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Sublingual therapeutic immunization with a polyvalent bacterial preparation in patients with recurrent respiratory infections: immunomodulatory effect on antigen-specific memory CD4$^+$ T cells and impact on clinical outcome

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Summary

Recurrent respiratory tract infections (RRTIs) are common clinical conditions in individuals with alterations of the immune function. A prospective open pilot study in a cohort of patients with RRTIs has been performed to assess whether sublingual immunization with a polyvalent bacterial vaccine could exert an immunomodulatory effect on the antigen-specific immunological responses and have an impact on the clinical outcome. Seventeen patients with RRTIs were recruited. An oral polyvalent bacterial preparation (Bactek®) was administered to all patients daily for 6 months. Immunological assessment was performed at baseline and at the end of immunization. Immunological measurements included: T cell-specific proliferations of CD3$^+$CD4$^+$ and CD3$^+$CD8$^+$ to Bactek® antigens, total immunoglobulin levels, antibodies to pneumococcal polysaccharide and tetanus toxoid and B, T and natural killer (NK) cell subsets. There was a significant increase in the proliferative capacity of CD3$^+$CD4$^+$ T cells specific to Bactek® antigens at month 6 in comparison to baseline ($P < 0{\cdot}0001$). A significant increase in total CD3$^+$ T cells was also observed ($P < 0{\cdot}05$). No significant differences were observed between baseline and month 6 in levels of total immunoglobulins, specific antibodies and B, T or NK cell subsets. A significant reduction in the patient's rate of RRTIs was observed compared with 1 year prior to initiation of therapy ($P < 0{\cdot}0001$). The results demonstrate that long-term administration of a sublingual polyvalent bacterial preparation in patients with RRTIs exerts an immune stimulating effect on CD4$^+$ T helper cell responses to bacterial antigens which could be associated with clinical benefit.

Keywords: bacterial preparation, CD4 T cell proliferation, immunomodulation, recurrent respiratory tract infections, sublingual immunization

Introduction

Recurrent respiratory tract infections (RRTIs) are a major health-care problem associated with significant morbidity and mortality. It is also associated with the spread of resistance to antibiotics in patients with deficient humoral and cellular immune functions, such as primary and secondary immunodeficiencies and chronic obstructive pulmonary disease [1,2]. Immunostimulating agents of bacterial origin have been used as an adjunct treatment in these patients because of their immunomodulatory properties, increasing immune responses and boosting the innate immune system [3]. The polyvalent bacterial preparations that have been used to treat RRTIs contain different formulations of bacterial strains, which are found frequently in the upper and lower respiratory tract. These vaccines may contain whole inactivated microorganisms, lysates or defined cellular components [4]. A number of studies have shown that the oral administration of bacterial immune stimulants ameliorates RRTIs in adults and children by reducing the number, duration and severity of infectious clinical episodes [3–10]. However, the use of immunomodulators in susceptible patients with RRTIs is still a matter of debate due to the contradictory results reported in several studies conducted with different bacterial preparations and in different clinical settings [4]. Uncertainty about the immune mechanisms involved in protection and clinical improvement also hampers the wide use of bacterial immune stimulants in daily practice [11].

Recently, the sublingual route of administration of bacterial preparations has been proposed as a safer and effective immunotherapy to stimulate strong and long-lasting systemic and mucosal antigen-specific humoral and cell-mediated immunity. The generation of antigen-specific memory $CD4^+$ T cells by sublingual immunization could up-regulate T helper type 1 (Th1) immune responses enhancing more efficient anti-microbial defences, in comparison with other routes of immunization. Moreover, the induction of immunological memory to the specific pathogen is critical to maintain long-lasting immune surveillance upon vaccination using the sublingual immunization strategy [12–33].

In the present pilot study we have evaluated prospectively whether the daily administration of a polyvalent bacterial preparation via the sublingual route could stimulate a T helper-specific immune response with impact on the clinical outcome of patients with RRTIs.

Materials and methods

Patient population and study design

Seventeen patients, five males and 12 females, mean age 46·6 years (range 21–77), referred to the Clinical Immunology Unit of the Hospital General Universitario Gregorio Marañón of Madrid for immunodeficiency evaluation, were included in this study. All patients had suffered recurrent upper and lower RRTIs which fulfilled the primary immunodeficiency warning signs of the Jeffrey Modell Foundation during the 12 months prior to recruitment into the study [34]. All the subjects recruited into this pilot study were required to meet the following criteria: three or more episodes of rhinitis, pharyngitis and tonsillitis in the previous 12 months (according to medical records from out-patient clinics); two or more new ear and/or sinus infections and/or bronchitis within 1 year in the absence of allergy; one pneumonia episode per year for more than 1 year; persistent chronic obstructive pulmonary disease (COPD) exarcebations by RRTIs; recurrent viral infections (colds and herpes); recurrent need for oral and/or intravenous antibiotic courses and/or hospitalizations to clear infections in the previous 12 months. Among the exclusion criteria were: treatment with immunosuppressants, immunostimulants, gamma globulins within the previous 12 months; patients who had laboratory or clinical criteria for lymphoproliferative disorders or non-respiratory chronic infections; episodic asthma treated with inhaled or systemic corticosteroids; persistent thrush or fungal infection on skin or elsewhere; chronic infection with tuberculosis-like bacteria; and a family history of primary immunodeficiency. All patients who met the inclusion criteria started the immunizations with Bactek® when they had asymptomatic clinical status. Patients were assessed every 3 months and every time they had respiratory tract symptoms. If the patients had bronchitis, pneumonia or exacerbated COPD, the number of days to clinical cure, the number of days that antibiotics or oral steroids were used and the number and duration of hospitalizations were recorded. RRTIs [rhinitis, pharyngitis, tonsillitis, otitis, sinusitis, bronchitis, viral infections (colds and herpes) and pneumonia] were defined by the presence of diagnostic symptoms for at least 48–72 h. Multiple illnesses were counted only if the patient was without symptoms for at least 72 h between the end of one episode and the beginning of another. Clinical details of the RRTIs suffered by each patient and the courses of treatment administered are summarized in Table 1.

The immunodeficiency evaluation of the patients, prior to being enrolled into this study, included the specific antibody response to immunization against pneumococcal polysaccharide and tetanus toxoid antigens. This prospective pilot study was designed to assess whether the daily sublingual administration (throughout a period of 6 months) of a polyvalent bacterial preparation could stimulate a T helper-specific immune response with impact on the clinical outcome of patients with RRTIs. The study was approved by the Ethics and Clinical Research Committee of the Hospital General Universitario Gregorio Marañón and written informed consent was obtained from each patient prior to the initiation of the study. The study was conducted between November 2008 and November 2009, including a 6-month treatment and a 6-month follow-up period.

Bacterial preparation. Bactek® is a commercially available polyvalent bacterial preparation (Inmunotek Laboratories, Madrid, Spain). It contains different species of inactivated bacteria at 10^9 bacteria/ml which are frequently present in the respiratory tract: *Staphylococcus aureus* (15%), *S. epidermidis* (15%), *Streptococcus pneumoniae* (60%), *Klebsiella pneumoniae* (4%), *Branhamella catarrhalis* (3%) and *Haemophilus influenzae* (3%). It was administered through the sublingual route to all 17 patients using the following schedule: days 1 and 2, one and two sublingual drops, respectively, and thereafter, up to day 180, three sublingual drops daily taken in the early morning hours.

Clinical assessment

The clinical follow-up period for each patient started at initiation of immunization at day 0. All patients were monitored closely for their safety and clinical evaluation at each study visit (day 0, month 3, month 6, and thereafter every 3 months until 1 year). Clinical status and total number of infectious respiratory episodes that had occurred in the previous year to immunization were recorded for each patient by their attending physician. The number of infectious respiratory episodes prior and after Bactek® treatment was considered the main variable for the clinical outcome (Table 1). Patients were treated with antibiotics only for controlling their RRTI at physician criteria.

Table 1. Diagnosis, clinical manifestations and number of episodes of upper and lower respiratory tract infections in our cohort of patients with recurrent respiratory tract infections (RRTIs) before and after Bactek® treatment (BT).

Patients	Diagnosis	Clinical manifestations	Episodes before Bactek® (pre-BT)	Episodes after Bactek® (post-BT)
1	RRTIs	Pneumonia	3	0
2	RRTIs Mild hypogammaglobulinaemia	Pharyngitis, tonsillitis and bronchitis	2	0
3	RRTIs	Severe pharyngitis and tonsillitis	8	0
4	RRTIs	Pneumonia	3	0
5	RRTIs	Bronchitis	5	0
6	RRTIs IgA immunodeficiency	Pharyngitis	8	1
7	RRTIs	Otitis and sinusitis	5	0
8	RRTIs IgG4 immunodeficiency	Pneumonia	2	0
9	RRTIs	Severe pharyngitis and tonsillitis	5	1
10	COPD Mild hypogammaglobulinaemia	Pharyngitis and exacerbations of COPD Pneumonia	8 2	1 0
11	RRTIs Mild hypogammaglobulinaemia	Pharyngitis	4	0
12	RRTIs Bronchiectasis	Pharyngitis and tonsillitis Pneumonia	5 2	0 0
13	RRTIs	Sinusitis, otitis and bronchitis	5	0
14	RRTIs Autoimmune thyroiditis	Pharyngitis and tonsillitis	6	0
15	RRTIs IgG4 immunodeficiency	Tonsillitis, pharyngitis and otitis	5	0
16	RRTIs Mild hypogammaglobulinaemia	Bronchitis	5	0
17	RRTIs	Pharyngitis and tonsillitis Labial and nasal HSV infection	10 12	0 3

Pre-BT: number of episodes of upper and lower respiratory tract infections scored 1 year prior to immunization with Bactek®; post-BT: number of episodes of upper and lower respiratory tract infections scored throughout 12 months after initiation of therapy with Bactek®. RRTIs, recurrent respiratory tract infections; HSV, herpes simplex virus; COPD, chronic obstructive pulmonary disease; HSV, herpes simplex virus; Ig, immunoglobulin.

Immunological assessment

Measurement of immunoglobulins and antibodies. Patients' serum samples were assessed at baseline and 6 months after initiation of Bactek® treatment. Serum levels of immunoglobulin (Ig)G, IgA and IgM, as well as IgG subclasses and salivary IgA, were determined by nephelometry (Beckmann Coulter, Miami, FL, USA). Anti-pneumococcal polysaccharide and anti-tetanus antibody (IgG) levels were quantified by enzyme-linked immunosorbent assays (ELISA; The Binding Site, Birminghan, UK) at baseline and 6 months after initiation of Bactek® treatment.

Measurement of B, T and natural killer (NK) cell subsets. Peripheral blood mononuclear cell (PBMC) samples were obtained before the initiation of treatment (baseline) and after 6 months of therapy with Bactek®. T lymphocytes ($CD3^+/CD4^+$ and $CD3^+/CD8^+$), B lymphocytes ($CD19^+$) and NK cells ($CD3^-CD56^+$) were measured as described elsewhere [35,36] using monoclonal antibodies (Tritest™) and Trucount™ tubes (BD Biosciences, Madrid, Spain) and multiparametric flow cytometry [fluorescence activated cell sorter (FACS)Calibur; BD Biosciences, Spain].

Antigen-specific $CD3^+/CD4^+/CD8^+$ T cell proliferation by CFSE loading assay. $CD3^+$, $CD4^+$ and $CD8^+$ $CFSE^-$ T cell proliferation specific to bacterial antigens was measured *in vitro* by carboxyfluorescein succinimidyl ester (CFSE) assay [37,38] at baseline and after 6 months of treatment. The bacterial antigens used in culture were the same inactivated bacteria of Bactek®, prepared as a sterile saline suspension without excipients (Inmunotek SL, Madrid, Spain). Phytohaemagglutinin (PHA) mitogen was used as positive control. Influenza antigen (Sanofi Pasteur-MSD, Madrid, Spain) was used as an unrelated stimulus. The PBMCs from each patient were isolated by Ficoll and loaded with CFSE (Molecular Probes, Eugene, OR, USA) as described elsewhere [35,36]. Briefly, CSFE-labelled cells were cultured in complete RPMI-1640

medium (Biochrom AG, Berlin, Germany) at 10^6 cells/ml without any antigenic stimuli (negative control), or with the following stimuli: Bactek® (1:10 dilution), PHA (2 μg/ml) and influenza antigen (1:10 dilution). After 6 days of incubation at 37°C and 5% CO_2, cells were collected and stained with anti-CD3 [allophycocyanin (APC)], anti-CD8 [peridinin chlorophyll (PerCP)] and anti-CD4 [phycoerythrin (PE)] (BD Biosciences). A total of 100 000 events were analysed by flow cytometry in the lymphocyte gate. Percentages of $CD3^+$, $CD4^+$ and $CD8^+$ $CFSE^{low}$ were evaluated simultaneously, gating on $CD3^+$ T cells and measuring sequentially proliferating $CD8^+$ and $CD4^+$ subsets within the $CD3^+$ cells. Net percentages of $CFSE^{low}$ were calculated subtracting the negative control values. Lymphoproliferative rates scoring greater than 0·2% of $CD3^+$ $CFSE^{low}$ cells were deemed positive. Cell cultures with healthy donors ($n = 10$) were conducted simultaneously as controls.

Statistics

The Excel spreadsheet (Microsoft, Inc., Redmond, WA, USA) and the statistical software SPSS version 11·0 (SPSS, Inc., Chicago, IL, USA) were used. The results were analysed for normality (Shapiro–Wilk), showing that all the outcomes did not follow a normal distribution. Descriptive statistics for all *in vitro* outcomes were expressed as the median with the first and third interquartile ranges (IQR). The Wilcoxon test was used for comparative statistics. The Hodges–Lehmann estimator (with 95% lower and upper confidence limits) was used to measure the effect size of the differences between the determinations at the two time-points.

Results

Patient features

The immunological and clinical baseline characteristics of the patients are shown in Tables 1 and 2. Seven of 17 (41%) patients with RRTIs showed alterations of the humoral immune response (one selective IgA deficiency, two selective IgG4 subclass deficiencies and four mild IgG hypogammaglobulinaemia). However, all patients showed a conserved antibody response to polysaccharide and protein antigens and normal levels of B, T and NK cells (Table 2). Bactek® that was administered daily through the sublingual route during 6 months was well tolerated without adverse reactions.

Immunological response

The levels of immunoglobulins (IgG, IgA and IgM) in serum, salivary IgA or titres of IgG antibodies against pneumococcal polysaccharide and tetanus toxoid antigens were not modified after 6 months of treatment with Bactek®

Table 2a. Descriptive statistics (median and 1st and 3rd quartiles) of plasma and salivary levels of humoral parameters before and after treatment.

	Baseline		6 months			
Serum (mg/dl)	Median	IQ range	Median	IQ range	H–L	*P* (Wilcoxon)
IgG	995·00	728·00–1275·00	966·00	637·00–1240·00	58 (−229, 342)	0·307
IgA	219·00	94·23–328·50	184·00	82·40–344·00	28 (−81, 156)	0·221
IgM	118·50	93·50–178·50	122·00	94·10–160·00	0·20 (−42, 47)	0·733
IgG1	772·00	585·00–862·00	640·00	421·00–766·00	97 (−78, 344)	0·184
IgG2	199·00	173·00–412·00	205·50	176·00–299·00	22 (−68, 132)	0·917
IgG3	43·23	33·70–53·50	37·05	24·90–54·13	2·40 (−11, 20)	0·675
IgG4	14·10	8·41–23·60	12·23	7·06–24·85	−0·47 (−14, 12)	0·861
Anti-PPC antibody	24·90	8·20–23·78	26·40	8·60–27·00	−1 (−14, 2)	0·530
Anti-TT antibody	4·10	0·33–7·00	5·10	0·90–7·00	0 (−2, 1)	0·878
Salivary IgA	13·25	7·83–20·03	10·40	5·88–18·35	2 (−8, 11)	0·866

Anti-PPC: levels of anti-pneumococcal polysaccharide antibodies [immunoglobulin (Ig)G]; anti-T: levels of anti-tetanus antibodies (IgG). Comparative statistics [Hodges–Lehmann (H–L) and Wilcoxon tests] were used to compare all these pairs of variables. IQ, interquartile.

Table 2b. Descriptive statistics (median and 1st and 3rd quartiles) of absolute numbers of lymphocytes subsets in peripheral blood before and after the treatment. Comparative statistics [Hodges–Lehmann (H–L) and Wilcoxon tests] were used to compare all these pairs of variables.

	Baseline		6 months			
Cells/μl	Median	IQ range	Median	IQ range	H–L	*P* (Wilcoxon)
CD3	1422·00	1226·00–1560·00	1494·00	1242·75–1877·50	−145 (−502, 13)	0·049
CD4	900·00	783·00–1057·00	1052·00	783·00–1179·50	−142 (−339, 85)	0·079
CD8	483·00	360·00–592·00	539·00	428·50–729·00	−83 (−232, 80)	0·148
CD19	198·00	162·00–269·00	194·50	177·25–262·00	−15 (−76, 47)	0·173
NK	198·00	160·00–299·50	245·00	185·25–303·50	−35 (−122, 63)	0·289

IQ, interquartile; NK, natural killer.

Table 3. Descriptive statistics (median and 1st and 3rd quartiles) of proliferating T cells specific to Bactek® antigens (T $CD3^+$ 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE), T $CD4^+$ CFSE and T $CD8^+$ CFSE) before (baseline) and after 6 months of treatment with Bactek®. Comparative statistics [Hodges–Lehmann (H–L) and Wilcoxon tests] were used to compare all these pairs of variables.

	Baseline		6 months			
	Median	IQ range	Median	IQ range	H–L	*P* (Wilcoxon)
$\%CFSE^{low}$ $CD3^+$						
Control	1·83	0·67–4·32	1·98	1·23–2·98	−0·22 (−1·2, 1·64)	0·831
PHA	82·80	67·52–91·09	73·72	58·96–80·98	9·87 (−1·00, 21·60)	0·113
Bactek®	0·98	0·69–1·34	8·00	6·22–11·30	**−7·02 (−9·30, −5·50)**	**<0·001**
$\%CFSE^{low}$ $CD3^+CD4^+$						
Control	1·04	0·40–2·92	1·24	1·05–2·34	−0·22 (−0·90, 0·86)	0·981
PHA	47·72	40·76–58·90	42·14	33·92–54·20	4·52 (−6·80, 15·00)	0·332
Bactek®	0·76	0·40–1·20	7·59	4·87–8·48	**−6·72 (−7·70, −4·20)**	**<0·001**
$\%CFSE^{low}$ $CD3^+CD8^+$						
Control	0·31	0·10–1·44	0·52	0·32–1·22	−0·12 (−0·60, 0·43)	0·981
PHA	32·80	21·43–49·56	21·39	18·99–32·10	8·07 (−3·40, 19·20)	0·062
Bactek®	0·12	0·00–0·47	0·91	0·00–1·12	−0·37 (−1·00, 0·05)	0·100

IQ, interquartile; PHA, phytohaemagglutinin.

(Table 2A). No significant differences were detected among the absolute numbers of $CD4^+$, $CD8^+$, B and NK cells in blood at baseline or after treatment (Table 2b).

Table 3 shows the *in vitro* proliferative response of blood lymphocytes from the patients under study. After 6 months of therapeutic immunization with Bactek® all patients showed a significant increase in the percentage of proliferating $CD3^+$ CFSE cells in response to Bactek® in comparison to baseline ($P < 0{\cdot}001$). This was accompanied by a significant increase in the percentage of proliferating $CD4^+$ CFSE cells specific to Bactek® ($P < 0{\cdot}001$) but not $CD8^+$ CFSE cells ($P = 0{\cdot}1$). The lymphoproliferative response to PHA was similar before and after treatment with Bactek® (Table 3). Figure 1 is a representative example showing the percentage of proliferating $CD3^+$ CFSE and $CD4^+$ CFSE from one patient at baseline and after 6 months of treatment with Bactek®.

Interestingly, when the same cell cultures were stimulated with influenza antigen, a robust enhancement in the percentage of proliferating $CD3^+CD4^+$ cells was also observed after treatment with Bactek® (baseline = 0·6; month 6 = 4·70; $P < 0{\cdot}001$) as well as the proliferating $CD3^+CD8^+$ cells (baseline = 0·02; month 6 = 0·67; $P < 0{\cdot}005$).

Clinical response

Clinical assessment throughout the study showed a significant reduction of the total number of upper and lower respiratory tract infections episodes after treatment with Bactek® compared with the number of RRTIs scored throughout 12 months prior to treatment ($P = 0{\cdot}0001$) (Table 1). In addition to the lower frequency of RRTIs, clinical improvement was also observed regarding the severity and duration of the infectious respiratory event (e.g. patient 10 showed an exacerbation of COPD which was resolved in 10 days with ambulatory antibiotic therapy).

Discussion

In this pilot study, we have observed a remarkable reduction in the frequency of upper and lower respiratory tract infectious episodes in a cohort of patients with RTTIs treated for 6 months with Bactek®, a polyvalent bacterial preparation. This was observed over a 12-month period after initiation of therapeutic immunization, in comparison to the number of RRTIs prior to treatment. These results are in agreement with other studies showing an association between immunization with polyvalent bacterial preparations and clinical improvement of infectious respiratory diseases [3,5,9,12,14,15,21,23–31,33,39–41]. Our cohort of patients showed a higher frequency of RRTIs, 1 year prior to initiation of treatment with Bactek®, in comparison with other cohorts. The good clinical response obtained, in comparison to other studies, is in agreement with the data reported in a placebo-controlled study in children, showing that the treatment with bacterial preparations is more effective in selected patients reporting a larger number of RRTIs in the year prior to therapy [10].

We have identified one of the major immunomodulatory processes triggered by the polivalent whole bacterial preparation; that is, a significant increase in the proliferating capacity of the antigen-specific memory $CD3^+CD4^+$ T cells. However, this is not the only immunological setting which may be affected following sublingual immunization with Bactek®. Additionally, it is possible that an innate immune response triggered by the immunostimulatory properties of the bacterial-derived product antigens might also have contributed to the strong antigen-specific cell-mediated immunity. Taking into account the expression of receptors for bacterial moieties on cells of the innate immune system, it is highly likely that components of the innate immune system are also involved in the beneficial effects observed

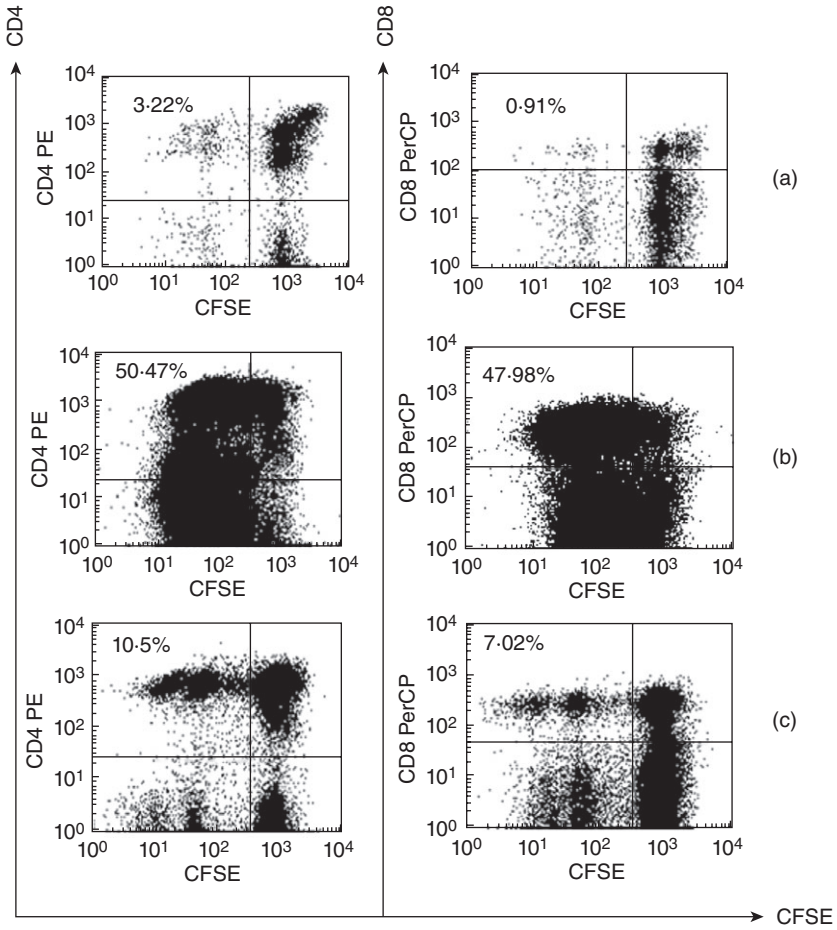

Fig. 1. Representative sample shown here demonstrates percentages of proliferating $CD4^+$ and $CD8^+$ T cell subsets from one Bactek®-immunized patient. Peripheral blood mononuclear cells (PBMC) were labelled with 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) and then stimulated with culture medium alone (negative control) (a), phytohaemagglutinin (PHA) (positive control) (b) and Bactek® antigens (c). Events were evaluated in a lymphocyte and then in a $CD3^+$ T cell gate.

after immunization with Bactek® in the clinical outcome of patients with RRTIs. However, those non-specific immune mechanisms need to be elucidated further, mainly in the context of the induction of a specific immune response which might also have an important role in enhancing immune effector mechanisms of the innate system [4,12,20,21,25,30–32].

It has been suggested that bacterial-derived products may act as non-specific immune stimulants enhancing the innate immunity. Immune stimulation might be ascribed to immune cell activation by means of Toll-like receptors recognizing molecular patterns of bacterial origin, stimulation of phagocytosis by whole inactivated bacteria and many others that may have a reflection in the further specific immune response [4,42,43]. A number of clinical studies have shown that the oral administration of a polyvalent bacterial preparation in patients with COPD is capable of improving impaired immune functions, such as alveolar macrophage activity and interferon-gamma production [7,25,44–46]. However, there are still contradictory results in relation to the effectiveness of immune stimulants of bacterial origin for the reduction of respiratory infections in a susceptible patient population [7,25,44,46]. It should be noted that commercially available bacterial preparations may be widely different. Bactek® preparation differs from others in its formulation, concentration and/or route of administration (sublingual), and delivers whole inactivated bacteria instead of the most common bacterial lysates.

In our study we have also observed an augmentation of the proliferating capacity of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells specific to influenza antigens after 6 months of treatment with Bactek®. The fact that our patients had been vaccinated with influenza, or underwent influenza infection, at least within the 2 years prior to treatment with Bactek®, presents the possibility that the bacterial preparation may have induced an immune stimulating effect which, in turn, might have enhanced the ongoing cellular immune responses to viral antigens [47–51]. This could be related to the existence of a dependence on $CD4^+$ helper activity for the expansion of viral antigen-specific $CD4^+$ and $CD8^+$ T cells [52] and with the induction of $CD4^+$ helper T cells associated with effective immune responses in long-term therapeutic immunizations [4,7,11,42,43,53,54].

This study shows the potential clinical benefit of a bacterial preparation administered by the sublingual route in a reduced number of patients. However, further prospective double-blind, placebo-controlled, randomized clinical trials, with a larger number of patients, are warranted to determine

more clearly the clinical impact of bacterial immune stimulants in patients with RRTIs. Considering the high prevalence and management high cumulative cost of respiratory infections, as well as the frequent failure of conventional therapies, bacterial immunostimulation could be an effective management strategy to reduce costs and the frequency, severity and duration of such episodes in adults and children suffering of chronic respiratory tract infections.

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Disclosure

The authors have no conflicts of interest regarding this article.

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