ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2003) 21: 223-230

An Open-Label, Prospective Study of an Oral Polyvalent Bacterial Lysate (Luivac®) in the Treatment of Recurrent Respiratory Tract Infections in Thai Patients

Perapun Jareoncharsri, Chaweewan Bunnag, Prayuth Tunsuriyawong, Paraya Assanasane, Siriporn Voraprayoon and Bangon Pinkaew

SUMMARY **An open-label, non-comparative study was performed in the Department of Otolaryngology, Siriraj Hospital, Bangkok, Thailand, to assess the safety, tolerability, acceptability and efficacy of an oral polyvalent bacterial lysate (Luivac®) in the treatment of recurrent respiratory tract infections (RTIs) in Thai patients. Thirty-three patients were included in this study, 18 males and 15 females, with a mean age of 34.0 ± 14.7 years. The mean number of RTIs during the 12-month period preceding the study was 9.5 per patient. During the study each patient received one tablet of Luivac® daily for 28 days followed by a treatment-free period of 28 days. This was followed with another 28 days on Luivac®, after which there was a 28-day treatment-free follow-up period. This study lasted 4 months with five scheduled patient visits (V1-V5). Laboratory studies were done at baseline (V1) and after treatment (V4), which included complete blood count and serum immunoglobulins (IgA, IgE, IgG and IgM). The incidence of all adverse events was 15.2% and no case was related to the studied drug. There were no clinical relevant changes in laboratory parameters after treatment. The reduction rate of RTIs per month at the end of the study period was 63.5% when compared to the average RTIs rate per month during the 12 months preceding the study. A comparison of the first study period (V1-V3) and the second study period (V3-V5) showed a reduction in duration of RTIs (23.1%), in the clinical infection score (17.5%), in the number of antibiotics used (2.1%), in the number of symptomatic treatments (3.5%), and in the number of days absent from school or work (50.0%). Overall tolerability and acceptability were assessed as very good and good in 96.8% of the patients. This study suggests that oral polyvalent bacterial lysate (Luivac®) was safe and also showed a tendency to be effective in preventing RTIs in Thai patients with or without risk factors for recurrent RTIs. Other clinical advantages were reduction in the severity and duration of infection as well as in reduction of the cost of treatment and the number of days absent from school or work.**

Respiratory tract infections (RTIs) are the most common diseases worldwide. Both upper and lower RTIs can cause considerable morbidity, absence from school and work and create a heavy socioeconomic burden. In the United States, over $2 billion are spent annually on the purchase of OTC (over the counter) medications for RTIs, and the economic burden for the outpatient management of acute RTIs is over $10 billion per year.[1]

Viral upper respiratory tract infection (viral URI) or the common cold is a very frequent disease occurring in both children and adults around the world. The viruses responsible for the common cold are rhinoviruses (50-60%), corona viruses (10-20%), followed by influenza viruses, respiratory syncytial viruses, adenoviruses, and parainfluenza viruses.[2] Rhinoviruses do not destroy airway epithelial cells, but cause only functional problems such as ciliostasis. However, influenza and parainfluenza viruses can cause destruction of the airway epithelium of both the upper and lower tracts, possibly promoting secondary bacterial infections.[2,3]

Complications of viral URI occur often, especially in children.

From the Rhinology & Allergy Division, Department of Otolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.
Correspondence: Perapun Jareoncharsri

These complications, which are usually associated with bacterial infections, include acute otitis media, acute rhinosinusitis, pharyngitis, tonsillitis, bronchitis and pneumonia.

Viral infections can precipitate and aggravate the symptoms of asthma in both children and adults.[4,5] Bacterial infections of the respiratory tract may play a role in the development of bronchial hyperresponsivenes, bronchospasm, and worsening of chronic obstructive airway disease.[6] Viral infections can cause alteration in mucosal immunity and in the host response to bacterial colonization,[7,8] which may predispose the individual to recurrent RTIs. Individuals with recurrent RTIs during childhood and adolescence-adulthood have a high prevalence of respiratory symptoms, respiratory diseases and low lung function values.[9]

Patients with increased susceptibility to recurrent RTIs may have an impaired mucosal immune response. The first line defense in the airway epithelium is formed by the cilia of the intact airway epithelium and by secretory IgA and IgG leaking from the serum.[3] Having impaired or slightly defective mucosal immune defense mechanisms may be a predisposing factor for recurrent RTIs, and such individuals may be more susceptible to infections, even with weakly virulent bacteria or opportunistic organisms.

The treatment of RTIs consists of symptomatic-supportive medications and antimicrobial therapy, but this cannot prevent the recurrence of the diseases. Vaccines are not available for most etiologic RTI pathogens. The polyvalent bacterial lysate LW50020 (Luivac®) is an oral immunomodulator or immunostimulator, which exerts its pharmacodynamic effects by stimulating the mucosal immune system. The concept of a common mucosal immune system is based on the fact that the mucosa-associated lymphoid tissue represents the largest area of interaction for environmental organisms with the immune systems and the lymphocyte redistribution between distinct mucosal lymphoid tissues. Presentation of an antigen at one mucosal site may lead to stimulation of an immune response at a distant mucosal site, and subsequently to an increase in the number of IgA secreting cells.[10-12]

Animal studies have shown that oral administration of polyvalent bacterial lysate leads to an increase in non-specific as well as specific immunity in all mucosal tissues as well as to proliferation and differentiation of the immune cells.[13-17] The expected clinical results of the stimulation of the gut-associated lymphoid tissue with these obligate facultative bacteria are an enhanced inhibition of bacterial adherence, inactivation of pathogens in the respiratory mucosa, and a decrease in susceptibility to recurrent RTIs. Prospective, double-blind, placebo-controlled studies have shown that the administration of oral polyvalent bacterial lysate (Luivac® or Paspat oral®) results in a reduction of the number, duration and severity of RTIs in children and adults.[18-20] These studies were done mainly in Europe, South America and Central America, in which the climates and the seasonal variation of RTIs in the population are different from Thailand. Therefore, the objective of this study was to assess the safety, tolerability, acceptability and efficacy of the oral bacterial lysate immunomodulator (Luivac®) in Thai patients with recurrent RTIs.

PATIENTS AND METHODS

This was an open-label, non-comparative controlled study, conducted at the Department of Otolaryngology, Siriraj Hospital, Bangkok, Thailand, from January-December 1997. The study was approved by the Ethical Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Siriraj Hospital, Mahidol University.

Children and adults of both sexes were included if they were older than 3 years of age and had a history of recurrent respiratory tract infections (RTIs) (i.e. rhinitis, sinusitis, otitis media, pharyngitis, laryngitis, bronchitis, or a combination of these infections), which required medical treatment. The required number of infections during the 12 month period preceding the study to be included was as follows: 8 infections for 3-to-6-year-old children, 6 for 7-to-11-year-old children, and 4 for 12-to-18-year-old adolescents and adults. In addition, children or adults who had at least three severe infections (i.e. duration of two weeks or more) during the preceding 12 months were also included.

Patients with chronic RTIs, i.e. chronic sinusitis, chronic otitis media, chronic bronchitis; severe immunodeficiency, or other serious diseases were excluded. Pregnant women were also excluded.

Study medication

The study medication was

Luivac® (Sankyo Pharma GmbH, Munich, Germany). Each tablet contained 3 mg of a bacterial lysate mixture from seven different American Tissue Culture Collection (ATCC) strains (*Staphylococcus aureus, Streptococcus mitis, Streptococcus pyogenes, Streptococcus pneumoniae, Klebsiella pneumoniae, Branhamella catarrhalis,* and *Haemophilus influenzae*; which contained at least 10^9 microorganisms of each strain). Excipients were mannitol, microcrystalline cellulose, sodium starch glycollate, magnesium stearate, and colloidal anhydrous silica.

At inclusion each patient received one tablet of Luivac® daily for 28 days. Then a treatment-free period of 28 days was followed by another 28-day Luivac® course, after which there was again a 28-day follow-up period. The study lasted 4 months with five scheduled visits as follows: at inclusion (visit 1 = V1), at the end of the 4^{th}, 8^{th}, 12^{th} weeks (visits 2, 3 and 4 = V2, V3 and V4, respectively), and at the end of the study (visit 5 = V5). The first study period included V1-V3 (first treatment course and treatment-free interval); the second study period included V3-V5 (second treatment course and treatment-free follow-up).

Study endpoints

The primary endpoint was safety, which encompassed the number of adverse events (AEs)/adverse drug reactions (ADRs), the effect of Luivac® on laboratory variables and the overall tolerability.

Secondary endpoints were acceptability and efficacy of therapy. The following variables were assessed for efficacy: number, duration, severity (three-point scale; mild = 1, moderate = 2, severe = 3) and clinical infection score (product of duration and severity of RTIs), treatment of infections (with antibiotics or symptomatic agent), and number of days absent from school or the inability to work. The clinical infection score was calculated by multiplying the duration by the severity to derive a total for all infections in the respective period.

Comparison of these parameters between the first period of study (V1-V3) and the second period of study (V3-5) was performed by calculating the percentage of change. A comparison was done between the number (rate) of RTIs per month experienced during the study period (V1-V5) and the preceding 12 months.

Data collection

Demographic data, number of infections during the past 12 months, and risk factors for RTIs (predisposition to eczema/neurodermatitis, known allergies, hay fever/chronic rhinitis, conjunctivitis, predisposition to bronchitis/recurrent cough, hyperactive bronchial system/asthma and positive skin tests for allergy) were documented at V1.

Laboratory studies were done at V1 (baseline values) and at V4 (post-treatment values), which included hemoglobin, hematocrit, white blood cell counts, differential counts, platelets, and serum immunoglobulins (IgA, IgE, IgG and IgM).

Patients who developed acute RTIs during the study were requested to contact their physician to document the severity, duration, and treatment of the infection. After the end of each infection, a final diagnosis was rendered as regards to location, i.e. upper or lower respiratory tract or ear. Any other symptoms or conditions occurring during the study or complaints related to the patients' general health status and well-being were documented as AEs. The diagnosis and details relating to severity, treatment, outcome, and possible causal relationship to the study medication were noted. An AE assessed as possibly or probably related to the study medication was classified as an ADR. There were no specific criteria for early termination of the study. In general, patients were free to decide whether they were willing to continue the study or not, and if severe AEs occurred, the investigators were responsible for justifying whether these AEs were related to the studied drug and the patients should be withdrawn or not.

Compliance was checked by counting the Luivac® tablets remaining at the end of the treatment cycles (V2 and V4). Overall tolerability and acceptability was assessed by the investigators at the patient's last visit (V5).

RESULTS

The study started in March 1997, which is a summer month in Thailand and not the peak incidence of RTIs. Patients were included continuously, and the last patient was included in August 1997, which was in the rainy season and with the peak incidence of RTIs. The study ended in December of the same year, which was the cool month and another season of

peak incidence of RTIs. The number of patients included each month was as follows: 5 patients in March, 6 patients each in April, May, June and July and 4 patients in August.

A total of 33 patients were included, with a mean age of 34.0 ± 14.7 years old (range 4-65 years). There were 18 males and 15 females. Thirty patients (90.9%) were adolescents and adults. Six patients (18.2%) were attending a kindergarten or other school. Ten patients (30.3%) had a history of exposure to passive smoke and 4 patients (12.1%) were active smokers. The details of the demographic and baseline characteristics are shown in Table 1. One patient did not come back for follow - up after the V2 period for an unknown reason. Another patient had acute rhinopharyngolaryngitis during the 9th week and discontinued the medication prematurely. The number of patients at the end of the study was, therefore, 31.

History of allergies and risk factors

Positive skin tests for allergies were found in 79.3% of the patients. About one third of the patients had a history of bronchitis and recurrent cough. Other risk factors were hay fever/chronic rhinitis/conjunctivitis (78.8%), known allergies (65.6%), liability to eczema/neurodermatitis (9.1%), and a hyperactive bronchial system/asthma (6.3%).

History of recurrent RTIs

During the 12 months preceding entry into the study, the patients experienced a mean of 9.5 infections of the upper, lower respiratory tract and ear (range 4-24 infections), and an average of 3.1 severe infections (range 0-12 infections). The number of infections decreased with age (Table 2).

Primary endpoint: safety

AEs and ADRs

AE occurrence with or without

Table 1 Demographic and baseline characteristics of the patients

	N (%)
Sex	
Male	18 (54.5)
Female	15 (45.5)
Total no. of patients	33 (100)
Age (years)	
Mean ± SD	34.0 ± 14.7
Median	37.0
Range	4 - 65
Age group (years)	
3-6	2 (6.1)
7-11	1 (3.0)
12-18	3 (9.1)
> 18	27 (81.8)
Height (cm)	
Mean ± SD	154.3 ± 14.9
Range	101 - 173
Weight (kg)	
Mean ± SD	52.7 ± 13.4
Range	16 - 75
Attending kindergarten/school	6 (18.2)
Working person	27 (81.8)
Passive smokers	10 (30.3)
Active smokers	4 (12.1)

Table 2 Mean number of infections by age during the past 12 months

AGE (years)	Mean no. of infection
Children (3 - 6)	15.0
Children (7 - 11)	7.0
Adolescent (12 - 18)	8.3
Adults (> 18)	9.3
All patients	9.5

suspected causal relationship to Luivac® in this study population was 15.2% (5 in 33). Upon analysis none of the AEs were found to be related to the Luivac® treatment. One patient had nose bleed at V3, which was caused by a nasal steroid spray. One patient was bitten by a dog. Two other patients experienced nausea, vomiting, abdominal pain and diarrhea during V3 and V4 which was diagnosed as viral gastroenteritis associated with upper respiratory tract infection. Another patient had a high fever, headache, sore throat and hoarseness at the beginning of the 8th week and the Luivac® was discontinued prematurely. This patient was diagnosed as having acute rhinopharyngolaryngitis, which was considered as an infection unrelated to the study medication. All these AEs were resolved after treatment, and did not recur.

Laboratory evaluations

There were no relevant clinical shifts of any parameters investigated. Mild changes in some parameters, i.e. neutrophil count, did occur, but while statistically significant, they were within normal limits (Table 3).

Mean IgE values were slightly decreased, but without a statistically significant change. There was no change in mean IgM values. Mean IgA and IgG values were statistically significantly decreased, but were still within normal range (Table 3).

Overall evaluation of tolerability

Overall tolerability was assessed for patients who completed the study at their final visit (V5). A total of 31 patients were evaluated. Tolerability was rated as very good (77.4%) and good (19.4%) in 96.8%, and as moderate in 3.2% of patients.

Secondary endpoints

Acceptance of therapy

The acceptability was very good

Table 3 Laboratory study comparison between baseline value (V1) and post-treatment value (V4)

Blood parameters (normal value)	V1		V4		95% CI		*p* value
	Mean	SD	Mean	SD	Lower	Upper	
Hemoglobin (12.0-18.0 g/dl)	13.90	1.19	13.87	1.34	-0.2582	0.2995	0.88
Hematocrit (37-52%)	42.79	3.66	40.16	7.39	-0.3387	5.5870	0.08
White blood cells (4,000-11,000 x 10^6/mm^3)	6,859.3	1,568.50	6,563.4	1,530.11	-462.69	1,054.4	0.19
Plateletes (150-440 x 10^3/mm^3)	316.74	100.05	320.81	96.34	-29.58	21.43	0.75
Neutrophils (40-47%)	48.01	9.74	53.39	7.56	-9.14	-1.61	0.007*
Eosinophils (0-7%)	2.88	2.31	2.67	1.66	-0.76	1.19	0.66
Basophils (0-1.5%)	2.13	5.41	0.78	0.55	-0.76	3.47	0.20
Lymphocytes (19-48%)	39.94	10.62	35.52	9.77	-0.32	9.16	0.07
Monocytes (3.4-9.0%)	4.11	2.47	4.77	2.34	-1.99	0.66	0.31
IgA mg/dl (70-350 mg/dl)	262.96	83.75	234.01	72.69	10.28	47.62	0.006*
IgE IU/ml (25-100 IU/ml)	139.88	181.32	114.30	128.44	-8.49	59.64	0.14
IgG mg/dl (700-1,700 mg/dl)	1,314.2	393.54	1,054.3	468.12	45.82	491.84	0.02*
IgM mg/dl (70-210 mg/dl)	128.57	58.19	127.94	99.41	-38.21	39.47	0.98

*$p \leq 0.05$

and good in 96.8% and poor in 3.2% of the patients. One patient rated the acceptability as poor because he felt that his sore throat did not improve, however he had no RTIs during the study period and his coughing and sneezing symptoms improved.

Efficacy: number, duration and severity of infections

The mean rate of RTIs per month during the study period (V1-V5) was 63.5% lower than during the 12 months preceding the study (Table 4). A comparison between the first study period (V1-V3) and the second study period (V3-V5) showed that the rate of RTIs increased by 8.7%, the duration of RTIs decreased by 23.1%, and the clinical infection score (which partly reflects the severity of infection) decreased by 17.5% (Table 5).

The number of antibiotic and symptomatic treatments decreased by 2.1% and 3.5% respectively, and the number of days with inability to work or to go to school decreased by 50.0% (Table 5).

DISCUSSION

The mean number of RTIs recorded per year in the general population was 4.9 in the first 2 years of life, 2.8 for those 5-19 years old, 2.2 in adolescents - adults, and 1.6 in adults over 40 years old.[21] The patients included in this study had a higher RTI rate in the 12 months preceding the study (mean = 9.5/patient) than was seen in the general population (Table 2). The presence of a high percentage of patients who had allergies, rhinitis and bronchitis indicated that the study sam-

Table 4 Number of RTIs, percentage of RTIs per month of the study population, and the relative change in RTIs before and during the study period

		Start treatment							
	n = 33	n = 33	n = 33	n = 32	n = 31	n = 31	Mean % (V1-V5)	Difference	% Change
	Preceding 12 months	V0	V1	V2	V3	V4	V5		
Total no. of RTIs	312	8	11	7	10	10			
No. of RTIs/ month (%)	26 (78.8)	8 (24.2)	11 (33.3)	7 (21.9)	10 (32.3)	10 (32.3)	28.8	50.0	63.5

Table 5 Relative reduction in efficacy endpoints, comparison of the V1-V3 and V3-V5 periods

	V1 (n = 33)	V 2 (n = 33)	V 3 (n = 32)	V 4 (n = 31)	V 5 (n = 31)	Mean % (V1-V3)	Mean % (V3-V5)	Difference	% Change
No. of RTIs (%)	8/33 (24.2)	11/33 (33.3)	7/32 (21.9)	10/31 (32.3)	10/31 (32.3)	26.5	28.8	+2.3	+8.7
Antibiotics use (%)	8/8 (100.0)	6/11 (54.5)	4/7 (57.1)	8/10 (80.0)	7/10 (70.0)	70.5	69.0	-1.5	-2.1
Symptomatic treatment (%)	8/8 (100.0)	11/11(100.0)	6/7 (85.7)	9/10 (90.0)	10/10(100.0)	95.2	91.9	-3.3	-3.5
Duration of infection (days)	1.88 ± 0.71	3.36 ± 1.32	2.56 ± 1.12	2.06 ± 0.63	1.42 ± 0.45	2.6	2.0	-0.6	-23.1
No. of days with inability to work	0	0.06 ± 0.06	0	0.03 ± 0.03	0	0.02	0.01	-0.01	-50.0
Clinical infection score	3.33 ± 1.25	4.76 ± 1.57	3.94 ± 2.07	3.52 ± 1.13	2.39 ± 0.94	4.0	3.3	-0.7	-17.5

ple represented a population sample who had a high risk of recurrent RTIs.

Adverse events in this study were found in 15.2% of patients, which was slightly higher than in another study (7.2%).[22] None of the adverse events were related to the study medication, so the adverse drug reaction rate was 0% in our study, which was similar to the results of a previous study (0-3.6%).[22] The adverse drug reactions reported in another study included gastrointestinal symptoms, skin reactions, and respiratory symptoms which were mild and resolved spontaneously. The safety results of this study and other studies[18-21] showed that the use of this oral immunomodulator in patients with known allergies, positive skin tests, hay fever, rhinitis, conjunctivitis, bronchitis, asthma and hyperactive bronchial system was safe. The overall tolerability and acceptability observed in this study were similar to that of other studies.[18-21]

The reduction in the monthly RTI rate during the study period (as compared to the 12 months preceding the study) was 63.5%, which was similar to the result of other double-blinded, placebo-controlled studies.[18-20] But when a comparison of RTI rates within the period of study was done (V1-V3 vs. V3-V5), the RTI rate increased slightly during the second period (8.7%) (Table 5). This may be due to several factors, i.e. the number of patients was too small and the study period was not long enough, i.e. it should include an entire 1-year period in order to account for seasonal variations in the incidence of RTIs. In a large open prospective, multinational study of polyvalent bacterial lysate in the treatment of RTIs, which included nearly 5,000 patients from 14 countries and lasted about 2 years, the reduction rate of RTIs between the study periods (V1-V3 vs. V3-V5) was 52%; the duration of the RTIs, the clinical infection score, the use of antibiotic and symptomatic treatments, and the number of days absent from school or work decreased between 50-65%.[22] From this same study, the reduction of at least 1.7 infections and 14.3 days of infection per patient per year was calculated, which in turn caused a saving of 0.9 antibiotic and 1.1 symptomatic treatments per year per patient.[22] In a long-term double-blind study with polyvalent bacterial lysate, the reductions in number and duration of the infections remained stable over 1 year.[19]

In our study, after 4 months treatment the reduction in the number of antibiotics used (2.1%) and in the symptomatic treatments given (3.5%) was not as high as in the large multinational study,[22] but the reduction in the number of days absent from school or work was nearly the same (50.0%), and the duration of RTIs and the clinical infection score decreased by about half (17.5 - 23.1%) (Table 5).

The reduction in the RTI rate, duration of infection, number of days absent from school or work, as well as the decrease in antibiotic and symptomatic treatments have great cost- saving benefits when applied to a large population, and may decrease the problem of overuse of antimicrobial agents and the resulting increased bacterial resistance. However, the duration of this study was only 4 months which was too short too observe the incidence of RTIs of the whole year. Ideally, the study should cover at least 1 year to include all the seasonal variations of RTIs.

In conclusion, this study demonstrated that the administration of two courses of an oral polyvalent bacterial lysate (Luivac®) during a 4-months period in patients with recurrent respiratory tract infections was safe and the overall tolerability and acceptability were good in 96.8% of patients. The study also showed a tendency of the effectiveness in preventing RTIs in patients with or without risk factors for recurrent RTIs. Other clinical advantages were reduction in the severity and duration of infection as well as a reduction of the cost of treatment and the number of days absent from school or work.

ACKNOWLEDGEMENTS

The authors would like to thank Miss Tippawan Inchukul for her assistance in preparing the manuscript. We also thank Sankyo Pharma, GmbH, Munich, Germany for providing the oral polyvalent bacterial lysate (Luivac®) used in this study, and Ms. Michelle Miller for editing the manuscript.

REFERENCES

1. Shapiro ED. Epidemiology of acute respiratory infections. Semin Pediatr Infect Dis 1998; 9: 31-6.
2. van Cauwenberge PV, Ingels K. Effect of viral and bacterial infection on nasal and sinus mucosa. Acta Otolaryngol (Stockh) 1996; 116: 316-21.
3. Barclay WS, Al-Nakib W, Higgin PG, Tyrrell DAJ. The time course of the humoral immune response to rhinovirus infection. Epidem Infect 1989; 103: 659-69.
4. Busse WW. The role of respiratory infections in airway hyperresponsiveness and asthma. Am J Respir Crit Care Med 1994; 150: 77-9.

5. Newman-Taylor A. Environmental determinants of asthma. Lancet 1995; 345: 296-9.
6. Cazzola M, Matera MG, Rossi F. Bronchial hyperresponsiveness and bacterial respiratory infection. Clin Ther 1991; 13: 157-71.
7. Clancy R, Pang G, Dunkley M, Taylor D, Cripps A. Acute or chronic bronchitis: a model of mucosal immunology. Immunol Cell Biol 1995; 73: 414-7.
8. Taylor DC, Clancy RL, Cripps AW, Butt H, Bartlett L, Murree-Allen K. An alteration in the host-parasite relationship in subjects with chronic bronchitis prone to recurrent episodes of acute bronchitis. Immunol Cell Biol 1994; 72: 143-51.
9. Paoletti P, Prediletto R, Carrozzi L, *et al.* Effects of childhood and adolescence-adulthood respiratory infections in a general population. Eur Respir J 1989; 2: 428-36.
10. Duijvestijn A, Hamann A. Mechanisms and regulation of lymphocyte migration. Immunol Today 1989; 10: 23-7.
11. Hapson LA, Brandtzaeg P. The mucosal defence system. In: Stiehem ER, ed., Immunological disorders infants and children, WB Saunders, Philadelphia, 1989; p. 116.
12. McGhee JR, Mestecky J, Dertzbaugh MT, Dldridge JH, Hirasawa M, Kiyono H. The mucosal immune system: from fundamental concepts to vaccine development. Vaccine 1992; 10: 75-9.
13. Helmberg A, Bock G, Wolf H, Wick G. An orally administered bacterial immunomodulator primes rabbit neutrophils for increased oxidative burst in response to opsonized zymosan. Infect Immun 1989; 57: 3576-80.
14. Van Daal GJ, Beusenberg FD, So KL, *et al.* Protection against influenza virus infection in mice by oral immunization with a polyvalent bacterial lysate. Int J Immunopharmacol 1991; 13: 831-40.
15. Van Daal GJ, van't Veen A, So KL, *et al.* Oral immunization with polyvalent bacterial lysate and infection with *Streptococcus pneumoniae*: influence on interferon-gamma and PMN-elastase concentrations in murine bronchoalveolar lavage fluid. Int Arch Allergy Immunol 1992; 97: 173-7.
16. Ruedl CH, Albin B, Bock G, Wick G, Wolf H. Oral administration of a bacterial immunomodulator enhances murine intestinal lamina propria and Peyer's patch lymphocyte traffic to the lung: possible implications for infectious disease prophylaxis and therapy. Int Immunol 1993; 5: 29-36.
17. Ruedl CH, Frühwirth M, Wick G, Wolf H. Immune response in the lungs following oral immunization with bacterial lysates of respiratory pathogens. Clin Diagn Lab Immunol 1994; 1: 150-4.
18. Riedl-Seifert RJ, van Aubel A, Kämmereit A, Elsasser U. Recurrent infections during childhood - are they still a "crux medicorum"? Results of a placebo-controlled double-blind study with an oral bacterial lysate in children prone to infections. J Pharmacol Ther 1993; 3: 108-17.
19. Ruah SB, Abel S, Binder M, van Aubel A, Elsasser U, Kämmereit A. Paspat oral in Portugal-prevention and therapy of respiratory tract infections in infection-prone children. A double-blind, placebo-controlled, multicenter study, Study No. FK-09/92. Research Report. Munich, Germany, Luitpold Pharma, 1996.
20. Rutishauser M, Pitzke P, Grevers G, van Aubel A, Elsasser U, Kämmereit A. Use of a polyvalent bacterial lysate in patients with recurrent respiratory tract infections: results of a prospective, placebo-controlled, randomized, double-blind study. Adv Ther 1998; 15: 330-41.
21. Monto AS, Sullivan KM. Acute respiratory illness in the community. Frequency of illness and the agents involved. Epidemiol Infect 1993; 110: 145-60.
22. Grevers G, Palacios OA, Rodriguez B, Abel S. Treatment of recurrent respiratory tract infections with a polyvalent bacterial lysate: results of an open, prospective, multinational study. Adv Ther 2000; 17: 103-16.