) and suspended in a phosphate-buffered saline, 0.1% Tween 20 solution. Conidia concentrations were evaluated by measurement of the optical density of the suspension at 600 nm, with a 0.6 optical density corresponding to 2 × 107 conidia/ml. The suspension was then diluted as needed in order to reach the desired concentration.

**Cell Culture and Stimulation Conditions**—The human bronchial epithelial cell line BEAS-2B obtained from the American Type Cell Collection (Manassas, VA) was maintained in serial passage in F-12K culture medium supplemented with 10% fetal calf serum, 1% penicillin and streptomycin, 1% glutamine, and 10 mM HEPES in 75-cm2 culture flasks and seeded at 5 × 104 on 24-well plates 3 days before. In all experiments, except for the kinetic study, BEAS-2B cells were stimulated during 15 h with conidia (3 × 107) in 300 μl of medium without penicillin and streptomycin.
β-actin were determined by real time quantitative PCR. QuantiTect SYBR Green PCR kit (Qiagen) was used following the manufacturer’s instructions. The PCR mixture was composed of 12.5 µl of QuantiTect SYBR Green Mix (2x), 5 µl of cDNA (125 ng of total RNA) and corresponding primers (IL-8 forward, AGAGACAGCAGAGCACAAC; IL-8 reverse, TTATGCACTCTTGCCAAAAC; β-actin forward, GGAAATCTGTCGTGACTAC; β-actin reverse, TGGCGTACAGGTGCTTTTG) (final concentration 300 nM) and then distilled water was added to a final volume of 25 µl. PCR was done using model 7500 real time PCR systems (Applied Biosystems, Foster City, CA) according to the manufacturer’s suggestions following thermal cycling program (enzyme activation 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 45 s, and extension at 72 °C for 45 s). Relative gene expression was calculated by comparing the number of thermal cycles that were necessary to generate threshold amounts of product (CT). CT was calculated for the IL-8 gene and for the housekeeping gene β-actin. For each cDNA sample, the CT for β-actin was subtracted from the CT for IL-8 to give the parameter ΔCT, thus normalizing the initial amount of RNA used. The amount of IL-8 mRNA was calculated as 2^{-ΔΔCT}, where ΔΔCT is the difference between the ΔCT of the two cDNA samples to be compared.

Cytokine Measurements and Cytotoxicity of Cultured Cells during A. fumigatus Infection—IL-8 concentrations in cell culture supernatants were determined using Duo-Set ELISA kits. To test the viability of the infected and uninfected cells, 50 µl of supernatants were determined using Duo-Set ELISA kits. IL-8 concentrations were measured by ELISA. Each point is the mean ± S.E. of three experiments performed in triplicate. *, p < 0.05, significantly different from base-line values.

RESULTS

A. fumigatus Induces IL-8 Synthesis by Respiratory Epithelial Cells—Confluent monolayers of BEAS-2B cells were infected with 3 × 10^5 conidia/0.3 ml/well. At different time intervals postinfection, culture supernatants were collected and assayed for IL-8 concentrations. A time-dependent increase in IL-8 secretion was noted and reached significance after 8 h compared with unstimulated cells (Fig. 1), a time point at which all spores are swollen, as observed by light microscopy. To confirm that only the germinated state of A. fumigatus is able to induce IL-8 production, CEA17, a mutant strain of A. fumigatus unable to germinate, was tested under the conditions used with the wild type strain. As anticipated, 15 h postinfection, IL-8 synthesis was not triggered by this mutant (Fig. 2).

In order to assess whether the release of soluble components were involved in the induction of IL-8 production, 3 × 10^5 conidia/0.3 ml were incubated for 15 h at 37 °C in complete F12K medium. After incubation, fungal cultures were centrifuged, and supernatants were filtered and added to BEAS-2B cells for 15 h. The supernatants did not induce IL-8 synthesis (data not shown).

Taken together, these results indicate that the germinated form of A. fumigatus and not resting spores or released components induces IL-8 production by epithelial cells.

To determine whether A. fumigatus has any cytotoxic effect on the cells, we measured the release of the cytosolic enzyme LDH into the supernatant after 15 h of stimulation. There was no difference in LDH release between infected and uninfected cells (data not shown).
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A. fumigatus Induces NF-κB Activation in Respiratory Epithelial Cells—NF-κB is a critical transcriptional activator involved in the expression of many inflammatory genes of the innate immune response, including IL-8 (33). To determine whether the interaction between epithelial cells and A. fumigatus induced NF-κB activation, we measured the transcription of a luciferase reporter gene containing in its promoter copies of the consensus sequence of NF-κB elements (NF-κB-Luc). Transfected cells were stimulated for 15 h with 3 x 10^5 conidia or with MALP-2 (30 ng/ml), a TLR2 agonist used as positive control of NF-κB activation. As presented in Fig. 3, A. fumigatus and MALP-2 stimulation resulted in a 3- and a 6-fold increase of luciferase activity, respectively, indicating that NF-κB is activated by A. fumigatus.

IL-8 Gene Expression Induced by A. fumigatus Is NF-κB-Dependent—Since A. fumigatus is able to induce IL-8 production and NF-κB activation in epithelial cells, we assessed whether NF-κB activation is involved in IL-8 production. In a number of studies (33–36), it was found that a sequence spanning nucleotides −1 to −133 within the 5’-flanking region of the IL-8 gene is essential and sufficient for transcriptional regulation of the gene. This promoter element (short promoter) contains binding sites for NF-κB, AP-1, and NF-IL-6. To determine the implication of each site in the context of A. fumigatus stimulation, cells were transiently transfected with a luciferase reporter gene driven by the IL-8 promoter (−133) containing site-specific mutations and stimulated for 15 h with 3 x 10^5 conidia. As shown in Fig. 4, the NF-κB and AP-1 site mutation reduced the IL-8 promoter activity by 99 and 40%, respectively. In contrast, the NF-IL-6 site had no significant effect.

A. fumigatus Induces Activation of PI3K, p38 MAPK, and ERK1/2—The transmission of extracellular signals to their intracellular targets is mediated by a network of interacting proteins that relay biochemical messages and thus control multiple cellular processes. Several related intracellular signaling pathways, collectively known as protein kinase signaling cascades, have been demonstrated to play a role in many systems.

To determine whether A. fumigatus was able to activate PI3K, p38 MAPK, and ERK1/2, kinases that have been implicated in IL-8 production (33), we examined the phosphorylation of the Ser/Thr kinase Akt, identified as an important target of PI3K (37, 38), and of p38 MAPK and ERK1/2. Phosphorylation was measured at different time intervals (3, 8, 10, and 13 h) postinfection by immunoblotting of whole cell lysates with a phosphospecific antibody that recognizes the phosphorylated form of ERK1/2.

PI3K, p38 MAPK, and ERK1/2 Pathways—To investigate the implication of PI3K, p38 MAPK, and ERK1/2 in the production of IL-8, specific inhibitors were tested. The PI3K (LY294002), p38 MAPK (SB203580), and ERK1/2 (PD98059) inhibitors, incubated 1 h before and during the 15 h of stimulation with A. fumigatus, diminished IL-8 production in a concentration-dependent manner; LY294002 and SB203580 inhibited IL-8 synthesis by around 50% and by more than 95% at concentrations of 3 and 30 μM, respectively. The ERK1/2 inhibitor (PD98059) was less potent and inhibited the IL-8 synthesis by only 20% at 3 μM and 75% at 30 μM (Fig. 6). It is of note that each one of these inhibitors prevents the phosphorylation of its own target without affecting the phosphorylation of the target of the other inhibitors (data not shown). No difference in LDH release was found between treated and untreated infected cells (data not shown).
Implication of PI3K, p38 MAPK, and ERK1/2 in the Activation of the IL-8 Promoter—To understand the involvement of the protein kinases in the activation of the IL-8 promoter, cells were transfected with a luciferase reporter gene driven by the long IL-8 promoter (−1481) and then treated with concentrations of kinase inhibitors that inhibited by around 80% IL-8 synthesis (i.e. 10 μM for LY294002 and SB203580 and 30 μM for PD98059). As previously, kinase inhibitors were incubated 1 h before and during the 15 h of stimulation with A. fumigatus. Luciferase activity, IL-8 mRNA, and protein productions were then measured. Although all kinase inhibitors diminished IL-8 mRNA and protein productions by around 80% (Fig. 8, B and C), only PD98059, the inhibitor of ERK1/2, blocked the luciferase activity of the IL-8 reporter gene (Fig. 8A).

These data indicate that, apart from NF-κB, another transcription factor is necessary for IL-8 gene expression and that the activation of this factor is controlled by ERK1/2.

MyD88 Is Involved in NF-κB Activation Induced by A. fumigatus—To determine whether TLRs were implicated in NF-κB activation of epithelial cells stimulated by A. fumigatus, we stably transfected BEAS-2B cells with a vector expressing a dominant negative form of MyD88 (MyD88-DN), a common adaptor molecule to the intracellular domain of all TLRs (except TLR3). For each particular experiment, cells were transiently transfected with the vector expressing NF-κB-Luc and stimulated for 15 h with 3 × 10^5 conidia, and luciferase activity was measured. As shown in Fig. 9, luciferase activity was deeply inhibited in cells expressing the MyD88-DN and stimulated by A. fumigatus compared with control cells expressing the empty control plasmid (pcDNA3). As expected, the luciferase activity was similar in the two cell lines when stimulated with TNF-α used as a negative control and was inhibited in MyD88-DN-expressing cells when stimulated with MALP-2 used as positive control. From these experiments substantiating a role for MyD88, we speculate that possibly TLR pathways, although receptors for IL-1 and IL-18 operate also through MyD88, are important for the activation of NF-κB in epithelial cells stimulated with A. fumigatus.

MyD88 Is Not Involved in IL-8 Synthesis by A. fumigatus—Because our results implicated NF-κB in IL-8 production by A. fumigatus-activated epithelial cells, we next evaluated whether MyD88 was involved in this production. IL-8 was measured in supernatants of BEAS-2B cells expressing MyD88-DN or pcDNA3 alone and stimulated for 15 h with A. fumigatus. Surprisingly, no difference was observed for IL-8 production between the two cell lines stimulated with A. fumigatus (Fig. 10) or TNF-α used as negative control, whereas MALP-2-induced IL-8 production was completely abolished in the MyD88-DN cell line. These data indicate that whereas MyD88 is implicated...
in the signaling pathway leading to NF-κB activation and NF-κB is involved in IL-8 synthesis (see above), MyD88 is not implicated in the production of this chemokine by epithelial cells stimulated with A. fumigatus.

**DISCUSSION**

The dogma of the anti-A. fumigatus innate response is that the key cells are alveolar macrophages and neutrophils, two types of phagocytes, which eliminate conidia and hyphae, respectively (3–5). For a long time, the respiratory epithelial layer was described only as a physical inert barrier between the internal and external environments. However, recently, numerous studies have demonstrated that respiratory epithelial cells have a great deal of importance in the initiation of the host innate immune response by sensing the pathogens and secreting inflammatory mediators (6–8).

In the present study, we observed that germinating conidia are able to induce IL-8 synthesis, whereas resting conidia or mutant conidia that are unable to germinate did not. This result is in accordance with a previous study showing that production of IL-8 significantly increased only when epithelial cells were stimulated with hyphae (26). The activation results from direct contact between epithelial cells and germinating conidia and not from released soluble molecule(s), since A. fumigatus culture supernatants are not able to induce IL-8 production. In contrast to our observations, others have shown that proteases obtained from filtrates of A. fumigatus cultures induced the release of IL-8 by respiratory epithelial cell lines (25). This discrepancy may be explained by the fact that concentrations of fungal proteases obtained from A. fumigatus culture filtrates were probably higher than those possibly produced in our experimental conditions that are more closely related to an in vivo infection process. This hypothesis is reinforced by the observation of epithelial cell detachment when stimulated with the proteases (24), whereas under our conditions, direct stimulation with A. fumigatus did not result in either detachment or lysis of the cells.

These data show that respiratory epithelial cells play an active role in the antifungal innate immune response, by producing IL-8, a chemokine that contributes to the effective recruitment and activation of neutrophils at the site of infection (39, 40). However, it is commonly thought that inflammation must be controlled much more tightly in the lungs than in other organs, in order to preserve a high degree of functionality allowing sufficient gas exchange. To this aim, the epithelial cell
response may be somewhat restricted to the presence of crucial pathogens to avoid frequent inflammation. Our results are in accordance with this view, since respiratory epithelial cell responses differ from macrophages. Alveolar macrophages are able to recognize and eliminate resting conidia (3, 5), whereas epithelial cells do not sense resting conidia but are able to recognize A. fumigatus during the first step of conidia swelling, the pathogenic state of A. fumigatus development. This delayed epithelial response may be useful to limit the inflammatory response. Indeed, conidia are first eliminated by resident alveolar macrophages, whereas epithelial cells remain in an inactive state. However, as soon as the capacity of alveolar macrophages to eliminate conidia is overwhelmed, free conidia swell and begin to be recognized by epithelial cells. This interaction induces IL-8 production, which contributes to the recruitment of neutrophils that are able to eliminate hyphae.

Since the molecular mechanism by which A. fumigatus induces IL-8 secretion by respiratory epithelial cells had not been studied, we examined the activation and the involvement of the transcriptional factor NF-κB of protein kinases, such as PI3K, p38 MAPK, and ERK1/2. NF-κB is a ubiquitous transcription factor playing a key role in the immune response to infections. In unstimulated cells, NF-κB dimers are sequestered in the cytoplasm by a family of inhibitors, called IκB. Activation of the IκB kinase induces the phosphorylation and degradation of IκB and allows translocation of NF-κB to the nucleus, where it can bind to specific sequences of DNA and regulate gene transcription. The activation of these genes leads to the expression of a number of mediators, including many cytokines, chemokines, and cell adhesion molecules that are involved in the inflammatory response (41, 42). In a number of studies, it was found that the IL-8 gene transcription requires activation of the combination of NF-κB and either AP-1 or NF-IL-6, depending on the cell type (33, 36). Using transient transfection of cells by the short IL-8 promoter-driven reporter gene containing the binding site, mutated or not, for NF-κB, AP-1, and NF-IL-6, we observed that NF-κB and AP-1 are implicated in the activation of the promoter.

It is well known that many stimuli able to induce IL-8 production activate a number of protein kinases (43, 44). We therefore examined the contribution of PI3K, p38 MAPK, and ERK1/2. First, we observed that they were all activated upon A. fumigatus challenge. We then used specific inhibitors and observed that the blockade of PI3K, p38 MAPK, and ERK1/2 pathways inhibited IL-8 synthesis, indicating their involvement. However, inhibition of PI3K and p38 MAPK blocked neither the NF-κB transactivation nor the IL-8 promoter activity, whereas inhibition of ERK1/2, which also had no effect on the NF-κB transactivation, did block the activation of the IL-8 reporter gene. These data imply that neither PI3K, p38 MAPK, nor ERK1/2 is involved in the nuclear translocation of NF-κB and the upstream signaling pathways, since NF-κB is able to activate the promoter NF-κB-Luc. The present results also indicate that PI3K and p38 MAPK are not involved in the IL-8 promoter reporter gene activation, although they are necessary for the IL-8 protein production. It is now well recognized that protein kinases can control the transcriptional process in many ways, in particular through their ability to remodel chromatin structure by phosphorylation of histone (45–48). Under our experimental conditions, this activity cannot be highlighted, since the transfected IL-8 promoter gene is not wrapped in nucleosomes or within the

FIGURE 9. Involvement of MyD88 in A. fumigatus-induced NF-κB activation in respiratory epithelial cells. BEAS-2B cells stably transfected either with vector expressing a dominant negative form of MyD88 (MyD88-DN) or with the empty plasmid (pcDNA3) used as a control were cultured in 24-well plates and transiently transfected with an NF-κB-driven luciferase gene (NF-κB-Luc). After 24 h, cells were incubated for 15 h with 3 × 10⁵ conidia, 100 ng/ml MALP-2, or 0.05 ng/ml TNF-α. Then cells were lysed, and the luciferase activity was measured. Each histogram, expressed in -fold increase over the nonstimulated cells, is the mean ± S.E. of five experiments performed in triplicate. **, p < 0.01; *, p < 0.05, significantly different from pcDNA3 values.

FIGURE 10. Involvement of MyD88 in A. fumigatus-induced IL-8 synthesis in respiratory epithelial cells. BEAS-2B cells stably transfected either with vector expressing a dominant negative form of MyD88 (MyD88-DN) or with the empty plasmid (pcDNA3) used as a control were cultured in 24-well plates. Cells were incubated for 15 h with 3 × 10⁵ conidia, 100 ng/ml MALP-2, or 0.05 ng/ml TNF-α. Then supernatants were collected for IL-8 measurement. Each histogram, expressed in ng/ml, is the mean ± S.E. of five experiments performed in triplicate. **, p < 0.01, significantly different from pcDNA3 values.
native chromatin environment and consequently cannot recapitulate the specificity of the endogenous gene. Taking these data into account, our results suggest on the one hand that PI3K and p38 MAPK may play a role in the downstream NF-kB translocation events like epigenetic modifications of chromatin or also possibly in mRNA stabilization (49–51) and on the other hand that ERK1/2 is involved in the translocation of one or several transcriptional factors other than NF-kB that are essential for the IL-8 gene expression.

Human TLRs are a family of 10 receptors, expressed in lung epithelial cells (30, 52, 53), that trigger innate immune reactions in response to various microbial products and are the first step of a signaling cascade that leads to the activation of various nuclear factors and, in particular, of NF-kB. Recent studies have demonstrated a crucial involvement of TLRs in the recognition of A. fumigatus. In vitro studies suggest that TLR2 is clearly involved in the recognition of A. fumigatus by alveolar macrophages and neutrophils (19, 54–56), whereas the contribution involved in the recognition of respiratory epithelial cells by TLR-associated adaptor molecules, it appeared that TLR2 and TLR4, and p38 MAPK may play a role in the downstream NF-kB translocation events like epigenetic modifications of chromatin or also possibly in mRNA stabilization (49–51) and on the other hand that ERK1/2 is involved in the translocation of one or several transcriptional factors other than NF-kB that are essential for the IL-8 gene expression.

In summary, taking advantage of a cell line that can be easily transfected, our results suggest the existence of two independent signaling pathways in respiratory epithelial cells activated by A. fumigatus. One does not result in IL-8 synthesis and is controlled by MyD88 and NF-kB, implicating one or several TLRs. The other, which is probably the most important observation of our study, since it does not fit with the classical paradigm (33–36), is MyD88- and NF-kB-independent. This latter pathway leads to IL-8 synthesis through different kinase pathways, including PI3K, p38 MAPK, and ERK1/2. It is difficult to speculate on the nature of the receptor that recognizes A. fumigatus and that triggers this second intracellular transduction. Indeed, Dectin-1, which ligates β1–3-glucans expressed by germinating conidia (60, 61), would be the most obvious candidate, but it seems not to be expressed by epithelial cells (62). DC-SIGN is a C-type lectin that mediates the binding and capture of A. fumigatus. Nonetheless, its expression is restricted to dendritic cells and subsets of macrophages (63).

Whatever it could be, it also remains to be demonstrated that this pathway is operative in primary cells in culture and under in vivo situation when conidia invade the air spaces. In short, our results showing that the synthesis of IL-8 by epithelial cells in response to a fungal challenge is not controlled by the TLR-MyD88-NF-kB pathway have to be extended in order to identify the host cell receptor(s) and its ligand(s), the downstream signaling pathways, and the genes regulated by the different kinase pathways, to allow the search of potential therapeutic targets able to manipulate the innate immune response of immunosuppressed patients suffering from invasive pulmonary aspergillosis.

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