

TREATMENT OF RELAPSING CHRONIC INFECTIONS OF THE RESPIRATORY TRACT. A COMPARATIVE STUDY OF THE EFFECTIVENESS OF NON SPECIFIC IMMUNOTHERAPY WITH THE IMMUNOADJUVANT P40 AND OF VACCINOTHERAPY

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S U M M A R Y

The treatment of relapsing chronic infections (RCI) encounters many difficulties. In the present study, the use of the immuno adjuvant P40 either alone or in association with vaccinotherapy for the treatment of RCI turned out to be very effective, whereas vaccinotherapy alone was not. It is hypothesized that cell-mediated immunity may play a major role in controlling RCI, since the clinical improvement of the patients kept up with the positivation of previously negative skin tests carried out with either the specific infecting agent or with recall-antigens.

I N T R O D U C T I O N

Presently, the treatment of relapsing chronic infections (RCI) relies mainly on the administration of anti-microbial drugs given either alone or in association with anti-inflammatory drugs. Although acute phases of RCI can usually be controlled by such treatments, these are most often unable to prevent relapses. In order to avoid relapses, treatments have to be pursued for long periods of time which can possibly be harmful to the patients. It now appears very likely that relapses may originate from the patient's inability to mount an adequate cell-mediated reaction toward the infecting agent¹⁰. Since attempts to identify the infecting agent have in many cases been unsuccessful, in particular in RCI of the respiratory tract, this explains why immunization of patients with specific vaccines could not be carried out. This fact also accounts for the use of polymicrobial vaccines, the effects of which are most often unpredictable because of their lack of specificity. Therefore it has appeared to us that stimulation of the patient's

natural defenses might substitute for specific immunization^{12,15}. In the present study we have tested the effectiveness of the new immuno-adjuvant P40 deriving from *Corynebacterium granulorum*³ in the treatment of patients suffering from various RCI. The efficiency of the treatment with P40 alone was compared with that of the treatment by either vaccinotherapy alone or by vaccinotherapy in association with P40.

P A T I E N T S A N D M E T H O D S

Patients — Hundred and ten patients suffering from RCI of various origins were selected for this study. These patients were affected by RCI of either the upper respiratory tract (otitis, angina, rhinopharyngitis, sinusitis) or the lower respiratory tract (chronic bronchitis, infected asthma). All these patients had in common the following features: 1) iterative infectious episodes in spite of the administration of varying regimens of antibiotics and/or anti-

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inflammatory drug; 2) either exaggerated, or normal, or reduced skin delayed hypersensitivity responses to the infecting agent (when identified) or to the usual recall-antigens (when the etiology of the infection had not been elucidated).

Before P40 has become available for this study, all patients had repeatedly received courses of vaccinotherapy. This is why these patients were not randomized. When P40 could be used, were allotted to either Group II or Group III those patients who: 1) have had a history of previous treatment with antibiotics and/or anti-inflammatory drugs which had lasted for more than 5 years and 2) had not responded to vaccinotherapy alone.

Immunoadjuvant P40 — The preparation of the immunoadjuvant P40 was described in detail elsewhere³. Briefly, killed *C. granulorum* whole cells were delipidated and disintegrated mechanically. Bacteria which had not been disrupted were removed by low speed centrifugation. The supernatant which contained the disrupted bacteria was fractionated by ammonium sulfate precipitation and the particulate fraction precipitated to a concentration of ammonium sulfate corresponding to 40% saturation was collected. After exhaustive dialysis against several changes of distilled water, the fraction was lyophilized. It was designated as P40 fraction. Suspensions of P40 in saline at various concentrations were sterilized in the autoclave at 120°C for 20 min without loss of activity.

Skin-tests — They were carried out by administering intradermally under the volume of 0.1 ml on the ventral part of the forearm various bacterial preparations: *St. aureus*, *H. influenzae*, *N. catarrhalis*, *K. pneumoniae*, *Str. pneumoniae*, streptococcal exoproteins (Service des Allergènes, Institut Pasteur, Paris), varidase (Lederle, U.S.A.) or of the auto-vaccine when the infecting organism had been identified. All tests were read 48 h after the challenge injection.

Treatments

a) **Vaccinotherapy alone** — 23 patients (Group I) were injected with either mono — or poly-microbial preparations (consisting of one of the above indicated microorganisms or of commercially available mixtures of them (Di-

vasta, C.C.B., Institut Pasteur or M.R.V., Laboratoire des Stallergènes). Patients showing both strong skin and systemic reactions were first desensitized using a 1/100th dilution of a dose of preparation eliciting a positive skin reaction. The treatment consisted of weekly subcutaneous injections of increasing doses of antigen, starting with 0.1 ml, the dose being increased by 0.1 ml at each injection so as to reach a dose of 1 ml after 10 weeks. After 10 weeks the treatment was interrupted. In patients showing normal or decreased skin reactions, the treatment was initiated with a concentration of 10⁹ microorganisms per ml and it proceeded as specified above.

b) **Vaccinotherapy + immunoadjuvant P40** — in 38 patients (Group II) to the vaccinotherapy was associated the administration of the immunoadjuvant P40. The treatment was performed as specified for the vaccinotherapy alone, except that P40 was included in the vaccine.

c) **Immunoadjuvant P40 alone** — 49 patients (Group III) received P40 alone as a weekly subcutaneous injection of 0.1 ml (0.20 mg/ml) for 10 weeks and the treatment was interrupted.

In addition, those patients of all 3 groups who, after being improved by the treatment, relapsed, received subsequent series of injections at fixed doses.

Criteria for evaluating the efficiency of treatments — treatment efficiency in patients with RCI of the respiratory tract was scored as either good (+) or bad (—) on the basis of 3 criteria:

1) the time-interval between 2 relapses had to be at least twice as long as in natural infection; 2) the administration of antibiotics and anti-inflammatory drugs could be suppressed during the span between 2 relapses; 3) occupation (school for children) was not interrupted during the span between 2 relapses.

It must be stressed that all patients had repeatedly presented in the past relapses at regular time-intervals.

In RCI of the skin, of uro-genitry tract and of various origins, only criteria 1 and 2 were taken into account.

X-ray examination of patients with RCI of the respiratory tract was not used as a crite

tion, since it was not changed by the treatment. The same holds true for humoral modifications.

RESULTS

Group I — this group of 23 patients receiving vaccinotherapy alone included only patients with respiratory RCI. Seven of them were found to be improved by the treatment (30.04% good results (+)).

Group II — this group receiving vaccinotherapy + P40 included 21 patients with RCI of the respiratory tract and 5 patients with RCI of various origins. Among the former 18 patients out of 21 (85.7% good results (+)) proved to be improved by the treatment. 14 Out of these 18 patients had previously been treated unsuccessfully with vaccinotherapy alone (C.C. B., M.R.V.).

Among the patients with RCI of various origins there were: a) 1 patient with furunculosis who was improved by the combined treatment, whereas the previous administration of Divasta alone had proved to be inefficient; b) 2 patients with relapsing *E. coli* cystitis. When skin-tested with the auto-vaccine they failed to respond to the particular strain of *E. coli* isolated from their urines. Treatment of these 2 patients by the combination auto-vaccine + P40 resulted in the positivation of the skin-test with the auto vaccine at the same time as the relapses were prevented; c) 2 patients with suppurative wounds. One of them with chronic epididymitis (*Proteus*) had regularly been relapsing for the last 20 years. The suppuration was completely healed up by a 6-week treatment associating vaccinotherapy + P40. Relapses have not been observed since 2 years. The other patient suffered from *Staph. aureus* tibial osteitis which had kept on suppurating in spite of repeated surgical sanitation of the wound and antibiotherapy. Cicatrization of the wound was achieved in 10 weeks with the association Divasta + P40. Divasta alone had previously proved to be inefficient.

Group II also included 12 patients with mucocutaneous herpes. These patients received an association of P40 with various recall-antigens so as to elicit strong delayed hypersensitivity reactions. Six of the patients were improved in this way (50% good results (+)).

Group III — this group of 49 patients was treated with P40 alone. It consisted of: a) 17 patients with RCI of the respiratory tract. 14 Of them were improved (82.3% good results (+)). Before initiation of the treatment, 10 of these 17 patients were found not to respond when skin-tested with bacteria isolated from the respiratory tract. At the end of the treatment 7 of these patients were improved and responded positively when tested with the same bacterial antigens, in particular with *H. influenzae*. In contrast, patients who were not improved by the treatment were still unresponsive to skin-tests; b) 24 patients with relapsing herpes. 18 good results (75% (+)) were obtained with P40 treatment; c) 8 patients with relapsing vaginal candidiasis. All these patients were unresponsive when skin-tested with candidin. The presence of *C. albicans* could also be demonstrated in the vaginal secretions. After treatment 7 out of 8 patients (87.5% good results (+)) were improved according to criteria 1 and 2. In improved patients skin-tests to candidin were also positive and *C. albicans* was absent from the vaginal secretions. The only patient who was not improved by P40 treatment was still unresponsive to candidin and the presence of *C. albicans* was ascertained in the vaginal secretions.

A large enough number of patients with RCI of the respiratory tract in Groups I, II and III also permitted the statistical evaluation by the Student t-test of the comparative effectiveness of the various treatments in these patients. The results summarized in Table I clearly show that Group II treatment (vaccinotherapy + P40) and Group III treatment (P40 alone) are more effective than Group I treatment (vaccinotherapy alone), the difference being highly significant ($\alpha < 0.001$). In contrast, there is no statistically significant difference between the effectiveness of Groups II and III treatments. This further substantiates that vaccinotherapy is of no great value for the treatment of patients with RCI of the respiratory tract.

DISCUSSION

Enhancement of natural resistance to infections appears to be one effective way of how to prevent relapses in patients with RCI. For this purpose, the use of polymicrobial vaccines

T A B L E I

Statistical evaluation of the comparative effectiveness of vaccinotherapy alone (Group I), of vaccinotherapy + P40 (Group II) and of P40 alone (Group III) in the treatment of patients with relapsing chronic infections of the respiratory tract

Treatments	No. of patients	Proportion of patients improved according to the defined criteria	Percentage of patients improved
Vaccinotherapy alone (Group I)	23	7/23	30.04
Vaccinotherapy + P40 (Group II)	21	18/21	85.71
P40 alone (Group III)	17	14/17	82.35

Statistical differences between the various groups of treatments:

Group I — II:	$\alpha < 0.001$ (highly significant)
Group I — III:	$\alpha < 0.001$ (highly significant)
Group II — III:	$0.9 < \alpha > 0.5$ (not significant)

has not been very successful probably because of the weak immunogenic potency of such vaccines. This also explains why polymicrobial vaccines have to be administered for long periods of time in order for them to exercise their possible therapeutic effects. In fact, polymicrobial vaccines were used unsuccessfully for the treatment of infectious asthma by several Authors^{1,2,8,14} and in¹⁷. However, MUELLER et al.¹⁶ showed that asthma in which the microbial factor is essential, in comparison with those in which it is occasionally involved, could be improved by the administration of polymicrobial vaccines. In a few instances, improvement of the patients, as assessed clinically, was found not to be correlated with the results of *in vitro* tests. In this respect, the use of auto-vaccines⁷ or of vaccines containing the bacterial species involved in infection⁴ proved to be rather effective. Therefore, the question remains open as to whether vaccinotherapy is able to determine antipyrogenic responses in patients with RCI.

The effect of P40 seems likely to be mediated by a qualitative rather than a quantitative modification of the host's immune response resulting in enhancement of cell-mediated responses. This view is supported by reports in the literature in which protection of mice against *S. typhimurium* infection, as afforded by BCG^{5,18}, and *S. enteritidis* as afforded by *C. parvum*², were found not to result from an increased production of specific antibodies. Protection of mice against *L. monocytogenes* infec-

tion could also be achieved with P40 (unpublished results). In *L. monocytogenes* infection the predominating role of macrophages in protection is well documented. Consequently, the protective effect of P40 against infection might possibly be mediated by macrophage activation. In fact, it has been shown that P40 increased the enzymatic activity of macrophages (K. Masek, Institute of Pharmacology, Prague: personal communication).

Although macrophages may be involved, it appears most likely that cell-mediated reactions are of paramount importance in the control of RCI. In fact, it could be ascertained in the present study that patients skin-tested with preparations of the infecting agent, when it had been isolated and identified, were, as a matter of fact, unresponsive. In contrast, clinically improved patients were found to have become responsive.

In herpes, the effect of P40 can also tentatively be ascribed to enhancement of delayed-type hypersensitivity to the virus.

The precise mode of action of P40 is still to be elucidated. The route of injection and dosage were arbitrarily chosen with the purpose of avoiding the formation of a granuloma⁹. At the dosages used in this study, undesirable reactions, either local and/or systemic, were not observed. However, the dosage selected by us might have been too low for effectively stimulating strongly immunocompromised patients. This fact could explain why clinical im-

provement of a few patients was of short duration, whereas relapses could be prevented by resuming the treatment.

The present study clearly shows that the use of an immunoadjuvant, such as P40, can be highly effective in controlling RCI. It appears probable that the effect is mediated by enhancement of cell-mediated reactions, since clinically improved patients were found to be responsive to skin-tests performed with antigens to which they were previously unresponsive.

RESUMO

Tratamento de infecções crônicas recidivantes do aparelho respiratório. Estudo comparativo da eficácia da imunoterapia inespecífica através do imunoadjuvante P40 e da vacinoterapia.

O tratamento de infecções crônicas recidivantes (I.C.R.) encontra muitas dificuldades. No presente estudo, o uso do imunoadjuvante P40, quer isolado, quer associado a vacinoterapia no tratamento de I.C.R., demonstrou ser muito eficaz, ao passo que a vacinoterapia isoladamente não o foi. Foi aventada a hipótese de que a imunidade mediada por células pode desempenhar papel importante no controle das I.C.R., uma vez que a melhora clínica dos pacientes foi acompanhada de positividade de testes cutâneos anteriormente negativos, testes estes realizados quer com o agente infeccioso específico; quer com antígeno inespecífico de reforço.

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