The Journal of Laryngology and Otology
September 1995, Vol. **109**, pp. 859–862

Broncho-alveolar lavage in chronic upper respiratory tract infections

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Abstract
The relationship between upper and lower respiratory tract infections has been demonstrated previously, although the effect of chronic infection of one tract on the other has not been well studied. This work analyses the broncho-alveolar lavage fluid of patients with chronic purulent rhino-sinusitis and reveals an increase in total and neutrophil cell counts as well as an increase in immuno-globulin A levels. The associated increase in the neutrophil nitro-blue tetrazolium dye reduction test positivity provides evidence for increased phagocytosis to compensate for the increased contamination of the lower respiratory tract.

Key words: Rhinitis; Sinusitis; Bronchoalveolar lavage fluid; Nitro-blue tetrazolium; Phagocytosis

Introduction

The relationship between upper and lower respiratory tract infections has been recognized and well documented since Quinn and Meyer (1929) noted the prevalence of sinusitis in association with bronchiectasis. More recently, Cole (1981) quoted an incidence of 42 per cent of rhino-sinusitis in 200 patients with chronic purulent sputum production, most of whom had proven bronchiectasis. This association was also demonstrated in patients with acute exacerbation of chronic bronchitis (Ogilvie, 1941) and those suffering from recurrent bronchitis (Hogg and Brock, 1951).

The close relationship of the upper to the lower respiratory tract is not only due to their anatomical continuity and physiological similarity but also to the common defence mechanisms they share (Mackay and Cole, 1987) (see Figure 1), thus any derangement will be reflected in an infection of the upper, lower or both of the respiratory tracts. Depressed protective reflexes (Huxley *et al.*, 1978), abnormalities of the mucociliary clearance mechanism (Penderson and Mygind, 1982) and immune deficiencies (Bachmann, 1965) have been repeatedly shown.

In addition, there are hints that an infection in one region affects the others. Although well proven in viral upper respiratory tract infections (Harford and Hara, 1950; Jacab and Dick, 1973) which impair the mucociliary clearance and inhibit the alveolar phagocytic activity in the lower respiratory tract, the effect of chronic purulent rhino-sinusitis on the lower respiratory tract has not been well studied.

This project aimed at studying some changes in the pulmonary defence mechanisms, including immuno-globulin A (IgA) levels and neutrophil cell function in patients with chronic purulent upper respiratory tract infections.

Patients and methods

This study included 15 adult patients suffering from chronic or recurrent rhino-sinusitis listed for sinus surgery at our ENT Department. Patients selected were: (1) not smokers or alcoholics; (2) free from any other disorder that might influence the immune system, such as diabetes mellitus, chronic renal failure or malignancy; (3) clear of any

Local mechanisms
(1) Non-specific
 reflexes (cough, sneeze)
 mucociliary system
 epithelial integrity and lining fluid
 pulmonary macrophage
(2) Specific
 immunoglobulin (secretory IgA, IgE)
 lymphocytes

Systemic mechanisms
(1) Non-specific
 serum factors (e.g. opsonisns, complement components)
 granulocyte phagocytes
 mononuclear phagocytes
(2) specific
 serum immunoglobulins
 lymphocytes

FIG. 1
Systems of defences of the respiratory tract (after Mackay and Cole, 1987).

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This work was undertaken at Alexandria University, Egypt.
Accepted for publication: 20 May 1995.

medications that might affect the immune system such as corticosteroids or immunosuppressive drugs; (4) non-atopic as proven on clinical examination and on Radio immuno absorbent test; (5) with normal serum immunoglobulins G, A and M; (6) without a history of chronic or recurrent lower respiratory tract infections or a recent acute infection.

All patients underwent a full medical examination, chest X-ray and pulmonary function tests to exclude an associated lower respiratory tract infection.

The study also included 10 adult volunteers (mainly members of staff), with no history of chronic respiratory disease (upper or lower), nor recent infections, who fulfilled the above criteria.

Properly consented candidates, during a remission of infection, underwent a broncho-alveolar lavage (BAL) to a subsegment of the lingula. The lavage was performed using a standard technique (Reynolds, 1987) through a fibreoptic bronchoscope with five aliquots, each 20 ml, of sterile physiological saline at room temperature. The initial aliquot was discarded, and the rest pooled in a sterile glass container, measured and transported to the laboratory on ice.

Our preference for small volumes for BAL is an attempt to minimize the discomfort and the chance of complications. We also avoided a second right-sided lavage, as no variation in the lavage analysis according to side was expected, although the remote chance of an early unilateral pathology undetected by examination and on radiology could not be totally excluded.

A blood sample was taken at the same sitting for differential cell counting. In the laboratory, total cell count was undertaken in a haemocytometer on unconcentrated BAL fluid, followed by differential counts from a cytocentrifuge cell preparation stained with Wright's Geimsa stain.

Then, the BAL fluid underwent low grade centrifugation to pellet the cells which were resuspended and washed. Prepared neutophils' function was tested by the histochemical method of nitro-blue tetrazolium dye (NBT) reduction test.

NBT test (one of two neutrophil function tests which has received wide acceptance (Babior, 1992) is an example of tests used to detect a defect in the intracellular killing function of phagocytes including neutrophils, it should be noticed that a defect in intracellular killing may result from a defect in any of the phagocyte functions including mobility, recognition, ingestion, degranulation or killing. The principle of the test relies on the fact that neutrophils can reduce the clear, yellow, water soluble compound (NBT) into formazan, a deep blue dye (Park, 1971) subsequent to the metabolic burst generated through the hexose monophosphate shunt following ingestion of latex or other particles.

As this phenomenon is closely related to metabolic events, in the neutrophils following ingestion, NBT reduction is a useful means of assaying overall metabolic activity of the neutrophils (Segal, 1974). The NBT test was performed using a Sigma Kit No. 840, based on a modification of the method of Feigin *et al.* (1971) which is derived from the reference method described by Park *et al.* (1968). Test values were reported in terms of percentage positive (formazan-containing) neutrophils.

The liquid phase of the BAL fluid was then separated, aliquoted and frozen at −70°C for subsequent analysis. Later, total IgA and albumin were measured by single radial immunodiffusion as described by Mancini *et al.* (1962) using immunodiffusion plates (Behringwerke AG, Marburg-Lahen, Germany).

The paired '*t*'-test was used to compare samples at a level of significance of 5 per cent.

The mean BAL fluid concentration of albumin did not differ significantly between controls and patients. Thus expressions of IgA as proportions of albumin and absolute values were similar, consequently, only the last variable was adopted in the results.

TABLE I
BRONCHO-ALVEOLAR LAVAGE DATA IN 15 PATIENTS WITH CHRONIC PURULENT UPPER RESPIRATORY TRACT INFECTION

No. of case	Total yield of fluid (ml)	Total cells (10^3 cell/ml)	NBT (%)	IgA value (mg/ml)
1	45	32	35	0.30
2	40	38	42	0.31
3	32	45	35	0.22
4	40	25	45	0.29
5	40	28	29	0.25
6	35	17	23	0.24
7	32	21	41	0.31
8	35	05	29	0.33
9	42	16	33	0.12
10	30	14	41	0.13
11	40	25	30	0.17
12	45	17	21	0.35
13	35	23	26	0.28
14	37	14	22	0.24
15	36	26	18	0.25
X	37.6	23.1	31.3	0.25
SD	4.6	10.2	8.4	0.07

X = mean value.
SD = standard deviation.

https://doi.org/10.1017/S0022215100131500 Published online by Cambridge University Press

TABLE II

BRONCHO-ALVEOLAR LAVAGE DATA IN 10 ADULT VOLUNTEERS WITH NO HISTORY OF RESPIRATORY TRACT DISEASE

No. of case	Total yield of fluid (ml)	Total cells (10^3 cell/ml)	NBT (%)	IgA value (mg/ml)
1	35	09	23	0.13
2	34	16	24	0.27
3	43	14	22	0.17
4	43	18	25	0.29
5	33	12	19	0.12
6	47	24	27	0.20
7	41	11	16	0.11
8	37	08	17	0.23
9	32	20	28	0.12
10	49	21	29	0.11
X	39.4	15.3	23	0.17
SD	5.7	5.12	4.3	0.07

X = mean value.
SD - standard deviation.

Results

This study included 15 patients suffering from chronic or recurrent rhino-sinusitis and 10 volunteers, both groups with no clinical evidence of lower respiratory tract infection. The patients' ages ranged between 16 and 53 years (mean 24.3 ± 12.2), while that of the controls had a mean of 34.2 ± 7.3 years. Seven of the patients were males and eight were females, while a 1:1 sex ratio was selected for the controls.

The patients had had symptoms of chronic rhino-sinusitis for a period ranging between two to 20 years (mean 8.5 ± 6.3). In association with this 10 of the patients showed manifestations of chronic pharyngitis, six of chronic tonsillitis and one of chronic laryngitis.

Plain sinus and chest X-rays confirmed the involvement of one or more of the paranasal sinuses and a normal chest in all patients. X-rays were avoided in the controls.

Both patients and controls had a normal full blood count. Although a higher white blood cell was noticed in the patients it did not reach statistical significance, neither did the differential cell counts.

The difference between the mean volume of BAL fluid collected from patients (37.6 ml ± 4.6) and from controls (39.4 ml ± 5.7), was not statistically significant.

Tables I and II, present the BAL fluid total cell counts, NBT positivity percentage, and total IgA values for the 15 patients involved in the study and the 10 volunteers, while Table III compares their mean results with the mean results of the controls.

Differential cell counts for BAL fluid of patients showed a statistically significant increase in neutrophils (18 per cent) in comparison to two per cent for the controls. Macrophage, lymphocyte, basophil and eosinophil cell counts were not significantly different.

Discussion

This study included 15 patients suffering from chronic upper respiratory tract infection in the form of rhino-sinusitis, in association with chronic pharyngitis, laryngitis or tonsillitis. These patients did not however reveal any symptoms, signs, pulmonary functions or radiological findings suggesting the extension of infection to the lower respiratory tract.

We did not detect any predisposing factors to the chronicity of their infection, except for the marked

TABLE III

COMPARISON BETWEEN PATIENTS AND CONTROLS AS REGARDS ALBUMIN CONTENT, TOTAL CELL COUNT, NBT POSITIVITY AND TOTAL IGA IN BAL FLUID

	Albumin (mg/ml)	Total cell count (10^3 cell/ml)	NBT (%)	IgA (mg/ml)
Patients				
X	2.97	23.1	31.3	0.25
SD	1.58	10.2	8.4	0.07
Controls				
X	2.54	15.3	23.0	0.17
SD	0.45	5.1	4.3	0.07
Paired *t*-test	0.83	2.3*	2.9*	2.99*

*Statistically significant (p = 0.02, 0.01, 0.01).
X = mean value.
SD - standard deviation.

deviation in their nasal septum (the significance of which is debatable). Atopy and immune-deficiency was excluded on a clinical and laboratory basis. While primary mucociliary abnormalities was not suspected clinically. Although, these patients had symptoms of variable severity and duration they were not further subgrouped to achieve statistical significance.

Differential blood counts, during a period of remission of infection, for these patients, did not reveal any abnormality, while their BAL fluid analysis data confirmed the stimulation of the lower tract defence mechanisms, as evidenced by an increase in total cell count especially of neutrophils and NBT positivity.

In our patients the increase in NBT test positivity of neutrophils collected from BAL fluid signifies that phagocytosis and killing of bacteria occur in a higher proportion than in the control group. The fact that the lower respiratory tract is immunologically stimulated is also demonstrated in the significantly higher IgA levels, which may be due to an increase in the synthetic capacity of immunoglobulin-producing cells, or their count, or due to an increased transudation of IgA from the circulation. However all mechanisms may interplay.

The response of the lower respiratory tract demonstrated by this study differed from the systemic reaction reported by Humbert *et al.* (1971) on serum NBT in cases of indolent bacterial infection in four patients with chronic respiratory tract infections. The neutrophil and NBT positivity percentages were normal.

We may speculate that the change in the lower respiratory tract is due to its continuous contamination by bacteria descending in a post-nasal drip (Huxley *et al.*, 1978). Alternatively the neutrophils could in fact originate in the upper respiratory tract. A further possibility is that the cytokines are being elaborated in the upper respiratory tract and are then reaching the lung via a post-nasal drip.

Conclusion

This study offers evidence for the stimulation of the lower respiratory tract in cases of chronic purulent upper respiratory tract infections, for which the immunological and phagocytic defence mechanisms compensate, thus preventing a clinically obvious lower respiratory tract infection.

Acknowledgement

I would like to thank Ms P. Robinson, Consultant ENT Surgeon, Manor Hospital, Walsall, for her assistance and advice in preparing this paper.

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