Inhibitory Effect of Low Density Lipoprotein on the Inflammation-Inducing Activity of Calcium Pyrophosphate Dihydrate Crystals

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ABSTRACT. ***Objective.*** It has been proposed that low density lipoprotein (LDL) plays a role in the self-limiting nature of pseudogout inflammation. We investigated changes of LDL concentration in rat air pouch fluid during periods of acute and subsiding inflammation to evaluate whether LDL contributes to inhibiting inflammation of pseudogout. We examined whether LDL binds to calcium pyrophosphate dihydrate (CPPD) crystals as a possible mechanism for reduction of inflammation.

Methods. In this *in vivo* study, 5 mg suspensions of CPPD crystals and saline were injected into the rat air pouch. Fluid samples were taken from rat air pouch at 0, 3, 6, 12, 24, and 48 h after injection. White blood cells in the samples were counted; the remaining fluid was centrifuged and concentrations of ß-glucuronidase and PGE_2 in the supernatant were measured as inflammatory markers. LDL in the supernatant was immunochemically identified by Western blotting, then pellets containing crystals were examined by the same technique.

Results. LDL was identified in the air pouch 3 h after CPPD crystal injection, and its concentration increased and reached a peak level after 24 h. Inflammatory markers reached maximal level from 6 to 12 h, then decreased after 24 h. In the pellets containing crystals, LDL could not be identified in every specimen.

Conclusion. LDL in the rat air pouch increased during the inflammatory course induced by CPPD crystal and the inflammation subsided as the LDL increased. Since some reports indicate LDL was related to reduction of crystal induced inflammation such as gout or pseudogout, we concluded that LDL could contribute to the resolution of acute pseudogout arthritis *in vivo* with or without binding to CPPD crystals. (J Rheumatol 2001;28:2674–80)

Key Indexing Terms:
CALCIUM PYROPHOSPHATE DIHYDRATE CRYSTAL ARTHRITIS
LOW DENSITY LIPOPROTEIN REMISSION

Arthritis induced by calcium pyrophosphate dihydrate (CPPD) crystals is diagnosed as pseudogout, with various clinical features including a common complication of osteoarthritis and acute articular inflammation. However, it is not proven that attacks of pseudogout lead to severe progressive joint degeneration[1].

Inflammation induced by monosodium urate crystals also shows diagnostic features similar to those of pseudogout, but it is clinically characterized by its ability to enter remission in a short period of time without therapy[1]. Mechanisms involving this natural remission are not fully understood.

Terkeltaub, *et al*[2] examined the possible *in vivo* associations of proteins with monosodium urate crystals. Among the proteins adhering to crystals, they observed that lipoproteins adhering to crystals in the tophus of patients with gout suppressed the rate of lactate dehydrogenase release from neutrophils, a marker for inflammation. Ortiz-Bravo, *et al*[3] showed by immuno-electron microscopy examination that among the proteins adhering to monosodium urate crystals, relative increase in the amount of apolipoprotein B bound to crystals paralleled the extent of reduction in inflammation accompanied by a decrease in the amount of IgG bound to crystals. Burt, *et al*[4] showed that lipoproteins (low, LDL, and high density, HDL) adhering to CPPD crystals *in vitro* suppressed the activity of CPPD crystals in inducing neutrophil associated inflammation. Onuma[5] determined the concentrations of LDL and VLDL in inflammatory synovial fluid from humans. Then, to assess the suppressive effectiveness of lipoproteins on inflammation, CPPD crystals were mixed with LDL and VLDL at various concentrations around the physiological values previously determined and an aliquot of the mixture in various combinations was injected into the rat air pouch. They concluded that lipoproteins, especially LDL in inflammatory synovial fluid, played a important role in the recovery from CPPD crystal induced arthritis. Thus, LDL is suspected to attenuate the inflammatory activity of CPPD

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crystals by interacting with the crystals. However, it is not yet known at what stage of inflammation LDL accumulated in synovial membrane and how adhesion of LDL to CPPD crystals is temporally associated with pathogenesis and remission of inflammation. We evaluated the quantitative change of LDL during the course of inflammation in the rat air pouch, and evaluated the relationship with reduction of the inflammation induced by CPPD crystals. We also tried to observe the presence *in vivo* of LDL-CPPD crystal complexes formed in the articular cavity, as suggested by Burt, *et al*[4].

MATERIALS AND METHODS

Preparation of CPPD crystals. CPPD crystals of monoclinic system were prepared according to the procedure described by Cheng, *et al*[6]. Briefly, 308.5 mg of $CaCl_2$ (Wako Pure Chemical Co., Tokyo, Japan) and 193.5 mg of $MgCl_2$ $6H_2O$ (Wako) were dissolved into 600 ml distilled water, and pH was adjusted to 7.4 by adding 0.1 M NaOH. Then 120.5 mg of $Na_4P_2O_7$ $10H_2O$ (Wako) and 14.742 g of NaCl (Wako) were dissolved into 1200 ml distilled water and the pH was adjusted to 7.4 by adding 0.1 M HCl. These 2 solutions were mixed and the pH was adjusted to 7.4 by adding 0.1 M NaOH. CPPD crystals were formed during 3 weeks' incubation at 37°C in a water bath. The pH value in the mixed solution was periodically adjusted to 7.4 during incubation.

The crystals were thoroughly washed with distilled water and dried at 37°C in an incubator. Those CPPD crystals were confirmed to be monoclinic by an x-ray crystallographic examination.

Preparation of air pouch as a model of pseudo-synovial space. According to Edwards, *et al*[7], 20 ml aliquots of sterile air obtained by passing air through a filter (0.22 μm pore size) were injected into the back region of 7-week-old male Wistar rats (180–200 g). Three days after injection, 10 ml aliquot of sterile air was additionally injected into the primary site. Air pouches produced a week after the primary injection were used for experiments.

Injection of CPPD crystals and collection of air pouch fluids. Five milligrams of CPPD crystals were mixed with 10 ml physiological saline solution. After the CPPD crystal suspension was thoroughly mixed by a reciprocal shaker, an aliquot was injected into an air pouch. At 0, 6, 9, 12, 24, and 48 h after injection, air pouch fluids were collected each time by washing within the cavity with 5 ml physiological saline. Immediately after collection, the samples were assessed for total white blood cell (WBC) count and subjected to centrifugation at 3000 rpm for 20 min. Supernatant fractions were assessed for ß-glucuronidase and and prostaglandin E_2 (PGE_2) activity as a chemical mediator of neutrophil associated inflammation, using a ß-glucuronidase assay kit (Sigma, St. Louis, MO, USA) and a PGE_2 kit (AMI, USA). Values (n = 5) were compared among those obtained at the indicated times, using Fisher's exact probability test.

Purification of LDL. LDL was purified from rat plasma according to Lindegren, *et al*[8] as described below.

Solution A was prepared by dissolving 11.40 g NaCl and 0.1 g EDTA·Na_2 into 1003 ml distilled water; 1.0 ml of 1 M NaOH solution was also added. The specific gravity was 1.006 g/ml. For preparation of solution B, 24.98 g NaBr were dissolved into 100 ml of solution A. The specific gravity was 1.182 g/ml.

Three milliliters of rat serum sample containing EDTA Na_2 at a concentration of 1 mg/ml were overlaid with 1.5 ml of solution A in a centrifuge tube (5PA tube, Hitachi) and centrifuged at 20,000 rpm for 30 min at 16°C in an RPS65T rotor (Hitachi) with an ultracentrifuge CP70G (Hitachi). After centrifugation, a 3 ml sample was removed from the bottom of each tube and transferred to a separate tube. This sample was overlaid with 1.5 ml of solution A and centrifuged at 40,000 rpm for 16 h at 16°C. After centrifugation, the upper layer was discarded and the remaining 3 ml bottom layer was mixed with 1.5 ml of solution B, followed by centrifugation at 40,000 rpm for 20 h at 16°C. Finally, 0.75 ml samples containing LDL were recovered from the upper layer.

Immunoelectrophoretic assessment of lipoproteins in air pouch fluids. Ten milliliters of LDL preparation as above were diluted with 40 ml of distilled water, and 10 ml aliquots were used for each gel electrophoretic analysis according to Laemmli[9] using a 4–16% polyacrylamide gradient gel containing 0.1% sodium dodecyl sulfate (SDS). Immunoblotting was performed as described by Towbin[10].

To transfer protein materials in the gel to a membrane, gels were immersed in buffer (0.1 M Tris-HCl, pH 7.4, 0.192 M glycine, 20% methanol) and proteins were electrophoretically transferred to the nitrocellulose membrane (Schleicher and Schuel, Germany) in the buffer using an electroblotting apparatus. For blocking nonspecific antibody binding, the blotted membranes were immersed in a blocking buffer consisting of 0.7% skim milk, 10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, and 0.2% Tween 20 and incubated at room temperature for 1 h followed by washing 4 times with blocking buffer using 3 min incubation each time. For reaction with primary antibodies, membranes to which proteins had been transferred were incubated overnight at room temperature with rabbit antibodies against rat apolipoprotein B isoforms (1:1000 dilution with the blocking buffer), which was kindly donated by Dr. Takeshima[11]. For reaction with secondary antibodies, the membranes were washed 4 times with the blocking buffer, each time with 3 min incubation, and incubated with peroxidase tagged goat antibodies against rabbit IgG (1:1000 dilution with the blocking buffer; Bio-Rad, USA) at room temperature for 1 h. For color development, membranes were washed 4 times with blocking buffer with 3 min incubation each time, then immersed in 100 ml phosphate buffer (pH 7.4) containing 50 mg of diaminobenzidine (Sigma), 0.03% $CoCl_2$, and 0.03% ammonium nickel sulfate for 1 h. Finally, 10 ml of 1% hydrogen peroxide was added for color development.

Biochemical analysis of the precipitate fractions. Precipitate fractions obtained from air pouch fluid following ultracentrifugation were washed 3 times with phosphate buffer (pH 7.4) and then dissolved into an equal volume of solution containing 0.1 M phenylmethanesulfonyl fluoride and 4 M guanidine-HCl by gently stirring the mixture overnight at 4°C. Proteins in 100 μl aliquot solution were precipitated by adding 1.4 ml ethanol. Precipitated materials were suspended in 50 μl of sample-dissolving buffer for electrophoresis. Proteins in the buffer solution were dissolved by heating at 95°C for 10 min, and then subjected to immunoelectrophoresis.

Assessment of CPPD crystals in precipitate fractions. CPPD crystals in the precipitate fractions were visualized by staining with Alizarin red and also by polarized light microscopy. Submicroscopic features of the crystals were examined by transmission electron microscopy. For electron microscopy, samples were placed on collodion covered grids coated with carbon.

In vitro examination of LDL adhesion to CPPD crystals. Supernatant fractions of fluids collected from the air pouches in which inflammation had entered remission were mixed with CPPD crystals *in vitro* to immunoelectrophoretically determine whether LDL in the supernatant could adhere to the crystals. It was also determined whether the extent of adhesion depends on the amount of CPPD crystals.

Supernatant fractions of fluids were collected from the air pouch 48 h after 5 mg of CPPD crystals were injected. Then 100 μl aliquots of supernatant were mixed with 10, 50, and 100 μg and 1 mg of CPPD crystals and incubated 3 h at 37°C in a water bath with reciprocal shaking 60 times/min. The reaction mixtures were centrifuged at 11,000 rpm for 10 min, and the resulting precipitates were washed 3 times with phosphate buffer (pH 7.4), followed by mixing with 60 μl of sample dissolving buffer for electrophoresis. The mixtures were heated at 95°C to solubilize proteins that adhered to crystals. Supernatant fractions obtained by centrifugation at 11,000 rpm for 10 min were subjected to electrophoresis, and LDL bands were examined immunochemically.

RESULTS

Numbers of white blood cells in air pouch after injection of CPPD crystals. The number of WBC increased sharply at 6 h after injection and reached at a maximum level 12 h after injection ($29.46 \pm 4.29 \times 10^6$), followed by decreases at 24 h

(20.52 ± 02.64 × 10^6) and at 48 h (16.60 ± 1.54 × 10^6). The difference between 12 and 48 h is significant ($p < 0.01$) (Figure 1).

ß-Glucuronidase concentration in air pouch after injection of CPPD crystals. ß-Glucuronidase concentration also increased sharply at 6 h after injection and reached at maximum level 12 h after injection (39.84 ± 4.88 unit/ml), followed by significant decreases in concentration at 24 h (28.18 ± 3.27 unit/ml) and 48 h (21.04 ± 2.84 unit/ml). The difference between 12 and 24 h is significant ($p < 0.05$) and as well as between 12 and 48 ($p < 0.01$) (Figure 1).

PGE_2 concentration in air pouch after injection of CPPD crystals. PGE_2 concentration reached a maximum level 6 h after injection (57.92 ± 11.67 ng/ml) and sharply declined after 12 h followed by significant decreases at 24 h (3.55 ± 1.67 ng/ml) and 48 h (0.93 ± 0.13 ng/ml). The difference between 6 and 12 h is significant ($p < 0.01$) (Figure 1).

Time course changes in LDL concentrations in air pouch fluids. We investigated whether LDL was present in the air pouch after injection of CPPD crystals and examined changes in LDL in air pouch fluid during periods of acute and subsiding inflammation. LDL in 500 and 240 kDa isoforms were detected by electrophoresis. These isoforms specifically reacted in Western blot analysis using the antibody against rat apolipoprotein B isoform. Thus we assumed that these isoforms detected in the gel quantitatively represented LDL

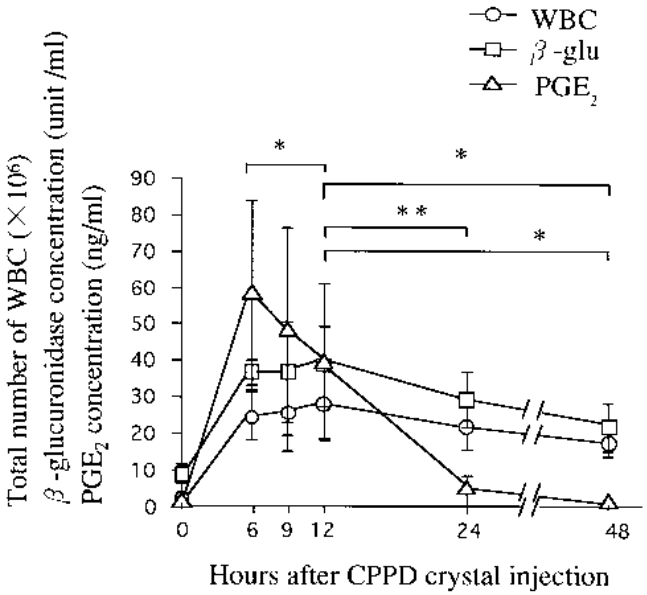


Figure 1. Total numbers of white blood cells (WBC), ß-glucuronidase concentration, and PGE_2 concentration in an air pouch after injection of CPPD crystals. Values are mean ± SD; n = 5. The number of WBC reached maximum level 12 h after injection. The difference between 12 and 48 h is significant, *$p < 0.01$. ß-glucuronidase concentration reached maximum level 12 h after injection; difference between 12 and 24 hours is significant, **$p < 0.05$, and also between 12 and 48 h, *$p < 0.01$. PGE_2 concentration reached maximum level 6 h after injection and declined sharply after 12 h; difference between 6 and 12 h is significant, *$p < 0.01$.

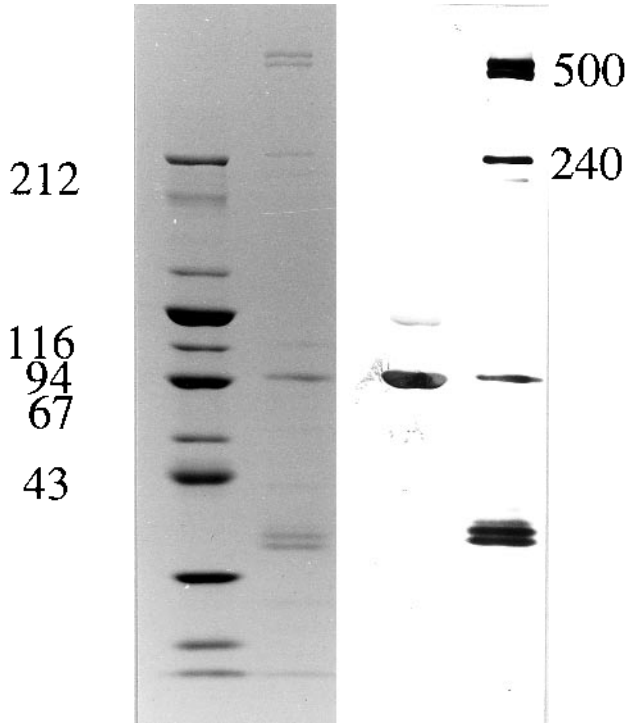
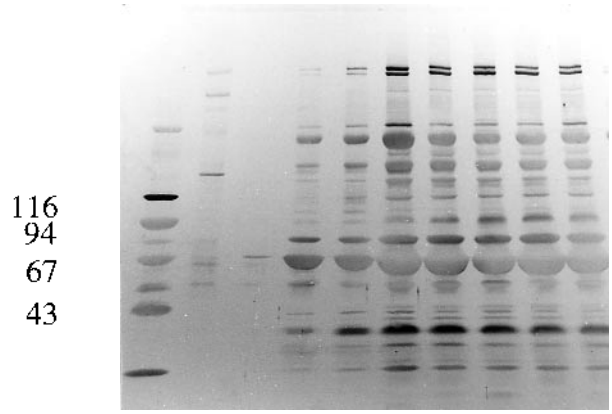


Figure 2. SDS-PAGE of LDL in rat plasma and immunoblotting with anti-rat apolipoprotein B. Lane 2: SDS-PAGE stained by Coomassie brilliant blue R-250. Lane 4: Western blot analysis using antibody against rat apolipoprotein B isoform. Numbers on the left indicate molecular weight standards in kDa (LMW Kit E and HMW Kit E, Carbamylate, USA). Lanes 1 and 3 show the molecular weight marker.

(Figure 2). The 500 and 240 kDa isoforms were first detected in the air pouch at 3 h after injection of CPPD crystals followed by the gradual increase in concentrations. Quantitative analysis of LDL by digitizing densitometric scans showed its concentration reached a maximum 24 h after injection of CPPD crystals, followed by significant decreases (NIH Image 1.55 on Macintosh computer) (Figure 3). In the control group, in which 10 ml saline alone was injected into an air pouch, LDL isoforms were not identified at any time after injection (Figure 4).

Assessment of CPPD crystals in pellets. CPPD crystals were identified by staining with Alizarin red and transmission electron microscopy during time course collection of pellets in the air pouch. Although the amount of crystals decreased at 72 h, crystals still remained in the air pouch. CPPD crystals in the pellets were not morphologically different from those before injection (data not shown).

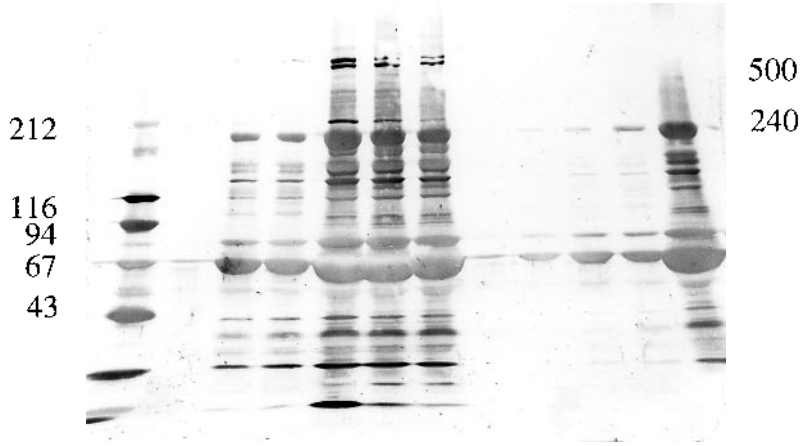
In vitro examination of LDL adhesion to CPPD crystals. LDL isoforms were detectable from at least 10 mg of CPPD crystals after incubation with supernatant of fluid collected from the air pouch at 48 h after injection of CPPD crystals. The extent of LDL adhesion to CPPD crystal depends on the amount of CPPD crystal (data not shown).



1 2 3 4 5 6 7 8 9 10

Density(%)									
500KDa	0	7	27	100	77	72	57	52
240KDa	0	10	29	100	41	39	35	42

Figure 3. Western blot analysis using antibody against rat apolipoprotein B isoform and densitometric analysis of LDL. Lane 3: LDL in an air pouch at 0 h after injection of CPPD crystals. Lane 4: 3 h; Lane 5: 6 h; Lane 6: 24 h; Lanes 7 and 8: 48 h; Lanes 9 and 10: 72 h after injection. Numbers on the left indicate molecular weight standards in kDa (LMW Kit E and HMW Kit E, Carbamylate, USA). Lane 1 shows the molecular weight marker. LDL concentrations were expressed quantatively by digitizing densitmetric scans. LDL concentration reached a maximum level 24 h after injection.



1 2 3 4 5 6 7 8 9 10 11 12

Figure 4. Western blot analysis using antibody against rat apolipoprotein B isoform in the control group (only 10 ml saline injection into an air pouch). Lane 2: LDL in an air pouch at 0 h after injection of CPPD crystals. Lane 3: 3 h; Lane 4: 6 h; Lane 5: 24 h; Lane 6: 48 h; Lane 7: 72 h after injection. Lane 8: 0 h after injection of saline; Lane 9: 3 h; Lane 10: 6 h; Lane 11: 24 h; Lane 12: 48 h after injection. Numbers on the left indicate molecular weight standards in kDa (LMW Kit E and HMW Kit E, Carbamylate, USA). Lane 1 shows the molecular weight marker. Lanes 8 to 12: LDL was not identified in air pouch.

Analysis of proteins in pellets from pouch fluids. We analyzed the pellets from air pouch fluid by immunoblotting. LDL in the pellets was not identified in any specimen (data not shown).

DISCUSSION

Pseudogout is clinically characterized by its ability to enter remission without therapy. However, mechanisms involving this natural remission are not fully understood. Inflammation associated with pseudogout is induced by white blood cells, mainly polymorphonuclear leukocytes, gathering in the articular cavity, where they phagocytose CPPD crystals deposited in the cavity fluid[12]. Shin *et al*[13] used an air pouch model of pseudo-synovium in which CPPD crystals were injected. They assessed time course changes in total WBC count in the pouch and compared findings with those in arthritic synovial lesions. The results showed that inflammatory remission was associated with synovial uptake of CPPD crystals. McCarty, *et al*[14] observed that when monosodium urate crystals, a causative substance for gout, were injected into the articular cavity of normal rabbits, the crystals were dissolved and absorbed by nonarticular tissues. Sakamoto[15] demonstrated that CPPD crystals injected into an air pouch in rats were partially dissolved due to the activity of inflammatory cells and the decreased pH of the synovial fluid caused by inflammation. However, CPPD crystals are relatively insoluble and the rate of solubilization was only about 20 μg per 1 mg of crystals during the remission period starting 24 h after onset of inflammation[15]. Indeed, residual CPPD crystals were observed clinically in the synovial fluid even after inflammation entered remission. Thus, decrease in the amount of CPPD crystals or merely partial solubilization of the crystals may not fully explain the mechanisms causing autoremission of inflammation. Alternatively, an unknown factor(s) that attenuates the inflammatory nature of CPPD crystals may be responsible for the remission.

Burt, *et al*[4] showed that the rate of lactate dehydrogenase release from neutrophils, a marker for inflammation, was decreased by 50% when the cells were fed CPPD crystals to which lipoproteins (LDL and HDL) adhered. Onuma[5] reported that VLDL at concentrations higher than those found in the synovial fluid was needed for suppression, whereas LDL at concentrations similar to those found in the synovial fluid was sufficient for suppression. They concluded that LDL may play a more important role than VLDL in bringing arthritis induced by CPPD crystals into autoremission. Thus, LDL is suspected to attenuate inflammatory activity of CPPD crystals by interacting with the crystals.

In our study, rabbit antibodies against rat apolipoprotein B isoforms were used as primary antibodies for immunoblot detection of LDL. Apolipoprotein B isoforms are major structural constituents of LDL[16]. Onuma[5] demonstrated that the concentration of the VLDL in the inflammatory synovial fluid was remarkably lower than that of LDL (VLDL 20 mg/dl, LDL 560 mg/dl). Therefore we assumed that apolipoprotein B-100 (500 kDa) and apolipoprotein B-48 (240 kDa) detected in the gel quantitatively represented LDL.

Time course changes in inflammatory events. During progression of inflammation, CPPD crystals derived from articular cartilage and deposited in the articular cavity are first phagocytosed by synovial macrophages and fibroblasts. In response to phagocytosis, these cells release PGE_2, which stimulates vascular permeability. As a consequence, polymorphonuclear leukocytes penetrate the articular cavity[17]. Polymorphonuclear leukocytes also stimulate inflammation in response to phagocytosing the crystals. On lysis of polymorphonuclear leukocytes associated with inflammation, ß-glucuronidase, a lysozyme, is released. ß-glucuronidase, as well as total WBC count, is a quantitative marker for inflammation. Thus we adopted total WBC count, ß-glucuronidase, and PGE_2 as inflammation markers in this study. The results showed that inflammation progressed to a peak between 6 and 12 h after CPPD crystal injection followed by a decline toward the autoremission stage between 48 and 72 h. Shin, *et al*[18] reported that in the air pouch system the number of total WBC reached a maximum 6 h after injection of CPPD crystals. Gordon, *et al*[17] showed that concentrations of PGE_2 peaked before the number of total WBC reached a maximum, in agreement with our results.

Crystal selection. Watanabe, *et al*[19] demonstrated that monoclinic CPPD crystal induced more inflammation than triclinic crystal. We used only the monoclinic form because we expected it would give clear data with its ability to create strong inflammation. Moreover, it was more difficult to prepare a uniform size crystal with the triclinic than with the monoclinic form, which might have given more varied results due to the various crystal sizes.

Time course changes in LDL concentrations in the supernatant fractions of articular fluid. We first detected LDL in the air pouch 3 h after CPPD crystal injection followed by a gradual increase in concentrations. When the time course changes in LDL concentration were compared to those of inflammatory markers including total WBC count, ß-glucuronidase concentration, and PGE_2 concentration, LDL started to increase in coordination with the start of inflammation and remained at higher levels even after inflammation showed a peak intensity. Normal synovial membrane possesses a barrier that allows only proteins of relatively small molecular size to pass through into the articular cavity. However, under arthritic conditions, the barrier is destroyed and plasma proteins are allowed to pass through. Consequently, the protein constitution of synovial fluid becomes similar to that of blood plasma[20]. Indeed, LDL is a large molecule of $2–3 \times 10^6$ kDa[13], and only a small amount was detected in healthy synovial fluid[5]. Thus, it seemed that LDL was released from circulating blood into synovial fluid due to destruction of the barrier caused by CPPD crystals in inflammatory synovium.

Role of LDL in suppressing inflammation. Inflammation induced by CPPD crystals in an air pouch eventually enters

remission without therapy. Increased levels of LDL seemed to be associated with remission of inflammation, since LDL started to increase in concert with the onset of inflammation, reached a maximum, and remained elevated 24 h after injection of CPPD crystals, when inflammation started to subside.

Since Onuma[5] described suppression of the inflammatory activity of CPPD crystals in the presence of LDL, our observations that LDL increased prior to signs of inflammatory remission and remained elevated during the remission period would strongly suggest that LDL plays an integral role in inducing autoremission of inflammation.

Limitations of this study in detecting adhesion of LDL to CPPD crystals. Burt, *et al*[4] demonstrated adhesion of lipoproteins that were purified from blood plasma to CPPD crystals when the 2 were mixed. In our study, when purified LDL fractions from rat blood plasma or supernatant fractions of air pouch fluids collected 48 h after injection of CPPD crystals were mixed with CPPD crystals, adhesion of LDL was detected when more than 10 μg of CPPD crystal was used (data not shown). However, LDL was not detectable from CPPD crystals that were recovered during the collection of precipitate samples in the air pouch. Injection of excess amounts (20 mg) of CPPD crystals into the air pouch also failed to reveal LDL adhered to the crystals (data not shown). In studies of uric acid crystals, adhesion of LDL was observed *in vivo* by immunoelectron microscopy[3] and electrophoretic analysis[21]. Failure to observe LDL adhesion to CPPD crystals in our study might be due to a decrease in CPPD crystals below a threshold amount at which a detectable amount of LDL adheres to the crystals. The other possibility is that LDL was stripped from the surface of CPPD crystals during the process of ultracentrifugation for collecting crystals.

Terkeltaub, *et al*[22] suggested that hyaluronic acid present in synovial fluid interferes with adhesion of LDL to monosodium urate crystals. Further study of mixing LDL with CPPD crystals *in vitro* in the presence of hyaluronic acid would be necessary to evaluate this possibility.

Other possible mechanisms of LDL related autoremission. Burt, *et al*[4] found that adhesion of lipoproteins to CPPD crystals attenuated the inflammation-inducing nature of CPPD crystals. However, we were unable to observe adhesion of LDL to the crystals. Bonneau, *et al*[23] reported that LDL suppressed activities of polymorphonuclear leukocytes including migration, phagocytosis, and proliferation by interacting with the leukocytes. They also described that LDL suppressed the complement pathway. Immunosuppressive activity of LDL has also been reported[24,25]. Thus the antiinflammatory activity of the LDL could be explained not only by adhesion of LDL to CPPD crystals[5], but also by its interaction with inflammatory cells and complement pathway.

Along with LDL, transforming growth factor produced by synovial tissue has been suggested to act as a suppressor of arthritis induced by monosodium urate crystals[26,27]. Uptake of the crystals by synovium may also play a role in bringing inflammation into remission[18]. Thus, various factors have been proposed to be involved in the mechanisms of autoremission in inflammation; these factors are not yet fully understood. Among these factors, we have found that changes in LDL concentrations in synovial fluid were closely associated with the onset and reduction of inflammation induced by CPPD crystals and suggest that LDL may play an integral role in bringing inflammation into autoremission.

REFERENCES

1. Ryan LM, McCarty DJ. Calcium pyrophosphate crystal deposition disease; pseudogout; articular chondrocalcinosis. In: McCarty DJ, editor. Arthritis and allied conditions. A textbook of rheumatology. 11th ed. Philadelphia: Lea & Febiger; 1989:1711-36.
2. Terkeltaub R, Tenner AJ, Kozin F, Ginsberg MH. Plasma protein binding by monosodium urate crystals. Analysis by two-dimensional gel electrophoresis. Arthritis Rheum 1983;26:775-83.
3. Ortiz-Bravo E, Sieck MS, Schumacher HR Jr. Changes in the proteins coating monosodium urate crystals during active and subsiding inflammation. Immunogold studies of synovial fluid from patients with gout and of fluid obtained using the rat subcutaneous air pouch model. Arthritis Rheum 1993;36:1274-85.
4. Burt HM, Jackson JK, Rowell J. Calcium pyrophosphate and monosodium urate crystal interactions with neutrophils: Effect of crystal size and lipoprotein binding to crystals. J Rheumatol 1989;16:809-17.
5. Onuma S. A role for lipoproteins in the recovery from CPPD crystal-induced arthritis. Nippon Seikeigeka Gakkai Zasshi 1994;68:953-60.
6. Cheng PT, Pritzker KPH. Pyrophosphate, phosphate ion interaction — Effects on calcium pyrophosphate and calcium hydroxyapatite crystal formation in aqueous solutions. J Rheumatol 1983;10:769-77.
7. Edwards JCW, Sedgwick AD, Willoughby DA. The formation of a structure with the features of synovial lining by subcutaneous injection of air — An in vivo/tissue culture system. J Pathol 1981;134:147-56.
8. Lindgren FT, Nichols AV, Wills RD. Fatty acid distributions in serum lipids and serum lipoproteins. Am J Clin Nutr 1961;9:13-23.
9. Laemmli UK. Cleavage of structure proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-5.
10. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets — Procedure and some applications. Proc Natl Acad Sci USA 1979;76:4350-4.
11. Takeshima M, Hara T. Purification of low density lipoprotein from rat plasma and preparation of its antibody. Nakamura Gakuen Kenkyu Kiyo 1990;22:123-8.
12. McCarty DJ. Pathogenesis and treatment of crystal-induced inflammation. In: McCarty DJ, editor. Arthritis and allied conditions. A textbook of rheumatology. 10th ed. Philadelphia: Lea & Febiger; 1985:1494-514.
13. Scanu AM. Plasma lipoproteins. In: Scanu AM, editor. The biochemistry of atherosclerosis. New York, Basel: Marcel Dekker; 1979:3-8.
14. McCarty DJ, Palmar DW, James C. Clearance of calcium pyrophosphate dihydrate crystals in vivo. II. Studies using triclinic crystals doubly labeled with ^{45}Ca and ^{85}Sr. Arthritis Rheum 1979;22:1122-31.
15. Sakamoto H. The solubility of calcium pyrophosphate dihydrate crystals. Nippon Seikeigeka Gakkai Zasshi 1995;69:484-92.
16. Assmann G. Biochemistry of lipoproteins. In: Schattauer FK, editor. Lipid metabolism and atherosclerosis. Stuttgart: Verlag; 1982:14- 53.
17. Gordon TP, Kowanko IC, James M, Roberts-Thomson PJ. Monosodium urate crystal-induced prostaglandin synthesis in the rat

subcutaneous air pouch. Clin Exp Rheumatol 1985;3:291-6.
18. Shin YM, Sidgwick AD, Moore A, Willoughby DA. Studies on the clearance of calcium pyrophosphate crystals from facsimile synovium. Ann Rheum Dis 1984;43:487-92.
19. Watanabe W, Baker DG, Schumacher HR Jr. Comparison of the acute inflammation induced by calcium pyrophosphate dihydrate, apatite and mixed crystals in the rat air pouch model of a synovial space. J Rheumatol 1992;19:1453-7.
20. Hamanishi C. Blood-synovial barrier. Seikeigeka MOOK 1984; 32:58-70.
21. Terkeltaub R, Dyer CA, Martin J, Curtiss LK. Apolipoprotein (Apo) E inhibits the capacity of monosodium urate crystals to stimulate neutrophils. J Clin Invest 1991;87:20-6.
22. Terkeltaub R, Martin J, Curtiss LK, Ginsberg MH. Glycosaminoglycans alter the capacity of low density lipoprotein to bind to monosodium urate crystals. J Rheumatol 1990;17:1211-6.
23. Bonneau C, Couderc R, Roch-Arveiller M, Giroud JP, Raichvarg D. Effects of low-density lipoproteins on polymorphonuclear leukocyte functions in vitro. J Lipid Mediat Cell Signal 1994;10:203-12.
24. Hui D Y, Harmony JAK, Innerarity TL, Mahley RW. Immuno regulatory plasma lipoproteins — Role of apoprotein E and apoprotein B J Biol Chem 1980;255:11775-81.
25. Pepe MG, Curtiss LK. Apolipoprotein E is a biologically active constituent of the normal immunoregulatory lipoprotein, LDL-In. J Immunol 1986;136:3716-23.
26. Liote F, Prudhommeaux F, Schiltz C, et al. Inhibition and prevention of monosodium urate monohydrate crystal-induced acute inflammation in vivo by transforming growth factor ß1. Arthritis Rheum 1996;39:1192-8.
27. Ortiz-Bravo E, Schumacher HR Jr. Components generated locally as well as serum alter the phlogistic effect of monosodium urate crystals in vivo. J Rheumatol 1993;20:1162-6.