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## Redaktion

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## Macrophage depletion with clodronate-containing liposomes affects the incidence and development of rheumatoid arthritis

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by chronic arthritis leading to irreversible joint destruction [1]. The pathogenesis of RA is not yet fully understood, and most RA models are limited to pathogenic microbial infection. Unfortunately, specific approaches aimed at preventing cell influx and neovascularization or normalizing fibroblast differentiation are not yet available. Moreover, trials targeting T and B cells in RA have yielded disappointing results.

Evidence accumulated in recent years strongly suggests that the presence of synovial macrophages is essential for initiation and maintenance of arthritis, and that the number of synovial macrophages in the target organ may predict joint destruction. Tissue damage induced by autoantibodies and cell necrosis lead to in situ proliferation and differentiation of mononuclear cells/macrophages [2], and activated macrophages release pro-inflammatory cytokines and present antigen information to T and B cells. Effector T and B cells further damage the tissue, necrotic cells again activate macrophage proliferation and differentiation, and a series of vicious cycles are established. Anti-autoimmune cells, cell necrosis, and macrophages constitute a vicious circle in which macrophages play a key role and can promote the progress of rheumatoid arthritis. Therefore, we hypothesized that following macrophage depletion,

when microorganisms carrying heterophilic antigen enter the body to produce autoantibodies and cause cell necrosis, there will be no further induction of in situ macrophage proliferation, and effector T and B cells will not be able to further damage the tissue, thus breaking the vicious cycle described above.

The current study was designed to verify the key role of macrophages in this cycle and investigate the impact of macrophages on the initiation and maintenance of RA by depleting macrophages. We infer that macrophage depletion could be more effective than blocking single macrophage-derived mediators.

Herein, the effects of macrophage depletion with clodronate-containing liposomes (Clo-lip) on the initiation and maintenance of RA was investigated. After depleting macrophage with Clo-lip [3], the rate of creation of the RA model was evaluated. Histological features and inflammatory cells were observed by HE (haematoxylin and eosin) staining. The study may provide new research perspectives and perhaps therapeutic approaches.

### Materials and methods

#### Mice and reagents

BALB/c mice were obtained from the Affiliated Zhongshan Hospital of Dalian University. Ethical approval was obtained from local institutional review boards (Affiliated Zhongshan Hospital

of Dalian University decision number DW2014-109). The monoclonal antibodies (mAbs) and fluorescein isothiocyanate (FITC)-labeled anti-mouse CD68 were purchased from eBioscience (eBioscience, ThermoFisher, Waltham, Massachusetts, USA). Clo-lip were purchased from Qifa (Qifa, Shanghai City, China). Biotin-streptomycin immunohistochemical assay kit was purchased from Zhongshanjinjiao Biotechnology (Beijing, China).

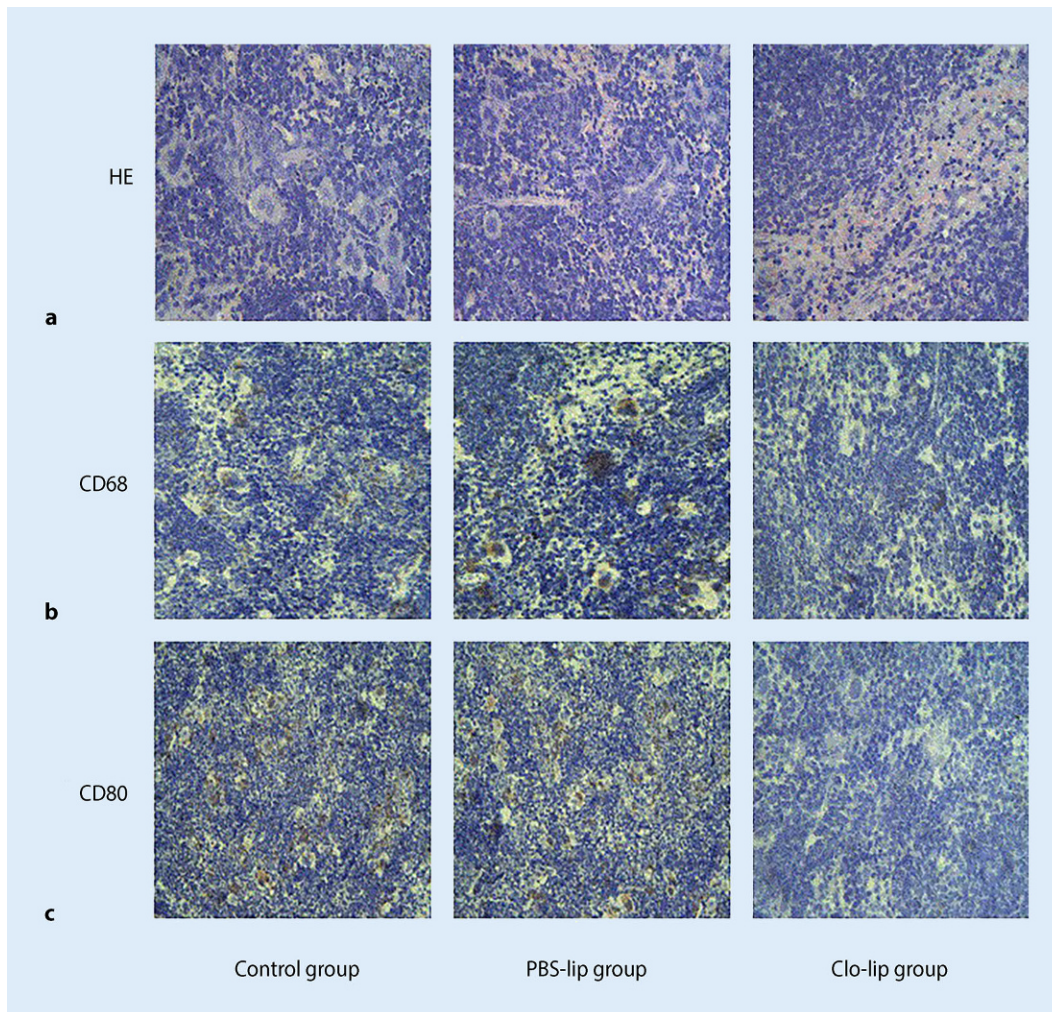
#### Macrophage depletion with clodronate-containing liposomes

Firstly, 0.2 mm of Clo-lip was injected into the peritoneal cavity of BALB/c mice, which were sacrificed 48 hours later by cervical dislocation. The spleen was removed. The effect of macrophage depletion was evaluated by HE and immunohistochemical staining.

#### Preparation of an RA model after macrophage depletion

Mice were randomly divided three groups: control group (A), phosphate-buffered saline (PBS)-lip group (B), and Clo-lip group (C). The groups were administered normal saline, PBS-lip, and Clo-lip, respectively, intraperitoneally at the following timepoints: the 1st day, 5th day, 9th day, 13th day. The RA model mice were established at the following timepoints: the 3th day, 10th day, 17th day. Type II collagen was dissolved in

R. Yuan contributed equally to the manuscript.



**Fig. 1** ◀ Effect of macrophage depletion with clodronate-containing liposomes (*Clo-lip*) administered via intraperitoneal injection. **a** HE (haematoxylin and eosin) staining indicated less or no horse-shoe-shaped nuclei—the bulk of typical polynuclear macrophages—in the *Clo-lip* group, with many in control and phosphate-buffered saline (PBS)-lip groups. **b** immunohistochemical (IHC) staining indicated less or no brown tag-CD68 macrophages in the *Clo-lip* group, with many in the control and PBS-lip group. **c** IHC staining indicated less or no brown tag-CD80 macrophages in *Clo-lip* group, with many in the control and PBS-lip group. (a–c: 40×,  $p < 0.05$ )

0.1 mol/L acetic acid, stirred and adjusted to a concentration of 2 mg/mL overnight at 4 °C. The inactivated BCG (Bacillus Calmette – Guerin) was placed in liquid paraffin, which was prepared into 2 mg/mL complete Freund’s adjuvant (CFA). The mixture of type II collagen and the same volume of CFA were prepared for injection. Mice were injected with the above mixture at 3–4 points in the joint via subcutaneous injection, once more after 1 and 2 weeks. The successfully established experimental mice had red swelling on the upper limbs after 3 weeks.

### Evaluation of joints and degree of arthritis after macrophage depletion

The toes and joints of each group of mice were observed from the 15th day after pri-

mary immunization and every day thereafter for 7 consecutive weeks. We quantified the experimental mice. The arthritis score in mice was as follows: 0 point: normal appearance, 1 point: slight red swelling, 2 points: severe redness, affecting the entire claw, 3 points: claw deformation or joint rigidity or loss of function. The degree of arthritis score was evaluated by accumulating four-paw scores: the score of RA mice was the sum of all the diseased joint scores, with a maximum score of 12. The higher the arthritis score, the more severe the joint symptoms.

### HE staining after macrophage depletion

Mice were sacrificed by cervical dislocation on the 49th day after primary immunization. Upper limbs of mice were used

for HE staining to observe the degree of the infiltration by inflammatory cells.

### Immunofluorescence after macrophage depletion

The synovial cell suspension was collected on the 49th day after primary immunization and fixed with 4% paraformaldehyde for 5 min at room temperature (RT) and permeabilized with 0.5% Triton X-100 (Amresco, USA) for 10 min on ice. The cells were blocked for nonspecific binding by immersion in blocking solution (PBS containing 0.1% BSA) for 30 min at RT. The cell suspension was then incubated with FITC-labeled anti-mouse CD68 overnight at 4 °C. Cells were resuspended in PBS buffer and washed twice. After final washing, cells were fixed and analyzed using a confocal laser scanning microscopy.

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## Macrophage depletion with clodronate-containing liposomes affects the incidence and development of rheumatoid arthritis

### Abstract

**Objective.** This study investigated the effect of macrophage depletion with clodronate-containing liposomes (Clo-lip) on the incidence and development of rheumatoid arthritis (RA).

**Methods.** The effect of macrophage depletion with Clo-lip in the spleen was assessed by HE (haematoxylin and eosin) staining and immunohistochemistry (IHC). Thirty BALB/c mice were randomly divided into three groups, which were administered PBS-lip, Clo-lip, or normal saline. RA model mice were then created and the appearance of the paws was observed. Expression of CD68 by macrophages was examined by immunofluorescence on the 49th day. Forty-five RA model mice

were created and randomly divided into three groups. The experiment group was administered Clo-lip at different timepoints. The degree of arthritis score was recorded during the administration. Histological features were detected by HE staining on the 84th day.

**Results.** Compared to controls, horseshoe-shaped nuclei and multi-core large cells were reduced in the experimental group (HE stain;  $p < 0.05$ ). Brown tag-CD68 and tag-CD80 macrophages were fewer in the experimental group than in the control group (immunohistochemistry;  $p < 0.05$ ). Furthermore, the degree of arthritis score in the experimental group was significantly

decreased ( $p < 0.05$ ). HE staining showed that there was no or less inflammatory cell infiltration in the articular cavity in mice in the experimental group, and that the percentage of CD68<sup>+</sup> macrophage cells in synovial cells was significantly lower than in the control group ( $p < 0.05$ ).

**Conclusion.** Macrophage depletion with Clo-lip can affect the incidence and development of RA.

### Keywords

T lymphocytes · B lymphocytes · Autoimmune diseases · Spleen · Mice

## Einfluss des Makrophagenrückgangs mittels clodronathaltiger Liposomen auf die Inzidenz und Entwicklung einer rheumatoiden Arthritis

### Zusammenfassung

**Ziel.** In der vorliegenden Studie wurden die Auswirkungen des Makrophagenrückgangs bei Gabe clodronathaltiger Liposomen (Clo-lip) auf die Inzidenz und Entstehung einer rheumatoiden Arthritis (RA) untersucht.

**Methoden.** Die Auswirkungen des Makrophagenrückgangs unter Clo-lip in der Milz wurden anhand der HE-Färbung und immunhistochemischer Untersuchungen (IHC) ermittelt. Dazu wurden 30 BALB/c-Mäuse randomisiert in 3 Gruppen unterteilt, denen PBS-lip, Clo-lip oder normale Kochsalzlösung verabreicht wurde. Dann wurden RA-Modell-Mäuse erzeugt und das Erscheinungsbild der Pfoten beobachtet. Mittels Immunfluoreszenz wurde am 49. Tag die Expression von CD68 bei Makrophagen untersucht. Außerdem

wurden 45 RA-Modell-Mäuse erzeugt und randomisiert in 3 Gruppen unterteilt. Der Versuchsgruppe wurde Clo-lip zu verschiedenen Zeitpunkten verabreicht. Während der Verabreichung wurde die Höhe des Arthritiscores dokumentiert. Histologische Merkmale wurden anhand der HE-Färbung am 84. Tag ermittelt.

**Ergebnisse.** Im Vergleich zu den Kontrollen waren hufeisenförmige Kerne und mehrkernige große Zellen in der Versuchsgruppe reduziert (HE-Färbung;  $p < 0,05$ ). Braune CD68- und CD80-markierte Makrophagen waren in der Versuchsgruppe weniger vorhanden als in der Kontrollgruppe (Immunhistochemie;  $p < 0,05$ ). Darüber hinaus war die Höhe des Arthritiscores in der Versuchsgruppe

signifikant vermindert ( $p < 0,05$ ). In der HE-Färbung zeigte sich, dass es keine oder eine geringere Entzündungszellinfiltration in die Gelenkhöhle bei Mäusen in der Versuchsgruppe gab und dass der Anteil an CD68-positiven Makrophagen bei den Synovialzellen signifikant niedriger als in der Kontrollgruppe war ( $p < 0,05$ ).

**Schlussfolgerung.** Ein Makrophagenrückgang unter Clo-lip-Gabe kann die Inzidenz und Entstehung einer RA beeinflussen.

### Schlüsselwörter

T-Lymphozyten · B-Lymphozyten · Autoimmunerkrankungen · Milz · Mäuse

## Macrophage depletion with clodronate-containing liposomes after rheumatoid arthritis induction

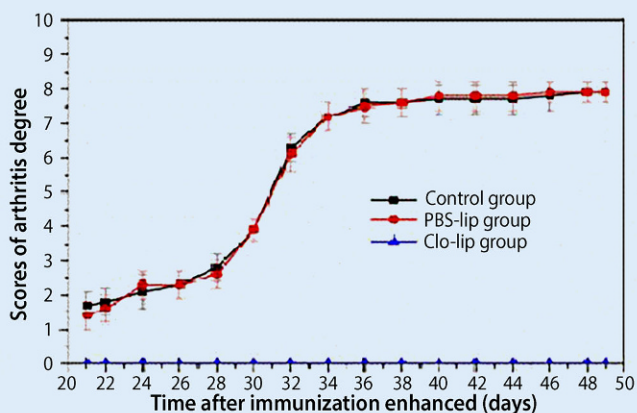
RA mice were randomly divided into three groups: A group (control group), B group (PBS-lip group), and C group (Clo-lip group), which were administered normal saline, PBS-lip, and Clo-lip, respectively, at the following timepoints: 42nd day, 45th day, 49th day, 53rd day,

56th day, 59th day, 63rd day, 66th day, 70th day, 74th day, 77th day, 80th day, 84th day. The degree of arthritis score was recorded during administration.

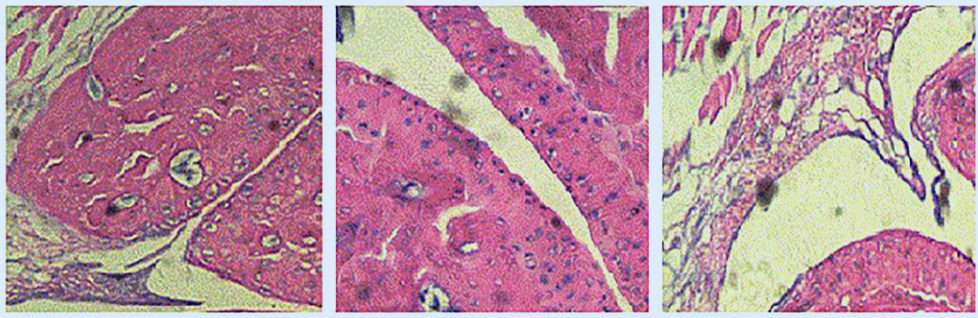
## Evaluation of joints and degree of arthritis

On the 84th day, 15 mice from each group were randomly selected and given treatment by macrophage depletion with Clo-lip after induction of RA. Mice were sacri-

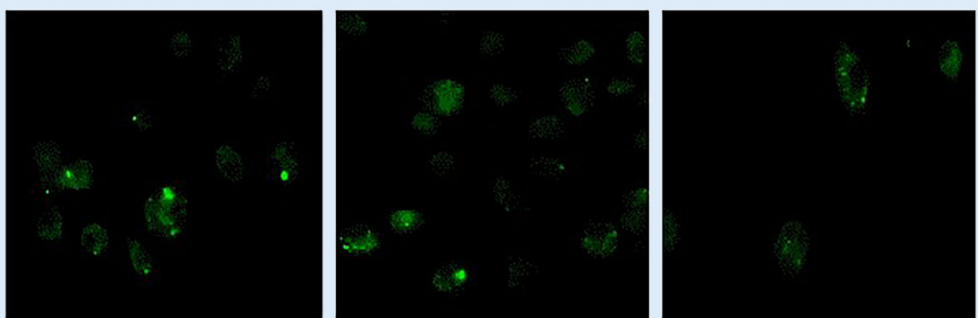
ficed by cervical dislocation. Synovial tissue was fixed with 4% paraformaldehyde. Sections made from paraffin-embedded tissues were deparaffinized in xylene and dehydrated by passing through a series of graded ethanol. Sections were used for HE staining to observe the degree of infiltration by inflammatory cells and tissue structure.



a



b



c

Control group

PBS-lip group

Clo-lip group

**Fig. 2** ◀ Effect of macrophage depletion with clodronate-containing liposomes (*Clo-lip*) on the incidence of rheumatoid arthritis. Mice were sacrificed by cervical dislocation on the 49th day after primary immunization. **a** Degree of arthritis score beginning at the 21st day in the three groups. **b** HE (haematoxylin and eosin) staining of infiltrating inflammatory cells of the articular cavity. **c** Immunofluorescence analysis of CD68 expression by macrophages in synovia. Cells were stained for CD68-FITC. **a**, **c** Values are mean  $\pm$  SD of 10 mice. Results were considered highly statistically significant for  $p < 0.05$

**Statistical analysis**

The GraphPad Prism (GraphPad Software Inc., CA, USA) statistical analysis system was used for data statistical analysis.  $p < 0.05$  was considered statistically significant.

**Results**

**Effect of macrophage depletion with clodronate-containing liposomes**

In order to investigate the effect of macrophage depletion with *Clo-lip* administered by intraperitoneal injection, the spleen was removed and subjected

to HE and immunohistochemical staining. HE staining revealed that horse-shoe-shaped nuclei—the bulk of typical polynuclear macrophage cells—were significantly reduced in the *Clo-lip* group compared to the control and PBS-lip groups ( $p < 0.05$ ). Meanwhile, brown tag-CD68 macrophage and tag-CD80 macrophages in the *Clo-lip* group were significantly less than in control and PBS-lip groups according to immunohistochemical staining ( $p < 0.05$ ). These results clearly indicate that intraperitoneal injection of *Clo-lip* can deplete macrophages (Fig. 1).

**Effect of macrophage depletion on the rate of RA model creation, joints, and degree of arthritis**

To determine whether macrophage depletion with *Clo-lip* could influence the rate of establishment of the RA model, mice were sacrificed by cervical dislocation on the 49th day after primary immunization. There was no death before the 49th day. The rate of establishment of the RA model was zero in the *Clo-lip* group; however, all mice successfully created the RA model in the control and PBS-lip groups. In order to investigate the effect of macrophage depletion with *Clo-lip* on joints and the degree of arthritis, we observed the appearance after the first

**Table 1** Comparison of the degree of arthritis score beginning at the 21st day in the three groups. ( $\bar{x} \pm s$ ;  $n = 10$ )

Group	d21	d22	d24	d26	d28	d30	d32	d34	d36	d38	d40	d42	d44	d46	d48	d49
Clo-lip	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	1.7 ± 0.4 <sup>a</sup>	1.8 ± 0.4 <sup>a</sup>	2.1 ± 0.5 <sup>a</sup>	2.3 ± 0.4 <sup>a</sup>	2.8 ± 0.4 <sup>a</sup>	3.9 ± 0.3 <sup>a</sup>	6.3 ± 0.4 <sup>a</sup>	7.2 ± 0.4 <sup>a</sup>	7.6 ± 0.4 <sup>a</sup>	7.6 ± 0.4 <sup>a</sup>	7.7 ± 0.4 <sup>a</sup>	7.7 ± 0.4 <sup>a</sup>	7.7 ± 0.4 <sup>a</sup>	7.8 ± 0.4 <sup>a</sup>	7.9 ± 0.3 <sup>a</sup>	7.9 ± 0.3 <sup>a</sup>
PBS-lip	1.4 ± 0.4 <sup>b</sup>	1.6 ± 0.4 <sup>b</sup>	2.3 ± 0.4 <sup>b</sup>	2.3 ± 0.4 <sup>b</sup>	2.6 ± 0.4 <sup>b</sup>	3.9 ± 0.3 <sup>b</sup>	6.1 ± 0.5 <sup>b</sup>	7.2 ± 0.4 <sup>b</sup>	7.5 ± 0.5 <sup>b</sup>	7.6 ± 0.4 <sup>b</sup>	7.8 ± 0.4 <sup>b</sup>	7.8 ± 0.4 <sup>b</sup>	7.8 ± 0.4 <sup>b</sup>	7.9 ± 0.3 <sup>b</sup>	7.9 ± 0.3 <sup>b</sup>	7.9 ± 0.3 <sup>b</sup>

*Clo-lip* clodronate-containing liposomes, *PBS* phosphate-buffered saline,  $\bar{x} \pm s$  mean + standard deviation  
<sup>a</sup>Compared to Clo-lip group:  $t = 8.578, p < 0.05$   
<sup>b</sup>Compared to Clo-lip group:  $t = 8.343, p < 0.05$

**Table 2** Changes in the degree of arthritis score in the different groups ( $\bar{x} \pm s$ ;  $n = 15$ )

Group	d42	d45	d49	d53	d56	d59	d63	d66	d70	d74	d77	d80	d84
Clo-lip	7.4 ± 0.4	7.0 ± 0.2	6.4 ± 0.4	6.2 ± 0.4	5.8 ± 0.4	5.6 ± 0.4	5.5 ± 0.4	5.3 ± 0.4	4.9 ± 0.4	4.7 ± 0.4	4.3 ± 0.4	4.1 ± 0.3	4.0 ± 0.2
Control	7.6 ± 0.4 <sup>a</sup>	7.7 ± 0.4 <sup>a</sup>	7.7 ± 0.4 <sup>a</sup>	7.8 ± 0.4 <sup>a</sup>	7.9 ± 0.2 <sup>a</sup>	8.0 ± 0.2 <sup>a</sup>	7.9 ± 0.2 <sup>a</sup>	8.0 ± 0.2 <sup>a</sup>	8.0 ± 0.2 <sup>a</sup>	8.1 ± 0.3 <sup>a</sup>	8.2 ± 0.4 <sup>a</sup>	8.2 ± 0.4 <sup>a</sup>	8.3 ± 0.4 <sup>a</sup>
PBS-lip	7.5 ± 0.4 <sup>b</sup>	7.6 ± 0.4 <sup>b</sup>	7.6 ± 0.4 <sup>b</sup>	7.6 ± 0.4 <sup>b</sup>	7.7 ± 0.4 <sup>b</sup>	7.7 ± 0.4 <sup>b</sup>	7.7 ± 0.4 <sup>b</sup>	7.8 ± 0.4 <sup>b</sup>	7.8 ± 0.4 <sup>b</sup>	8.0 ± 0.3 <sup>b</sup>	8.0 ± 0.5 <sup>b</sup>	8.1 ± 0.3 <sup>b</sup>	8.1 ± 0.3 <sup>b</sup>

$\bar{x} \pm s$  mean + standard deviation, *Clo-lip* clodronate-containing liposomes, *PBS* phosphate-buffered saline  
<sup>a</sup>Compared to Clo-lip group:  $t = 8.072, p < 0.05$   
<sup>b</sup>Compared to Clo-lip group:  $t = 7.536, p < 0.05$

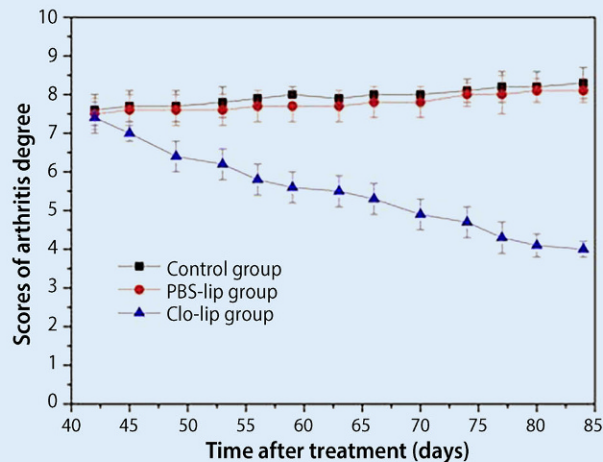
immunization and recorded the degree of arthritis score beginning at the 21st day. The toe joint had no change in inflammation and a normal appearance from the first immunization to the end of the observation period on the 49th day in the Clo-lip group; however, in control and PBS-lip groups, after the 21st day, single toe joints began to swell and the degree of arthritis gradually began to become serious. The degree of arthritis score in the Clo-lip group was significantly lower ( $p < 0.05$ ) compared to the control and PBS-lip group (■ Fig. 2; ■ Table 1).

### The effect of macrophage depletion on inflammatory cells infiltrating the articular cavity

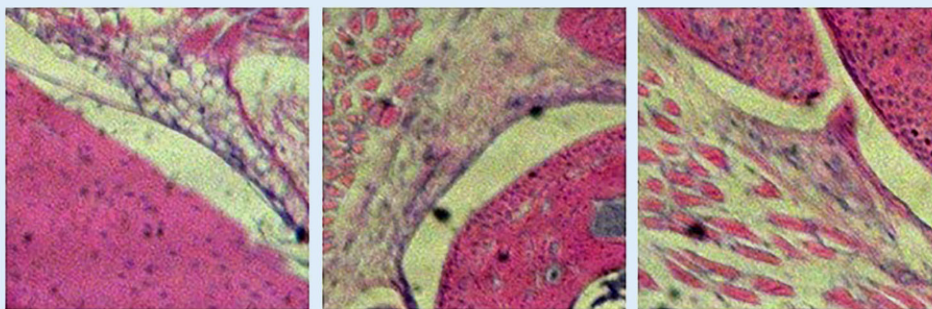
In order to investigate the effect of macrophage depletion with Clo-lip on inflammatory cells infiltrating the articular cavity, mice were sacrificed by cervical dislocation on the 49th day after primary immunization. The upper limbs of mice were used for HE staining to observe the degree of infiltration by inflammatory cells. HE staining showed a clear articular cavity, synovial membrane cells arranged orderly, and no inflammatory cell infiltration in synovial tissue; the articular cartilage surface was smooth in the Clo-lip group. However, there was serious inflammation in subcutaneous soft tissue, clear congestion, edema, and inflammatory cell infiltration—which included a lot of polymorphonuclear leukocytes, monocytes, and lymphocytes—in the control and PBS-lip groups. Cartilage and bone damage were serious and a lot of inflammatory cells could be seen in the joint cavity in control group and PBS-lip group mice (■ Fig. 2).

### The effect of macrophage depletion on CD68 expression by macrophages in synovia

In order to investigate the effect of macrophage depletion with Clo-lip on CD68 molecular expression by macrophages in synovia, mice were sacrificed by cervical dislocation on the 49th day after primary immunization. The synovial cell suspension was collected.



a



Control group

PBS-lip group

Clo-lip group

b

**Fig. 3** Effect of macrophage depletion with clodronate-containing liposomes (*Clo-lip*) on development of RA. **a** Three groups were differentially administered normal saline, phosphate-buffered saline (PBS)-lip, or *Clo-lip* after creation of the RA model at the various timepoints. During administration, the degree of arthritis score was recorded. The degree of arthritis score was lower in the *Clo-lip* group than in the other two groups. **b** Mice were sacrificed by cervical dislocation on the 84th day. There were still inflammatory cells and bone destruction in control and PBS-lip groups. Less inflammatory cell infiltration was present in the *Clo-lip* group

The results showed that the expression rate of FITC-labeled anti-mouse CD68 in the *Clo-lip* group was significantly lower than in the other groups ( $p < 0.05$ ; [Fig. 2](#)).

### The effect of macrophage depletion on RA development

In order to investigate the effect of macrophage depletion with *Clo-lip* on RA development, the three groups were differentially administered normal saline, PBS-lip, or *Clo-lip* after the creation of the RA model at the various timepoints. During the administration, the degree of arthritis score was recorded. Results revealed that symptoms relieved and the degree of arthritis score reduced gradually. The degree of arthritis score was significantly lower in the *Clo-lip* group than the other two groups ( $p < 0.05$ ; [Fig. 3](#); [Table 2](#)).

### The effect of treating macrophage depletion on inflammatory cells infiltrating the articular cavity

In order to investigate the effect of treatment with *Clo-lip* to deplete macrophages on inflammatory cells infiltrating the articular cavity, 15 mice were randomly selected in each group at the 84th day. Results indicated that there were still inflammatory cells and bone destruction in control and PBS-lip groups, whereas less inflammatory cell infiltration was present in the *Clo-lip* group ([Fig. 3](#)).

### Discussion

Rheumatoid arthritis is a chronic autoimmune disease characterized by articular synovial inflammation. Many macrophages infiltrate the articular synovial fluid and tissue. Publications in the literature have reported that this is related to inflammatory reaction, pannus,

and increased fibrous tissue and joint fibrosis, which imply that macrophages plays a key role in the development and progression of rheumatoid arthritis [4–13].

The macrophage is an important immune cell, and new studies have confirmed that macrophages can proliferate and differentiate in tissue in situ under appropriate factors [14, 15]. In a previous study, we noticed that some ascitic cells isolated from melanoma patients survived, proliferated, and differentiated into giant phagocytes after the other cells died. In the present study, the effects of dying cells on abdominal exudate cells and the biological characteristics of the differentiated cells were studied. Morphologic studies have revealed that the large phagocytes possessed characteristics of macrophages [1]. This discovery not only helps to deepen understanding of the pathogenesis of RA but also provides a new approach for its treatment.

When microenvironmental conditions change, macrophages can polarize and differentiate into M1 or M2 cells [16–18]. The M1 macrophage is a cell with immune functions, which contribute to promoting inflammation responses and eliciting specific immune responses [19]. M1 macrophages in the articular synovia of mice phagocytose dead cells and present antigen information to T and B cells; effector T and B cells aggravate damaged tissue and cell necrosis further strengthens and induces macrophage proliferation, which forms a vicious circle. However, the M2 macrophage is anti-inflammatory cell. Recent studies have shown that M2 macrophages are also involved in wound healing or tissue remodeling, formation of pannus, rheumatoid nodules, and joint fibrosis. The study of RA and clinical treatment practice support our hypothesis. We deplete alone effector B and T cells, but the macrophages proliferate in the articular cavity in situ and present antigen to newborn B and T cells. These newborn B and T cells were stimulated to be differentiated into producing autoantibodies T and B cells, thus further damaging the tissue. We deplete the macrophage with Clo-lip, observing an effect on the incidence and development of RA.

To evaluate the effect of macrophage depletion with Clo-lip intraperitoneal injection, the spleen was removed for evaluation by HE and immunohistochemical staining. HE staining revealed that horse-shoe-shaped nuclear—the bulk of typical polynuclear macrophage cells—were significantly reduced in the Clo-lip compared to the control and PBS-lip groups. Meanwhile, brown tag-CD68 tag-CD80 macrophages, as assessed by immunohistochemical staining, were significantly fewer in the Clo-lip group than in control and PBS-lip groups. These results clearly illustrate the efficacy and stability of the Clo-lip effect. The degree of arthritis score was statistically significantly lower in the Clo-lip group than in the other two groups. Upper limb joint tissue sections of mice showed a clear articular cavity, synovial membrane cells arranged orderly, no inflammatory cell infiltration in synovial tissue, and that the articular cartilage surface was smooth in the

Clo-lip group. There was serious inflammation in subcutaneous soft tissue, clear congestion, edema, and infiltration of inflammatory cells such as polymorphonuclear leukocytes, monocyte, and lymphocytes in the control and PBS-lip groups; cartilage and bone damage was serious and a lot of inflammatory cells could be seen in the joint cavity. The expression rate of CD68 as assessed by immunofluorescence in synovial cells in the Clo-lip group was less than in the other groups, and the difference was statistically significant. Whether or not macrophages are present affects the incidence of RA. We conclude that microorganisms carrying heterophilic antigen enter the body, the production of autoantibodies leads to synovial cell necrosis, but necrotic synovial cells cannot induce the proliferation of macrophage in situ because of the depletion with Clo-lip. Furthermore, effector T cells cannot further damage the synovial tissue, which hampers the incidence of RA.

The three groups were administered normal saline, PBS-lip, or Clo-lip after the establishment of the RA model at the following time points: 42nd day, 45th day, 49th day, 53rd day, 56th day, 59th day, 63rd day, 66th day, 70th day, 74th day, 77th day, 80th day, 84th day. During administration, the degree of arthritis score was recorded. The degree of arthritis score was lower in the Clo-lip group than in the other two groups and the difference was statistically significant. There was less inflammatory cell infiltration of synovial tissue in mice in the Clo-lip group on the 84th day. We conclude that macrophages influence the development of RA. Macrophage depletion after RA development can alleviate infiltration of inflammatory cells and relieve joint symptoms.

Because of the reduced bioavailability of chlorine phosphonic acid with the long duration, the depletion of macrophages after clodronate liposome administration is reversible. We injected the phosphonic acid salt intermittently to deplete the macrophages. In short, depletion of macrophage influences the incidence of RA. Despite this temporary effect, a single administration of these liposomes is effective in prophylactic settings and

has therapeutic effects in chronic ongoing arthritis. Whether the newborn macrophage affect its formation or not will need to be studied in future investigations.

## Conclusion

Depleting macrophages affects the incidence and development of RA in mice, which provides a new research perspective for the mechanism of RA and also a potential new target for its prevention.

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## Compliance with ethical guidelines

**Conflict of interest.** Q. Zhang, R. Yuan, C. Li, W. Wei, W. Shen, Y. Cui, and X. Yuan declare that they have no competing interests.

This article does not contain any studies with human participants. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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## Immer mehr Kliniken geht es schlecht

**Viele deutsche Krankenhäuser schweben in Insolvenzgefahr. Die wirtschaftliche Lage der Kliniken hat sich insgesamt verschlechtert – wohl auch deswegen, weil Patienten seltener stationär behandelt werden mussten.**

Nach mehreren Jahren des Anstiegs ist die Umsatzrendite der deutschen Krankenhäuser im Jahr 2017 um 0,5 Punkte gegenüber dem Vorjahr auf 1,7% gefallen. Als Hauptgrund nennt der „Krankenhaus Rating Report 2019“ erstmals seit langem wieder gesunkene stationäre Fallzahlen (minus 0,5% gegenüber 2016). Als mögliche Gründe dafür nennt das Papier, das von einem Team um den Gesundheitsökonom Prof. Boris Augurzky vom RWI – Leibniz-Institut für Wirtschaftsforschung erarbeitet wurde, zunehmende Ambulantisierung der Medizin, Fachkräftemangel, hohen Sättigungsgrad bei kardiologischen und orthopädischen Leistungen, gesteigerten Informationsstand der Patienten und intensivere MDK-Prüfungen. Für 2018 erwarten die Experten eine Stagnation der Fallzahl. Der Report, der 877 Krankenhäuser mit einem am Umsatz gemessenen Marktanteil von 70 Prozent erfasst, zeigt dabei im Ländervergleich erhebliche Unterschiede bei den Ertragskennziffern der Krankenhäuser.

### „Osten ist die Benchmark“

Die besten Umsatzrenditen erwirtschaften demnach die Kliniken in Ostdeutschland (Mittelwert 3,1%). „Der Osten ist die Benchmark“, sagt Co-Autor Dr. Sebastian Krolop.

Die Häuser in Sachsen-Anhalt und Thüringen kommen auf Renditen von 3,8%, gefolgt von Sachsen mit 3,1% und Berlin, Brandenburg und Mecklenburg-Vorpommern mit jeweils 2,6%. Von den West-Ländern schaffen nur Nordrhein-Westfalen (1,9%) und Niedersachsen sowie Bremen (je 1,8%) einen besseren Wert als der gesamtdeutsche Durchschnitt von 1,7%. Darunter liegen Bayern und Hessen mit je 1,6% Umsatzrendite, Schleswig-Holstein und Hamburg mit 1,3% sowie Rheinland-Pfalz und das Saarland mit 0,8%. Einen negativen Wert weist gar Baden-Württemberg auf mit minus 0,6%.

### Private Krankenhäuser weisen größte Umsatzrendite aus

Auch beim Blick auf die Trägerschaft zeigen sich große Diskrepanzen. Die größte Umsatzrendite erwirtschafteten im Jahr 2017 private Krankenhäuser mit 5,8% (2016: 5,8%). Den zweiten Platz belegten die freigemeinnützigen Kliniken mit 1,3% (2,6%). Schlusslicht sind die Häuser in öffentlicher Hand mit minus 0,3%. Im Vorjahr hatten sie noch 0,9% erwirtschaftet. Frappierend ist dort die Entwicklung der Zahl der Häuser mit negativem Jahresergebnis: Waren 2016 noch mehr als ein Viertel der Öffentlich-Rechtlichen im Minus, schrieben 2017 mit 51% mehr als die Hälfte rote Zahlen. Aber auch bei den Freigemeinnützigen lag mehr als ein Viertel im Minus.

### Große regionale Unterschiede

Der Anteil der Kliniken, die 2017 ein negatives Jahresergebnis erwirtschafteten, betrug in:

- Baden-Württemberg (51%)
- Bayern (26%)
- Berlin, Brandenburg, Mecklenburg-Vorpommern (zusammen 26%)
- Bremen, Niedersachsen (zusammen 26%)
- Hamburg, Schleswig-Holstein (26%)
- Hessen (32%)
- Nordrhein-Westfalen (23%)
- Rheinland-Pfalz, Saarland (zusammen 43%)
- Sachsen (18%)
- Sachsen-Anhalt, Thüringen (zusammen 13%)

**Quelle: Ärzte Zeitung  
(www.aerztezeitung.de)**