Acta Paediatrica Hungarica, 27 (3), pp. 205—210 (1986)

Immunoglobulin levels in bronchoalveolar lavage fluid of children with recurrent obstructive bronchitis

B Nagy, L Maródi, Judit Jezerniczky, L Karmazsin

Department of Paediatrics, University Medical School, Debrecen, Hungary

Bronchoalveolar lavage was performed in 22 children with recurrent obstructive bronchitis and the recovered lavage fluid samples were analysed for concentration of IgA, IgG, IgM, IgE and C_3. Previously a significant influx of exudate macrophages and persistence of bacteria on the bronchoalveolar surface were detected in these patients and a severe mucosal inflammation was observed bronchoscopically. The relative lavage fluid levels of immunoblobulins to albumin were significantly higher than in serum, indicating a local production of these proteins. The elevated levels of C_3 indicated a high activity of the macrophages and the complement system. It is concluded that the mucosal inflammation in patients with recurrent obstructive symptoms cannot be attributed to a deficiency of immunoglobulins either in blood or in bronchial secretions.

Bronchoalveolar lavage (BAL) fluid contains a wide variety of cells and proteins which play an integral role in lung host defence and inflammatory reactions [7]. In children with recurrent obstructive bronchitis we have determined the morphological, cytochemical and functional characteristics of the lavaged cells [6]. In these cases a severe chronic mucosal inflammation was observed bronchoscopically with a high frequency of positive bacteriologic cultures (80% of all lavages) in the recovered fluid, and a considerable influx of exudate macrophages were found without any sign of acute inflammation even in symptom-free periods.

Immunoglobulins (Igs) may protect against inhaled proteins or viral and microbial organisms in the lung. So, local levels of immunoglobulins in the lung lining fluid have a pathogenetic importance as their absence predisposes to infection or to persistence of bacteria on the alveolar surface [7]. There are scattered reports of patients with recurrent pulmonary infections having selective IgA deficiency detected in bronchial fluid [5].

Therefore, levels of immunoglobulins were measured in the lung lining fluid and serum of children with recurrent obstructive bronchitis and compared with data of normal healthy volunteers.

Materials and Methods

Twenty-two children from 2 to 6 years of age with recurrent obstructive bronchitis were selected on the basis of various criteria as described previously [16]. The children were subjected to bronchoscopy

for the diagnostic purpose to exclude bronchial malformations and other disorders which might be associated with obstructive symptoms. BAL was performed after bronchoscopic examination in each patient. The procedure has been described in detail earlier [16].

The recovered lavage fluid was centrifuged at 800 $\times$ *g* for 10 min at 4°C after having been seperated from the mucus. The fluid supernatant was decanted and stored at —4°C until assayed.

Total protein concentration of each specimen of bronchial washing was determined by the method of Lowry et al [10]. Concentrations of IgA, IgG, IgM and C_3 were determined in bronchial fluid and serum samples by Beckman Immunochemistry Analyser II ICS (USA). IgE measurements were made with paper stabilized radioimmunoassay kit (IgE PRIST; Pharmacia Diagnostics, Uppsala, Sweden). The assay was sensitive to nanogram amounts of IgE (2 ng/ml $\approx$ 1 U/ml) [1, 21]. Albumin contents of the recovered fluid and serum were quantified by bromcresolgreen photometry [19, 20] and used as a reference standard. The bronchial fluid samples were left unconcentrated in view of the very sensitive methods used. The mean immunoglobulin and C_3/albumin ratios in bronchial fluid and serum were compared to reported data of young healthy adults [14, 17, 18]. Because of ethical problems it was not possible to have a better control group of children.

Results

The average recovered volume was 46 $\pm$ 4.6% of the instilled fluid. BAL was performed in all patients without difficulty or complications. No fluid samples had blood contamination.

Quantitative values for total protein, albumin, C_3 and immunoglobulins found in serum and bronchial lavage fluid were not compared directly to other results (Table I) because procedures used by different authors were different; so the amount of recovered protein depended on the lavage volume.

The Ig content in BAL expressed as Ig/albumin ratio, each of them was significantly greater than that present in serum (Table II). Assuming that albumin in BAL is derived exclusively from serum by nonspecific transudation [13], this disproportionate increase in IgA and IgG/albumin ratios was strong evidence of these immunoglobulins being locally synthesised in the lung, and that transudation was partially responsible for the Ig content in BAL.

IgM in BAL was also present to a significantly greater extent than in serum. Because of its large molecular weight, IgM molecules do not move freely from serum to the alveolar surface under normal conditions [5, 17, 18, 23]. In our cases signs of severe chronic mucosal inflammation were found bronchoscopically, so we had to reckon with greater fluxes of immunoglobulins across epithelial and alveolar membranes than in normal individuals.

Levels of IgE in the lavage fluid were increased compared to their serum levels.

C_3 levels in bronchial specimens may be the result of an increased local production but the source of the complement components within the lung is not known [3]. Complement activity was certainly lost with the

TABLE I

Concentrations of total protein, albumin, IgA, IgG, IgM and C_3 are expressed as g/l in serum and mg/l in lavage fluid. All concentrations of IgE are expressed as U/ml $\approx$ 2 ng/ml
Data represent mean $\pm$ SE.
Concentrations of protein species in serum and lavage fluid

	Total protein	Albumin	IgA	IgG	IgM	IgE	C_3
Children with recurrent obstructive bronchitis n = 22							
Serum	70.3±2.0	45.0±0.6	1.92±0.1	13.75±0.2	1.1±0.02	110±4.4	1.32±1.2
Lavage	93.8±4.5	26.6±2.6	22.8±2.0	41.5±4.8	9.9±1.2	0.92±0.1	11.0±0.8

TABLE II

Relative concentrations of protein species in serum and lavage fluid
Values represent mean concentrations of protein species divided by albumin concentration. Values for Ig species and C_3 are mg/mg albumin, and values for IgE are expressed as ng/μg albumin

		IgA/alb.	IgG/alb.	IgM/alb.	IgE/alb.	C_3/alb.
Children with recurrent obstructive bronchitis n = 22	Serum	0.042	0.30	0.024	0.0048	0.029
	Lavage	0.85	1.56	0.37	0.091	0.41
Merill et al. (14) Young healthy subjects n = 17	Serum	0.04			0.0045	
	Lavage	0.319			0.017	
Rankin et al. (17) Healthy volunteers n = 11	Serum	0.042	0.189	0.018		
	Lavage	0.28	0.195	0.006		
Reynolds and Newball (18) Healthy volunteers n = 5	Serum	0.05	0.23			
	Lavage	0.72	0.12			

lavage but the sensitivity of our assay was high, thus the concentrating procedure with its harmful, denaturing effect could be omitted. The C_3/albumin ratios in serum were not significantly different from results of other authors [18].

DISCUSSION

Of the major immunoglobulins, IgA, IgT and IgE are all present on the bronchoalveolar surface in normal individuals but IgM is not detected or is present in very low amounts using

a sensitive assay technique [4, 7, 9]. There are at least two potential sources for immunoglobulins found in the lung lining fluid: transudation from serum and in situ production by lung lymphocytes in the submucosa and the airway lumina [2, 7, 8, 15, 17, 18, 23]. The alveolar structures are permeable to molecules of low molecular weight and are comparatively impermeable to very large molecules [18]. The leakage of immunoglobulins is probably more significant during an inflammatory process due to changes in alveolar, epithelial membrane permeability.

In various inflammatory lung diseases immunoglobulin production is markedly increased at sites of disease activity but not in blood [5, 6, 8]. Rankin et al reported on some correlation between the number of IgG secreting cells and the IgG/albumin ratio in BAL fluid of patients with sarcoidosis, and the ratio of IgG/albumin was significantly greater in BAL than in serum [17]. This suggests that the increase in local Ig production in sarcoidosis is primarily responsible for the elevated IgG levels in BAL fluid. Both sources are important and contribute to the maintenance of the immunoglobulin levels in the airspaces.

The present study was done on children with recurrent obstructive bronchitis with protracted cough and wheezing without signs of acute infection. Diseases which cause obstructive symptoms were previously excluded but signs of chronic mucosal inflammation were bronchoscopically observed throughout months in the symptom-free periods, too. A previous study showed the persistence of bacteria on the bronchoalveolar surface in 80% of cases, and a significant influx of exudate macrophages was detected without polymorphonuclear leukocyte accumulation [16]. This inflammation might be intensified by various products of exudate macrophages, and changes of the inflamed mucosa were considered to be the morphological basis of the frequent and permanent obstructive symptoms [22, 24].

The present results have shown that the levels of all major immunoglobulins are increased in BAL fluid of children; this was markedly supported by the ratios of Ig/albumin in bronchial lavages and serum. It is therefore reasonable to assume that the local production of immunoglobulins predominates. We cannot distinguish between monomeric and dimeric forms of IgA by the method used but most of the IgA in lavage is certainly in the dimeric form [12]. It may be assumed that at least some of the lung monomeric IgA is derived from serum [18].

The point of view that children with recurrent obstructive bronchitis have a local deficiency of secretory IgA and so an increased susceptibility to lung infections and atopic diseases [24] cannot be accepted.

Both the classical and alternative complement pathways are represented on the alveolar epithelial surface of normal individuals [3, 7, 8]. The complement components are probably

derived from serum and their local production is also possible [7, 18]. The levels of C_3 synthesised by mononuclear phagocytes were increased in our patients indicating the activity of macrophages and the complement system.

The relative lavage levels of IgE were increased compaired to their serum concentrations. The patients had no positive skin test to environmental antigens nor a history of atopy, and their serum IgE levels were normal. This chronic inflammation of the mucosa may play an important role in the early hypersensibilization altering the affinity of the mast cell membranes for IgE [11], but answers to this question await further study.

ACKNOWLEDGEMENT

We are indebted to Dr G Balla for expert technical assistance.

REFERENCES

1. Bazaral M, Hamburger RN: Standardization and stability of immunoglobulin E (IgE). J Allergy Clin Immunol 49: 189, 1972
2. Bienenstock J, Clancy RL, Perey DYE: Bronchus associated lymphoid tissue (BALT): Its relationship to mucosal immunity In: Lung Biology in Health and Disease Ed: C Lenfant Vol 5. Respiratory Defense Mechanism Part II. M Dekker, New York 1977. pp. 29—58
3. Colton HR, Einstein LP: Complement metabolism: Cellular and humoral regulation. Transplant Rev 32: 3, 1976
4. Deuschl H, Johansson SGO: Immunoglobulins in tracheo-bronchial secretion with special reference to IgE. Clin Exp Immunol 16: 104, 1974
5. Falk GA, Okinaka AJ, Siskind GW: Immunoglobulins in the bronchial washings of patients with chronic obstructive pulmonary disease Am Rev Resp Dis 105: 14, 1972
6. Gwynn CM, Ingram J, Almousawi T, Stanworth DR: Bronchial provocation test in atopic patients with allergen-specific IgG_4 antibodies. Lancet 1: 254, 1982
7. Hunninghake GW, Gadek JE, Kawanami O, Ferrans VJ, Crystal RG: Inflammatory and immune processes in the human lung in health and disease: Evaluation by bronchoalveolar lavage. Am J Pathol 97: 149, 1979
8. Josephs SH: Immunologic mechanisms in pulmonary disease. Pediatr Clin North Am 31: 919, 1984
9. Lawrance EC, Theodore BJ, Martin RR: Modulation of pokeweed-mitogen-induced immunoglobulin secretion by human bronchoalveolar cells Am Rev Resp Dis 126: 248, 1982
10. Lowry OH, Rosebraugh MJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193: 263, 1951
11. Merrill WW, Naegel GP, Reynolds HY: Reaginic antibody in the lung lining fluid J Lab Clin Med 96: 494, 1980
12. Merrill WW, Goodenberger D, Strober W, Matthay RA, Naegel GP, Reynolds HY: Free secretory component and other proteins in human lung lavage. Am Rev Resp Dis 122: 156, 1980
13. Merrill WW, O'Hearn E, Rankin JA, Naegel GP, Matthay RA, Reynolds HY: Kinetic analysis of respiratory tract proteins recovered during a sequential lavage protocol Am Rev Resp Dis 126: 617, 1982
14. Merrill WW, Naegel GP, Olchowski JJ, Reynolds HY: Immunoglobulin G subclass proteins in serum and lavage fluid of normal subjects Am Rev Resp Dis 131: 581, 1985
15. Murphy S, Florman AL: Lung defenses against infection: A clinical correlation. Pediatrics 72: 1, 1983
16. Nagy B, Maródi L, Karmazsin L: Characterization of cells in bronchoalveolar lavage fluid from children with recurrent obstructive bronchitis Acta Paediatr Hung 27: 211, 1986
17. Rankin Ja, Naegel GP, Schrader CE, Matthay Ra, Reynolds HY: Air-space immunoglobulin production and levels in bronchoalveolar lavage fluid of normal subjects and patients with sar

coidosis Am Rev Resp Dis 127: 442, 1983
18. Reynolds HY, Newball HH: Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage J Lab Clin Med 84: 559, 1974
19. Rodkey FL: Determination of albumin in plasma and serum Clin Chem 10: 643, 1964
20. Rodkey FL: Spectrophotometric determination of albumin in human serum Clin Chem 11: 478, 1965
21. Rowe DS: Measurements of concentrations of human serum immunoglobulins C i n Exp Immunol 9: 695, 1971
22. Székely E, Farkas E: Pediatric Bronchology. Akadémiai Kiadó, Budapest 1978. p. 401
23. Warr GA, Martin RR, Sharp PM, Rossen RD: Normal human bronchial immunoglobulins and proteins Am Rev Resp Dis 116: 25, 1977
24. Weingärtner L, Dietzsch HJ: Chronische Bronchitis im Kindesalter. Georg Thieme, Leipzig 1975. p. 67

Received 2 October 1985

B Nagy MD
Pf 32
4012 Debrecen, Hungary