ELSEVIER
Journal of Cystic Fibrosis 3 (2004) 179–183
Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Anti-neutrophil cytoplasmatic antibodies and lung disease in cystic fibrosis ☆

Ludger Dorlöchter[a,*], Malin Carlsson[b], Edda J. Olafsdottir[a], Ola D. Røksund[a], Karen Rosendahl[c], Gjermund Fluge[a]

[a] *Department of Paediatrics, Haukeland University Hospital, N-5021 Bergen, Norway*
[b] *Department of Clinical Immunology, University Hospital, S-22362 Lund, Sweden*
[c] *Section of Paediatric Radiology, Haukeland University Hospital, N-5021 Bergen, Norway*

Received 13 February 2003; accepted 23 April 2004
Available online 19 July 2004

Abstract

Background: Bactericidal-permeability-increasing protein (BPI) is a potent anti-microbial protein produced by neutrophil granulocytes. Anti-neutrophil cytoplasmatic antibodies (ANCA) directed against BPI have been detected in up to 91% in patients with cystic fibrosis (CF). We aimed to evaluate the prevalence of BPI-ANCA in our CF patients and to determine whether presence of BPI-ANCA is correlated with organ damage. **Methods:** Twenty-four patients performed respiratory function testing and pulmonary high-resolution computed tomography (HRCT). HRCT was scored by using a modified Bhalla method. Serum samples were analysed by direct binding enzyme-linked immunosorbent assay for BPI-ANCA. **Results:** The prevalence of anti-BPI-IgG was 71% and anti-BPI-IgA 33%. Twenty-nine percent of our patients were positive for both BPI-ANCA isotypes. Mean HRCT score was 8.0 ranging from 0 to 22, bronchiectasis presented the most common finding (79%). There was a significant correlation between BPI-ANCA and both HRCT score and FEV_1 ($p < 0.01$). High levels of BPI-ANCA were correlated to chronic *Pseudomonas aeruginosa* lung infection ($p < 0.01$). **Conclusions:** BPI-ANCA was common in our study group. Highly significant correlations between BPI-ANCA and parameters to evaluate lung disease in CF may be a consequence of the inflammation process, or it may indicate a pathogenic role of BPI-ANCA levels in the development of lung disease. More research is needed and the clinical significance of our findings needs further evaluation.

Keywords: Autoantibodies; X-ray tomography; Spirometry; Disease progression

1. Introduction

Bactericidal-permeability-increasing protein (BPI) is a 55-kDa membrane associated protein found in the azurophilic granules of neutrophil granulocytes. BPI is the most potent anti-microbial-granule protein identified so far and it consists of two similar domains. The N-terminal domain binds to lipopolysaccarides in the membrane of gram-negative bacteria, for example *Pseudomonas aeruginosa* (*PSA*), and has both endotoxin neutralising and bactericidal effect. The C-terminal domain is important for the opsonization of bacteria [1].

Anti-neutrophil cytoplasmatic antibodies (ANCA) are autoantibodies directed against different proteins in the cytoplasm of neutrophil granulocytes, and they have been used in diagnostic work-up as serological markers of vasculitides since 1982 [2,3]. Recently, BPI has been found to represent a major ANCA target antigen as well [4]. Moreover, BPI-ANCA are detected in up to 91% in patients with CF and they are claimed to worsen lung inflammation and pulmonary function [5,6]. Because more than 90% of all deaths are related to pulmonary disease in cystic fibrosis (CF), early detection and longitudinal assessment of lung parenchyma damage is a major issue in managing CF. Pulmonary high-resolution computed tomography (HRCT) has recently been considered as the most promising imaging technique to assess

☆ This work was supported by grants from the Norwegian Cystic Fibrosis Society.
* Corresponding author. Children's Hospital, Haukeland University Hospital, 5016 Bergen, Norway. Tel.: +47-55975200; fax: +47-55975147.
E-mail address: ludger.dorlochter@helse-bergen.no (L. Dorlöchter).

1569-1993/$ - see front matter © 2004 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jcf.2004.04.005

pulmonary architectural abnormalities in patients with CF [7–9].

The aim of this study has been to evaluate the prevalence of BPI-ANCA in our study group and whether presence of BPI-ANCA is correlated with organ damage in patients with cystic fibrosis. We have therefore correlated BPI-ANCA (isotype IgG and IgA) values to a common HRCT score for cystic fibrosis [10] and to pulmonary function measured as forced expiratory volume in one second (FEV_1).

2. Materials and methods

Haukeland University Hospital serves as a regional centre for patients with CF in Western Norway. All 29 CF patients more than 6 years of age were invited to take part in the study, one adult patient refused, two sets of HRCT scans and two sets of blood samples were lost leaving 24 patients enrolled in the study (13 female, 11 male). The median age was 13 years (mean 16.6 years, S.D. 9.1, range 6–40 years).

The CF diagnosis was made by demonstration of repeated elevated sweat chloride concentrations (mean 99.5 mmol/l, S.D. 26.1) and typical clinical manifestations in all patients. The mean forced vital capacity was 92.7% of predicted (S.D. 19.3); the mean forced expiratory volume in one second was 79.3% of predicted (S.D. 23.4). Eight patients were found to be homozygous for the dF508 mutation (33%); the remaining 16 patients either had two other mutations (4 patients), 1 detectable mutation (5 patients) or no detectable mutation (7 patients). We consider our CF patient cohort to be representative of the Norwegian CF population.

All patients had received conventional therapy for CF for at least 6 months and they were in clinically stable condition.

Data were collected between February 1999 and August 2000. Informed consent was obtained from patients and/or their families. The regional ethics committee approved our study protocol.

Our patients attend the outpatient department every second–third month, or more often when needed. Chronic lung infection with *PSA* was defined as two or more precipitins or antibodies against *P. aeruginosa* (ELISA) and repetitive sputum cultures of *PSA* within 6 months [11]. Eight patients (33%) had chronic lung infection with *PSA* and they have been given 12-day courses of intravenous antibiotic treatment every third month.

Sixteen patients (67%) have been taking pancreatic enzyme supplementation and the amount of lipase was between 5000 and 10,000 IE/kg bodyweight/day.

Serum samples of all patients were analysed by direct binding enzyme-linked immunosorbent assay (ELISA) for BPI-ANCA isotypes IgG and IgA. The analysis was performed at Wieslab AB, Lund (Sweden) as previously described [12]. In short, antigens were coated in microtiter plates at a concentration of 1 μg/ml in a carbonate buffer. All samples were diluted 1/80 and incubated for 1 h. Bound antibodies were detected using alkaline phosphatase-conjugated goat anti-human IgG (Sigma, Stockholm, Sweden) and IgA (Eurodiagnostica, Malmö, Sweden). Anti-BPI-IgG was quantified from a calibrator curve of serum that was serially diluted and results expressed in arbitrary units (U). For anti-BPI-IgA the results were expressed as optical density (OD). In each ELISA analysis, one positive and one negative control was analysed. Anti-BPI-IgG and anti-BPI-IgA cut off was calculated from the results of 42 normal sera + 3 S.D. Cut-off values were defined at 0.14 OD for IgA and 27 U/ml for IgG.

Pulmonary HRCT (General Electrics, HiSpeed CT) was performed using 1-mm thin sections and 10-mm intervals during inspiration, followed by 1-mm thin sections with 20-mm intervals during expiration. A paediatric radiologist evaluated the HRCT images and classified the findings according to a modified scoring system as described by Bhalla et al. [10]. The morphological criteria were based on description of localisation and degree of pathological changes. According to Bhalla, we evaluated bronchiectasis, peribronchial thickening, mucus plugging, sacculations, bullae, emphysema and collapse or consolidation. The scoring system was extended in terms of mosaic perfusion according to Helbich and colleagues. The obtainable score ranged from 0 to maximal 27 points, the latter represented extensive parenchymal damage. Standard spirometry was assessed by Sensor Medics Vmax equipment (CA, USA). FEV_1 was chosen as a representative variable and it was expressed as percent of predicted according to European Coal and Steel reference set [13].

Data analysis was performed using a commercially available software package (SPSS). Descriptive statistics included mean, minimum and maximum values, standard deviations and median. The relationship between BPI-ANCA values and HRCT score and forced expiratory volume in one second, respectively, was assessed by partial correlations, adjusting for age, gender and chronic *PSA* lung infection. The correlation between BPI-ANCA and *PSA* was evaluated by Kendall's tau-b. All reported p-values are two-tailed, $p < 0.05$ was considered significant and $p < 0.01$ was considered highly significant.

3. Results

Prevalence of anti-BPI-IgG in our study group was 71% (17 out of 24 patients) with a mean value of 72.4 U/ml (range 4–320 U/ml) and median of 36 U/ml. Anti-BPI antibodies of IgA isotype were positive in 33% (8 out of 24 patients) with a mean of 0.26 OD (range 0–1.28 OD) and median of 0.06 OD. Seven patients (29%) were positive for both BPI-ANCA isotypes (Table 1).

The mean HRCT score in our CF group was 8 ± 6.6 (S.D.) ranging from 0 to 22 (median 6.5). Bronchiectasis

Table 1
Individual patient data, mean values and standard deviation of 24 patients with cystic fibrosis listed as age, BPI-ANCA values, HRCT score, FEV_1 (% predicted), serum immunoglobulin G levels, chronic *PSA* lung infection and maldigestion

Age (years)	Anti-BPI-IgA (OD)[a]	Anti-BPI-IgG (U/ml)[a]	HRCT score	FEV_1 (% predicted)	Serum-IgG (g/l)	*PSA*[b]	Exocrine pancr. insufficiency[c]
6	0.02	74	5	99	9.3	–	Yes
7	0.02	55	0	91	15.8	–	Yes
8	0.02	26	1	85	8.8	–	Yes
8	0.03	62	7	78	8.2	–	No
8	0.36	41	10	73	14.6	+	Yes
9	0.07	46	8	82	11.2	–	Yes
10	0.03	17	3	95	10.3	–	Yes
12	0.05	39	7	71	10.4	–	No
12	0.72	320	15	55	18.1	+	Yes
12	0.74	56	15	65	15.8	+	Yes
13	0	4	1	98	6.5	–[d]	Yes
13	0.01	27	5	101	15	–	No
13	0.01	17	1	112	15.3	–	Yes
13	0.04	32	3	99	10.7	–	No
17	0.29	29	9	69	16	+	Yes
17	1.24	320	22	25	18.9	+	Yes
21	0.08	33	3	91	12.7	–	Yes
22	0.09	13	4	84	8.1	–	No
23	1.28	320	15	37	21.2	+	Yes
24	0.01	30	1	114	10.1	–	No
26	0.31	94	13	76	17.2	+	Yes
30	0.01	2	6	102	11.1	–	No
34	0.13	74	19	61	15.4	+	Yes
40	0.70	6	19	40	17.3	–	No
Mean = 16.6	0.26	72.4	8	79.3	13.3		
S.D. = 9.1	0.39	98.4	6.6	23.4	4.0		

[a] Cut-off values were defined as 0.14 (OD) for IgA and 27 (U/ml) for IgG.
[b] *PSA* = chronic *PSA* lung infection.
[c] Faecal elastase-1 < 200 μg/g faeces.
[d] Intermittently *PSA* lung infection.

was the most common finding (19 of 24 patients, 79%). Bronchiectases were graded as mild in 10, moderate in 6 and severe in 3 of these 19 patients. Peribronchial thickening was the second most common finding and was found in 17 patients (71%); all of them were graded as mild. Mucus plugging was present in 15 patients (63%), 8 patients had mucus plugging in 1–5 broncho-pulmonary segments, 2 patients had mucus plugging in 6–9 segments and 5 patients in more than nine segments. Moreover, we detected consolidations in 12 patients, sacculations in 7 patients, mosaic perfusion in 8 patients and bullae in 4 patients. One patient had emphysema, none had abscesses.

Individual results of age, BPI-ANCA values, HRCT score, FEV_1 (% predicted), serum immunoglobulin G levels, chronic *PSA* lung infection and maldigestion are presented in Table 1.

There was a significant correlation between BPI-ANCA values and HRCT score ($r = 0.67$, $p < 0.01$ for IgA; $r = 0.48$, $p = 0.029$ for IgG). High titers of BPI-ANCA were correlated with a high HRCT score. Fig. 1 shows the correlation between anti-BPI-IgA and HRCT score.

Furthermore, there was a significant correlation between BPI-ANCA and FEV_1 (% predicted, $r = -0.75$, $p < 0.01$ for IgA; $r = 0.54$, $p = 0.012$ for IgG). High titers of BPI-ANCA were correlated with impaired pulmonary function as measured by FEV_1 (Fig. 2).

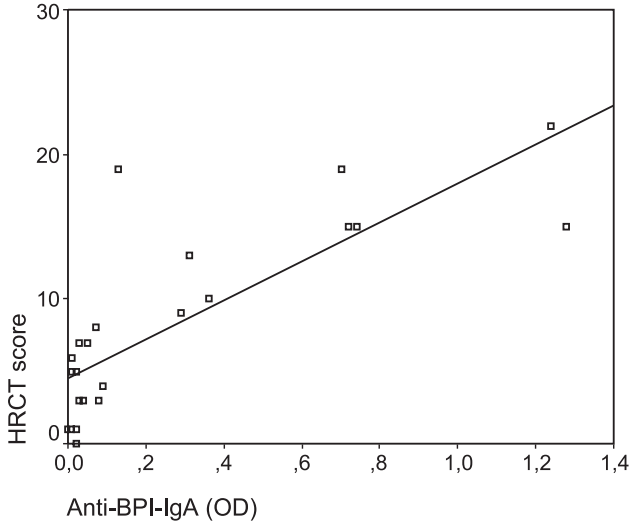

Fig. 1. Correlation between anti-BPI-IgA (OD) and HRCT score in 24 patients with cystic fibrosis ($r = 0.67$, $p < 0.01$); the graph reflects a regression line.

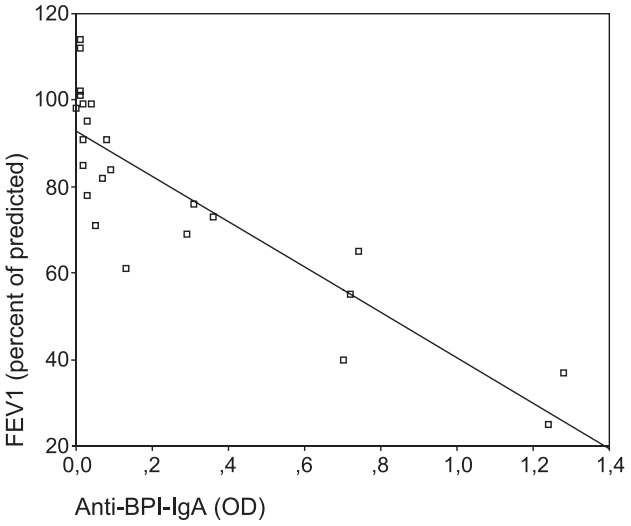

Fig. 2. Correlation between **anti-BPI-IgA** (OD) and FEV_1 (% predicted) in 24 patients with cystic fibrosis ($r = -0.75$, $p < 0.01$); the graph reflects a regression line.

High levels of BPI-ANCA were strongly correlated to chronic *PSA* lung infection ($r = 0.650$, $p < 0.01$ for IgA; $r = 0.532$, $p < 0.01$ for IgG) as shown in Table 1.

4. Discussion

ANCA have been used as serological markers in routine diagnostic work-up of patients with autoimmune disorders like Wegener's granulomatosis or microscopic polyangitis for many years [2]. Recently, BPI-ANCA (isotypes IgG and IgA) have gained focus because of high prevalence in cystic fibrosis patients [6,14].

In the present study, 71% patients with CF were positive for anti-BPI-IgG and 33% were positive for anti-BPI-IgA. Levels of anti-BPI-IgA were expressed as optical density. It is possible that the high absorbance values are not confined to the linear part of the curve but possibly on the upper, flattened part of the curve. These values therefore possibly should have been even higher.

We have found a highly significant correlation between BPI-ANCA and pulmonary HRCT findings, scored with a modified Bhalla score. In this study, we have for the first time shown that BPI-ANCA are not only closely related to pulmonary function tests [4,15] and colonization by *PSA* [15], but to an established HRCT score that evaluates lung destruction. HRCT scans confer detailed information of the distribution and severity of lung damage [8] and scoring of the pathological findings carry the opportunity to quantify structural lung damage. Our findings may contribute to a better understanding of the significance of BPI-ANCA levels and severity of lung disease in CF.

Thirty-three percent of patients had chronic *PSA* lung infection and one additional patient was intermittently *PSA* infected. The prevalence of chronic *PSA* lung infection is low in Norway (35%) [16] compared to Denmark (45%) [17] or USA (60%) [18]. In accordance with previous investigations [5,15,19], high levels of BPI-ANCA in our study group were strongly correlated to chronic *PSA* lung infection. It is striking that the Norwegian *PSA* prevalence is almost identical with the prevalence of anti-BPI-IgA in our study group. Prevalence of anti-BPI-IgG was even higher and all patients with chronic PSA lung infection were positive for anti-BPI-IgG. Sediva et al. [14] found prevalences comparable to ours in their Czech studygroup (77% anti-BPI-IgG and 29% anti-BPI-IgA), although they do not comment their *PSA* prevalence. In Schultz material, the *PSA* prevalence was 37%, with prevalence of 48% for anti-BPI-IgG and 11% for anti-BPI-IgA [20]. Neither Sediva nor Schultz could demonstrate a significant correlation between BPI-ANCA and clinical data like pulmonary function tests or *PSA* colonization.

The finding of BPI-ANCA in CF infants [14] even before colonization by *PSA* may indicate a primary role of BPI-ANCA in the pathogenesis of CF. Due to the design of the present study, our youngest patients were six respectively seven years and both were positive for anti-BPI-IgG without *PSA* colonization. However, we did not find a correlation between age and BPI-ANCA.

All but one patient positive for anti-BPI-IgA had chronic *PSA* lung infection. This patient was the oldest of our study group (40 years old). She showed extensive pulmonary parenchyma damage (Bhalla score 19) and impaired pulmonary function (FEV_1 40% of predicted). Interestingly, this patient had normal values for anti-BPI-IgG. Moreover, she was one of the seven patients with preserved exocrine pancreatic function.

Regarding pancreatic function, we found a significant difference between patients with exocrine pancreatic insufficiency and patients having patent pancreatic function in terms of anti-BPI-IgG values ($p = 0.009$, data not presented here), reflecting higher anti-BPI-IgG titers in patients with exocrine pancreatic insufficiency. We have no explanation for this finding. One could speculate whether this is an epiphenomenon, which reflects the more common advanced lung disease in CF patients with pancreatic malfunction.

A substantial and persistent inflammatory response is elicited by chronic *PSA* lung infection, although incapable of clearing the infection. Mechanisms responsible for this incompetence remain unclear [21]. Increased antibody production as response to repetitive bacterial infections is demonstrated by hypergammaglobulinemia in infected CF patients and develops gradually during the course of the disease [22]. In our study group, there was a close correlation between serum levels of total immunoglobulin G and BPI-ANCA (data not shown). High levels of immunoglobuline in many CF patients lead to increased levels of immune complexes, and finally tissue damage is established via phagocytosis and the complement pathway [22,23]. The pathogenetic effect of BPI-ANCA is probably different and more specific. One may speculate that BPI-ANCA reduce

the endotoxin neutralising and bactericidal effect of BPI and thereby contribute to the incompetence in clearing the infection. BPI and other ANCA antigens origin from polymorphonuclear leucocytes (PMN) and are exposed on the PMN cell surface after release. ANCA binding to such a membrane bound antigen is thought to activate the PMN and result in release of proinflammatory cytokines, which trigger lung inflammation further [24].

In a recent report, Sediva et al. [25] describe that destruction of *PSA,* mediated by normal human neutrophiles was titer-dependent inhibited in the presence of BPI-ANCA. Still, the role of BPI-ANCA in worsening lung disease, lung infection and inflammation in CF is complex and not fully understood.

Our findings may have clinical relevance, as BPI-ANCA positive patients with CF have poorer clinical outcome, demonstrated by the close correlations to pulmonary function and lung parenchyma destruction and thus may prove useful as a prognostic marker of disease activity. Our findings may contribute to a better understanding of the significance of BPI-ANCA levels and severity of lung disease in CF.

Further investigation is needed. Markers of inflammation e.g. interleukin-1, interleukin-8 and tumour necrosis factor-α (TNF-α) correlated to BPI-ANCA would be of particular interest, and we intend to look at these aspects in near future. Longitudinal studies of BPI-ANCA in infants and young CF patients and correlating these findings with pulmonary status could further elucidate the role of these antibodies in the disease process.

Acknowledgements

We thank Tore Wentzel-Larsen, MSc at Centre for Clinical Research, Haukeland University Hospital for planning and control of our statistical analysis.

References

[1] Elsbach P, Weiss J. Role of the bactericidal/permeability-increasing protein in host defence. Curr Opin Immunol 1998;10:45–9.
[2] Gross WL, Schmitt WH, Csernok E. ANCA and associated diseases: immunodiagnostic and pathogenetic aspects. Clin Exp Immunol 1993;91:1–12.
[3] Segelmark M, Westman K, Wieslander J. How and why should we detect ANCA? Clin Exp Rheumatol 2000;18:629–35.
[4] Zhao MG, Jones SJ, Lockwood CM. Bactericidal/permeability-increasing protein (BPI) is an important antigen for antineutrophil cytoplasmatic autoantibodies (ANCA) in vasculitis. Clin Exp Immunol 1995;103:397–402.
[5] Mahadeva R, Dunn AC, Westerbeek RC, et al. Anti-neutrophil cytoplasmic antibodies (ANCA) against bactericidal/permeability-increasing protein (BPI) and cystic fibrosis lung disease. Clin Exp Immunol 1999;117:561–7.
[6] Schultz H, Weiss J, Carroll SF, Gross WL. The endotoxin-binding bactericidal/permeability-increasing protein (BPI): a target antigen of autoantibodies. J Leukoc Biol 2001;69:505–12.
[7] Mason AC, Nakielna BEM. Newly diagnosed cystic fibrosis in adults: pattern and distribution of brochiectasis in 12 cases. Clin Radiol 1999; 54:507–12.
[8] Helbich TH, Heinz-Peer G, Eichler I, et al. Cystic fibrosis: CT assessment of lung involvement in children and adults. Radiology 1999; 213:537–44.
[9] Demirkazik FB, Ariyürek OM, Öycelik U, Göcmen A, Hassanabad HK, Kiper N. High resolution CT in children with cystic fibrosis: correlation with pulmonary functions and radiographic scores. Eur J Radiol 2001;37:54–9.
[10] Bhalla M, Turcios N, Aponte V, et al. Cystic fibrosis: scoring system with a thin-section CT. Radiology 1991;179:783–8.
[11] Johansen HK, Hoiby N. Seasonal onset of initial colonisation and chronic infection with *Pseudomonas aeruginosa* in patients with cystic fibrosis in Denmark. Thorax 1992;47:109–11.
[12] Rasmussen N, Sjolin C, Isaksson B, Bygren P, Wieslander J. An ELISA for the detection of anti-neutrophil cytoplasm antibodies (ANCA). J Immunol Methods 1990;127:139–45.
[13] Quanjer PH, Tammeling GJ, Cotes JE, et al. Symbols, abbreviations and units. Working party standardization of lung function tests, European community for steel and coal. Eur Respir J Suppl 1993; 16:85–100.
[14] Sediva A, Bartunkova J, Kolarova I, et al. Antineutrophil cytoplasmic autoantibodies (ANCA) in children with cystic fibrosis. J Autoimmun 1998;11:185–90.
[15] Aebi C, Theiler F, Aebischer CC, Schoeni MH. Autoantibodies directed against bactericidal/permeability-increasing protein in patients with cystic fibrosis: association with microbial respiratory tract colonization. Pediatr Infect Dis J 2000;19:207–12.
[16] Fluge G, Ojeniyi B, Hoiby N, et al. Typing of *Pseudomonas aeruginosa* strains in Norwegian cystic fibrosis patients. Clin Microbiol Infect 2001;7:238–43.
[17] Johansen HK, Kovesi TA, Koch C, Corey M, Hoiby N, Levison H. *Pseudomonas aeruginosa* and *Burkholderia cepacia* infection in cystic fibrosis patients treated in Toronto and Copenhagen. Pediatr Pulmonol 1998;26:89–96.
[18] Fritzsimmons SC. Cystic Fibrosis Foundation Patient Data Registry Annual Data Report;1996. Bethesda, Maryland.
[19] Carlsson M, Eriksson L, Erwander I, Wieslander J, Segelmark M. Pseudomonas-induced lung damage in cystic fibrosis correlates to bactericidal-permeability increasing protein (BPI)-autoantibodies. Clin Exp Rheumatol 2003;21:S95–100.
[20] Schultz H, Csernok E, Schuster A, Schmitz TS, Ernst M, Gross WL. Anti-neutrophil cytoplasmatic antibodies directed against the bactericidal/permeability-increasing protein (BPI) in paediatric cystic fibrosis patients do not recognize N-terminal regions important for the antimicrobial and lipopolysaccharide binding activity of BPI. Pediatr Allergy Immunol 2000;11:64–70.
[21] Bals R, Weiner DJ, Wilson JM. The innate immune system in cystic fibrosis lung disease. J Clin Invest 1999;103:303–7.
[22] Döhring G, Bellon G, Knight R. Immunology of cystic fibrosis. In: Hodson ME, Geddes DM, editors. Cystic fibrosis 2000. p. 109–40. Arnold, a member of the Hodder Headline Group London.
[23] Pressler T, Jensen ET, Espersen F, Pedersen SS, Hoiby N. High levels of complement-activation capacity in sera from patients with cystic fibrosis correlate with high levels of IgG3 antibodies to *Pseudomonas aeruginosa* antigens and poor lung function. Pediatr Pulmonol 1995; 20:71–7.
[24] Bartunkova J, Tesar V, Sediva A. Diagnostic and pathogenic role of antineutrophil cytoplasmatic autoantibodies. Clin Immunol 2003;106: 73–82.
[25] Sediva A, Bartunkova J, Bartosova J, Jennette C, Falk RJ, Jethwa HS. Antineutrophil cytoplasmatic antibodies directed against bactericidal/permeability increasing protein detected in children with cystic fibrosis inhibit neutrophil-mediated killing of *Pseudomonas aeruginosa*. Microbes Infect 2003;5:27–30.