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Similarity of Gene Expression Patterns in Human Alveolar Macrophages in Response to *Pseudomonas aeruginosa* and *Burkholderia cepacia*

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To determine if differences in the severity of pulmonary infection in cystic fibrosis seen with late isolates of *Pseudomonas aeruginosa* and *Burkholderia cepacia* are associated with differences in the initial response of alveolar macrophages (AM) to these pathogens, we assessed gene expression changes in human AM in response to infection with a laboratory strain, early and late clinical isolates of *P. aeruginosa*, and *B. cepacia*. Analysis of gene expression changes at the RNA level using oligonucleotide microarrays, following exposure to laboratory *P. aeruginosa* strain PAK, showed significant ($P < 0.01$) >2.5-fold upregulation of 42 genes and >2.5-fold downregulation of 45 genes. The majority of the changes in gene expression involved genes as part of inflammatory pathways and signaling systems. Interestingly, similar responses were observed following exposure of AM to early and late clinical isolates of *P. aeruginosa*, as well as with *B. cepacia*, suggesting that the more severe clinical outcome of infections with late clinical isolates of *P. aeruginosa* or with *B. cepacia* cannot be explained by differences in the early interactions of these organisms with the human AM, as reflected by the similarity of gene expression changes in response to exposure of AM to these pathogens.

Cystic fibrosis (CF), a hereditary disorder caused by mutations of the CF transmembrane conductance regulator (CFTR) gene, is characterized by chronic infection of the respiratory epithelium by *Pseudomonas aeruginosa* (6, 14, 33). While the early *P. aeruginosa* isolates from CF patients are planktonic, free-floating organisms, *P. aeruginosa* isolates from the respiratory tract later in the disease often produce a mucoid biofilm and are difficult to eradicate (6, 14, 16, 33). In some cases of CF, there is superinfection with *Burkholderia cepacia*, a pathogen with a distant relationship to *P. aeruginosa* (16, 37). *B. cepacia* superinfection is an ominous sign in CF, as it is invariably associated with a progressive downhill course and death (14, 16, 37).

Although the airway epithelial cells are the dominant cell type affected by CFTR dysfunction in the CF lung, the interaction of organisms with cells of the pulmonary host immune system may be important in determining the extent of the inflammatory responses to these pathogens and the lack of bacterial clearance from the airways that characterizes the disease (5, 14, 18, 20, 21, 30). Alveolar macrophages (AM), the resident mononuclear phagocytes in the respiratory tract, are the first line of cell-mediated defenses against inhaled organisms (4, 18, 25). Besides their scavenger function upon interaction with pathogens, AM release a variety of mediators that play a central role in the inflammatory response to organisms, such as the recruitment of polymorphonuclear phagocytes to the respiratory epithelium (4, 18, 25, 26). The phagocytic contribution of AM to the defense against *P. aeruginosa* is thought to be negligible (9), and studies with mice have shown that temporary elimination of AM did not affect the course of *P. aeruginosa* pulmonary or systemic infection; however, it affected cytokine and chemokine production (13). AM likely play a role in the control and maintenance of the pulmonary inflammatory response to *P. aeruginosa* (9, 20, 21, 23, 27, 41). As AM express only negligible amounts of the CFTR gene, mutations in CFTR associated with CF are unlikely to be associated with AM dysfunction per se (44). This has also been confirmed by studies with transgenic G551D mice expressing the human CFTR gene in lung epithelium or AM, with an increase in pulmonary clearance of *P. aeruginosa* and a decrease in inflammatory cytokines in epithelial cell-corrected, but not macrophage-corrected, mice (28). However, the epithelial abnormalities associated with CFTR mutations present the AM with the challenge of clearing a large, persistent burden of *P. aeruginosa* and, in some late cases, *B. cepacia*. AM isolated from patients with CF produce larger amounts of inflammatory cytokines compared to non-CF AM (21, 30). The inability of AM to efficiently clear organisms like *P. aeruginosa* and *B. cepacia* from the lung, in combination with an exacerbated inflammatory milieu, may be important factors in the interaction of these pathogens with the host defense system in CF (5, 7, 9, 14, 15, 20, 41). One clue to the inability of AM to efficiently clear *P. aeruginosa* is the observation that AM are more susceptible than epithelial cells to *P. aeruginosa*-induced apoptosis (11, 12, 17, 40, 42, 43).

Since the clinical outcome of infection with late strains of *P.*

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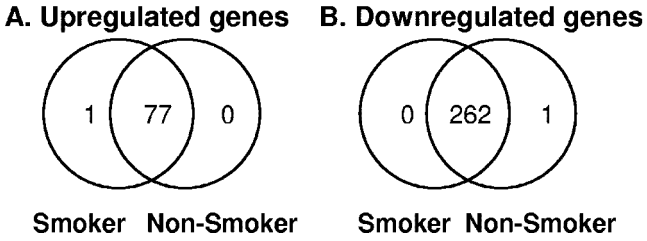

FIG. 1. Venn diagram representation of gene expression changes following infection with PAK in AM derived from smokers and nonsmokers. AM were obtained by bronchoalveolar lavage from smokers ($n = 8$) or nonsmokers ($n = 4$) and infected in vitro with laboratory *P. aeruginosa* strain PAK at 5 CFU/cell. Gene expression changes in PAK-infected AM were compared to uninfected AM. Differences were evaluated using a nonparametric test ($P < 0.05$) with Benjamini-Hochberg correction.

aeruginosa and with *B. cepacia* is more severe than with early isolates of *P. aeruginosa*, we hypothesized that these differences could be due to a differential response of the AM upon exposure to these different pathogens. To understand the interaction of human AM with *P. aeruginosa* and *B. cepacia*, we used an unbiased approach based on microarray technology to assess the early changes in human AM gene expression in response to short time points of exposure to two clinical strains of *P. aeruginosa* (one early and one late isolate) and a strain of *B. cepacia*.

Changes in gene expression in AM infected with *P. aeruginosa* strains and *B. cepacia*. The *P. aeruginosa* strains used included laboratory strain PAK (provided by A. Prince, Columbia University, New York, NY) and two clinical strains isolated from the sputum of an individual with CF, AD2A and AD15B (provided by J. Burns, University of Washington, Seattle). AD2A is an early clinical isolate, and AD15B is a late clinical isolate; both were derived from the same individual. The *B. cepacia* strain was J2315, genomovar 3 (kindly provided by John LiPuma, University of Michigan). Human AM were obtained by bronchoalveolar lavage of healthy, nonsmoking or smoking volunteers (36). AM represented >95% of the cells in all cases. Cell viability (always >90%) was determined by trypan blue exclusion. The yield of AM derived from individuals with a history of smoking was four to five times higher compared to nonsmokers. After 3 h of adherence, the cells were infected with the bacteria at a dose of 5 CFU/cell in RPMI 1640–25 mM HEPES, pH 7.4, for 4 h. The viability of the cells following 4 h of infection was >85% as assessed by trypan blue exclusion for all bacterial strains. All analyses were carried out with the Affymetrix HuGeneFL chip using the protocols from Affymetrix (Santa Clara, CA) and the GeneSpring software (Silicon Genetics, Redwood City, CA). As the yield of AM derived from nonsmoking individuals was not sufficient to allow assessment of more than two conditions (e.g., control and infection with PAK) and the preliminary analysis of gene expression changes in AM infected with PAK demonstrated that the results were independent of whether the AM were derived from smokers or nonsmokers (Fig. 1), all subsequent experiments were performed with AM derived from smokers, which by virtue of their increased numbers (2, 4, 38) allowed infection with PAK, AD2A, AD15B, and *B. cepacia* within the same experiment and sample.

There were a total of 87 genes differentially expressed in PAK-infected cells versus uninfected controls, with a *P* value of <0.01 and a fold change of >2.5-fold (up- or downregulated). Gene expression changes in AM following exposure to PAK (the laboratory *P. aeruginosa* strain) for 4 h showed significant upregulation ($P < 0.01$, >2.5-fold change) of a total of 42 genes, which were categorized into the following five functional groups: secreted proteins, nuclear proteins, metabolism and enzymes, signal transduction and growth control, and cell surface proteins (Table 1). The category with the greatest number of upregulated genes (15 out of 42) was secreted proteins, including cytokines and chemokines known to be induced by bacterial stimulation of macrophages (25, 26, 34, 35). The strongest upregulation was seen for interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) (102- and 62-fold compared to controls, respectively). The second largest functional group consisted of genes coding for factors related to signal transduction and growth control (12 out of 42). The majority of these are known to be induced by cytokine signaling (e.g., TNF receptor-associated factor IκB). Some of the upregulated genes in this category, such as Jagged, have not been previously reported as associated with bacterial stimulation of macrophages. A smaller group of upregulated genes codes for nuclear proteins involved in transcriptional regulation, including the enhancer of zeste homolog 2 (EZH2), also not previously associated with bacterial activation of macrophages.

A total of 45 genes were significantly ($P < 0.01$) downregulated by >2.5-fold in PAK-infected AM samples (Table 2). In contrast to the genes upregulated following infection with PAK, none of these were genes coding for secreted proteins. The majority were in the category of genes coding for factors involved in signal transduction and growth control (11 out of 45), as well as metabolism and enzymes (12 out of 45). Another functional category with several members was that of genes coding for nuclear proteins (8 out of 45). Overall, decreases in gene expression levels following *P. aeruginosa* infection of AM were of lesser magnitude than the changes observed for upregulated genes. The two largest decreases in gene expression levels were for Lyl-1 and docking protein 1.

The *n*-fold change for the 87 genes up- or downregulated in AM in response to PAK infection were also assessed following infection of AM with *P. aeruginosa* clinical isolates AD2A and AD15B and with *B. cepacia*. The *n*-fold changes were calculated individually for each strain as the ratio of the average expression level in infected AM samples to that of uninfected controls (Tables 1 and 2). Similar changes were observed following infection with AD2A, AD15B, and *B. cepacia*. The 42 upregulated genes and the 45 downregulated genes selected by comparison of PAK-infected cells with uninfected controls were also upregulated or downregulated following exposure of AM to both *P. aeruginosa* clinical isolates, as well as to *B. cepacia*, and the extent of upregulation was similar for all of the strains. Infection with AD2A appeared to lead to higher induction levels for the upregulated genes, especially in the categories of nuclear proteins, metabolism and enzymes, and cell surface proteins (Table 1), and to a less pronounced decrease in all categories (Table 2). However, none of these differences were statistically significant. To visualize the similarities of the overall patterns of gene expression changes fol-

TABLE 1. Upregulation of genes in AM following infection with *P. aeruginosa* strains and *B. cepacia*

Category and gene	Description	*n*-fold upregulation[a]				*P* value[b]
		PAK-infected AM	2A-infected AM	15B-infected AM	*B. cepacia*-infected AM	
Secreted proteins						
Y00081	IL-6	101.8	145.4	138.8	104.0	0.0009
X02910	TNF	61.9	69.9	68.8	54.6	0.0003
M69203	Cytokine (presumed)	21.03	23.6	29.5	35.7	<0.0001
M27436	Thromboplastin	17.35	74.5	46.2	19.9	0.0054
J04130	MIP-1β	15.48	11.2	14.5	20.6	<0.0001
M57731	MIP-2α	14.76	15.4	14.5	12.3	0.0024
M23178	MIP-1α	14.53	11.3	15.4	18.1	0.0000
U64197	MIP-3α	13.63	27.3	19.9	16.1	0.0009
X13967	Leukemia inhibitory factor	13.00	33.8	18.8	10.1	0.0008
X53800	MIP-2β	11.54	17.7	10.3	9.4	0.0026
J05008	Endothelin 1	10.99	20.1	7.0	12.0	0.0013
X04500	IL-1β	9.35	5.4	7.9	12.1	<0.0001
M28130	IL-8	3.45	4.4	4.0	3.7	0.0048
D14874	Adrenomedullin	3.07	10.3	3.3	3.7	0.0031
M65290	IL-12B (p40)	2.96	3.4	2.2	3.4	0.0075
Nuclear proteins						
X97748	Pentaxin-related gene	16.21	22.3	22.4	19.4	0.0070
J04102	E26 oncogene homolog 2	3.30	6.0	3.6	2.9	0.0040
D79994	Kidney ankyrin repeat-containing protein	2.99	4.7	4.2	4.3	0.0047
U61145	Enhancer of zeste homolog 2	2.61	4.0	3.1	2.6	0.0012
Metabolism/enzymes						
D10522	Myristoylated alanine-rich protein kinase C substrate	7.71	56.3	14.7	12.5	0.0017
X68277	Dual-specificity phosphatase 1	4.71	9.8	5.5	3.5	0.0048
M90657	Transmembrane 4 superfamily member 1	4.08	7.0	1.2	4.4	0.0050
U15932	Dual-specificity phosphatase 5	3.62	8.5	4.6	3.9	0.0055
U19523	GTP cyclohydrolase 1	2.91	16.3	6.3	4.4	0.0021
M13792	Adenosine deaminase	2.89	4.6	4.2	3.9	0.0026
Signal transduction/ growth control						
S81914	Immediate early response 3	18.72	22.2	22.2	21.6	0.0007
U19261	TNF receptor-associated factor 1	6.28	7.9	6.9	7.1	0.0015
M59465	TNF-induced protein 3	5.54	12.3	7.7	4.8	0.0097
M69043	IκBα	4.76	5.5	4.8	3.6	0.0030
U61276	Jagged-1	4.37	6.7	5.5	5.1	0.0044
L40379	Thyroid receptor interacting protein 10	3.40	5.8	3.7	3.3	0.0096
X61123	B-cell translocation gene 1	3.68	6.2	2.9	2.8	0.0045
D79206	Ryudocan core protein	3.50	9.3	7.5	3.4	0.0015
X07743	Pleckstrin	2.98	5.7	4.8	3.7	0.0070
D30755	TNFAIP3 interacting protein 1	2.69	8.9	3.1	2.7	0.0009
S59049	Regulator of G-protein signalling 1	2.55	3.7	2.4	2.0	0.0097
AF005775	CASP8 and FADD-like apoptosis regulator	2.51	14.4	3.6	2.3	0.0025
Cell surface proteins						
AF014958	Chemokine receptor-like 2	5.08	10.7	11.0	5.4	0.0002
M92357	TNF-induced protein 2	4.78	7.8	6.9	5.2	0.0006
X68486	Adenosine A2b receptor	3.86	5.0	3.8	3.8	0.0047
M29696	IL-7 receptor	2.99	4.7	4.2	4.3	0.0014
Z37987	Glypican	2.72	17.1	7.2	3.0	0.0097

[a] Ratio of average gene expression level of infected AM samples/uninfected AM samples; $n = 8$ for PAK, $n = 3$ for clinical *P. aeruginosa* isolates AD2A and AD15B, and $n = 6$ for *B. cepacia.*

[b] *P* value based on comparison of PAK-infected AM with uninfected controls.

lowing infection with the different *P. aeruginosa* strains and *B. cepacia*, the *n*-fold gene expression changes for the genes up- or downregulated by more than 2.5-fold in AM following infection with PAK (ordered by gene identification number within each functional category) were plotted for all strains (Fig. 2A and B).

Relevant to CF, studies of the response of the host to the different forms of *P. aeruginosa*, as well as *B. cepacia*, have focused primarily on pathogen interaction with epithelial cells (19, 24). Differences in the induction of cytokine responses have been observed with different *P. aeruginosa* isolates or *B. cepacia* (31). *B. cepacia* is also thought to be located mostly

TABLE 2. Downregulated genes in AM following infection with *P. aeruginosa* strains and *B. cepacia*

Category and gene	Description	*n*-fold downregulation[a]				*P* value[b]
		PAK-infected AM	2A-infected AM	15B-infected AM	*B. cepacia*-infected AM	
Nuclear proteins						
M22638	Lyl-1	11.40	5.4	13.2	4.9	0.0003
L41067	Nuclear factor of activated T cells	4.62	1.7	4.2	2.9	0.0001
U81556	Nuclear LIM interactor-interacting factor 2	3.28	1.7	2.3	2.2	0.0016
X70683	SRY box 4	3.12	3.4	6.4	2.9	0.0008
D21852	R3H domain	3.02	1.2	1.6	1.6	0.0093
L25931	Lamin B receptor	2.98	1.6	2.3	2.1	0.0008
U73524	ATP/GTP-binding protein	2.66	1.1	2.7	1.6	0.0037
Z48633	Retrotransposon	2.63	1.9	2.4	2.6	0.0025
Metabolism/enzymes						
U78190	GTP cyclohydrolase I regulatory protein	3.69	2.3	1.8	3.3	0.0019
X62055	Protein tyrosine phosphatase 6	3.42	2.5	2.6	2.9	0.0007
U46689	Aldehyde dehydrogenase 10	2.98	1.9	1.5	2.0	0.0020
U72342	Platelet-activating factor acetylhydrolase	2.96	1.4	1.7	1.3	0.0009
U81375	Nucleoside transporter 1	2.95	1.9	1.7	2.5	0.0012
U75370	Mitochondrial RNA polymerase	2.86	2.3	2.2	2.0	0.0039
L40401	Acyl coenzyme A thioesterase	2.85	2.3	2.0	2.0	0.0011
J03459	Leukotriene A4 hydrolase	2.73	2.0	1.5	2.1	0.0027
X74008	Protein phosphatase 1	2.71	0.8	1.5	2.1	0.0028
J03909	Lysosomal thiol reductase	2.59	1.5	1.4	1.8	0.0009
D63876	ARF binding protein 3	2.58	1.2	1.8	2.4	0.0017
D83782	SREBP cleavage-activating protein	2.55	2.5	1.9	3.3	0.0004
Signal transduction/growth control						
U70987	Docking protein 1	6.12	3.5	6.5	4.2	0.0000
L13738	p21cdc42Hs kinase	4.35	2.0	3.3	3.6	0.0034
D89077	Src-like adaptor	3.75	0.9	3.0	3.6	0.0011
AF015913	Skb 1 homolog	3.37	0.8	1.9	2.1	0.0008
Z48541	Phosphotyrosine phosphatase U2	3.23	2.3	2.6	2.6	0.0059
D16227	Hippocalcin-like 1	3.13	0.9	2.2	1.2	0.0057
AB002382	Catenin delta 1	3.07	1.3	2.2	1.1	0.0018
J05614	Proliferating cell nuclear antigen	3.01	1.7	2.6	2.4	0.0037
X91809	Regulator of of G-protein signalling 19	2.75	1.6	1.7	2.0	0.0001
X16416	v-abl homolog 1	2.75	1.2	1.8	2.0	0.0004
U16811	BCL2 antagonist 1	2.66	1.6	2.8	2.3	0.0000
Cell surface proteins						
U79288	Cadherin 13	4.63	3.0	2.0	2.1	0.0005
D50683	Transforming growth factor beta receptor II	3.63	1.4	2.6	3.3	0.0078
U90546	Butyrophilin	3.60	1.0	4.3	2.1	0.0087
X84709	Fas associated via death domain	3.23	1.6	2.6	2.2	0.0001
X13334	CD14	2.98	1.9	1.8	2.1	0.0073
X60299	Kallmann syndrome I gene	2.64	1.6	1.5	1.4	0.0072
U25956	Selectin P ligand	2.55	1.8	2.2	2.5	0.0065
Not classified						
D43947	KIAA0100 gene product	4.44	0.8	2.0	2.4	0.0014
D86961	HMGIC fusion partner like 2	3.85	1.0	3.3	4.6	0.0013
D29642	KIAA0053 gene product	3.10	1.6	2.5	3.3	0.0006
U95740	Hypothetical gene BC008967	3.06	1.7	4.9	2.4	0.0002
U06631	Homolog to PC326	2.97	2.6	1.9	3.2	0.0019
Y09022	Not56-like protein	2.68	2.0	2.3	2.3	0.0030
U68494	Expressed sequence tag	2.57	0.7	1.9	1.8	0.0033

[a] Ratio of average gene expression level of uninfected AM samples/infected AM samples; $n = 8$ for PAK, $n = 3$ for clinical *P. aeruginosa* isolates AD2A and AD15B, and $n = 6$ for *B. cepacia*.

[b] *P* value based on comparison of PAK-infected AM with uninfected controls.

intracellularly, in contrast to the extracellular location of *P. aeruginosa* (8). *P. aeruginosa* and *B. cepacia* also seem to differ in their abilities to induce cytolysis or apoptosis in cells with which they interact, important features in the creation of an inflammatory environment.

As an important component of the innate immune system in the lung, AM are in close contact with pulmonary pathogens such as *P. aeruginosa* and *B. cepacia* and are found in abundance in the inflamed local milieu of the CF lung (20, 21). Studies with mice suggest that AM are not directly involved in

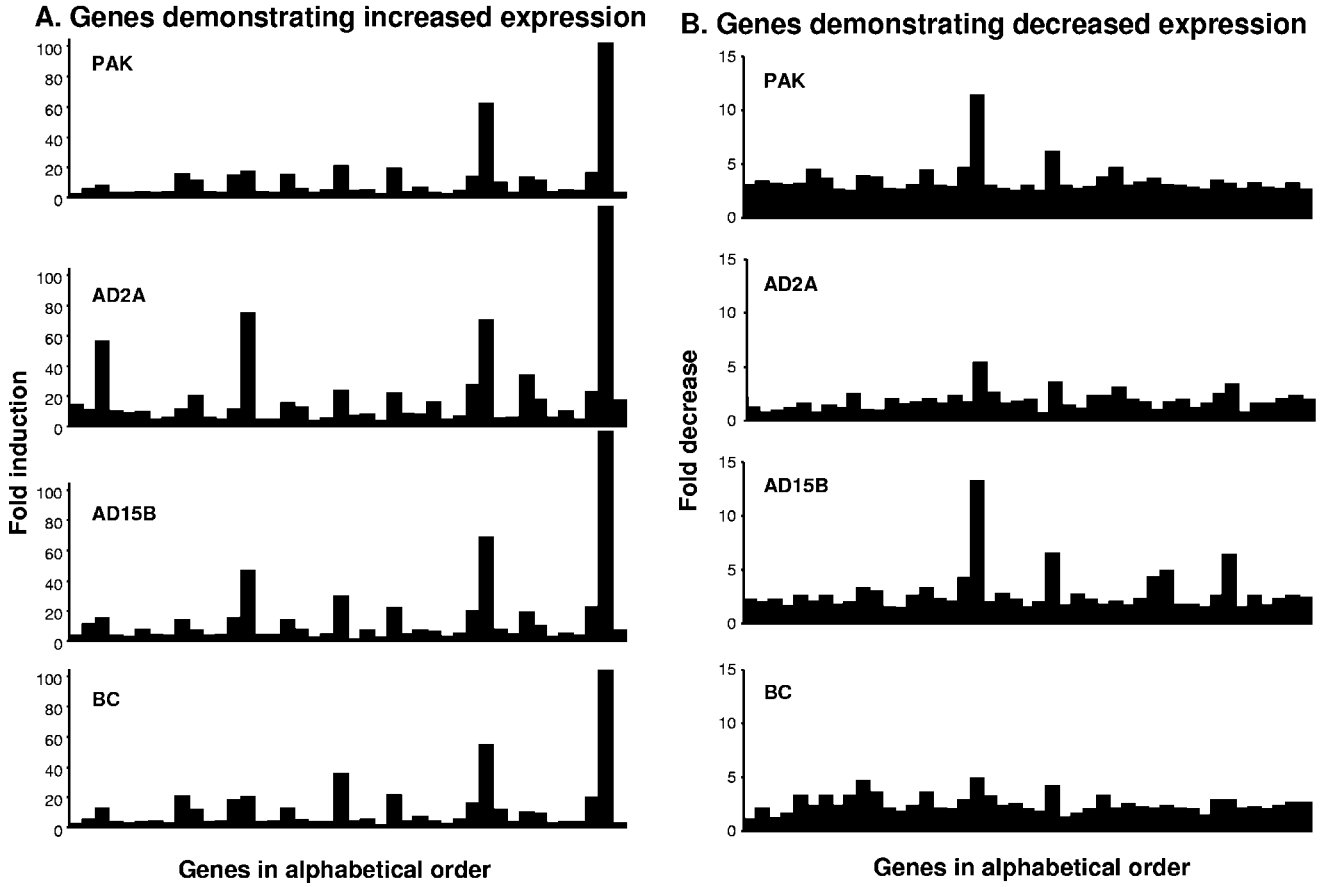

FIG. 2. Gene expression changes in human AM following infection with laboratory *P. aeruginosa* strain PAK, clinical *P. aeruginosa* isolates AD2A (early) and AD15B (late), and *B. cepacia*. The ordinate shows the *n*-fold up- or downregulation in infected AM for all genes listed in Tables 1 and 2. The abscissa shows the individuals genes, ordered by gene identification number within each functional category for the genes increased (A) or decreased (B) following infection.

the clearance of *P. aeruginosa* (21). However, since the AM response to the bacteria contributes to the composition of the inflammatory milieu on the airway epithelial surface, it is relevant to ask whether there is variability of the response of AM to different *P. aeruginosa* strains or *B. cepacia*. Interestingly, the present study demonstrates that the human AM response to short-term exposure of various *P. aeruginosa* isolates or *B. cepacia* is relatively uniform. Although mRNA levels for a variety of cytokines and chemokines were rapidly induced following exposure of the AM to the various *Pseudomonas* strains and *B. cepacia*, the short-term response of AM to *P. aeruginosa* isolates and *B. cepacia* was, at least in vitro, relatively homogeneous. These homogenous responses are dominated by the induction of inflammatory cytokines and chemokines, which are important in the host responses that lead to elimination of the bacteria in the healthy host, with TNF-α and IL-6 showing the highest levels of induction (3, 26). Similarly, signal transduction pathways known to be related to cytokine activation, especially those mediated by transcription factor NF-κB (1, 3, 26), were rapidly induced to similar extents following exposure to the different *P. aeruginosa* isolates and *B. cepacia*. Future experiments need to identify the specificity of the gene expression changes with those observed with other, not lung or CF-related, gram-negative pathogens.

Confirmation by TaqMan real-time reverse transcription (RT)-PCR of selected genes. Most of the genes up- or downregulated in AM exposed to the *P. aeruginosa* strains or to *B. cepacia* corresponded to genes coding for inflammatory mediators or other proteins involved in pathways previously described as part of the response of AM to pathogens (25, 26, 34, 35). The agreement between the microarray data presented here and previous studies using other techniques to measure mRNA levels is in itself confirmation of the validity of the approach (10). Therefore, we chose to focus the validation of the results obtained by microarray analysis on four genes that had not been previously described in association with AM activation or response to pathogens: Jagged, EZH2, Lyl-1, and DOK1. The gene expression changes observed by microarray analysis were independently confirmed by TaqMan real-time RT-PCR as follows: Jagged was upregulated (PAK, 21.1-fold ± 6.7-fold; *B. cepacia*, 21.3-fold ± 3.6-fold; Fig. 3A), EZH2 was upregulated (PAK, 4.3-fold ± 0.4-fold; *B. cepacia*, 5.1-fold ± 0.6-fold; Fig. 3B), DOK1 was downregulated (PAK, 5.3-fold ± 0.4-fold; *B. cepacia*, 3.4-fold ± 0.8-fold; Fig. 3C), and Lyl-1 was downregulated (PAK, 4.1-fold ± 1.3-fold; *B. cepacia*, 4.8-fold ± 0.7-fold; Fig. 3D). Compared to the gene expression changes observed by microarray analysis, the magnitude of the changes was higher for the upregulated Jagged and EZH2

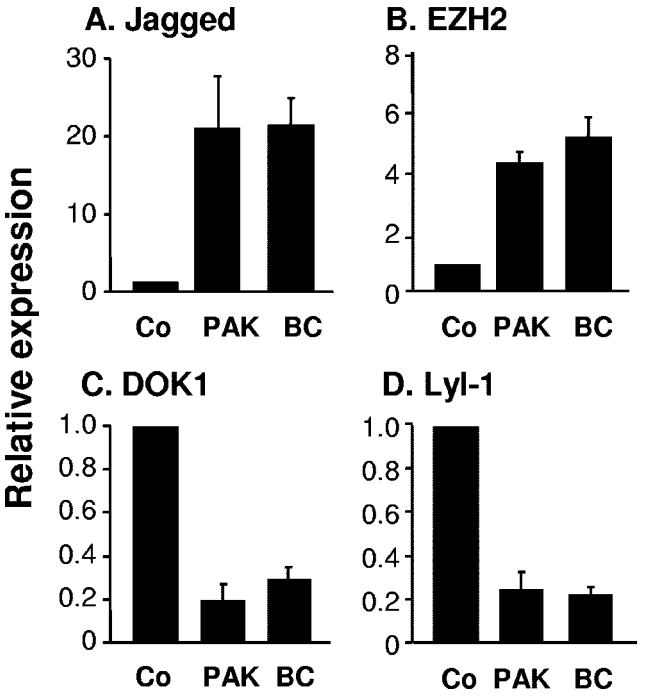

FIG. 3. Confirmation of microarray results by TaqMan real-time RT-PCR. Human AM were infected in vitro with PAK or *B. cepacia* (5 CFU/cell) for 4 h. RNA levels for four genes were measured by quantitative real-time RT-PCR. A, Jagged; B, EZH2; C, DOK1; D, Lyl-1. Relative expression levels in the infected samples were calculated using the ΔΔCt method, using 18S rRNA as an internal normalization control (Co), and the expression levels in the uninfected controls (naive) as the calibrator levels, set at 1. Shown is the mean ± the standard error of four independent experiments, using four individual AM samples, split for the three conditions used: uninfected, PAK infected, and *B. cepacia* infected.

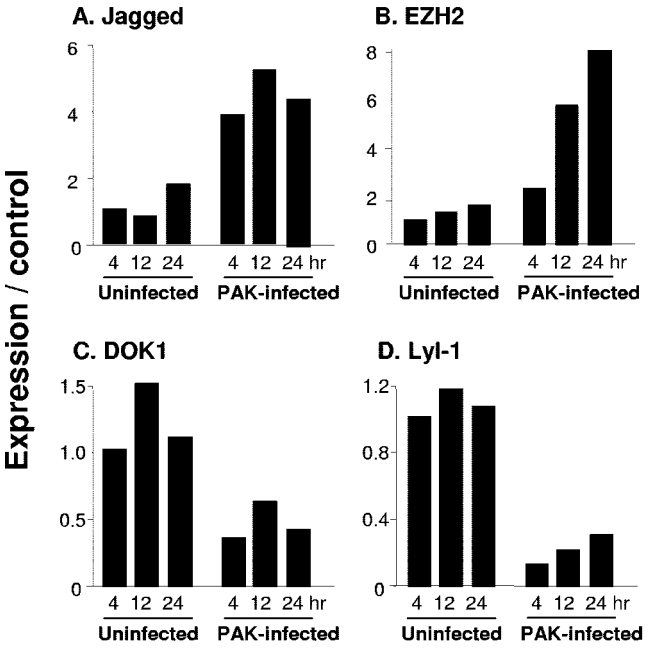

FIG. 4. Time course of mRNA levels of Jagged, EZH2, Lyl-1, and DOK1 in AM following infection with *P. aeruginosa*. Human AM were infected in vitro with PAK (5 CFU/cell) for 4 h, medium was then changed, and gentamicin was added. RNA was analyzed by quantitative real-time PCR for the mRNA expression levels of Jagged (A), EZH2 (B), DOK1 (C), and Lyl-1 (D) and normalized to levels of 18S rRNA at 0, 12, and 24 h after the initial infection. Data are presented as the expression level of the sample divided by the expression of uninfected control AM within the same experiment. Expression levels of uninfected AM of each experiment were set as 1. Shown are representative results of three independent experiments.

genes and lower for the downregulated Lyl-1 and DOK1 genes. Two-way analysis of variance confirmed that for each of the four genes there was a statistically significant effect of infection with PAK ($P < 0.001$) or *B. cepacia* ($P < 0.001$), but not methodology ($P > 0.9$ for PAK, $P > 0.6$ for *B. cepacia*).

To follow the gene expression of Jagged, EZH2, Lyl-1, and DOK1 at later time points, AM were infected for 4 h with PAK and then analyzed 0, 12, and 24 h following the initial infection by TaqMan real-time RT-PCR. The expression levels of Jagged and EZH2 were higher at all time points tested compared to the uninfected cells (Fig. 4A and B). The expression levels of Lyl-1 and DOK1 were decreased at all time points evaluated compared to the uninfected controls (Fig. 4C and D).

Jagged, one of the ligands for the notch receptor, is known to influence hematopoietic cell fate decisions (22). The Jagged-notch interaction plays a role in the survival and differentiation of stem and T cells and the induction of antigen-specific regulatory T cells (45). EZH2, a member of the polycomb group of proteins, functions as a transcriptional repressor and plays a role as an epigenetic regulator in the development of B cells (39). Similarly to Jagged, upregulation of EZH2 in AM may play a role in the activation of pulmonary immune responses. Of the downregulated genes, docking protein 1, originally found constitutively tyrosine phosphorylated in hematopoietic progenitors isolated from patients with leukemia, has been postulated to be a critical substrate for p210(bcr/abl) and acts as an adaptor protein that links the activin receptors with Smad proteins, factors involved in apoptosis (29). Lyl-1 is a helix-loop-helix DNA binding protein with lineage and differentiation-specific properties (32). No known function related to bacterial activation of myeloid cells has been described so far. Docking protein 1 and Lyl-1 may both be related to apoptosis induced by *P. aeruginosa*, to which macrophages have been shown to be more susceptible too.

Taken together, relevant to the pathogenesis of *Pseudomonas* and *Burkholderia* infection in CF, the observations in the present study are consistent with the concept that the AM play an important role in contributing to the inflammatory milieu of the respiratory epithelium. However, while *Pseudomonas* clinical isolates late in the course and superinfection with *Burkholderia* are both associated with progressive deterioration (14, 37), the responses of human AM to different strains of *Pseudomonas*, as well as *Burkholderia*, seem to be remarkably similar, suggesting that AM are not responsible for the worsening of CF in association with different strains of bacteria.

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