REPRINT

Oral immunization with bacterial extracts for protection against acute bronchitis in elderly institutionalized patients with chronic bronchitis

B. Orcel, B. Delclaux, M. Baud, J.Ph. Derenne

THE EUROPEAN RESPIRATORY JOURNAL

OFFICIAL JOURNAL OF THE
EUROPEAN RESPIRATORY SOCIETY

Eur Respir J, 1994, 7, 446–452
Printed in UK - all rights reserved

European Respiratory Journal
ISSN 0903 - 1936

Oral immunization with bacterial extracts for protection against acute bronchitis in elderly institutionalized patients with chronic bronchitis

B. Orcel, B. Delclaux, M. Baud, J.Ph. Derenne

Oral immunization with bacterial extracts for protection against acute bronchitis in elderly institutionalized patients with chronic bronchitis. B. Orcel, B. Delclaux, M. Baud, J.Ph. Derenne. ©ERS Journals Ltd.

ABSTRACT: Acute bronchitis is a major source of morbidity in elderly patients. The purpose of this study was to assess the preventive effects of oral immunisation with a bacterial extract.

Three hundred and fifty four patients with chronic bronchitis, living in institutions for the elderly (aged >65 yrs), were included in a randomized, placebo-controlled, double-blind study. The purpose of the study was to assess preventive effects of OM-85 BV (an immunostimulating agent consisting of lyophilized fractions of eight of the most common pathogens isolated in respiratory tract infections) against acute lower respiratory tract infections. Two hundred and ninety patients completed the study (143 taking placebo and 147 taking OM-85 BV).

There was a 28% reduction in the number of lower respiratory tract infections in the patients treated with OM-85 BV; this was entirely due to 40% reduction in the number of episodes of acute bronchitis ($p<0.01$), with no difference in the number of episodes of pneumonia and bronchopneumonia. A larger number of patients in the OM-85 BV group were free of acute bronchitis throughout the 6 month study period (96 *vs* 69) and there was a 28% reduction in the number of antibiotic prescriptions in the OM-85 BV treated group.

These results suggest that OM-85 BV has a protective effect against acute bronchitis in elderly patients living in institutions.

Eur Respir J., 1994, 7, 446–452.

Service de Pneumologie, Groupe Hospitalier Pitié-Salpêtrière, France.

Correspondence: J.Ph Derenne
Service de Pneumologie et Réanimation
Groupe Hospitalier Pitié-Salpêtrière
47 boulevard de l'Hôpital
75013 Paris
France.

Keywords: Chronic bronchitis
elderly patients
immunostimulation

Received: January 13 1993
Accepted after revision October 5 1993

It is generally accepted that the major bacterial pathogens in infectious exacerbations of chronic bronchitis are *S. pneumoniae* and *H. influenzae*, although many other micro-organisms have been isolated from the bronchi during such episodes [1]. Since acute bronchitis is a major cause of morbidity and mortality in patients with chronic bronchitis, particularly in old age, prevention of respiratory tract superinfection is an important goal in clinical practice [2]. However, numerous studies involving various oral immunizing agents have yielded conflicting results [3, 4].

We report positive results obtained in a randomized, placebo-controlled, double-blind study with the administration of OM-85 BV (Broncho-Vaxom®), an immunostimulating agent composed of extracts of eight of the most frequent bacterial species found in the respiratory tract, in protection against bronchial superinfections in a large population of elderly patients with chronic bronchitis

OM-85 BV is an extract obtained by submitting eight micro-organisms (*Diplococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumonia, Klebsiella ozaenae, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus viridans* and *Moraxella catarrhalis*, in equal portions) to progressive alkaline lysis. Experimental studies indicate that the compound has an immunopotentiating effect on both cellular and humoral responses [5–10].

It spontaneously enhances tumour necrosis factor-α (TNF-α) and interleukin-2 (IL-2) production in peripheral blood mononuclear cells obtained from human volunteers [5] When cells are cultured in the presence of phytohaemagglutinin, there is an increased production of TNF-α, IL-2 and interferon-γ (IFN-γ) [5]. In murine cells it increases IL-1 production [6], and activates the cytotoxic properties of peritoneal and bone marrow derived macrophages [7]. It stimulates the killing activity of rabbit polymorphonuclear leucocytes [10].

OM-85 BV enhances the expression of adhesion molecules on normal human blood monocytes and granulocytes in a dose-dependent manner [11]. Since adhesion molecules play a pivotal role in antibacterial defence [12], these results suggest that the clinical interest of OM-85 BV could be related to the upregulation of adhesion molecules induced by the bacterial extract. In patients with chronic bronchitis, EMMERICH *et al.* [8] reported that the

Broncho-Vaxom®, Laboratoires OM, 1217 Meyrin, 2/Genève, Switzerland.

administration of OM-85 BV increases the helper/suppressor T-lymphocyte ratio, improves alveolar macrophage activity, and increases the concentration of IFN-γ in bronchoalveolar lavage fluid.

Recently LUSUARDI *et al.* [9] have studied 20 patients with chronic bronchitis, randomly treated with OM-85 BV or a placebo. There was no modification in the placebo group. In contrast, alveolar macrophages collected by bronchoalveolar lavage of the OM-85 BV group, showed a significant increase of random migration, of chemotactic response to formyl-methionyl-leucyl-phenylalanine (FMLP) 10^{-7} M and of O_2^- release in basal conditions and after stimulation with opsonized zymosan. No modification in systematic immunity was ever observed. This indicates that OM-85 BV can increase immune defences in the respiratory tract of patients with chronic bronchitis without interfering with systemic immunity.

Materials and methods

Definition of terms

The terms used in this study are defined in a classical way [13–15]. Chronic bronchitis was defined as a history of excessive sputum expectoration occurring on most days during at least three consecutive months for not less than two consecutive years. Acute bronchitis was defined as an acute infectious episode associating cough, increased purulent sputum of short duration, without modification of chest X-ray. Wheeze, dyspnoea and fever were not consistently observed. There were no fixed inspiratory crackles, but occasional crackles eliminated by cough could be heard. Pneumonia associated infection with fever, malaise, dyspnoea and cough. Inspiratory crackles persisting after cough were consistently observed. Chest X-ray showed pulmonary infiltrate or consolidation. Bronchopneumonia associated signs of pneumonia with wheeze. Acute infection of the lower respiratory tract included acute bronchitis, pneumonia and bronchopneumonia.

Patients

The study involved 396 patients with chronic bronchitis [13] recruited in 25 institutions for the elderly and disabled in Greater Paris (France). They were all over 65 yrs of age. All of the patients had had at least four documented acute infections of the lower respiratory tract, treated with antibiotics, in the reference period in the previous year (six months between October 1, 1986 and April 30, 1987).

Patients with asthma or bullous emphysema, a history of cancer, progressive infectious or immune diseases, left heart failure, and renal or hepatic insufficiency were excluded from the study. Similarly, patients who had received immunosuppressive treatment in the previous 3 months, or another immunomodulating agent or antipneumococcal vaccine in the previous 6 months, were excluded. All patients were immunized against influenza 8 days before the study.

Since the patients were not routinely seen by a chest specialist, the charts were reviewed independently by two chest physicians, who were unaware of what the patients were receiving. They withdrew 42 patients because of insufficient documentation or associated disease. Therefore, the population considered consisted of 354 patients.

There were 88 smokers, 90 ex-smokers and 175 nonsmokers. Smoking status was unknown in one patient. The distribution of smokers, ex-smokers and nonsmokers was similar in both groups.

There was a number of associated diseases and pathologies listed in table 1. There was a larger number of associated diseases in the OM-85 BV treatment group (403 *vs* 331). Essentially this was due to a larger number of cardiovascular disorders (201 *vs* 137) in the patients receiving the product ($p<0.01$).

Lung volumes

Forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were measured with a portable spirometer (Fukuda Sanyo Spiro Analyser ST90) calibrated twice a day. A single medical investigator visited

Table 1. – Associate disease and pathologies in the population studied

Diagnosis	Placebo n=174	OM-85 BV n=180	Total patients	Statisitical significance
Mental disorders	54	47	101	NS
Diseases of the nervous system and sense organs	36	28	64	NS
Diseases of the circulatory systems	137	201	338	p<0.01
Diseases of the digestive system	29	27	56	NS
Disease of the mucoskeletal system and connective tissue	21	25	46	NS
Symptoms, signs and ill-defined conditions	20	36	56	NS
Endocrine, nutritional and metabolic diseases	11	17	28	NS
Diseases of genito-urinary system	12	10	22	NS
Others	11	12	23	NS
Total*	331	403	734	NS

NS: nonsignificant. *: total number of associated diseases.

each institution and made all measurements. The patients were first shown how to make a forced expiration. After one or two training manoeuvres without the apparatus, they made a series of 2–4 forced expirations. The best values were kept for analysis. Reliable FEV_1 and FVC measurements were obtained in 221 patients.

Since it is often difficult for elderly patients to breathe in a closed circuit, because of psychological or dental problems, we chose to measure total lung capacity (TLC) by means of a radiographic method. X-ray pictures were taken (anteroposterior (AP) and lateral) at maximum inspiratory volume. TLC was computed by using the method of LLOYD *et al.* [16]. All spirometric values were compared to the normal values of QUANJER [17].

Arterial blood gases

Arterial blood gases were measured by the same medical investigator, on patients sitting or semi-recumbent and breathing room air. Arterial blood samples were obtained in a disposable preheparinized system from radial or brachial artery, and processed in less than 5 min in a Corning 178 blood gas analyser.

Trial design

A prospective, double-blind, placebo-controlled trial was conducted over a 6 month period (October 19, 1987 to May 17, 1988). The patients were allocated to either OM-85 BV or placebo treatment by a centralized randomization procedure, stratified for each centre. The first group took capsules containing 7 mg of OM-85 BV. The second group took similar capsules containing only the excipients. Both groups took one capsule every morning for the first 10 days of the first 3 month period. The capsules were tested for chemical and biological quality according to the requirements of the French National Health authorities.

A baseline examination was performed by the physicians of the institution in which the patients were hospitalized. This included a clinical examination and a standard, comprehensive modified Medical Research Council (MRC) questionnaire [18]. Further assessments were made on days 30, 60, 90, 135 and 180, and included a clinical examination and a questionnaire including the number, duration and type of respiratory events, together with the nature, dosage and duration of treatments. All other medical events and untoward side-effects were also noted.

Blood samples were taken on days 0, 90 and 180 for measurement of immunoglobulins A, G, M and E (IgA, IgG, IgM and IgE), red and white blood cells, serum glutamic oxalo-acetic transaminase (SGOT), serum glutamic pyruvic-transaminase (SGPI), gamma-glutamyl transferase (γ-GT) and alkaline phosphatase, urea, creatinine, proteins and glucose. Urinary sugar and proteins were measured on the same occasions.

Statistical analysis

Qualitative and discrete quantitative variables were analysed using the chi-squared test. Continuous variables were tested using analysis of variance to one factor (treatment) or the Wilcoxon non-parametric test when an heteroscedasticity was found with Bartlett's test. All values are given as mean±standard deviation.

Ethics

All patients gave oral consent, after having the purpose of the study described by the physician in charge of the trial in the presence of a third party. The study was approved by the Ethics Committee of the Saint Antoine Faculty of Medicine (Paris).

Results

Data analysis

Two hundred and ninety (143 taking placebo and 147 taking OM-85 BV) out of 354 patients completed the study. The physical characteristics of the 290 patients are shown in table 2, together with lung function tests values absolute volumes and percentages of predicted values [14], arterial blood gases, white and red blood cell counts, and serum immunoglobulin concentrations. At inclusion, all values were within the normal range, and there was no significant difference in these parameters between the two groups of patients.

Table 2. – Characteristics of the patients completing the study (n=290)

Parameters		Placebo n=143	OM-85 BV n=147	Statistical significance
Sex	M/F	67/76	65/82	NS
Age	yrs	82±8	82±7	NS
Height	cm	161±9	161±9	NS
Weight	kg	60.1±13.4	60.0±12.2	NS
FEV_1	*l*	1.02±0.50	1.04±0.51	NS
	% pred	55±23	55±24	NS
FVC	*l*	1.61±0.72	1.60±0.26	NS
	% pred	65±22	62±25	NS
TLC	*l*	4.91±1.63	5.02±1.70	NS
	% pred	92±23	94±24	NS
Pao_2	torr	75.7±10.8	74.9±10.8	NS
	kPa	10.1±1.4	10.0±1.4	
$Paco_2$	torr	40.6±5.8	40.1±6.1	NS
	kPa	5.4±0.8	5.3±0.8	
pH		7.42±0.04	7.43±0.03	NS
WBC	mm^{-3}	5487±1572	5270±1369	NS
RBC	$10^6/mm^3$	4.30±0.58	4.41±0.50	NS
IgA	$g \cdot l^{-1}$	3.32±1.52	3.28±1.51	NS
IgG	$g \cdot l^{-1}$	13.11±3.64	12.30±3.30	NS
IgM	$g \cdot l^{-1}$	1.30±0.91	1.20±1.27	NS
IgE	$\mu g \cdot l^{-1}$	128±257	83±167	NS

All values are given as mean±SD. Spirometric measurements are expressed in absolute volumes and as % of predicted values [8]. NS: nonsignificant difference; M: male; F: female; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; TLC: total lung capacity; Pao_2: arterial oxygen tension; $Paco_2$: arterial carbon dioxide tension; WBC: white blood cells; RBC: red blood cells: Ig: immunoglobulin.

Table 3. – Death related to respiratory infections

	Placebo n=174	OM-85 BV n=180
Pulmonary embolism with respiratory infection	2	
Pneumonia-bronchopneumonia	5	4
Acute bronchitis + acute respiratory failure*	4	1
Total	11	5

*: multiorgan failure in four patients (three placebo, on OM-85 BV).

Thirty nine patients died, 21 in the placebo group and 18 in the OM-85 BV group. There were more deaths related to acute infectious respiratory disease in the placebo group (n=11) than in the OM-85 BV group (n=5) (table 3), whereas the contrary was true for deaths unrelated to respiratory causes (10 *vs* 13). There were more deaths in the older patients (18% of those ≥90 yrs *vs* 10% of those <90 yrs).

Twenty five patients did not complete the study for other reasons, including transfer to another hospital, failure to follow the protocol, or return to their family home (10 placebo, 15 OM-85 BV)

Incidence of lower respiratory tract infections

The results are shown in figure 1. The number of acute infections of the lower respiratory tract was smaller in the OM-85 BV group (112 in 147 patients) than in the placebo group (156 in 143 patients), a 28% reduction ($p<0.05$).

A finer analysis of the data showed that a larger number of patients in the OM-85 BV group were free of acute bronchitis during the study period (96 *vs* 69; $p<0.01$); similarly, the absolute number of episodes of acute bronchitis was 40% lower in the group taking OM-85 BV ($p<0.01$). This was associated with a number of antibiotic prescriptions for the acute infections of the lower respiratory tract, 28% lower in the OM-85 BV group ($p<0.05$). In contrast, there was no difference in the number of episodes of pneumonia and bronchopneumonia. The number of acute respiratory tract infections was lower in both groups than in the reference period for the previous year.

The frequency of respiratory signs in the two groups (dyspnoea, cough, phlegm, rales) showed a similar tendency to decrease over the study period.

Respiratory medications

More than a third of the patients completing the study received respiratory medications when entering the study (table 4). There were no significant differences between the patients with the active compound and those receiving a placebo.

Respiratory medications taken by the patients during the study are also shown in table 4. We observed an overall reduction in the treatment group, that was not statistically significant except for mucolytics ($p<0.01$) and miscellaneous medications (paracetamol, corticosteroid and others) ($p<0.001$).

Biological effects

The measured biological parameters remained stable in both groups throughout the study. In particular, there was no change in serum immunoglobulin concentrations.

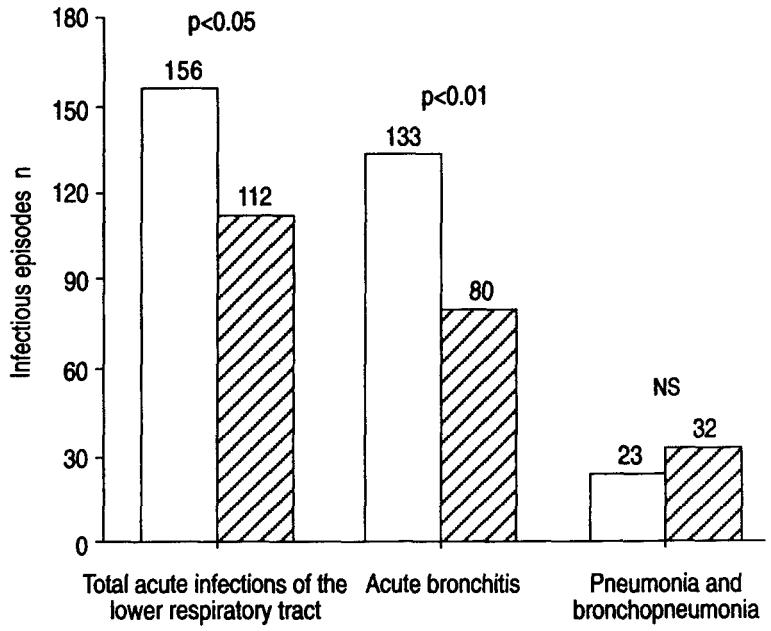

Fig. 1. – Number of infectious episodes of the lower respiratory tract. □: placebo (n=143); ▨: OM-85 BV (n=147). NS: nonsignificant.

Table 4. – Respiratory medication in patient entering the study (left hand side) and during the study (right hand side)

	Respiratory medications at the start of the study		Statistical significance	Respiratory medications during the study		Statisitical significance
	Placebo n=143	OM-85 BV n=147		Placebo n=143	OM-85 BV n=147	
Theophylline	30	31	NS	12	5	NS
Mucolytitics	26	22	NS	50	31	p<0.01
B_2-agonist	7	5	NS	7	3	NS
Almitrine	4	6	NS	0	0	
Miscellaneous	7	6	NS	53	19	p<0.001
Paracetamol	0	0		22	8	
Corticosteroid	1	0		13	2	
Others	6	6		18	9	
Physiotherapy	7	10	NS	6	4	NS
Oxygen	1	3	NS	3	3	NS

NS: nonsignificant.

Table 5. – Intercurrent medical events

Diagnosis	Placebo n=143	OM-85 BV n=147	Total	Statistical significance
Mental disorders	4	4	8	NS
Diseases fo the nervous system and sense organs	2	6	8	NS
Diseases of the circulatory system	8	7	15	NS
Diseases of the digestive system	14	4	18	NS
Disease of the musculoskeletal system and connective tissue	1	4	5	NS
Symptoms, signs and ill-defined conditions	10	11	21	NS
Endocrine, nutritional and metabolic disease	2	3	5	NS
Diseases of genito-urinary system	7	19	26	NS
Others	29	30	59	NS
Total	77	88	165	NS

NS: nonsignificant.

Untoward effects and intercurrent events

None of the patients complained of specific side-effects. There was a large number of intercurrent events, a common finding in this age group: 77 in the placebo group and 88 in the OM-85 BV group (NS) (table 5). Lower urinary tract infections were slightly more frequent in the treated group; but the difference was not significant.

Discussion

We report the first large-scale, double-blind, placebo-controlled, clinical trial of an immunostimulating agent in the prevention of acute bronchitis in elderly patients with chronic bronchitis. The results show that OM-85 BV, an agent containing extracts from eight of the most frequent bacterial species found in the respiratory tract, has a protective effect against acute infection of the lower respiratory tract, particularly acute bronchitis in elderly subjects with chronic bronchitis.

The aetiology of exacerbations of chronic bronchitis remains controversial, although several attempts have been made to correlate clinical status with virological and bacterial findings. The reported frequency of viral infections varies from 4.4–63.3% (review [19]). In addition, GUMP *et al.* [20] have shown that viruses can be isolated from the airways of symptom-free patients with chronic bronchitis .

The concept that viruses are the primary agents of acute bronchitis has led to the suggestion that immunization procedures might reduce the rate of recurrent episodes of acute illness; nonetheless, the results of viral immunization have so far been disappointing [4].

An alternative theory holds that the immediate cause is a shift in the host-bacterial balance, which results in bacterial infection. The role of bacteria in exacerbations of chronic bronchitis is still under debate [2], and comparative trials of prophylactic antibiotic therapy have provided no clear-cut evidence for a substantial benefit (reviews [2, 19]). Similarly, the curative efficacy of antibiotics is still controversial [2, 21–25]. However,

there seems to be a tendency for antibiotics to be more effective than a placebo in a significant number of randomized, placebo-controlled trials assessing antimicrobial therapy of exacerbations of chronic obstructive pulmonary disease (review [2]).

The theoretical basis for oral immunization is that the bacterial fractions administered can be recognized by gut-associated lymphoid tissue (GALT), thus priming bronchial-associated lymphoid tissue (BALT) through cooperation and cell traffic between these two systems [26, 27].

Although the mechanisms involved in the immunostimulating properties of bacterial extracts are not fully understood, there is some experimental evidence that they act on cells of the immune system and on mediators in man and in experimental animals [7–10, 28, 29]. In addition, there is increased antibody secretion in the respiratory tract after oral administration of OM-85 BV in man [8].

In recent years, there has been a series of attempts to demonstrate *in vivo* efficacy of bacterial extracts for the prevention of chronic bronchitis in adults [2–4, 30]. However, the results of clinical trials have been inconsistent, and opposing conclusions have been drawn. DAHAN *et al.* [3] carefully analysed the results of eight double-blind studies of RU 41740, a glycoprotein extract of *K. pneumoniae* K_2O_1 strain. Four of these trials led to positive conclusions and the other four to negative ones. Furthermore, two of the four positive studies were associated with a high risk of false positives. DAHAN *et al.* [3] showed that the discrepancies were due to inadequate analysis of the relationship between the occurrence of the disease during the trial, the sample size, and the estimated magnitude of the benefit of the drug.

A common characteristic of these studies was an insufficient number of subjects, carrying a risk of falsely negative (lack of power) and falsely positive results. In consequence, we selected a population of subjects having had a large number of acute respiratory infections in the previous year, as well as a sufficiently large number of subjects to avoid false-positive results [31] and to provide sufficient statistical power.

We chose to test OM-85 BV in elderly subjects with chronic bronchitis, as acute bronchitis is a major cause of morbidity in this population. We did not analyse the bacterial flora in the throat or sputum, because of the poor correlation between the micro-organisms isolated and those identified in the bronchi of patients with chronic bronchitis [19].

In addition, the treatment was administered directly to the patients, to ensure compliance; and follow-up was facilitated by the fact that the patients were in hospitals or institutions for the chronically disabled.

There was a decrease in the number of acute respiratory tract infections in both groups of patients during the trial relative to the reference period in the preceding year. These differences may have been related to a much warmer winter in 1987–1988 than in 1986–1987, or to the fact that all of the patients had been vaccinated against influenzae before the study. PROFETA *et al.* [32] reported that RU 41740 significantly increased the HI-specific antibody response to A/Chile/83 and A/Philippines/82 after influenzae vaccination. However, such an effect of OM-85 BV has not yet been proven. Finally, it is well-known that participation in a study can have a powerful placebo effect, particularly on old people [33], and when the treatment is directly administered by nurses or doctors [34].

In conclusion, OM-85 BV administration was associated with a 28% decrease in the number of lower respiratory tract infections, and a one third decrease in the use of antibiotics. This was entirely due to a 40% decrease in the number of episodes of acute bronchitis. Therefore, it seems that OM-85 BV had a protective effect on the immunological system in the airways. On the other hand, OM-85 BV did not protect against pneumonia or bronchopneumonia. This improvement in clinical status was not associated with any significant change in circulating immunoglobulin concentrations.

References

1. Tager I, Speizer FE. Role of infection in chronic bronchitis. *N Engl J Med* 1975; 292: 563–571.
2. Murphy TF, Sethi S. State of the Art. Bacterial infection in chronic obstructive pulmonary disease. *Ann Rev Respir Dis* 1992; 146: 1067–1083.
3. Dahan R, Constantini D, Caulin C, Yagello M. Clinical trials with C 1740, an immunomodulator compound proposed for prevention of acute infectious exacerbations in chronic bronchitis. *Meth Find Exptl Clin Pharmacol* 1986; 8: 41–43.
4. Bignon J, Lambre C, Delepierre F. Immunomodulation et bronchite chronique. *Med Hyg* 1988; 46: 172–181.
5. Wybran J, Libin M, Schandene L. Activation on natural killer cells and cytokine production in man by bacterial extracts. *Immunopharmac Immunotoxicol* 1989; 11: 17–32.
6. Bottex C, Cristau B, Corazza JL, Mougin B, Fontanges R. Effects of two bacterial extracts, OM 89 and Broncho-Vaxom, on IL-1 release and metabolic activity of a murine macrophage cell-line. *Int J Immunother* 1988; 4: 203–212.
7. Mauel J, Pham TV, Kreis B, Bauer J. Stimulation by a bacterial extract (Broncho-Vaxom) of the metabolic and functional activities of murine macrophages. *Int J Immunopharmac* 1989; 11 (6): 637–645.
8. Emmerich B, Emslander HP, Packmann K, Hallek M, Milatovic D, Busch R. Local immunity in patients with chronic bronchitis and the effects of a bacterial extract, Broncho-Vaxom, on T-lymphocytes, macrophages, gamma-interferon and secretory immunoglobulin A in bronchoalveolar lavage fluid and other variables. *Respiration* 1990; 57: 90–99.
9. Lusuardi M, Capelli A, Carli S, Spada EL, Spinazzi A, Donner CF. Local airways immune modifications induced by oral bacterial extracts in chronic bronchitis. *Chest* 1993; 103: 1783–1791
10. Nauek M, Matthys H, Emmons LR, *et al.* The immunomodulators Broncho-Vaxom and Uro-Vaxom stimulate the bacterial killing and oxidative metabolism of polymorphonuclear leukocytes by the activation of phosphatidylinositol turnover. *Int J Exp Clin Chemother* 1991; 4: 1–11.
11. Duchow J, Marchant A, Delville JP, Schandene L, Goldman M. Upregulation of adhesion molecules by Broncho-Vaxom on phagocytic cells. *Int J Immunopharmac* 1992; 14: 761–766.

12. Anderson DC, Springer TA. Leucocyte adhesion deficiency: an inherited defect in the MAC-1, LFA-1 and pl 50,95 glycoproteins. *Ann Rev Med* 1987; 38: 175–194.
13. Medical Research Council. Definition and classification of chronic bronchitis for clinical and epidemiological purposes. A report to the Medical Research Council by their Committee on the aetiology of chronic bronchitis. *Lancet* 1965; i: 775–779.
14. Duffell GM. Acute bronchitis. *In*: Hurst JW, ed. Medicine for the Practicing Clinician. Woburn, Butterworth, 1983.
15. Perlino CA. Pneumonia. *In*: Hurst JW, ed. Medicine for the Practicing Clinician. Woburn, Butterworth, 1983.
16. Lloyd HM, String ST, Dubois AB. Radiographic and plethysmographic determination of total lung capacity. *Radiology* 1966; 86: 7–14.
17. Quanjer PhH. Standardized lung function testing. *Bull Eur Physiopathol Respir* 1983; 19 (Suppl. 5): 1–95.
18. Medical Research Council. Standardised questionnaires on respiratory symptoms. *Br Med J* 1960; 2: 1665.
19. Sachs FL. Chronic Bronchitis *In*: Pennington JE, ed. Respiratory Infections: diagnosis and Management. New York, Raven Press, 2nd edn, 1983; pp. 113–124.
20. Gump DW, Phillips CA, Forsyth BR, *et al.* Role of infection in chronic bronchitis. *Am Rev Respir Dis* 1976; 113: 465–474.
21. British Thoracic and Tuberculosis Association. A comparison of trimethoprim sulphamethoxazole compound and tetracycline in exacerbations of chronic bronchitis. *Br J Dis Chest* 1972; 66: 199–206.
22. Nicotra MB, Rivera M, Awe RJ. Antibiotic therapy of acute exacerbations of chronic bronchitis. A controlled study using tetracycline. *Ann Intern Med* 1982; 97: 18–21.
23. Pines A, Raafat H, Greenfield JSD, Linsell WD, Solari ME. Antibiotic regimens in moderately ill patients with purulent exacerbations of chronic bronchitis. *Br J Dis Chest* 1972; 66: 107–115.
24. Anthonisen NR, Manfreda J, Warren CPW, Hershfield ES, Harding GKM, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann Intern Med* 1987; 106: 196–204.
25. Editorial. Antibiotics for exacerbations of chronic bronchitis? *Lancet* 1987; ii: 23–24.
26. Bienenstock J. The derivation, distribution and function of intestinal mucosal lymphocytes. *Monogr Allerg* 1981; 17: 233–249.
27. Mestecky J, McGhee JR, Arnold RR, Michalek SM, Prince SJ, Babb JL. Selective induction of an immune response in human external secretions by ingestion of bacterial antigen. *J Clin Invest* 1978; 61: 731–737.
28. Wood CD, Moller G. Influence of RU 41.740, a glycoprotein extract from Klebsiella pneumoniae, on the murine immune system. I. T-independent polyclonal B-cell activation. *J Immunol* 1984; 132 (2): 616–621.
29. Rajagopalan P, Dournon E, Vilde JL, Pocidalo JJ. Direct activation of human monocyte-derived macrophages by a bacterial glycoprotein extract inhibits the intracellular multiplication of virulent *Legionella pneumophilia* serogroup 1. *Infect Immunol* 1987; 55(9): 2234–2239.
30. Farine JC, Meredith M. Clinical evaluation of a bacterial immunomodulator in chronic bronchitis. *Adv Immunomod* 1988; 38: 403–408.
31. Staquet MJ, Rozencweig M, Von Hoff DD, Muggia FM. The delta and epsilon errors in the assessment of cancer clinical trials. *Cancer Treat Rep* 1979; 63 (11–12): 1917–1921.
32. Profeta ML, Guidi G, Meroni PL, *et al.* Influenza vaccination with adjuvant RU 41740 in the elderly (letter). *Lancet* 1987; i: 973.
33. Lasagna L, Mosteller F, Von Felsinger JM, Beecher HK. A study of the placebo response. *Am J Med* 1954; 16: 770–779.
34. Feldman PE. Personal element in psychiatric research. *Am J Psychiatr* 1956; 113: 52–54.