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Treatment with Selemax®, a selenium-enriched yeast, ameliorates experimental arthritis in rats and mice

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disease that mainly targets the synovial membrane, cartilage and bone. It affects 1 % of the population and is associated with significant morbidity and increased mortality. Se is an essential trace element with antioxidant properties and the ability to modulate the immune responses. Selemax® is an inactive yeast (*Saccharomyces cerevisiae*) enriched with organic Se. The aim of the present study was to investigate the effects of Selemax® administration in models of an antigen-induced arthritis (AIA) in C57BL/6 mice, and of an adjuvant-induced arthritis (AdIA) in Holtzman rats. As control, the animals were treated with the same inactivated yeast species that was not enriched for Se. In the AIA model, treatment with different doses of Selemax® (0·01, 0·1, 1 and 10 % added to food) significantly decreased the number of inflammatory cells recruited to the knee cavity, essentially by reducing the number of neutrophils. Levels of proinflammatory cytokines, including TNF-α, IL-1β and chemokine (C-X-C motif) ligand 1/keratinocyte chemoattractant (CXCL1/KC), were also reduced in the peri-articular tissue of mice treated with Selemax® at the tested dose (1 %). In the AdIA model in rats, Selemax® treatment decreased paw oedema and hypernociception. This reduction was associated with inhibition of the influx of proinflammatory cells. Therefore, treatment with Selemax® is associated with amelioration of several inflammatory and functional parameters in models of arthritis, suggesting that this Se-enriched yeast should be evaluated further in patients with RA.

Key words: ***Saccharomyces cerevisiae*: Selenium: Antigen-induced arthritis: Adjuvant-induced arthritis: Rheumatoid arthritis**

Rheumatoid arthritis (RA) is a chronic and autoimmune inflammatory disease that affects 1 % of the population and is associated with significant morbidity and increased mortality[1]. It is characterised by tissue inflammation, including infiltration of organs by inflammatory cells, chronic secretion of inflammatory cytokines (TNF-α, IL-1β, interferon-γ, IL-6) and tissue destruction and dysfunction[2–4]. Furthermore, leucocytes interact in the joints with resident cells and matrix and produce reactive oxygen species (ROS), which have a role in RA by inducing tissue damage associated with inflammation[5].

There have been some major improvements in the knowledge of mechanisms of disease in the context of RA in the last 20 years, and several new therapeutic options are now being tested or have been approved, including drugs which modify the function of cluster of differentiation (CD) 20 (rituximab), cytotoxic T-lymphocyte antigen 4 (CTLA4) (abatacept), TNF-α (etanercept, infliximab, adalimumab, certolizumab and golimu-mab), IL-1β (anakinra) and IL-6 (tocilizumab)[6]. The clinical efficacy of these biological agents has provided support for the importance of immune pathways in the

Abbreviations: AdIA, adjuvant-induced arthritis; AIA, antigen-induced arthritis; CXCL1, chemokine (C-X-C motif) ligand 1; KC, keratinocyte chemoattractant; LPS, lipopolysaccharide; MPO, myeloperoxidase; RA, rheumatoid arthritis; ROS, reactive oxygen species.

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pathogenesis of RA. However, treatments are usually expensive, need parenteral routes, response rates vary between patients and undesirable side effects may be significant.

Se is an essential trace element with antioxidant properties and has been shown to modulate inflammatory and immune responses[7]. Se attenuates cellular immune responses, especially by dampening oxidative stress. While this may potentially lead to an increased risk of viral and bacterial infections, the inflammatory response does not induce such a great damage to the host tissue[7,8]. In addition, Se deficiency seems to worsen the outcome of autoimmune disorders. Parnham *et al.*[9] demonstrated that in adjuvant-induced arthritis (AdIA) in rats, arthritic manifestations significantly worsened after 6 and 12 weeks of application of an Se-deficient diet. Interestingly, Se supplementation to RA patients did not decrease the arthritic score but improved some symptoms of the disease[10].

Selemax® is an inactive yeast enriched with organic Se. This product is a selected pure culture of *Saccharomyces cerevisiae* capable of absorbing inorganic Se and converting it into organic Se (patent no. 4530846). The aim of the present study was to investigate the effects of Se supplementation in the form of Selemax® in two different models of arthritis: antigen-induced arthritis (AIA) in mice and AdIA in rats.

Materials and methods

Animals

Male C57BL/6 mice (8–10 weeks) and female Holtzman rats (8 weeks) were used throughout this study. The animals were kept in cages (maximum of five animals per cage) in an animal house facility with controlled temperature (23°C) and on a 12 h light–12 h dark cycle. Water and food were provided *ad libitum*. The animals were purchased from the animal facility of the Federal University of Minas Gerais and kept at the animal room of the laboratory after arthritis induction. All procedures described here had prior approval from the Ethical Committee for Animal Experimentation of the Federal University of Minas Gerais (CETEA/UFMG) with the protocol no. 166/2006. All experimental procedures were carried out according to the standards set forth by the Brazilian College for Animal Experimentation (Colégio Brasileiro de Experimentação Animal)[11].

Arthritis induction

Murine AIA was inducted according to reported methodology[12]. Briefly, mice were immunised subcutaneously with 100 µl of an emulsion containing 500 µg of methylated bovine serum albumin (Sigma) in PBS mixed with an equal volume of Freund's complete adjuvant (Sigma) on day 0. Then, 14 d later, AIA was induced by intra-articular injection (10 µl) of methylated bovine serum albumin in PBS (10 mg/ml) into the knee (stifle) joint. For control, the same volume of PBS was injected into the joint of immunised mouse. Mice were killed 24 h after antigen challenge and the knee cavity was washed with 3% bovine serum albumin in PBS and the peri-articular tissue was removed from the joint for evaluation of cytokines and myeloperoxidase (MPO) activity.

For AdIA in rats, the animals were anaesthetised intraperitoneally with a ketamine and xylazine mixture (3·2 and 0·16 mg/kg, respectively) and then injected subcutaneously with a single dose of 0·2 ml mineral oil–water emulsion (10:1, v/v) containing 400 µg of dried *Mycobacterium butyricum* into the dorsal root of the tail, as previously described[13]. Control animals were those injected subcutaneously with a single dose of 0·2 ml mineral oil–water emulsion (10:1) without *M. butyricum* in the same location. The time of adjuvant injection is referred to as day 0.

Selemax treatment

Selemax® and Se-free yeast were obtained from Biorigin as lyophilised powder. Treatments with Selemax® (organic Se combined yeast) or with the Se-free yeast were carried out by mixing in commercial chow with different percentage (0·01, 0·1, 1 and 10 %, w/w) of both products. Mice were fed with the supplemented diets for 7 d after arthritis induction, challenged

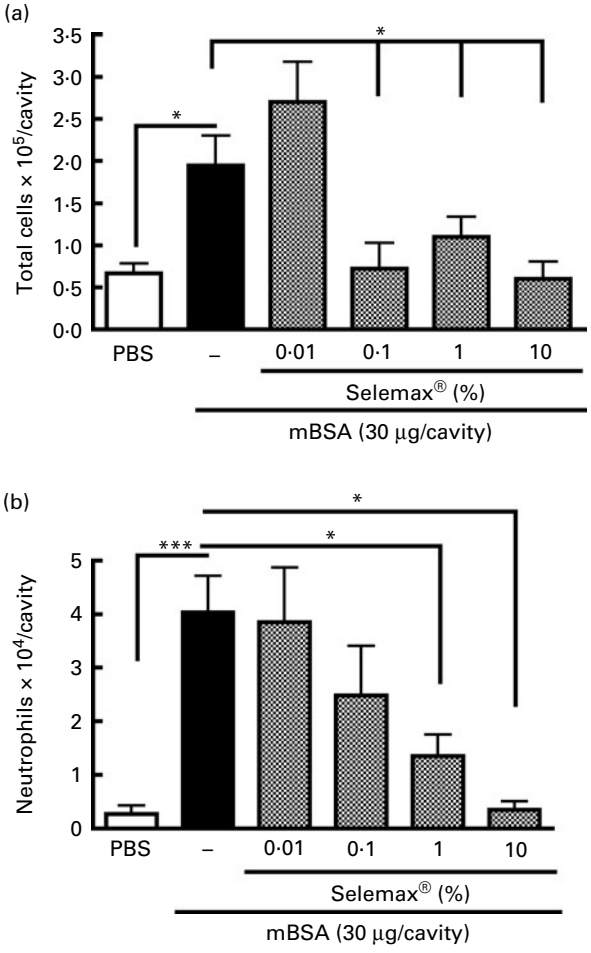

Fig. 1. Dose–response of Selemax® (0·01, 0·1, 1 and 10 %) in the number of (a) total and (b) neutrophil cells from the intra-articular fluid from control mice (□, PBS-treated), arthritic mice (■) and arthritic mice treated with different doses of Selemax® (▣). Values are means of five animals in each group, with their standard errors represented by vertical bars. Mean values were significantly different: $^{*}P<0·05$, $^{***}P<0·001$. mBSA, methylated bovine serum albumin.

and killed, as shown in the text, after AIA induction. In the AdIA model, rats were fed with supplemented diets for 10 d after the arthritis induction when the animals were killed. The dose of 1 % Selemax® delivered a final Se concentration of 45–50 μg/d.

Differential cell count

The total number of leucocytes in the fluid of the knee cavity wash was determined by counting the leucocytes in a Neubauer chamber after staining the samples with Turk's solution. Differential counts were obtained from cytospin preparations (Shandon III; Thermo Shandon) by evaluating the percentage of each leucocyte on a slide stained with Giemsa and May-Grumwald stains. For the blood leucocytes count, mice were killed by cervical displacement and 20 μl of blood were collected from the brachial plexus. A blood smear was prepared and stained as described previously.

Myeloperoxidase concentrations

The extent of neutrophil accumulation in the peri-articular tissue and right knee was measured by assaying MPO activity, as previously described[14]. Briefly, the peri-articular tissue was removed and snap-frozen in liquid N_2. Upon thawing and processing, the tissue was assayed for MPO activity by measuring the change in optical density at 450 nm using tetramethylbenzidine. The results were expressed as the neutrophil index that denotes the activity of MPO related with casein-elicited murine peritoneal neutrophils processed in the same way.

Measurement of cytokines in peri-articular tissue

The concentration of TNF-α, IL-1β and chemokine (C-X-C motif) ligand 1/keratinocyte chemoattractant (CXCL1/KC) in mice was measured in peri-articular tissue of the animals. In rats, the levels of neutrophil chemoattractant-1-inducing cytokine (CINC-1), TNF-α and IL-1β were evaluated in tarsotibial joint tissue. These assays were conducted using sandwich ELISA according to the procedures supplied by the manufacturer (R&D Systems). The tissue was homogenised in PBS (0·4 M-NaCl and 10 mM-$NaPO_4$) containing anti-proteases (0·1 mM-phenylmethylsulphonyl fluoride, 0·1 mM-benzethonium chloride, 10 mM-EDTA and 20 Kallikrein inhibitor units of aprotinin A) and 0·05 % Tween 20. This solution was added in the following proportion: 1 ml of solution per each 100 mg of tissue. The samples were then centrifuged during 10 min at 3000 ***g*** and the supernatant immediately used for ELISA assays at 1:3 dilution in PBS.

Selenium determination

After Selemax® treatment, the liver, blood and knee were weighted, macerated and lyophilised for Se analysis. The

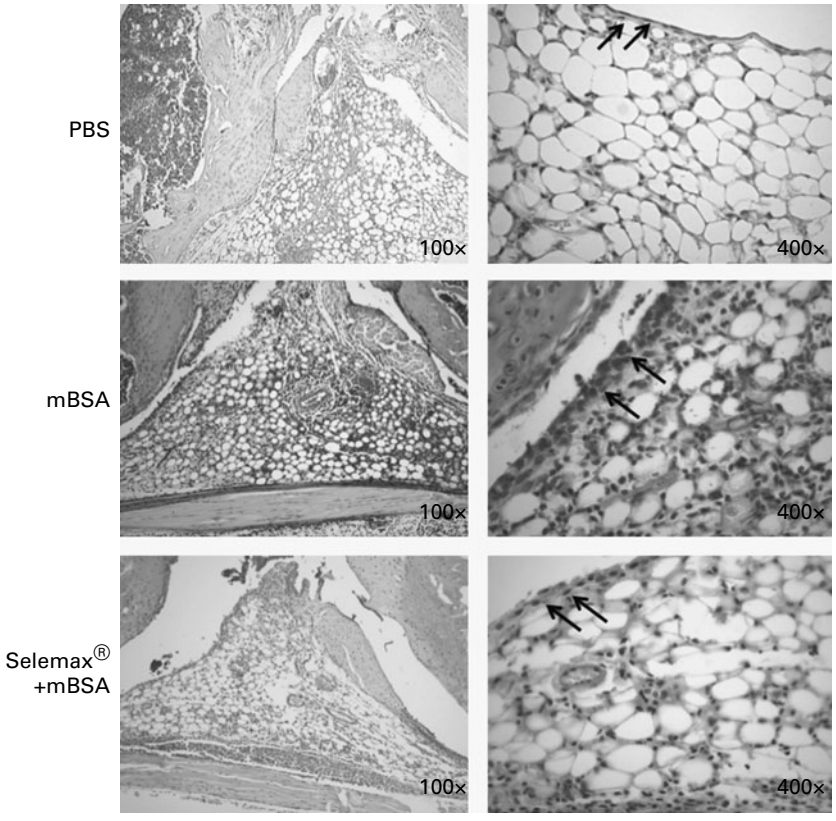

Fig. 2. Histological evaluation on the knee joint from mice. Selemax® (1 %) was added in the diet 7 d before the induction of arthritis. Sections of the knee joints were stained with haematoxylin and eosin to assess the histopathological features in the control animals (PBS), arthritic animals (methylated bovine serum albumin; mBSA) and Selemax®-treated arthritic animals (Selemax + mBSA) 24 h after induction of arthritis or injection of sterile PBS as control. Original magnification 100 × and 400 × . Representative results are shown. Arrows indicate synovial membranes.

pellet was scraped, transferred to appropriate tubes and dried at 70°C for 2 d. The neutron activation analysis, k_0-method, was applied to determine the Se concentrations in the samples. The cells were weighed in the irradiation vials accompanied by standards of Se and were irradiated for 8 h in the TRIGA MARK I IPR-R1 research nuclear reactor, at 100 kW; the average thermal neutron flux is $6{\cdot}6 \times 10^{11}$ n/cm^2 per s. The γ-spectroscopy was performed on an high-purity germanium (HPGe) detector with 15 % efficiency and the characteristic peak of Se radionuclide was used to calculate the elemental concentration according to the methodology previously described[15].

Measurement of hypernociception

The method for measuring hypernociception has been previously described elsewhere[16,17]. Briefly, the response of control (naive) and arthritic rats to five flexions of the tarsotibial joints of both hindpaws was tested daily for 16 d starting from day 0 and maintained until day 16. The results are reported as the means of arthritis nociception index, with their standard errors. The index was calculated by evaluating the number of vocalisations obtained following five flexions of the hindlimb tarsotibial joints. The local animal ethics committee approved the procedures described previously.

Measurement of oedema

Hindpaw volume was used as an indicator of paw oedema and was measured daily using an Ugo Basile hydroplethysmometer (model 7150). The results are reported as changes in paw volume (ml). All measurements were obtained at the same time of the day.

Histology

The knee joint was removed and fixed during 24 h with 10 % paraformaldehyde (pH 7·2). The joints were then incubated in 20 % EDTA at pH 7·2 for 5 d at room temperature to bone decalcification. Samples were washed with PBS and dehydrated. After being embedded in paraffin, the joints were sliced into 3 μm-thick sections that were stained with haematoxylin and eosin. The slides were coded and examined by a single pathologist who was unaware of the experimental conditions of each group.

Macrophage culture

The mice were treated with Selemax® or Se-free yeast after injection of 3 % thioglycolate solution (intraperitoneally). After 3 d, the cells were harvested by peritoneal lavage in ice-cold

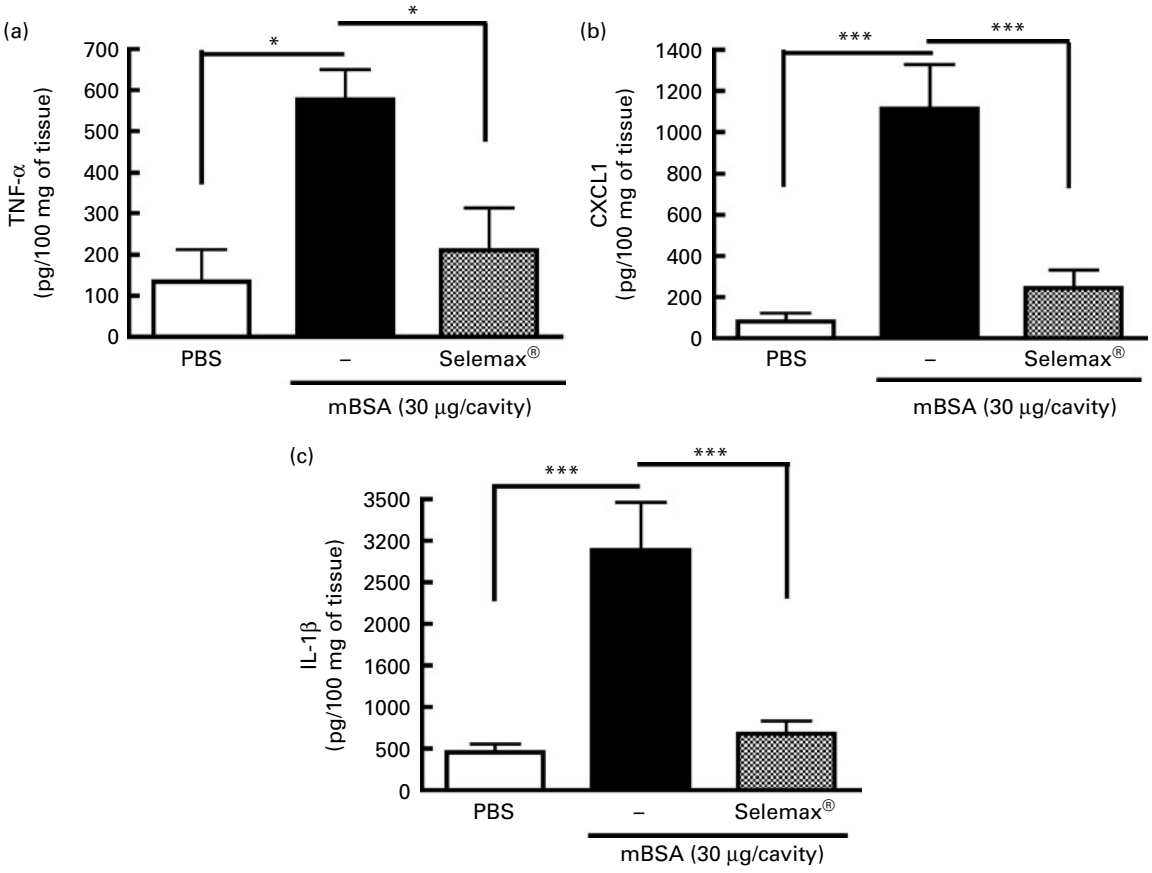

Fig. 3. Effects of Selemax® on cytokine production in peri-articular tissue of mice. Treatment with Selemax® (1 %) decreases (a) TNF-α, (b) chemokine (C-X-C motif) ligand 1/keratinocyte chemoattractant (CXCL1/KC) and (c) IL-1β levels in the antigen-induced arthritis model. Values are means of five animals in each group, with their standard errors represented by vertical bars. Mean values were significantly different: *$P<0{\cdot}05$, ***$P<0{\cdot}001$. □, Control mice; ■, arthritic mice; ▩, arthritic mice treated with Selemax®. mBSA, methylated bovine serum albumin.

PBS and plated at a density of 10^6 cells/ml in a twenty-four-well tissue culture dishes. Non-adherent cells were removed after 3 h. Macrophages were cultured overnight in Roswell Park Memorial Institute (RPMI) (Gibco®; Life Technologies Company) supplemented with 10 % heat-inactivated fetal calf serum, 25 mM-HEPES, 50 mM-2-mercaptoethanol, 100 μg/ml penicillin and 100 μg/ml streptomycin, and 2 mM-L-glutamine. All cells were stimulated in serum-free RMPI with 100 ng/ml of ultrapure lipopolysaccharide (LPS from *Escherichia coli* serotype 0111:B4; Sigma Chemical Company) for 16 h (or with PBS as a control). Apocynin (Sigma Chemical Company) was added at a 10 mM concentration to the group of cells as an antioxidant control. Cell culture supernatants were collected for cytokine determinations as described previously.

Reactive oxygen species assay

Peritoneal macrophages were isolated from Selemax® (1 %)- or control-treated mice and were cultured in ninety-six-well plates (0·5 × 106 cells/well) using RPMI medium without phenol-red (Gibco) supplemented with 10 % heat-inactivated fetal calf serum (Gibco) for 24 h in a humidified incubator at 37 °C with 5 % CO_2. The cells were then loaded for 30 min with ROS-specific fluorescent probe 2′,7′-dichlorofluorescin diacetate (20 μM final concentration; Sigma), washed twice with pre-heated medium and exposed to LPS (100 ng/ml). Fluorescence was assessed at 5 min intervals within 95 min with a spectrofluorimeter (Synergy 2 – BioTek) equipped with a fluorescein isothiocyanate filter (excitation: 485 nm; emission: 538 nm).

Statistical analysis

Results are shown as the means with their standard errors. Differences were evaluated by using ANOVA followed by Student–Newman–Keuls *post hoc* analysis. Results with $P<0·05$ were considered significant.

Results

Selemax® treatment inhibited neutrophil recruitment into the knee cavity during antigen-induced arthritis in a dose-dependent manner

In order to choose the best dose of Selemax® for the treatment of animals, four different concentrations (0·01, 0·1, 1 and 10 %)

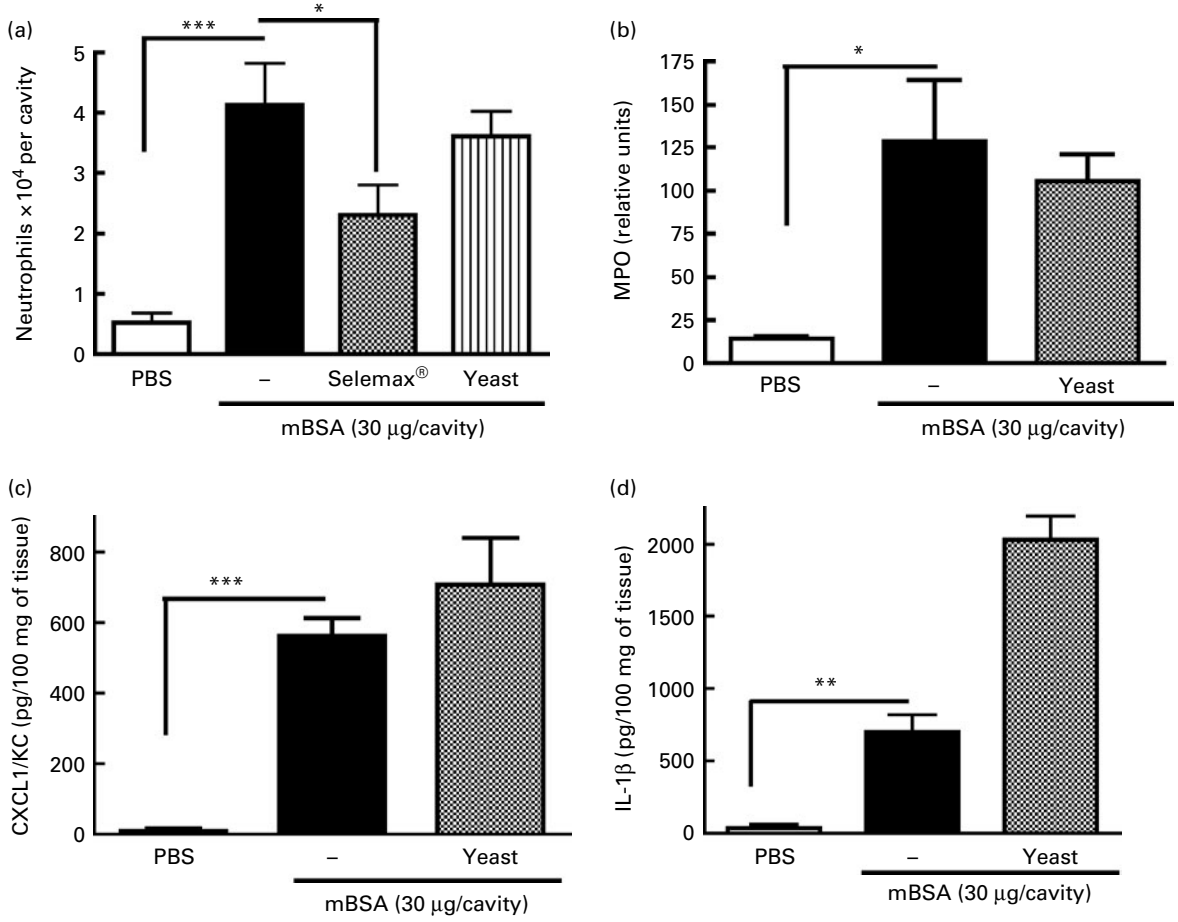

Fig. 4. Effect of selenium-free yeast on antigen-induced arthritis in mice. (a) Number of neutrophils was counted in fluid of peri-articular cavity. (b) Myeloperoxidase (MPO) and (c) chemokine (C-X-C motif) ligand 1/keratinocyte chemoattractant (CXCL1/KC) were analysed in peri-articular tissues. Values are means of five animals in each group, with their standard errors represented by vertical bars. Mean values were significantly different: *$P<0·05$, **$P<0·01$, ***$P<0·001$. mBSA, methylated bovine serum albumin.

Table 1. Effect of Selemax® in the presence of selenium in the liver, blood and knee in control and treated-mice*
(Mean values with their standard errors)

		Se (μg/g tissue)	
Group	Organ	Mean	SEM
Control	Liver	ND	
Selemax® (1 %)	Liver	11	1
Control	Blood	ND	
Selemax® (1 %)	Blood	4	0·4
Control	Knee cavity	ND	
Selemax® (1 %)	Knee cavity	ND	

ND, not detected.
* In Selemax®, Se content was found to be 940 (SEM 10) μg/g product. No Se was detected in the Se-free product.

were tested in murine AIA. Antigen challenge induced a significant increase in the number of cells recruited to the knee cavity. There was a decrease in the total number of leucocytes in mice that received 0·1, 1 and 10 % Selemax® (Fig. 1(a)). This was mostly due to significant reduction of neutrophils in the knee cavity (Fig. 1(b)). However, no differences in the total number of leucocytes and neutrophils were observed in the intra-articular cavity of arthritic mice treated with 0·01 % of Selemax® (Fig. 1(a) and (b)). Maximal reduction was obtained for Selemax® concentrations approximate to 1 % and for this reason, this concentration was used for all subsequent experiments. We preferred not to use 10 % Selemax as this would mean that a substantial proportion of the diet was composed of yeast and could represent too much exposure to Se.

Effect of Selemax® on murine antigen-induced arthritis

Histological evaluation of RA lesions was performed in mice subjected to AIA. As shown in Fig. 2, joint tissues of the methylated bovine serum albumin-injected group showed typical pathological alterations of arthritis, including synovial hyperplasia, damaged joint structures and tissue lymphocyte infiltration. However, there was reduction in joint destruction, synovial hyperplasia and inflammatory cell infiltration in the Selemax®-treated group (Fig. 2). Induction of arthritis in mice resulted in an increase in the levels of neutrophils ($P<0·05$; Fig. 1(b)), TNF-α (Fig. 3(a)), CXCL1/KC (a mouse orthologue of human IL-8; Fig. 3(b) and IL-1β; Fig. 3(c)) in peri-articular tissues. In animals treated with 1 % Selemax®, MPO activity (an index of neutrophil accumulation) was decreased, and the levels of TNF-α, KC and IL-1β were similar to those of non-arthritic animals.

To confirm whether the effects of Selemax® were due to the presence of organic Se, the same experimental model was performed using an Se-free formulation of the product. As seen in Fig. 4, the Se-free yeast-treated animals had neutrophil recruitment in the cavity (Fig. 4(a)), MPO activity (Fig. 4(b)) and chemokine (Fig. 4(c)) production that were similar to those observed in non-treated arthritic mice.

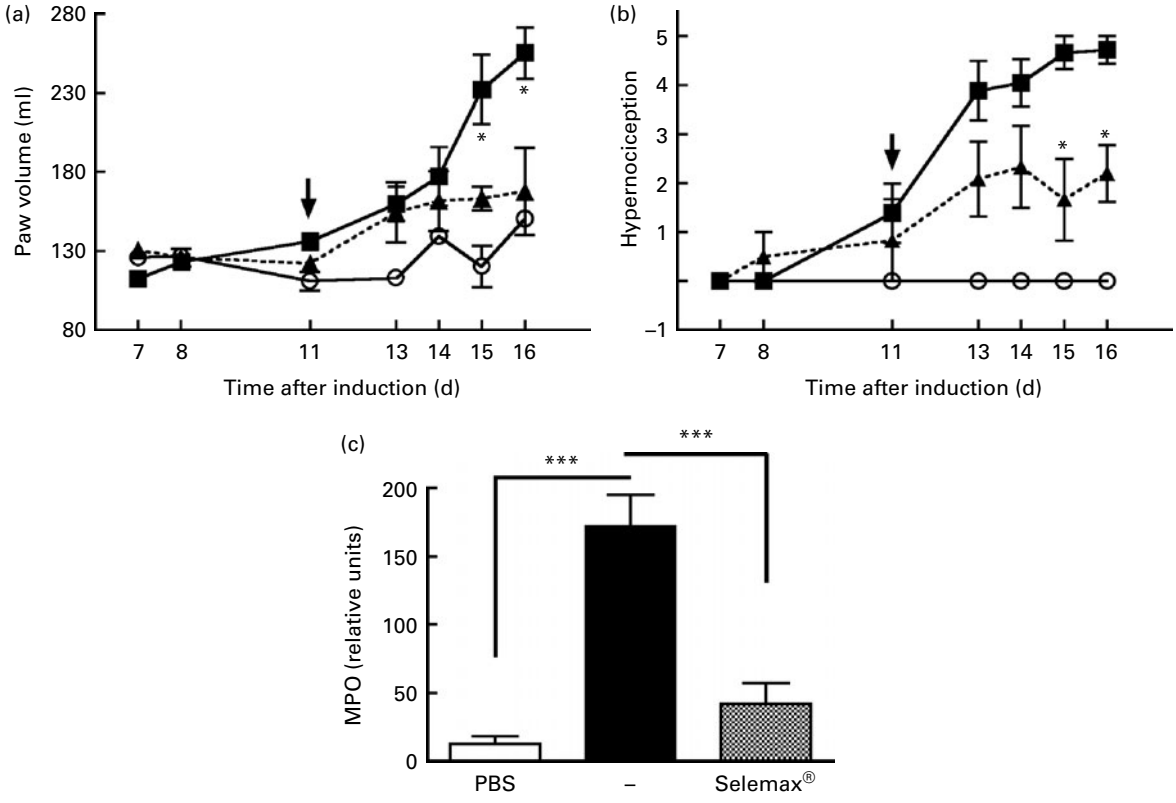

Fig. 5. Effect of treatment with Selemax® in a model of adjuvant-induced arthritis in rats. Treatment with Selemax® until 10 d after induction of disease diminished (a) paw volume, (b) hypernociception and (c) myeloperoxidase (MPO) in arthritic rats. Values are means of five animals in each group, with their standard errors represented by vertical bars. Mean values were significantly different: *$P<0·05$, ***$P<0·001$. (a,b) -○-, PBS; -■-, VEHICLE; -▲-, Selemax®. (c) □, PBS; ■, arthritis; ▩, arthritis + Selemax®.

However, IL-1β levels were higher in arthritic mice treated with Se-free yeast than in arthritic mice (Fig. 4(d)). This effect could be due to the yeast itself, since glucan components (Zymozan) present in the yeast wall may have a proinflammatory effect. We quantified Se levels in the organs of Selemax®-treated animals by neutron activation analysis in lyophilised organs (Table 1). In control mice, Se was not detectable in the organs or blood, as expected. In animals fed Selemax®, Se was found in blood, showing that treatment with Selemax® was effective in elevating systemic levels of Se.

Effect of Selemax® on adjuvant-induced arthritis in rats

To evaluate the effects of Selemax® on AdIA in rats, the morphological and clinical aspects of arthritic animals, including hindpaw swelling, hypernociception and neutrophil accumulation and cytokine production (CINC, TNF-α, IL-1β and IL-10) were analysed after disease induction. To mimic a potentially relevant clinical situation and to avoid any effect on the sensitisation phase of arthritis induction, treatment with Selemax® started on day 10 after disease induction. Hindpaw swelling reflects both inflammatory and arthritic changes occurring in rats with AdIA. The volume of swollen hindpaws in arthritic rats on days 15 and 16 after induction was about twice that found in healthy controls (Fig. 5(a)). When the animals were treated with Selemax®, the volume of swollen hindpaws was similar to that of animals in the control group (no-arthritis-induced mice; Fig. 5(a)). Treatment of rats with Selemax® also reduced inflammatory hypernociception (Fig. 5(b)) and neutrophil influx (Fig. 5(c)) compared to non-treated arthritic mice. There was also significant inhibition of peri-articular levels of TNF-α (Fig. 6(b)), IL-1β (Fig. 6(c)) and even IL-10 (Fig. 6(d)) cytokines. Selemax® treatment did not affect the levels of CINC (Fig. 6(a)). These data demonstrate that Selemax® treatment was also effective in reducing arthritis disease in the AdIA model in rats.

Ex vivo effects of treatment with Selemax® in macrophages

High levels of ROS production were detected in LPS-stimulated macrophages *in vitro* (Fig. 7). Peritoneal macrophages harvested from mice treated with Selemax® (1%), and stimulated *in vitro* with LPS, showed significant reduction of ROS production (Fig. 7). Furthermore, the levels of CXCL1 (Fig. 8(a)) and TNF-α (Fig. 8(b)) were also elevated in macrophages stimulated with LPS, but a reduction in the production of these cytokines was observed when macrophages stimulated *in vitro* with LPS were obtained from mice treated with Selemax®. Macrophages from mice treated with Selemax® also showed a decreasing production of TNF-α and CXCL1 (Fig. 8(a) and (b)) when treated with apocynin, an antioxidant drug. These results, together, suggest that the protective effect of Selemax® could be due to the antioxidant effects of Se on the immune cells.

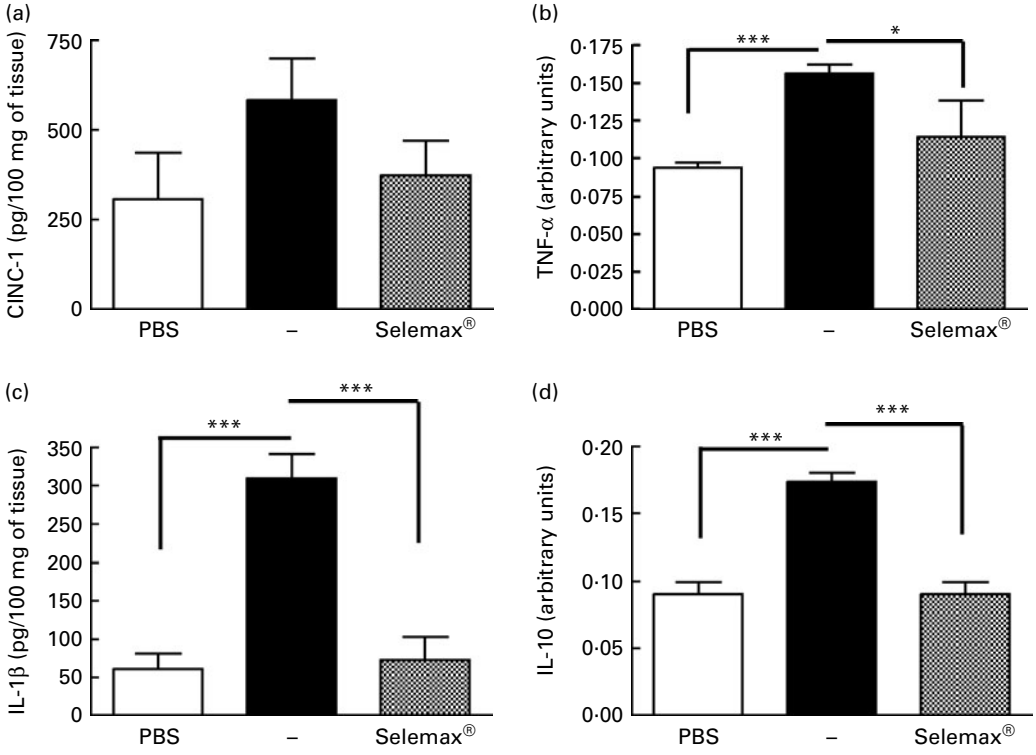

Fig. 6. Effect of Selemax® on adjuvant-induced arthritis (AdIA) in rats. Treatment with Selemax® until 10 d after induction of the disease decreased the levels of (b) TNF-α, (c) IL-1β, (d) IL-10, but not (a) neutrophil chemoattractant-1-inducing cytokine (CINC-1) in the AdIA model. Values are means of five animals in each group, with their standard errors represented by vertical bars. Mean values were significantly different: $^{*}P<0{\cdot}05$, $^{***}P<0{\cdot}001$. □, PBS; ■, arthritis; ▩, arthritis + Selemax®.

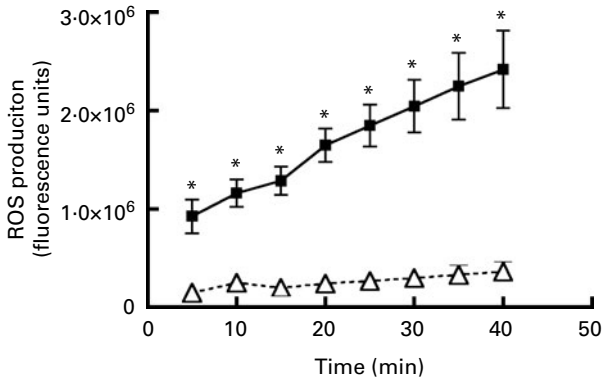

Fig. 7. Kinetic of reactive oxygen species (ROS) production in mice peritoneal macrophages. Macrophages, pooled from mice treated or not with Selemax® (1 %; -△-) were plated (3×10^5 cells/well) and stimulated with lipopolysaccharide (100 ng/ml) for 16 h. ROS production was measured in the supernatant of macrophage culture. Values are means of five animals in each group, with their standard errors represented by vertical bars. *All the results obtained with the macrophages from non-treated mice were statistically different from those of treated mice ($P<0·05$). -■-, Control.

Discussion

In the present study, we highlight three major findings: (i) treatment with Selemax® reduced inflammation in both experimental models of rat and murine arthritis; (ii) the efficiency of treatment with Selemax® was associated with increased systemic levels of Se; (iii) the principal valuable effect of treatment with Selemax® was to reduce ROS production that consequently decreased proinflammatory cytokines production and infiltration of leucocytes at the site of inflammation.

Se, as an essential component of selenocysteine-containing protein, is involved in several aspects of cell biochemistry and function, and may act as an antioxidant[18]. Treatment with a diet rich in Selemax® was able to decrease the total number of cells and neutrophils in the synovial cavity of mice subjected to AIA. The decrease of neutrophils was confirmed by their reduction in the knee cavity of arthritic mice treated with Selemax®. This effect was observed not only in an acute model of AIA in mice, but also in a chronic model of AdIA in rats as measured by MPO activity in the knee tissue. Neutrophils are an important source of proinflammatory mediators, including cytokines, ROS and enzymes, and are relevant in mediating tissue injury associated with the studied arthritis model. Indeed, prevention of polymorphonuclear (PMN) cell recruitment prevented tissue oedema, injury and functional responses in the models of arthritis[19–22]. The potential relevance of TNF-α, IL-1β and CXCL1/KC on arthritis has been suggested[23]. The decreased cell recruitment and production of proinflammatory mediators, including TNF-α, IL-1β and CXCL1, observed in arthritic animals treated with Selemax® could explain the overall prevention of tissue destruction in both arthritis models. Therefore, amelioration of arthritis observed in Selemax®-treated mice seems to be secondary to reduction in neutrophil recruitment and the consequent prevention of cytokine release and tissue injury.

The involvement of Se in arthritis has been previously emphasised. Previous studies[24,25] showed that synovial fluid and plasma Se concentrations in patients with RA were significantly lower than those in healthy individuals. This can be a sign, of depletion or redistribution of Se from the plasma pool into other tissues as a defence mechanism, that it might be modulated by proinflammatory and immunoregulatory cytokines. We measured the concentration of Se in mice treated or not with Selemax® and we observed an increase of Se levels in the liver and blood of animals after treatment with Selemax®. So, treatment with Selemax® increased the systemic levels of Se and, in the context of arthritis, treatment with Selemax® could supply the deficiency of Se, as demonstrated in previous studies[9]. However, it was still possible that the observed effects could be due to the presence of the inactive yeast or parts of the yeast cell wall[26–28]. Refuting this hypothesis, we

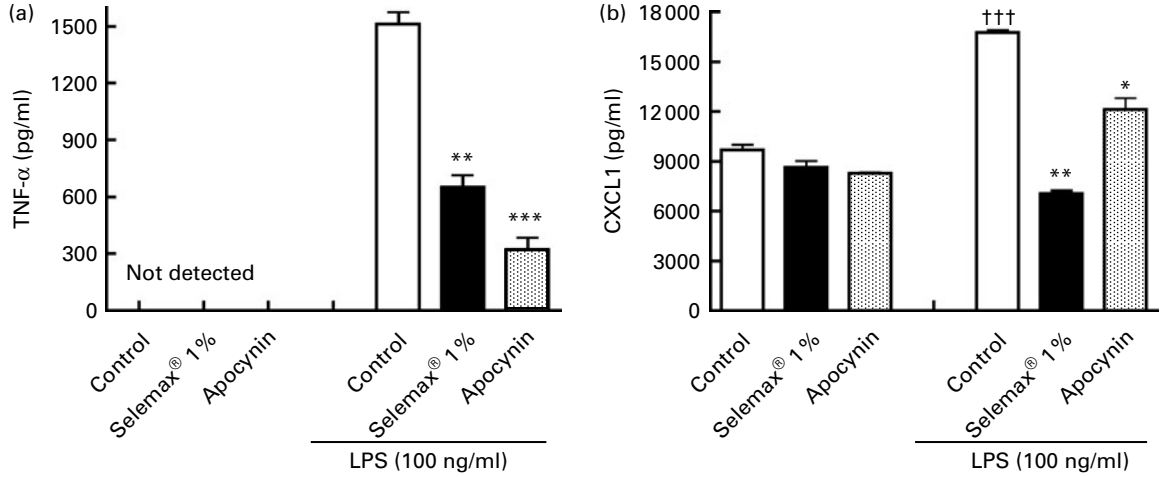

Fig. 8. Influence of Selemax® treatment on cytokine production from isolated macrophages. Macrophages were harvested from mice treated or not with Selemax® (1 %), plated (3×10^5 cells/well) and stimulated with lipopolysaccharide (LPS; 100 ng/ml) for 16 h. (a) TNF-α and (b) chemokine (C-X-C motif) ligand 1/keratinocyte chemoattractant (CXCL1/KC) were measured in the supernatant of macrophage culture by ELISA. Values are means of five animals in each group, with their standard errors represented by vertical bars. *Mean values were significantly different from those of the macrophage stimulated with LPS without treatment: *$P<0·05$, **$P<0·01$, ***$P<0·001$. †††Mean values were significantly different from that of the cells not stimulated with LPS ($P<0·001$).

have shown that the treatment with Se-free yeast failed to show the anti-inflammatory effect in arthritic animals. This finding highlights the role of Se in the protective effect of Selemax® in arthritis.

Se is an important natural antioxidant that is essential in many metabolic processes in humans and animals. It is found in nature in two forms, inorganic and organic. Organic Se is related to amino acids such as methionine and cysteine. Outdoor-living animals that eat plants take Se in the form of selenomethionine. Due to the mentioned metabolic processes, there is a constant need for supplementation of mostly inorganic Se to the animal diet. Taking into consideration Se toxicity, a limit of inorganic Se supplementation was determined[29]. This compelled scientists to produce Se in organic form, which is metabolised as bound to methionine and better used by an organism.

Incorporation of Se into selenoproteins is crucial for the important functions in various inflammatory aspects. Vunta *et al.*[30] demonstrated previously that supplementation of Se to macrophages leads to a significant decrease in the LPS-induced expression of important proinflammatory genes, such as cyclo-oxygenase-2 and TNF-α, via the inhibition of the mitogen-activated protein kinase pathway. In agreement with these previous findings, our results showed that treatment with Selemax® reduced the macrophage production of TNF-α and CXCL1 levels. These cytokines play an important role in the recruitment of neutrophils in arthritis[2,12]. Moreover, TNF-α stimulates IL-8 which induces the migration of neutrophils to the site of inflammation by increasing the molecules of adhesion[31]. Beside this, the activity of TNF-α seems to be dependent on the generation of intracellular ROS. In the present study, we observed that macrophages from mice treated with Selemax® and stimulated with LPS had a reduced generation of ROS and consequently reduced TNF-α and CXCL1 production. All these results together suggest that Selemax® treatment inhibits the neutrophil migration by an antioxidant effect of Se on macrophage.

Se-deficiency in mice exacerbated the LPS-mediated infiltration of macrophages into the lungs, suggesting that Se status was a crucial host factor that regulates inflammation. Along the same lines, other studies have shown that Se supplementation of macrophages decreased the expression of proinflammatory genes, cyclo-oxygenase-2 and inducible NO syntheses, via the inactivation of NF-κB, whereas the expression of cyclo-oxygenase-1 was unaffected[32,33]. Therefore, Se is able to modulate the inflammatory activation of leucocytes and this activity may be relevant for the observed anti-inflammatory activity of Selemax® in arthritic animals.

Based on the data presented earlier and taking into account the antioxidative properties of organic Se, it is possible to suggest that an enriched diet with organic Se could ameliorate the injury caused by arthritis. These findings highlight the contribution of Se in modulating the pathophysiology of RA and suggest that Selemax® is a new promising and effective adjunct treatment in patients with arthritis due to an efficient form of presentation of a rich organic Se content which produced anti-inflammatory effects in the experimental models of arthritis.

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