Mod Rheumatol (2003) 13:135–138

ORIGINAL ARTICLE

Akihiro Honda · Tomihisa Koshino · Takeshi Hayashi
Toshitaka Takagi · Renzo Okamoto · Tomoyuki Saito

CD44 expression in synovial lining and cartilage of rat knees induced by intraarticular injection of synthetic lipid A

Received: April 4, 2002 / Accepted: August 28, 2002

Abstract To study the effect of synthetic lipid A on the expression of the adhesion molecule CD44 in synovium and cartilage, synthetic lipid A (10 μg/ml, 0.5 ml) diluted in 0.025% triethylamine (TEA) was injected into the left knee of male Wistar rats. The equivalent volume of 0.025% TEA was injected simultaneously into the right knee of the same rat as a control. The numbers of infiltrating neutrophils and mononuclear cells in the synovium were counted, and the expression of CD44 was detected immunohistochemically. Infiltration of neutrophils in the synovium of the knee reached a maximum at 12 h after intraarticular injection of lipid A (78.4 ± 5.6 cells/400× field), and had subsided at 7 days after injection (2.1 ± 0.6). CD44 expression in the lining cells of the synovium was detected 24 h after injection (2.5 ± 0.3 cell layers), and it lasted 7 days after injection (2.6 ± 0.4 cell layers). CD44 expression in cartilage started at 24 h (69.0% ± 4.6% positive area of specimen) and lasted 7 days after injection (27.3% ± 3.3%). Intraarticular injection of lipid A was proven to induce acute arthritis and CD44 expression in the synovial lining layers and articular cartilage.

Key words Acute inflammation · CD44 · Intraarticular injection · Lipid A

Introduction

CD44 is a cell-surface glycoprotein known to mediate cell attachment to extracellular matrix components and specific cell-surface ligands.[1,2] Takagi et al. suggested that CD44 might play an important role in joint destruction in rheumatoid arthritis (RA).[3] Rheumatoid synovitis is associated with upregulation of CD44 in the joint cartilage and synovial lining layer.[4–11] Sato et al. demonstrated expression of CD44 in the synovial lining and articular chondrocytes of mouse knee joints with collagen-induced arthritis.[12]

Repeated injection of lipopolysaccharide (LPS) resulted in arthritis, accompanied by elevation of serum rheumatoid factor, in rats.[13] Synthetic lipid A, the bioactive moiety of LPS, has been used to induce inflammation,[14,15] and administration of lipid A in a subcutaneous air pouch induced expression of CD44 on lining cells of the pouch wall with infiltrating cells in the exudate.[16]

The onset and the duration of CD44 expression is on synovial lining cells or chondrocytes in the process of inflammation is still unclear. In this study, intraarticular injection of lipid A into rat knees was carried out and CD44 expression was studied in the synovium and cartilage in the early phase of arthritis.

Materials and methods

Experimental animals

Male Jcl Wistar rats aged 8–11 weeks old, weighing 180–200 g, were used in this experiment.

Injection of lipid A

Synthetic *Escherichia coli*-type lipid A was purchased from Daiichi Kagaku Yakuhin, Tokyo, Japan. It was dissolved in 0.025% triethylamine (TEA) at 0.01 mg/ml, and 0.5 ml diluted lipid A was injected into the left knee of rats; 0.5 ml 0.025% triethylamine was injected into the right knee of the same rats simultaneously. The rats were killed at 2, 12, or 24 h or 7 days after injection.

Histological examination

After sacrifice, the knee joints were quickly removed, fixed in 10% neutral buffered formalin, and decalcified in 0.5 M

A. Honda (✉) · T. Koshino · T. Hayashi · T. Takagi · R. Okamoto · T. Saito
Department of Orthopaedic Surgery, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan
Tel. +81-45-787-2655; Fax +81-45-781-7922

ethylenediaminetetraacetic acid (EDTA) (pH 7.5) for 6 weeks. Decalcified specimens were embedded in paraffin and sectioned sagittally along the longitudinal axis of the knee joint. Then, sections were stained with hematoxylin and eosin (H&E). Inflammatory cell infiltration in the synovium was quantified. The numbers of neutrophils and mononuclear cells were counted in three randomly selected 400× microscopic fields. The mean cell number was calculated and expressed as the representative value for each specimen.

Immunohistochemical staining

Sections from the paraffin blocks were first treated with phosphate-buffered saline (PBS) and then with 5% normal goat serum for 30 min to reduce nonspecific background staining. After washing with PBS three times, the sections were incubated overnight with rat antimouse CD44 monoclonal antibodies (5 μg/ml) (Pharmingen, San Diego, CA, USA) at 4°C, and then at room temperature for 1 h. After washing with PBS three times, the sections were incubated with biotinylated purified antirat IgG antibodies, then rinsed with 0.05 M tris-HCl buffer. The sections were then incubated with peroxidase-conjugated avidine–biotin complex, followed by the addition of diaminobenzidine (Dojindo, Kumamoto, Japan) to develop the color, and washed in tap water.

Loss of metachromasia in the cartilage

To observe the structure of the cartilage, toluidine blue staining was performed.

Microscopic analysis

The maximum number of cell layers in the CD44-positive synovial lining cell layer using immunohistochemical staining was counted in a 400× visual field. The number of chondrocytes was counted in three random 400× visual fields. The percentage of CD44-positive chondrocytes out of all chondrocytes per 400× field was calculated. The data were expressed as the average percentage of three fields.

Statistical analysis

Student's *t* test was used to test the statistical significance of differences between the correlations. *P* values less than 0.05 were considered to be statistically significant.

Results

Histological findings showed that synovial lining cells were proliferated, and moderate numbers of infiltrating cells were found in the synovial tissue. The structure of articular cartilage was shown to be maintained with metachromasia by staining with toluidine blue. In our specimens, only a few layers of synovial lining were observed, but the surface of synovial membrane was irregular or wavy. The interstitial matrix of the synovium was edematous. The structure of the cartilage was maintained for 7 days after injection, which was defined as the extreme acute phase of arthritis.

Neutrophil cell infiltration was observed in the synovium of knees injected with lipid A (10 μg/ml, 0.5 ml). The mean number of infiltrating neutrophils was 43.0 ± 9.3 at 2 h after injection (range, 28–60), which was significantly greater than that in the contralateral knees injected with TEA (5.7 ± 2.3) (range, 2–10) ($P < 0.05$). Neutrophil number reached a maximum of 78.4 ± 5.6 at 12 h after injection (range, 45–110) and then gradually decreased to 2.1 ± 0.6 by 7 days after injection (range, 0–5) (Table 1).

CD44 expression in the synovial lining was detected at 24 h after injection, and the mean number of CD44-positive synovial lining cell layers was 2.5 ± 0.3 (range, 1–5). The expression lasted 7 days after injection, and the mean number of positive cell layers was 2.6 ± 0.4 (range, 0–4) (Table 2).

CD44 expression in the articular cartilage was found after 24 h, and the mean percentage of positive areas of cartilage was 69.0% ± 4.6% (range, 40%–90%). CD44-positive expression lasted for 7 days (Table 3).

Discussion

An important effect of LPS in the process of inflammation is activation of monocytes/macrophages, resulting in the

Table 1. Inflammatory cell infiltration in the synovium of rat knees injected with lipid A or triethylamine

Time after injection	Lipid A[a]		Triethylamine[b]	
	Neutrophils	Mononuclear cells	Neutrophils	Mononuclear cells
2 h ($n = 3$)	43.0 ± 9.3*	4.0 ± 1.0	5.6 ± 2.3	4.0 ± 0.6
12 h ($n = 11$)	78.4 ± 5.6**	7.6 ± 1.3	10.2 ± 2.2	2.2 ± 0.4
24 h ($n = 11$)	57.5 ± 7.5**	5.4 ± 1.2	7.4 ± 1.3	3.0 ± 0.5
7 days ($n = 11$)	2.1 ± 0.6	5.8 ± 1.1	2.1 ± 0.6	3.2 ± 0.5

Data are number of cells per 400× fields
[a] Lipid A (10 μg/ml, 0.5 ml) was injected into the left knee
[b] Triethylamine (0.025%, 0.5 ml) was injected into the right knee as a control
* $P < 0.05$ versus triethylamine injection; ** $P < 0.01$ versus triethylamine injection

Table 2. CD44 expression in the lining of rat knees after lipid A or triethylamine injection

Time after injection	Lipid A (10 μg/ml), 0.5 ml	Triethylamine (0.025%), 0.5 ml
2 h ($n = 3$)	0	0
12 h ($n = 11$)	0.7 ± 0.2	0.4 ± 0.2
24 h ($n = 11$)	2.5 ± 0.3*	0.6 ± 0.2
7 days ($n = 11$)	2.6 ± 0.4*	0.8 ± 0.2

Data are number of CD44-positive cell layers
* $P < 0.01$ versus triethylamine injection

Table 3. CD44 expression in the joint cartilage of rat knees after lipid A or triethylamine injection

Time after injection	Lipid A (10 μg/ml), 0.5 ml	Triethylamine (0.025%), 0.5 ml
2 h ($n = 3$)	0	0
12 h ($n = 10$)	0	0
24 h ($n = 10$)	69.0 ± 4.6*	25.0 ± 2.2
7 days ($n = 10$)	27.2 ± 3.3*	6.0 ± 2.7

Data are percentage of CD44-positive areas
* $P < 0.01$ versus triethylamine injection

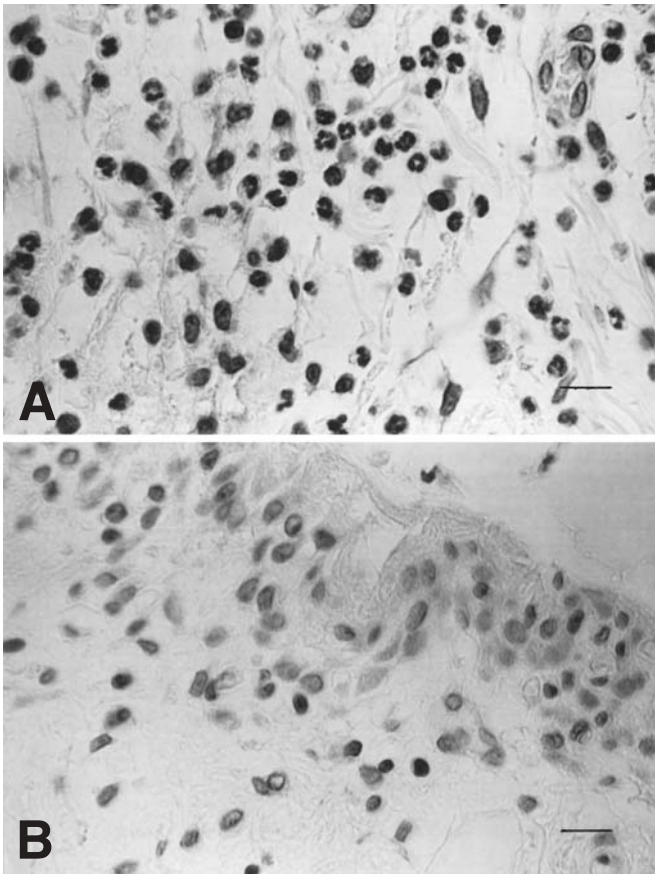

Fig. 1. Histological sections of synovium of rat knee 12 h after injection with lipid A (10 μg/ml, 0.5 ml) (**A**) and triethylamine (0.025%, 0.5 ml) (**B**). H&E staining. **A** Note the marked infiltration of neutrofils and mononuclear cells. **B** Note the scattered infiltration of mononuclear cells. *Bar* 20 μm

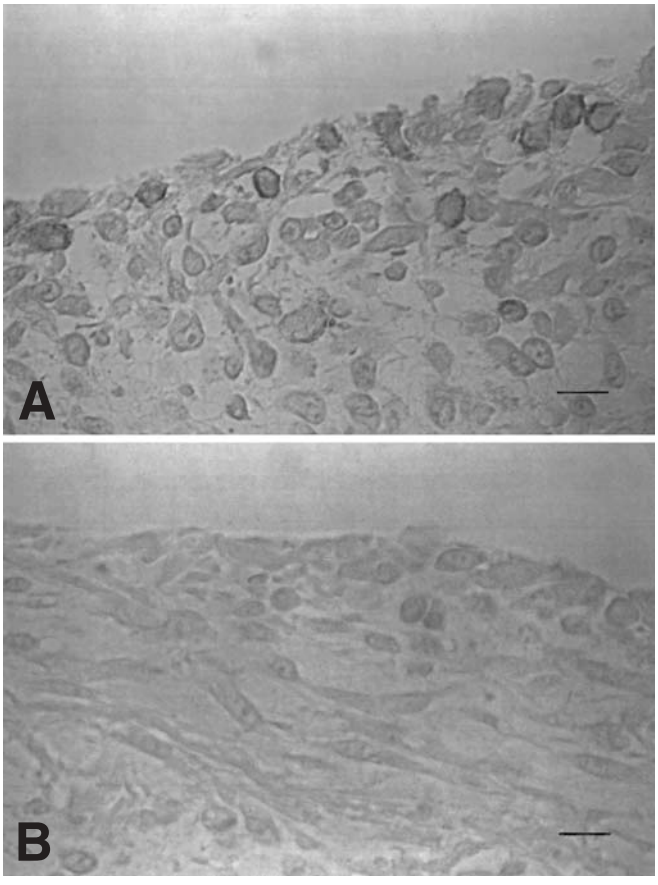

Fig. 2. Histological sections of synovium of rat knee 7 days after the injection with lipid A (**A**) or triethylamine (**B**). Anti-CD44 immunostaining. **A** Note the CD44-positive lining two cells deep. **B** Note the negative staining of the lining. *Bar* 20 μm

release of inflammatory mediators such as IL-1, IL-6, IL-8, and tumor necrosis factor (TNF).[17–20] Noyori et al.[13] reported that systemic injection of LPS obtained from *E. coli* O:14 into rats induced arthritis with elevation of anti-O:14 antibodies and rheumatoid factor in serum and upregulation of IL-1 in the synovium. Intra-air pouch injection of lipid A induced inflammation of the inner wall associated with proliferation of the lining layers with expression of CD44,[21] suggesting that lipid A might stimulate synovial cells to induce IL-1, which in turn interacts with synovial lining cells to induce the expression of CD44. CD44 might play a role as an anchor of leukocytes to promote their adhesion to synovial cells. Adhering to these cells, leukocytes produce cytokines such as IL-1 and TNF. Furthermore, signal transduction through CD44 in mononuclear cells was reported to upregulate IL-1 synthesis.[22]

Stoop et al. described increased resistance to collagen-induced arthritis (CIA) in CD44-deficient DBA/1 mice.[23] According to their study, CD44-deficient mice exhibited significant reductions in both the incidence and severity of CIA. They concluded that CD44 seems to play an important role in the development of arthritis, most likely by directing leukocyte traffic to the site of inflammation.

There are a few reports[24,25] suggesting that intraarticular LPS injection could induce acute arthritis. In the current study, we used direct injection of synthetic lipid A into the rat knee joint with simultaneous injection of TEA into the contralateral knee. Lipid A induced inflammatory cell infiltration in the injected knees, compared to the contralateral knees injected with the vehicle alone. These results suggest that cell infiltration was not induced by a systemic immunological pathway but by a local reaction.

Concerning the time course of CD44 expression and arthritis, Sato et al. reported that CD44 was detected in synovial lining cells and chondrocytes were detected in knee joints 3 weeks after immunization, and that expression increased in a time-dependent manner in mice with CIA.[12] In the current study, acute inflammatory cell infiltration reached a peak at 12 h and had subsided 7 days after injection; CD44 expression in synovial lining cells and chondrocytes started at 24 h, accompanied by infiltration of polymorphonuclear cells. Even after polymorphonuclear cells decreased, CD44 expression persisted.

Several studies have reported that CD44 expression was detected in animal models of chronic inflammation.[12,16,21] In the present study, the results indicated that CD44 expression appeared in the acute phase of inflammation and was maintained for the following period, which suggests that CD44 also might play an important role in the acute phase of inflammation.

References

1. Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. CD44 is the principal cell surface receptor for hyaluronate. Cell 1990;61:1303–13.
2. Miyake K, Underhill CB, Lesley J, Kincade PW. Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. J Exp Med 1990;172:69–75.
3. Takagi T, Suzuki K, Okamoto R, Koshino T. Expression of CD44 on human articular chondrocytes (abstract). Arthritis Rheum 1994;37(suppl):S191.
4. Brennan FR, Mikecz K, Glant TT, Jobanputra P, Pinder S, Bavington C, et al. CD44 expression by leukocytes in rheumatoid arthritis and modulation by specific antibody: implications for lymphocyte adhesion to endothelial cells and synoviocytes in vitro. Scand J Immunol 1997;45:213–20.
5. Chow G, Knudson CB, Homandberg G, Knudson W. Increased expression of CD44 in bovine articular chondrocytes by catabolic cellular mediators. J Biol Chem 1995;270:27734–41.
6. Croft DR, Dall P, Davies D, Jackson DG, McIntyre P, Kramer IM. Complex CD44 splicing combinations in synovial fibroblasts from arthritic joints. Eur J Immunol 1997;27:1680–4.
7. Fujii K, Tanaka Y, Hubscher S, Saito K, Ota T, Eto S. Cross-linking of CD44 on rheumatoid synovial cells up-regulates VCAM-1. J Immunol 1999;162:2391–8.
8. Haynes BF, Hale LP, Patton KL, Martin ME, McCallum RM. Measurement of an adhesion molecule as an indicator of inflammatory disease activity. Arthritis Rheum 1991;34:1434–43.
9. Henderson KJ, Edwards JCW, Worrall JG. Expression of CD44 in normal and rheumatoid synovium and cultured synovial fibroblasts. Ann Rheum Dis 1994;53:729–34.
10. Johnson BA, Haines GK, Harlow LA, Koch AE. Adhesion molecule expression in human synovial tissue. Arthritis Rheum 1993;36:137–46.
11. Mikecz K, Dennis K, Shi M, Kim JH. Modulation of hyaluronan receptor (CD44) function *in vivo* in a murine model of rheumatoid arthritis. Arthritis Rheum 1999;42:659–68.
12. Sato MI, Koshino T, Takagi T. CD44 expression on chondrocytes in knees of DBA/1 mice with type II collagen-induced arthritis. Clin Exp Rheumatol 1999;17:185–90.
13. Noyori K, Okamoto R, Takagi T, Hyodo A, Suzuki K, Koshino T. Experimental induction of arthritis in rats immunized with *Escherichia coli* O:14 lipopolysaccharide. J Rheumatol 1994;21:484–8.
14. Galanos C, Luderitz O, Rietschel ET, Westphal O, Brade H, Brade L, et al. Synthetic and natural *E. coli* free lipid A express identical endotoxic lipid A. Eur J Biochem 1985;148:1–5.
15. Rietschel ET, Brade L, Brandenburg K, Flad HD, De Jong-Leuveninck J, Kawahara K, et al. Chemical structure and biologic activity of bacterial and synthetic lipid A. Rev Infect Dis 1987;9(suppl):S527–36.
16. Kurosaka N, Takagi T, Koshino T. Effects of hyaluronate on CD44 expression of infiltrating cells in exudate of rat air pouch, induced by sensitization with lipopolysaccharide. J Rheumatol 1999;26:2186–90.
17. Dayer J-M, Demczuk S. Cytokines and other mediators in rheumatoid arthritis. Springer Semin Immunopathol 1984;7:387–413.
18. Flad HD, Loppnow H, Rietschel ET, Ulmer AJ. Agonists and antagonists for lipopolysaccharide-induced cytokines. Immunobiology 1993;187:303–16.
19. Lei MG, Chen TY, Morrison DC. Lipopolysaccharide/lipid A receptors on lymphocytes and macrophages. Int Rev Immunol 1990;6:223–35.
20. Loppnow H, Brade L, Brade H, Rietschel ET, Kusumoto S, Shiba T, et al. Induction of interleukin 1 by bacterial and synthetic lipid A. Eur J Immunol 1986;16:1263–7.
21. Matsukura Y, Takagi T, Okamoto R, Koshino T. Upregulation of CD44 in the inflamed mouse air pouch injected with synthetic lipid A. J Rheumatol 1998;25:539–45.
22. Denning SM, Le PT, Singer KH, Haynes BF. Antibodies against the CD44 p80 lymphocyte homing receptor molecule augment human peripheral blood T cell activation. J Immunol 1990;144:7–15.
23. Stoop R, Kotani H, McNeish JD, Otterness IG, Mikecz K. Increased resistance to collagen-induced arthritis in CD44-deficient DBA/1 mice. Arthritis Rheum 2001;44:2922–31.
24. Akahoshi T, Endo H, Kondo H, Kashiwazaki S, Kasahara T, Mukaida N, et al. Essential involvement of interleukin-8 in neutrophil recruitment in rabbits with acute experimental arthritis induced by lipopolysaccharide and interleukin-1. Lymphokine Cytokine Res 1994;13:113–6.
25. Bliven ML, Eskra JD, Otterness IG. Limitation of activity in an acute model of arthritis: effect of drug treatment. Inflamm Res 1997;46:491–5.