

Effect of Ribosomal Immunotherapy on the Clinical Condition and Plasma Levels of Cytokines IL-4, IL-5, IL-12 and IFN γ and Total IgE in Patients with Seasonal Allergy during the Pollen Season

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Regulation of the activity of T helper-1 cell (TH1) and T helper-2 cell (TH2) lymphocytes is considered to be the most important factor in the origin and development of allergic disease in predisposed individuals. Factors increasing TH1 by mediated immune response (mainly the production of interferon gamma [IFN γ]) are related to suppression of the development of allergic disease.^[1] Bacterial infection, bacterial lysates and bacterial immunomodulation preparations are very effective activators of macrophages for production of interleukin (IL)-12,^[2,3] which is the principal activator of the production of IFN γ from T lymphocytes and natural killer (NK)-cells. It has been known for some time from experimental studies that bacterial immunomodulators can, in addition, directly stimulate NK-cells and T lymphocytes for the production of IFN γ .^[4-6]

The objective of the present study was to validate whether the bacterial immunomodulator Ribomunyl® (Pierre Fabre Médicament, France)¹ is able to influence the levels of these cytokines in allergic patients in the course of their seasonal dis-

turbances, and whether a reduction in these levels can reduce seasonal allergic reactions.

1. Patients and Methods

A group of 13 volunteers, grass-pollen allergy patients (six males and seven females aged 15–46 years, average age 27.2 years) were treated with Ribomunyl® (according to the usual regimen, see below) during the grass-pollen season (April–August).

Ribomunyl® is an immunomodulatory preparation composed of ribosomal fractions isolated from *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae* and *S. pyogenes* (0.75mg) combined with *K. pneumoniae* membrane proteoglycans (1.125mg). Ribomunyl® therapy (usual regimen) consists of one triple-dose tablet once daily in the morning for 4 days per week for 3 weeks, followed by maintenance treatment for 4 days per month for 2–5 months.

Inclusion criteria were: a positive case history (duration >3 years) of seasonal allergic disease with clinical manifestations of rhinoconjunctivitis or

1 The use of trade names is for product identification purposes only and does not imply endorsement.

mild bronchial asthma and with a positive reaction in a skin-prick test (Allyostal, Stalergenes, France) to grass and/or cereal pollens (wheat larger than 5mm) and total immunoglobulin E (IgE) values over 100 IU/mL.

Exclusion criteria were: age <15 years and >50 years; gravidity; other diseases needing any continual therapy; allergic patients with other allergy types (for example allergy to mites, pets, etc.); patients with allergen immunotherapy; allergic patients requiring treatment with systemic corticosteroids, antileukotrienes and continual therapy with antihistamines (cetirizine, loratadine, terfenadine or ketotifen). During the allergic season (if required) patients received occasional temporary symptomatic treatment (antihistamines in total and/or local administration, sodium cromoglicate in the form of ophthalmic drops or nasal spray, or intranasal corticosteroids).

Before study enrolment, each patient was informed regarding the benefits and/or risks of study participation and written informed consent was obtained. The study was conducted in compliance with the Tokyo amendment of the Declaration of Helsinki.

Laboratory examinations consisted of the determination of plasma values of cytokines IL-4, IL-5, IL-12 and IFN γ in peripheral blood by the ELISA method using the commercial sets of Immunotech Comp. Absorbance of samples was measured on a photometer Tecan Spectra at a wavelength of 450nm. Evaluation was carried out using the KIM E program. IgE values were measured turbidimetrically.

Prior to the commencement of immunotherapy and after 10 and 20 weeks, clinical checks and laboratory examinations were performed.

Statistical evaluation was provided by ANOVA analysis, with *p*-values <0.05 showing statistical significance.

2. Results

Tolerability of Ribomunyl® was excellent in all allergic or asthmatic volunteers during the study period and no adverse effects were observed. All

volunteers completed the study without any complications. The main aim of the study was to evaluate the effect of the bacterial immunomodulator Ribomunyl® on the plasma levels of cytokines and total IgE, which are the important regulatory mechanisms that influence the development and allergic inflammation course. The laboratory examination results are presented in table I.

The total IgE level in the course of the pollen season in patients in our study was typically increasing in comparison with the laboratory status prior to the allergic season, and the administration of ribosomal immunotherapy was not influenced.

Ribosomal immunotherapy statistically significantly increased plasma values of IFN γ (*p* < 0.05) and these increased levels still persisted at the examination after the termination of seasonal reactions and decreased IL-4 (statistically insignificant, *p* = 0.4). IFN γ levels were very markedly increased in the group of seven clinically improved patients (markedly better + better; table II) in comparison with the group of six patients without clinical improvement (IFN γ : 0.147 vs 0.089 ng/L after 10-week treatment; table I shows a mean value of 0.109 ng/L for all patients).

The clinical effect of treatment is presented in table II. None of the patients in the study experi-

Table I. Summary of laboratory examination results

	IL-4 (ng/L)	IL-5 (ng/L)	IL-12 (ng/L)	IFN γ (ng/L)	IgE (IU/mL)
Prior to treatment					
Mean	7.79	1.85	13.27	0.084	278
SD	9.60	0.18	13.32	0.003	298
SE	2.66	0.05	3.69	0.0009	82
After 10 weeks					
Mean	6.10	2.72	14.93	0.109 ^a	365 ^b
SD	8.59	3.09	14.11	0.08	318
SE	2.38	0.05	3.91	0.02	88
After 20 weeks					
Mean	4.48	2.17	14.39	0.115 ^a	426 ^b
SD	2.06	1.08	13.22	0.08	376
SE	0.57	0.29	3.67	0.02	104

a Significance at the 0.05 level.

b Significance at the 0.005 level.

IFN γ = interferon-gamma; IgE = immunoglobulin E; IL = interleukin; SD = standard deviation; SE = standard error.

Table II. Clinical effect of ribosomal immunotherapy in patients with seasonal allergy during the pollen season

Comparison with previous season	Markedly better	Better	No improvement
Clinical manifestation in the season under study	No reactions Symptomatic treatment not needed	Mild reactions Occasional, temporary symptomatic treatment	Common reactions Continual symptomatic treatment
No. of patients	4	3	6

enced worse seasonal reactions compared with the previous season.

3. Discussion

On the basis of data from the literature,^[2-4,7-9] we assumed that the administered bacterial antigenic stimulus could induce release of IL-12 and IFN γ , which we attempted to detect in peripheral venous blood. The stimulus employed was oral ribosomal immunotherapy (Ribomunyl®), which combined the efficacy of a nonspecific stimulus of the proteoglycan of the bacterial cell membrane from *K. pneumoniae* with a specific induction activity of purified ribosomes of selected bacterial strains: *S. pyogenes*, *S. pneumoniae*, *K. pneumoniae* and *H. influenzae*.^[2] The use of commercial oral bacterial immunomodulators to influence the course of allergy has to date not been reported in the literature; there are only experimental studies using bacterial endotoxin in rats sensitised with ovalbumin. The experiments revealed that timely administration of bacterial lipopolysaccharide (endotoxin) in aerosol prior to or shortly after sensitisation acted preventively on the development of allergic inflammation in the respiratory tracts of the animals under study.^[10] Similar considerations concerning a protective effect of bacterial stimuli against the development of disease, particularly in childhood, are the basis of the 'hygienic hypothesis' of the increase in allergies in recent decades.^[5,6] However, these existing data state that in individuals with already developed allergic disease (allergic rhinoconjunctivitis and asthma), exposure to bacterial endotoxins can cause a deterioration in the manifestations of their allergic disease.^[11,12] No data are available in the literature concerning the safety or undesirable effects of commercially produced oral bacterial immunomodulators in allergic patients, but our experience

over many years of use allows us to state that they are very safe preparations, and in relation to their administration to allergic patients (with clinical manifestations of an allergic rhinoconjunctivitis or asthma) no signs of activation of allergic inflammation were observed as long as they were administered during the period of stabilised allergic disease for the prevention of recurring infections of the respiratory tract.

Controlled studies dealing with the chronological relationship of exposures to bacterial stimuli and allergens as well as the dependence on the quantity of these stimuli or specificity of individual bacterial species that act on the immune system through motifs contained in RNA are needed to explain the interactions between bacterial immunomodulators that are formed by the proteoglycans of bacterial walls, lysates of whole bacterial bodies or just bacterial ribosomes, or combinations of these agents. In any case, it is possible to state that the components of bacterial entities rank among the most physiological modulators of the human immune system, which is readily able to identify them and respond to them appropriately.

4. Conclusion

Ribomunyl® therapy increased IFN γ levels in patients with seasonal allergy during the course of the pollen season in a statistically significant way. The increase in plasma IFN γ levels was more pronounced in patients who had a better or markedly better clinical course in the pollen season compared with the previous year with the same cover from symptomatic antiallergic treatment. In this treatment regimen (symptomatic therapy + ribosomal immunotherapy), a better outcome during the pollen season was observed in more than half of the patients under study.

Our findings suggest that in atopic patients there are some who through the action of bacterial antigens (e.g. in the form of bacterial immunomodulators) respond more easily by increasing IFN γ production, and this may be the cause of blocking, or at least alleviation, of allergic reactions during the allergic season. In patients who did not respond to the bacterial antigenic stimulus with sufficient production of IFN γ , the intensity of their allergic reaction was not influenced. There still remain, however, many questions (for example, the kind of bacterial immunomodulator: its composition, administration regimen, and the number of bacterial antigenic components, the length of immunotherapy, possible combination of immunomodulation by bacterial antigens with allergen immunotherapy) that have yet to be answered.

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