The basophil surface marker CD203c identifies *Aspergillus* species sensitization in patients with cystic fibrosis

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Background: Colonization by *Aspergillus fumigatus* in patients with cystic fibrosis (CF) can cause *A fumigatus* sensitization and/or allergic bronchopulmonary aspergillosis (ABPA), which affects pulmonary function and clinical outcomes. Recent studies show that specific allergens upregulate the surface-expressed basophil marker CD203c in sensitized subjects, a response that can be readily measured by using flow cytometry.
Objective: We sought to identify *A fumigatus* sensitization in patients with CF by using the basophil activation test (BAT).
Methods: Patients with CF attending Beaumont Hospital were screened for study inclusion. BAT was used to identify *A fumigatus* sensitization. Serologic (total and *A fumigatus*–specific IgE), pulmonary function, and body mass index measurements were performed.
Results: The BAT discriminates *A fumigatus*–sensitized from nonsensitized patients with CF. Persistent isolation of *A fumigatus* in sputum is a significant risk factor for *A fumigatus* sensitization. Levels of the *A fumigatus*–stimulated basophil activation marker CD203c inversely correlated with pulmonary function and body mass index in *A fumigatus*–sensitized but not nonsensitized patients with CF. Total and *A fumigatus*–specific IgE, but not IgG, levels are increased in *A fumigatus*–sensitized patients with CF and ABPA when compared with those in *A fumigatus*–sensitized and nonsensitized patients with CF without ABPA. Itraconazole treatment did not affect *A fumigatus* sensitization.
Conclusion: Combining the BAT with routine serologic testing allows classification of patients with CF into 3 groups: nonsensitized, *A fumigatus*–sensitized, and ABPA. Accurate and prompt identification of *A fumigatus*–associated clinical status might allow early and targeted therapeutic intervention, potentially improving clinical outcomes. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Allergic bronchopulmonary aspergillosis,* Aspergillus fumigatus*, basophil activation test, body mass index, CD203c, cystic fibrosis, flow cytometry, FEV_1, itraconazole, sensitization*

Cystic fibrosis (CF) is an inherited disorder characterized by recurrent polymicrobial pulmonary exacerbations and chronic neutrophil-dominated inflammation. Dysfunctional CF transmembrane conductance regulator protein leads to impaired mucociliary clearance and colonization of the lungs of patients with CF by bacteria and fungi. Much focus has been given to the role of bacteria in the airways of patients with CF; however, an increasing recognition of fungi has emerged.[1-3] *Aspergillus fumigatus* is the most commonly isolated fungus in patients with CF, with a prevalence of up to 60%.[4] The fungus is associated with a range of manifestations in patients with CF, most commonly allergic bronchopulmonary aspergillosis (ABPA) and, less commonly, aspergillomas and invasive pulmonary aspergillosis.[5-7]

ABPA affects between 2% and 15% of patients with CF, and recurrent episodes affect pulmonary function.[1,8-11] It manifests as an allergic, hypersensitive, T_H2 $CD4^+$ cell–driven response to *A fumigatus*, and the diagnosis of ABPA in patients with CF is particularly challenging because of overlapping clinical, immunologic, and radiologic features that are similar to those of a pulmonary exacerbation. To address this, consensus conference criteria were published to aid clinicians in diagnosing CF with ABPA.[12] Despite this, inherent weaknesses in these criteria exist. They can be difficult to use in the CF setting, and many patients with CF-ABPA do not fulfill these criteria despite a good response to treatment. The criteria have not been updated for 12 years despite advances in both the understanding and treatment of CF with ABPA. Hence there is a growing need for a simplified updated classification to robustly diagnose ABPA, facilitating earlier intervention to improve clinical outcomes.

Sensitization is an immunologic phenomenon defined by the production of specific IgE (sIgE). It can arise from a combination of genetic predisposition and allergen exposure.[13,14] Recent reports show that *A fumigatus* sensitization is associated with a greater decrease in lung function and an increased rate of pulmonary exacerbations in patients with CF.[15-17] The basophil

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Abbreviations used
ABPA: Allergic bronchopulmonary aspergillosis
BAT: Basophil activation test
BMI: Body mass index
CF: Cystic fibrosis
GM: Galactomannan
ROC: Receiver operating characteristic
sIgE: Specific IgE
sIgG: Specific IgG
SPT: Skin prick test

activation test (BAT) is a novel technique that measures upregulation of CD203c on stimulation with the specific allergen to which a patient is sensitized.[18] CD203c is an ectonucleotide pyrophosphatase/phosphodiesterase expressed on basophil surfaces, which are important effector cells in type II immune responses.[18,19] CD203c can be rapidly measured by means of flow cytometry and has been proposed as a diagnostic tool in patients with atopic disease, including peanut, drug, and wasp venom allergy.[20-22]

It has been shown that basophils are primed and hyperresponsive to *A fumigatus* allergen stimulation in patients with CF and ABPA.[18] In the current study the BAT to *A fumigatus* was used to identify *A fumigatus* sensitization in a CF cohort, and results were correlated with key CF clinical measurements. Furthermore, *A fumigatus*–stimulated CD203c, in conjunction with commonly available immunologic parameters, was examined for use in the classification of patients with CF into nonsensitized, sensitized, and ABPA groups.

METHODS

Patient recruitment

We prospectively recruited 48 patients with CF to the study between October 2012 and October 2014. As control subjects, 11 healthy volunteers without CF were also recruited. Ethical approval was obtained from our institutional review board. CF was confirmed by sweat chloride levels (>60 mmol/L) and genotyping. Pulmonary function testing (see the Methods section in this article's Online Repository at www.jacionline.org) and serum sampling were performed on the day of the BAT. Total circulating IgE, sIgE, and specific IgG (sIgG) levels to *A fumigatus* were determined by using the ImmunoCAP assay (Phadia, Uppsala, Sweden). Patients' demographics are outlined in Table E1 in this article's Online Repository at www.jacionline.org. Quarterly sputum samples were routinely collected for standard microbiological evaluation, including fungi.[23] Exclusion criteria were pregnancy, lung transplantation, peanut allergy, and age less than 16 years.

Cohort characterization

The CF with ABPA cohort were given diagnoses per previously published consensus criteria. sIgE levels to *Aspergillus* species were used as an alternative to skin prick tests (SPTs), which are interchangeable in consensus criteria.[12] To differentiate between *A fumigatus*–sensitized and nonsensitized patients with CF, an arbitrary cutoff (1.36) was set at 3 SDs greater than the mean value[24] of the stimulation index of the healthy control population, with the stimulation index defined as the ratio between *A fumigatus*– and PBS-stimulated CD203c values.

BAT

Samples were processed, as previously described.[18,25,26] Briefly, venous blood was collected in S-Monovette EDTA blood tubes (Sarstedt, Numbrecht, Germany) and centrifuged at 400*g* for 10 minutes at 4°C. The supernatant was further centrifuged at 3000*g* for 10 minutes at 4°C, and 97 μL of platelet-free plasma was added to 100 μL of erythrocyte/leukocyte pellet and incubated for 30 seconds at 37°C. Samples were incubated with 3 μL of *A fumigatus* extract (Mediwiss Analytic GmbH, Moers, Germany) for 10 minutes at 37°C. PBS and peanut extract (Mediwiss Analytic GmbH) were used as controls. Untreated basophils were also evaluated. After washing, cells were stained with fluorescence isothiocyanate–labeled mouse anti-human CD3, HLA-DR, CD41a, and CD66b; peridinin-chlorophyll-protein complex-Cy5.5–labeled mouse anti-human CD123 (BD Biosciences, San Jose, Calif); and phycoerythrin-labeled mouse anti-human CD203c (BioLegend, San Diego, Calif) at saturating concentrations. The LIVE/DEAD FixableNear-IR Dead Cell stain kit (Invitrogen, Carlsbad, Calif) was used to distinguish between live and dead cells. After washing, erythrocytes were lysed, and leukocytes were fixed with Lyse/Fix buffer (BD Biosciences) for 30 minutes on ice. After centrifugation at 490*g* for 5 minutes, cells were resuspended in 2.5 mmol/L EDTA containing 5% FCS and analyzed by means of flow cytometry on a BD FACSCalibur (BD Biosciences) equipped with BD CellQuest Pro Software. Compensation was performed with Calibrite Beads (BD Biosciences), and at least 200 basophils per sample were analyzed. Data were analyzed with FlowJo software (TreeStar, Ashland, Ore).

Statistical analysis

Statistical analysis was performed with GraphPad PRISM 4.0 software (GraphPad Software, San Diego, Calif). Data were tested for normality by using the Kolmogorov-Smirnoff test. Normal data were compared by using a 2-tailed independent Student *t* test, and for nonnormal data, the Mann-Whitney *U* test was performed. Differences were considered significant at a *P* value of less than .05.

RESULTS

The BAT discriminates between nonsensitized and *A fumigatus*–sensitized patients with CF

Flow cytometry was used to measure basophil activation in response to *A fumigatus*. Basophils were gated as the Live/Dead$^-$CD3$^-$HLA-DR$^-$CD41a$^-$CD66b$^-$CD123$^+$ population and evaluated for CD203c expression after *A fumigatus* extract stimulation (see Fig E1 in this article's Online Repository at www.jacionline.org). PBS and peanut extract were used as nonoffending and immunogenic controls, respectively. An arbitrary cutoff of 1.36 was determined by using the stimulation index of the control subjects without CF (mean ± SD, 1.018 ± 0.114; n = 11) to differentiate between *A fumigatus*–sensitized and nonsensitized patients with CF. A positivity threshold of 3 SDs greater than the healthy mean of the stimulation index was determined as before.[24] By using this cutoff, 23 (47.9%) of the 48 recruited patients with CF were *A fumigatus* sensitized. The ability of sIgE to distinguish between sensitized and nonsensitized subjects has been previously proposed by using a cutoff of 0.35 kU/L.[16] The diagnostic performance of the BAT was examined against sensitization status determined with sIgE (*A fumigatus* sensitization cutoff, 0.35 kU/L; ImmunoCAP assay)[16] by using receiver operating characteristic (ROC) curve analyses. The area under the ROC curve was 0.9134 ($P < .0001$, see Fig E2 in this article's Online Repository at www.jacionline.org), indicating the excellent discriminative ability of the BAT between nonsensitized and *A fumigatus*–sensitized patients with CF and corroborating the strength of *A fumigatus*–stimulated CD203c levels as an indicator of *A fumigatus* sensitization in patients with CF.

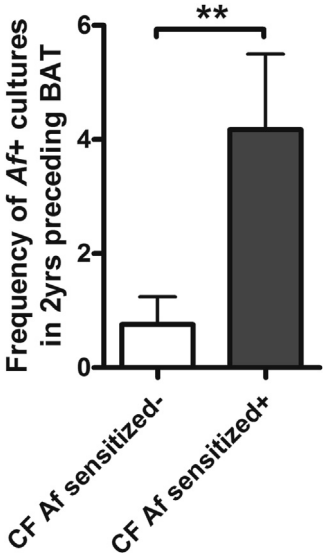

FIG 1. Frequency of *A fumigatus (Af)*–positive sputum cultures in nonsensitized patients with CF (*CF Af sensitized*−; n = 25) and *A fumigatus*–sensitized patients (*CF Af sensitized*+; n = 23) in the 2-year period preceding the BAT. Patients with CF were screened for *A fumigatus* in sputum as part of routine care. Data are presented as means ± SEMs. $^{**}P < .01$.

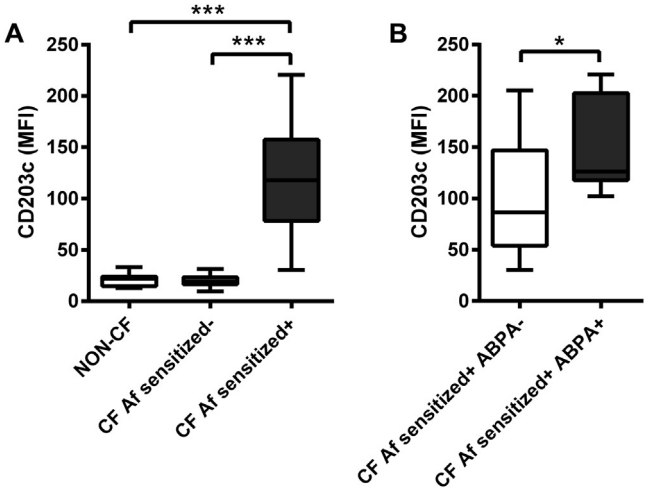

FIG 2. *A fumigatus (Af)*–stimulated CD203c levels in nonsensitized patients, *A fumigatus*–sensitized patients, and patients with CF and ABPA. **A,** Patients with CF were classified as nonsensitized (n = 25) or *A fumigatus* sensitized (n = 23). **B,** The *A fumigatus*–sensitized cohort was further divided into those with (*CF Af sensitized*+ *ABPA*+; n = 7) or without (*CF Af sensitized*+ *ABPA*−; n = 16) ABPA. Data are presented as Tukey box plots, and the median is represented by the *middle line*. *MFI*, Mean fluorescence intensity. $^{*}P < .05$ and $^{***}P < .001$.

A fumigatus–sensitized patients with CF have higher frequencies of *A fumigatus*–positive sputum cultures compared with nonsensitized patients with CF

Patients with CF were screened for the presence of *Aspergillus* species in their quarterly sputum samples as part of routine care, amounting to 8 sputum samples from each patient in the 2-year period preceding the BAT. Fourteen (60.9%) of 23 *A fumigatus*–sensitized patients, but only 6 (24.0%) of 25 nonsensitized patients with CF grew *A fumigatus* on at least 1 occasion in the 2-year period preceding the BAT. *A fumigatus*–sensitized patients with CF displayed higher frequencies of *A fumigatus* sputum isolation compared with nonsensitized patients with CF ($P = .0038$, Fig 1). *Candida albicans* colonization was also assessed to verify the specificity of the BAT to *A fumigatus*. In contrast to *A fumigatus*, 19 (82.6%) of 23 *A fumigatus*–sensitized and 17 (68.0%) of 25 nonsensitized patients with CF grew *C albicans* at least once in the 2 years preceding the BAT. There was no significant difference in the frequency of *C albicans* isolation between groups (see Fig E3 in this article's Online Repository at www.jacionline.org), illustrating the specificity of the BAT to *A fumigatus*. Similar results were observed when colonization with *Pseudomonas aeruginosa* was investigated (see Fig E4 in this article's Online Repository at www.jacionline.org). No significant difference in *A fumigatus*–stimulated CD203c levels between patients with nonmucoid ($P = .3263$) or mucoid ($P = .2351$) *P aeruginosa* compared with their noncolonized counterparts was noted. In summary, BAT to *A fumigatus* is specific, and patients with CF with an increased frequency of *A fumigatus* in sputum are more likely to have sensitization to *A fumigatus*.

A fumigatus–stimulated CD203c levels are increased in blood basophils from *A fumigatus*–sensitized compared with nonsensitized patients with CF

A fumigatus–sensitized patients with CF had significantly higher *A fumigatus*–stimulated CD203c values compared with healthy control subjects ($P < .0001$) and nonsensitized patients with CF ($P < .0001$; Fig 2, *A*). Interestingly, *A fumigatus*–stimulated CD203c values were significantly higher in *A fumigatus*–sensitized patients with CF with ABPA than *A fumigatus*–sensitized patients with CF without ABPA ($P = .0408$; Fig 2, *B*). This demonstrates that the BAT to *A fumigatus* distinguishes *A fumigatus* sensitization from nonsensitization along a quantitative spectrum and could also be potentially used in the diagnosis of patients with ABPA within the *A fumigatus*–sensitized cohort.

CD203c expression levels inversely correlate with FEV_1 and body mass index of *A fumigatus*–sensitized patients with CF

A fumigatus sensitization has previously been shown to be associated with worse lung function in patients with a variety of pulmonary diseases, including asthma, chronic obstructive pulmonary disease, and CF.[16,17,27,28] Therefore we explored the relationship of *A fumigatus*–stimulated CD203c levels with lung function (FEV_1) and body mass index (BMI), both established determinants of mortality in patients with CF.[29] We observed a significant correlation of *A fumigatus*–stimulated CD203c levels with decreasing FEV_1 in *A fumigatus*–sensitized patients (n = 16, $r^2 = 0.6409$, $P = .0002$; Fig 3, *A*). Interestingly, no correlation between *A fumigatus*–stimulated CD203c values and FEV_1 in nonsensitized patients with CF was detected (n = 25, $r^2 = 0.0198$, $P = .5027$; Fig 3, *A*). Patients with CF with ABPA receiving corticosteroid treatment were excluded from this analysis because of the potential interference of corticosteroid therapy with CD203c levels (see Figs E5 and E6 in this article's Online Repository at www.jacionline.org). BMI was also shown to inversely correlate with *A fumigatus*–stimulated CD203c levels in *A fumigatus*–sensitized (n = 16, $r^2 = 0.3009$, $P = .0278$) but not nonsensitized patients with CF

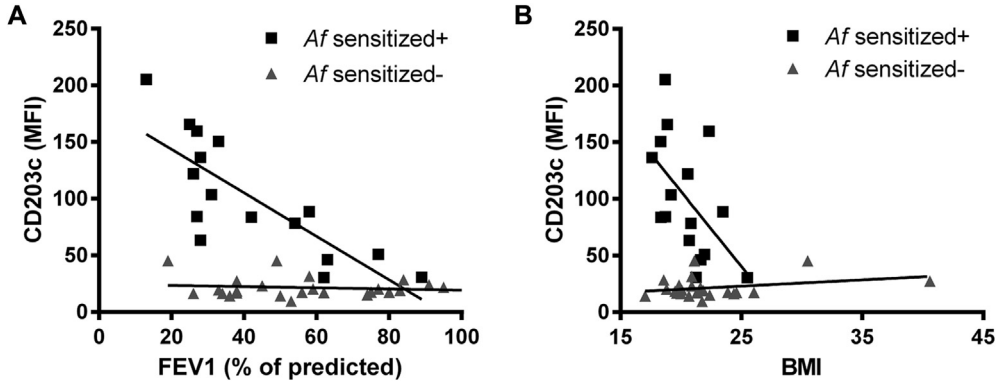

FIG 3. Correlation of *A fumigatus (Af)*–stimulated CD203c levels with FEV_1 and BMI. *A fumigatus*–stimulated CD203c levels correlated with FEV_1 ($r^2 = 0.6409$, $P = .0002$; **A**) and BMI ($r^2 = 0.3009$, $P = .0278$; **B**) in *A fumigatus*–sensitized (n = 16) but not nonsensitized patients with CF (FEV_1: n = 25, $r^2 = 0.0198$, $P = .5027$; BMI: $r^2 = 0.0905$, $P = .1439$). *MFI*, Mean fluorescence intensity.

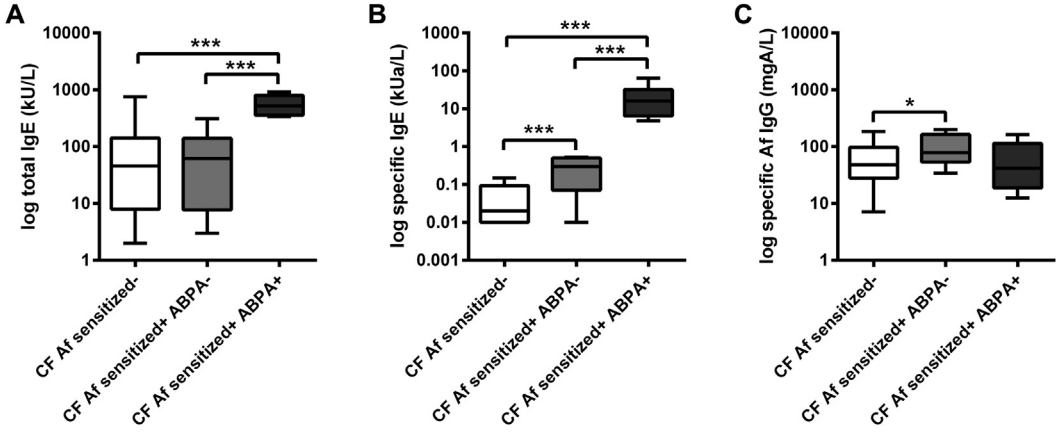

FIG 4. Serologic immune parameters in patients with CF according to *A fumigatus (Af)* sensitization status. Total IgE **(A)**, sIgE **(B)**, and sIgG **(C)** serum levels were measured in nonsensitized patients (n = 25), *A fumigatus*–sensitized patients (n = 16), and patients with CF with ABPA (n = 7). Data are presented as Tukey box plots, and the median is represented by the *middle line*. $*P < .05$ and $***P < .001$.

(n = 25, $r^2 = 0.0905$, $P = .1439$; Fig 3, *B*). Together, these data illustrate that *A fumigatus*–stimulated CD203c levels correlate with key clinical parameters in patients with CF, including FEV_1 and BMI, confirming the clinical effect of the *A fumigatus*–sensitized state.

Total IgE and *A fumigatus* sIgE, but not *A fumigatus* sIgG, levels are increased in sera of *A fumigatus*–sensitized patients with ABPA

Total IgE and *A fumigatus* sIgE and sIgG levels were evaluated in serum samples from nonsensitized patients, *A fumigatus*–sensitized patients, and patients with CF-ABPA. As described above, the stimulation index threshold was used to distinguish sensitized (>1.36) from nonsensitized (<1.36) patients. The patients with CF-ABPA were defined as those meeting classical consensus criteria for ABPA.[12] Total IgE levels were significantly higher in patients with CF-ABPA (n = 7) when compared with those in nonsensitized and *A fumigatus*–sensitized patients with CF (n = 25, $P < .0001$ and n = 16, $P < .0001$, respectively; Fig 4, *A*). Similarly, when sIgE levels were assessed, significantly higher levels were observed in patients with CF-ABPA than in nonsensitized and *A fumigatus*–sensitized patients with CF ($P = .0004$ and $P < .0001$, respectively; Fig 4, *B*). *A fumigatus*–sensitized patients with CF without ABPA had higher sIgE levels than nonsensitized patients with CF ($P < .0001$). No increase in sIgG levels was observed in the CF-ABPA group when compared with the *A fumigatus*–sensitized and nonsensitized patients with CF; however, *A fumigatus*–sensitized patients with CF without ABPA had higher sIgG levels than nonsensitized patients with CF ($P = .0302$; Fig 4, *C*). Therefore total serum IgE and sIgE levels, but not sIgG levels, are useful in aiding the detection of CF-ABPA and in differentiating it from *A fumigatus* sensitization.

Antifungal therapy does not alter *A fumigatus*–stimulated CD203c expression levels on basophils

Recent work by our group has shown that elimination of *A fumigatus* bioburden with itraconazole (400 mg/d for 6 weeks) improves clinical outcome.[23] BAT to *A fumigatus* was performed to investigate the effect of fungal eradication on *A fumigatus* sensitization. Although we have previously shown that itraconazole reduces the fungal load in sputum,[23] no change in

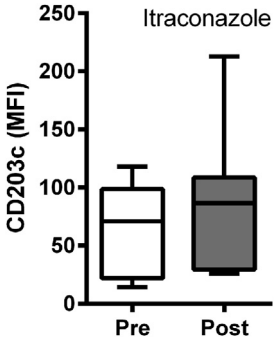

FIG 5. Effect of itraconazole treatment on *A fumigatus (Af)*–stimulated CD203c levels in patients with CF. *A fumigatus*–stimulated CD203c levels in blood basophils from patients with CF before and after 6 weeks of treatment with oral itraconazole (n = 8, $P = .4028$) are presented as Tukey box plots, and the *line in the middle of the box* represents the median. *MFI*, Mean fluorescence intensity.

CD203c levels before and after itraconazole treatment was observed (n = 8, $P = .4028$; Fig 5). Additionally, no difference in baseline CD203c expression was observed (n = 8, $P = .3829$; see Fig E5, *A*). This shows that decreasing the fungal burden in patients with CF undergoing antifungal therapy with itraconazole does not affect *A fumigatus* sensitization once it is established.

Additionally, in a small number of patients (n = 4) receiving monthly pulses of high-dose intravenous methylprednisolone for ABPA (10-15 mg/kg for 3 days),[30] a trend toward efficacy of treatment was observed, with *A fumigatus*–stimulated CD203c values halving (57.6%) 1 week after treatment initiation ($P = .1604$, see Fig E6). However, this trend decreased to 30% after 1 month and before the next infusion of corticosteroids (n = 4, $P = .2672$; see Fig E6), which is suggestive of the transient effect of corticosteroid therapy with time.

TABLE I. Simplified classification of *Aspergillus* species–associated disease in patients with CF

	A fumigatus–stimulated CD203c	sIgE	Total IgE
Cutoff	1.36*	1.45 kU_A/L†	185 kU/L‡
Nonsensitized group	↓	↓	↓
A fumigatus–sensitized group	↑	↓	↓
CF with ABPA group	↑	↑	↑

*Determined as described in the Methods section.
†Local cutoff value.
‡As proposed by Baxter et al.[11]

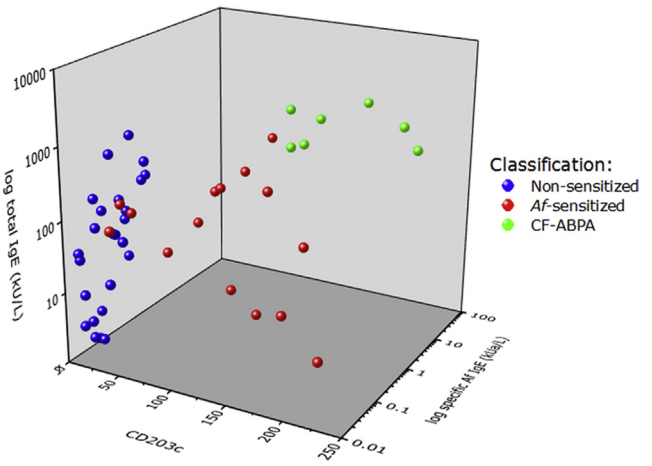

FIG 6. Graphic representation of a simplified classification of *Aspergillus* species–associated disease by using a combination of *A fumigatus (Af)*–stimulated CD203c and total IgE and sIgE levels. The *blue, red,* and *green dots* denote nonsensitized patients (n = 25), *A fumigatus*–sensitized patients (n = 16), and patients with CF-ABPA (n = 7), respectively, as depicted with the 3-dimensional scatter plot.

DISCUSSION

The role of bacteria in the lungs of patients with CF has been extensively studied, and potential roles for fungi are beginning to emerge. Recently, much focus has been given to the wide spectrum of *A fumigatus*–associated morbidities in patients with CF, including *A fumigatus* sensitization and ABPA. Both of these clinical states are associated with poorer clinical outcomes.[16,31] For this reason, identifying patients with *A fumigatus* sensitization with or without ABPA is important to ensure appropriate and timely therapeutic intervention and minimize pulmonary effect.[30] In this study we focused on the basophil activation marker CD203c as an indicator of *A fumigatus* sensitization and assessed its effect on important clinical measures of CF disease.

Sensitization to *A fumigatus* has previously been associated with poorer lung function,[16,17] a finding confirmed by this study. Gernez et al[18] recently reported that basophils from patients with CF and ABPA are primed and hyperresponsive to stimulation with *A fumigatus* extract. In the current study we used the BAT to identify the *A fumigatus* sensitization status of our CF cohort. From a logistic viewpoint, the BAT to *A fumigatus* can be performed in less than 4 hours by using a maximum of 2 mL of whole blood. This can be taken in the same blood draw as the routinely measured immunologic investigations minimizing discomfort for the patient. A flow cytometer and appropriately trained staff are required to perform the BAT, which would represent the only limitations to its applicability in routine CF center practice.

Almost half of the patients studied were *A fumigatus* sensitized, which is in accordance with previous studies (31% to 61%) that used serology and SPTs alone.[15-17] Increased serum IgE levels to *A fumigatus* and SPTs are considered the gold standards for routine laboratory investigations and are part of the established consensus criteria for the diagnosis of ABPA in patients with CF.[12] Nevertheless, both of these tests harbor disadvantages. Measuring levels of sIgE to *A fumigatus* is an easily accessible test for the clinician and convenient for the patient. However, it has been shown that sIgE levels tend to change over time and have to be interpreted along with the constellation of other clinical and laboratory variables and patient history to reach a diagnosis.[32,33] SPTs are often laborious and time consuming because the full investigation can take up to 72 hours to complete. Furthermore, subcutaneous injection of the antigen will induce swelling, itching, and reddening at the site of injection in an allergic patient and is therefore a source of discomfort to the patient. The SPTs can be subjective for the attending clinician and open to interpretation because some suggest a wheal diameter of greater than 3 mm and others recommend a diameter of greater than 4 mm for a positive result.[32] Accumulating reports have

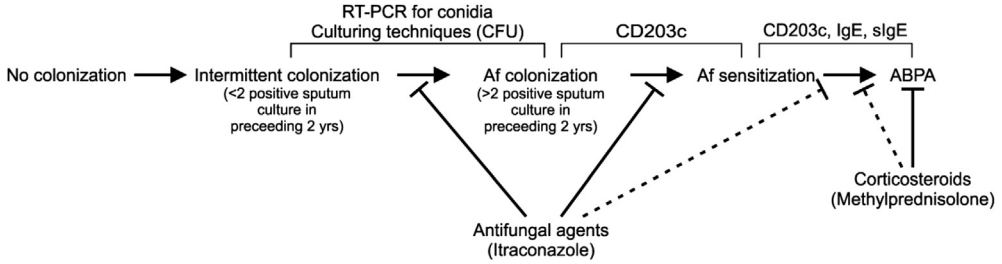

FIG 7. A schematic diagram illustrating the potential stages of *A fumigatus (Af)*–associated CF disease with detection methods and possible treatment interventions at each stage. *Solid* and *hashed lines* represent established and suggested treatment regimens, respectively.

shown striking differences in individual patients between SPT responses and serologic test results, suggesting an important role for the effector cells of immediate hypersensitivity (mast cells and basophils) and their activation during an allergic response.[32,34-37] Therefore there is a great need for a reliable *in vitro* method to complement the routine laboratory tests for sensitization/allergy when the latter show discrepancies or are not feasible.[33] Of note, *A fumigatus* sIgE levels correlated significantly with CD203c levels (see Fig E7 in this article's Online Repository at www.jacionline.org). The BAT is a specific and reliable measure of IgE-dependent response in sensitized subjects because (1) the mast cells are tissue bound, (2) basophil accessibility is easy, and (3) that the upregulation of basophil surface markers can be conveniently measured by means of flow cytometry. By comparing CD203c levels with the already established cutoff sIgE value of 0.35 kU_A/L[16] to distinguish between sensitized and nonsensitized patients, ROC curve analysis further confirmed the usefulness of the BAT as an indicator of *A fumigatus* sensitization in patients with CF. Persistent and prolonged exposure to the fungus is a significant risk factor for *A fumigatus* sensitization because sensitized patients display a higher frequency of *A fumigatus* isolation from sputum culture. Although a study by Baxter et al[15] showed no correlation between *A fumigatus* colonization and sensitization, it is conceivable that these different outcomes might be related to methodological differences in defining *A fumigatus* sensitization, colonization, and the microbiological techniques used. Interestingly, the *A fumigatus*–sensitized ABPA cohort had higher *A fumigatus*–stimulated CD203c values than *A fumigatus*–sensitized patients without ABPA, illustrating that levels of measured CD203c might allow differentiation between *A fumigatus* sensitization and CF with ABPA.

We and others have previously shown that persistent carriage of *A fumigatus* in the airways of patients with CF is associated with radiologic abnormalities, including more severe bronchiectasis and mosaic pattern perfusion, and has an effect on pulmonary function.[16,38] *A fumigatus* sensitization is also associated with lung function decrease and increased duration of intravenous antibiotic treatments.[15,17] In line with this, our data illustrate a significant inverse correlation between *A fumigatus*–stimulated CD203c values with FEV_1 and BMI. These findings are in concordance with previously published studies suggesting a negative effect of *A fumigatus* sensitization in patients with CF.[15-17] *A fumigatus* sensitization likely facilitates CF with ABPA, and multiple factors, including degree of fungal exposure, mucus viscosity, immune status, atopy, and age, might also have interdependent roles.[12,32,39] Other factors, such as HLA-DR/HLA-DQ subtypes and the presence of single nucleotide polymorphisms in the IL-4 binding site of IL-4 receptor α have also been described to associate with ABPA development.[40-42] Longitudinal studies are needed to confirm that *A fumigatus* sensitization routinely precedes ABPA.

Baxter et al[11] have recently proposed a novel classification of aspergillosis in adults with CF. Using a combination of serologic (total IgE and *A fumigatus* sIgE and sIgG), RT-PCR, and galactomannan (GM) data, they distinguish between nondiseased subjects, patients with CF with ABPA, patients with *A fumigatus* sensitization, and patients with *A fumigatus* bronchitis. In accordance with their findings, we found that patients with CF-ABPA had higher total IgE and *A fumigatus* sIgE levels compared with nonsensitized and *A fumigatus*–sensitized patients without ABPA. Our study showed no difference in sIgG levels between patients with CF with or without ABPA. Additionally, when applied to our CF population, both the GM assay and RT-PCR for *A fumigatus* detected a high number of *A fumigatus*–positive sputum samples (data not shown), preventing adequate differentiation between the cohorts according to the aforementioned classification. This high positivity might be due to the fact that RT-PCR identifies both live and dead organisms[15] and that GM assays can yield false-positive results in the presence of penicillin-derived antibiotics, to which many of our patients with CF are exposed.[43,44] This limits its utility in the current study.

For the purposes of the current study, we adopted a novel simplified classification that allows differentiation between nonsensitized subjects, *A fumigatus*–sensitized patients, and patients with CF-ABPA. We used a combination of *A fumigatus*–stimulated CD203c and total IgE and *A fumigatus* sIgE levels (Table I) to identify ABPA, avoiding the need for RT-PCR, GM, or *A fumigatus* sIgG testing that is not always accessible in CF centers. Increased *A fumigatus*–stimulated CD203c values (>1.36) combined with increased serum sIgE (>1.45 kU_A/L) and total IgE (>185 kU/L) levels correctly identified all cases of CF with ABPA based on the consensus conference criteria. Of note, the sIgE cutoff (1.45 kU_A/L) used in the proposed classification system for identification of CF with ABPA is higher than the sIgE cutoff (0.35 kU_A/L) routinely used to identify sensitization alone. Furthermore, the total IgE cutoff herein has been considerably reduced compared with the consensus values (minimum, 500 kU/L), which is in line with a previously published ROC curve analysis demonstrating that the optimum level for ABPA diagnosis is greater than 185 kU/L, producing 91% sensitivity and 90% specificity.[11]

The inclusion of the reduced level in our proposed classification system was validated when 1 patient meeting our classification criteria for CF with ABPA (ie, increased CD203c, total IgE, and *A fumigatus* sIgE levels), but not the consensus conference criteria (total IgE >500 kU/L), had ABPA, as defined by consensus conference criteria shortly after the study conclusion. A graphic representation of our classification is depicted in Fig 6.

Our previous work has shown that itraconazole alleviates fungal burden in patients with CF colonized with *A fumigatus*.[23] In this study itraconazole administration did not influence *A fumigatus*–stimulated CD203c levels, suggesting that fungal eradication from the lung does not affect *A fumigatus* sensitization once established. Taking into consideration that persistent *A fumigatus* colonization is a significant risk factor for sensitization, early itraconazole intervention might be warranted to clear colonization, reduce exposure, and thereby minimize the risk of sensitization to *A fumigatus*. Acute ABPA flares can be treated with intravenously administered pulsed methylprednisolone at 10 to 15 mg/kg/d for a 3-day period every month.[30,45] Methylprednisolone was administered to 4 patients with CF with ABPA in this study, and a trend toward reduction in *A fumigatus*–stimulated CD203c values was observed after treatment. However, this trend was not significant ($P = .1604$), warranting future longitudinal study with increased numbers to examine the effect of corticosteroid treatment on *A fumigatus* sensitization and potentially the use of the BAT to monitor treatment responses. Additionally, future work should also examine the effect of anti-IgE and anti–IL-13 strategies in the context of CD203c levels and CF with ABPA.

In conclusion, we propose a novel and simplified means of identifying sensitization to *A fumigatus* by using *A fumigatus*–stimulated CD203c values. Using the BAT, we show that increased incidence of *A fumigatus* colonization is associated with *A fumigatus* sensitization and that the latter state affects lung function. The practical applicability of the BAT to *A fumigatus* in the clinical setting includes an evaluation of *A fumigatus*–stimulated CD203c values for patients positive for *A fumigatus* on at least 2 occasions within the preceding 2 years (Fig 7). If the BAT result is negative, itraconazole treatment can be offered for fungal eradication; however, a positive result indicates sensitization and a potential increased future risk of ABPA.[10,16] Consequently, sensitized patients should have their serologic results closely monitored for increases in total IgE or *A fumigatus* sIgE levels that might indicate ABPA. Despite the lack of effect of azole treatment on the sensitization state, eradication therapy to reduce fungal bioburden can be recommended. If sensitization is a prerequisite for ABPA, corticosteroids can be considered at the early sensitization stage to reduce the likelihood of having ABPA. A longitudinal study with adequate numbers should be performed to assess the benefits of systemic corticosteroid administration on clinical outcomes in *A fumigatus*–sensitized patients without ABPA.[17] Timely detection of *A fumigatus* sensitization and CF with ABPA equips clinicians to deliver a targeted approach to CF *A fumigatus* therapy to improve clinical outcomes.

We sincerely thank the patients with cystic fibrosis and healthy volunteers for taking part in this study.

Key messages

- **The BAT can identify *A fumigatus*–sensitized patients in the population with CF.**
- **CD203c levels inversely correlate with pulmonary function and BMI in *A fumigatus*–sensitized patients with CF.**
- **Prompt identification of *A fumigatus* sensitization might improve the management of *A fumigatus*–associated disease in patients with CF.**

REFERENCES

1. Chotirmall SH, McElvaney NG. Fungi in the cystic fibrosis lung: Bystanders or pathogens? Int J Biochem Cell Biol 2014;52C:161-73.
2. Pohl K, Hayes E, Keenan J, Henry M, Meleady P, Molloy K, et al. A neutrophil intrinsic impairment affecting Rab27a and degranulation in cystic fibrosis is corrected by CFTR potentiator therapy. Blood 2014;124:999-1009.
3. Delhaes L, Monchy S, Fréalle E, Hubans C, Salleron J, Leroy S, et al. The airway microbiota in cystic fibrosis: a complex fungal and bacterial community—implications for therapeutic management. PLoS One 2012;7:e36313.
4. Mortensen KL, Jensen RH, Johansen HK, Skov M, Pressler T, Howard SJ, et al. *Aspergillus* species and other molds in respiratory samples from patients with cystic fibrosis: a laboratory-based study with focus on *Aspergillus fumigatus* azole resistance. J Clin Microbiol 2011;49:2243-51.
5. Sabino R, Ferreira JAG, Moss RB, Valente J, Veríssimo C, Carolino E, et al. Molecular epidemiology of *Aspergillus* collected from cystic fibrosis patients. J Cyst Fibros 2015;14:474-81.
6. Chotirmall SH, Mirkovic B, Lavelle GM, McElvaney NG. Immunoevasive *Aspergillus* virulence factors. Mycopathologia 2014;178:363-70.
7. Chotirmall SH, Al-alawi M, Mirkovic B, Lavelle G, Logan PM, Greene CM, et al. *Aspergillus*-associated airway disease, inflammation and the innate immune response. Biomed Res Int 2013;2013:723129.
8. Amin R, Dupuis A, Aaron SD, Ratjen F. The effect of chronic infection with *Aspergillus fumigatus* on lung function and hospitalization in patients with cystic fibrosis. Chest 2010;137:171-6.
9. Tunnicliffe G, Schomberg L, Walsh S, Tinwell B, Harrison T, Chua F. Airway and parenchymal manifestations of pulmonary aspergillosis. Respir Med 2013;107:1113-23.
10. Nepomuceno IB, Esrig S, Moss RB. Allergic bronchopulmonary aspergillosis in cystic fibrosis: role of atopy and response to itraconazole. Chest 1999;115:364-70.
11. Baxter CG, Dunn G, Jones AM, Webb K, Gore R, Richardson MD, et al. Novel immunologic classification of aspergillosis in adult cystic fibrosis. J Allergy Clin Immunol 2013;132:560-6.e10.
12. Stevens DA, Moss RB, Kurup VP, Knutsen AP, Greenberger P, Judson MA, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis—state of the art: Cystic Fibrosis Foundation Consensus Conference. Clin Infect Dis 2003;37(suppl 3):S225-64.
13. Knutsen AP, Bush RK, Demain JG, Denning DW, Dixit A, Fairs A, et al. Fungi and allergic lower respiratory tract diseases. J Allergy Clin Immunol 2012;129:280-93.
14. Alesina R, De Amici M, Ciprandi G. Serum IgE discriminates allergy from sensitisation better than skin testing. Allergol Immunopathol (Madr) 2014;42:171-3.
15. Baxter CG, Moore CB, Jones AM, Webb AK, Denning DW. IgE-mediated immune responses and airway detection of *Aspergillus* and *Candida* in adult cystic fibrosis. Chest 2013;143:1351-7.
16. Fillaux J, Brémont F, Murris M, Cassaing S, Rittié J-L, Tétu L, et al. Assessment of Aspergillus sensitization or persistent carriage as a factor in lung function impairment in cystic fibrosis patients. Scand J Infect Dis 2012;44:842-7.
17. Peetermans M, Goeminne P, De Boeck C, Dupont LJ. IgE sensitization to *Aspergillus fumigatus* is not a bystander phenomenon in cystic fibrosis lung disease. Chest 2014;146:e99-100.
18. Gernez Y, Dunn CE, Everson C, Mitsunaga E, Gudiputi L, Krasinska K, et al. Blood basophils from cystic fibrosis patients with allergic bronchopulmonary aspergillosis are primed and hyper-responsive to stimulation by aspergillus allergens. J Cyst Fibros 2012;11:502-10.
19. Sokol CL, Medzhitov R. Emerging functions of basophils in protective and allergic immune responses. Mucosal Immunol 2010;3:129-37.

20. Santos AF, Douiri A, Bécares N, Wu SY, Stephens A, Radulovic S, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. J Allergy Clin Immunol 2014;134:645-52.
21. Rodríguez-Trabado A, Cámara-Hijón C, Ramos-Cantariño A, Porcel-Carreño SL, Jiménez-Timón S, Pereira-Navarro G, et al. Basophil activation test for the in vitro diagnosis of nonsteroidal anti-inflammatory drug hypersensitivity. Allergy Asthma Proc 2008;29:241-9.
22. Balzer L, Pennino D, Blank S, Seismann H, Darsow U, Schnedler M, et al. Basophil activation test using recombinant allergens: highly specific diagnostic method complementing routine tests in wasp venom allergy. PLoS One 2014;9: e108619.
23. Coughlan CA, Chotirmall SH, Renwick J, Hassan T, Low TB, Bergsson G, et al. The effect of *Aspergillus fumigatus* infection on vitamin D receptor expression in cystic fibrosis. Am J Respir Crit Care Med 2012;186:999-1007.
24. Bergin DA, Reeves EP, Hurley K, Wolfe R, Jameel R, Fitzgerald S, et al. The circulating proteinase inhibitor α-1 antitrypsin regulates neutrophil degranulation and autoimmunity. Sci Transl Med 2014;6:217ra1.
25. Gernez Y, Tirouvanziam R, Yu G, Ghosn EEB, Reshamwala N, Nguyen T, et al. Basophil CD203c levels are increased at baseline and can be used to monitor omalizumab treatment in subjects with nut allergy. Int Arch Allergy Immunol 2011;154:318-27.
26. Gernez Y, Tirouvanziam R, Reshamwala N, Yu G, Weldon BC, Galli SJ, et al. Modulation of mTOR effector phosphoproteins in blood basophils from allergic patients. J Clin Immunol 2012;32:565-73.
27. Bafadhel M, McKenna S, Agbetile J, Fairs A, Desai D, Mistry V, et al. *Aspergillus fumigatus* during stable state and exacerbations of COPD. Eur Respir J 2014;43:64-71.
28. Menzies D, Holmes L, McCumesky G, Prys-Picard C, Niven R. *Aspergillus* sensitization is associated with airflow limitation and bronchiectasis in severe asthma. Allergy 2011;66:679-85.
29. Sharma R, Florea VG, Bolger AP, Doehner W, Florea ND, Coats AJ, et al. Wasting as an independent predictor of mortality in patients with cystic fibrosis. Thorax 2001;56:746-50.
30. Cohen-Cymberknoh M, Blau H, Shoseyov D, Mei-Zahav M, Efrati O, Armoni S, et al. Intravenous monthly pulse methylprednisolone treatment for ABPA in patients with cystic fibrosis. J Cyst Fibros 2009;8:253-7.
31. Kousha M, Tadi R, Soubani AO. Pulmonary aspergillosis: a clinical review. Eur Respir Rev 2011;20:156-74.
32. Chotirmall SH, Branagan P, Gunaratnam C, McElvaney NG. *Aspergillus*/allergic bronchopulmonary aspergillosis in an Irish cystic fibrosis population: a diagnostically challenging entity. Respir Care 2008;53:1035-41.
33. Chirumbolo S. Basophil activation test in allergy: time for an update? Int Arch Allergy Immunol 2012;158:99-114.
34. Bousquet J, Lebel B, Dhivert H, Bataille Y, Martinot B, Michel FB. Nasal challenge with pollen grains, skin-prick tests and specific IgE in patients with grass pollen allergy. Clin Allergy 1987;17:529-36.
35. Niederberger V, Stübner P, Spitzauer S, Kraft D, Valenta R, Ehrenberger K, et al. Skin test results but not serology reflect immediate type respiratory sensitivity: a study performed with recombinant allergen molecules. J Invest Dermatol 2001; 117:848-51.
36. Schoos AM, Chawes BL, Følsgaard NV, Samandari N, Bønnelykke K, Bisgaard H. Disagreement between skin prick test and specific IgE in young children. Allergy 2015;70:41-8.
37. Valent P, Hauswirth AW, Natter S, Sperr WR, Bühring HJ, Valenta R. Assays for measuring in vitro basophil activation induced by recombinant allergens. Methods 2004;32:265-70.
38. McMahon MA, Chotirmall SH, McCullagh B, Branagan P, McElvaney NG, Logan PM. Radiological abnormalities associated with Aspergillus colonization in a cystic fibrosis population. Eur J Radiol 2012;81:e197-202.
39. Denning DW, Pashley C, Hartl D, Wardlaw A, Godet C, Del Giacco S, et al. Fungal allergy in asthma-state of the art and research needs. Clin Transl Allergy 2014;4: 14.
40. Chauhan B. Knutsen A p, Hutcheson PS, Slavin RG, Bellone CJ. T cell subsets, epitope mapping, and HLA-restriction in patients with allergic bronchopulmonary aspergillosis. J Clin Invest 1996;97:2324-31.
41. Chauhan B, Santiago L, Kirschmann DA, Hauptfeld V, Knutsen AP, Hutcheson PS, et al. The association of HLA-DR alleles and T cell activation with allergic bronchopulmonary aspergillosis. J Immunol 1997;159:4072-6.
42. Knutsen AP, Kariuki B, Consolino JD, Warrier MR. IL-4 alpha chain receptor (IL-4Ralpha) polymorphisms in allergic bronchopulmonary aspergillosis. Clin Mol Allergy 2006;4:3.
43. Zandijk E, Mewis A, Magerman K, Cartuyvels R. False-positive results by the platelia *Aspergillus* galactomannan antigen test for patients treated with amoxicillin-clavulanate. Clin Vaccine Immunol 2008;15:1132-3.
44. Boonsarngsuk V, Niyompattama A, Teosirimongkol C, Sriwanichrak K. False-positive serum and bronchoalveolar lavage *Aspergillus* galactomannan assays caused by different antibiotics. Scand J Infect Dis 2010;42:461-8.
45. Chmiel JF, Aksamit TR, Chotirmall SH, Dasenbrook EC, Elborn JS, LiPuma JJ, et al. Antibiotic management of lung infections in cystic fibrosis. II. Nontuberculous mycobacteria, anaerobic bacteria, and fungi. Ann Am Thorac Soc 2014;11:1298-306.

METHODS

Pulmonary function testing

Results of pulmonary function tests, including FEV_1, were determined by using the MicroLab Spirometer (CareFusion, San Diego, Calif) after appropriate calibration and reported as relative measurements (percent predicted). Three representative pulmonary function tests meeting American Thoracic Society/European Respiratory Society criteria[E1] were performed at each study visit, and the averages were used for analyses.

REFERENCE

E1. Brusasco V, Crapo R, Viegi G. Coming together: the ATS/ERS consensus on clinical pulmonary function testing. Eur Respir J 2005;26:1-2.

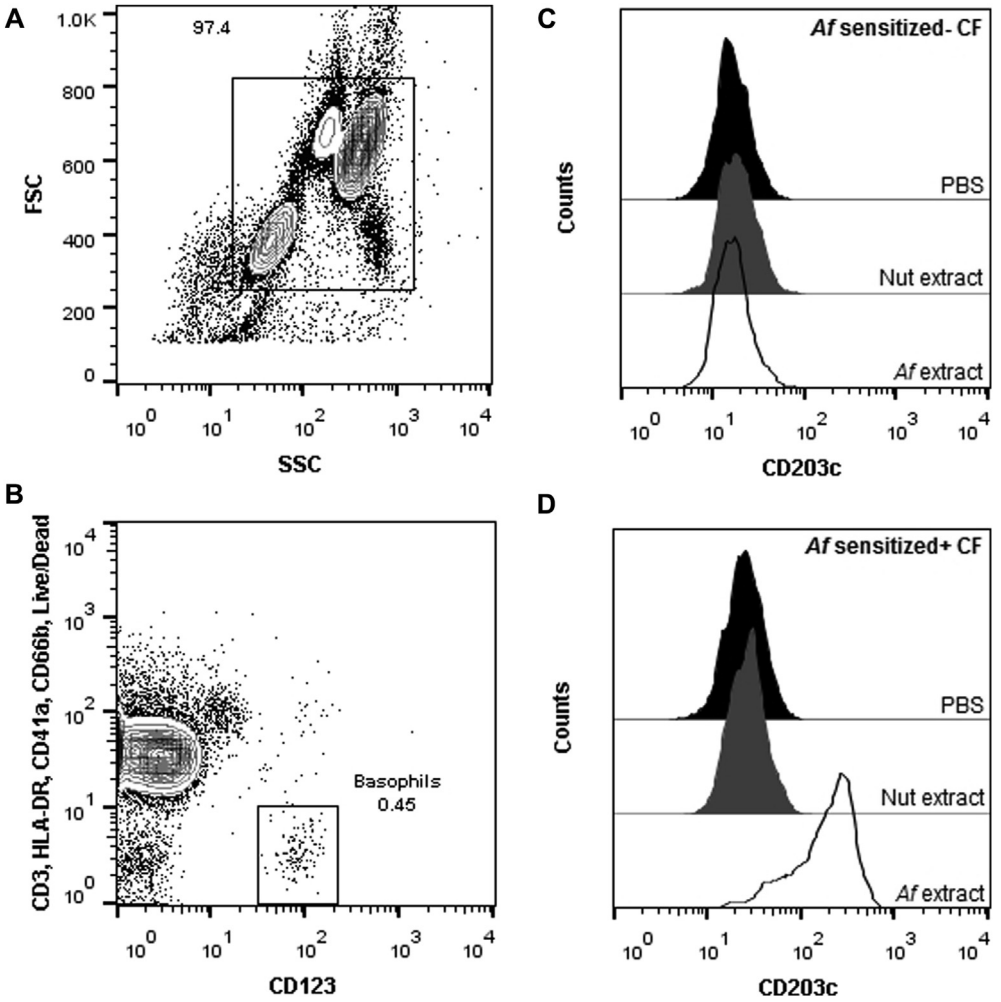

FIG E1. Gating strategy for monitoring cell-surface expression of the basophil activation marker CD203c. **A,** Leukocytes were chosen based on forward scatter *(FSC)* and side scatter *(SSC)*. **B-D,** Basophils were selected as the $CD3^{-}$HLA-$DR^{-}CD41a^{-}CD66b^{-}$Live/$Dead^{-}$ and $CD123^{+}$ population (Fig E1, *B*) and monitored for CD203c expression levels after 10 minutes of stimulation with PBS, peanut, and *A fumigatus (Af)* extract (Fig E1, *C* and *D*). Shown are representative histograms from nonsensitized (Fig E1, *C*) and *A fumigatus*–sensitized patients with CF (Fig E1, *D*). All samples were adequate for flow gating strategy and analysis.

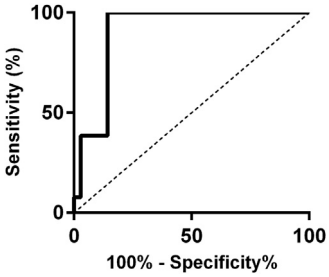

FIG E2. ROC curve analyses of the diagnostic performance of the BAT against sensitization status, as determined by sIgE levels. *A fumigatus* sIgE levels of greater than 0.35 kU/L have been considered indicative of sensitization, and this cutoff value was used to bisect our cohort into sensitized and nonsensitized patients based on their sIgE levels. The area under the ROC curve of 0.9134 ($P < .0001$) indicates the excellent discriminating ability of the BAT between nonsensitized (n = 33) and *A fumigatus*–sensitized (n = 15) patients with CF who were defined by using the *A fumigatus* sIgE levels (cutoff, 0.35 kU/L).

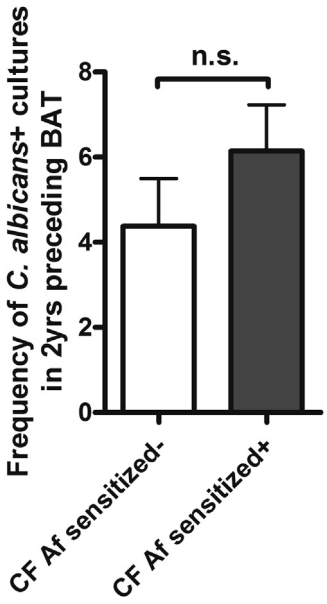

FIG E3. Frequency of *Candida albicans*–positive cultures in nonsensitized (*CF Af sensitized*−; n = 25) and *A fumigatus (Af)*–sensitized patients with CF (*CF Af sensitized*+; n = 23) in the 2-year period preceding the BAT. Patients with CF were screened for the presence of *C albicans* in their sputum samples as part of routine care. Culture data presented here were collected in the 2-year period preceding the BAT, and the differences between the 2 groups were nonsignificant ($P = .0981$). Data are presented as means ± SEMs. *n.s.,* Not significant.

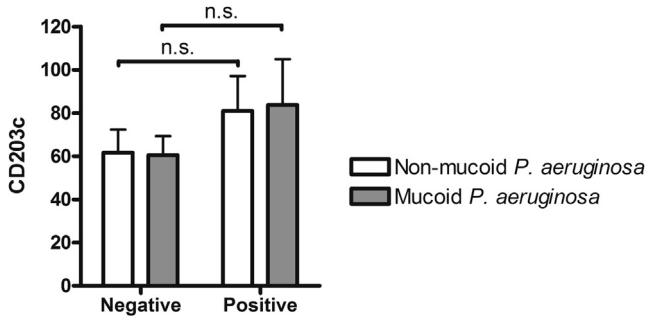

FIG E4. Effect of *Pseudomonas aeruginosa* colonization on *A fumigatus*–stimulated CD203c levels. Patients with CF (n = 48) were screened for the presence of nonmucoid and mucoid *P aeruginosa* in their sputum as a part of routine care. There was no significant difference in *A fumigatus*–stimulated CD203c levels between patients with nonmucoid ($P = .3263$) or mucoid ($P = .2351$) *P aeruginosa* compared with their noncolonized counterparts. Data are presented as means ± SEMs. *n.s.*, Not significant.

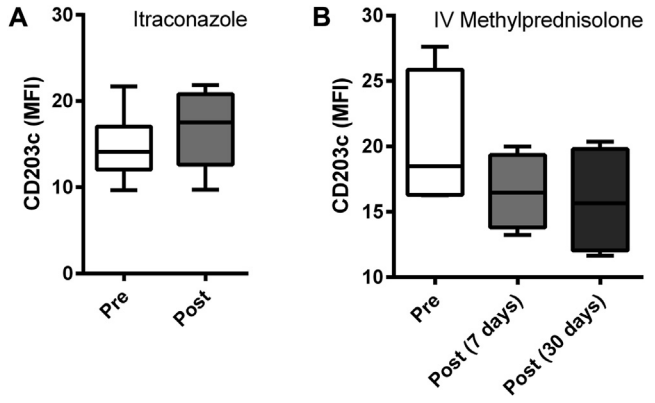

FIG E5. Baseline CD203c levels in blood basophils before and after treatment with itraconazole or methylprednisolone. Baseline surface CD203c levels in blood basophils were evaluated by means of flow cytometry before and after 30 days of treatment with oral itraconazole ($P = .3829$, n = 8; **A**) and before and 7 ($P = .3402$, n = 4) and 30 days (and $P = .1660$, n = 4) after treatment with intravenous methylprednisolone **(B)**. Data are presented as Tukey box plots, and the *middle line* represents the median. *IV*, Intravenous; *MFI*, mean fluorescence intensity.

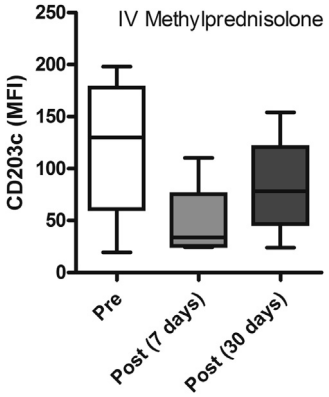

FIG E6. *A fumigatus*–stimulated CD203c levels in blood basophils before and after treatment with methylprednisolone. *A fumigatus*–stimulated surface CD203c levels in blood basophils were evaluated by means of flow cytometry before and 7 days (P = .1604, n = 4) and 30 days (P = .2672, n = 4) after treatment with intravenous *(IV)* methylprednisolone. Data are presented as Tukey box plots, and the *middle line* represents the median. *MFI*, Mean fluorescence intensity.

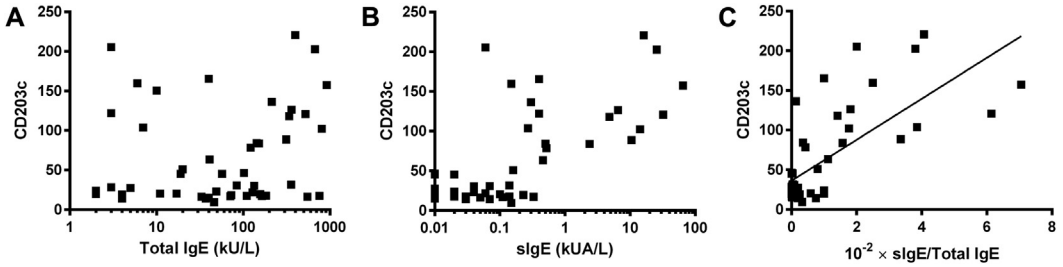

FIG E7. Correlation of total IgE levels **(A)**, sIgE levels **(B)**, and specific/total IgE ratios **(C)** with CD203c levels in our CF cohort (n = 48). Total and sIgE levels are shown in logarithmic scale.

TABLE E1. Characteristics of the patients enrolled in this study

Baseline clinical characteristics (n = 48)	
Age (y)	28 ± 10
Sex	
Male	29 (60.4%)
Female	19 (39.6%)
ΔF508 homozygous	20 (41.7%)
ΔF508 heterozygous	25 (52.1%)
BMI (kg/m^2)	21 ± 4
CFRD	12 (25.0%)
IGT	7 (14.6%)
FEV_1 (% predicted)	54 ± 23
FVC (% predicted)	75 ± 25

Data are presented as numbers (percentages) or means ± SDs.
CFRD, CF-related diabetes; *FVC*, forced vital capacity; *IGT*, impaired glucose tolerance.