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IL-10 Controls *Aspergillus fumigatus*– and *Pseudomonas aeruginosa*–Specific T-Cell Response in Cystic Fibrosis

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ABSTRACT

Up to 90% of patients with cystic fibrosis (CF) are chronically colonized with *Pseudomonas aeruginosa*, and 10% to 50% of CF patients are colonized with *Aspergillus fumigatus*. Despite an extensive inflammatory reaction, patients cannot eliminate the microorganisms. The present study demonstrates that an IL-10 mediated T-cell tolerance to major infectious agents *A. fumigatus* and *P. aeruginosa* plays an important role in the control of T-cell–mediated inflammatory responses in CF. Peripheral blood mononuclear cells of CF patients secreted significantly higher amounts of IL-10. T-cell response against recombinant *A. fumigatus* antigens rAsp f 3, rAsp f 4, rAsp f 6, and heat-inactivated *P. aeruginosa* was controlled by IL-10. Proliferation and interferon-γ production was significantly increased when endogenous IL-10 was blocked in aspergillus and pseudomonas antigen-stimulated cells of CF patients. The role of IL-10 was further documented by increased spontaneous proliferation of peripheral blood mononuclear cells of CF patients after preincubation with antisense oligonucleotides blocking the synthesis of IL-10 receptor-associated kinases janus tyrosine kinase 1 and tyrosine kinase 2. Together, these data demonstrate an important role of IL-10–mediated peripheral T-cell tolerance to *P. aeruginosa* and *A. fumigatus* in the control of the intensity of the inflammatory T-cell response in CF. **(*Pediatr Res* 53: 313–319, 2003)**

Abbreviations

CF, cystic fibrosis
ABPA, allergic bronchopulmonary aspergillosis
IFN-γ, interferon-γ
PBMC, peripheral blood mononuclear cells
rAsp, f 3/4/6 recombinant *Aspergillus fumigatus* antigen 3/4/6
BAL, bronchoalveolar lavage
Jak 1, Janus tyrosine kinase 1
Tyk 2, tyrosine kinase 2

The chronic pulmonary infection and the ensuing destructive inflammatory process is still the major cause of morbidity and mortality in cystic fibrosis (CF) patients despite great progresses in antimicrobial therapy (1). Efforts are made to understand the inflammatory mechanisms in the CF lung and to find out why microorganisms such as *Pseudomonas aeruginosa* can escape the definitive elimination by the immune system and therefore cause an endless inflammation. *Aspergillus fumigatus* is a ubiquitous mold found in up to 50% of CF respiratory secretions (2). The interaction between *A. fumigatus* and the host organism ranges from simple colonization to allergic bronchopulmonary aspergillosis (ABPA), which may rarely lead to invasive aspergillosis in immune-compromised individuals (3). Characterization, cloning, and production of some recombinant allergens from *A. fumigatus* improved the diagnostic possibilities (4–7). At present, several recombinant antigens are available for determination of specific IgE against *A. fumigatus* (7–9). Distinct patterns of specific antibodies were found in patients with and without ABPA. Whereas patients sensitized to *A. fumigatus* show specific IgE antibodies to rAsp f 1 and rAsp f 3, patients with ABPA also show specific antibodies to two intracellular antigens of *A. fumigatus*, rAsp f 4 and/or rAsp f 6 (10). In patients who develop ABPA, *A. fumigatus* contributes undoubtedly to the severe course of disease, and like *P. aeruginosa*, *A. fumigatus* cannot be eliminated effectively from the lung in most patients. Elevated levels of proinflammatory cytokines such as IL-1β, IL-6, IL-8, and tumor necrosis factor-α were found in serum (11, 12) and in bronchoalveolar lavage fluid (BAL) of CF patients (13). Alveolar macrophages are the predominant phagocytes recov-

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ered by BAL in healthy individuals. In stable CF patients or in very young children and infants with little clinical evidence of lung problems and mild illness, mostly neutrophils are recovered from BAL (14, 15). Besides perpetuating the inflammatory response, neutrophils themselves may directly injure the lung by releasing oxygen metabolites as well as lysosomal enzymes and proteases (16).

IL-10, a major regulatory cytokine of inflammatory responses, was identified originally as an inhibitor of IFN-γ and IL-2 synthesis in T-cells. Evidence demonstrates that IL-10 is a general inhibitor of activation and cytokine production in T-cells, neutrophils, and macrophages. IL-10 is produced by macrophages (17, 18), natural killer cells (19), and both Th1- and Th2-type lymphocytes (20). IL-10 plays an important role in T-cell behavior as demonstrated in different mouse models (21), in natural antigen exposure, and in increased allergen administration during allergen-specific immunotherapy (22, 23). The suppressor effect of IL-10 on T-cells has recently been demonstrated to be primarily directed to block the costimulatory signal for activation in T-cells. IL-10 inhibits CD28 signaling cascade and subsequent phosphatidylinositol 3-kinase activation in T-cells (23). IL-10 receptor contains α and β chains, and triggering of the IL-10 receptor (R) results in phosphorylation of the receptor-associated protein tyrosine kinases Jak 1 and Tyk 2 (24, 25) and the following activation of signal transduction and activation of transcription 1α and 3 (24). Because IL-10 has been shown to inhibit T-cell activation and proinflammatory cytokine production, changes in IL-10 secretion may efficiently contribute to the inflammatory response in CF. In addition, IFN-γ was studied as a Th1 type and IL-13 as a Th2 type cytokine to give a better insight into the immunologic mechanisms in CF. Thus, the present study demonstrates that IL-10 is essential in regulation of the lung inflammatory response. In CF patients, *A. fumigatus*– and *P. aeruginosa*–induced increased amounts of IL-10 controls the aspergillus- and pseudomonas-specific T-cell responses.

METHODS

Subjects. Seventeen patients with CF (mean age: 20.0 ± 10.2 y; range: 8–44.5 y; median: 17.5 y) were recruited from the CF outpatient clinics of the University Children's Hospital in Berne, Switzerland. Their mean forced expiratory volume in 1 s was 60.1 ± 24.3% of the value predicted for sex and height (range: 30–100%; median: 55%; lung function of patients older than 18 y were based on adult predicted values), and the mean forced vital capacity was 74.1 ± 22.0% of predicted values (range: 36–105%; median: 74%). Seven patients (mean age: 17.9 ± 1 0.9 y; range: 8–39.8 y; median: 17.5 y) received a diagnosis of ABPA in the last 12 mo according to Nelson's criteria (2). Their immunologic values in serum were as follows (mean ± SD): total IgE 2150.7 ± 1758.8 kU/L (range: 500–5662 kU/L; median: 1912.0 kU/L), *A. fumigatus* RAST class 4.3 ± 0.8 (range: 3–5; median: 4.0). Specific IgE against recombinant *A. fumigatus* antigens was measured as rAsp f 1: 128 ± 94 EU/L (range: 46–278 EU/L; median: 80.0 EU/L); rAsp f 3: 597 ± 807 EU/L (range: 48–2303 EU/L; median: 319.0 EU/L); rAsp f 4: 46 ± 41 EU/L (range: 2–118 EU/L; median: 44.0 EU/L); rAsp f 6: 54.9 ± 57 EU/L (range: 1–165 EU/L; median: 48.0 EU/L). Reference values (12) were rAsp f 1: <9.6 EU/L; rAsp f 3: <13.2 EU/L; rAsp f 4: <8.4 EU/L; rAsp f 6: <7.2 EU/L, respectively. Specific IgG against *A. fumigatus* was 215 ± 123 kU/mL (range: 94–394 kU/L; median: 170.0 kU/L); reference value was <20 kU/mL. Thirteen of 17 patients were chronically colonized with *P. aeruginosa* (two positive sputum samples within 6 mo). The patients were on their regular therapeutic regimen, which includes inhalation of β-2-mimetics, chest physiotherapy, high caloric intake, and enzyme and vitamin substitution, but none of them received systemic corticosteroids 1 mo before blood samples were taken. In all patients, *A. fumigatus* was isolated at least once in sputum. A group of 17 healthy children and adults (mean age: 30.6 ± 14.9 y) served as controls (Table 1). The study was approved by the local ethical committee of the University of Bern, Switzerland, and written informed consent was obtained from all patients and/or their parents.

Antigens. IgE binding properties and characterization of recombinant *A. fumigatus* antigens were previously described (6–8); rAsp f 3, a protein homologous to peroxisomal proteins of *Candida boidinii*, rAsp f 4, an allergen with unknown biologic function, and *A. fumigatus* manganese-dependent superoxide dismutase (rAsp f 6) were cloned from an *A. fumigatus* cDNA library and displayed on pJuFo-phage surface. The regions coding for the mature proteins were subcloned into the p[His]$_6$-DHFR high level expression vector. Constructs were verified by nucleotide sequence determinations and used to produce hexahistidine-tagged recombinant proteins in *Escherichia coli*. Purity and molecular size of the proteins were analyzed by polyacrylamide gradient gels and by Western blot analysis using standard procedure (8). *A. fumigatus* antigens were used alone or in combination at titrated doses between 0.1 and 1 μM concentrations. A *P. aeruginosa* strain, isolated from one CF patient (University Children's Hospital, Bern, Switzerland) was heat inactivated by 56°C for 30 min. Titrated doses of 0.26–26.6 μg/mL protein content were used. Tetanus toxoid(Serum Institute, Bern, Switzerland) was used at 10 μg/mL as control.

T-cell cultures. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll (Biochrom, Berlin, Germany) density gradient centrifugation. Cells were washed three times and resuspended in RPMI 1640 medium supplemented as described (22). Cytokine secretion of polyclonally stimulated cells was determined in supernatants of 5×10^5 cells, in 500 μL of medium, in 48-well plates. The cells were stimulated with an MAb mixture containing 0.5 μg/mL of each of the anti-CD2 (4B2 and 6G4), anti-CD28 (15E8; CLB, Amsterdam, The Netherlands), and anti-CD3 (CRL 8001; American Type Culture Collection, Manassas, VA) MAb. Supernatants of triplicate cultures were taken after 72h. Five $\times 10^5$ cells/500 μL were stimulated in a 48-well plate with recombinant *A. fumigatus* antigens, rAsp f 3, rAsp f 4, rAsp f 6, and *P. aeruginosa*. Supernatants were harvested on day 5 for cytokine detection. Proliferation was measured in parallel cultures 20 h after the addition of 1 μCi of [^{3}H]thymidine/well in 96-well plates (DuPont/New England Nuclear, Boston, MA). Incorporation of the labeled nucleotide was determined in an LKB beta plate

Table 1. *Clinical and serological characteristics*

	All patients	non-ABPA	ABPA	Control group (reference values)
Male/female	9/8	5/5	5/2	7/10
Age (y)	20.0 ± 10.2 (8–44.5)	21.4 ± 10.1 (12.6–44.5)	17.9 ± 10.9 (8.0–39)	30.6 ± 14.9 (10–50)
FEV1 (% predicted)	60.1 ± 24.3 (30–100)	52.7 ± 23.5 (30–95)	71.0 ± 23.1 (30–100)	>80
FVC (% predicted)	74.1 ± 22.0 (36–105)	65.0 ± 20.8 (36–99)	87.1 ± 17.4 (57–105)	>80
P. aeruginosa in sputum	13	8	5	0
A. fumigatus in sputum	10	3	7	0
Total IgE	926.1 ± 1508.8 (8–5662)	68.9 ± 47.3 (8–145)	2150.7 ± 1758.8 (500–5662)	<70
A. fumigatus–specific IgE RAST class	1.9 ± 2.1 (0–5)	0.2 ± 0.4 (0–1)	4.3 ± 0.8 (3–5)	(0–2)
A. fumigatus–specific IgG (ELISA, kU/L)	133.0 ± 109.4 (18–384)	95.1 ± 106.6 (18–341)	215.4 ± 123.5 (94–384)	(<20)
rAsp f 1–specific IgE (U/mL)	71.1 ± 79.1 (0–278)	30.8 ± 31.8 (0–106)	128.5 ± 94.1 (46–278)	(<9.6)
rAsp f 3–specific IgE (U/mL)	261.0 ± 573.8 (3–2303)	25.4 ± 17.1 (3–51)	597.5 ± 807.9 (48–2303)	(<13.2)
rAsp f 4–specific IgE (U/mL)	20.4 ± 33.5 (0–118)	2.3 ± 1.9 (0–5)	46.3 ± 40.7 (2–118)	(<8.4)
rAsp f 6–specific IgE (U/mL)	26.0 ± 43.6 (0–165)	5.8 ± 11.5 (0–38)	54.8 ± 56.7 (1–165)	(<7.2)

reader (Wallac-Pharmacia, Turku, Finland). IL-10 was neutralized in cultures with 5 μg/mL rat anti–IL-10R (3F9–2) and anti–IL-10 MAb (JES3–19F1) (23). Rat IgG (Sigma Chemical Co., St. Louis, MO) or mouse IgG1 (Coulter Corp., Miami, FL) served as controls. The synthesis of IL-10R–associated kinases Jak 1 and Tyk 2 was blocked by preincubation of PBMC with antisense oligonucleotides Jak 1 (AS): GGTTGCATCTGGAATCTTT and Tyk 2 (AS): CCAACTTTATGTGCAATGTG; Jak 1 scrambled: TTGTGAACTGCCTGTGATT and Tyk 2 sense: CACATTGCACATAAAGTTGG, were used as control (26). All oligonucleotides were optimized in titrated doses and finally used at 1-μM doses.

Quantification of cytokines. The solid-phase ELISA for IFN-γ, IL-10, and IL-13 was performed as described previously (27, 28). Intracytoplasmic IFN-γ, IL-10, and IL-13 were detected in PBMC stimulated with 50 ng/mL phorbol ester and 500 ng/mL Ca^{++} Ionophore (both from Sigma Chemical Co.) for 5 h, 2 μM of monensin was added to cultures during the last 4 h. Cells were incubated with PE-labeled anti–IL-10, anti–IL-13, and FITC-labeled anti–INF-γ antibodies (all from PharMingen)) for 40 min at room temperature. FITC- or PE-labeled rat IgG1 and mouse IgG1 were used as isotype control antibodies. The multicolor analysis was performed on an Epics Profile Flow cytometer (Coulter Corp.) with Argon laser (488 nm).

Statistical analysis. Nonparametric statistical comparison of cytokine production and proliferation of cells from each individual was performed by Wilcoxon signed-rank test. Nonparametric statistical comparison between different groups was performed by Mann Whitney *U* test.

RESULTS

PBMC of CF patients produce high IL-10 in comparison with healthy controls. A dysregulated cytokine response seems to be an important regulating event in the inflammatory response in CF. For analysis of their role in CF, IL-10, IFN-γ, and IL-13 were measured in 5-d supernatants of PBMC stimulated with anti-CD2, anti-CD3, and anti-CD28 MAb combination (Fig. 1*A*). CF patients showed significantly higher IL-10 production compared with the controls ($p < 0.05$). There was no difference in IL-13 and IFN-γ production. For supporting these findings, PBMC were stimulated with phorbol ester and Ca^{++} ionophore and intracytoplasmic IL-10 was stained in fixed and permeabilized cells. CF patients' T-cells expressed higher IL-10 in comparison with controls. The increase in intracytoplasmic IL-10 content between unstimulated and stimulated cells was significantly higher in CF patients compared with controls ($p = 0.003$; Fig. 1*B*). There was no difference in intracytoplasmic IL-13 and IFN-γ expression between patients and controls (data not shown).

IL-10 regulates specific T-cell proliferation to A. fumigatus and P. aeruginosa. For determining antigenic properties of recombinant *A. fumigatus* antigens and heat-inactivated *P. aeruginosa*, PBMC of CF patients and controls were stimulated with *P. aeruginosa* and rAsp f 3, rAsp f 4, and rAsp f 6. Figure 2 shows mean and SE of stimulation indexes of patients and controls. Significantly increased T-cell proliferation with these antigens (stimulation index >2) was observed in 69% of patients and 17% of healthy controls. Tetanus toxoid stimulated T-cell proliferation in all patients and controls as a control antigen.

For analysis of the role of endogenous IL-10 in the control of specific immune response against *A. fumigatus* and *P. aeruginosa*, the IL-10R was blocked and the released IL-10 was neutralized. Figure 3 depicts the proliferation under stimulation with a mixture of rAsp f 3, rAsp f 4, rAsp f 6, and *P. aeruginosa* as well as under blocking the influence of endogenous IL-10 with MAb specific to IL-10 and IL-10R. Com-

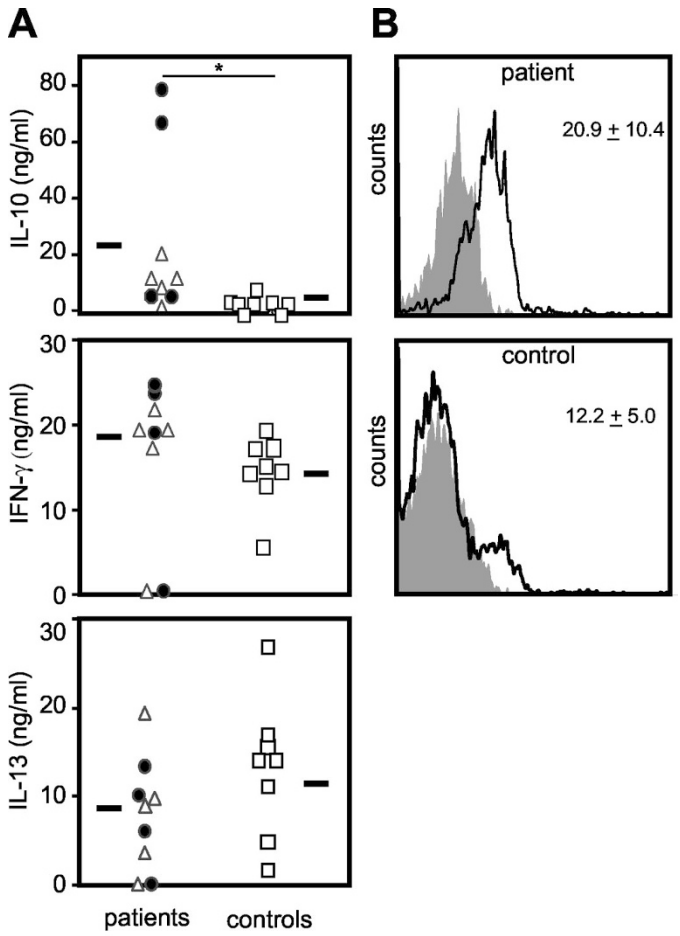

Figure 1. Increased IL-10 production in PBMC of CF patients. (*A*) IL-10, IFN-γ, and IL-13 production of CF patients ($n = 9$) and controls ($n = 8$) were measured in 5-d supernatants after stimulation by anti-CD2, anti-CD3, anti-CD28 MAb. Triangles indicate ABPA patients. CF patients produced significantly more IL-10 compared with controls. No difference was detected between ABPA and non-ABPA patients. *$p < 0.05$. (*B*) PBMC of patients and controls were stimulated for 5 h with phorbol ester and Ca^{++} ionophore and for 4 h with monensin. Intracytoplasmic IL-10 was stained in fixed and permeabilized cells. CF patient's T-cells expressed higher IL-10 increase in comparison to controls. FACS analysis of cells from one patient and one control is shown representative of six patients and six controls. Mean intracellular IL-10 of patients and controls is given with SD in the upper right quadrant.

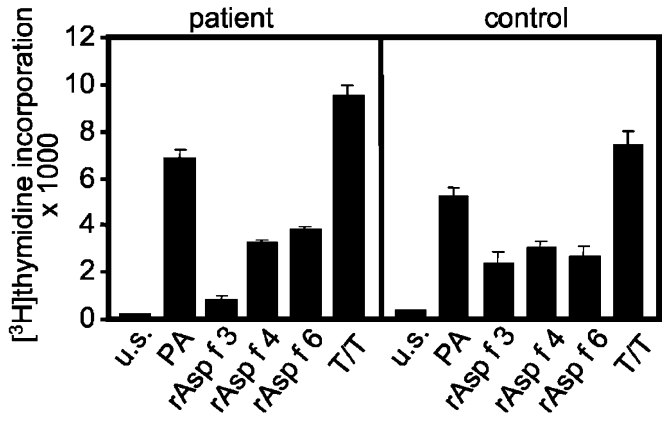

Figure 2. T-cell proliferation by *P. aeruginosa* and recombinant *A. fumigatus* antigens. PBMC of CF patients and controls were stimulated with titrated doses of heat-inactivated *P. aeruginosa* (PA) and recombinant *A. fumigatus* antigens rAsp f 3, rAsp f 4, and rAsp f 6. [^{3}H]thymidine incorporation was measured after 5 d in triplicate. Data are shown as mean and SE of patients and controls at optimal doses of rAsp f 3, rAsp f 4, rAsp f 6 (1 μM), *P. aeruginosa* (26 μg/mL), and tetanus toxoid (10 μg/mL) from PBMC; u.s., unstimulated.

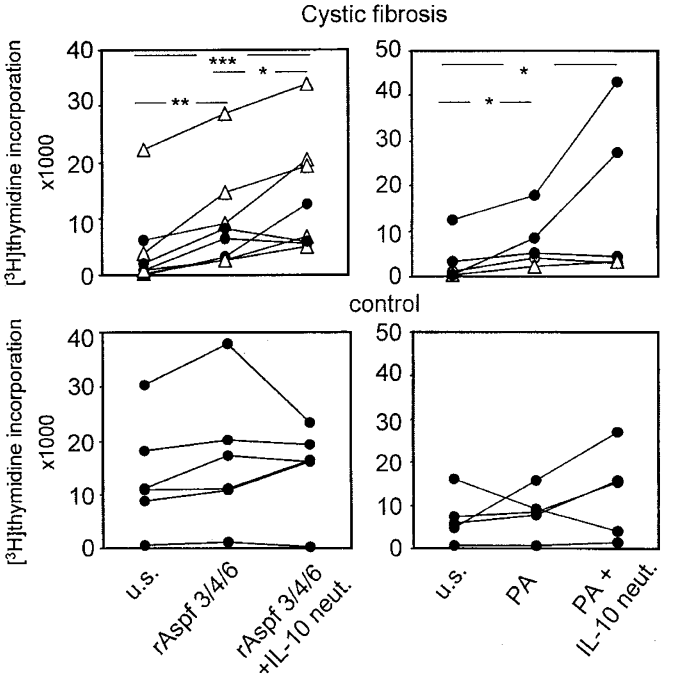

Figure 3. Regulation of T-cell response to *A. fumigatus* and *P. aeruginosa* by IL-10. IL-10R was blocked by anti–IL-10R MAb, and released IL-10 was neutralized by anti–IL-10 MAb (IL-10 neut.) in rAsp f 3, rAsp f 4, rAsp f 6 mixture (each 0.1 μM; eight patients, six controls) and *P. aeruginosa* (2.6 μg protein/mL) stimulated PBMC (five patients, six controls). △, ABPA patients; ●, non-ABPA patients. Results of [^{3}H]thymidine incorporation after 5 d of stimulation are given as mean ± error bars of triplicates. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

pared with unstimulated cells, PBMC showed significantly increased proliferation when stimulated with rAsp f 3, rAsp f 4, and rAsp f 6 ($p = 0.002$), which showed a further increase when the effect of endogenous IL-10 was blocked ($p = 0.035$). A significant T-cell proliferation was observed by *P. aeruginosa* stimulation ($p = 0.02$), as well as under IL-10 blocking conditions ($p = 0.043$). There was no significant difference in specific *A. fumigatus*– or *P. aeruginosa*–induced T-cell proliferation between CF patients with and without ABPA. Healthy individuals did not show significant proliferation by *A. fumigatus* and *P. aeruginosa* stimulation as well as under IL-10 blocking conditions. These data suggest that aspergillus- and pseudomonas-specific proliferation of T-cells in CF is under the suppressive control of endogenous IL-10.

Endogenous IL-10 suppresses IFN-γ response to A. fumigatus and P. aeruginosa in CF. Cytokine production of specifically stimulated cells was studied in PBMC of CF patients that were stimulated with rAsp f 3, rAsp f 4, rAsp f 6, and *P. aeruginosa*. A significant increase in IL-10 production by *A. fumigatus* and *P. aeruginosa* antigens ($p < 0.05$) but no significant change in IFN-γ was observed (Fig. 4). Interestingly, IFN-γ significantly increased by suppressing endogenous IL-10 in *A. fumigatus*– and *P. aeruginosa*–stimulated

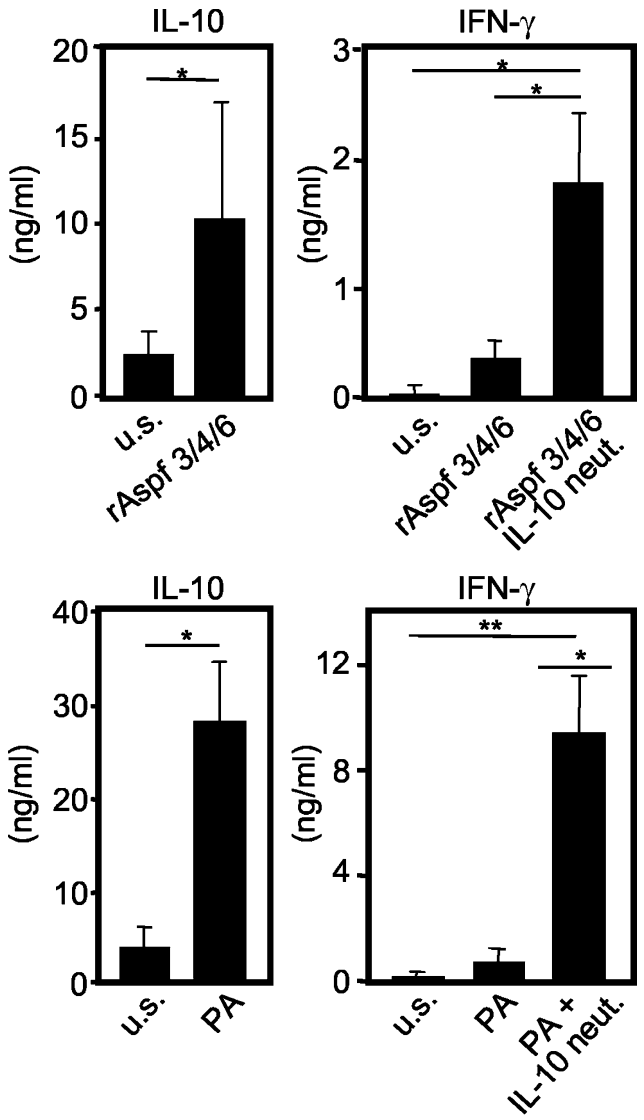

Figure 4. IL-10 controls IFN-γ response to *A. fumigatus* and *P. aeruginosa* in CF patients. IL-10 and IFN-γ production was measured in 5-d supernatants of PBMC of CF patients stimulated with rAsp f 3, rAsp f 4, rAsp f 6 (0.1 μM) ($n = 7$), and *P. aeruginosa* (2.6 μg protein/mL; $n = 5$) as well as IL-10 and IL-10R blocking conditions (IL-10 neut.); $*p \leq 0.05$, $**p \leq 0.01$.

cells compared with nonstimulated cells ($p < 0.05$ and $p < 0.01$, respectively). It is known that the release of proinflammatory cytokines correlates with the intensity of inflammation, and these data demonstrate that *P. aeruginosa*– and *A. fumigatus*–induced IFN-γ production is strongly controlled by IL-10. This mechanism may play a decisive immunoregulatory role in the control of the inflammatory response in CF.

Regulation of T-cell proliferation by IL-10R–associated kinases Jak 1 and Tyk 2. The regulatory role of endogenous IL-10 in the control of T-cell response was demonstrated by blocking the IL-10R–associated kinases Jak 1 and Tyk 2 using antisense oligonucleotides. Because polyclonal stimulation of PBMC showed significantly high IL-10 secretion, we determined whether background proliferation of unstimulated PBMC is controlled by IL-10R–mediated signals. PBMC of three CF patients were preincubated for 24 h with antisense oligonucleotides to Jak 1 and Tyk 2. Scrambled sense oligonucleotides of Jak 1 and sense oligonucleotides of Tyk 2 were used as controls. After 24 h of incubation with antisense oligonucleotides to IL-10R–associated kinases Jak 1 and Tyk 2, the proliferation of resting PBMC significantly increased compared with sense and scrambled oligonucleotide controls (Fig. 5). Similarly blocking of the IL-10R also increased the background proliferation by PBMC. Accordingly, background proliferation of PBMC, which represents *in vivo*–activated T-cell response, is actively suppressed by IL-10. Blocking of the receptor as well as inhibition of IL-10R–associated kinases leads to increase in T-cell proliferation.

DISCUSSION

The present study demonstrates that IL-10 plays an essential role in the control of *A. fumigatus*– and *P. aeruginosa*–induced T-cell responses in CF patients. IL-10 is a potent anti-inflammatory cytokine, which acts by inhibiting the synthesis of proinflammatory cytokines of monocytes/macrophages (18, 29), T-cells (30), neutrophils (31), eosinophils (32), mast cells (33), and dendritic cells (34, 35) as well as human alveolar macrophages (36). In addition, IL-10 down-regulates the antigen-presenting capacity of monocytes by down-regulating MHC class II molecules and costimulatory ligands for T-cells (18). The present data demonstrate that PBMC of CF patients secrete more IL-10 than healthy individuals after polyclonal T-cell stimulation. Thus, IL-10–mediated suppressor pathways may be relevant in the regulation of the inflammatory response to microbial agents in CF.

Usually, *A. fumigatus* is found within the mucus, impacted in the airways without evidence of tissue invasion, and it is frequently isolated from CF respiratory secretions (2, 37). Although there is evidence for an immunologic response in many patients with *A. fumigatus*–positive cultures, they may

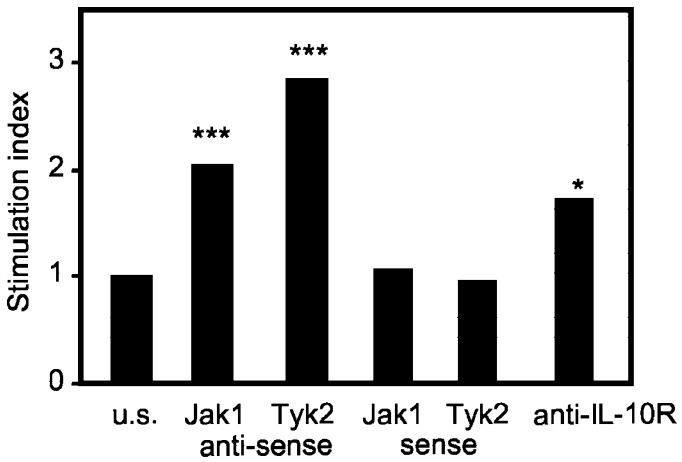

Figure 5. Regulation of T-cell proliferation by IL-10R–associated kinases Jak 1 and Tyk 2. The synthesis of IL-10R–associated kinases Jak 1 and Tyk 2 was blocked in PBMC of three CF patients, which were preincubated for 24 h with antisense (AS) oligonucleotides Jak 1 AS and Tyk 2 AS. As controls, Jak 1 scrambled sense and Tyk 2 sense were used. Stimulation index of [^{3}H]thymidine incorporation of one patient is shown. The experiment was repeated three times with similar results. The proliferation of resting unstimulated T-cells significantly increased after blocking the IL-10R–associated kinases and IL-10R; $*p < 0.05$, $***p < 0.001$.

not necessarily have ABPA, because immunologic response may vary over time. Why the immune system is not able to eliminate definitively the fungus from the bronchial tree is still not clear. The presence of a tolerance mechanism would, at least in part, explain this clinical observation. In the present study, specific T-cell suppressive effects of endogenous IL-10 could clearly be shown by a significant increase of cell proliferation after the endogenous IL-10 influence was inhibited. A significant increase in IFN-γ production after blocking endogenous IL-10 was detected after stimulation with recombinant *A. fumigatus* antigens. Previously, a Th2-like cytokine pattern described in *A. fumigatus* stimulated PBMC from patients with ABPA (38). *A. fumigatus*–specific cell lines secreted predominantly IL-4 after 48 h of culture, and IFN-γ and IL-2 were not detectable (39). Apparently, endogenous IL-10 is able to control IFN-γ levels in CF efficiently. This explains the low quantities of IFN-γ production, previously observed in CF patients by different stimulatory conditions (40). The role of IL-10 in the immune response of mice to *A. fumigatus* was studied in a murine ABPA model (41). IL-10–deficient mice showed a high mortality, and their lung cells produced exaggerated amounts of IL-4, IL-5, and IFN-γ compared with wild-type mice, demonstrating IL-10 as a natural suppressor of cytokine production and inflammation.

Binding of IL-10 to the extracellular domain of the IL-10R activates the phosphorylation of the receptor-associated Jak 1 and Tyk 2 for transcription (42). Therefore, the influence of IL-10 on T-cell proliferation was additionally studied by incubating the cells with antisense oligonucleotides, which block the transcription of Jak 1 and Tyk 2 and thus the IL-10 action. By preincubation with antisense oligonucleotides, PBMC of CF patients showed increased proliferation. This provides additional evidence for the control of T-cell responses by IL-10 in microbial infection. Unfortunately, the antisense approach does not allow generation of antigen-specific cultures because of decreased antigen-presenting cell capacity of monocytes and B-cells in PBMC because of plastic adhesion and decreased viability of these cells, as a result of incubation with oligonucleotides.

CF patients have a bronchial tree chronically colonized with *P. aeruginosa* from early childhood on, despite intact defense mechanisms against microorganisms outside the lung. Current management of pulmonary disease in CF is directed as a consequence of infection and inflammation (1). Despite an intense host inflammatory reaction with progressive suppurative pulmonary disease resulting from *P. aeruginosa* colonization (43), the bacteria are not eliminated. Thus, one may argue that tolerance mechanisms against this microorganism may develop. Indeed, PBMC of CF patients produced high amounts of IL-10 by *P. aeruginosa* stimulation. Blocking of endogenous IL-10 leads to high IFN-γ production and T-cell proliferation, indicating that IL-10 is actively down-regulating the ongoing inflammatory response against *P. aeruginosa*. The control of *P. aeruginosa*–induced inflammation by IL-10 was furthermore demonstrated in IL-10–deficient mice, which showed an increased area of lung inflammation and more severe weight loss after inoculation of *P. aeruginosa*–containing agar beads into their lung. In contrast, IL-10–treated mice showed less severe weight loss, fewer BAL neutrophils, and smaller areas of lung inflammation (44).

The present study dissects *A. fumigatus*–specific immune response by using recombinant antigens. Previous studies were performed with aspergillus extracts, which may contain a nonstandard mixture of antigens, nonantigenic proteins, and cell toxic proteins (45). However, a crude *P. aeruginosa* antigen was used because recombinant protein antigens are currently not available. This extract contains lipopolysaccharide and other Gram-negative cell-wall products and DNA. In addition to cell wall products, bacterial DNA may display stimulatory effects on mammalian immune cells, and CpG oligonucleotide-containing DNA of *P. aeruginosa* may contribute to the stimulatory effects on PBMC of CF patients and controls (46). Together, these data demonstrate that IL-10 plays an essential role in tolerance against *A. fumigatus* and *P. aeruginosa* in the lungs of CF patients. Moreover, severe destructive effects of the immune response against antigens of *A. fumigatus* and lipopolysaccharide, lipopolysaccharide-like cell-wall products, and CpG oligonucleotide-containing DNA of *P. aeruginosa* are efficiently controlled by endogenous IL-10. Moreover, this mechanism may provide an explanation for the existential survival strategy of certain microorganisms in the lungs of CF patients.

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