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Original article

Expression of cannabinoid receptor 2 and its inhibitory effects on synovial fibroblasts in rheumatoid arthritis

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Abstract

Objective. Recent studies have suggested immunomodulatory and anti-inflammatory effects of cannabinoid receptor 2 (CB2R) activation, which shows no psychoactivity. However, it is still unclear whether CB2R is expressed in fibroblast-like synoviocytes (FLS) of RA. In this study we investigated whether CB2R is expressed in FLS of RA, and whether CB2R activation modulates the function of RA-FLS.

Methods. Expression of CB2R in synovial tissue and FLS was studied by immunohistochemistry, western blotting and RT-PCR. mRNA expression levels of CB2R, IL-6 and MMPs were analysed by quantitative real-time RT-PCR. The protein concentrations of IL-6 and MMPs in culture supernatants were determined by ELISA. The protein levels of signal transducing molecules were assayed by western blotting.

Results. Both mRNA and protein expression of CB2R were found in synovial tissue and cultured FLS with slightly higher levels in RA patients than in OA patients. In cultured RA-FLS, the expression level of CB2R was up-regulated by stimulation with IL-1β, TNF-α or lipopolysaccharide. *In vitro*, HU-308, a selective CB2R agonist, inhibited IL-1β-induced proliferation of RA-FLS as well as IL-1β-induced production of MMP-3, MMP-13 and IL-6 in RA-FLS in a dose-dependent manner. HU-308 also suppressed IL-1β-induced activation of extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase in FLS.

Conclusion. In RA-FLS, proinflammatory mediators up-regulate the expression of CB2R, which negatively regulates the production of proinflammatory cytokines and MMPs. These data suggest that CB2R may be a potential therapeutic target of RA.

Key words: cannabinoid receptor 2, fibroblast-like synoviocytes, interleukin 6, matrix metalloproteinases, rheumatoid arthritis.

BASIC SCIENCE

Introduction

RA is an immune-mediated inflammatory disease of unknown aetiology that is characterized by chronic inflammatory infiltration of the synovium, leading to eventual cartilage and bone destruction [1]. In spite of significant improvements in the treatment of RA, there is still a need for the identification of new pathways involved in the modulation of inflammation in order to further increase efficacy, particularly in patients in whom the disease does not respond to current therapies.

Recently, discovery of the endocannabinoid system, especially two subtypes of cannabinoid receptors, has elicited a great deal of interest in inflammatory diseases, including multiple sclerosis and RA [2]. Two types of cannabinoid receptors—cannabinoid receptor 1 (CB1R) and cannabinoid receptor 2 (CB2R)—were discovered in the early 1990s [3, 4]. CB1R exists primarily on central and peripheral neurons and is associated with the

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psychoactive effects of cannabinoids. CB2R is predominantly expressed by cells of haematopoietic origin and is thought to mediate cannabinoid-induced immune modulation [5]. This type of distribution supports the prospect that anti-inflammatory and immunosuppressive CB2R-selective drugs without psychoactivity can be developed for the management of chronic inflammatory diseases such as RA.

In 2008 the endocannabinoids anandamide and 2-arachidonoylglycerol were identified in the synovial fluids of RA patients; neither of these were detected in the synovial fluids of normal volunteers [6]. This finding makes the endocannabinoid system an attractive therapeutic target of RA. Fibroblast-like synoviocytes (FLS) are recognized as both propagators of the immune response and the engine of joint damage in RA [7, 8]. However, it remains unclear whether CB2R is expressed in FLS.

In the present study, we revealed the expression of CB2R and its up-regulation by proinflammatory mediators in RA-FLS and demonstrated the inhibitory effects of CB2R activation on the production of proinflammatory cytokine IL-6 and MMPs.

Materials and methods

Reagents

HU-308, a selective agonist for CB2R [9], was purchased from Tocris Bioscience (Bristol, UK), lipopolysaccharide (LPS) from Sigma (St Louis, MO, USA) and human IL-1β and TNF-α from R&D Systems (Minneapolis, MN, USA). Collagenase type II was purchased from Worthington Biochemical (Lakewood, NJ, USA). Recombinant rabbit-polyclonal antibodies to human CB2R, monoclonal antibodies to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and α-tubulin, and HRP-conjugated goat anti-rabbit IgG were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Monoclonal antibodies against extracellular signal-regulated kinase 1/2 (ERK1/2), phospho-ERK1/2, p38 mitogen-activated protein kinase (MAPK) and phospho-p38 MAPK were purchased from Cell Signalling Technology (Boston, MA, USA). IRDye-conjugated donkey anti-rabbit IgG and goat anti-mouse IgG were purchased from Rockland Immunochemicals (Gilbertsville, PA, USA). Alexa Fluor 488-conjugated donkey anti-rabbit IgG was purchased from Life Technologies (Carlsbad, CA, USA). Trizol, oligo $d(T)_{18}$ primers, dNTP mixture, ribonuclease inhibitor, reverse transcriptase M-MLV, premix Ex Taq and SYBR premix Ex Taq were all purchased from TaKaRa Biotechnology (Dalian, China).

Immunohistochemistry

Synovial tissues were obtained at the time of knee joint replacement surgery or synovectomy from RA or OA patients (see supplementary Table S1, available at *Rheumatology* Online). All the diagnoses of the patients met the ACR criteria for RA [10] or OA [11], respectively. Informed consent was obtained from each patient according to the Declaration of Helsinki. The study design was approved by the ethics committee of Changhai Hospital, Second Military Medical University, Shanghai.

Fresh synovial tissues were fixed in 4% paraformaldehyde for 48 h and then embedded in paraffin. Serial sections (4 μm) were incubated overnight at 4°C with anti-CB2R antibodies and then incubated with HRP-conjugated goat anti-rabbit IgG as a secondary antibody for 1 h at room temperature. The peroxidase activity was visualized with 3,3′-diaminobenzidine and then the sections were counterstained with haematoxylin. Negative controls were stained with isotype-matched non-specific IgG at the same concentration.

For immunocytochemistry, cultured RA-FLS were incubated overnight at 4°C with anti-CB2R antibodies and then incubated with Alexa Fluor 488-conjugated donkey anti-rabbit IgG as a secondary antibody for 1 h at room temperature. 4′,6-diamidino-2-phenylindole (DAPI) solution was applied for 5 min for nuclear staining. The staining was photographed with a CKX41 inverted fluorescence microscope (Olympus, Tokyo, Japan).

Isolation and culture of human FLS

Human FLS were isolated and cultured as previously [12]. After careful removal of the adipose and fibrous tissue, fresh synovial tissue was minced and then digested overnight with 1.0 mg/ml collagenase in serum-free DMEM at 37°C on a horizontal shaker. The cell suspensions were filtered through a stainless steel filter to remove the undigested tissue. The filtrate was centrifuged and the cell precipitation was washed twice with PBS. After that, the cells were resuspended in complete media containing 10% fetal calf serum (FCS), 100 U/ml of penicillin, 100 μg/ml streptomycin and 100 μg/ml amphotericin B. The cells were counted, seeded into culture flasks (2.5×10^4 cells/cm^2) and cultured overnight in a humidified, 5% CO_2 atmosphere at 37°C. The non-adherent cells were then washed out. Adherent cells were cultured in complete medium, and the culture medium was replaced every week. Upon confluence, cells were dispersed with trypsinization and then transferred to new plastic dishes in a split ratio of 1:3. Passages 3–8 of the FLS were used in subsequent experiments, at which time they were a homogeneous population of fibroblasts. Before each experiment, the FLS were starved for 12 h with DMEM containing 1% FCS.

RT-PCR and quantitative real-time RT-PCR

Total RNA was extracted from cultured FLS with Trizol. RNA was evaluated spectrophotometrically for quantity and purity. After reverse transcription, complementary DNA was used as templates for PCR. PCR amplification was performed using specific primers (Table 1). The constitutively expressed gene encoding GAPDH was used as an internal control in RT-PCR to normalize the amounts of mRNA in each sample. The PCR products were analysed by electrophoresis in 2% agarose gels stained with ethidium bromide and bands were visualized and photographed under ultraviolet excitation. Quantitative real-time RT-PCR was carried out on an Applied Biosystems

Table 1 Sequences of specific PCR primers

Molecule	Primer (5′-3′)
CB2R	fw: TGG CAG CGT GAC TAT GAC
	rv: AAA GAG GAA GGC GAT GAA
IL-6	fw: CCA GGA GAA GAT TCC AAA GAT G
	rv: GGA AGG TTC AGG TTG TTT TCT G
MMP-3	fw: TAT GGA CCT CCC CCT GAC TCC
	rv: AGG TTC AAG CTT CCT GAG G
MMP-13	fw: GCT GCC TTC CTC TTC TTG A
	rv: TGC TGC ATT CTC CTT CAG GA
GAPDH	fw: GAA GGT CGG AGT CAA CGG
	rv: GGA AGA TGG TGA TGG GAT T

CB2R: cannabinoid receptor 2; fw: forward; rv: reverse; MMP: matrix metalloproteinase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase.

7500 Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). The housekeeping gene of GAPDH was used to normalize all tested genes and quantification of the mRNA level was performed using the $\Delta\Delta$Ct method. The value of each control sample was set at one and used to calculate the fold change of target genes.

Western blotting

Cultured human FLS were lysed with lysis buffer. Protein concentrations were determined using the Bradford method. The lysates were fractionated by Tris-glycine-buffered 10% SDS-polyacrylamide gel electrophoresis, transferred onto nitrocellulose membranes and incubated overnight at 4°C with antibodies against CB2R, ERK1/2, phospho-ERK1/2, p38, phospho-p38 and GAPDH or α-tubulin. After washing, the membranes were incubated with IRDye-conjugated secondary antibodies and then scanned using the Odyssey Infrared Imaging System (LI-COR, Lincoln, NE, USA). In some cases, densitometry of the signal bands was analysed with Image Gauge version 4.0 software (Fuji Photo Film, Tokyo, Japan).

ELISA

Cultured FLS were pre-treated with HU-308 (1–10 μM) for 15 min followed by 24-h stimulation with IL-1β (1 ng/ml). In the collected culture supernatants, the concentrations of human IL-6, total MMP-3 and pro-MMP-13 were determined with ELISA kits purchased from R&D Systems (Minneapolis, MN, USA).

Proliferation assay with cell counting kit 8

Cultured RA-FLS (5×10^3) were pre-treated with different concentrations of HU-308 for 30 min prior to the addition of IL-1β (1 ng/ml) and then incubated for 48 h. Cell proliferation was measured as previously described using cell counting kit 8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) [13]. Briefly, 10 μl of CCK-8 reagent were added to each well and 4 h later the absorbance at 450 nm was determined using an ELISA plate reader (Multiskan MK3, Labsystems, Vantaa, Finland).

Statistical analysis

Data were analysed using SPSS 16.0 software (IBM, Armonk, NY, USA). One-way analysis of variance with Tukey's post-test for multiple comparisons was used to determine the statistical significance of comparisons. *P*-values <0.05 were considered statistically significant.

Results

Higher expression level of CB2R in RA than OA

We first investigated the expression of CB2R in the synovium of RA patients. Synovial membranes were obtained from RA and OA patients and stained with anti-CB2R antibodies. Positive staining of anti-CB2R antibodies was evident in all RA tissues as well as OA tissues examined in the lining layer and in the interstitial sublining layer areas (Fig. 1A). Furthermore, the expression level in RA samples was slightly higher than that in OA samples.

We next examined CB2R expression in cultured FLS. As a result, constitutive expression of CB2R in resting FLS was confirmed at the mRNA level by RT-PCR (Fig. 1B) and at the protein level by western blotting (Fig. 1C). Compared with the FLS from OA patients, a slightly higher level of CB2R expression in the FLS from RA patients was also found. The expression of CB2R protein in RA-FLS was further confirmed by IF staining of anti-CB2R antibodies (Fig. 1D).

Up-regulated expression level of CB2R by proinflammatory mediators

To determine whether an inflammatory environment modulates the expression level of CB2R, cultured RA-FLS were stimulated with proinflammatory mediators, such as TNF-α (25 ng/ml), IL-1β (10 ng/ml) or LPS (100 ng/ml). As a result, real-time RT-PCR analysis showed that all of these proinflammatory mediators significantly enhanced the mRNA expression level of CB2R by 3.3–7.5-fold (Fig. 2).

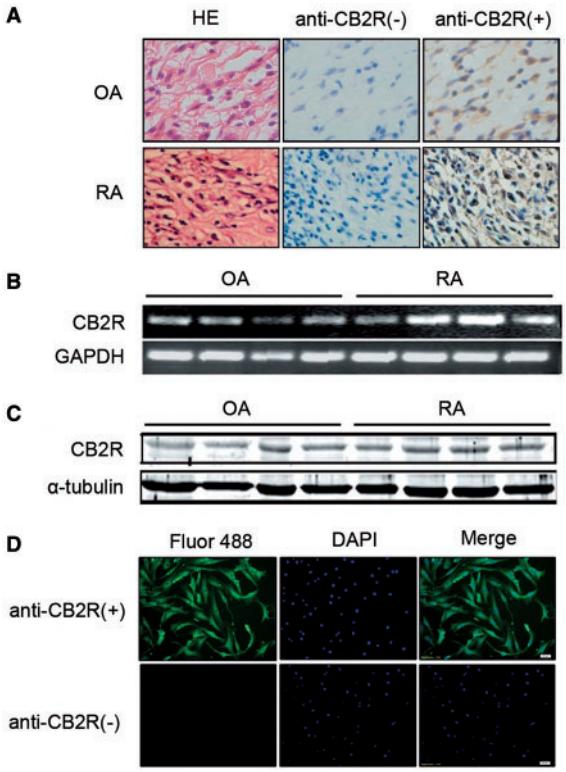
Inhibitory effects of HU-308 on IL-1β-induced proliferation of RA-FLS

In this step we tested the biological function of HU-308, a selective CB2R agonist [9], in cultured RA-FLS. IL-1β is present in RA synovial fluid and participates in joint inflammation and joint destruction. As expected, stimulation with IL-1β (1 ng/ml) resulted in an enhanced proliferation rate of RA-FLS. Pre-treatment with HU-308 (1–10 μM) significantly inhibited the IL-1β-induced proliferation of RA-FLS in a dose-dependent manner (Fig. 3).

Inhibitory effects of HU-308 on IL-6, MMP-3 and MMP-13 production in RA-FLS

To determine whether HU-308 can modulate cytokine and MMP production in human RA-FLS, cells were incubated with IL-1β (1 ng/ml) in the presence or absence of HU-308. In IL-1β-stimulated RA-FLS, HU-308 (1–10 μM) inhibited

Fig. 1 Expression of CB2R in RA synovium and FLS



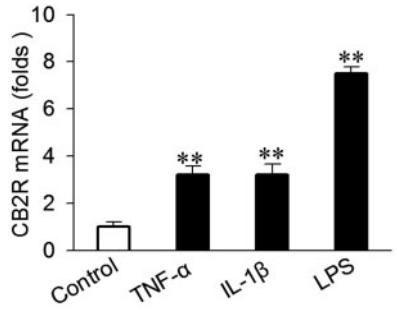
(**A**) Synovial membranes from the patients with RA or OA were stained with anti-CB2R antibodies or isotype-matched control IgG. The left lane shows haematoxylin and eosin (HE) staining and the other two lanes show immunohistochemical staining (original magnification 400×). (**B**) Expression of CB2R mRNA in FLS isolated from four OA patients and four RA patients, as determined by RT-PCR. (**C**) Expression of CB2R protein in FLS isolated from the above patients, as determined by western blotting. (**D**) The expression of CB2R protein in RA-FLS was detected by IF staining of Alexa Fluor 488-conjugated anti-CB2R antibodies. The nuclei were counterstained with DAPI (original magnification 200×).

the mRNA expression levels of IL-6, MMP-3 and MMP-13 in a dose-dependent manner (Fig. 4A). Moreover, HU-308 also significantly decreased the amounts of IL-6, MMP-3 and MMP-13 proteins released into the culture medium of IL-1β-stimulated RA-FLS in a dose-dependent manner (Fig. 4B).

Inhibitory effect of HU-308 on IL-1β-induced activation of ERK1/2 and p38 in RA-FLS

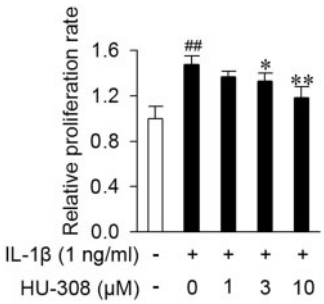
In cultured RA-FLS, IL-1β (1 ng/ml) enhanced intracellular protein levels of both ERK1/2 and phosphorylated ERK1/2 (p-ERK1/2), as well as levels of both p38 MAPK and phosphorylated p38 (p-p38) MAPK. HU-308 (10 μM) blunted the IL-1β-induced activation of ERK1/2 for >2 h and temporarily inhibited IL-1β-induced activation of p38 MAPK for <30 min (Fig. 5).

Fig. 2 Proinflammatory mediators up-regulated the expression level of CB2R mRNA in RA-FLS



Cultured RA-FLS were stimulated with TNF-α (25 ng/ml), IL-1β (10 ng/ml) or LPS (100 ng/ml) for 6 h and the expression level of CB2R mRNA was analysed by quantitative real-time RT-PCR. Data are expressed as the mean (S.D.) ($n = 4$). Compared with the control group, $^{**}P < 0.01$.

Fig. 3 Inhibitory effects of HU-308 on the proliferation of IL-1β-stimulated RA-FLS



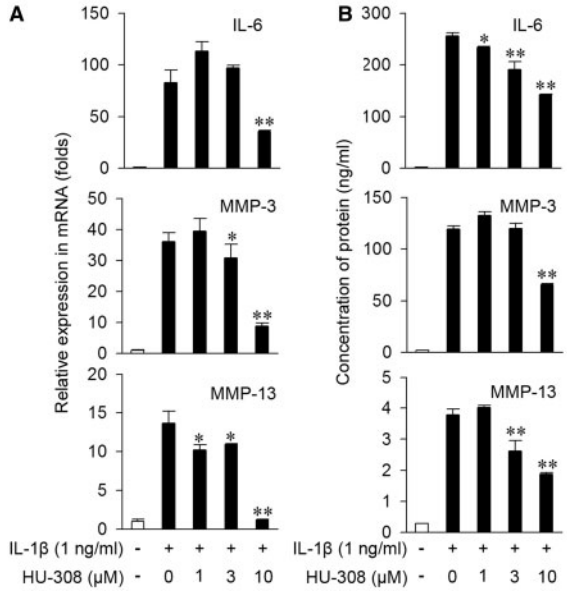
Cultured RA-FLS were pre-treated with different concentrations of HU-308, a selective agonist for CB2R, for 30 min and then incubated with IL-1β (1 ng/ml) for 48 h. Cell proliferation was measured using CCK-8. Data are expressed as the mean (S.D.) ($n = 4$). $^{*}P < 0.05$; $^{**}P < 0.01$ *vs* IL-1β alone; $^{\#\#}P < 0.01$ *vs* negative control (absence of IL-1β).

Discussion

The results reported here demonstrate for the first time that RA-FLS constitutively express CB2R, which may be up-regulated by inflammation. Moreover, activation of CB2R inhibits the production of IL-6, MMP-3 and MMP-13 by attenuating the activation of ERK1/2 and p38 MAPK in FLS. These data offer a promising therapeutic target for the development of novel pharmacological approaches to treat RA.

CB2R is expressed by all immune cells, but its expression level varies among immune cell populations and activation states [14]. Recently CB2R has been identified

FIG. 4 Inhibitory effects of HU-308 on the production of IL-6 and MMPs in IL-1β-stimulated RA-FLS



Cultured RA-FLS were pre-treated with different concentrations of HU-308 for 30 min and then incubated with IL-1β (1 ng/ml) for 3 or 24 h. (**A**) Expression levels of mRNA assayed by quantitative real-time RT-PCR 3 h after IL-1β stimulation. (**B**) Protein concentrations in culture supernatants determined by ELISA 24 h after IL-1β stimulation. Data are expressed as the mean (S.D.) ($n = 4$). *$P < 0.05$; **$P < 0.01$ *vs* IL-1β alone.

molecularly and pharmacologically in numerous other cell types, including articular chondrocytes [15], osteocytes, osteoblasts and osteoclasts [16]. In the present study, immunohistochemical studies showed localization of CB2R in the lining layer and in the interstitial sublining layer of all RA synovial tissues. This finding is consistent with a previous report in which CB2R protein in the homogenates of RA synovial tissues was revealed by western blotting [6]. In RA synovium infiltrated with massive immune cells, the existence of CB2R is not uncommon. In the following experiments we confirmed the expression of CB2R in RA-FLS at both the mRNA and protein levels by RT-PCR, western blotting and immunocytochemical studies. Furthermore, we demonstrated that proinflammatory mediators, e.g. TNF-α, IL-1β and LPS, significantly up-regulated the expression level of CB2R in FLS. We also found that the expression level of CB2R in RA synovial tissues and FLS was higher than that in OA. These data suggest that the up-regulated expression of CB2R may play a counteracting role in the process of RA synovitis, which is also supported by increased concentrations of endocannabinoids in RA synovial fluid [6]. In addition, it has also been demonstrated that the expression level of CB2R can be increased under certain conditions and disease states [14]. For example, increased CB2R has been found in multiple sclerosis [17], allergic contact dermatitis [18] and acute and chronic bladder inflammation [19]. The increasing receptor number is also important for increasing the efficacy of CB2R agonists.

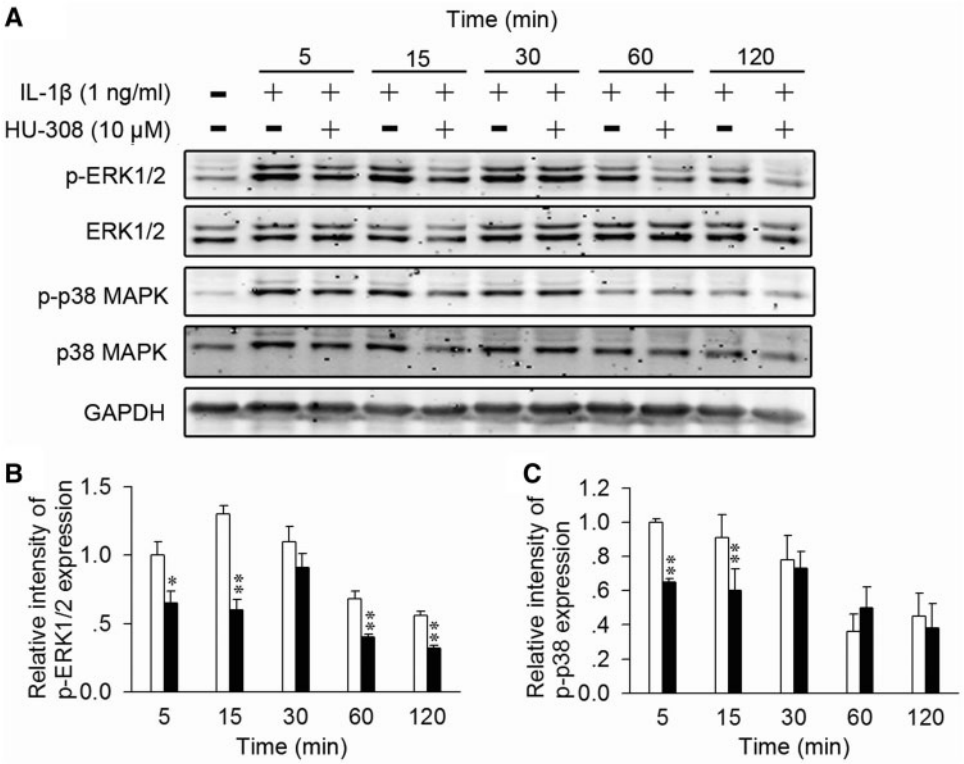
HU-308 is a synthetic cannabinoid, and the affinity of HU-308 binding to CB2R is almost 300 times the affinity to CB1R. Functional studies show it inhibits forskolin-stimulated cyclic adenosine monophosphate (cAMP) production in CB2R-transfected cells, whereas it shows little effect in CB1R-transfected cells. So HU-308 is a specific agonist of CB2R [9].

In bone marrow–derived primary monocytic cultures derived from wild-type mice, HU-308 dose-dependently suppressed the formation of osteoclasts. The deficiency of CB2R completely abolished the inhibitory effect of HU-308 on osteoclast formation [16]. *In vivo*, HU-308 also attenuated ovariectomy-induced bone loss through suppression of osteoclast formation. No protective effects of HU-308 were observed in ovariectomized CB2R knockout mice [20]. These data further support that CB2R mediates the effect of HU-308, and also imply that selective activation of CB2R may inhibit bone erosion caused by osteoclasts in RA.

Accumulating evidence demonstrates that proinflammatory cytokines such as TNF-α and IL-6 play pivotal roles in the physiopathology of RA, and the biologics of TNF inhibitors and anti-IL-6 receptor monoclonal antibody have shown dramatic efficacy in RA treatment [1, 21, 22]. TNF-α and IL-6 in RA synovitis joints are produced mainly from macrophage-like synoviocytes and FLS. In the present study, the *in vitro* effect of HU-308 on proinflammatory cytokine production was investigated in RA-FLS. HU-308 significantly decreased IL-1β-induced production of IL-6 from RA-FLS in a dose-dependent manner. In addition, HU-308 significantly inhibited LPS-induced IL-6 and TNF-α production from peritoneal macrophages isolated from wild-type mice, whereas HU-308 failed to suppress LPS-induced cytokine production from CB2R gene knockout macrophages (unpublished data). Taken together, these results indicate that HU-308 inhibits the inflammatory process by decreasing the production of proinflammatory cytokines in RA synovitis, which is mediated by CB2R.

In a previous study, two synthetic cannabinoids—CP55940 (10 μM) and Win55212-2 (10 μM)—were reported to attenuate IL-6 and IL-8 secretion from IL-1β-stimulated RA-FLS, and the inhibitory effects were not counteracted by CB1R antagonist AM-281 (80 μM) or CB2R antagonist AM-630 (80 μM). So it was concluded that CP55940 and Win55212-2 exert a potent anti-inflammatory effect on RA-FLS via a non-CB1R/CB2R-mediated mechanism [23]. Although AM630 was used as a CB2R antagonist, 10 μM of AM-630 was also an inverse agonist at CB2R and a partial agonist at CB1R [24]. So up to 80 μM of AM-630 is not a solely specific antagonist of CB2R, and may also activate CB1R. In some studies, SR 144528 was used as a selective antagonist of CB2R [25], but there was evidence for the inverse agonist property of SR 144528 and the constitutive activation of CB2R

FIG. 5 Suppression of HU-308 on IL-1β-induced signalling pathways in FLS



(**A**) Western blotting analysis of the intracellular levels of ERK1/2 and p-ERK1/2, as well as the levels of p38 and p-p38 MAPK in the presence of IL-1β and/or HU-308. Densitometry analysis of the western blotting bands of (**B**) p-ERK1/2 and (**C**) p-p38 MAPK from three independent experiments. Data are expressed as the mean (S.D.) ($n = 3$). *$P < 0.05$; **$P < 0.01$ *vs* IL-1β alone.

in Chinese hamster ovary–expressing cells [26]. At this time there is no pure specific antagonist of CB2R, so we did not use any pharmacological antagonists to interrupt the binding of HU-308 to CB2R in the present study.

In RA, the MMP family is related to the invasiveness of FLS and the erosion of cartilage [27]. In the present study, HU-308 significantly inhibited the IL-1β-induced production of MMP-3 and MMP-13 in RA-FLS. Our results were consistent with the finding that Win-55212-2, a non-selective agonist to CB1R and CB2R, significantly inhibited IL-1-induced proteoglycan and collagen breakdown in bovine cartilage explants [15]. These data suggest that HU-308 may protect cartilage from damage in RA by inhibiting the production of MMPs. In addition, we also found that HU-308 inhibited IL-1β-induced proliferation of FLS.

The cellular processes that contribute to the pathogenesis of RA are regulated by three families of MAPKs, namely, ERK1/2, JNK, and p38 [7, 28]. In the present study, IL-1β stimulated phosphorylation of ERK1/2 and p38 MAPK, but did not affect JNK in RA-FLS, which is consistent with previous reports that IL-1β stimulated FLS via activating intracellular ERK1/2 and p38 MAPK [29, 30]. In the present study, HU-308 effectively inhibited IL-1β-induced activation of ERK1/2 and p38 MAPK in RA-FLS. This finding was indirectly supported by the finding that HU-308 dose-dependently attenuated TNF-α-induced activation of ERK1/2 and p38 MAPK in vascular smooth muscle cells [31]. However, it was also reported that the activation of CB2R results in activation of ERK1/2 and p38 MAPK in monocytes [32]. These data infer that signalling of CB2R activation may vary substantially depending on cell type and stimulus.

In conclusion, CB2R is expressed in RA synoviocytes, including FLS, and its expression is up-regulated by proinflammatory mediators. Selective activation of CB2R suppresses the production proinflammatory cytokines and MMPs from FLS.

Rheumatology key messages

- Cannabinoid receptor 2 (CB2R) is expressed by RA synovium, including fibroblast-like synoviocytes (FLS), and is up-regulated by inflammation.
- Activation of CB2R inhibits the proliferation and production of proinflammatory cytokines and MMPs in RA-FLS.
- Activation of CB2R inhibits IL-1β-induced activation of extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase in RA-FLS.

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Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* Online.

References

1 Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet 2010;376:1094–108.

2 Klein TW. Cannabinoid-based drugs as anti-inflammatory therapeutics. Nat Rev Immunol 2005;5:400–11.

3 Matsuda LA, Lolait SJ, Brownstein MJ *et al*. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 1990;346:561–4.

4 Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61–5.

5 Howlett AC, Barth F, Bonner TI *et al*. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 2002;54:161–202.

6 Richardson D, Pearson RG, Kurian N *et al*. Characterisation of the cannabinoid receptor system in synovial tissue and fluid in patients with osteoarthritis and rheumatoid arthritis. Arthritis Res Ther 2008;10:R43.

7 Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. Immunol Rev 2010; 233:233–55.

8 Neumann E, Lefevre S, Zimmermann B *et al*. Rheumatoid arthritis progression mediated by activated synovial fibroblasts. Trends Mol Med 2010;16:458–68.

9 Hanus L, Breuer A, Tchilibon S *et al*. HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. Proc Natl Acad Sci USA 1999;96:14228–33.

10 Arnett FC, Edworthy SM, Bloch DA *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315–24.

11 Altman R, Asch E, Bloch D *et al*. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986;29: 1039–49.

12 Zhang W, Cong XL, Qin YH *et al*. IL-18 upregulates the production of key regulators of osteoclastogenesis from fibroblast-like synoviocytes in rheumatoid arthritis. Inflammation 2013;36:103–9.

13 Ishiyama M, Tominaga H, Shiga M *et al*. A combined assay of cell viability and in vitro cytotoxicity with a highly water-soluble tetrazolium salt, neutral red and crystal violet. Biol Pharm Bull 1996;19:1518–20.

14 Miller LK, Devi LA. The highs and lows of cannabinoid receptor expression in disease: mechanisms and their therapeutic implications. Pharmacol Rev 2011;63: 461–70.

15 Mbvundula EC, Bunning RA, Rainsford KD. Arthritis and cannabinoids: HU-210 and Win-55,212-2 prevent IL-1alpha-induced matrix degradation in bovine articular chondrocytes in-vitro. J Pharm Pharmacol 2006; 58:351–8.

16 Ofek O, Karsak M, Leclerc N *et al*. Peripheral cannabinoid receptor, CB2, regulates bone mass. Proc Natl Acad Sci USA 2006;103:696–701.

17 Maresz K, Pryce G, Ponomarev ED *et al*. Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. Nat Med 2007;13:492–7.

18 Karsak M, Gaffal E, Date R *et al*. Attenuation of allergic contact dermatitis through the endocannabinoid system. Science 2007;316:1494–7.

19 Merriam FV, Wang ZY, Guerios SD *et al*. Cannabinoid receptor 2 is increased in acutely and chronically inflamed bladder of rats. Neurosci Lett 2008;445:130–4.

20 Sophocleous A, Landao-Bassonga E, Van't Hof RJ *et al*. The type 2 cannabinoid receptor regulates bone mass and ovariectomy-induced bone loss by affecting osteoblast differentiation and bone formation. Endocrinology 2011; 152:2141–9.

21 McInnes IB, O'Dell JR. State-of-the-art: rheumatoid arthritis. Ann Rheum Dis 2010;69:1898–906.

22 Buch MH, Emery P. New therapies in the management of rheumatoid arthritis. Curr Opin Rheumatol 2011;23: 245–51.

23 Selvi E, Lorenzini S, Garcia-Gonzalez E *et al*. Inhibitory effect of synthetic cannabinoids on cytokine production in rheumatoid fibroblast-like synoviocytes. Clin Exp Rheumatol 2008;26:574–81.

24 Ross RA, Brockie HC, Stevenson LA *et al*. Agonist-inverse agonist characterization at CB1 and CB2 cannabinoid receptors of L759633, L759656, and AM630. Br J Pharmacol 1999;126:665–72.

25 Rinaldi-Carmona M, Barth F, Millan J *et al*. SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. J Pharmacol Exp Ther 1998;284: 644–50.

26 Portier M, Rinaldi-Carmona M, Pecceu F *et al*. SR 144528, an antagonist for the peripheral cannabinoid receptor that behaves as an inverse agonist. J Pharmacol Exp Ther 1999;288:582–9.

27 Tolboom TC, Pieterman E, van der Laan WH *et al*. Invasive properties of fibroblast-like synoviocytes: correlation with growth characteristics and expression of MMP-1, MMP-3, and MMP-10. Ann Rheum Dis 2002;61:975–80.

28 Cohen P. Targeting protein kinases for the development of anti-inflammatory drugs. Curr Opin Cell Biol 2009;21: 317–24.

29 Suzuki M, Tetsuka T, Yoshida S *et al*. The role of p38 mitogen-activated protein kinase in IL-6 and IL-8 production from the TNF-alpha- or IL-1beta-stimulated rheumatoid synovial fibroblasts. FEBS Lett 2000;465: 23–7.

30 Kirchmeyer M, Koufany M, Sebillaud S *et al*. All-trans retinoic acid suppresses interleukin-6 expression in interleukin-1-stimulated synovial fibroblasts by inhibition of ERK1/2 pathway independently of RAR activation. Arthritis Res Ther 2008;10:R141.

31 Rajesh M, Mukhopadhyay P, Hasko G *et al*. CB2 cannabinoid receptor agonists attenuate TNF-alpha-induced human vascular smooth muscle cell proliferation and migration. Br J Pharmacol 2008;153:347–57.

32 Gertsch J, Schoop R, Kuenzle U *et al*. Echinacea alkylamides modulate TNF-alpha gene expression via cannabinoid receptor CB2 and multiple signal transduction pathways. FEBS Lett 2004; 577:563–9.

Clinical vignette

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Polymyalgia rheumatica or lymphoma recurrence? Positron emission tomography/computed tomography is a specific imaging technique that helps differential diagnosis

In November 2011 a 72-year-old retired surgeon was diagnosed with Hodgkin's lymphoma. Fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT) showed cervical lymph node uptake (Fig. 1A, arrow), which quickly disappeared after two cycles of adryamicin, bleomycin, vinblastine sulphate, and dacarbazine (ABVD) (Fig. 1B). In October 2012 the patient complained of pain in the girdles of 75 days duration, worsening at night, and accompanied by fatigue and morning stiffness lasting 3 h. He had lost 1 kg in 1 month, but denied fever, headache or vision impairment. Clinical examination revealed active elevation of the arms below 90°, slight pain on passive motion of the shoulders, stiffness of the hips and slight swelling and tenderness of two MCP joints. ESR was 120 mm/h, CRP was 50.6 mg/dl and IgM RF and anti-cyclic citrullinated peptides were absent. The appearance of systemic symptoms raised the suspicion of a lymphoma relapse, in view also of the short course of chemotherapy. A third FDG-PET/CT scan showed involvement of the shoulders (Fig. 1C, arrowheads), trochanteric bursae (open arrowheads) and ischiatic bursae, without large-vessel vasculitis. This pattern is typical of PMR [1]. After the diagnosis of PMR, treatment with 0.2 mg/kg prednisone was started with prompt and complete resolution of symptoms.

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Reference

1 Camellino D, Cimmino MA. Imaging of polymyalgia rheumatica: indications on its pathogenesis, diagnosis and prognosis. Rheumatology 2012;51:77–86.

Fig. 1 Positron emission tomography/computed tomography, coronal view: the three different scans are shown