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ORIGINAL ARTICLE

**Satoshi Miyazaki · Toshikazu Yoshikawa
Akira Hashiramoto · Ryoji Yamada · Yasunori Tsubouchi
Masataka Kohno · Yutaka Kawahito · Motoharu Kondo
Hajime Sano**

ACTH expression in synovium of patients with rheumatoid arthritis and Lewis rats with adjuvant arthritis

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Abstract Adrenocorticotropic hormone (ACTH) and another pro-opiomelanocortin-derived neuropeptide, β-endorphin (β-End), are stimulated by corticotropin-releasing hormone (CRH) at the anterior pituitary. CRH and β-End have predominantly proinflammatory effects in peripheral inflammatory sites. We have supposed that inflammatory stimuli develop ACTH as well as β-End. In this study, we investigated the expression of ACTH in inflamed synovial tissue from patients with rheumatoid arthritis (RA) and osteoarthritis (OA), and at inflammatory joints with adjuvant-induced arthritis (AA) in female Lewis (LEW/N) rats. The expression of ACTH immunostaining was significantly greater in synovium of RA patients than in that of OA patients ($P < 0.0001$), and correlated with the extent of inflammatory mononuclear cell infiltration. Extensive and intense intracellular ACTH immunostaining, which correlated with the advance in arthritis score, was observed in the synovial lining layer, inflammatory mononuclear cells, and fibroblast-like cells of synovium and chondrocytes in LEW/N rats with AA. In addition, we performed double immunostaining of the same sections from arthritic joints in rats with anti-ACTH and anti-CRH antibodies. ACTH and CRH colocalized in inflammatory mononuclear cells and fibroblast-like cells. ACTH may play a role in the pathogenesis of RA as well as CRH.

Key words Adjuvant arthritis · Adrenocorticotropic hormone (ACTH) · Corticotropin releasing hormone (CRH) · Lewis rat · Rheumatoid arthritis (RA)

Introduction

Adrenocorticotropic hormone (ACTH) is a 39-amino acid peptide from the anterior pituitary, which stimulates the production of glucocorticoids at the adrenal cortex. Corticotropin-releasing hormone (CRH) stimulates the pituitary production of ACTH and other pro-opiomelanocortin (POMC)-derived neuropeptides such as β-endorphin (β-End).[1,2] It has previously been shown that CRH is locally secreted at inflammatory sites in several animal models, and in arthritic joints of patients with rheumatoid arthritis (RA).[3–5] The extent and intensity of immunoreactive CRH expression at inflammatory sites correlated with the extent and intensity of mononuclear cell infiltration.[5,6] More recently, we reported CRH receptor expression in the synovial lining cell layer, subsynovial stromal cells, blood vessel endothelial cells, and inflammatory mononuclear cells.[7]

We have also reported that β-End is also locally secreted at inflammatory sites of arthritis in model rats and in arthritic joints of patients with RA.[8] We also reported that intraperitoneal administration of anti-CRH antibody and anti-β-End antibodies suppressed the development of adjuvant-induced arthritis of Lewis (LEW/N) rats.[8] These findings suggested that CRH and β-End have predominantly proinflammatory effects in peripheral inflammatory sites. Immunoreactive ACTH and its receptor are also present in the cells of several human tissues outside the brain, such as the spinal cord, adrenal medulla, lung, liver,[9] pancreas,[10] duodenum,[11] and placenta.[12] It has been reported that lymphocytes produce ACTH and β-End by the stimulation of lipopolysaccharide-like *Escherichia coli* endotoxin in mice.[13] However, the presence of ACTH at peripheral inflammatory sites has not yet been examined.

In this study, we have demonstrated intense expression of ACTH in synovial cells from patients with RA, as well as

S. Miyazaki · T. Yoshikawa · A. Hashiramoto · R. Yamada · Y. Tsubouchi · M. Kohno · Y. Kawahito · M. Kondo · H. Sano (✉)
First Department of Internal Medicine, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kamigyo-ku, Kyoto 602-8566, Japan
Tel. +81-75-251-5505; Fax +81-75-252-3721
e-mail: hsano@koto.kpu-m.ac.jp

in inflammatory joints of rats with adjuvant-induced arthritis (AA).

Materials and methods

Human synovial tissue specimens

Synovial specimens were intraoperatively obtained from the knees of 20 patients with RA and 12 patients with osteoarthritis (OA) at arthroscopic biopsy or total joint replacement.[5] All 20 patients with RA met the 1987 revised criteria for the classification of RA.[14] No attempt was made to segregate RA or OA patients on the basis of disease duration or clinically assessed disease activity. The specimens were preserved in 10% formalin, embedded in paraffin, and sectioned (6 μm) onto gelatin-coated slides.

Adjuvant-induced arthritis specimens

Specific pathogen-free female LEW/N rats, 6 weeks of age, were inoculated intradermally in the right hind foot with 0.2 μl *Mycobacterium butyricum* in a suspension of paraffin oil, or with paraffin oil only (control animals).[4] The animals were weighed daily, and at the same time the diameter of the tarsal joint of the limbs was measured. The degree of erythema, swelling, and distortion was graded as a score on a scale of 0 to 4. The score of each extremity was summed as the arthritis score.

On days 0, 3, 5, 12, 16, and 18 after the injection of adjuvant, the arthritic rats and the oil-injected control rats were killed, and the hind feet were cut off at the tibio-tarsal joint. The feet were fixed in 10% formalin, decalcified in 10% ethylenediaminetetraacetic acid (EDTA), embedded in paraffin, and sectioned onto gelatin-coated slides for immunohistochemical analyses.

Immunohistochemistry

Immunoperoxidase staining was done with a Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) as described previously.[3–6,8] All procedures were performed at room temperature. The sections were deparaffinized, and endogenous peroxidase activity was exhausted by incubating in 0.3% peroxide in methanol for 45 min. The sections were preincubated with 0.1% bovine serum albumin (Sigma, St. Louis, MO, USA) in phosphate buffered saline (PBS) for 20 min, and with diluted goat serum (1:66.7 dilution; Vector) in PBS for 20 min. This was followed by incubation in a humid chamber with 1:200 dilution of rabbit polyclonal anti-rat ACTH antibody (ICN), anti-human ACTH antibody (Sigma), or normal rabbit serum (Vector). After 40 min, the sections were washed in PBS and incubated with biotinylated goat antirabbit IgG (Vector) for 30 min. The sections were further washed in PBS and incubated with an avidin-DH:biotinylated horseradish peroxidase H complex (Vector) for 45 min. Finally, the sections were washed, and color was developed by immersing them in a solution of 0.05% (wt/vol) 3,3′-diaminobenzidine tetrahydrochloride (Sigma), 0.04% (wt/vol) nickel chloride, and 0.01% hydrogen peroxide in 0.05 M Tris, pH 7.4, for 5 min. The sections were counterstained with 0.5% light green SF (Sigma).

Positive staining was indicated by brownish-black deposits, and the background staining was green.

For each tissue specimen, the extent and intensity of staining with anti-ACTH antibody was graded on a scale of 0 to 4+ by two independent observers on two separate occasions using coded slides, and an average score was calculated.[4–7] Grade 4 implies that all staining was maximally intense throughout the specimen, whereas grade 0 implies that staining was absent throughout the specimen.

Double antibody immunostaining

Double antibody immunostaining was performed with the Vectastatin ABC kit (Vector).[15,16] Sections were stained with anti-rat ACTH antibody (1:200 dilution) using the peroxidase method, or 25 μg/ml anti-CRH antibody (Santa Cruz) using the peroxidase method without using 0.04% (wt/vol) nickel chloride. Control staining was obtained with normal rabbit serum (Vector). Counterstaining was done with 0.5% light green.

For immunostaining with anti-rat ACTH antibody, positive staining was indicated by black deposits for the peroxidase method. For immunostaining with anti-CRH antibody, positive staining was indicated by brown deposits for the peroxidase method without using nickel chloride. Positive staining for double antibody staining was indicated by dark brownish-black deposits.

Statistical analysis

Statistical analyses of the data were performed using analysis of variance (ANOVA).

Results

Expression of ACTH in the synovial tissues of patients with RA and OA

To determine the expression of ACTH in the synovial tissues of patients with RA and OA, we immunohistochemically stained synovial tissues. Diffuse and intense ACTH staining was observed in the lining cell layer infiltrating mononuclear cells and fibroblast-like cells of inflamed synovial specimens from patients with RA (Fig. 1A,C). Control immunostaining with normal rabbit serum was completely negative in all cases (Fig. 1B). In contrast to the staining of RA specimens, synovial tissue sections from patients with OA, stained very weakly on the synovial lining cell layer and synovial cells (Fig. 1D).

The extent and intensity of staining of the synovial lining cell layer and synovial cells with ACTH antibody were

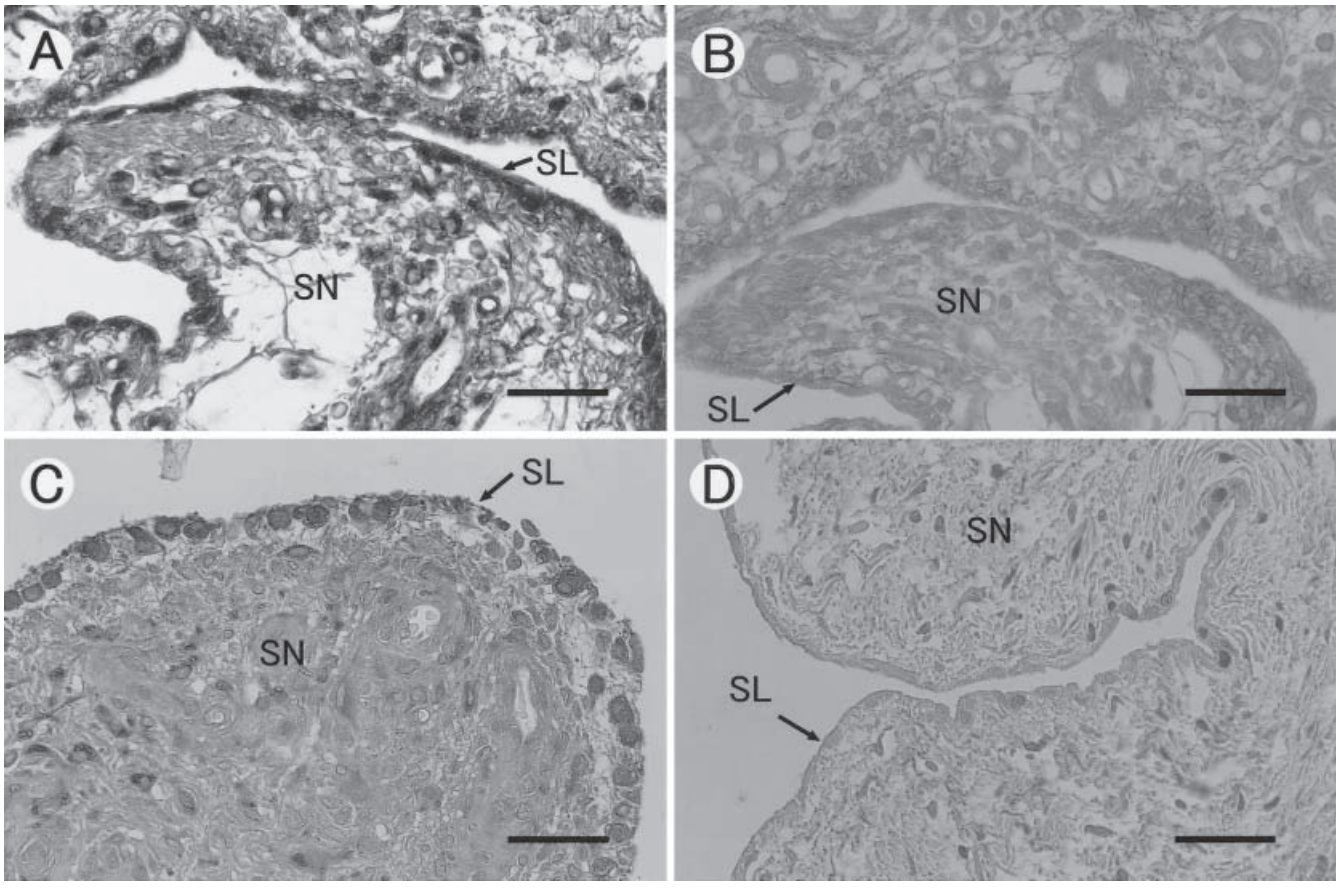

Fig. 1. Representative adrenocorticotropic hormone (ACTH) immunoperoxidase-stained sections of inflamed synovial tissue specimens from patients with rheumatoid arthritis (RA) or osteoarthritis (OA). Synovia from RA and OA patients were immunostained with ACTH antibody as described. Positive staining is indicated by black deposits. **A**, **C** Representative RA synovium stained with ACTH antibody. **B** RA synovium stained with normal rabbit serum. **D** Representative OA synovium stained with ACTH antibody. *SN*, synovium; *SL*, synovial lining layer. *Bar* 5 μm

graded 0–4+ by two independent observers on 20 RA and 12 OA specimens (Fig. 2). In a statistical analysis using ANOVA, ACTH staining was significantly more extensive and intense in synovial specimens from RA patients ($n = 20$) than on specimens from OA patients ($n = 12$) ($P < 0.0001$).

Expression of ACTH in the synovium of female Lewis rats with adjuvant-induced arthritis

Chronic polyarthritis clinically appears in female Lewis rats on days 10–12 after adjuvant injection (Table 1).

ACTH immunostaining was negative in synovial cells of Lewis rats on day 1 after the injection of adjuvant (Fig. 3A). Diffuse and intense ACTH staining was observed in the synovial lining cell layer, inflammatory mononuclear cells, fibroblast-like cells of inflamed synovium, and chondrocytes in Lewis rats on day 18 after the injection of adjuvant (Fig. 3B). Control immunostaining with anti-ACTH antibody preabsorbed with ACTH polypeptides or normal rabbit serum was completely negative in all cases (Fig. 3C).

Development of the expression of ACTH in cartilage after the injection of adjuvant in female Lewis rats

ACTH staining of the cartilage on days 3 and 5 after the injection of adjuvant was very weakly developed (Fig. 4A,B). ACTH staining of chondrocytes became more intense on day 12 (Fig. 4C), and strongest on day 18 (Fig. 4D). The arthritis score increased from day 12, and the expression of ACTH in the synoviocytes and chondrocytes also developed from day 12 to day 18 (see Table 1).

Double antibody immunostaining with ACTH and CRH in arthritic joints of female Lewis rats

To determine the specific localization of ACTH and CRH, we performed double immunostaining on day 18 in the same sections of joint tissues from rats with adjuvant-induced arthritis. Staining with anti-ACTH and anti-CRH antibodies showed intense dark brownish-black deposits in some of the inflammatory mononuclear cells and fibroblast-like cells (Fig. 5A). Staining with anti-ACTH antibody and normal rabbit serum showed black deposits only in some

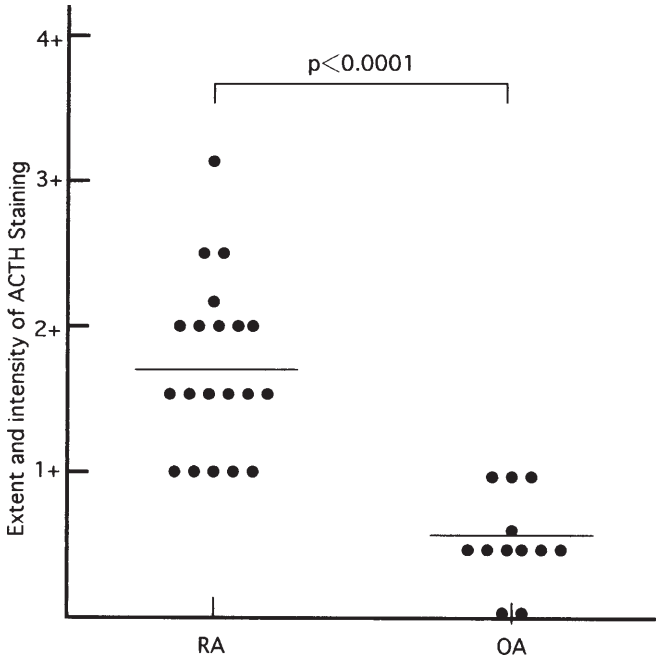

Fig. 2. Statistical analysis of ACTH immunostaining of synovial cells from patients with RA or OA. The extent and intensity of immunostaining with anti-ACTH antibody in synovial cells from RA and OA patients were graded 0–4 by two independent observers. ACTH immunostaining was significantly more extensive and intensive in synovial cells from RA patients ($n = 20$) than in those from OA patients ($n = 12$) by analysis of variance ($P < 0.0001$)

mononuclear inflammatory cells (Fig. 5B). Staining with anti-CRH antibody and normal rabbit serum showed brown deposits only (Fig. 5C). Control staining with normal rabbit serum was uniformly negative (Fig. 5D). These findings showed the colocalization of ACTH and CRH in synovial cells.

Discussion

ACTH production and secretion from POMC is promoted by CRH. We have previously reported the expression of CRH in the joints of RA patients, and the proinflamatory effect of CRH in the synovial cells of RA patients.[4,5] However, the existence and participation of ACTH in the joints of RA patients has not been reported.

In this study we showed the existence of ACTH in the synovial lining cell layer, mononuclear cells, and fibroblast-like cells of the inflamed synovium and in chondrocytes of female LEW/N rats with adjuvant-induced arthritis, as well as in inflamed synovial cells from patients with RA. The intense immunoreactive (ir)- ACTH was expressed after the injection of complete Freund's adjuvant, and the most intensive staining was expressed in the synovial lining cell layer, mononuclear cells, fibroblast-like cells, and chondrocytes on day 18. A comparison of the ACTH staining of synoviocytes and chondrocytes and the arthritis score

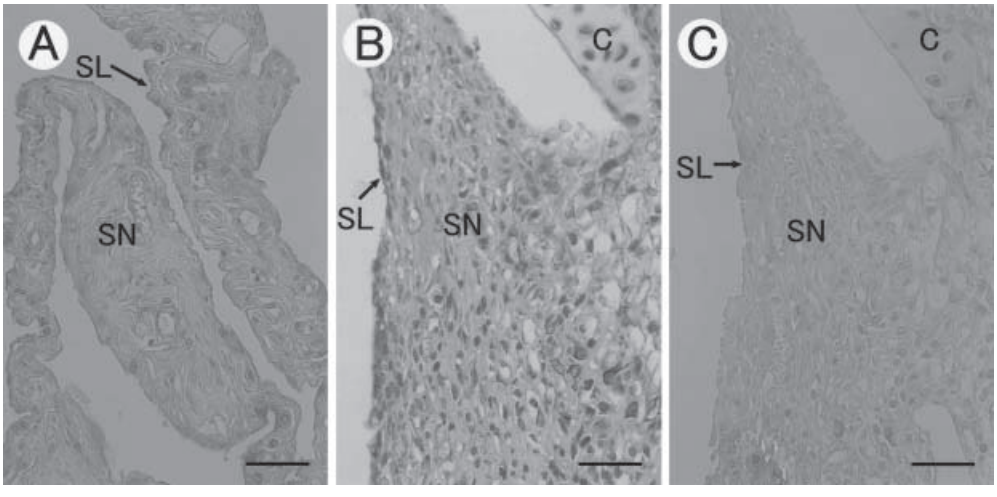

Fig. 3. Representative ACTH immunoperoxidase-stained sections of inflamed synovial specimens from female Lewis (LEW/N) rats with adjuvant-induced arthritis (AA) on days 1 and 18. **A** Synovium stained with ACTH antibody on day 1 after the injection of adjuvant in Lewis rats. **B** Representative inflamed synovium stained with ACTH antibody on day 18 after the injection of adjuvant in Lewis rats. **C** Representative inflamed synovium stained with ACTH antibody preabsorbed with ACTH on day 18 after the injection of adjuvant in Lewis rats. *C*, cartilage. *Bar* 5 μm

Table 1. Relationship between arthritis score and ACTH expression in joints of adjuvant-injected rats

	Day 1	Day 3	Day 5	Day 7	Day 12	Day 14	Day 16	Day 18
Arthritis score[a]	1.0	1.5	1.5	3.0	6.0	9.0	10.0	12.0
ACTH expression[b]								
Synoviocytes	0.0	0.5	0.5	0.5	2.5	3.25	3.5	3.5
Chondrocytes	0.0	0.5	1.0	1.0	1.2	3.0	3.0	3.0

[a] The degree of erythema, swelling, and distortion was graded on a scale of 0–4. The score for each extremity was summed to obtain the arthritis score (0–16)
[b] The extent and intensity was graded from 0 to 4 by two independent observers. The average score for ACTH expression is shown

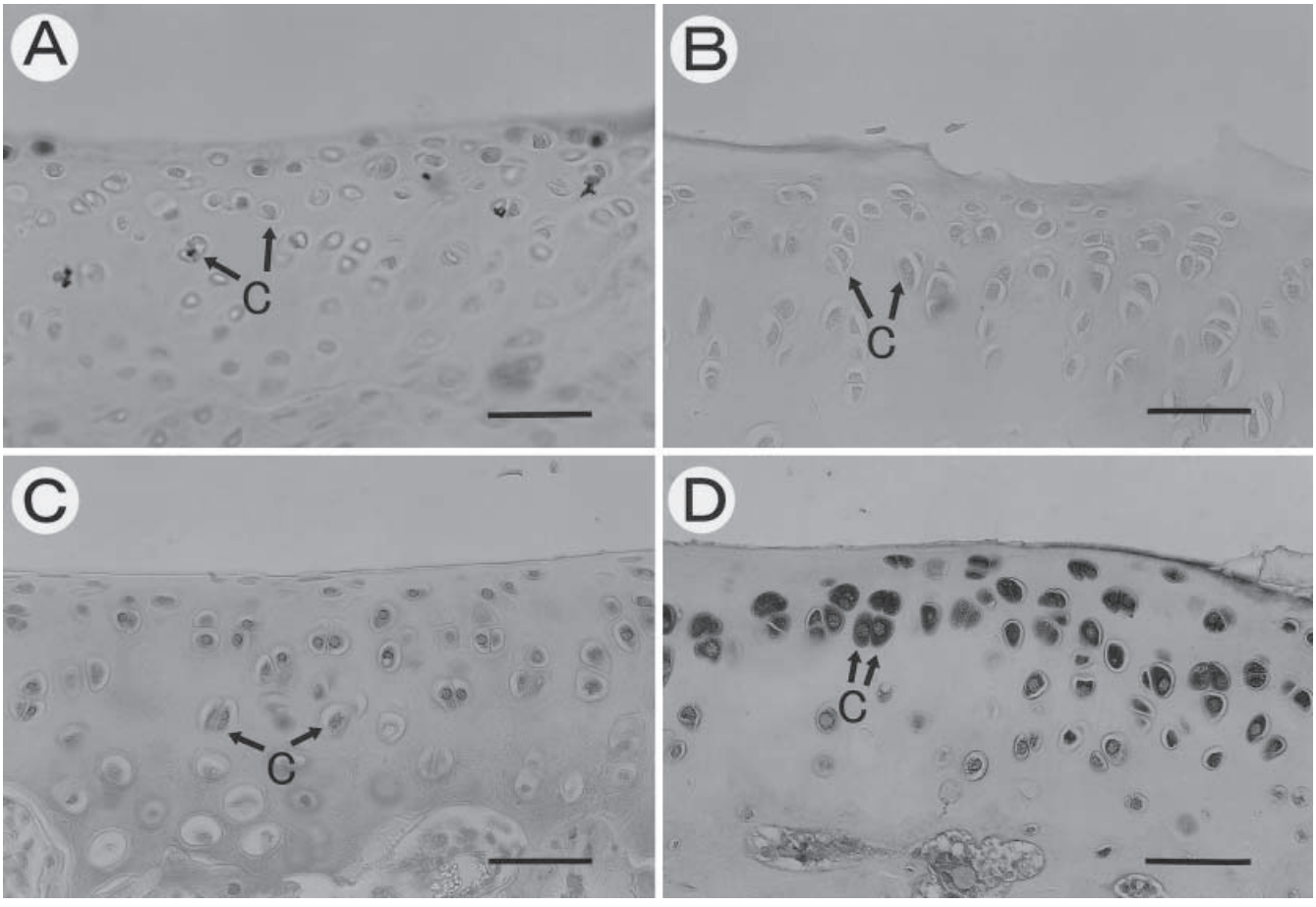

Fig. 4. Representative ACTH immunoperoxidase-stained sections of cartilage specimens from adjuvant-injected Lewis rats on days 3, 5, 12, and 18. **A** Representative cartilage stained with ACTH antibody on day 3 after the injection of adjuvant. **B** Representative cartilage stained with ACTH antibody on day 5. **C** Representative cartilage stained with ACTH antibody on day 12. **D** Representative cartilage stained with ACTH antibody on day 18. *Bar* 5 μm

showed that both the extent and intensity of ACTH staining was correlated with arthritis score.

These data suggest that the production of ACTH increases during inflammation, as does the production of CRH. We also showed the expression of both ACTH and CRH in some inflamed synoviocytes in LEW/N rats with AA.

It has been reported ACTH influences the immune system. Mitogen-activated lymphocytes release ir-ACTH that stimulates the in vitro release of corticosterone from rat adrenal cells.[17] It is suggested that ACTH released from immunomodulatory cells such as mitogen-activated lymphocytes may exert an anti-inflammatory effect throughout the whole body during immune activation such as the activation of adrenal cells.[18] Lolait et al.[19,20] have also reported that β-End and ACTH may be synthesized in a subpopulation of mouse spleen macrophages. These, or related, peptides may modulate lymphocyte function in the specific microenvironment of the spleen. Harbour et al.[13] reported that CRH induces B lymphocytes from C3HeB/FeJ bacterial lipopolysaccharide (LPS) (i.e., *Escherichia coli* endotoxin)-sensitive mice to produce ir-ACTH 1-39 and β-End, whereas LPS induces these lymphocytes to process and produce ir-ACTH 1-23 to 1-26, and α- or γ-End from ACTH 1-39 and β-End.

Cells of the immune system also produce ACTH in humans.[21] Mononuclear cells in human peripheral blood synthesize and release ACTH and endorphin-like peptides when stimulated with some substances.[22–26] ACTH-like immunoreactivity was detected in some cell lines derived from patients with lymphoid and myeloid malignancies.[21]

It has been reported that leukocytes in human peripheral blood produce ir-ACTH 1-39 and β-End after stimulation with Newcastle disease virus or CRH, and they also produce ir-ACTH 1-24 and α- or γ-End by stimulation with LPS.[22] B lymphocytes from C3H/HeJ LPS-resistant mice expressed but did not process POMC after LPS or CRH treatment.[13,22] It was hypothesized that LPS induces a proteolytic enzyme that cleaves ACTH 1-39 and β-End into the smaller ACTH and endorphin species.[27] These POMC peptides have different immunoregulatory activities.[13] β-End is an enhancer of in vitro antibody production, while α-End is a suppressor of the in vitro antibody response. ACTH 1-39 suppresses the antibody response, but ACTH 1-24 has no effect. It was hypothesized that the processing and producing of different POMC peptides such as ACTH 1-39, ACTH

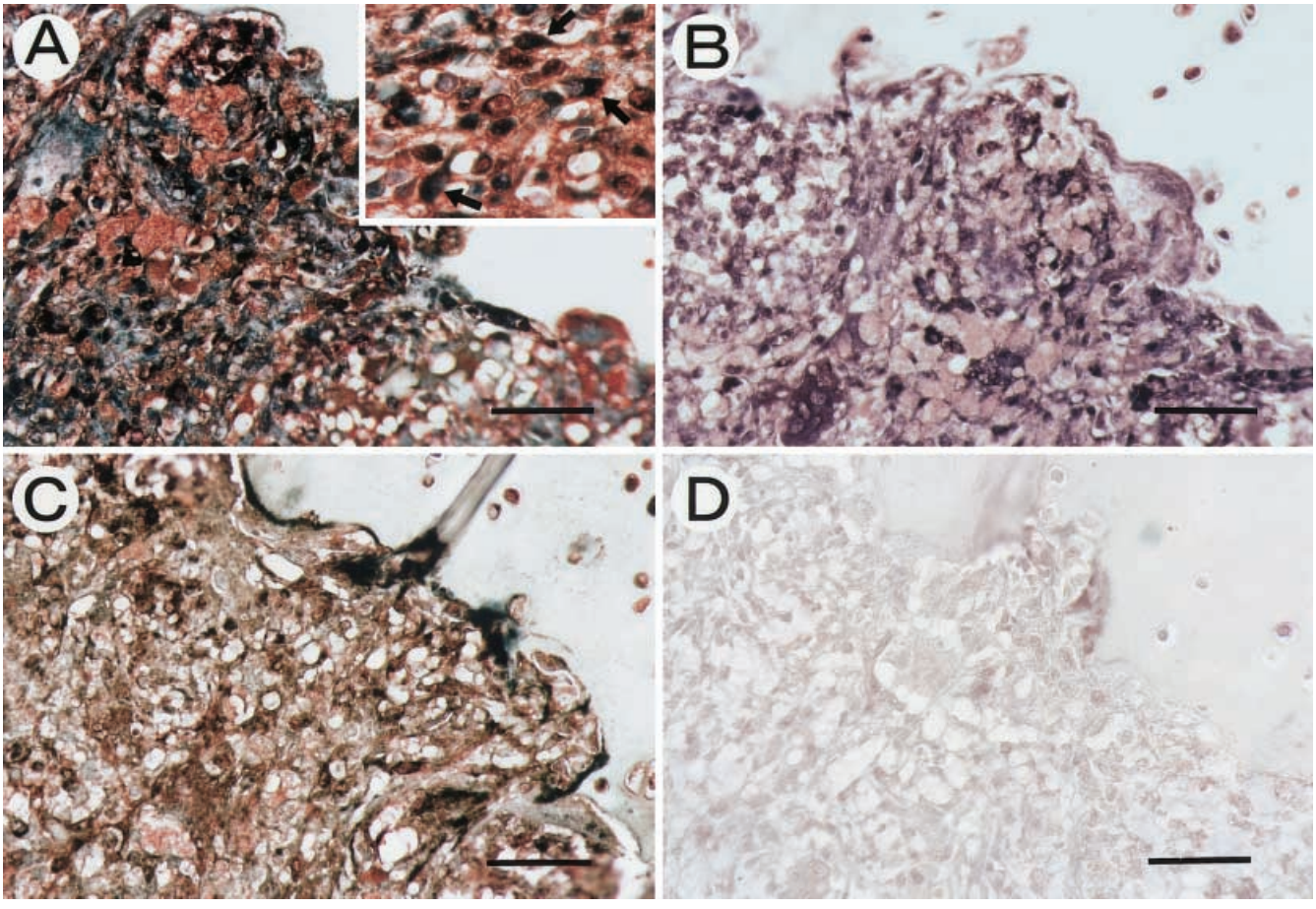

Fig. 5. Representative double antibody immunostaining of inflamed joint specimens from female Lewis rats with AA on day 18 after the injection of aduvant. ACTH and CRH were immunostained in adjuvant-injected Lewis rats on day 18, as described. **A** Representative inflamed joint specimens stained with ACTH and CRH antibodies (brownish-black deposits). The cells indicated by *arrows* show double staining with ACTH and CRH antibodies. **B** Representative inflamed joint specimens stained with ACTH antibody and normal rabbit serum (black deposits). **C** Representative inflamed joint specimens stained with CRH antibody and normal rabbit serum (brown deposits). **D** Representative inflamed joint specimens stained with normal rabbit serum. *Bar* 5 μm

1-24, β-End, and α-End, caused by various stimuli such as CRH, might play an important role in modulating the immune response in RA.

These findings suggest the possibility of immunomodulatory effects of the ACTH expressed in the synovial cells of RA and AA in LEW/N rats.

However, Jessop et al.[28] have reported that, unlike the hypothalamus–pituitary–adrenal (HPA) axis, immune POMC activation is not necessarily consequent in immune CRH in the spleen of rats with adjuvant-induced arthritis. They showed an increase in thymic CRH on day 11, which was followed by an increase in ACTH on day 14. Conversely, changes in splenic ACTH content were evident by day 3 after the injection of adjuvant, whereas the increase in CRH became apparent only by day 14. It was considered that splenic POMC activation is probably independent of CRH and does not require stimulation by CRH, but it is not yet clear whether all of the POMC-related peptides released from immunomodulatory cells have the ability to respond to CRH.

Recently, we reported that CRH receptor and urocortin (in the CRH family) are expressed in synovial cells, and that urocortin stimulates IL-1β and IL-1 secretion by human peripheral mononuclear cells.[7] In this study, we showed colocalization of ACTH and CRH in the synoviocytes of arthritic joints. However, the immunological role of ACTH in arthritis is still unclear. ACTH may play an important role in the pathogenesis of arthritis as well as CRH.

References

1. Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. Science 1981;213:1394–7.
2. Turkelson CM, Arimura A, Culler MD, Fishback JB, Grott K, Konda M, et al. In vivo and in vitro release of ACTH by synthetic CRF. Peptides 1981;2:425–31.
3. Karalis K, Sano H, Redwine J, Listwak S, Wilder RL, Chrousos GP. Autocrine or paracrine inflammatory actions of corticotropin-releasing hormone in vivo. Science 1991;254:421–3.
4. Crofford LJ, Sano H, Karalis K, Webster EL, Goldmuntz EA, Chrousos GP, et al. Local secretion of corticotropin-releasing hormone in the joints of Lewis rats with inflammatory arthritis. J Clin Invest 1992;90:2555–64.

5. Crofford LJ, Sano H, Karalis K, Friedman TC, Epps HR, Remmers EF, et al. Corticotropin-releasing hormone in synovial fluids and tissues of patients with rheumatoid arthritis and osteoarthritis. J Immunol 1993;151:1587–96.
6. Crofford LJ, Sano H, Karalis K, Webster EA, Friedman TC, Chrousos GP, et al. Local expression of corticotropin-releasing hormone in inflammatory arthritis. Ann NY Acad Sci 1995;771:459–71.
7. Kohno M, Kawahito H, Tsubouchi Y, Hashiramoto A, Yamada R, Inoue K, et al. Urocortin expression in synovium of patients with rheumatoid arthritis and osteoarthritis: relation to inflammatory activity. J Clin Endocrinol Metab 2001;86:4344–52.
8. Sano H, Kawahito Y, Hashiramoto A, Miyazaki S, Yamada R, Fukui W, et al. Hypothalamic–pituitary–adrenal axis function in rheumatoid arthritis – CRH and β-endorphin (in Japanese). Clin Immunol (Japanese) 1997;29:555–65.
9. Suda T, Tomori N, Tozawa F, Mouri T, Demura H, Shizume K. Distribution and characterization of immunoreactive corticotropin-releasing factor in human tissues. J Clin Endocrinol Metab 1984;59:861–6.
10. Petrusz P, Merchenthlaler I, Maderdrut JL, Vigh S, Shally AV. Corticotropin-releasing factor (CRF)-like immunoreactivity in the vertebrate endocrine pancreas. Proc Natl Acad Sci USA 1983;80:1721–5.
11. Kriseman ACN, Linton EA, Lowry PJ, Rees LH, Besser GM. Corticotropin-releasing factor immunoreactivity in human gastrointestinal tract. Lancet 1982;2:1245–6.
12. Shibasaki T, Odagiri E, Shizume K, Ling N. Corticotropin-releasing factor-like activity in human placental extracts. J Clin Endocrinol Metab 1982;55:384–6.
13. Harbour DV, Smith EM, Blalock JE. Novel processing pathway for pro-opiomelanocortin in lymphocytes: endotoxin induction of a new prohormone-cleaving enzyme. J Neurosci Res 1987;18:95–101.
14. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
15. Sano H, Engleka K, Mathern P, Hla T, Crofford LJ, Remmers EF, et al. Coexpression of platelet-derived growth factor-B, and fibroblast growth factor-1 in situ in synovial tissues of patients with rheumatoid arthritis and Lewis rats with adjuvant or streptococcal cell wall arthritis. J. Clin Invest 1993;91:553–65.
16. Zhou M, Grofova I. The use of peroxidase substrate Vector VIP in electron microscopic single and double antigen localization. J Neurosci Methods 1995;62:149–58.
17. Clarke BL, Gebhardt BM, Blalock JE. Mitogen-stimulated lymphocytes release biologically active corticotropin. Endocrinology 1993;132:983–8.
18. Pepper GM, Maimon J, Schneider BS. Quantification and characterization of ACTH-related peptides produced by human peripheral blood mononuclear cells. Lymphokine Cytokine Res 1991;10:133–40.
19. Lolait SJ, Lim AT, Toh BH, Funder JW. Immunoreactive β-endorphin in a subpopulation of mouse spleen macrophages. J Clin Invest 1984;73:277–80.
20. Lolait SJ, Clements JA, Markwick AJ, Cheng C, McNally M, Smith AI, et al. Pro-opiomelanocortin messenger ribonucleic acid and posttranslational processing of beta-endorphin in spleen macrophages. J Clin Invest 1986;77:1776–9.
21. Buzzetti R, McLoughlin L, Lavender PM, Clark AJL, Rees LH. Expression of pro-opiomelanocortin gene and quantification of adrenocorticotropic hormone-like immunoreactivity in human normal peripheral mononuclear cells and lymphoid and myeloid malignancies. J Clin Invest 1989;83:733–7.
22. Smith EM, Morrill AC, Meyer WJ III, Blalock JE. Corticotropin-releasing factor induction of leukocyte-derived immunoreactive ACTH and endorphins. Nature 1986;321:881–2.
23. Smith EM, Blalock JE. Human lymphocyte production of corticotropin and endorphin-like substances: association with leukocyte interferon. Proc Natl Acad Sci USA 1981;78:7530–4.
24. Harbour-McMenamin D, Smith EM, Blalock JE. Bacterial lipopolysaccharide induction of leukocyte-derived corticotropin and endorphins. Infect Immun 1985;48:813–7.
25. Johnson HM, Smith EM, Torres BA, Blalock JE. Regulation of the in vitro antibody response by neuroendocrine hormones. Proc Natl Acad Sci USA 1982;79:4171–4.
26. Galin FS, LeBoeuf RD, Blalock JE. Corticotropin-releasing factor upregulates expression of two truncated pro-opiomelanocortin transcripts in murine lymphocytes. J Neuroimmunol 1991;31:51–8.
27. Harbour DV, Galin FS, Hughes TK, Smith EM, Blalock JE. Role of leukocyte-derived pro-opiomelanocortin peptides in endotoxin shock. Circulatory Shock 1991;35:181–91.
28. Jessop DS, Renshaw D, Lightman SL, Harbuz MS. Changes in ACTH and β-endorphin immunoreactivity in immune tissues during a chronic inflammatory stress are not correlated with changes in corticotropin-releasing hormone and arginine vasopressin. J Neuroimmunol 1995;60:29–35.