ELSEVIER
Journal of Cystic Fibrosis xx (2017) xxx – xxx
Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Short Communication

IgG avidity to *Pseudomonas aeruginosa* over the course of chronic lung biofilm infection in cystic fibrosis

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Received 18 July 2017; revised 20 September 2017; accepted 20 September 2017
Available online xxxx

Abstract

Background and objectives: The mechanisms leading to low effectiveness of the humoral immune response against *P. aeruginosa* in cystic fibrosis (CF) are poorly understood. The aim of the present study was to assess the avidity maturation of specific antipseudomonal IgG before and during the development of chronic lung infection in a cohort of Danish CF patients.
Methods: Avidity maturation was assessed against a pooled *P. aeruginosa* antigen (St-Ag) and against *P. aeruginosa* alginate in 10 CF patients who developed chronic lung infection and 10 patients who developed intermittent lung colonization, using an ELISA technique with the thiocyanate elution method. Avidity was quantitatively determined by calculating the avidity Constant (Kav).
Results: IgG avidity to St-Ag significantly increased at the onset (Median Kav = 2.47) and one year after the onset of chronic infection (Median Kav = 3.27), but did not significantly changed in patients who developed intermittent colonization. IgG avidity against alginate did not significantly change over the years neither in patients who developed chronic lung infection (Median Kav = 3.84 at the onset of chronic infection), nor in patients who developed intermittent colonization.
Conclusion: IgG avidity to *P. aeruginosa* alginate does not significantly enhance as chronic lung infection progresses. This probably plays a role in the difficulty to mount an effective opsonophagocytic killing to clear mucoid *P. aeruginosa* infection in CF.

Keywords: Cystic fibrosis; *Pseudomonas aeruginosa*; Biofilm; Humoral response; IgG avidity; Chronic infection

1. Introduction

The high response of specific anti-*P. aeruginosa* IgG antibodies is a hallmark during chronic lung infection in CF [1]. However, this response is not associated with clinical improvement [2,3], but rather leads to formation of circulating immune complexes, which are deposited in the lower airway tissue, triggering tissue damage and long term deterioration of lung function [4]. The mechanisms leading to low effectiveness of the humoral immune response against *P. aeruginosa* are poorly understood. A low opsonizing capacity of CF antibodies have been evidenced and some reports point to their low avidity [5–7]. Nevertheless, there is a gap of almost two decades regarding to this subject and very few studies have addressed the humoral mechanisms of the CF immune response since then. Thus, the aim of the present study was to assess the avidity maturation of specific antipseudomonal IgG before and

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https://doi.org/10.1016/j.jcf.2017.09.004
1569-1993© 2017 Published by Elsevier B.V. on behalf of European Cystic Fibrosis Society.

during the development of chronic biofilm lung infection in a cohort of Danish CF patients.

2. Methods

Serum samples from 20 Danish patients followed at the Copenhagen CF reference center (Rigshospitalet, University of Copenhagen) who developed *P. aeruginosa* chronic lung infection ($n = 10$) were selected for measuring the avidity of specific *P. aeruginosa* IgG to a pooled *P. aeruginosa* antigen (St-Ag) [8] and to *P. aeruginosa* alginate, extracted from the mucoid alginate-producing *P. aeruginosa strain* DK1-NH57388A (isolated from CF sputum) [9,10], from two years before until two years after the onset of chronic lung infection. Chronic infection was defined by following the Copenhagen criteria – *P. aeruginosa* presence in the monthly microbiological respiratory culture for six or more months and/or two precipitating antibodies against *P. aeruginosa* [1,8]. For comparison purposes, a five-year prospective assessment was made with results from patients who did not develop chronic lung infection, but did develop intermittent lung colonization [1,8] ($n = 10$). Chronically infected patients are treated everyday by colistin inhalation; also, they are treated in the CF center every three month with intravenous antibiotic combinations (e.g. meropenem and tobramycin) and oral azithromycin for two weeks (maintenance therapy). Intermittently colonized patients are treated for *P. aeruginosa* eradication with colistin inhalation and oral ciprofloxacin.

Serum IgG levels against *P. aeruginosa* St-Ag and alginate were measured by an ELISA technique [10], and IgG avidity was measured by and ELISA technique using the previously described thiocyanate elution method [11], where the eluent agent potassium thiocyanate (KSCN) was added to the microtiter plate wells (after addition of the serum samples) at increasing concentrations (from 0.75 to 6.0 mol/L). The plate was then incubated before washing and addition of the conjugate antibody. After washing and substrate addition, the optical densities (ODs) were generated by an ELISA reader and the data were fitted into a graphic of $\log_{10}$(OD) versus KSCN molarity by linear regression analysis, and the avidity constant (Kav) representing the molar concentration of KSCN required to reduce the initial optical density (without KSCN) by 50% was estimated.

The Wilcoxon test was used for comparison between two related samples and the Mann-Whitney test was used for comparison between two independent samples, respectively. The statistical analyses were made using SPSS 20 for Windows (IBM, Armonk, United States). A result was considered statistically significant when the significance value was 5% ($p \leq 0.05$).

3. Results and discussion

Patients who developed *P. aeruginosa* chronic lung infection [Median age = 18.5 years old (8.5–38.9), 07 females] had significantly higher IgG levels against St-Ag in all periods, when comparing to patients who developed intermittent colonization. On the other hand, IgG levels against alginate were significantly higher only one year before and two years after the onset of chronic infection (Table 1). The Kav values against St-Ag were low in the two years prior the onset of chronic infection, with a significant increase during the onset, and one year after, and apparently plateaued two years after the onset of chronic infection (Table 1, Fig. 1a).

In patients who developed only intermittent lung colonization [Median age = 14.0 years old (9.3–19.8), 07 females], IgG levels against both St-Ag and alginate did not significantly increase over the five years of follow-up, and the Kav pattern against St-Ag was shown to be constant in the first three years of follow-up, followed by a little but not significant increase in the fourth year and by a plateau in the fifth year (Table 1, Fig. 1a). This is an important finding, since differentiation between chronic infection and intermittent colonization remains a dilemma in CF. Culture-based methods often fail to detect *P. aeruginosa* in respiratory samples due to the lack of a representative sputum specimen [12], and serological analyses may show overlapping results of serum IgG concentrations between chronically infected and intermittently colonized patients [13]. Thus, an increase in IgG avidity may be supportive for this differentiation. However, it is important to

Table 1
Median IgG levels in U/mL and avidity values (expressed as Kav) against *P. aeruginosa* St-Ag and alginate in CF patients who developed chronic lung infection (values prospectively measured from two years before until two years after the onset of chronic infection) and CF patients who developed intermittent lung colonization (prospectively followed in parallel for five years).

History of *P. aeruginosa* colonization/infection	Measure	Antigen	*P. aeruginosa* colonization/infection status	
			Chronic infection	Intermittent colonization
−2 years/year 1	Median [IgG] (U/mL)	St-Ag	17.2 *	7.1
		ALG	6.7	8.8
	Kav (mol/L KSCN)	St-Ag	1.62	1.12
		ALG	3.47	2.90
	% Mucoid *P.a.*		25	None
−1 year/year 2	Median [IgG] (U/mL)	St-Ag	17.2 *	7.0
		ALG	13.4 *	8.5
	Kav (mol/L KSCN)	St-Ag	1.56	1.19
		ALG	3.50	3.37
	% Mucoid *P.a.*		20	None
Onset/year 3	Median [IgG] (U/mL)	St-Ag	21.6 *	9.2
		ALG	10.0	9.1
	Kav (mol/L KSCN)	St-Ag	2.80 *	1.17
		ALG	3.84	3.17
	% Mucoid *P.a.*		50	None
+1 year/year 4	Median [IgG] (U/mL)	St-Ag	29.9 *	12.7
		ALG	7.7	6.9
	Kav (mol/L KSCN)	St-Ag	3.27 *	1.41
		ALG	2.87	2.80
	% Mucoid *P.a.*		50	None
+2 years/year 5	Median [IgG] (U/mL)	St-Ag	23.6 *	9.7
		ALG	10.6 *	5.7
	Kav (mol/L KSCN)	St-Ag	3.52	1.37
		ALG	3.11	2.28
	% Mucoid *P.a.*		60	None

* $p < 0.05$ when compared with the intermittently colonized group; *P.a.* = *Pseudomonas aeruginosa*.

Please cite this article as: Mauch RM, et al, IgG avidity to *Pseudomonas aeruginosa* over the course of chronic lung biofilm infection in cystic fibrosis, J Cyst Fibros (2017), https://doi.org/10.1016/j.jcf.2017.09.004