Local Airways Immune Modifications Induced by Oral Bacterial Extracts in Chronic Bronchitis*

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Bacterial extracts can act as immune stimulants and in some instances have been used, rather empirically, to prevent recurrent infections in the nonimmunocompromised host. Some agents are administered via oral route with the goal to increase airways immune defenses. In animal models and in normal humans, gut-associated lymphoid tissue (GALT) stimulation is able to induce a generalized response by the whole mucosal-associated lymphoid tissue (MALT). The aim of this placebo-controlled, double-blind, parallel-group study was to evaluate whether the stimulation of the GALT through oral administration of a polyvalent bacterial extract (BE) could lead to significant immune modifications either systemically or locally in the respiratory tract in patients suffering from chronic bronchitis. We selected 20 subjects (5 nonsmokers, 6 smokers, and 9 ex-smokers) for at least 3 years. According to a balanced-block randomization method, ten patients received active treatment and ten received placebo. Either drug or placebo was to be taken as one capsule daily the first 10 days of 3 consecutive months. Each capsule of the active product contained 7 mg of a BE obtained from eight different bacterial strains. On entry (T0) and 90 days after beginning of treatment (T90), all patients underwent bronchoalveolar lavage (BAL) and peripheral blood withdrawal to assay BAL fluids and serum samples for immune parameters. The BAL recoveries, cellularity, cell differentials, and lymphocyte subsets (CD19, CD3, CD4, CD8) did not show significant differences. IgG/albumin and IgA/albumin values were not significantly different, but IgA/albumin was significantly increased in the treatment (T0 = 0.14, 0.01 to 0.27, median and range, T90 = 0.15, 0.08 to 0.45, p = 0.025) vs the placebo group when data from current smokers were excluded. Functional tests on alveolar macrophages (AM) (leading front stimulated motility and superoxide anion -O2−-release) showed a significant increase of random migration (T0 = 10.6, 7.0 to 23.6, T90 = 13.4, 8.1 to 25.8 μm, p = 0.02) and of stimulated motility after FMLP 10−7 M (T0 = 13.2, 8.3 to 46.4, T90 = 18.3, 8.4 to 49.6 μm, p = 0.04), a significant increase of O2− release in basal conditions (T0 = 6.0, 1.7 to 30.5 nM/106 AM/105, T90 = 11.1, 5.5 to 24.5, p = 0.05) and after stimulation with opsonized zymosan (T0 = 17.7, 4.7 to 35.2, T90 = 22.1, 13.8 to 53.3, p = 0.009) in the treatment group only. Data were not significantly different in the placebo group between T0 and T90. No modifications in systemic immunity were ever observed. Our data demonstrate that oral administration of a BE can increase immune defenses in the respiratory tract of patients with chronic bronchitis, without apparently altering systemic immunity. This confirms the possibility of a preferential traffic of immune information across the MALT and supports a rationale for experimental trials with oral treatments using BEs in the prevention of chronic bronchitis exacerbations.

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Prevention of lower airways recurrent infections is one of the main goals in the treatment of chronic bronchitis, since their individual and socioeconomic costs are a matter of particular concern. Specific immunization against influenza is recommended, but the extent of protection is limited.1 Polyvalent pneumococcal vaccine is efficacious but not widely used.2 Vaccines against Klebsiella pneumoniae and Haemophilus influenzae are available, too, but their efficacy needs to be further substantiated before extensive use. The prophylactic use of antibiotics has no proven efficacy3 and has almost been abandoned. The use of nonspecific immunomodulators is currently suggested for clinical use in all those situations characterized by enhanced susceptibility to infections in the nonimmunocompromised host. A good cost/benefit ratio seems to be offered by immunobiotherapeutics such as bacterial extracts (BES), which are the strongest natural exogenous immunomodulating agents.4 A number of immunostimulating agents of bacterial origin are available on the European market for prevention and treatment of recurrent infections, including chronic bronchitis exacerbations. A few double-blind clinical trials employing different BEs against placebo showed a significant reduction of the incidence of upper and lower respiratory tract recurrent infections in both pediatric and adult-age patients.5-8 Notwithstanding, an accurate demonstration of BE biologic effects related to an increase of general and/or local defenses against microorganisms is lacking at present in patients with chronic bronchitis.

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In in vitro conditions, BEs are able to stimulate functional activities of macrophages and lymphocytes, to activate natural killer cells and the release of cytokines from human peripheral blood mononuclear cells.

In the animal treated in vivo with BEs, bacterial killing is enhanced and immunoglobulin production and secretion can be augmented in definite conditions. Resistance to experimental infection in animal models is increased.

In healthy humans, the ingestion of killed bacteria can lead to a selective stimulation of immune response in external secretions without modifications of serum antibody levels, but to our knowledge, the effect of oral BE has not been tested in healthy subjects.

The oral route of administration is generally preferable for prolonged and/or extensive treatments. The possibility of "absorbing immunizing, bactericidal and vaccinating substances through the digestive tract" was reported as experimentally demonstrated in man many years ago. The existence of a protective local immune system functioning fairly independently of systemic immunity was proposed by Besredka in 1919 when he demonstrated that rabbits, after oral immunization with killed Shiga bacillus, were protected against fatal dysentery irrespective of the serum antibody titer. The interest in local immunity was significantly revived in the 1960s when it was reported by Tomasi and Zigelbaum that the predominant immunoglobulin in external body fluids was immunoglobulin A, but an accurate functional evaluation of the mucosa-associated lymphoid tissue and of the effects of oral immunization with BEs started in the 1970s.

The aim of our study was to evaluate the effects of the oral administration of a BE on systemic and airways immunity in patients suffering from chronic bronchitis to provide a possible rationale for the use of bacterial-derived immunostimulants in clinical trials for the prevention of recurrent respiratory tract infections.

METHODS

Study Population

From March 1988 to February 1990, we selected 20 patients (5 nonsmokers, 6 smokers, and 9 ex-smokers) affected by chronic bronchitis, diagnosed according to the American Thoracic Society (ATS) standard criteria. All ex-smokers had stopped smoking for at least three years. Patients with respiratory disorders other than chronic bronchitis, including asthma, immunodeficiency conditions, autoimmune disorders, malignancies, or clinically significant cardiovascular, neurologic, endocrine, and hematologic disorders were excluded. Patients having received any drug with known effects on the immune system within the previous 180 days were also ineligible to participate. Only steady-state treatment with bronchodilators was admitted. No signs of exacerbation were present at the admission to the trial. Institutional Review Board approval and informed consent were obtained before study start and patient entry, respectively.

Study Design and Drugs

This study was a placebo-controlled, double-blind, parallel-group clinical trial. Since a balanced-block randomization method was used, ten patients were assigned to receive the active product and ten were assigned to receive placebo.

One capsule of either the BE or placebo were administered the first 10 days of 3 consecutive months, according to manufacturer's recommendations. We evaluated gelatin capsules containing a BE consisting of a lyophilized lysate of fractions of the following bacterial strains: H influenzae, D pneumoniae, K pneumoniae, K ozensa, M aureus, S pyogenes, S viridans, N catarrhalis (Broncho-vaxom, Byk Gulden Italia, Italy). Placebo capsules were of identical appearance to active product.

Bronchoalveolar Lavage and Processing of Specimens

To evaluate local airways immunity, bronchoalveolar lavage (BAL) was performed just before the first administration (T0) and 90 days after (T90). During fiberoptic bronchoscopy, three 50-ml boluses of 37°C prewarmed sterile saline solution were injected, immediately recovered with a syringe, and kept separate. Fluids were filtered through a monolayer of sterile gauze.

Before centrifugation, cytocentrifuge (Cytospin II, Shandon, London, UK) slide preparations on native fluids were made in duplicate for each recovery and stained with May-Grunwald-Giems. A total amount of at least 500 cells per each slide were examined at a 1,000 X magnification. Individual cell differentials were determined as the weighed mean of the three recoveries. Bronchoalveolar cells were separated by refrigerated (4°C) centrifugation (Beckman TJ 6, Beckman, Fullerton, Calif), 400 g for 15 min, and resuspended in cell culture medium RPMI 1640 (Boehringer Mannheim GmbH, Mannheim, Germany).

B (CD19+) lymphocytes, total T (CD3+) lymphocytes, helper/inducer (CD4+), and suppressor/cytotoxic (CD8+) T-lymphocyte subpopulations were determined with a microsopic direct immunofluorescence assay using monoclonal antibodies of the Leu series labeled with fluorescein-isothiocyanate and phycoerythrin in a simultaneous test (Simultest T and B Test and Simultest T Helper/Suppressor Test, Becton and Dickinson, Mountain View, Calif).

Supernatants were immediately frozen at -80°C and assays were done within 15 days. We evaluated the levels of albumin, IgG, IgA, IgM, C3, and C4 in BAL fluid with a modified immunoturbidimetric method (reagent kits: URIN-PAK immuno MICROALB, for albumin; SERA-PAK immuno, for the other substances, Miles Italiana, Cavenago Brianza, MI, Italy). All BAL fluid samples were unconcentrated. The immunoturbidimetry reaction required an incubation time of 30 min for all the assays; the absorbance readings were performed at 340 nm using a spectrophotometer (COMFUR M 2000 CS2, Bayer Diagnostic-Electronic, Munich, Germany). All samples were tested in duplicate.

For the analysis of macrophage activities, the first aliquot recovered was discarded. Separation procedures with a Ficoll-Hypaque (Kit Histopaque 1077, Sigma Chemicals, St. Louis) gradient centrifugation at 4°C were adopted when neutrophils exceeded 3 percent of total cells. The BAL mononuclear cells were subsequently washed three times in phosphate-buffered saline solution and finally resuspended in RPMI 1640+10 percent fetal calf serum.

Viability as assessed with the trypan blue dye exclusion test was always greater than 95 percent.

Superoxide anion release was assessed spectrophotometrically (550 nm) both in basal conditions and after opsonized zymosan phagocytosis as superoxide dismutase inhabitable reduction of ferricytochrome C. Macrophage stimulated motility was evaluated with the leading front method, reading microscopically (400 X) seven fields at least.

In a modified Boyden chamber with a 8-μm pore nitrocellulose membrane (Millipore Corp, Bedford, Mass), 500 μl of cell suspension with 5 x 10^6 macrophages were put in the upper chamber.
**Table 1—BAL Cell Differentials**

<table>
<thead>
<tr>
<th></th>
<th>Cells/ml, $\times 10^6$</th>
<th>Mac, %</th>
<th>Ly, %</th>
<th>Neu, %</th>
<th>Eos, %</th>
<th>Bas, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T90</td>
<td>T0</td>
<td>T90</td>
<td>T0</td>
<td>T90</td>
</tr>
<tr>
<td>BE</td>
<td>mean</td>
<td>3.05</td>
<td>4.28</td>
<td>85.9</td>
<td>76.3</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>$\pm$ SEM</td>
<td>0.45</td>
<td>0.57</td>
<td>1.9</td>
<td>4.9</td>
<td>1.6</td>
</tr>
<tr>
<td>PL</td>
<td>mean</td>
<td>2.59</td>
<td>2.89</td>
<td>80.7</td>
<td>76.8</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>$\pm$ SEM</td>
<td>0.3</td>
<td>0.37</td>
<td>3.5</td>
<td>5.6</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*Cellularity and percent cell differentials assessed on bronchoalveolar lavage (BAL) fluids. BE = subjects treated with bacterial extract; PL = subjects treated with placebo. MAC = macrophages; Ly = lymphocytes; Neu = neutrophils; Eos = eosinophils; Bas = basophils.

FMLP (formyl-methionyl-leucyl-phenylalanine) $10^{-7}$ was used as a chemotactic stimulant. Incubation in CO$_2$ 5 percent at 37°C was prolonged for 90 min.

**Blood Investigations**

To evaluate systemic immunity, heparinized blood samples were taken by venipuncture at T0 and T90. Total leukocyte count was carried out with a standard hemocytometric procedure. Cell differentials were assessed microscopically after staining with May-Grunwald-Giemsa.

Mononuclear and polymorphonuclear cells were separated with a single and double density gradient centrifugation, respectively, using Ficoll-Hypaque (Kit Histopaque 1077 and 1119, Sigma Chemicals, St. Louis). Viability was >95 percent. Residual red blood cells were lysed with a 0.1 percent KHCO$_3$ and 0.83 percent NH$_4$Cl solution.

Lymphocyte subpopulations were assessed as described in the previous paragraph. Superoxide generation was evaluated in blood granulocytes obtained with a Ficoll-Hypaque double gradient centrifugation (all reagents from Sigma Chemicals, St. Louis).

Granulocyte stimulated motility was studied with the previously described method, but using 3-μm pore nitrocellulose membranes. Serum immune components (Immunglobulin G, A, and M, and C3 and C4 complement fractions) were determined spectrophotometrically using commercially available standard immunoturbidimetric kits (SERA-PAK immuno, Mils Italiana, Cavenago Brianza, MI, Italy).

**Statistical Analysis**

All data are reported as mean and standard error of the mean ($\pm$ SEM) unless specified differently. The BAL data concerning protein fractions and cell activities are reported also as median and range. The $t$ test for unpaired observations was performed to compare the study groups with respect to age, weight, height, and pulmonary function tests (FVC, FEV$_1$). Fisher's exact test was used to compare study groups as regards proportion of patients with respect to sex, smoking habits, and season of inclusion. The Wilcoxon signed-rank test and the Mann-Whitney $U$ test were used for examining differences in BAL and serum data in paired and independent samples, respectively, since the distribution of these data did not appear to be normal. A level greater than 95 percent probability was considered to be statistically significant.

Two matched subgroups of nonsmoking/ex-smoking subjects (seven from each group) was considered apart to repeat the statistical analysis eliminating present tabagism as a variability factor.

**RESULTS**

**Patients**

The two groups of patients were matched for sex (eight men + two women vs six men + four women), age (61.2 ± 2.2 vs 66.2 ± 1.3 years), weight (76.4 ± 4.2 vs 75.2 ± 4.0 kg), height (169.9 ± 3.5 vs 166.1 ± 2.8 cm), smoking habits (three smokers and five ex-smokers vs two smokers, four ex-smokers), clinical status (FVC 73.7 ± 5.0, SEM, FEV$_1$, 63.8 ± 4.2 percent predicted, vs FVC 77.7 ± 4.8, FEV$_1$, 67.0 ± 5.6 percent predicted), and season of inclusion (five placebo and five active treatment cases were studied in spring/summer, two and three in summer/autumn, one and one in autumn/winter, one and one in winter/spring, and one placebo case in winter).

No exacerbations were registered in the follow-up period. Only two patients per group experienced a slight deterioration of dyspnea for a few days, well controlled by aerosol bronchodilators.

Tolerance of BAL was good for all the subjects. Recoveries were 74 ± 3.8 ml (T0) and 72 ± 6.7 ml (T90) in the BE group, and 76 ± 3.3 ml (T0) and 62 ± 4.9 ml (T90) in the placebo group.

**Table 2—Alveolar Lymphocyte Subpopulations**

<table>
<thead>
<tr>
<th></th>
<th>CD19, %</th>
<th>CD3, %</th>
<th>CD4, %</th>
<th>CD8, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T90</td>
<td>T0</td>
<td>T90</td>
</tr>
<tr>
<td>BE</td>
<td>mean ±</td>
<td>1.92</td>
<td>1.67</td>
<td>79.1</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.62</td>
<td>0.62</td>
<td>1.6</td>
</tr>
<tr>
<td>PL</td>
<td>mean ±</td>
<td>1.30</td>
<td>1.23</td>
<td>81.4</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.40</td>
<td>0.35</td>
<td>1.7</td>
</tr>
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</table>

*Bronchoalveolar lavage lymphocyte subpopulations, as a percentage of total lymphocytes. BE = subjects treated with bacterial extract; PL = subjects treated with placebo.
**BAL Cell Data**

Cellularity, percentage of cell differentials (Table 1), and lymphocyte subsets (Table 2) did not show statistically significant intragroup and intergroup differences. The same applies to the comparison of absolute values (data not shown). The bacterial lysate acted as a stimulant of alveolar macrophage random migration that changed significantly (T0 = 10.6, 7.0 to 23.6 μm, median and range, T90 = 13.4, 8.1 to 28.8 μm, p = 0.02) while no changes occurred in the placebo group (T0 = 13.2, 5.4 to 18.6 μm, T90 = 11.6, 5.3 to 20.1 μm) (mean and SEM values reported in Fig 1). There were no significant intergroup (BE → placebo) variations in motility after FMLP stimulation, while intragroup (T0 → T90) variations were significant in the BE group only (T0 = 13.2, 8.3 to 46.4 μm, T90 = 18.3, 8.4 to 49.6 μm, p = 0.04; placebo T0 = 15.3, 7.5 to 42.0 μm, T90 = 19.5, 6.4 to 27.4 μm) (see Fig 1 for mean ± SEM values).

Also, superoxide anion release from alveolar macrophages challenged with opsonized zymosan was increased by the drug (T0 = 17.7, 4.7 to 35.2 nM/10^6 min, T90 = 22.1, 13.8 to 53.3, p = 0.009; placebo: T0 = 19.3, 6.0 to 87.4, T90 = 14.1, 6.6 to 32.5, p = NS; intergroup analysis p = 0.049) (Fig 2). Basal values did not show significant differences (Fig 2) at the intergroup comparison, but again, T90 value in the BE group (11.1, 5.5 to 24.5) was significantly higher than at baseline (6.0, 1.7 to 30.5, p = 0.05); placebo T0 = 6.3, 0 to 18.2, T90 = 6.4, 0 to 17.7.

Cytologic data regarding currently nonsmoking patients were similar to the global case series, the only exception being a stimulated motility not significantly modified (p = 0.12).

**BAL Biochemical Data**

Considering absolute levels of the protein fractions evaluated in our study, a significant increase of IgA was found in the BE group (T0 = 0.5 ± 0.06, T90 = 0.78 ± 0.06, mg/dl, vs placebo, T0 = 0.62 ± 0.12 and T90 = 0.6 ± 0.11, p = 0.002) (Fig 3), but no significant variations were found for the other parameters. Individual protein fractions to albumin ratios were not significantly different (see Table 3 and Fig 3 for albumin, IgG, and IgA values; other data not shown). In the subgroup of nonsmoking/ex-smoking subjects treated with the BE, T90 average IgA/albumin value was significantly increased in comparison with baseline (p = 0.028), though intergroup variations did not appear significant (Fig 4).

**Blood Data**

We were not able to find any significant differences
FIGURE 2. Superoxide anion release from alveolar macrophages in basal conditions and after challenge with opsonized zymosan (nM/10^6/10 min) at baseline (T0) and after treatment (T90) in bacterial extract (BE) and placebo (PL) groups. Values reported as individual data and mean ± SEM.

FIGURE 3. IgA levels in bronchoalveolar lavage supernatants at baseline (T0) and after treatment (T90) in bacterial extract (BE) and placebo (PL) groups. Data are reported as absolute values (upper graphs) and as a ratio to albumin levels (lower graphs) (individual data and mean ± SEM).
as for blood constituents: cell differentials, lymphocyte subsets, immunoglobulins, granulocyte stimulated motility, and superoxide anion generation, which were all in the normal range. Immunoglobulin values, granulocyte stimulated motility and $O_2^-$ release are reported in Tables 4 and 5.

**DISCUSSION**

Patients affected by chronic bronchitis usually experience recurrent airways inflammatory exacerbations with important deterioration of respiratory function. Periodic courses of antibiotics as a preventive measure were in use until recently, but clinical trials have demonstrated that they are not significantly effective.3 Systemic immune defects have never been definitely demonstrated in the average chronic bronchitic patient. In two studies, defects of chemotaxis, phagocytosis, and Candida killing were described,20,24 but enhanced chemotaxis was reported by other authors.25 A recent study reconsidered the topic of neutrophil function in chronic bronchitis concluding with a rare occurrence of constitutional defects with a possible, but not definitely proven, exception concerning the release of $O_2$ metabolites.26 A possible correlation between IgG subclass deficiencies and development/progression of respiratory damage in chronic obstructive lung disease was described in a recent article.27 Knowledge about local immune defenses is even in a more preliminary phase.28 Bronchoalveolar lavage IgG and IgA levels in our patients were comparable to or higher than values found in control normal subjects and published in a previous report.29

Currently, there is a prevailing tendency to stimulate the immune system to prevent exacerbations, either specifically (eg, with influenza and pneumococcal vaccines) or with nonspecific treatments, such as the administration of BEs.4

According to our results, oral BEs can locally modify nonspecific cellular defenses, but the ability to affect the specific immune components such as lymphocyte subsets and immunoglobulins is controversial. The absence of significant variations of lymphocytes and standardized Ig data could be explained, at least in

![Figure 4. IgA levels in bronchoalveolar lavage supernatants at baseline (T0) and after treatment (T90) in bacterial extract (BE) and placebo (PL) groups, excluding data from currently smoking subjects. Data are reported as a ratio to albumin levels (individual data and mean ± SEM).](image-url)
Table 4—Immunoglobulins and C Fractions*

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>C3</th>
<th>C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial extract, mean ± SEM</td>
<td>1,317 ± 183</td>
<td>317 ± 54</td>
<td>135 ± 17</td>
<td>179 ± 27</td>
<td>42 ± 27</td>
</tr>
<tr>
<td>Placebo, mean ± SEM</td>
<td>1,198 ± 125</td>
<td>333 ± 69</td>
<td>144 ± 35</td>
<td>19 ± 19</td>
<td>45 ± 19</td>
</tr>
</tbody>
</table>

*Immunoglobulins and complement fractions assessed in serum. Serum values reported in milligrams per deciliter.

part, by the presence in our case series of both smoker and nonsmoker as well as ex-smoker subjects. That smoking habits influence BAL immune parameters is well known. Velluti et al. found a significant threefold to fourfold increase of both IgG and A in healthy smokers compared with nonsmokers. Costabel et al. demonstrated a T-lymphocyte helper-suppressor imbalance in smokers, with a significant reduction in the ratio due to an increase of the CD8+ lymphocytes. The analysis of our data performed after exclusion of smokers seems in part to confirm this point of view, since the IgA/alb levels were significantly increased in the BE group at T90 vs T0 but spontaneous variations in both Ig and albumin levels could have significantly biased our data.

Also, methodologic problems have to be considered, since using the BAL technique, we took a picture of the lung microenvironment in two definite moments (T0 and T90), which allows us only a gross evaluation of the kinetics of different immune parameters potentially occurring at the lung level during the trial. When materials to be studied are readily and repeatedly obtainable (eg, saliva), immune modifications induced by BE appear better assessed at short periods.

Furthermore, the exact determination and expression of BAL biochemical data are still controversial.

Also, in animal models, BEs increase macrophage activities. In rats, it is somehow able to augment the specific antibody general and local response to inhaled antigens, such as ovalbumin, administered after BE pretreatment, thus showing an adjuvant-like activity, but very high dosages of the agent were used.

In brief, BEs seem to have a bacterial lytopolysaccharide-like activity without antigenic and endotoxic properties, maintaining only a stimulatory effect. From a recent report on specific oral vaccination against H influenzae in chronic bronchitis, it is possible to argue that, similar to BE, oral bacteria can activate specific defenses rather than induce a specific response. The results would even be preferable if a significant protection could be extended toward different bacterial species. In this case, duration of the immune state should be investigated accurately. A reduction of the bronchial colonization rate of bacterial species noncross-reactive with H influenzae described in that article can probably be explained by mechanisms such as a stimulation of phagocytosis as we have obtained with BE.

Patients with chronic bronchitis have chronic hypersecretion and expectoration, with a persistent bacterial colonization of the airways and thus an important bacterial contamination of sputum even in periods of clinically stable phase. Since a part of the bronchial secretion is normally swallowed, we must suppose that bacterial remnants resulting from gastric digestion can reach and stimulate GALT in a way similar to oral BEs. It may be argued that patients with chronic bronchitis are already self-immune-stimulated and that the response to oral BE is dependent on a priming effect on MALT by the long-term swallowing of contaminated sputum. At any rate, it is possible to calculate that the global bacterial lipo-glyco-protein burden reaching the gut is on the average not greater than 1/1,000 of the dosage of each capsule (7 mg). Secondly, the clinical value of our findings is confirmed by the double-blind random design. Data in literature confirm the possibility of stimulating human MALT in

Table 5—Blood Granulocyte Activities*

<table>
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<th></th>
<th>O&lt;sub&gt;2&lt;/sub&gt;basal, nM/10&lt;sup&gt;9&lt;/sup&gt;/10&lt;sup&gt;−&lt;/sup&gt;</th>
<th>O&lt;sub&gt;2&lt;/sub&gt;zymosan, nM/10&lt;sup&gt;9&lt;/sup&gt;/10&lt;sup&gt;−&lt;/sup&gt;</th>
<th>Stimulated migration FMLP µm</th>
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<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T90</td>
<td>T0</td>
</tr>
<tr>
<td>Bacterial extract</td>
<td>4.7 ± 1.2</td>
<td>11.1 ± 4.3</td>
<td>40.2 ± 5.0</td>
</tr>
<tr>
<td>Placebo</td>
<td>7.8 ± 1.7</td>
<td>7.5 ± 2.5</td>
<td>37.3 ± 5.3</td>
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</tbody>
</table>

*Blood granulocyte activities (O<sub>2</sub>− release in basal conditions and after opsonized zymosan phagocytosis and stimulated migration after FMLP 10<sup>−9</sup>). Values are given as mean ± SEM.
normal subjects with bacterial agents. It may be interesting to evaluate the possibility of associating BE pretreatment to specific vaccinations in man; it could eventually enhance the protection against those agents such as influenza viruses, Pneumococcus, and Haemophilus for which vaccines are available, but with a variable degree of protection. Alternatively, in case of viral infections that are known to depress macrophage activities, thus favoring bacterial superinfections, BE could help in preventing macrophage phagocytosis defects.

Our study further confirms that in humans it is possible to stimulate the gut-associated lymphoid tissue (GALT) to obtain activation of immune defenses in other organs sharing the same embryonal origin and possessing a mucosal associated lymphoid tissue, such as BALT in airways. A preferential traffic of immune information across mucosal-associated lymphoid tissues seems evident from our data with regard to those activities altered in alveolar cells but unaltered in blood, which reflects systemic immunity, but a number of parameters, such as monocyte activities and cytokine levels, were not considered.

Further studies would be necessary to evaluate the duration of the stimulatory effect on the immune system by BE and the fine mechanisms of activation with particular regard to the exchange of immune information between lymphocytes and phagocytic cells (lymphokines and other mediators). With regard to chemotactic factor release, we did not find a net recruitment of inflammatory cells since BAL cell populations did not differ significantly at T90 vs T0, but direct assessment of chemotaxis was not performed. In the same time it would be extremely important to be precise about the nature and mechanisms of the relative immune defects affecting COPD patients rendering them so prone to respiratory infections but that have not yet been adequately characterized.

In our trial, the small number of patients and the short period of observation did not allow us to correlate our laboratory findings with the occurrence of chronic bronchitis exacerbations or other aspects of clinical relevance. Despite that, we can conclude that the capability of oral BE to increase alveolar macrophage activities, and probably to enhance IgA synthesis, at least in nonsmokers, should foster a less empirical use of these agents in experimental clinical trials for the prevention of chronic bronchitis exacerbations.

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