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Journal of Cystic Fibrosis 17 (2018) 631 – 635

www.elsevier.com/locate/jcf

Short Communication

Mast cell tryptase changes with *Aspergillus fumigatus* – Host crosstalk in cystic fibrosis patients

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Received 26 September 2017; revised 26 January 2018; accepted 31 January 2018
Available online 15 February 2018

Abstract

Pulmonary and systemic antifungal immunity influences quality of life and survival of people with cystic fibrosis. *Aspergillus fumigatus* (*Af*) induces specific IgG and IgE. Mast cells respond to IgE, IgG and direct interactions with *Af*. Mast cells are the source of the protease tryptase. We aimed at evaluating serum baseline tryptase as a potential biomarker of the *Af*-host interaction in cystic fibrosis patients. Serum baseline tryptase, IgE and IgG directed to *Af* extract and *Af* molecular allergens were measured in 76 cystic fibrosis patients. The main findings were (i) lower levels of serum baseline tryptase in patients displaying specific IgE to *Af* ($p < 0.0001$) and (ii) an association between tryptase levels and IgE or IgG responses to *Af* and ribotoxin (Asp f 1). These findings suggest that serum baseline tryptase is influenced by *Af*-host interactions and thus might be a marker for mast cell regulation and pulmonary immune defenses.

Keywords: *Aspergillus fumigatus*; Cystic fibrosis; Immunoglobulin E; Lung transplantation; Mast cell tryptase

1. Introduction

Quality of life and survival in people with cystic fibrosis (CF) mainly depend on pulmonary status [1]. *Aspergillus fumigatus* (*Af*) is a major threat in CF patients. *Af*-host interaction is modulated by immune and environmental factors [2]. *Af* antigens induce specific immunoglobulin (Ig) G (*Af* IgG) or IgE (*Af* IgE) [3,4] and engage in direct interactions with mast cells (MCs) [5]. Lung MCs are involved in antifungal defenses and fungal allergic inflammation [5–8]. Tryptase and chymase are MC-specific proteases [9]. Normal airway mucosa hosts tryptase-producing MCs (MCT), while chymase- and tryptase-producing MCs (MCTC) reside in the submucosa [6]. Lung MCT and MCTC differ in functional phenotype, relative abundance and behavior during *Af* and CF-driven bronchial inflammatory processes [6–8,10]. Tryptase can be readily measured in peripheral blood [9,11]. We set out to describe serum baseline tryptase (sbT) in CF patients and evaluate it as a potential biomarker of the *Af*-host interaction.

2. Patients and Methods

Seventy-six CF patients were studied (Table 1).

Abbreviations: Af, *Aspergillus fumigatus*; CF, cystic fibrosis; CI, confidence interval; IQR, interquartile range; Ig, immunoglobulin; LTx, lung transplantation; MC(s), mast cell(s); sbT, serum baseline tryptase.

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https://doi.org/10.1016/j.jcf.2018.01.012

Table 1
Demography and laboratory results of the CF sample population. Seventy-six CF patients from the Adult and Pediatric Regional Centers for Cystic Fibrosis of Marseille were studied during routine follow-up visits. CF patients presented with (n = 39, *Af* IgE+) or without (n = 37, *Af* IgE-) detectable IgE to *Af* extract. Three clinical groups were defined: adult patients with native lungs, adult LTx recipients, and children. There was no significant difference in age and sex-ratio between *Af* IgE+ and *Af* IgE- groups. Bold characters with asterisks indicate significant differences between the *Af* IgE+ and *Af* IgE- groups ($p < 0.04$). Minimum and maximum values for tryptase measurements are presented in Fig. 1. IQR, interquartile range; LTx, lung transplantation.

Group	*Af* IgE statuss	n	Age (median, range)	Sex ratio (M/F)	Serum baseline tryptase (µg/L) (median, IQR)	Serum specific IgE (kUA/L) (median, IQR)			
						Af extr.	Asp f 1	Asp f 2	Asp f 3
All	*Af* IgE+	39	23 (2–59)	1	**2.8*** (2.4–4.2)	3.6 (1.7–11.1)	0.5 (0.3–2.3)	0.5 (0.1–3.1)	0.0 (0.0–0.8)
	Af IgE–	37	26 (0–56)	0.9	**4.2** (3.2–6.0)	<0.10	NA	NA	NA
Adult, native lungs	*Af* IgE+	12	24 (18–54)	1.0	**2.8*** (2.5–3.5)	5.1 (2.4–13.2)	1.5 (0.4–4.4)	1.6 (0.3–2.8)	0.0 (0.0–1.3)
	Af IgE–	11	28 (18–56)	0.8	**4.0** (3.06–5.6)	<0.10	NA	NA	NA
Adult, LTx	*Af* IgE+	12	44 (22–59)	0.7	**4.1***(2.6–5.2)	3.5 (2.0–7.0)	0.5 (0.3–1.0)	0.2 (0.1–1.0)	0.0 (0.0–0.7)
	Af IgE–	11	40 (19–48)	1.2	**5.0** (4.1–6.2)	<0.10	NA	NA	NA
Pediatric	*Af* IgE+	15	10 (2–14)	1.5	**2.5*** (2.2–3.8)	3.7 (0.8–9.3)	0.4 (0.2–2.1)	0.8 (0.1–5.1)	0.0 (0.0–0.2)
	Af IgE–	15	11 (0–17)	0.9	**3.7** (3.3–4.5)	<0.10	NA	NA	NA

3. Methods

sbT, total IgE, *Af* IgE, *Af* IgG and specific IgE and IgG to recombinant allergens Asp f 1, 2, 3, 4 and 6 were measured with ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden) [12]. Positivity thresholds were 1 µg/L (sbT), 0.10 kU_A/L (IgE), 2 mg_A/L (IgG) [13]. *Af* sensitization (*Af* IgE+) was defined as IgE to *Af* extract ≥ 0.10 kU_A/L. Patients with IgE < 0.10 kU_A/L to *Af* extract were considered as not sensitized (*Af* IgE-).

3.1. Ethics statement

IgE and IgG determination was part of routine investigations. SbT was measured on excess serum. Patients received written laboratory reports. The study was based on a retrospective review of medical charts and laboratory results. Under the French law, ethics committee approval and patient consent were not required for this type of non-interventional study, provided the patients had received information and retained the right to oppose the use of excess serum and anonymized medical data [14–15].

3.2. Data expression and statistical analysis

The results were presented as median and range or interquartile range (IQR), in Table 1 and Fig. 1. Intergroup comparison of sbT values was performed with the Mann-Whitney test. sbT, IgE and IgG levels were log-transformed for normalization for further univariate and multivariate statistical analysis.

3.3. Multivariate analysis

A stepwise selection was performed to retain the most parsimonious model including the covariates that displayed an independent statistically significant ($p < 0.05$) effect on sbT levels. The analyses, including Pearson's correlation coefficient and Wald chi-2 test, were computed with the SAS 9.2 (SAS Institute Inc., Cary, NC, USA) software. Two-sided p values < 0.05 were considered statistically significant.

4. Results

4.1. sbT distribution

Median sbT was 3.6 µg/L (1–21.6), mean 4.0 ± 2.9. sbT was below 11.5 µg/L in 73/76 patients (96%), and higher in 3*Af* IgE- patients (2 months, 4 years, 17 years). The highest value,s 21.60 µg/L (17 years, *Af* IgE-), was excluded from further statistical analysis. sbT was below 1 µg/L in two patients (17, 22 years; *Af* IgE-, *Af* IgE+) (Fig. 1).

4.2. sbT levels and Af sensitization

sbT levels were higher in *Af* IgE- compared to *Af* IgE+ patients in the whole population (Table 1) and between clinical groups (Table 1, Fig. 1). Lung transplantation (LTx) by itself did not affect sbT: median 3.2 (range 1–8.6) in adult patients with native lungs *versus* 4.4 (range 1.9–10.5) in LTx recipients, $p > 0.1$. Potential confounding factors did not differ between clinical groups (Supplementary Table 1).

4.3. sbT levels and IgG or IgE to Af extract and allergens

In *Af* IgE- patients, sbT levels were negatively correlated with IgG to *Af* extract (−0.59, p 0.02 and Asp f 1 (−0.63, p 0.01). There was no significant correlation between sbT and *Af* IgG levels in the *Af* IgE+ group. *Af* IgE+ adult patients with native lungs displayed a positive correlation of sbT with IgE to *Af* extract and Asp f 1 levels (0.48 and 0.59, $p < 0.01$). In *Af* IgE+ LTx

Serum specific IgE (kUA/L) (median, IQR)		Serum specific IgG (mgA/L) (median, IQR)						Serum total IgE (kIU/L) (median, IQR)
Asp f 4	Asp f 6	*Af* extr.	Asp f 1	Asp f 2	Asp f 3	Asp f 4	Asp f 6	
0.0 (0.0–0.5)	0.0 (0.0–0.0)	**37.5*** (20.9–63.8)	4.5 (2.8–7.2)	2.6 (0.0–4.8)	0.0 (0.0–0.0)	0.0 (0.0–2.6)	0.0 (0.0–2.9)	**194*** (88–506)
NA	NA	**25.8** (9.8–50.0)	4.9 (2.5–7.7)	0.0 (0.0–3.5)	0.0 (0.0–2.2)	0.0 (0.0–2.2)	0.0 (0.0–2.9)	**158** (5–38)
0.1 (0.0–0.8)	1.0 (0.0–0.3)	41.9 (24.6–51.6)	5.0 (3.1–7.3)	2.7 (0.0–5.5)	0.0 (0.0–0.0)	0.0 (0.0–0.6)	2.39 (0.0–2.9)	**236*** (139–356)
NA	NA	46.7 (22.8–63.1)	5.3 (4.9–8.1)	2.7 (0.0–3.9)	2.2 (0.0–3.4)	2.2 (0.0–3.6)	2.8 (0.0–3.3)	**16** (4–30)
0.0 (0.0–0.2)	0.0 (0.0–0.2)	28.0 (24.8–63.8)	2.8 (2.5–5.4)	1.5 (0.0–4.4)	0.0 (0.0–0.5)	0.0 (0.0–0.6)	0.0 (0.0–0.8)	**113*** (80–287)
NA	NA	31.8 (19.5–46.8)	5.9 (4.2–9.6)	2.4 (0.0–3.3)	0.0 (0.0–2.2)	2.9 (0.0–6.2)	2.2 (0.0–3.0)	**30** (3–37)
0.0 (0.0–0.6)	0.0 (0.0–0.0)	**37.5*** (8.1–93.1)	**4.5*** (3.2–11.7)	2.1 (0.0–4.6)	0.0 (0.0–1.6)	0.0 (0.0–3.5)	0.0 (0.0–3.0)	**490*** (109–800)
NA	NA	**10.5** (1.7–26)	**2.2** (0.0–3.5)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	**15** (7–92)

recipients, sbT levels showed a negative correlation with IgE to *Af* extract and Asp f 1 (−0.56 and −0.61, p 0.03). There was no statistically significant correlation in the pediatric *Af* IgE+ group. Fig. 2 shows the correlations between log-transformed sbT and IgG or IgE in LTx recipients.

5. Discussion

We report here for the first time on sbT in CF patients and its relationship with humoral responses to *Af* and ribotoxin Asp f 1. Our main finding was lower sbT levels in *Af* IgE+ than in *Af* IgE- patients in adults with native lungs, adults with LTx, and children. The second finding was that sbT levels were associated with IgE and IgG responses to both *Af* extract and ribotoxin. Ribotoxin is highly specific of *Af* [2] and efficiently induces IgG and IgE responses. The interactions between sbT, IgE and IgG against *Af* extract and ribotoxin depend on the patients' *Af* sensitization and clinical status. The correlation between IgG to *Af* extract and Asp f 1 in *Af* IgE- patients, as well as between IgE to *Af* and Asp f 1 in LTx *Af* IgE+ patients, was negative; in

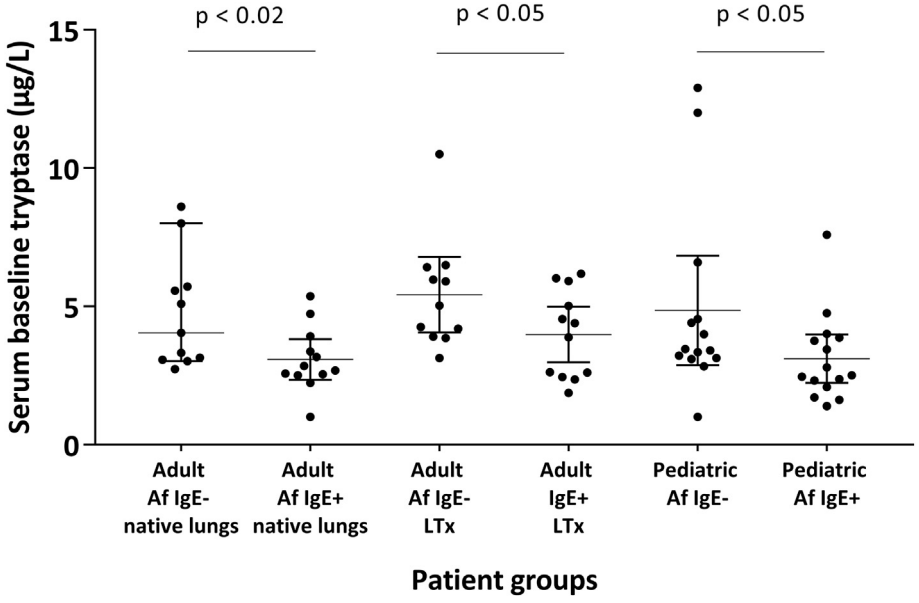

Fig. 1. Serum baseline tryptase results. Individual results for *Af* IgE+ and *Af* IgE- patients are plotted for clinical groups. Median and interquartile range are shown for each group. The outlier at 21.6 µg/L in the pediatric Af IgE- group was excluded. Af IgE-, patients without detectable IgE to *A. fumigatus* extract; Af IgE+, patients with detectable IgE to *A. fumigatus* extract; LTx, lung transplanted patients.

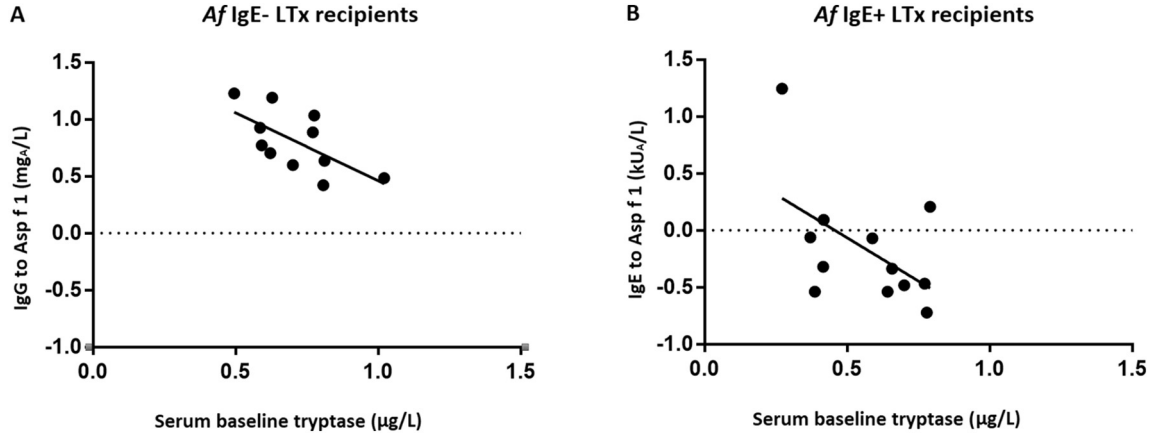

Fig. 2. Log-transformed serum baseline tryptase (sbT), *Af* IgG and *Af* IgE levels in *Af* IgE- and *Af* IgE+ lung transplant (LTx) recipients. A, IgG to Asp f 1 and sbT in *Af* IgE- LTx patients (log-transformed values, Pearson's coefficient of correlation r = −0.61); B, IgE to Asp f 1 and sbT in *Af* IgE+ LTx patients (log-transformed values, Pearson's coefficient of correlation r = −0.53).

contrast the correlation between IgE to *Af* and Asp f 1 in native lung *Af* IgE+ patients was positive. These findings suggest a stepwise progression in the *Af*-CF host interaction altering MC activity. We speculate the following steps [5,8,16–19]: (i) initial IgG downregulation of MC activity, followed by (ii) *Af*-induced, IgE-independent, chymase exocytosis inducing downregulation of IgE production and, finally (iii) IgG-IgE switch, sustained IgE production, loss of negative feedback mechanisms, and evidence for a classical loop of increased MC activity in the presence of increased IgE levels. The first step is illustrated in *Af* IgE- patients, the second in *Af* IgE+ LTx patients, and the third in *Af* IgE+ patients with native lungs. LTx might function as a reset to baseline point for *Af*-CF interaction, a restored lung environment allowing normal negative feedback mechanisms [16]. The clinical observation that IgE-driven *Af* disease including ABPA is infrequent in LTx recipients supports this hypothesis [20]. Further studies on larger samples of patients and controls, longitudinal analyses of pediatric patients and mechanistic experiments are needed. Limitations of this study include (i) a small number of patients in each group, preventing analysis of younger *versus* older children, of LTx allograft characteristics (duration, presence and stage of chronic lung allograft dysfunction), of other *Af* molecules which induce low prevalences of IgE and IgG, and (ii) the absence of age-matched controls for sbT.

In conclusion, we report here on sbT in CF patients and on its interplay with *Af* IgG and IgE. Our main finding was lower sbT levels in patients IgE-sensitized to *Af*. We hypothesize that *Af*-host interactions alter lung MCs activity, making sbT a potential systemic biomarker, provided further studies shed light on the mechanism and clinical implications of the *Af*-sbT connection.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcf.2018.01.012.

Funding

APHM, Marseille, France. ThermoFisher Scientific, R&D Department (Dr Jonas Lidholm): reagents for IgG to *Af* extract and molecules.

Conflict of interest statement

The authors have no conflict of interest to declare in this work.

Authors' Contributions

CG, SR, JLM and JV designed the research. CG, AC, MG, MRG and JCD collected clinical data. CG, SR and JV collected and analyzed data. CG, AC, JCD, SR and JV wrote the manuscript. All authors read and approved the final version.

Acknowledgments

The authors are thankful to Ms. Céline Chartier and Mr. Simon Pinchemel (IHU Méditerranée Infection) for technical assistance, to Pr Michel Arock (Paris 6 University, France) for his careful reading and constructive remarks, and to the three reviewers who substantially helped us improve the manuscript.

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