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Altered expression of inflammatory cytokines in primary osteoarthritis by human T lymphotropic virus type I retrovirus infection: a cross-sectional study

Yoshiki Yoshihara[1], Tomoo Tsukazaki[1], Makoto Osaki[1], Masahiro Nakashima[2], Kazuhisa Hasui[3] and Hiroyuki Shindo[1]

[1]Division of Orthopaedic Pathomechanism, Department of Developmental and Reconstructive Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[2]Tissue and Histopathology Section, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
[3]Department of Immunology, Course of Infection and Immunity, Course of Health Science, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

Corresponding author: Tomoo Tsukazaki, ttsuka@net.nagasaki-u.ac.jp

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Abstract

Human T cell leukaemia virus type I (HTLV-I) is known to be involved in late-onset chronic polyarthritis as HTLV-I-associated arthropathy. However, it is unclear whether HTLV-I infection could modify the pathophysiology of osteoarthritis (OA). In this study we compared several inflammatory cytokines, such as C-terminal parathyroid hormone-related peptide (C-PTHrP), soluble interleukin-2 receptor (sIL-2R) and interleukin (IL)-6, and an osteo-destruction marker, deoxypyridinoline, in synovial fluid (SF) samples obtained from 22 HTLV-I carriers and 58 control non-carrier patients with OA. These patients were diagnosed clinically and radiographically with primary OA affecting one or both knee joints, and were similar with regard to age, sex and clinical symptoms. We also performed histopathological examination as well as immunohistochemistry of HTLV-I-derived Tax protein in eight synovial tissues taken from carrier patients. C-PTHrP in SF was significantly higher in HTLV-I carriers (287 ± 280 pM) than in non-carriers (69 ± 34 pM), and the concentration in 13 carriers was above the upper range of OA. In HTLV-I carriers, the concentrations of sIL-2R (741 ± 530 IU/ml), IL-6 (55 ± 86 ng/ml) and deoxypyridinoline (3.1 ± 1.8 nM) were higher than in non-carriers (299 ± 303, 2.5 ± 4.0, 0.96 ± 1.0, respectively), and correlated positively with C-PTHrP. C-PTHrP, sIL-2R and IL-6 concentrations in SF positive for IgM antibody against HTLV-I antigen, a marker of persistent viral replication, were higher than of IgM-negative SF. Histologically, five and two synovia showed mild and moderate synovial proliferation with or without some degree of inflammatory reaction, respectively, and could not be distinguished from OA. Tax-positive synoviocytes were observed sparsely in all samples, and often appeared frequently in actively proliferating regions. Our results suggest that although HTLV-I infection does not necessarily worsen the clinical outcome and local synovitis, the virus can potentially modify the pathophysiology of OA by increasing the inflammatory activity in a subset of carrier patients, especially those with IgM antibody. Longitudinal studies are required to assess the association between HTLV-I infection and OA.

Keywords: Human T cell leukaemia virus type I, osteoarthritis, parathyroid hormone-related peptide, synovial fluid, Tax

Introduction

Retroviral infection is associated with various pathological conditions, including several cancers and immunological and neurological disorders [1]. Human T cell leukaemia virus type I (HTLV-I) is the causative virus of acute T-cell leukaemia (ATL) [2]. HTLV-I is estimated to infect more than 10 million people worldwide, and is endemic in several areas including southwestern Japan, especially in Kyusyu Island. Although most seropositive individuals are asymptomatic carriers, a proportion of these individuals develop ATL in adolescence. In addition, HTLV-I has also been shown to be involved in several immunological and inflam-

ATL = acute T-cell leukaemia; C-PTHrP = C-terminal parathyroid hormone; DPD = deoxypyridinoline; HAAP = HTLV-I-associated arthropathy; HTLV-I = human T lymphotropic virus type I; IL-6 = interleukin-6; OA = osteoarthritis; RA = rheumatoid arthritis; SF = synovial fluid; sIL-2R = soluble interleukin-2 receptor.

matory disorders, such as HTLV-I-associated myelopathy/tropical spastic paraparesis, bronchopneumonopathy, Sjögren syndrome and uveitis [3,4].

HTLV-I-associated arthropathy (HAAP) is also recognized as chronic arthritis caused by HTLV-I infection. In 1989, Nishioka and colleagues [5] reported 11 HTLV-I carriers with chronic oligoarthritis associated with ATL-like lymphocyte infiltration. The onset of HAAP often starts acutely in a relatively large joint such as the knee, wrist or shoulder, and the associated symptoms closely resemble those of rheumatoid arthritis (RA). The histopathological changes in HAAP include marked proliferation of synoviocytes in the synovial lining layer, gross infiltration of lymphocytes, and migration of atypical lymphocytes with nuclear indentation into the synovial fluid (SF) and/or synovial tissue.

Numerous studies have demonstrated that HTLV-I can alter the oncogenic and immunogenic properties of synovial cells and lymphocytes [6,7]. In addition, mice overexpressing Tax, the protein encoded by the HTLV-I pX region, develop RA-like chronic and systemic synovitis [8]. Furthermore, epidemiological studies have revealed a significant association of HTLV-I infection and RA in endemic areas in Japan [9,10], although studies in the USA, Europe and South Africa failed to link HTLV-I infection and RA [11-14]. These pieces of evidence link HTLV-I infection to synovial proliferation; however, several clinical issues remain unresolved. There is still no established criterion for the diagnosis of HAAP because of the lack of specific symptoms and/or laboratory markers. Moreover, it is also not clear whether HAAP could exhibit other phenotypes, such as monoarthritis instead of polyarthritis.

Osteoarthritis (OA) is a degenerative disorder caused by mechanical overload and/or a consequence of imbalanced biological events between cartilage degradation and synthesis [15]. In primary OA, age, obesity and malalignment are known as predisposing factors, but the association of virus infection has not yet been studied. In the present study we investigated a potential role of HTLV-I infection in the pathophysiology of primary OA. For this, we compared the concentrations of several inflammatory cytokines in SF taken from HTLV-I carriers and non-carrier patients who had been diagnosed with primary OA of one or both knee joints. We also studied the histopathological features of synovia of eight HTLV-I carrier patients, and determined the expression of Tax protein by immunohistochemistry.

Materials and methods

Patients and samples

Outpatients fulfilling the criteria of the American College of Rheumatology for the diagnosis of knee OA [16] and corresponding to OA grade II or higher by the radiographic criteria of Kellgren and Lawrence [17] were recruited to this cross-sectional study. Patients who fulfilled even one of the American College of Rheumatology criteria for RA during the later 4-year observation period were excluded. Patients with secondary arthritis, such as gout, pseudogout, purulent or traumatic arthritis or seronegative arthritis, were also excluded.

Peripheral blood and SF samples were obtained simultaneously from 22 HTLV-I carrier outpatients at the initial examination and were subjected to appropriate pretreatment as described previously [18]. As patient control, SF and serum were also obtained from 58 HTLV-I-negative OA patients. Comparison of HTLV-I carrier and non-carrier OA patients showed no obvious differences in age, sex, affected side and disease duration (Table 1). At the initial examination, 20 HTLV-I carriers and 55 non-carrier patients felt pain in the medial femorotibial joints, a common type in Japanese primary OA, whereas the remaining patients also complained of pain in the patellofemoral joint. Joint swelling, repeated hydrops and limited range of motion were also commonly observed in the enrolled patients, without obvious differences between HTLV-I carriers and non-carriers. The major radiographic findings in the 80 patients were osteophyte formation with or without narrowing of the joint space and sclerotic change of subchondral bone, and there was no significant difference in these radiographic features between HTLV-I carriers and non-carriers as defined by the Kellgren/Lawrence scoring method (Table 1). At the time of enrolment in the present study, patients were taking a variety of medications, including non-steroidal anti-inflammatory drugs, external splints and intra-articular injections of prednisone and/or hyaluronic acid.

Intra-articular injection was discontinued for at least 2 weeks before sample collection. The erythrocyte sedimentation rate, serum C-reactive protein and serum calcium concentrations were confirmed to be within the normal range in all patients. The study protocol was approved by the Human Ethics Review Committee of Nagasaki University School of Medicine, and a signed consent form was obtained from each subject.

ELISA and Western blotting of HTLV-I

Anti-HTLV-I antibody in sera and SF was screened by enzyme-linked immunosorbent assay (ELISA; Eitest-ATL kit; Eisai Inc., Tokyo, Japan) in accordance with the instructions provided by the manufacturer. This ELISA system is designed to detect IgG antibody. On the basis of this test, 22 patients with immunoreactivity in both serum and SF samples were defined as HTLV-I carriers.

To determine the epitope recognized by the antibody and to characterize the specificity of IgG and IgM antibodies, SF was subjected to Western blot analysis with the use of epitope-transferred membrane (Eitest-ATL WB kit; Eisai

Table 1

Clinical and radiographic background of human T lymphotropic virus type I carriers and non-carriers

Parameter	HTLV-I carriers		HTLV-I non-carriers
	IgM Ab+	IgM Ab-	
Number of patients	8	14	58
Age (years)[a]	68.8 (58–78)	72.0 (56–87)	68.6 (51–88)
Sex (M/F)	3/5	4/10	16/42
Disease duration (years)[a]	7.8 (4–15)	6.6 (4–10)	6.3 (3–13)
Unilateral/bilateral	2/6	5/9	17/41
K/L scale[b] (II/III/IV)	7/5/2	10/10/3	49/42/8

[a]Data are means (range); all other data are numbers of patients or affected joints. [b]Scoring was as follows: II, minimal osteophytes possibly with narrowing, cyst and sclerosis; III, moderate or definite osteophytes with moderate joint space narrowing; IV, severe with large osteophytes and definite joint space narrowing. Disease duration indicates the period from the first affected joint in cases with bilateral knee arthritis. Ab, antibody; F, female; HTLV-I, human T lymphotropic virus type I; K/L, Kellgren/Lawrence; M, male.

Inc.). Two envelope proteins and three core proteins derived from HTLV-I were fixed onto nitrocellulose membrane, and specific binding of IgG or IgM was distinguished by specific secondary antibody. The result of the Western blot was defined as positive when each antibody reacted with at least two antigens. In this Western blot analysis, IgG antibody was present in all 22 examined SF, whereas IgM class antibody, which is considered to be elevated in the acute phase of active viral replication [19], was detected in the SF of 8 carriers (Table 1). There was no difference in age, sex or disease duration between carrier patients with or without IgM antibody.

Measurements of C-terminal parathyroid hormone, sIL-2R, IL-6, chondrocalcin and deoxypyridinoline

The C-terminal region (amino acids 109–141) of parathyroid hormone-related peptide (C-PTHrP) was measured with a radioimmunoassay kit (Daiichi Radioisotopes Laboratory, Chiba, Japan) as described previously [18]. Soluble interleukin (IL)-2 receptor (sIL-2R) (Endogen Inc., Woburn, MA), IL-6 (Endogen Inc.), chondrocalcin (Teijin Co., Tokyo, Japan) and deoxypyridinoline (DPD) (PYRLINKS-D; Quidel Corporation, Santa Clara, CA) were determined by ELISA with the protocol recommended by each manufacturer.

Tissue samples and immunohistochemistry

The synovial tissues were obtained at the time of joint replacement surgery ($n = 4$), synovectomy ($n = 2$) or high tibial osteotomy ($n = 2$) from HTLV-I carriers, and subjected to routine histopathological and immunohistochemical examinations as described previously [20]. In brief, deparaffinized serial sections were preincubated with 3% H_2O_2 to remove endogenous peroxidase activity, and then incubated overnight at 4°C with monoclonal anti-HTLV-I Tax antigen (Lt-4, a gift from Dr Tanaka) antibody [21]. The protein expression was detected by the ImmunoMax/catalyzed signal amplification method with 3,3-diaminobenzidine tetrahydrochloride as a substrate [22]. The preparation incubated without the primary antibody served as the control. In accordance with our previous methods [20], the degree of proliferation of synovial lining cells was assessed in at least five points in an area of more than 1.5 cm × 1.5 cm as follows: -, 1 or 2 cells thick; +/-, 3 or 4 cells thick; ++, 5–9 cells thick; +++, more than 10 cells thick. The overall degree of inflammatory reaction was also semi-quantified as follows: -, no infiltration; +/-, minimal and partial infiltration; +, moderately diffuse or aggregated infiltration; ++, large number of aggregates, many demonstrating germinal centres. These histological findings were evaluated independently by two authors (TT and MN).

Statistical analysis

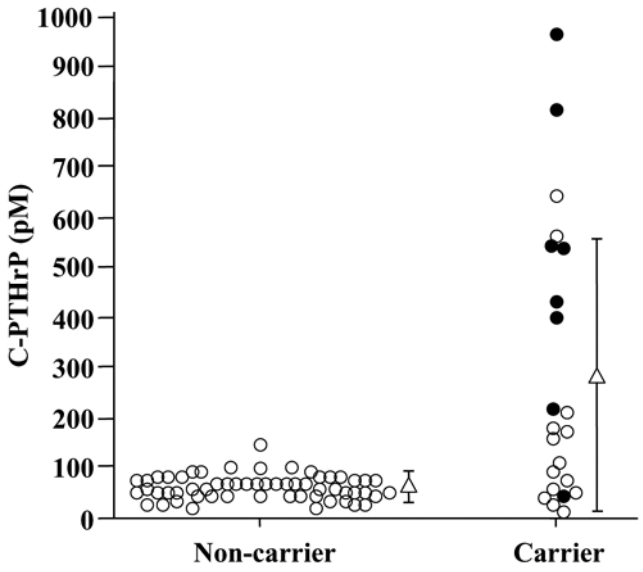
Data are expressed as means ± SD. Mann–Whitney test and χ^2 test with Yates's correction were used to compare data from two or three groups. Correlation coefficients were determined by Pearson linear regression analysis. $P < 0.05$ was considered significant.

Results

C-PTHrP in sera and SF

To investigate whether a distinct pathological state exists in HTLV-I-infected arthritis, we measured the concentrations of several inflammatory cytokines in SF from HTLV-I carrier and non-carrier OA patients. We have previously demonstrated that whereas C-PTHrP in SF of OA patients is within the low concentration of normal SF, C-PTHrP concentration in RA markedly increased with the severity of disease activity [18]. Consistent with the previous findings were our results that C-PTHrP concentrations in sera of HTLV-I carrier and non-carrier OA patients were low, ranging from 16 to 60 pM (carriers, 22 ± 12; non-carriers, 26 ± 16 pM; $P > 0.05$). In contrast, SF C-PTHrP in HTLV-I carriers (287 ± 280, range 19–955 pM) was significantly higher than in non-carrier OA patients (69 ± 34, range 22–

Figure 1



C-terminal parathyroid hormone (C-PTHrP) concentrations in synovial fluid samples of human T lymphotropic virus type I (HTLV-I) carrier patients and non-carrier patients with osteoarthritis. Synovial fluids were obtained from knee joints of HTLV-I carriers ($n = 22$) and non-carriers ($n = 58$) with primary osteoarthritis. The concentration of C-terminal (104–141) PTHrP was measured by radioimmunoassay. Filled circles, IgM-positive HTLV-I carriers; triangles and bars, means ± SD.

134 pM; $P < 0.001$) (Fig. 1). Furthermore, C-PTHrP was significantly higher in IgM-positive (494 ± 296, range 37–955; $n = 8$) than in IgM-negative (169 ± 195, range 19–644 pM; $P = 0.006$) HTLV-I carriers.

sIL-2R, IL-6, chondrocalcin and DPD in SF

We also evaluated sIL-2R and IL-6, which are known to increase in SF of inflammatory arthritis [23,24]. Both sIL-2R and IL-6 in SF of HTLV-I carriers (respectively 741 ± 530 IU/ml, range 211–1970, and 55 ± 86 ng/ml, range 0–333) were significantly higher than those of HTLV-I-negative OA patients (299 ± 303 IU/ml, range 0–1510, and 2.5 ± 4.0 ng/ml, range 0.1–13.9; $P < 0.001$ and $P < 0.05$, respectively). Furthermore, sIL-2R in IgM-positive carriers (1190 ± 556 IU/ml, range 493–1970) was also significantly higher than in IgM-negative carriers (440 ± 202 IU/ml, range 211–802; $P < 0.001$).

Concentrations of chondrocalcin (a marker of articular cartilage damage) and DPD (a marker of subchondral bone absorption) [25,26] were also examined in these samples. Although there was no significant difference in chondrocalcin concentration between carriers (3.0 ± 4.0 ng/ml, range 0–16.3) and non-carrier OA patients (3.3 ± 1.9 ng/ml, range 0–7.9; $P = 0.75$), DPD in HTLV-I carriers (3.1 ± 1.8 nM, range 0–6.8) was significantly higher than in non-carrier OA patients (0.96 ± 1.0 nM, range 0–3.6; $P < 0.001$).

To test whether the elevated C-PTHrP in SF reflected joint inflammation, the relationship between C-PTHrP and the above markers was examined in SF of HTLV-I carriers. C-PTHrP was positively correlated with sIL-2R ($r = 0.54$, $P = 0.01$), IL-6 ($r = 0.57$, $P = 0.001$) and DPD ($r = 0.60$, $P = 0.003$) (Fig. 2).

Histopathological and immunohistochemical examination for HTLV-I Tax

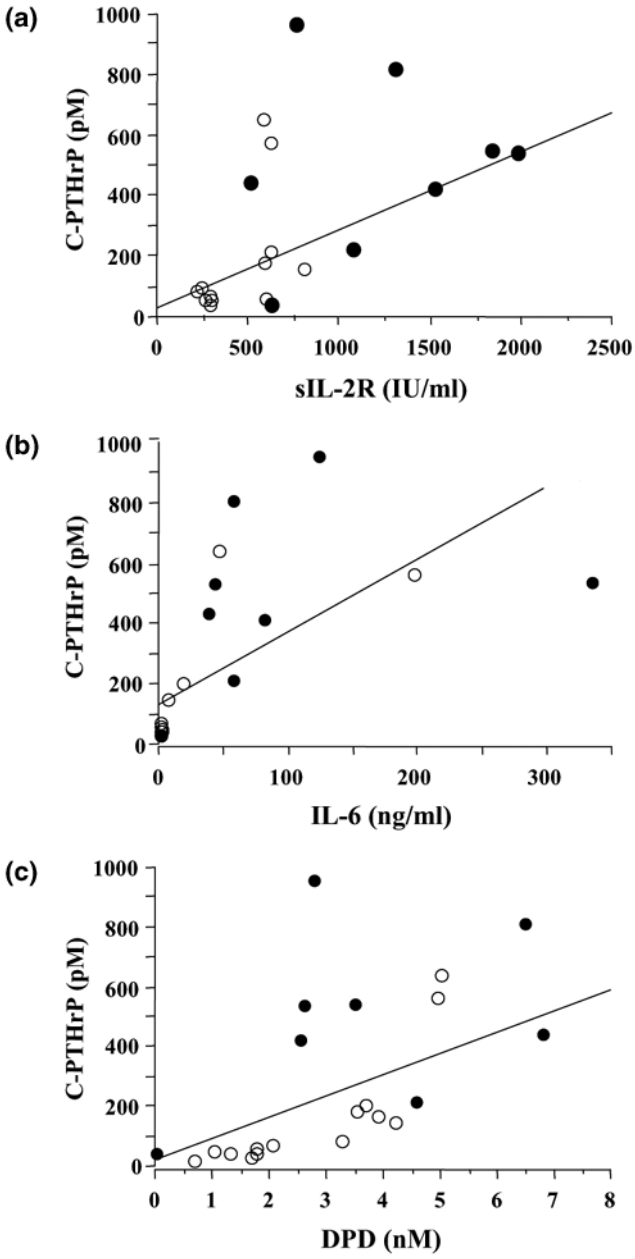
In eight synovial tissues obtained from HTLV-I carriers, six samples, including two IgM-positive carriers, showed histopathological features that are often observed in late-stage OA: synovial lining cells were stratified into only a few layers, and inflammatory reaction was subtle or minimal (Table 2). In contrast, the two synovia of IgM-positive patients consisted, in part, of papillary projected synovium with synovial lining cells stratified into more than five layers (Fig. 3a). Unlike RA, however, lymphocyte infiltration was focal and not significant, and the infiltrating lymphocytes never formed lymphoid follicles in the interstitium. There was no apparent relationship between histopathological findings, radiographic exacerbation and the concentration of each cytokine.

Immunohistochemistry demonstrated that Tax was expressed and that the number of Tax-positive cells varied in the samples (Fig. 3b). Although there was no correlation between Tax immunoreactivity and level of inflammation, the papillary projected region of IgM-positive carriers tended to contain a higher number of Tax-positive synoviocytes.

Discussion

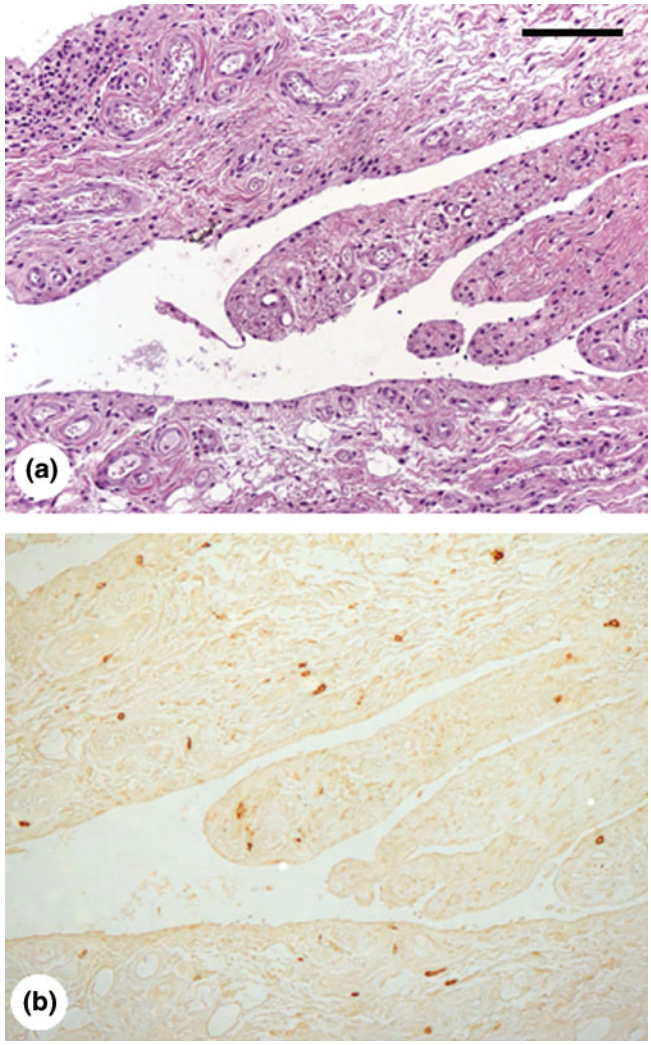
In the present study we compared the concentrations of several inflammatory cytokines in SF between HTLV-I carrier patients and HTLV-I-negative OA patients. By investigating OA patients only and excluding those with RA, we were able to demonstrate the involvement of HTLV-I infection in arthritis. Our results showed that C-PTHrP, sIL-2R and IL-6 were significantly higher in SF of HTLV-I carriers than in that of HTLV-I-negative OA patients. Furthermore, the concentrations of increased markers were higher in HTLV-I carriers positive for IgM antibody than those negative for the antibody. These results indicate that the joint inflammation is more severe in HTLV-I carriers than in HTLV-I-negative OA patients. However, we were unable to identify any differences in radiographic findings between the two groups. It is possible that the pathological changes in our carrier patients are under the limit of detection by the Kellgren scaling system, which addresses only the radiographic dimensions of arthritis. Alternatively, HTLV-I-associated arthritis might be only slowly progressive after onset, and the changes in disease status over a few years are often small and difficult to quantify.

Figure 2



Correlation between C-terminal parathyroid hormone (C-PTHrP) concentrations and **(a)** soluble IL-2 receptor (sIL-2R), **(b)** IL-6 and **(c)** deoxypyridinoline (DPD) in synovial fluid samples of human T lymphotropic virus type I (HTLV-I) carriers with osteoarthritis. The concentrations of sIL-2R, IL-6 and DPD were measured by enzyme-linked immunosorbent assay. Filled circles, IgM-positive HTLV-I carriers; open circles, IgM-negative HTLV-I carriers.

Figure 3



(a) Histopathological features and **(b)** immunohistochemical expression of Tax protein in synovial tissue obtained from a representative human T lymphotropic virus type I (HTLV-I) carrier with osteoarthritis (case 6). The synovium showed focal papillary proliferation together with hyperaemic dilated vessels and mild lymphocytic infiltration. Unlike in rheumatoid arthritis, however, neither stratified synovial lining cells nor lymphoid follicle formation were evident throughout the whole tissue. Tax-positive synoviocytes could be sparsely observed in the papillary projected area of the synovium. Scale bar, 100 μm.

With regard to the mechanism of HTLV-I infection-induced changes in immunogenic properties, previous studies reported that PTHrP, IL-2 receptor α subunit and IL-6 are cellular target genes of the Tax protein [27-29]. In particular, direct binding of Tax to the nuclear factor-κB sequence on the IL-6 promoter is important for HTLV-I-induced IL-6 secretion in cultured synoviocytes [30]. In our study we used immunohistochemistry to examine the expression of the Tax protein and showed the expression of Tax in synoviocytes in all samples examined. Although Tax expression was not correlated with cytokine concentration in SF, probably owing to the time discrepancy between SF aspiration and tissue preparation, Tax expression in synoviocytes might be responsible, at least in part, for the increased concentrations of PTHrP, sIL-2R and IL-6 in SF of carrier patients.

Table 2

C-terminal parathyroid hormone (C-PTHrP) concentration, radiographic changes, histopathological features and Tax expression in synovia of human T lymphotropic virus type Icarriers with knee-joint osteoarthritis

Case	IgM	C-PTHrP (pM)	Operation	Kellgren/Lawrence scale[a]		Interval (years)	Synovial proliferation	Inflammatory reaction	Tax expression[b]
				Initial	Before operation				
1	+	201	OST	IV	IV	3.4	+/-	-	+/-
2	-	154	TA	IV	IV	2.8	+	-	+
3	-	214	TA	IV	IV	1.2	+	+/-	+
4	+	439	TA	IV	IV	3.5	+	+/-	++
5	-	567	OST	III	IV	2.1	+	+/-	+
6	+	813	SYV	II	III	1.5	++	+	++
7	+	955	SYV	III	III	0.3	++	+	+
8	-	644	TA	IV	IV	0.5	+	+/-	+

The degrees of synovial proliferation and inflammatory reaction were semi-quantified as described in Materials and methods. [a]Scoring was as follows: II, minimal osteophytes possibly with narrowing, cyst and sclerosis; III, moderate or definite osteophytes with moderate joint space narrowing; IV, severe with large osteophytes and definite joint space narrowing. [b]Scoring was as follows: +/-, minimum staining in one area of the tissue; +, patchy staining involving several areas; ++, moderate diffuse staining. OST, osteotomy; SYV, synovectomy; TA, total joint arthroplasty.

Of the cytokines examined in our study, C-PTHrP behaved as a unique marker for HTLV-I carrier patients. PTHrP was first identified as a causative peptide of humoral hypercalcaemia of malignancy in ATL patients [31]. However, it is currently recognized that PTHrP is produced by many tissues and is involved in a variety of biological functions by binding to PTH/PTHrP receptor [32]. In RA, PTHrP is expressed in the proliferated synovium and such expression is correlated with the inflammatory activity [18,33]. PTHrP seems to act as a crucial mediator for inflammatory arthritis [34]. We previously demonstrated that PTHrP was also expressed in articular chondrocytes [35], and treatment of cultured chondrocytes with PTHrP inhibited chondrocyte differentiation [36]. Although the functional role of PTHrP in our carrier patients remains obscure, together with positive correlation of C-PTHrP with DPD, which is a marker for bone destruction, it is likely that PTHrP is also important in the degenerative process of the subchondral bone. Moreover, C-PTHrP in 13 carriers was elevated above the upper concentration in OA patients, indicating that C-PTHrP could be a potential marker of HTLV-I-associated arthritis, allowing it to be distinguished from primary OA. It should be noted here that the concentrations of C-PTHrP in our carrier patients were much lower than those in RA patients [18], and were not correlated with erythrocyte sedimentation rate or C-reactive protein, suggesting that the mechanism(s) involved in the activation of PTHrP in HTLV-I associated monoarthritis differ from that of RA.

The histopathological features of HAAP are thought to be indistinguishable from RA [5]. Despite the small number of patients in the present study, the synovia obtained from HTLV-I-infected patients showed relatively low inflammatory reaction and synovial proliferation, which could have been diagnosed as primary OA rather than RA. That synovia taken by synovectomy tended to accompany intense synovitis compared with others might be a result of the relatively short interval from onset to tissue preparation (average 0.9 versus 2.2 years; Table 2). Together with the relatively low expression of inflammatory cytokines compared with RA and no apparent differences in radiographic findings between HTLV-I carriers and non-carriers, we consider that the principal pathological features in the HTLV-I-infected synovia are equivalent to those of OA. Further studies with larger samples of synovial tissues are necessary to validate our hypothesis.

Taken together, our results suggest that HTLV-I infection can alter the pathophysiology of OA by increasing the expression of inflammatory cytokines, but this modification does not necessarily overcome the clinical outcome of simple OA except for IgM-positive patients, who could suffer from more severe symptoms as a result of the activated inflammation. Moreover, it is possible that the altered inflammatory activity is partial and transient during the natural course of OA and does not continue to the final stage. In contrast to the development of systemic arthritis in Tax transgenic mice [8], we consider that HTLV-I infection alone is not sufficient to cause the development of monoarthritis independently of OA. Further studies including the incidence of HTLV-I-modulated primary OA in seropositive patients, in addition to longitudinal studies, are required to enhance our understanding of the association between HTLV-I infection and arthritis.

Conclusions

The present study indicates that HTLV-I infection could modify the inflammatory activity of ordinary primary OA, whereby direct activation by Tax protein might account for the increased concentrations of PTHrP, sIL-2R, IL-6 and DPD in SF. Our results also suggest that C-PTHrP could be a potential marker to distinguish HTLV-I-associated OA from simple primary OA in carrier patients.

Competing interests

None declared.

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