Suppression of collagen-induced arthritis by lipopolysaccharide in DBA/1 mice

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Abstract. – OBJECTIVE: Injection of lipopolysaccharide (LPS) has both promotion and inhibition effects on the autoimmune disease. Given the variable roles of LPS in autoimmune diseases, the role of LPS played in collagen-induced arthritis (CIA, autoimmune disease) model remains to be further determined.

MATERIALS AND METHODS: CIA was induced by intradermal injection of collagen type II (CII) in DBA/1 mice (day 0) followed by a booster injection on day 21. Mice of CIA with LPS injection group (CIA+ LPS group) were intraperitoneally injected with 50 μg LPS on day 42. Tissues such as carpal joints and fingers were stained with hematoxylin and eosin (H&E) for histopathology analysis. Inflammation, pannus formation and bone resorption were monitored by a macroscopic scoring system. Serum level of IgG2a antibody was determined by enzyme-linked immunosorbent assay (ELISA).

RESULTS: The incidence of arthritis in CIA group was much higher than that in CIA+ LPS group (100%: 46.5%, $p < 0.05$), as same as the arthritis score (5.38:1.37, $p = 8.16 \times 10^{-6}$). Besides, the histopathologic score was also higher in CIA group than that in CIA+ LPS group (15.0:5.36). Compared with CIA group, mild synovial hyperplasia and no articular cartilage damage were observed in CIA+ LPS group. Besides, mice of CIA group produced a significantly higher level of IgG2a than CIA+ LPS group (3922 ng/ml: 2084 ng/ml, $p = 0.0333$) when arthritis developed.

CONCLUSIONS: Our findings showed that LPS might suppress CIA progression under special conditions, opening up a new understanding of the roles of LPS in arthritis and new possibilities for a clinical therapy of CIA.

Key Words:
  Collagen-induced arthritis, Lipopolysaccharide, Suppression, Endotoxin tolerance, Collagen type II.

Introduction

Collagen-induced arthritis (CIA) is a classical autoimmune model of rheumatoid arthritis (RA), including several pathological features, such as synovial hyperplasia, mononuclear cell infiltration, cartilage degradation and so on[1-3]. The CIA model has been widely used to identify potential pathogenic mechanism of autoimmunity, including the roles of individual cell types in disease onset and progression, as well as to design and test new therapeutics[4]. The development of CIA is strain-dependent, with H-2^q and H-2^r haplotypes showing the greatest degrees of susceptibility[5]. As the original "gold standard" of CIA, DBA/1 strain (H-2^q) is the most commonly used strain for pre-clinical testing of potential anti-arthritic drugs[6]. Collagen type II (CII), the major cartilage protein, is a relevant joint-specific autoantigen in the pathogenesis of RA[7]. Most of CIA were induced by using twice intradermal injection of CII emulsified in complete Freund's adjuvant (CFA) in susceptible DBA/1 mice[8]. CIA induced with CII/CFA results in a rapid and severe polyarthritis of the peripheral articular joints. The polyarthritis first appears around 3-4 weeks after disease challenge and becomes progressively worse for approximately 2-4 weeks before slowly waning[9].

Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, is composed of lipid A (or endotoxin), core polysaccharide and O-specific polysaccharide[10]. Many studies demonstrated that injection of LPS into normal mice would lead to the transient formation of autoantibody[11,12]. Thus LPS, a strong inducer of pro-inflammatory cytokines, always acts synergistically in the induction of arthritis by autoantibody[13]. On the other hand, in experimental autoimmune encephalomyelitis (EAE) model, treating neonatal rats with LPS during their first week of life significantly suppressed EAE-induced spinal cord damage[14]. In this model, the onset of EAE was delayed by LPS injected early in life, which can alter the predisposition to inflammation in adulthood[15]. One of the suppression mechanisms may be endotoxin toler-

ance. Innate immune cells exposed to minute amounts of endotoxin become refractory to subsequent endotoxin challenge[16]. So, endotoxin tolerance could suppress inflammatory diseases. LPS, containing endotoxin, can also make innate immune cells enter a transient unresponsive state at a low concentration and the innate immune cells are unable to respond to further challenge of LPS[17,18]. Thus, given the variable roles of LPS in autoimmune diseases, the role of LPS played in CIA model remains to be further determined.

In our study, DBA/1 mice were immunized by using CII/CFA. Occurrence of arthritis, histological assessment, inflammation and IgG2a analysis were conducted to assess the influence of LPS on CIA. Consequently, our findings suggested that LPS intraperitoneally injected into mice would suppress the development of CIA, which provided a new role of LPS in CIA model.

Materials and Methods

Animals

Male DBA/1 mice (6-8 weeks), provided by China National Rodent Laboratory Animal Resources, Shanghai Branch, were used for establishing arthritis model. All experimental procedures were approved by the local Ethical Review Process Committee. The mice were housed and bred in an individual ventilated caging system and were divided into 3 groups, as normal group (n = 8), CIA group (n = 8) and CIA with LPS injection group (CIA+ LPS group, n = 25).

Induction of CIA

To induce CIA, chicken type II (Sigma Chemical, St. Louis, MO, USA) was dissolved in 0.05 M acetic acid at 2 mg/ml and emulsified in equal volume of CFA (Sigma Chemical, St. Louis, MO, USA). Mice of CIA group and CIA+ LPS group were intradermally injected with the emulsion (100 μL) containing 100 μg CII (day 0). Twenty-one days later, a secondary injection was given at the same concentration used in primary immunization. 50 μg LPS from *Escherichia coli* 055:B5 (Sigma Chemical, St. Louis, MO, USA) were intraperitoneally injected into each mouse of CIA+ LPS group on day 42, just before the onset of arthritis[19]. Mice were examined 3-4 times a week for clinical signs of arthritis. The severity of arthritis is defined using an arthritis index which is the sum of the individual clinical scores for each mouse. The individual clinical score was determined as follows: 0 = no disease; 1 = mild swelling or redness of just one group of joints; 2 = swelling and redness of one whole foot which can move normally; 3 = moderate swelling and redness of one whole foot whose moving ability is partly limited; 4 = the joints are severe swelling, redness, and/or ankylosis, and the limb can't move at all[20].

Histological Assessment

Mice were sacrificed on day 90 after primary immunization. Four paws (including carpal joints, fingers, ankles, tarsal joints, and toes) were surgically removed from each animal. And all these tissues were fixed in paraformaldehyde, decalcified, and embedded in paraffin. Then, the sectioned tissues were stained with haematoxylin and eosin (H&E) for histopathology.

A histopathological score similar to Sims et al[20] was determined by levels of synovial proliferation, articular cartilage damage and inflammation. Inflammation was scored 0-4 according to the following criteria: 0 = normal; 1 = minimal inflammatory infiltration; 2 = mild infiltration; 3 = moderate infiltration with lymphoid aggregates; 4 = marked infiltration with lymphoid aggregates and edema. Pannus formation was defined as synovial proliferation adjacent to cartilage and filling the joint space and was scored 0-3 as follows: 0 = none; 1 = minimal; 2 = moderate (invasion of < 50% of the cartilage surface); 3 = severe (invasion of ≥ 50% of the cartilage surface). Bone resorption was scored 0-4 as follows: 0 = none; 1 = minimal (1-2 sites of resorption, visible only at high magnification); 2 = mild (at least 3 sites of resorption, visible only at high magnification); 3 = moderate (obvious foci of resorption, visible at low power); 4 = marked (large erosions extending to the marrow space). Diagnosis was assessed by an experienced pathologist.

IgG2a Analysis

Serum samples were collected when the mice were sacrificed on day 90, and stored at -80 °C before analysis. Serum levels of IgG2a antibody were determined by enzyme-linked immunosorbent assay (ELISA, Chondrex Inc., Redmond, WA, USA). Only 7 of 25 mice in one cage were selected for IgG2a antibody test in CIA+ LPS group.

Statistical Analysis

The appearance of arthritis between groups of mice were compared using the Chi-square test.

The clinical and histological severity of arthritis and IgG2a levels were analyzed by Student's *t*-test, assuming equal variances. $p < 0.05$ was considered significant. All data were expressed as the mean or mean ± standard deviation.

Results

Occurrence of CIA

No apparent arthritis was observed in normal group. The onsets of arthritis in CIA group and CIA+ LPS group were on day 44 and day 43 respectively. Although the onset of arthritis was a little late, all of DBA/1 mice in CIA group developed arthritis during 6-7 weeks. Average accumulative scores showed significant difference ($p = 2.08 \times 10^{-6}$) between CIA group and CIA+ LPS group (Table I). The frequency of arthritis of CIA group reached 100% on day 49, which was more than 50% higher compared with that of CIA+ LPS group ($p < 0.05$). The arthritis of CIA group was also much severer and longer-lasting than CIA+ LPS group during the observation time. In CIA+ LPS group, mice displayed overt toxic symptoms with dull eyes, loose hair, less movement and food intake during 48 hours after LPS intraperitoneal injection. After 72 hours, mice recovered from the toxic symptoms. Although LPS led to short term toxicity, only 28% (7/25) mice in CIA+ LPS group showed transient (1-3 days) mild arthritis during the first week after LPS injection.

Histological Analysis

Histologically, no inflammatory changes were observed in all mice of normal group. But partial and mild proliferative synovium were observed in a few mice (Table II, Figure 1, A). Accumulative scores of arthritis showed significant difference ($p = 8.16 \times 10^{-6}$) between CIA group and CIA+ LPS group (Table II). As for CIA group, infiltration of mononuclear inflammatory cells and proliferation of synovial cells were observed

in the synovium. Besides, articular cartilage damage was also observed in some joints (Figure 1, B). However, compared with the mice in CIA group, there was much less changes of inflammation of mice in CIA+ LPS group. The synovium was mild hyperplastic, and no articular cartilage damage was observed in CIA+ LPS group (Figure 1, C).

IgG2a Analysis

To assess the influence of LPS on CIA, IgG2a level of serum was measured. IgG2a level in normal group was 38 ng/ml, which was significantly lower than the CIA group ($p < 0.01$). Mice in CIA group produced significantly higher level of IgG2a (3922 ng/ml) when developed arthritis. In contrast, after LPS administration in CIA+ LPS group, the IgG2a level decreased to 2084 ng/ml, which was much lower than in CIA group ($p = 0.03$). The data was shown in Table III.

Discussion

Previous studies reported that LPS was usually used to accelerate CIA[21]. And LPS-triggered acceleration of joint inflammation is usually observed just 24-72 h after LPS administration in collagen immunized mice[22,23]. However, in the present study, LPS, the only different factor between CIA group and CIA+ LPS group, suppressed the onset and progression of CIA. So the results implied that the impact of LPS on CIA is variable depending on some unknown factors. It was not for the first time that the roles of LPS in autoimmune disease were variable. Previous studies described that the injection of LPS induced EAE in TCR-transgenic mice and relapsed encephalomyelitis in normal mice[24]. However, another study[25] found treatment with LPS before autoimmune encephalomyelitis induction delayed the onset of disease without affecting the peak severity. Specifically, mice exposed to LPS at 2 week of age showed a delayed onset and di-

Table I. Occurrences and average accumulative scores of arthritis.

Groups	Incidence %	Scores (mean)	n
Normal group	0	0	8
CIA group	100	5.38 ± 1.41	8
CIA+ LPS group	46.15*	1.37 ± 1.98**	25

*Means the significant difference ($p < 0.05$) of occurrences of arthritis between CIA group and CIA+ LPS group. **Means the significant difference ($p < 0.01$) of average accumulative scores between CIA group and CIA+ LPS group.

Table II. Average scores of arthritis at the histological level.

Groups	Inflammation	Pannus	Bone resorption	Accumulative scores	n
Normal group	0	0	0	0	8
CIA group	9.00 ± 1.60	5.00 ± 4.14	2.00 ± 2.83	15.00 ± 7.65	8
CIA+ LPS group	4.68 ± 3.40	0.68 ± 1.44	0	$5.36 \pm 3.96**$	25

**Means the significant difference ($p < 0.01$) of average accumulative scores between CIA group and CIA+ LPS group.

Table III. IgG2a levels in three groups.

	Normal group	CIA group	CIA+ LPS group
IgG2a (ng/ml)	$38 \pm 8**$	3922 ± 1483	$2084 \pm 1052*$
n	8	8	7

***Means the significant difference ($p < 0.05$) of IgG2a level between CIA group and CIA+ LPS group. **Means the significant difference ($p < 0.01$) of IgG2a level between CIA group and normal group.

minished severity of EAE. Thus, early life exposure to LPS suppressed EAE by promoting tolerogenic dendritic cells and regulatory T cells[26]. These findings illustrated the different roles of LPS in autoimmune diseases. In some cases, the injection of LPS induced autoimmune diseases, such as rheumatoid arthritis[27] and thyroiditis[28]. On the other hand, prior injection of mice with a sublethal dose of LPS protected them from a subsequent or lethal dose of LPS [18]. Maybe, in present study, exposure to bacterial LPS in early-life of the DBA/1 mice resulted in endotoxin tolerance in our experiment, which suppressed the CIA.

For investigating the mechanism of LPS suppressing CIA, IgG2a level of serum of the DBA/1 mice was tested. As was shown in present study, IgG2a level in CIA group and CIA+ LPS group was much higher than the normal and IgG2a in CIA group was also higher than that in CIA+ LPS group. The anti-CII antibodies in CIA are mainly the IgG2a subclass, and their levels are higher at the peak of arthritis. The binding and accumulation of anti-CII antibodies in particular region may initiate inflammatory responses [8, 29]. So it's reasonable that the decreasing incidence of arthritis by LPS administration was related to the decreasing IgG2a level. Nowak B et al[30] suggested that systemic administration of exopolysaccharide (EPS) markedly reduced IgG production and significantly ameliorated arthritis in the active models of CIA, especially, when LPS was used as an adjuvant alone. Similarly, Kojima F et al[31] got the same results. They demonstrated that serum IgG against CII, including subclasses IgG1,
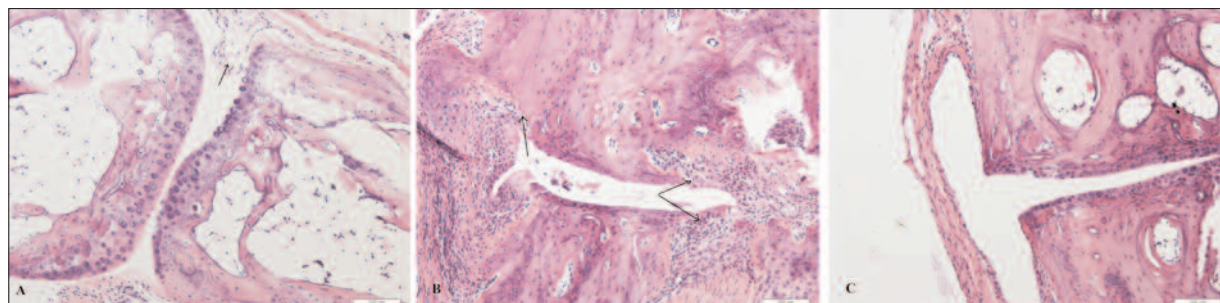

Figure 1. HE staining, original magnification × 100. **A,** Normal group. There was mild partial synovial proliferation and the surface of articular cartilage was smooth. **B,** CIA group. There were infiltration and synovial hyperplasia of inflammatory cells. The surface of articular cartilage was interrupted markedly. **C,** CIA+ LPS group. There were infiltration and synovial hyperplasia of mild inflammatory cells. The surface of articular cartilage was not interrupted.

IgG2a, IgG2b, IgG2c, and IgG3, was markedly reduced by microsomal PGE synthase-1 (mPGES-1), which correlated with the reduction inflammatory response and finally inhibited the development of CIA. All these researches suggested that the decrease of IgG level was closely related to the suppression of CIA, which was the same as our experiment results.

Conclusions

In present study, we compared arthritis procession between CIA group and CIA with LPS injection group in DBA/1 mice. The lower occurrence of CIA, fewer inflammatory changes and lower IgG2a level in CIA+ LPS group than those in CIA group suggested that LPS, to some extent, suppressed the development of CIA. The present study opens up a new understanding of the roles LPS played in arthritis and finds new possibility for clinical therapy of CIA.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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