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Serum immunoglobulins and immunoglobulin G subclasses in cystic fibrosis related to the clinical state of the patient

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Serum immunoglobulins and immunoglobulin G subclasses in cystic fibrosis related to the clinical state of the patient. M.E. Hodson, L. Morris, J.C. Batten.
ABSTRACT: Levels of serum immunoglobulins and immunoglobulin G subclasses were measured in 32 cystic fibrosis (CF) patients, 30 asthmatics and 27 controls. When compared with the asthmatic patients and controls, the CF patients had raised levels of all IgG subclasses as well as total IgG, IgM and IgA, but there was not a statistically significant increase in IgE. The levels of immunoglobulins in the CF patients were examined in relation to the clinical features of the disease. Raised levels of IgG4 were related to levels of IgE, but these raised levels of IgG4 appeared to be part of a general increase in total IgG and not an isolated feature. There was a significant correlation between the total IgG level and its subclasses, IgG1, IgG2, IgG3, IgG4 and IgA. IgG1 was significantly correlated with IgG2 and IgG4; IgG2 with IgG4; and IgG4 with IgE. Total IgG was the immunoglobulin most closely correlated with poor lung function. Serum IgA was higher in patients with positive immediate skin prick reactions to pollens ($p<0.005$) and death within two years of the study was related to high levels of total IgG ($p<0.01$), IgG3 ($p<0.001$), IgA ($p<0.001$), and IgE ($p<0.005$).
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Many workers have shown elevated immunoglobulins in the sera of patients with cystic fibrosis (CF) [1–5]. Two studies have shown low immunoglobulin levels, especially in children with less severe disease [6, 7]. There has been a suggestion that raised levels of serum immunoglobulins are significantly associated with poor clinical condition [8].

70% of patients with CF are atopic, 28% to multiple allergens and 50% to *Aspergillus fumigatus* [9]. 31% of patients have serum precipitins to *A. fumigatus* [10]. Although very many patients with CF are atopic, there is not a high incidence of hay fever or eczema and many of the patients with serum precipitins to *A. fumigatus* have no evidence of allergic bronchopulmonary aspergillosis. It has been postulated that IgG4 may be important in association with some forms of allergy. Indeed it has been shown that raised levels of IgG4 are present in 35% of asthmatic children [11]. Patients with CF, in addition to a high prevalence of positive skin tests, have many features of respiratory allergy including rhinitis, nasal polyps, cough, wheeze and lung overinflation. Abnormal bronchial lability [12] is well known in patients with CF, and many authors have reported increased airways responsiveness to bronchodilators in CF [13–17]. The prevalence of atopy in CF has been quoted as ranging from 10–88% [8]. Interest was therefore shown in the levels of serum immunoglobulin subclasses in patients with CF. Conflicting results have been found. SHAKIB *et al.* [18] showed that some patients with CF had elevated IgG4 levels, but CARSWELL *et al.* [19] did not confirm these findings. In few studies of serum immunoglobulins or their subclasses has an effort been made to relate the levels of immunoglobulins found with the clinical condition of the patients. This factor is very important as some patients can be clinically almost normal, whereas others have severe multi-system disease. Many of the previous studies were performed in children. The aim of the present study was to investigate the total levels of IgG, IgM, IgA and IgE, together with IgG1, IgG2, IgG3 and IgG4 in 32 adults using monoclonal anti-subclass antibodies which have recently become available. The earlier studies on immunoglobulin subclasses used the polyclonal anti-subclass antibodies. The results of immunoglobulins found in the CF patients will be compared with levels in asthmatic patients and healthy controls. The results obtained for the CF patients will be related to the various clinical features of the disease.

Patients and materials

Serum was collected from: i) 32 adults with CF, 21 males and 11 females, aged 17–49 yrs; ii) 30 asthmatics, 16 males and 14 females, aged 23–70 yrs; iii) 27 healthy controls, 12 males and 15 females, aged 15–60 yrs. The patients were diagnosed as having CF with a sweat sodium of >70 $mmol \cdot l^{-1}$. The following clinical data were recorded for all CF patients: age; sex; presence or absence of malabsorption, pneumothorax or diabetes mellitus; age at first chest symptoms; age at

commencement of daily sputum production; duration of chest symptoms and duration of daily sputum production. Their weight was recorded as percentage predicted for age and height, using control data from the Metropolitan Life Insurance Company [20] for patients over 18 yrs of age. For those under 18 yrs of age, the data produced by TANNER [21] were used. Respiratory function was recorded as peak expiratory flow rate (PEFR), forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) as percentage predicted. The normal values were taken from COTES [22]. Sputum samples of all patients were taken at intervals throughout the year, and the presence or absence of the following bacteria were noted: *Haemophilus influenzae, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Patients were recorded as having no evidence of the pathogen in any sputum sample during the year prior to the study, or continuous presence of the pathogen, *i.e.* it was present in every sample tested, or intermittent presence of the pathogen. Skin tests to common allergens including *Aspergillus fumigatus,* house-dust mite and pollens were performed on 28 patients. The serum of 31 patients was also examined for serum precipitins to *Aspergillus fumigatus.* The patients were followed-up for two years after the taking of the blood for the immunoglobulin level estimations. Survival for two years, or death within that period, was recorded.

Methods

Immunological methods 20 ml of venous blood was taken from the 32 CF patients, 30 asthmatic patients and 27 healthy controls. The blood was allowed to clot at 37°C for at least 60 min and the serum removed after centrifugation was stored at -20°C.

Quantitation of immunoglobulin classes and subclasses. The radial immunodiffusion method of MANCINI *et al* .[23] was used for the quantitative estimation of the total IgG, IgA and IgM immunoglobulin classes and the IgG heavy-chain subclasses. The IgE level of all serum samples was determined by a specific radioimmunoassay, the double antibody technique (Kallestad).

Determination of heavy-chain subclasses, IgG, IgA and IgM. 5 µl aliquots of patients' serum and the appropriate calibration standards (Seward-Immunostics) were dispensed into individual wells precision-cut into a layer of agarose gel containing the appropriate monospecific antiserum (Radial Immunodiffusion Plates, Seward Immunostics). The unknown protein concentration of the serum samples could be determined by comparison with the calibration curve drawn from the diameter of the precipitin rings of the known standard concentrations of immunoglobulins.

Plates were incubated for 48–72 h in a sealed container at room temperature and a specimen of known protein concentration was included as an internal control.

Determination of the heavy-chain subclasses IgG1 IgG2 and IgG4 using monoclonal antibodies (Seward-Immunostics). 1 ml of monoclonal antisera was incubated overnight at 4°C with 0.1 ml of 30% w/v polyethylene glycol (PEG) 6000 (BDH Chemicals) in 0.1 M Barbitone buffer, pH 8.6. 1.4% agarose (BDH) was prepared in 0.1 M Barbitone buffer, pH 8.6, containing 6% PEG 3000 and added to the dilution of centrifuged monoclonal anti-subclass antibody. The mixture was poured immediately onto 8x8 cm^2 glass plates to a depth of 1.5 mm. Wells of 2 mm diameter were punched into the agar and 5 µl of standard normal human reference serum (BR99-Seward) and patient serum at the appropriate dilution was added to each well. The plates were left at room temperature for 48 h, excess moisture was removed and then stained with Coomassie Brilliant Blue.

The relative protein concentration of unknown serum samples could be determined as described.

The Seward-Immunostics anti-IgG subclass monoclonal antibodies used were: IgG1 (BAM09, batch no. 2436) from clone JL512; IgG2 (BAM19, batch no. 2438) from clone GOM1; and IgG4 (BAM11, batch no. 2096B) from clone RJ4.

Determination of heavy-chain subclass IgG3. The monospecific antiserum BA37 (Batch 1636C) (Seward-Immunostics) was used to determine the concentration of IgG3 in serum samples. 1% agarose gels (BDH) containing 3% PEG 6000 in O.1'M Barbitone buffer, pH 8.6, were prepared and the procedure followed was the same as described above.

Statistical methods

The data for serum immunoglobulins previously published [24] appear to be distributed log-normally. A logarithmic transformation was therefore used for all calculations. An unpaired t-test was used to compare total IgG, IgA, IgM, IgE and IgG subclasses present in CF patients with levels found in asthmatics and normal controls. To compare levels of immunoglobulins with clinical and other laboratory data, correlation coefficients were calculated for continuous data, and unpaired t-tests were used for non-continuous data. Multiple regression analysis was undertaken to determine predictors of raised immunoglobulin levels. As many variables were examined it was likely that some would be significant by chance. Therefore, only those reaching the 1% level of significance were considered as being clinically important.

Results

Levels of IgG1, IgG2, IgG3, IgG4, total IgG, IgM, IgA and IgE are shown in table 1. When compared with asthmatics and the control group, patients with CF have higher levels of all immunoglobulin G subclasses, as well as total IgG, IgM and IgA, but not a significant increase in IgE. The percentage of CF patients whose values were 2 SD log normal or above the normal range were as follows: IgG1 56%; IgG2 34%; IgG3 47%; IgG4 13%; total IgG 69%; IgE 9%; IgA 28%; IgM 13%.

Table 1. – Levels of total IgG, IgG subclasses, IgE, IgA and IgM in controls, cystic fibrosis and asthmatic patients

	IgG $IU \cdot ml^{-1}$	IgE $IU \cdot ml^{-1}$	IgA $IU \cdot ml^{-1}$	IgM $IU \cdot ml^{-1}$	IgG1 $g \cdot l^{-1}$	IgG2 $g \cdot l^{-1}$	IgG3 $g \cdot l^{-1}$	IgG4 $g \cdot l^{-1}$
Cystic fibrosis n=32								
Mean	277	70	586	349	7.24	8.62	3.82	1.21
95% confidence limits	152–502	4–1122	169–2030	145–840	4.88–10.73	2.83–26.25	1.28–11.39	0.28–5.28
Controls n=27								
Mean	165	37	253	214	5.47	5.10	0.92	0.58
95% confidence limits	111–246	2–561	72–892	89–511	4.06–7.38	2.38–10.90	0.20–4.22	0.14–2.34
Cystic fibrosis compared with controls*	p<0.001	NS	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
Asthmatics n=30								
Mean	142	102	316	177	5.04	4.25	1.03	0.59
95% confidence limits	90–225	6–1622	121–828	33–957	3.20–7.94	1.24–14.55	0.39–2.73	0.22–1.57
Cystic fibrosis compared with Asthmatics*	p<0.001	NS	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

NS: not significant. All calculations on tables used log transformations. *unpaired t-test.

IgG

The level of IgG correlates with the levels of IgG1 (p<0.001), IgG2 (p<0.001), IgG3 (p<0.01) and IgG4 (p<0.001). It is also correlated closely with the level of IgA (p<0.01). There is a negative correlation between IgG and lung function as measured by forced vital capacity (p<0.01). The total IgG is higher in those patients who die within two years (mean 331 $IU \cdot ml^{-1}$) compared with those who survive (mean 258 $IU \cdot ml^{-1}$) (p<0.01). The multiple regression analysis which shows the best predictor of total IgG is IgG1 (p<0.001).

IgG1

The level of IgG1 correlates with total IgG (p<0.001), IgG2 (p<0.001), and IgG4 (p<0.001). The multiple regression analysis shows that the best predictor of the level of IgG1 is total IgG (p<0.001).

IgG2

The level of IgG2 correlates with IgG1 (p<0.001), IgG4 (p<0.01) and total IgG (p<0.001). The multiple regression analysis shows that the best predictor of the level of IgG2 is IgG1 (p<0.001).

IgG3

The level of IgG3 correlates with total IgG (p<0.01) and those patients who died within two years had a higher mean level of IgG3 ($5.97 g \cdot l^{-1}$) than those who survived (mean $3.21 g \cdot l^{-1}$). The multiple regression analysis reveals that the best predictor of the level IgG3 is the level of total IgG (p<0.01).

IgG4

The level of IgG4 correlates with the level of total IgG (p<0.001) and IgE (p<0.01). The multiple regression analysis reveals that the best predictors of the level of IgG4 were IgG1 (p<0.001) and IgE (p<0.01).

IgM

The level of IgM correlates with none of the continuous variables and was not related to any of the non-continuous variables. The multiple regression analysis reveals that the best predictor of IgM level is IgA (p<0.01).

IgA

The level of IgA correlates with the level of total IgG ($p<0.01$) and those patients dying within two years had a higher level (mean 935 $IU \cdot ml^{-1}$) compared with those who survived (mean 488 $IU \cdot ml^{-1}$) ($p<0.001$). IgA levels were higher, mean 817 $IU \cdot ml^{-1}$ in patients with positive immediate skin test reaction to pollen compared to those with negative skin tests to pollen, mean 444 $IU \cdot ml^{-1}$ ($p<0.01$). The multiple regression analysis showed that the best predictors of IgA were total IgG ($p<0.01$), and IgM ($p<0.01$).

IgE

The level of IgE was correlated with the level of IgG3 ($p<0.01$) and patients dying within two years had a higher mean level (218 $IU \cdot ml^{-1}$) compared with those surviving (45 $IU \cdot ml^{-1}$) ($p<0.005$). The multiple regression analysis showed that the best predictors of serum IgE were the duration of chest symptoms and presence of Haemophilus influenzae in the sputum. Those patients with a higher level of IgE had a shorter history of chest symptoms and were more likely to have Haemophilus influenzae in the sputum.

Discussion

These studies confirm previous findings that adult patients with CF have significantly raised levels of all serum immunoglobulins with the exception of IgE. These values appear to be of some prognostic significance in that those patients who died within two years of the study had higher levels of total IgG, IgG3, IgA and IgE than patients who survived. We did not examine specific IgE.

This study showed that all IgG subclasses are raised. In fact IgG4 was only raised in 13%, whereas IgG1 was raised in 56% and IgG3 in 47%. The raised level of IgG4 therefore appears to be part of a general increase in total IgG and not an isolated feature. It is interesting to note that the IgG1 and IgG3 subclasses are those with the most complement fixing activity [25]. This may be related to some of the pathological changes found in a CF lung. The total IgG level is indeed correlated with poor lung function. It would appear therefore that as pulmonary sepsis increases and lung function deteriorates the serum immunoglobulin level increases.

The relationship between IgG4 and IgE is of interest as they probably both reflect the atopic nature of patients with CF although neither of them correlated with positive skin reactions in this series. There are no particular clinical correlates with raised levels of serum IgG4. Shakib *et al.* [18] found that seven out of sixteen patients with CF had grossly elevated levels of IgG4 and suggested that this was related to immediate type hypersensitivity in their patients. 13% of our patients had IgG4 levels raised 2 SD above the control range. However, this did not appear to be related to atopy. We conclude that raised levels of IgG4 in the serum of CF patients are not of particular significance but are part of a generalized increase in immunoglobulin levels, particularly total IgG, IgG1 and IgG3, in response to bacterial infection.

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RÉSUMÉ: Les niveaux d'immunoglobulines sériques et des sous-classes d'immunoglobuline G ont été mesurés dans 32 cas de fibrose kystique, chez 30 asthmatiques et chez 27 sujets contrôles. Par comparaison avec les patients asthmatiques et les contrôles, les patients atteints de fibrose kystique ont des taux élevés de toutes les sous-classes d'immunoglobulines, ainsi que des IgG, IgM et IgA totales, mais il n'y a pas d'augmentation statistiquement significative des IgE. Les niveaux d'immunoglobulines chez les patients atteints de fibrose kystique ont été examinés en rapport avec l'état clinique de la maladie. Les niveaux élevés d'IgG4 sont en relation avec les niveaux d'IgE, mais ces niveaux élevés d'IgG4 sont une partie d'une augmentation générale des IgG totales et non pas un trait isolé. Il y a une corrélation significative entre le taux d'IgG totale et ses sous-classes IgG1, IgG2, IgG3, IgG4 et les IgA. L'IgG1 est en corrélation significative avec l'IgG2 et l'IgG4, l'IgG2 avec l'IgG4, et l'IgG4 avec l'IgE. L'IgG totale est l'immunoglobuline la plus directement corrélée avec un état fonctionnel peu satisfaisant. Les IgA sériques sont plus élevées chez les patients qui ont des réactions cutanées immédiates positives après prick-test aux pollens ($p<0.005$). Le décès, dans les deux années de l'étude, est en relation avec les niveaux élevés d'IgG totales ($p<0.01$), IgG3 ($p<0.001$), IgA ($p<0.001$), et d'IgE ($p<0.005$).