Effect of Immunostimulatory Ribomunyl on the Preventive Treatment of Rat Adjuvant Arthritis with Cyclosporine and Methotrexate

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ABSTRACT. ***Objective.*** To study the effects of ribomunyl, an oral ribosomal immunostimulant frequently used to prevent recurrent oropharyngeal and bronchopulmonary infections in children and adults, on adjuvant arthritis as well as its effect on methotrexate and cyclosporine treatment.

Methods. Rats with adjuvant induced arthritis were preventively treated orally with the following drugs: cyclosporin A (CSA, 2.5 mg/kg/day); ribomunyl (RIB, 25 mg/kg/day, 4 days a week); methotrexate (MTX, 0.6 mg/kg/week), and the combinations CSA + RIB, MTX + RIB, CSA + MTX, CSA + MTX + RIB for the period of 50 days from adjuvant application. Levels of serum albumin, serum nitrite/nitrate concentrations, hind paw swelling, arthrograms, and bone destruction were measured in rats as variables of the inflammation and destructive arthritis associated changes.

Results. Preventive treatment with low doses of CSA and MTX significantly inhibited both markers of inflammation and arthritis. The combination CSA + MTX was more effective on all evaluated measures than any of its components alone. RIB alone improved arthrogram scores and decreased serum nitrite/nitrate concentrations in arthritic rats. The combination of RIB with the low dose of CSA or MTX enhanced the beneficial effects of these drugs. The best preventive effect was observed with the combination of 3 agents, CSA + MTX + RIB.

Conclusion. Our results show ribomunyl to have the potential to improve some inflammation and arthritis associated changes in rat adjuvant arthritis and to enhance the preventive effect of MTX and CSA and their combination. (J Rheumatol 2003;30:2027–32)

Key Indexing Terms:
RIBOMUNYL CYCLOSPORINE METHOTREXATE ADJUVANT ARTHRITIS

The effectiveness of cyclosporine in some autoimmune diseases is assumed to be a consequence of the central role of the T cell immune responses in their pathogenesis. Cyclosporin A (CSA) inhibits the proliferation of T lymphocytes and selectively inhibits T cell mediated responses, including inhibition of antigen and mitogen induced secretion of interleukin 2 (IL-2), IL-3, and interferon gamma and other cytokines at the transcription level from naive T cells[1]. Although initially used in organ transplants, CSA has been effective as a single agent in rheumatoid arthritis (RA), psoriasis, and uveitis[2]. Although the clinical efficacy of CSA has been proven in clinical trials, its toxicity profile of renal function impairment and gastrointestinal intolerance has led to a search for combination therapies requiring lower levels of drugs while offering maintained or increased overall therapeutic efficacy. In adjuvant arthritis (AA) as well as in collagen induced arthritis (CIA), CSA can prevent the onset of arthritis when initiated at the time of immunization, but does not influence disease severity when initiated in established disease[3,4]. Methotrexate (MTX), widely used in RA therapy for its antiinflammatory and immunomodulatory effects, was administered in combination with CSA, both at relatively low dosage, in the CIA rat model with very good effect[5]. Clinical efficacy has since been reported for this combination therapy in RA[6].

Longterm application of immunosuppressive therapy has long been known to possibly result in disturbances of immunological homeostasis, including the development of resistance and recurrent secondary infections. Thus the options of immunosuppressive and also immunomodulatory therapies without the immunosuppressive adverse effects on the clinical conditions of patients and on cell mediated and nonspecific immunity function have been pursued. Combination of immunosuppressive and immunostimulant agents represents one such option of immunomodulatory therapy[7].

Many immunostimulants are of bacterial origin, e.g., whole bacteria, bacterial lysates, or bacterial extracts. Ribomunyl, frequently used to prevent recurrent oropharyngeal infections in children and adults, contains both proteoglycans from *Klebsiella pneumoniae* and ribosomes from 4 different bacterial strains[8]. The proteoglycans act as adjuvant and nonspecific immunostimulants. The immuno-

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Supported by Pierre Fabre Medicament, Castres Cedex.

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Submitted June 19, 2002; revision accepted February 3, 2003.

genicity of ribosome preparations can be attributed to either peptides naturally bound to ribosomes or to epitopes bound to membrane and cytoplasmic ribosomes[9]. Ribomunyl is able to initiate nonspecific immune responses by stimulating polymorphonuclear cells and macrophages[10,11]. Its specific immunostimulant properties have been described in both animals and humans, where specific antibody-forming B cells were found in tonsils after oral administration[12]. Ribomunyl has been shown to increase the production of many cytokines (IL-1, IL-6, IL-8, tumor necrosis factor-α, and colony-stimulating factors), leading to the activation of the cytokine network[13,14]. Ribomunyl was also reported to stimulate natural killer cells involved in antiviral immunity[15].

We investigated the effects of the immunostimulant oral vaccine ribomunyl on the development of AA in rats, as well as its effect on preventive immunosuppressive treatment with CSA and MTX and their combinations.

MATERIALS AND METHODS

Materials. MTX (Lachema, Brno, Czech Republic) was used as pure substance. Ribomunyl® oral vaccine (RIB) was a commercial preparation produced by Pierre Fabre Medicament, Castres Cedex. One bag contains 0.75 mg ribosomal fractions from *Hemophilus influenzae* (0.5 parts), *Streptococcus pneumoniae* (3.0 parts), *Streptococcus pyogenes* (3 parts), and *Klebsiella pneumoniae* (3.5 parts), supplemented with 1.125 mg of *K. pneumoniae* membrane proteoglycans, extended to make 500 mg with mannitol. Cyclosporine was applied in the form of Consupren®S (Galena a.s., Opava, Czech Republic) containing 100 mg/ml CSA. *Mycobacterium butyricum* was purchased from Difco Laboratories, Detroit, MI, USA, and incomplete Freund's adjuvant from Sigma-Aldrich Chemie GmbH (Tautkirchen, Germany).

Animals. Male Lewis rats (160–180 g; Charles River, Sulzfeld, Germany) were maintained during the experiment in standard animal facilities that comply with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The animals were fed pellet food and had free access to food and water. The experimental protocol and all procedures were approved by the State Veterinary Committee for Control of Animal Experimentation and by the National Institute of Rheumatic Diseases Animal Ethics Committee. Investigators were blinded to the control and treated animals.

Induction of arthritis. The rats were injected with 0.1 ml suspension of heat killed *M. butyricum* (12 mg/ml) in incomplete Freund's adjuvant intradermally at the base of the tail.

Treatment. CSA, MTX, and RIB were administered in corresponding doses from Day 0 (day of immunization) to Day 50. MTX solution in sterile saline 0.3 mg/kg was applied twice a week per os. CSA solution was diluted every day with olive oil to yield the desired concentration, and was applied per os in once-daily dosage of 2.5 mg/kg every day. RIB was administered orally 4 days a week, each dose freshly prepared and given in 0.1 ml saline solution containing 25 mg/kg body weight. The untreated groups received vehicle (olive oil, sterile saline) in the same manner daily for 50 days.

The animals were divided into the following 9 groups of 10. Group 1: nonarthritic untreated controls; Group 2: untreated rats with AA; Group 3: AA rats treated with MTX; Group 4: AA rats treated with CSA; Group 5: AA rats treated with RIB; Group 6: AA rats treated with the combination CSA + MTX; Group 7: AA rats administered the combination MTX + RIB; Group 8: AA rats treated with the combination CSA + RIB; and Group 9: AA rats treated with the combination CSA + MTX + RIB.

Evaluation measures. Hind paw swelling. The volume of the hind paw swelling was measured with an electronic water plethysmometer (UGO Basile, Comerio-Varese, Italy) on Days 14, 21, and 28.

Arthrogram score. The severity of arthritis was quantified by scoring each paw from 1 to 5, based on increasing levels of swelling and periarticular erythema. The sum of the scores for the limbs was calculated as the arthritic index, with the maximum possible score of 20 per rat. Arthrogram scores were evaluated on Days 14, 21, and 28.

Serum albumin concentrations. Serum albumin was measured on Days 14, 21, and 28 in rat serum by the spectrophotometric method, using a SYS 1 kit (BM/Hitachi, Boehringer-Mannheim, Germany) on a Hitachi 911 automatic biochemical analyzer.

Serum nitrite/nitrate. Nitrite/nitrate concentrations in deproteinized serum were determined as described[16] on Days 14, 21, and 28. Nitrate was reduced by Cu-coated cadmium granules in glycine buffer at pH 9.7 and the resulting nitrite was evaluated by Griess reaction.

Evaluation of bone destruction. Bone changes, indicated by destruction of tarsal and metatarsal bone structure of hind paws, were evaluated from radiographic prints from a computer controlled X-ray generator (Philips Super 80CP, Philips, Hamburg, Germany) on Day 50 after immunization, using the modified arthrogram scoring system[17]. Every hind paw radiograph was checked for matching to one of the 5 degrees of bone destruction: (1) osteoporosis of the distal part of the tibia and the tarsal bones; (2) hyperostosis with osteophytes in the tibiotarsal joint region; (3) degree (2) plus hyperostosis with osteophytes in the tarsometatarsal joint region; (4) degree (3) plus hyperostosis with osteophytes in the tarso-metatarsophalangeal joint region; (5) degree (4) plus deformation of the joint spaces.

Statistical analysis. One-way analysis of variance (ANOVA) was used for statistical analysis of the results and $p < 0.05$ was taken as the significance limit for all comparisons.

RESULTS

Hind paw swelling. Hind paw swelling indicates both arthritic and inflammatory changes in rats with AA. The volume of the swollen hind paws in arthritic rats was about twice that in healthy controls (Table 1). Statistically significant reductions were observed with MTX, CSA, with combinations MTX + RIB, CSA + RIB and CSA + MTX, and with the 3-agent combination CSA + MTX + RIB, on postimmunization Days 14, 21, and 28 compared to untreated arthritic controls. In combination with either CSA or MTX, RIB delayed the development of hind paw swelling, which was less on Day 14 in comparison with that observed for rats treated with either CSA or MTX alone. Such a difference could no longer be observed on Days 21 and 28. The combination CSA + MTX proved rather effective. In the group of animals receiving this combination, only 2 animals with a slightly swollen single paw were observed. The greatest reduction in hind paw swelling was observed with the 3-agent combination MTX + CSA + RIB. Animals receiving it exhibited no swelling. The administration of RIB alone had no effect on hind paw swelling.

Arthrogram scores. Arthrogram score is a more comprehensive variable that indicates the severity of arthritis. All treatments were associated with significant decreases of arthrogram scores (Table 2). Similarly to hind paw swelling, reductions in arthrogram scores were more pronounced for the groups treated with combinations. Again, the best beneficial effect was observed for the 3-agent combination CSA

Table 1. The effects of ribomunyl (RIB), cyclosporine (CSA), methotrexate (MTX), and their combinations on the volume of hind paw swelling (ml) in rats with AA.

Group	Day 14	Day 21	Day 28
Healthy controls	1.30 ± 0.10	1.38 ± 0.08	1.41 ± 0.06
AA controls	2.65 ± 0.18	2.83 ± 0.30	2.42 ± 0.29
AA preventive treated with			
CSA	2.02 ± 0.48**	2.15 ± 0.35***	2.00 ± 0.13***
MTX	2.30 ± 0.38*	2.39 ± 0.28**	2.12 ± 0.22*
RIB	2.44 ± 0.69	2.60 ± 0.39	2.27 ± 0.20
CSA + MTX	1.54 ± 0.16***‡‡††††	1.64 ± 0.25***‡‡‡†††	1.57 ± 0.21***‡‡‡††††
CSA + RIB	1.97 ± 0.43***	2.04 ± 0.36***‡	1.90 ± 0.15***
MTX + RIB	1.82 ± 0.35***††	2.34 ± 0.43**	2.06 ± 0.29*
CSA + MTX + RIB	1.33 ± 0.16***‡‡‡††††	1.48 ± 0.23***‡‡‡†††	1.42 ± 0.23***‡‡‡††††

AA rats were preventive treated with CSA 2.5 mg/kg/daily, MTX 0.6 mg/kg/week, RIB 25 mg/kg/4 days a week, or a combination in the same doses. Data represent mean values ± SD for 10 rats on a given day. Significance of differences vs arthritic control rats: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significance of differences vs arthritic rats preventive treated with CSA: ‡ $p < 0.05$, ‡‡ $p < 0.01$, ‡‡‡ $p < 0.001$. Significance of differences vs arthritic rats preventive treated with MTX: †† $p < 0.01$, ††† $p < 0.001$.

Table 2. The effects of ribomunyl (RIB), cyclosporine (CSA), methotrexate (MTX), and their combinations on arthrogram scores in rats with AA.

Group	Day 14	Day 21	Day 28
AA controls	21.60 ± 1.71	22.60 ± 1.58	19.60 ± 1.78
AA preventive treated with			
CSA	10.38 ± 3.53***	13.13 ± 4.17***	12.70 ± 3.16***
MTX	16.30 ± 2.21***	16.90 ± 2.28***	15.30 ± 3.13**
RIB	18.70 ± 3.47*	20.00 ± 2.26**	17.00 ± 2.16**
CSA + MTX	5.80 ± 2.39***‡‡††††	6.80 ± 2.90***‡‡‡†††	7.50 ± 2.46***‡‡‡††††
CSA + RIB	9.80 ± 4.24***	12.00 ± 3.59***	11.30 ± 3.16***
MTX + RIB	11.90 ± 3.63***††	16.10 ± 2.88***	14.10 ± 3.31***
CSA + MTX + RIB	3.60 ± 1.71***‡‡‡††††	4.70 ± 1.95***‡‡‡†††	4.90 ± 2.51***‡‡‡††††

See Table 1 notes. Significance of differences vs arthritic control rats: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significance of differences vs arthritic rats preventive treated with CSA: ‡‡ $p < 0.01$, ‡‡‡ $p < 0.001$. Significance of differences vs arthritic rats preventive treated with MTX: †† $p < 0.01$, ††† $p < 0.001$.

+ MTX + RIB. Interestingly, RIB alone also significantly decreased the arthrogram scores during the whole study.

Serum albumin concentrations. Serum albumin behaves like a negative acute phase reactant in rat as well as human arthritis. Lower levels of serum albumin corresponded to higher levels of inflammatory activity. MTX or CSA singly inhibited the serum albumin reduction to a similar extent (Table 3). More pronounced inhibition was observed for the combination CSA + MTX and for the 3-agent combination CSA + MTX + RIB. RIB alone had no significant effect on this inflammatory marker.

Serum nitrite/nitrate concentrations. Serum concentrations of nitrite/nitrate reflect nitric oxide (NO) production in various tissues and inflammatory responses. The clinical onset of AA was associated with a significant rise in nitrite/nitrate concentrations. CSA monotherapy decreased the serum nitrite/nitrate levels more effectively than MTX (Table 4). RIB enhanced the inhibitory effect of MTX. A significant decrease was also observed with the combinations CSA + MTX, MTX + RIB, and CSA + RIB. Values similar to those for healthy controls were found for arthritic rats treated with the combination CSA + MTX + RIB. RIB alone reduced nitrite/nitrate concentrations during the later phase of the disease (Day 28).

Evaluation of bone destruction. Except for RIB, all the treatments significantly reduced radiographic scores (Figure 1). CSA monotherapy was more effective than MTX, but less effective than the CSA + MTX combination. The maximum reduction of radiographic scores was obtained with the CSA + MTX + RIB combination. RIB alone had no effect, but the combination therapies with RIB manifested their beneficial effect by greater reductions in the radiographic scores.

DISCUSSION

These experiments in AA rats focused on the effects of the immunostimulatory vaccine ribomunyl on AA and on preventive treatment with CSA and MTX. Since the effects on radiographic scores were also investigated, the duration

Table 3. The effects of ribomunyl (RIB), cyclosporine (CSA), methotrexate (MTX), and their combinations on serum albumin concentrations (g/l) in rats with AA.

Group	Day 14	Day 21	Day 28
Healthy controls	31.60 ± 2.39	32.76 ± 2.07	31.88 ± 0.66
AA controls	24.29 ± 1.85	27.32 ± 1.05	25.89 ± 1.83
AA preventive treated with			
CSA	26.36 ± 1.26*	28.89 ± 2.04**	28.90 ± 1.65*
MTX	26.00 ± 0.64*	28.23 ± 0.76*	28.24 ± 0.55*
RIB	25.17 ± 0.94	26.58 ± 0.65	27.56 ± 1.50
CSA + MTX	27.01 ± 1.29**†	29.22 ± 0.95***††	29.50 ± 1.02***††
CSA + RIB	26.82 ± 0.96**	29.09 ± 1.39*	29.13 ± 1.69*
MTX + RIB	26.17 ± 0.99*	28.42 ± 1.15*	28.35 ± 0.83*
CSA + MTX + RIB	27.12 ± 1.41**†	29.35 ± 1.16***††	30.16 ± 1.35***†††

See Table 1 notes. Significance of differences vs arthritic control rats: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significance of differences vs arthritic rats preventive treated with MTX: † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$.

Table 4. The effects of ribomunyl (RIB), cyclosporine (CSA), methotrexate (MTX), and their combinations on serum nitrate/nitrite concentrations (nmol/ml) in AA rats.

Group	Day 14	Day 21	Day 28
Healthy controls	41.43 ± 9.27	43.89 ± 17.39	45.57 ± 4.67
AA controls	89.36 ± 5.83	76.75 ± 11.46	74.85 ± 13.49
AA preventive treated with			
CSA	63.59 ± 14.57***	55.26 ± 14.38**	56.02 ± 8.98**
MTX	76.24 ± 13.79*	65.46 ± 10.97*	61.77 ± 4.03**
RIB	88.99 ± 23.69	70.02 ± 12.25	57.76 ± 8.56**
CSA + MTX	55.99 ± 4.91***†††	54.45 ± 10.35***†	54.03 ± 11.26**
CSA + RIB	56.28 ± 14.15***	55.18 ± 17.56**	52.07 ± 14.70**
MTX + RIB	70.96 ± 15.11**	58.83 ± 11.87**	56.64 ± 12.64**
CSA + MTX + RIB	43.49 ± 10.10***‡‡††	43.26 ± 13.93***†††	41.42 ± 11.46***‡‡†††

See Table 1 notes. Significance of differences vs arthritic control rats: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significance of differences vs arthritic rats preventive treated with CSA: ‡‡ $p < 0.01$. Significance of differences vs arthritic rats preventive treated with MTX: † $p < 0.05$, ††† $p < 0.001$.

of the treatment was longer (50 days) than usually administered for AA (10–14 days following adjuvant administration) to prevent exacerbation of arthritis after discontinuation of the therapy. As well, ribomunyl was assumed to manifest its effect after longterm administration.

The results confirmed the previously reported effects of MTX and CSA obtained in rats with AA[4,17]. MTX at a dose of 0.6 mg/kg/week suppressed, but did not prevent, arthritis development. CSA at a dose of 2.5 mg/kg/day behaved similarly. In our study, both MTX and CSA significantly reduced hind paw swelling and arthrogram scores. The additive or synergistic effect of the combination treatment with low dose CSA + MTX agrees with the observations of Brahn, *et al*[5] in collagen induced arthritis. Ribomunyl alone had a positive, although statistically insignificant effect on hind paw swelling, and significantly reduced arthrogram scores throughout the study. It slightly potentiated the beneficial effect of MTX and CSA given alone or the 2-drug combination CSA + MTX, resulting in a more significant reduction of hind paw swelling and arthrogram scores throughout the treatment.

Serum albumin acts as a negative acute phase protein in rat arthritis, and the decrease of serum albumin levels signifies changes in the synthesis of this protein in the liver secondary to the activation of hepatic cells by inflammatory cytokines, mainly IL-1. Our results are in accord with the observation that CSA and MTX markedly prevent the albumin decrease in rat adjuvant arthritis[4]. When given alone, ribomunyl had no effect on this inflammatory marker, and its effect on MTX and CSA therapy was negligible.

NO, an unstable free radical produced by the action of the enzyme NO synthase (NOS) on L-arginine, is a mediator of multiple physiologic functions, and may also mediate local inflammation and tissue destruction[18]. Nitric oxide is involved in both initiation and development of adjuvant arthritis in rats[19]. Moreover, inhibitors of NOS have been shown to suppress arthritis in several animal models[20,21], and increased NO levels have been found in patients with RA[22,23]. Omata, *et al*[24] reported MTX to suppress *ex vivo* production of NO in macrophages from rats with adjuvant induced arthritis. Significant reductions of serum nitrite/nitrate concentrations by CSA were observed in both

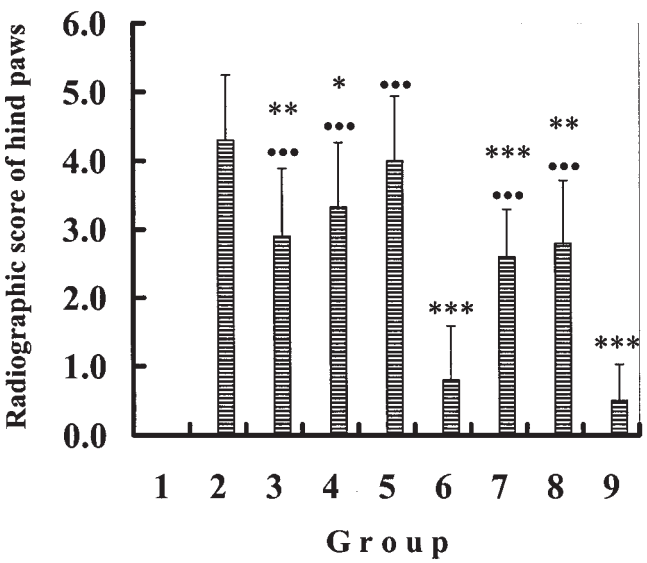

Figure 1. The effects of ribomunyl, cyclosporin A, methotrexate, and their combinations on radiographic scores in rats assessed on Day 50. Animals were grouped as follows: 1 = nonarthritic untreated controls; 2 = AA untreated controls; 3 = AA rats treated with CSA 2.5 mg/kg daily; 4 = AA rats treated with MTX 0.6 mg/kg/week; 5 = AA rats treated with ribomunyl 25 mg/kg, 4 days a week; 6 = AA rats treated with combination CSA + MTX; 7 = AA rats treated with combination CSA + RIB; 8 = AA rats treated with combination MTX + RIB; and 9 = AA rats treated with combination CSA + MTX + RIB. Data represent mean values ± SD for 10 rats. Significance of differences vs arthritic control rats: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Significance of differences between arthritic rats treated with the 3-agent combination CSA + MTX + RIB and rats receiving other treatment: ••• $p < 0.001$.

adjuvant and collagen induced arthritis in rats[25]. In our study, markedly increased serum nitrite/nitrate concentrations were measured for AA rats. RIB alone tended to decrease the nitrite/nitrate concentration and potentiated the effect of MTX and its combination with CSA.

Bone destruction of hind paws is a typical sign of chronic arthritis. Reduction of radiographic response in AA with MTX is dose dependent. Low doses of MTX of 0.1 or 0.2 mg did not normalize the radiographic findings in AA[26]. Morgan, *et al*[27] found that MTX given at 1 mg/kg/week resulted in mean total radiographic scores not different from healthy controls. Lower or higher doses of MTX were less effective. In our experiment, MTX 0.6 mg/kg/week significantly decreased the radiographic scores, but CSA and its combinations were more effective. Again, the best result was obtained with the 3-agent combination CSA + MTX + RIB. RIB alone was ineffective, although slightly potentiating the protective effect of MTX and CSA.

Ribomunyl has not yet been tested in RA in humans or animals. The immunomodulator OM-89 (Subreum), a slow acting antirheumatic drug, is an extract of selected *Escherichia coli* strains[28]. OM-89 suppresses adjuvant induced[29] and avridine induced polyarthritis in rats[30]. It significantly improves the clinical indicators of patients with RA, without serious side effects[31]. Similarly to OM-89,ribomunyl stimulates macrophage functions including phagocytic and metabolic activity[11,32], as well as natural killer cell activity[15,33,34]. Activation of nonspecific immunity by ribomunyl might help to induce body immune homeostasis, which is disturbed by the basic disease and immunosuppressive therapy.

Bacteria have been implicated in the pathogenesis of many type of inflammatory arthritides. Bacterial DNA of *H. influenzae, Bordetella*, and *Yersinia* was detected in synovial fluids of patients with RA and juvenile arthritis[35]. A single intraperitoneal injection of group A *S. pyogenes* cell wall causes chronic erosive polyarthritis in rats, as first described by Cromartie, *et al*[36]. In female Lewis rats severe disease develops that is T cell dependent and is comparable to human RA[37]. Peripheral blood mononuclear cells of the majority of patients with ankylosing spondylitis and some with RA, but not in healthy controls, included cells that proliferate in the presence of HSP60 of *K. pneumoniae*[38]. RA is also the most commonly reported host-related risk factor for septic arthritis. *Staphylococcus aureus* is the most common causative organism; *S. pneumoniae* causes 5% of all cases of septic arthritis and is more often responsible for polyarticular infections than other organisms[39].

Ribomunyl has specific immunostimulatory properties, described in animals and humans, where specific antibody-forming B cells secreting IgA and IgM antibodies were found in the tonsils after oral administration[40]. This suggests that antigens within ribomunyl (e.g., HSP65) may have homology with pathogenic antigens in AA, and anti-ribomunyl antibodies, particularly of the IgA and IgM class, may block these antigens being recognized by T cells.

Our results show that administration of ribomunyl alone as an immunostimulatory agent for preventive purposes did not worsen the clinical, inflammatory, or destructive markers of adjuvant arthritis. On the contrary, it had positive effects on arthrogram scores and serum nitrite/nitrate concentrations. It markedly enhanced some antiinflammatory and antiarthritic effects of MTX and CSA. The 3-agent combination CYA + MTX + RIB proved to be the most efficient combination for preventive treatment of AA.

Further studies are needed to verify the effect of ribomunyl and its combinations with cyclosporine and methotrexate on the therapy of adjuvant arthritis with respect to the possible clinical use of this vaccine in RA.

ACKNOWLEDGMENT

The authors thank Dusan Velic, PhD, for critical reading of the manuscript.

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