Pseudomonas aeruginosa LPS or Flagellin Are Sufficient to Activate TLR-Dependent Signaling in Murine Alveolar Macrophages and Airway Epithelial Cells

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Abstract

Background: The human lung is exposed to a large number of airborne pathogens as a result of the daily inhalation of 10,000 liters of air. Innate immunity is thus essential to defend the lungs against these pathogens. This defense is mediated in part through the recognition of specific microbial ligands by Toll-like receptors (TLR) of which there are at least 10 in humans. Pseudomonas aeruginosa is the main pathogen that infects the lungs of cystic fibrosis patients. Based on whole animal experiments, using TLR knockout mice, the control of this bacterium is believed to occur by the recognition of LPS and flagellin by TLRs 2,4 and 5, respectively.

Methodology/Principal Findings: In the present study, we investigated in vitro the role of these same TLR and ligands, in alveolar macrophage (AM) and epithelial cell (EC) activation. Cellular responses to P. aeruginosa was evaluated by measuring KC, TNF-α, IL-6 and G-CSF secretion, four different markers of the innate immune response. AM and EC from WT and TLR2, 4, 5 and MyD88 knockout mice were stimulated with the wild-type P. aeruginosa or with a mutant devoid of flagellin production.

Conclusions/Significance: The results clearly demonstrate that only two ligand/receptor pairs are necessary for the induction of KC, TNF-α, and IL-6 synthesis by P. aeruginosa-activated cells, i.e. TLR2,4/LPS and TLR5/flagellin. Either ligand/receptor pair is sufficient to sense the bacterium and to trigger cell activation, and when both are missing lung EC and AM are unable to produce such a response as were cells from MyD88−/− mice.

Introduction

Pseudomonas aeruginosa is an opportunist Gram-negative bacterium that is a frequent cause of acute pneumonia in patients who are being mechanically ventilated [1]. More notoriously, P. aeruginosa is also the main pathogen in cystic fibrosis, infecting 70% of the patients at an early age and contributing to the chronic lung destruction responsible for mortality. In fact, P. aeruginosa infection rarely occurs in healthy hosts due to efficient clearance of the pathogen by the innate immune response [2,3,4].

As a primary interface between pathogens and the host, epithelial cells (EC) lining the mammalian airways and the alveolar surface area are a crucial site for innate immune responses [5,6,7,8]. Also on the first line of the pulmonary defense against invading pathogens are alveolar macrophages (AM), which are mobile and capable of phagocytosis [9,10,11]. Thus, once P. aeruginosa enters the airways, there is direct encounter with these two cell types, which initiates a series of inducible host immune responses directed to bacterial eradication. This response includes the release of cytokines and chemokines to attract phagocytic cells to the site of infection [12]. Thus, efficient clearance of P. aeruginosa is considered to rely on the recognition of the pathogen by AM and EC, which mount intracellular signaling pathways responsible for triggering an innate response for host defense [2].

To accomplish this defense, the host makes use of Toll-like receptors (TLR) which are homologues of the Drosophila Toll protein, and which recognize conserved microbial structures, or pathogen-associated molecular patterns (PAMP) [13,14,15,16]. At present, 12 murine TLR and 10 human homologues have been cloned [17] and their ligand profile has been partially determined as reviewed [18,19]. P. aeruginosa expresses numerous PAMPs [20], among which are lipopolysaccharide (LPS) [21] and flagellin [22]. LPS is a glycolipid that constitutes the major portion of the outermost membrane of Gram-negative bacteria [23], while flagellin is a protein that arranges itself in a hollow cylinder to form the filament bacterial flagellum [24]. Using a mouse experimental model of P. aeruginosa-induced pneumonia, our laboratory has recently shown that the sensing of LPS by TLR4
and possibly TLR2 (as the atypical LPS of P. aeruginosa has been reported to be detected by the latter [25,26]), or of flagellin likely by TLR5 can effectively defend the lung from P. aeruginosa infection but that the absence of response by both results in hypersusceptibility to this infection [27]. This observation raised the question whether AM and EC were the effective cells of the TLR-mediated host innate immune response to P. aeruginosa infection? To address this question, we investigated in vitro the activation of these two cell types in the context of the LPS/TLR 2,4 and flagellin/TLR5 interactions. Cellular responses to P. aeruginosa were evaluated by measuring KC, TNF-α, IL-6 and G-CSF secretion, four different markers of the innate immune response observed in our previous in vivo study in which TLR2,4 have been implicated in the release of KC and TNF-α, but not of IL-6 and G-CSF [28].

Methods

Bacterial strains and growth conditions

The P. aeruginosa strain PAK, a widely studied strain of P. aeruginosa, was originally obtained from S. Lory (Harvard University, Boston, MA) [28]. This strain of P. aeruginosa is known to express a full complement of virulence factors, including pili, flagella, type II secreted enzymes, type III secreted exoenzymes S, T, and Y, exotoxin A, elastase and phospholipase and has a smooth LPS belonging to serotype 6. ΔflgC is a non-motile derivative of PAK in which the flgC gene encoding flagellin was deleted [29]. These strains were grown overnight in Luria Bertoni, purchased from Charles River Laboratories (L’Arbresle, France) and finally 30°C in LB and the OD600 nm was adjusted was washed twice with cold PBS. The pellet was suspended in one-culture was centrifuged at 3000 g for 15 min, and the cell pellet

Mouse strains

Males and females from several mouse strains were used for the experiments. TLR2−/−, TLR4−/−, TLR5−/− and MyD88−/− mice were a kind gift from S. Akira (Osaka University, Osaka, Japan) and were backcrossed eight times with C57BL/6J to ensure the approximate desired inbreds. The inoculum concentrations were verified by serial 10-fold dilutions of the bacterial suspensions and plating on LB agar [30]. To ensure that bacterial growth was identical for both strains of P. aeruginosa, in a parallel experiment a given concentration of each strain was incubated 4 hours at 37°C in LB and the OD600 nm was measured at the end of the incubation period.

Isolation, purification and culture of epithelial cells

Mouse AM were isolated as described [32]. Briefly, mice were euthanized by an intraperitoneal injection of a lethal dose of pentobarbital sodium (Ceva Santé Animale, Libourne, France), tracheas were cannulated, and lungs were washed several times with 0.7 mL PBS to provide 10 mL of broncho-alveolar lavages (BAL). Resident AM were collected from the BAL and centrifuged at 400 g for 15 min at 4°C. Pellets were then pooled in 1 mL lysis buffer (composed of NH4Cl 8.83 g/L, KHCO3 6.628 g/L, and EDTA(Na)2 1.25 g/L in H2O) for 5 min to eliminate red blood cells and then 10 mL PBS were added to stop the reaction. The solution was centrifuged again (400 g, 15 min, 4°C), Cell pellets were resuspended in RPMI 1640 medium (Invitrogen, San Francisco, CA) supplemented with 2 mM L-glutamine (Invitrogen) and 10% Fetal Calf Serum (FCS) (Hyclone, Logan, UT), counted and dispensed into 96-well tissue culture plates (TPP, Trasadingen, Switzerland). Cells (105 macrophages/well) were incubated in a 5% CO2 humidified atmosphere for 1 hour at 37°C for adhesion, before stimulation. Cells were then washed and incubated with different concentrations of bacteria suspended in the same medium, as indicated in the figure legends, and centrifuged (80 g, 4 min, 4°C) to increase the adherence between cells and bacteria, as well as to ensure similar contact between wild-type and non-motile P. aeruginosa. Conditioned media (250 μL) were collected after 4 hours of incubation, centrifuged (400 g, 5 min, 4°C) and stored at -20°C. In all cases, experiments were performed with a pool of cells collected from several mice, as indicated in the figure legends and all time points were performed in triplicate.
The majority of the cells were type I alveolar epithelial cells, in observations of the total cell population, indicating that the presence of type I cells after a 1 week of culture by examining the percentage of Clara cells, nonciliated bronchiolar cells in the mouse lung are Clara cells [40], none of our reagents were used as positive control to check the phenotype of the cell preparations. In the case of the comparison between wild-type and non-motile *P. aeruginosa*, LPS from *P. aeruginosa*, serotype 10 (purified by gel-filtration chromatography; Sigma-L8643) was also used, with exactly the same pattern of response as for *E. coli* LPS (data not shown), indicating that effects were comparable. Nonetheless, as *P. aeruginosa* LPS is far less efficient than *E. coli* LPS in terms of cell activation, and is far less purified and as such susceptible to display false positive response, we choose to employ the latter throughout the experiments.

**KC, TNF-α, IL-6 and G-CSF ELISA Assay**

Murine KC, TNF-α, IL-6 and G-CSF concentrations in cell culture supernatants were determined using DuoSet ELISA assay kits (R&D Systems, Minneapolis, MN) with TMB peroxidase substrate (KPL, Washington, D.C.).

**Statistical Analysis**

Cytokine levels were expressed as the mean±SEM. Differences between groups were assessed for statistical significance using the ANOVA test, followed by the Fisher test. A value of p<0.05 was considered statistically significant.

**Results**

**Alveolar Macrophages**

**Stimulation of WT and TLR2,4−/− alveolar macrophages by *P. aeruginosa***

We first tested whether TLR2 and TLR4 are critical for the secretion of KC, TNF-α, IL-6 and G-CSF induced by *P. aeruginosa* infection, by comparing the response of WT and TLR2,4−/− AM isolated from mouse BAL.

At the basal state (NS), cells from both strains of mice showed only a limited production of these mediators (Fig. 1). Exposure of WT AM for 4 hours to increasing concentrations of the wild-type PAK strain of *P. aeruginosa* elicited increasing productions of KC, IL-6 and TNF-α. LPS from *E. coli* and flagellin purified from *P. aeruginosa* also stimulated the production of these mediators, and acted as a positive control for the AM activation. Nevertheless, AM from both mouse strains did not produce any G-CSF (data not shown) neither in response to bacteria, nor to LPS or flagellin.

AM from TLR2,4−/− mice released a large amount of TNF-α, and KC which was slightly less than that released by WT AM, but not significantly different (Fig. 1). By contrast, the pattern of IL-6 production by TLR2,4−/− AM was clearly different. Indeed, above 5×10⁴ bacteria per well, the production was significantly decreased compared to that of WT AM. Finally, purified LPS did not induce the production of any of the cytokines, reflecting the lack of expression of TLR4 by these cells. On the contrary, flagellin still induced the production of the three cytokines, indicating that it was still recognized by AM.

These first results show that the recognition of LPS as it is expressed by live *P. aeruginosa* plays a limited role in the synthesis of TNF-α and KC, but was important for the synthesis of IL-6 by AM.

**Cytotoxicity and Total Cell Number Assay**

Cytotoxicity was measured with the CytoTox 96 Nonradioactive Cytotoxicity assay (Promega, Madison, WI), following the manufacturer’s protocol. The CytoTox 96 assay measures the lactate dehydrogenase (LDH) activity released from cells by the generation of a red colored product [42]. *P. aeruginosa* had no cytotoxic effect on the cells, at the concentrations and exposure time used.

This assay was also adapted to quantify the total cell number per well, at the end of the incubation periods. Briefly, cell samples were lysed by incubation with 200 μL per well of Lysis Solution (9% (v/v) Triton X-100 in water) at 37°C for 45 minutes. Fifty μL supernatant were then transferred to the enzymatic assay plate and mixed with 50 μL Substrate Mix. The enzymatic LDH activity was allowed to proceed for 30 minutes, protected from light, and the Stop Solution was added. The plate was read at 490 nm using an ordinary ELISA plate reader. The number of cells present is directly proportional to the absorbance values, which represent LDH activity [43].

**Reagents**

LPS from *E. coli*, serotype 0111:B4 (TLRgrade) (Alexis Biochemicals, Axxora, San Diego, CA) was used to stimulate both cell types. Recombinant *P. aeruginosa* flagellin was prepared as described before [44]. Phorbol 12-myristate 13-acetate (PMA, Sigma) was used in combination with ionomycine (Calbiochem, Darmstadt, Germany) as described before [45]. It is of note that all these reagents were used as positive control to check the phenotype of the cell preparations. In the case of the comparison of TLR2,4−/− with wild-type macrophages, LPS from *P. aeruginosa*, serotype 10 (purified by gel-filtration chromatography; Sigma-L8643) was also used, with exactly the same pattern of response as for *E. coli* LPS (data not shown), indicating that effects were comparable. Nonetheless, as *P. aeruginosa* LPS is far less efficient than *E. coli* LPS in terms of cell activation, and is far less purified and as such susceptible to display false positive response, we choose to employ the latter throughout the experiments.
Stimulation of WT and MyD88–/– alveolar macrophages by P. aeruginosa. The ligand recognition by all TLRs, except TLR3, initiates a cascade of signaling pathways involving the small adaptor protein MyD88 [46]. To determine whether P. aeruginosa-induced KC and TNF-α synthesis by AM is mediated by a TLR, the response of AM isolated from MyD88–/– mice was compared to the response of WT AM. As a result, MyD88-deficient AM did not produce any of the studied cytokines (KC, TNF-α, and IL-6), whatever the bacterial concentration used (Fig. 2). Purified LPS and flagellin did not activate these cells either. To verify that whatever the bacterial concentration used (Fig. 2). Purified LPS (1 μg/mL) and flagellin (20 ng/mL) were used as control agonists to stimulate the cells. Values represent means±SEM of three to five experiments performed in triplicate. *, p<0.05 when compared with the corresponding WT values. doi:10.1371/journal.pone.0007259.g002

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Stimulation of WT alveolar macrophages by P. aeruginosa and a ΔfliC mutant strain. To explore the importance of the TLR5/flagellin pathway, we employed two isogenic strains of P. aeruginosa, the wild-type strain and a ΔfliC mutant strain (flagellin mutant) to stimulate WT AM. When stimulated with the ΔfliC mutant strain, AM produced significantly less KC, TNF-α, and IL-6, in comparison with the wild-type bacterial strain (Fig. 3).

These data indicate that P. aeruginosa flagellin is sensed by AM and triggers their activation. TLR5, the receptor of flagellin, is thus possibly involved in the response of AM to P. aeruginosa. Nonetheless, this possible flagellin-TLR5 interaction does not fully account for the activation of AM, as the absence of flagellin does not lead to a complete lack of cytokine synthesis. Since in vivo an inability to control P. aeruginosa pulmonary infections is observed in the absence of recognition of both LPS and flagellin [27], we therefore examined the combined effect of these two ligands under in vitro situation.

Stimulation of WT and TLR2,4–/– alveolar macrophages by the ΔfliC mutant strain. TLR2,4–/– AM were stimulated by the ΔfliC mutant strain of P. aeruginosa. In response to this stimulation, TLR2,4–/– AM did not produce KC, TNF-α, nor IL-6 (Fig. 4).

Taken together, these data demonstrate that the recognition of P. aeruginosa by AM is mediated by flagellin, certainly via TLR5, but also by LPS via TLR2,4.

Stimulation of WT and TLR4,5–/– alveolar macrophages by P. aeruginosa. Finally, to confirm the role of TLR5 in the
recognition of *P. aeruginosa*, we used TLR4,5/2/2 AM, which were stimulated by the wild-type strain of *P. aeruginosa* (Fig. 5). As expected, productions of KC, TNF-α, and IL-6 were completely abolished in these cells. These results confirm the previous data obtained with the Δ*fliC* mutant strain, and show that the recognition of flagellin by AM is indeed mediated by TLR5.

The entire study thus demonstrates that only two ligand/receptor pairs are necessary for the activation of AM by *P. aeruginosa* in terms of KC, TNF-α, and IL-6 production, R2,4/LPS and TLR5/flagellin. Either one is sufficient and when both are missing AM are unable to produce the studied cytokines.

Epithelial Cells

**Stimulation of WT and MyD88−/− epithelial cells by *P. aeruginosa***. To better understand the mechanisms involved in the recognition of *P. aeruginosa* by the lung, we investigated the response of EC to this pathogen, since like AM, they are known to be on the first line of defense against invading pathogens. Primary EC purified from the lungs of mice were therefore stimulated with the same bacteria as for AM and the same cytokines were assayed.

At the basal state (NS), cells from both WT and MyD88−/− mice showed only a limited production of these mediators (Fig. 6). Exposure of EC from WT mice to increasing concentrations of the wild-type strain of *P. aeruginosa* for 4 hours elicited increasing amounts of KC and IL-6, but not of TNF-α or G-CSF (data not shown). Purified LPS and flagellin also stimulated the production of KC and IL-6, and acted as a control for the EC activation.

To determine whether the EC response to *P. aeruginosa* infection was mediated by a TLR, cells isolated from MyD88−/− mice were stimulated by the wild-type strain, in comparison with WT EC. Like for AM, MyD88-deficient EC did not produce KC or IL-6, whatever the bacterial concentration used (Fig. 6). Purified LPS and flagellin did not activate these cells either. To verify that MyD88−/− EC were nonetheless able to produce these two cytokines, we used the same combination of PMA and ionomycin as a positive control. PMA plus ionomycin added to MyD88−/− EC induced a 8-fold and 3-fold secretion of KC and IL-6, respectively. These secretions were similar to those obtained with the WT EC (data not shown).

These results show that i) at least one TLR signaling via MyD88 is involved in the recognition of *P. aeruginosa* by EC, and that ii) no MyD88-independent pathway is involved in the secretion of KC and IL-6.

**Stimulation of WT and TLR2,4−/− epithelial cells by *P. aeruginosa* and the Δ*fliC* mutant strain**. To search for the TLR that was implicated, we followed the same experimental approach with EC as we did with AM. TLR2,4−/− EC produced a large amount of KC and IL-6 in response to wild-type bacteria, though slightly reduced compared to WT EC (Fig. 7a). As seen
with AM, purified LPS did not induce the production of any of the cytokines, while flagellin did, reflecting the lack of expression of TLR4 by these cells. These experiments show that the recognition of *P. aeruginosa* LPS would only play a limited role in the activation of primary EC of the mouse and support studies done on established human EC lines [47].

We next stimulated WT EC with the ΔfliC mutant strain, in response to which they produced approximately the same amount of KC and IL-6, in comparison with the wild-type strain (Fig. 7b). These data indicate that flagellin as it is expressed by live *P. aeruginosa* does not seem to play an important role in the activation of EC in terms of KC and IL-6 production.

To verify the implication of two pathways, as we found for AM, TLR2,4/−/− EC were stimulated by the ΔfliC mutant strain of *P. aeruginosa*. In response to this stimulation, TLR2,4/−/− EC did not produce any KC and IL-6 (Fig. 7c).

Taken together, these data demonstrate that *P. aeruginosa* is sensed by EC through either LPS recognition by TLR2,4, or flagellin recognition probably by TLR5. 

**Stimulation of WT and TLR4,5−/− epithelial cells by *P. aeruginosa***. Finally, to confirm that the recognition of flagellin is mediated by TLR5, we used TLR4,5−/− EC, which were stimulated by wild-type *P. aeruginosa* (Fig. 8). As seen with AM, the production of KC and IL-6 were completely abolished in these cells.

These results confirm the previous data obtained with the ΔfliC mutant strain, and show that the recognition of flagellin by the EC is indeed mediated by TLR5. In summary, for EC as for AM, at least two pathways are at play for the sensing of *P. aeruginosa*, and either one is sufficient for an efficient recognition of these bacteria by EC.

**Discussion**

The message inferred from the present study is straightforward, *i.e.* AM and EC, the two cell types that first encounter *P. aeruginosa* during the process of lung infection, sense the bacterium and trigger an innate cellular response through a MyD88-dependent pathway and even exclusively through this pathway in the case of KC and IL-6 synthesis. It is of note that recently von Bernuth et al. [48] have shown that humans deficient in MyD88 have an increased susceptibility to *P. aeruginosa*. From these data one may consider the implication of MyD88-dependent but TLR-independent cascade, *i.e.* the IL-1 receptor (IL-1R) pathway [49]. However, it is unlikely that IL-1R is involved as we saw no cytokine response to challenge with this organism when the participation of certain TLR is prevented. In fact, in our hands at play are only two pairs of ligand-receptor, namely LPS-TLR2,4 and flagellin-TLR5. Moreover, we showed that these two pathways have a redundant role as suppression of only one of the two is not followed by a large significant loss of cell response, and that by contrast, when both are disrupted, there was a dramatic crippling of the responses that we examined.

These results obtained in *vitro* are in agreement with *in vivo* data from Feuillet et al. [50] showing that TLR4,5−/− mice are hypersusceptible to lung infection by wild-type *P. aeruginosa*, and from our laboratory [27] demonstrating that TLR4 and TLR5 play major but redundant roles in controlling bacterial replication during host defense against *P. aeruginosa* pneumonia. By contrast, they differ from the data reported by Skerrett et al. [51] showing that TLR2,4−/− mice were not hypersusceptible to a *P. aeruginosa*
strain lacking flagellin. As previously explained [27] this could simply be due to the use of a low bacterial load by the latter authors compared to the former. Nonetheless, the same group [51] reported that bone marrow cells lacking both TLR2 and TLR4 exhibited a reduced TNF-α production to heat-killed flagellated P. aeruginosa but no response to the heat-killed ΔfliC mutant. We extended their data using in vitro experimental conditions more closely related to the in vivo situation i.e. AM but not bone marrow-derived macrophages and live but not heat-killed bacteria. Thus, we report here on the synthesis of other mediators, KC and IL-6, the response of another cell type, EC, and the effect of another defect, TLR5 deficiency. Such a study was necessary as bone marrow-derived macrophages have been reported to be not responsive to flagellin [52,53] and that heat-killed bacteria do not release flagellin in the medium as do live bacteria (Ramphal, unpublished observation). The remarkable point is that AM and EC behave in almost the same way in response to P. aeruginosa, both using TLR2,4 and TLR5 for the recognition of the bacterium and to engage an innate immune response. Indeed, Ipaf could have been implicated as this intracellular receptor has been shown to sense flagellin [54,55], although it is implicated in the processing of immature to mature IL-1β and IL-18 [56] and not of IL-6 and KC. From the in vivo situation, it can be also deduced that Ipaf probably plays a minor role if any during lung infection as mice lacking both TLR4 and 5 are hypersusceptible to P. aeruginosa, dying within less than a day [50]. This lack of impact on the survival has been observed experimentally although Ipaf seems to be required for the early elimination of the bacterium in vivo [46]. Another aspect that needs to be discussed here is the involvement of the Cystic fibrosis transmembrane conductance regulator (CFTR) as a receptor of P. aeruginosa [57]. Indeed, Pier et al. have shown that CFTR may mediate the innate immune response to P. aeruginosa [7,58,59]. Thus, patients with non-functional CFTR, i.e. CF patients, are more susceptible to P. aeruginosa infection than other patients. Nevertheless, these patients develop chronic lung infections, which are

**Figure 7. Effect of TLR2,4 and flagellin expression on KC and IL-6 synthesis by lung epithelial cells challenged with P. aeruginosa.** Lung epithelial cells (EC) collected from mice were infected for 4 h with increasing concentrations (number of bacteria/well) of P. aeruginosa. Different combinations were used, i.e., WT and TLR2,4−/− mice cells stimulated with the wild-type bacteria strain PAK (7a); WT mice cells stimulated with the wild type bacteria strain PAK and its mutant ΔfliC (7b); WT and TLR2,4−/− mice cells stimulated with the ΔfliC mutant (7c). NS, non-stimulated cells cultured with medium only. LPS (1 μg/mL), and flagellin (20 ng/mL) were used as control agonists to stimulate the cells. Values represent means±SEM of three to five experiments performed in triplicate. *, p<0.05 when compared with either the corresponding WT mice or wild-type PAK strain values.

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immune response to synthesis of cytokines and chemokines would be more important BAL [48]. This suggests that modify the production of KC, TNF-\(\alpha\), and IL-6 in mice synthesize very low concentrations of IL-6 compared to those recovered from AM from WT mice (see Fig. 1). However, the difference is not so pronounced with EC (see Fig. 5). These data have to be compared to those observed in vivo [20]. Surprisingly, there is no correlation between the in vivo and in vitro experiments when comparing mediator production by WT and TLR2,4\(^{-/-}\) mice. Indeed, TLR2,4 have been implicated in the release of KC, TNF-\(\alpha\), but not of IL-6 in the in vivo experiments [20] while in the present study TLR2,4 expressed by either AM or EC are implicated in IL-6 synthesis but only marginally in that of KC (AM and EC) and TNF-\(\alpha\) (AM). These contradictory data poses a problem as to which cell types explain the in vivo data? Apparently it is neither AM nor EC. Several hypotheses can be raised such as the presence of dendritic cells, or the recruitment of polymorphonuclear neutrophils, or also a mechanism of cooperation between AM and EC. To answer this question, a thorough study will be necessary, in vivo by using co-culture of AM and EC, and in vitro by identifying the cell types producing a given mediator by immunocytochemistry. Another interesting question is the following. As TNF-\(\alpha\), KC, and IL-6 synthesis are all dependent on NF-kB nuclear translocation, which aspect of the signaling pathway(s) triggered by P. aeruginosa in TLR2,4\(^{-/-}\) AM leads to TNF-\(\alpha\) and KC and not to IL-6 formation?

One can wonder if the presently described redundant mechanism based on the recognition of LPS by TLR2,4 and of flagellin by TLR5 is operative in human cells. At least it is firmly established that human AM and EC express TLR2,4 and are activated by LPS. Also, both human cell populations express TLR5 and are activated by flagellin [64,65,66,67,22]. Redundancy is probably essential in humans given the fact that approximately 10% of Caucasians carry a polymorphism in the ligand-binding domain of TLR5 (stop codon) that acts in a dominant fashion to abolish flagellin signaling [68]. This polymorphism is associated with a slightly increased susceptibility to Legionnaires’ disease, but otherwise the carriers are apparently not more susceptible to infection with flagellated bacteria as demonstrated with Salmonella enterica [69]. At the opposite end, it has been recently reported that the TLR5 mRNA expression is increased in cystic fibrosis airway EC and that, as a probable consequence, these cells almost exclusively rely upon TLR5 to sense P. aeruginosa [70]. This phenotype would be beneficial in the frame of the chronic P. aeruginosa infection observed in these patients, although one can speculate that it could also be at the origin of the inflammatory status characterizing the lung of cystic fibrosis patients.

Author Contributions
Conceived and designed the experiments: ER VB MC. Performed the experiments: ER VB IGV. Analyzed the data: ER VB MC. Contributed reagents/materials/analysis tools: IGV LT RR. Wrote the paper: ER VB MC.

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