Expression and Regulation of Interferon-Related Development Regulator–1 in Cystic Fibrosis Neutrophils

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A genome-wide association study identified interferon-related development regulator–1 (IFRD1), a protein expressed by neutrophils, as a key modifier gene in cystic fibrosis (CF) lung disease. Here, we investigated the expression and regulation of IFRD1 in CF neutrophils. IFRD1 expression was quantified in peripheral blood and airway neutrophils from patients with CF, patients with non-CF lung disease, and healthy control subjects. The regulation of IFRD1 expression was analyzed using isolated neutrophils and *ex vivo* stimulation assays with CF airway fluids. IFRD1 single-nucleotide polymorphisms (SNPs) were analyzed in a CF cohort ($n = 572$) and correlated with longitudinal lung function and IFRD1 expression. Patients with CF expressed higher protein levels of IFRD1 in peripheral blood neutrophils compared with healthy or non-CF disease control subjects. Within patients with CF, IFRD1 protein expression levels in neutrophils were lower in airway fluids compared with peripheral blood. High IFRD1 expression was positively associated with the production of reactive oxygen species (ROS) in CF neutrophils. *In vitro* regulation studies showed that CF airway fluid and the CF-characteristic chemokines CXCL8 and CXCL2 down-regulated IFRD1 expression in neutrophils, an effect that was mediated through CXCR2. Genetic analyses showed that three IFRD1 SNPs were associated with longitudinal declines in lung function, and modulated IFRD1 expression. These studies demonstrate that IFRD1 expression is systemically up-regulated in human CF neutrophils, is linked to the production of ROS, and is modulated by chemokines in CF airway fluids, depending on the *IFRD1* genotype. Understanding the regulation of IFRD1 may pave the way for novel therapeutic approaches to target neutrophilic inflammation in CF.

Keywords: neutrophils; IFRD1; cystic fibrosis; inflammation; innate immunity

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CLINICAL RELEVANCE

These experiments demonstrate that the expression of interferon-related development regulator–1 (IFRD1), a key modifier gene of cystic fibrosis (CF) lung disease, is up-regulated in human CF neutrophils, is linked to the production of reactive oxygen species, and is modulated by chemokines in CF airway fluids, depending on the IFRD1 genotype. Understanding the regulation of IFRD1 may pave the way for novel therapeutic approaches in CF lung disease.

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, and is the most common lethal inherited disease in whites (1). The morbidity and mortality of patients with CF are substantially determined by chronic, progressive, neutrophil-dominated airway inflammation, bacterial infections, and airway remodeling (2–5). Despite evidence that CF is a monogenetic disease resulting from mutations within the CFTR gene, previous studies found only a poor association of CFTR genotypes with the severity of lung disease (6, 7). This inspired a broad search for non-CFTR modifier genes of CF lung disease (8–15). In a whole genome-wide study using single-nucleotide polymorphism (SNP) clustering, a novel candidate gene, interferon-related development regulator–1 (IFRD1) (16), was found to be associated with the severity of CF lung disease (17, 18).

IFRD1 is a protein expressed by mature neutrophils (17, 19), and it has been shown to interact with histone deacetylase (HDAC) enzymes, thereby modulating cell differentiation and oxidative stress (16, 20). Because CF lung disease is well known to be characterized by neutrophilic inflammation and increased oxidative stress (21), the IFRD1-mediated regulation of HDAC may play a key regulatory role in neutrophilic CF airway inflammation. To assess the functional impact of IFRD1 in CF lung disease, *Ifrd1* knockout mice were challenged with the CF-characteristic pathogen *Pseudomonas aeruginosa* (17). Those experiments showed that $Ifrd^{-/-}$ mice exhibited a reduced bacterial clearance, compared with wild-type mice. Further experiments demonstrated that neutrophils from $Ifrd^{-/-}$ mice featured an impaired production of reactive oxidative species (ROS) and proinflammatory cytokines and chemokines (17).

When viewed in combination, these studies strongly suggest that IFRD1 could represent both a genetic modifier and a key functional component of neutrophilic inflammation in CF lung disease. However, the expression levels and regulation of IFRD1 protein in human CF neutrophils, the key innate immune cells in CF lung disease (22), have not yet been studied, to the best of

our knowledge. To investigate the potential role of IFRD1 in neutrophilic lung disease in patients with CF, we studied (*1*) the expression of IFRD1 protein in CF, non-CF lung disease control, and healthy control blood and airway neutrophils; (*2*) the regulation of IFRD1 protein expression in neutrophils; and (*3*) the impact of single IFRD1 SNPs on neutrophil functionality and lung function in patients with CF.

MATERIALS AND METHODS

Study Populations

This study was approved by local ethics committees and by the Ethical Review Committees of the Ludwig-Maximilians University of Munich and the Universities of Tübingen and Frankfurt. Informed, written consent was obtained from all subjects in the study. In total, 572 patients with CF, three patients with non-CF bronchitis, and 25 healthy subjects were included (*see* Table E1 in the online supplement). Longitudinal values of forced expiratory volume in 1 second (FEV_1), calculated from a minimum of 5 consecutive years of CF patient data, were used to calculate the FEV_1 predicted at age 20 years, as previously reported by Schluchter and colleagues (23). For more details, please refer to the online supplement.

SNP Analyses

DNA was extracted from whole blood by a standard salting-out method (24), and SNP analysis was performed by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Sequenom, Hamburg, Germany), as described in detail elsewhere (25). Primer sequences are provided in Table E4.

Processing of Induced Sputum Samples

Induced sputum samples were obtained and mechanically processed as described previously (26).

Whole-Blood Neutrophil Isolation

Neutrophils were isolated from whole blood samples by Ficoll (GE Heathcare, Munich, Germany) gradient centrifugation and erythrocyte lysis, using ammonium chloride or the EasySep Human Neutrophil Enrichment Kit (Stem Cell Technologies, Grenoble, France), with negative selection using a tetrameric antibody complex recognizing cluster of differentiation (CD)2, CD3, CD9, CD19, CD36, CD56, glycophorin A, and dextran-coated magnetic particles, as indicated.

FACS Analyses

Blood neutrophils from patients with CF and healthy control subjects were measured for intracellular IFRD1 (antibody from Sigma-Aldrich, Munich, Germany) expression via flow cytometry (FACS Canto II; BD Biosciences, Heidelberg, Germany), as previously described (27). Where indicated, neutrophils were isolated for *ex vivo* stimulation experiments. For more details, please refer to the online supplement.

Statistical Analysis

IFRD1 concentrations between patients with CF and healthy subjects and between subjects with different IFRD1 gene variants were compared by one-way ANOVA with the Dunn multiple-comparison *post hoc* test. Associations between SNPs and qualitative outcomes were tested by using Pearson χ^2 and Fisher exact tests, according to a heterozygous genetic model (28). To account for multiple comparisons, a Bonferroni adjustment was performed.

For a more detailed description of the methods used in this study, please refer to the online supplement.

RESULTS

Expression of IFRD1 in Cystic Fibrosis Neutrophils

We quantified intracellular IFRD1 protein expression in peripheral blood and airway fluid (induced sputum) neutrophils of patients with CF, patients with non-CF lung disease, and healthy control subjects (Figure 1). We restricted our experimental analysis to intracellular IFRD1 protein, because confocal laser scanning microscopy (CLSM; Figure E1) and flow cytometry experiments provided evidence that IFRD1 protein was mainly localized at intracellular expression sites, which is consistent with previous studies of IFRD1 (29–31). Our experiments demonstrated that intracellular IFRD1 protein expression levels were robustly detectable in peripheral blood neutrophils. The neutrophilic expression of IFRD1 was further supported by the observation that neutrophil-like human promyelocytic leukemia cell line (HL-60) cells up-regulated intracellular IFRD1 protein expression upon differentiation into mature neutrophil-like cells (Figure E2), which is in line with a previous observation that IFRD1 mRNA expression increases upon neutrophilic HL-60 differentiation (17). IFRD1 protein expression was significantly higher in peripheral blood neutrophils from patients with CF, compared with those from non-CF lung disease and

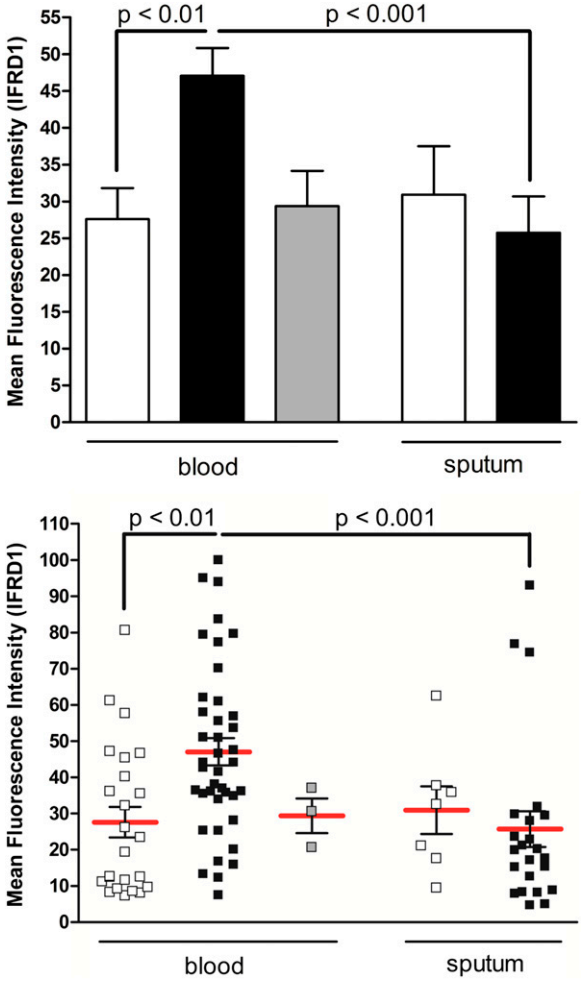

Figure 1. Expression of interferon-related development regulator–1 (IFRD1) in cystic fibrosis (CF) and control neutrophils. IFRD1 protein expression in blood and sputum neutrophils from patients with CF (*solid bars*), patients with non-CF lung disease (*shaded bars*), and healthy subjects (*open bars*) was measured by flow cytometry. Results are depicted as columns (*above*) or scatterplots (*below*). IFRD1 concentrations were significantly higher in blood neutrophils from patients with CF (*solid squares*) compared with patients with non-CF lung disease (*shaded squares*) and healthy control subjects (*open squares*). Results are presented as mean ± SEM.

healthy control subjects (Figure 1). Scatter blot analyses demonstrated that IFRD1 expression levels showed a substantial variation even within patient groups, suggesting the influence of genetic or microenvironmental/proinflammatory modifiers. In patients with CF, sputum neutrophils showed significantly lower protein expression levels compared with CF peripheral blood neutrophils (Figure 1). CLSM studies suggested a partial nuclear localization of IFRD1 protein in sputum, but not in peripheral blood neutrophils (Figure E1). When viewed in combination, these studies demonstrate that (*1*) IFRD1 protein expression is up-regulated in CF neutrophils compared with non-CF lung disease and healthy control neutrophils, (*2*) IFRD1 expression levels show a high variation within patient groups, and (*3*) IFRD1 expression is increased in CF peripheral blood versus CF airway neutrophils.

Regulation of IFRD1 Protein Expression in Neutrophils

After observing differential IFRD1 protein expression levels between circulating CF and non-CF and between circulating and airway neutrophils from patients with CF, we asked which factors (relevant to the proinflammatory CF disease phenotype) may regulate IFRD1 expression in neutrophils. Because IFRD1 has been linked to oxidative stress in murine neutrophils (17), immortalized HL-60 cells (17), and airway epithelial cells (35), we analyzed the relationship between IFRD1 expression and the production of reactive oxygen species (ROS) in human CF neutrophils. These experiments demonstrated that IFRD1 expression levels showed a positive association with ROS in CF neutrophils (Figure 2). Based on these findings, we asked whether dampening oxidative stress using antioxidants would exert an effect on IFRD1 expression. These experiments showed that the antioxidant reduced glutathione (GSH) exerted no significant effect on the IFRD1 protein expression of isolated neutrophils or neutrophils exposed to CF airway fluids (Table E2 and Figure E3). Similarly, protease inhibition exerted no effect on IFRD1 protein expression in these assays (Table E2 and Figure E3). Although GSH also showed no effect on baseline increased *IFRD1* mRNA expression levels in CF neutrophils, GSH pretreatment dampened the CF airway fluid-induced modulation of *IFRD1* mRNA expression (Figures E4 and E5). These experiments showed that IFRD1 protein expression is positively associated with ROS production in neutrophils, but that antioxidants are unable to modulate IFRD1 expression at the protein level.

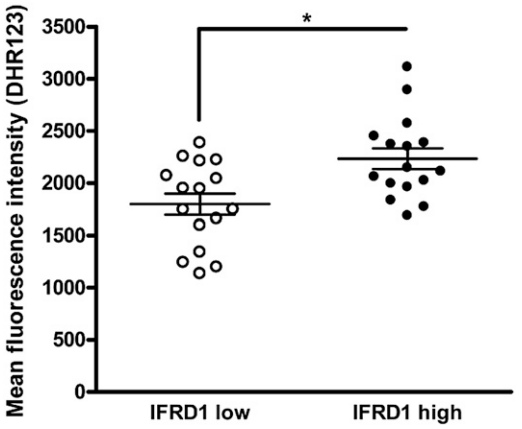

Figure 2. IFRD1 expression and reactive oxidative species (ROS) in neutrophils. Association of IFRD1 protein expression levels and ROS was measured by flow cytometry, using the dihydrorhodamine-123 (DHR123) method in CF neutrophils. "IFRD1 low" was defined as CF neutrophils with an IFRD1 expression below 44 mean fluorescence intensity (MFI) units, and "IFRD1 high" was defined as CF neutrophils with an IFRD1 expression above 44 MFI units.

Because IFRD1 protein expression in CF neutrophils was down-regulated in the airways compared with the circulation, we asked whether components in CF airway fluids are responsible for this effect. Therefore, we incubated peripheral blood isolated neutrophils with CF airway fluids (pooled CF sputum supernatants), and quantified the effects on IFRD1 protein expression. These experiments indicated that CF sputum supernatant decreased intracellular IFRD1 protein expression in neutrophils. By screening for several CF airway–related microenvironmental factors, we found that the chemokines CXCL8 (interleukin 8 [IL-8]) and CXCL2 growth-regulated protein-β (GRO-β), abundantly found in CF airway fluids (32), mimicked the effect of CF sputum supernatants and significantly down-regulated IFRD1 protein expression in peripheral blood isolated healthy control neutrophils (Figure 3A), as assessed by flow cytometry. These experiments also unexpectedly demonstrated that the kinetics and dynamics of CF airway fluid–induced down-regulation of IFRD1 protein expression on neutrophils were similar to those of recombinant CXCL8 at 10 ng/ml, whereas CXCL8 at 100 ng/ml or CXCL2 at 10 ng/ml or 100 ng/ml were different because IFRD1 expression levels returned almost to baseline after 120 minutes of stimulation. These similar characteristics may be attributable to the mean CXCL8 concentration of 11 ng/ml in our pooled CF airway fluids, and to the fact that CXCL8 can bind to CXCR1 and CXCR2 and activate ROS production (27) differentially in a concentration-dependent way. Proteases or exogenous oxidants, also abundantly present in CF sputum, were probably not involved in IFRD1 regulation in these assays, because a broad-spectrum protease inhibitor or the antioxidant GSH exerted no effect on the CF sputum–induced down-regulation of neutrophilic IFRD1 expression (Table E2 and Figure E3).

CXCL8 bioactivity is mediated through its two cognate G-protein–coupled chemokine receptors CXCR1 (IL-8RA, or CD181) and CXCR2 (IL-8RA, or CD182). We observed that the chemokine CXCL2, which exclusively binds CXCR2 but not CXCR1, exerted a down-regulating effect on IFRD1 similar to that of CXCL8 (Figures 3A and 3B), which led us to hypothesize that the CXCL8/CXCL2-induced effects on IFRD1 could be mediated through a CXCR2-dependent pathway. Blocking studies, using a small-molecule antagonist of CXCR2, showed that the effects of CXCL8 and CXCL2 on IFRD1 expression were almost completely abrogated when CXCR2 receptors were inhibited before chemokine stimulation. The stimulation of protein kinase C (PKC) pathways, using phorbol myristate acetate (PMA) as nonspecific neutrophil activator, or the stimulation of Toll-like receptor (TLR) pathways using LPS, exerted no effect (PMA) or only a nonsignificant effect (LPS) on IFRD1 protein expression, suggesting that IFRD1 expression in neutrophils is regulated through CXCL2 and CXCL8, rather than being affected by unspecific or TLR-dependent signaling (Figure 3B). When viewed in combination, these experiments provide evidence that (*1*) IFRD1 expression is positively associated with ROS production/oxidative bursts in CF neutrophils, (*2*) CF airway fluids down-regulate IFRD1 expression in neutrophils, and (*3*) the CF-characteristic chemokines CXCL8 and CXCL2 are involved in the regulation of IFRD1 protein expression in neutrophils through a CXCR2-mediated mechanism.

Association of *IFRD1* Polymorphisms with Protein Expression, Regulation, and CF Lung Disease

We studied the recently described *IFRD1* gene variants (17) in our CF population. We hypothesized that IFRD1 protein expression in neutrophils might be modulated by *IFRD1* SNPs.

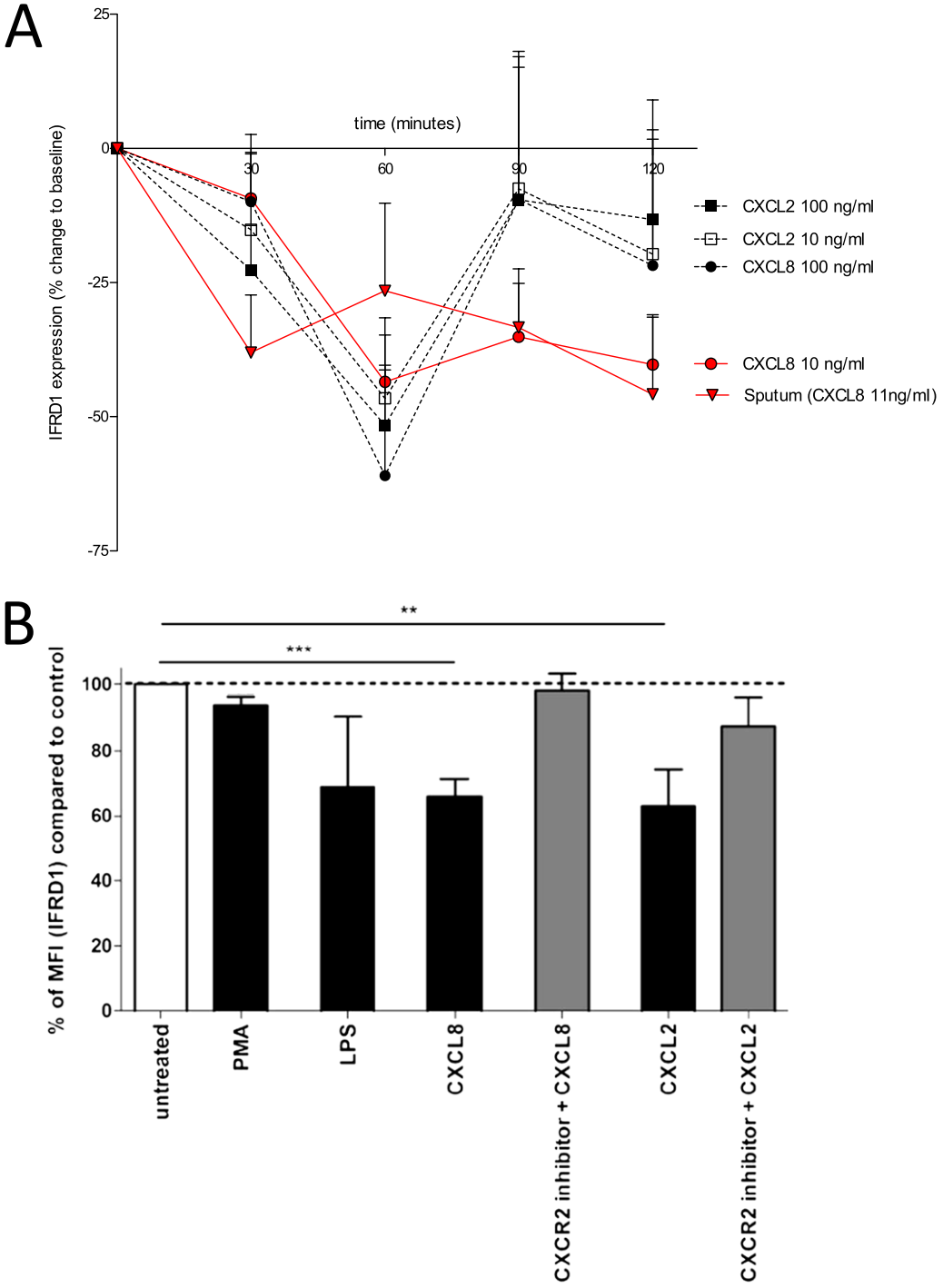

Figure 3. Role of CXCR2 chemokines in CF airway fluids in IFRD1 regulation. (*A*) Kinetics of CF sputum-induced/chemokine-induced modulation of IFRD1 expression in neutrophils. Isolated neutrophils were stimulated with pooled CF sputum supernatant from five patients with CF (containing a mean concentration of 11 ng/ml CXCL8), or 10 ng/ml or 100 ng/ml of recombinant human CXCL8 or CXCL2, and IFRD1 expression was measured after 30, 60, 90, and 120 minutes by flow cytometry ($n = 6$). Results are presented as percent change in MFI units over time, compared with time zero. (*B*) Isolated blood neutrophils were stimulated with phorbol myristate acetate (PMA; 10 μg/ml), LPS (1 μg/ml), CXCL8 (100 ng/ml), and CXCL2 (10 μg/ml) (*solid columns*) for 60 minutes at 37°C, and IFRD1 concentrations were measured by flow cytometry. Treatment with CXCL8 and CXCL2 led to significantly lower IFRD1 concentrations, compared with untreated control samples (*open column*; $P < 0.001$ and $P < 0.01$, respectively; $n = 6$). For stimulation experiments with recombinant human CXCL8/CXCL2, neutrophils were pretreated with the potent and selective nonpeptide antagonist of CXCR2 (SB225002; concentration, 100 nM; half-maximal inhibitory concentration, 22 nM) for 30 minutes at 37°C before stimulation (*gray columns*). Pretreatment with the CXCR2 inhibitor attenuated the effects of both CXCL8 and CXCL2 on IFRD1 expression. Data are presented as percent MFI units, compared with untreated control samples. All results are presented as mean ± SEM.

IFRD1 expression levels in peripheral blood neutrophils were significantly higher in CF patients heterozygous for SNPs rs11771128 and rs4727770, compared with carriers of the respective homozygous genotypes, whereas for rs3109105, a tendency without statistical significance was evident ($P < 0.05$; Figure 4). In addition, our experiments confirmed a significant association of three distinct *IFRD1* SNPs with the severity of CF lung disease (Table E3). In particular, the three intronic SNPs rs11771128, rs3109105, and rs4727770 were associated with the longitudinal course of FEV_1, according to Schluchter and colleagues (23). CF patients with a heterozygous genotype for these SNPs demonstrated a milder decline of lung function over time than did homozygous patients with CF. Other *IFRD1* SNPs showed no significant association with lung function data. None of the observed SNPs exerted significant effects on cross-sectional FEV_1 values (data not shown).

Next, we investigated the regulation of IFRD1 for gene variants for the SNPs rs11771128, rs4727770, and rs3109105, which showed the most significant association with the severity of CF lung disease in our cohort (Table E3). In flow cytometry analyses, neutrophils isolated from CF subjects with the heterozygous genotype GA for rs11771128 or the heterozygous genotype TA for rs4727770 showed a significant up-regulation of IFRD1 expression after stimulation with CXCL8, whereas neutrophils from homozygous patients with CF or from healthy control subjects with any genotype showed no up-regulation of cytosolic

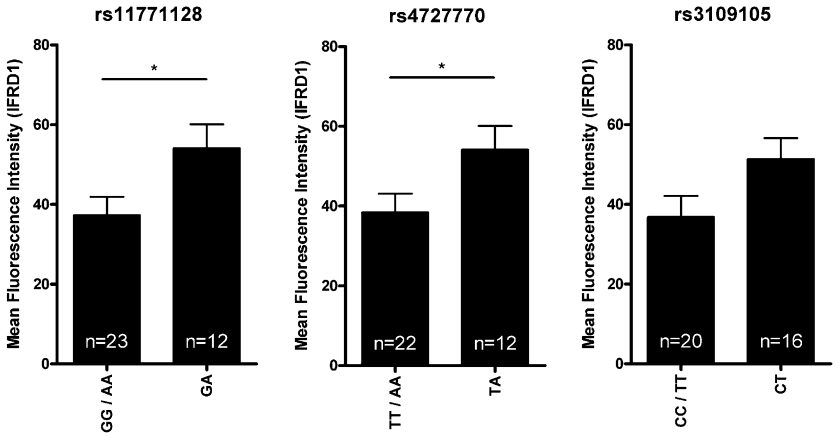

Figure 4. Effects of IFRD1 single-nucleotide polymorphisms (SNPs) on IFRD1 protein expression and regulation in CF neutrophils. IFRD1 concentrations of whole-blood neutrophils were measured in patients with CF by flow cytometry, and were stratified for IFRD1 genotypes. CF patients with the heterogenous genotype for rs11771128 (GA) and rs4727770 (TA) had significantly higher levels of IFRD1 protein expression in neutrophils, compared with homozygous (GG/AA and TT/AA, respectively) subjects. *$P < 0.05$.

IFRD1 expression after chemokine stimulation (Figure 5). When viewed in combination, these experiments demonstrate that in our CF cohort, three distinct *IFRD1* SNPs modulate the longitudinal course of CF lung disease and affect IFRD1 expression and the responsiveness of neutrophils toward CXCL8.

DISCUSSION

IFRD1 has been proposed as a key modifier gene and functional modulator of neutrophil-dominated CF lung disease, as supported by human genetic and murine functional studies (17). We followed this line of research by seeking to translate it into the human context of neutrophilic CF lung disease. Therefore, we characterized the expression and regulation of IFRD1 in peripheral and airway neutrophils from patients with CF, non-CF lung disease control subjects, and healthy control subjects. Our studies demonstrated that peripheral blood but not airway CF neutrophils featured increased IFRD1 expression levels compared with non-CF lung disease and healthy control neutrophils. Similar to the murine system (17), IFRD1 was also linked with oxidative stress in human neutrophils. In patients with CF, IFRD1 protein concentrations in neutrophils were decreased in airway fluid compared with peripheral blood, and further *in vitro* studies showed that CF airway fluid as well as the CF-characteristic chemokines CXCL8 and CXCL2 down-regulated IFRD1 expression in neutrophils in a CXCR2-dependent manner. Genetic analyses in a CF patient cohort provided evidence that three distinct IFRD1 SNPs modulated longitudinal lung function. Comparing different *IFRD1* alleles provided evidence that CXCL8 regulated IFRD1 expression, depending on the individual *IFRD1* genotype. Understanding the regulation of IFRD1 may pave the way for novel therapeutic approaches to target neutrophilic inflammation in CF.

In blood neutrophils, IFRD1 concentrations were significantly higher in patients with CF compared with healthy subjects. Intriguingly, the expression of IFRD1 in CF airway neutrophils was significantly lower compared with the expression in CF blood neutrophils, involving similar levels compared with healthy subjects, suggesting that proinflammatory factors present in the CF airway microenvironment down-regulate IFRD1. CF sputum supernatant decreased intracellular IFRD1 protein expression in neutrophils, an effect that was mimicked by the stimulation of neutrophils with the CXCR2 ligands CXCL8 and CXCL2, which are abundant in CF airway fluid. Based on these findings, we speculate that the CXCL8-mediated down-regulation of IFRD1 may explain the lower IFRD1 expression levels found in the airways, where high concentrations of CXCL8/CXCL2 are present compared with the CF circulation (27, 32, 33).

Blanchard and colleagues recently found decreased IFRD1 protein and increased IFRD1 mRNA expression levels in CF

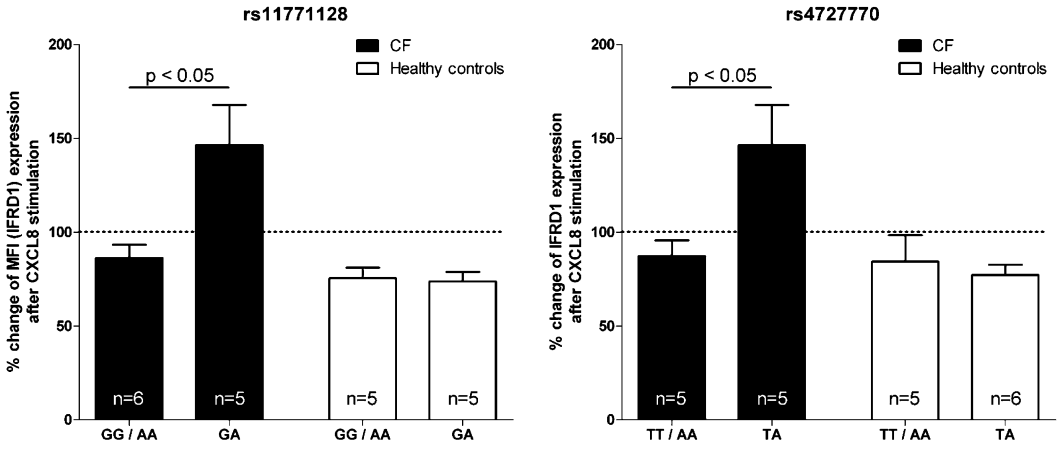

Figure 5. Effects of CXCL8 stimulation on IFRD1 protein expression, depending on the IFRD1 genotype. After stimulation with 100 ng/ml of CXCL8, IFRD1 expression in isolated neutrophils from patients with CF (*solid columns*) and healthy subjects (*open columns*), either homozygous (GG/AA or TT/AA) or heterozygous (GA or TA) for the SNPs rs11771128 or rs4727770, respectively, were quantified by means of flow cytometry. Patients with CF who were heterozygous carriers for this SNP showed an increase in IFRD1 concentrations after stimulation. In healthy subjects, no difference was evident between homozygous and heterozygous carriers. Data are presented as mean ± SEM.

airway epithelial cells, compared with control cells (35). Furthermore, that study found an effect of antioxidants on IFRD1 protein expression in epithelial cells. Inspired by those findings, we investigated the relationship between IFRD1 expression and oxidative stress in isolated neutrophils at the single-cell level. These experiments demonstrated that ROS production was positively associated with IFRD1 expression levels in isolated CF neutrophils, thereby confirming the previously described association between IFRD1 and oxidative stress in murine neutrophils (17) and immortalized HL-60 cells (17). Our experiments in human neutrophils also showed that antioxidants exerted no effect on baseline or CF airway fluid–stimulated IFRD1 protein expression, whereas an effect at the mRNA level was found, indicating that IFRD1 expression is differentially regulated (*1*) at the mRNA and protein levels, and (*2*) in neutrophils compared with epithelial cells. Given the relatively low regulatory transcriptional activity of neutrophils compared with other cell types, we focused on the characteristics of IFRD1 protein expression in our experiments. Similar to oxidants, proteases did not affect baseline or CF sputum–induced IFRD1 expression at the protein level. Beyond the association of IFRD1 and oxidative stress, the findings of Blanchard and colleagues (35) also imply that the expression and functionality of IFRD1 are not restricted to neutrophils, but may also regulate CF airway inflammation through the expression of IFRD1 in airway epithelial cells, a key cell type involved in the pathophysiology of CF lung disease. Further studies comparing IFRD1 expression and regulation in different CF-relevant cell subsets are warranted, to shed more light on the potential role of IFRD1 in the complex pathogenesis of CF lung disease.

In the next step, we investigated the role of *IFRD1* SNPs in our CF patient cohort. Although our data confirmed a significant role of *IFRD1* as modifier gene of CF lung disease severity, as proposed by Gu and colleagues (17), we found different SNPs within the *IFRD1* gene to be associated with longitudinal lung function in our CF cohort. This discrepancy between these two studies may be attributable to their different CF cohort study setups. Gu and colleagues (17) analyzed two homogeneous CF patient cohorts: (*1*) the Genetic Modifier Study Group Study, which enrolled patients homozygous for the ΔF508 mutation with extreme lung function parameters related to patient's age (34); and (*2*) the Cystic Fibrosis Twin and Sibling Study, which recruited twins and siblings suffering from CF with any *CFTR* genotype and their parents (12). Because the effects shown in these study cohorts were highly significant, we decided to investigate the relevance of *IFRD1* SNPs in a more heterogeneous CF study population. To investigate the molecular and biological effects of *IFRD1* SNPs on protein expression and regulation, we analyzed biological samples derived directly from both patients with CF and healthy subjects with distinct *IFRD1* genotypes, limiting the number of subjects to include. These studies demonstrated that patients with CF heterozygous for the SNPs rs11771128 and rs4727770 had significantly higher IFRD1 blood neutrophil protein levels, compared with homozygous individuals. Next, we investigated whether *IFRD1* SNPs would affect the regulation of IFRD1 protein expression in peripheral blood neutrophils isolated from patients with CF. IFRD1 protein levels in general decreased after stimulation with CXCL8 in peripheral blood neutrophils. However, in neutrophils from individuals with CF heterozygous for the SNPs rs1171128 or rs4727770, IFRD1 expression levels unexpectedly increased after stimulation with recombinant CXCL8 protein, an observation that underscores the complexity of CXCL8–IFRD1 regulation in CF neutrophils, and further cellular studies are required to dissect this interaction.

Regarding genetics, the main limitation of this study involves the low number of CF patients included. Consequently, these SNP results need to be confirmed by independent investigators in other CF populations. Nevertheless, our study was designed to match genetic data with protein expression and regulation profiles in neutrophils isolated from patients with CF and healthy control subjects. Therefore, we decided to limit the number of patients with CF, and to use freshly drawn blood for protein expression and regulation experiments. Viewing our genetic, expression, and regulation experiments in combination, our data indicate a more complex role of different *IFRD1* SNPs in affecting the decline in CF lung function and in modulating the IFRD1 protein expression of neutrophils.

In conclusion, our experiments corroborate a role for IFRD1 as a modifier of CF lung disease, and demonstrate that IFRD1 is differentially expressed and regulated in peripheral and airway neutrophils from patients with CF. These experiments also indicate that ROS and chemokines associate with and modulate IFRD1 expression in a CF-specific context in human neutrophils. Understanding the expression and regulation of IFRD1 in neutrophils may pave the way for novel therapeutic approaches to target neutrophilic inflammation in CF lung disease.

Author disclosures are available with the text of this article at www.atsjournals.org.

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