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Diminished expression of the ubiquitin-proteasome system in early treatment-naïve patients with rheumatoid arthritis and concomitant type 2 diabetes may be linked to IL-1 pathway hyper-activity; results from PEAC cohort

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Abstract

Objective Based on the recent evidence of IL-1 inhibition in patients with rheumatoid arthritis (RA) and concomitant type 2 diabetes (T2D), we evaluated the synovial tissue expression of IL-1 related genes in relationship to the ubiquitin–proteasome system and the effects of insulin on ubiquitinated proteins in fibroblast-like synoviocytes (FLSs).

Methods The synovial expression of IL-1 pathway genes was compared in early (< 1 year) treatment-naïve RA patients with T2D (RA/T2D n = 16) and age- and sex-matched RA patients without T2D (n = 16), enrolled in the Pathobiology of Early Arthritis Cohort (PEAC). The synovial expression of ubiquitin in macrophages and synovial lining fibroblasts was also assessed by Immunohistochemistry/immunofluorescence and correlated with synovial pathotypes. Finally, FLSs from RA patients (n = 5) were isolated and treated with human insulin (200 and 500 nM) and ubiquitinated proteins were assessed by western blot.

Results Synovial tissues of RA/T2D patients were characterised by a consistent reduced expression of ubiquitin–proteasome genes. More specifically, ubiquitin genes (*UBB, UBC,* and *UBA52*) and genes codifying proteasome subunits (*PSMA2, PSMA6, PSMA7, PSMB1, PSMB3, PSMB4, PSMB6, PSMB8, PSMB9, PSMB10, PSMC1, PSMD9, PSME1,* and *PSME2*) were significantly lower in RA/T2D patients. On the contrary, genes regulating fibroblast functions (*FGF7, FGF10, FRS2, FGFR3,* and *SOS1*), and genes linked to IL-1 pathway hyper-activity (*APP, IRAK2,* and *OSMR*) were upregulated in RA/T2D. Immunohistochemistry showed a significant reduction of the percentage of ubiquitin-positive cells in synovial tissues of RA/T2D patients. Ubiquitin-positive cells were also increased in patients with a lympho-myeloid pathotype

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compared to diffuse myeloid or pauci-immune-fibroid. Finally, in vitro experiments showed a reduction of ubiquitinated proteins in RA-FLSs treated with a high concentration of insulin (500 nM).

Conclusions A different IL-1 pathway gene expression was observed in the synovial tissues of early treatment-naïve RA/T2D patients, linked to decreased expression of the ubiquitin–proteasome system. These findings may provide a mechanistic explanation of the observed clinical benefits of IL-1 inhibition in patients with RA and concomitant T2D.

Keywords Rheumatoid arthritis, Type 2 diabetes, IL-1β, ubiquitin, Synovial biopsy, Pathobiology, Of Early Arthritis Cohort study (PEAC)

Introduction

The course of rheumatoid arthritis (RA), despite the clinical improvement associated with the introduction of biologic disease-modifying antirheumatic drugs (bDMARDs), is still burdened by accelerated atherosclerosis, resulting from the synergy between the pro-inflammatory process and "traditional" cardiovascular risk factors [1, 2]. In this context, a consistent connection has been increasingly highlighted between RA and aberrant glucose metabolism since the elevated frequency of concomitant insulin resistance (IR) and type 2 diabetes (T2D) [3]. Remarkably, interleukin-1β (IL-1β), IL-6, and tumour necrosis factor (TNF), which are well-known pathogenic mediators in RA, also play a pivotal role in the development of T2D [2, 4]. The inflammatory pathogenetic contribution of T2D has recently suggested new anti-diabetic therapeutic strategies by using bDMARDs, which are commonly used in RA, as effective therapies in improving glucose abnormalities [4, 5]. In a randomised controlled trial enrolling T2D patients, anakinra, a recombinant human interleukin-1 receptor antagonist, significantly reduced the glycated haemoglobin (HbA1c%) [6]. More recently, another trial investigated whether IL-1 inhibition could bidirectionally improve both glycaemic and inflammatory parameters in RA/T2D patients [7]. Interestingly, anakinra-treated patients had a significant reduction of HbA1c% which was not observed in control group of TNF inhibitors-treated patients. At the same time, a progressive reduction of RA disease activity was observed in both groups [7]. In addition, IL-1 inhibition showed to improve insulin sensitivity in RA/T2D patients [8]. The clinical benefits of IL-1 inhibition were also maintained in the long-term [9]. However, although these relevant clinical differences could suggest a possible pathogenic role for IL-1β in both RA and T2D, the underlying molecular mechanisms are not fully elucidated yet. The production and activity of IL-1β are strictly controlled, because of its strong inflammation-promoting capacity [10]. In this context, a role for the ubiquitin–proteasome system is proposed, consisting of a complex of enzymes inducing a post-translational modification by adding ubiquitin and tagging proteins for degradation by the proteasome [11]. Thus, the post-translational modification of proteins by ubiquitin plays a critical role in a variety of intracellular signalling pathways [11]. Interestingly, IL-1β may be regulated by ubiquitin–proteasome system; the precursor IL-1β may become inaccessible to caspase-1 by the addition of ubiquitin, consequently limiting its activation [12, 13]. Furthermore, the production of activated IL-1β may be enhanced by the lack of ubiquitin [12, 13]. However, the ubiquitin–proteasome system has not been investigated in RA/T2D patients.

On these bases, we aimed at evaluating the expression of IL-1 pathway genes and the relationship to the ubiquitin–proteasome system in the synovial tissues of early treatment-naïve RA patients with and without T2D. We also aimed at assessing the effects of high concentration of insulin and glucose in vitro on ubiquitinated proteins in RA-fibroblast-like synoviocytes (RA-FLSs).

Methods

Patients

Early (< 1 year) treatment-naïve RA/T2D patients (RA/T2D n = 16) were exploratory compared with age- and sex-matched RA patients without T2D (n = 16) among those enrolled in the Pathobiology of Early Arthritis Cohort (PEAC) [14]. The latter consists of consecutive patients with early treatment-naïve RA (disease duration < 1 year) recruited as part of a Medical Research Council–funded observational study. Synovial tissue specimens were obtained from all patients at study entry by ultrasound–guided synovial biopsy, as previously described [15, 16]. Patient demographic characteristics and clinical parameters collected at the time of assessment are reported in Table 1. Some potentially relevant features in this context, such as lipide profile, insulin levels, and HbA1c, were not included since PEAC cohort was not originally designed to assess these metabolic outcomes. All patients provided written informed consent, and the study received local ethics approval (PEAC LREC: 05/Q0703/198).

RNA sequencing analyses

Available synovial RNA sequencing was assessed and compared between RA/T2D patients (n = 8) with

Table 1 Descriptive characteristics of assessed patients

Clinical Variables	RA patients	RA/T2D patients	*P* values
Age, mean ± SD, years	54.7 ± 11.7	54.9 ± 11.5	0.952
Male Gender, n (%)	5 (31.5)	5 (31.5)	0.999
BMI, mean ± SD	24.7 ± 5.2	29.2 ± 5.0	**0.035**
Rheumatoid factor, n (%)	12 (75.0)	8 (50.0)	0.273
ACPA, n (%)	12 (75.0)	10 (62.5)	0.531
DAS28-CRP, mean ± SD	5.6 ± 0.9	5.4 ± 1.2	0.695
DAS28-ESR, mean ± SD	6.0 ± 1.5	5.9 ± 1.5	0.785
Tender joints, mean ± SD	14.1 ± 6.7	13.3 ± 6.7	0.766
Swollen joints, mean ± SD	7.8 ± 4.6	9.0 ± 4.8	0.485
VAS global health, mean ± SD	67.1 ± 28.1	56.1 ± 25.9	0.263
ESR, mm/h, mean ± SD	42.7 ± 33.8	44.2 ± 38.2	0.908
CRP, mg/L, mean ± SD	15.0 ± 13.1	15.8 ± 18.3	0.892

Abbreviations: *RA* Rheumatoid arthritis, *T2D* Type 2 diabetes, *BMI* Body mass index, *ACPA* Anti-citrullinated protein antibodies, *DAS28* Disease activity score in 28 joints, *CRP* C reactive protein, *ESR* Erythrocyte sedimentation rate, *VAS* Visual analogue scale

age- and gender-matched RA patients without T2D (n = 8). Considering their relevance in both diseases [17–20], IL-1, IL-6, TNF, and insulin pathway genes were specifically assessed (Supplementary material and methods 1). To compare the expression of the above genes in the two groups, we performed differential expressed genes analysis between RA/T2D and RA patients for the genes of interest using DeSeq2 (DESeq2, and a Wald test to compare variation between groups. Distributions of DEGs using nominal Wald test-derived *P* values and log2 fold changes were illustrated in a volcano plot. The analyses were performed in R (version 3.6.3 or higher).

RNA extraction and associated RNA sequencing were performed on synovial tissue samples, as previously extensively described [14]. The RNA-Seq dataset is deposited in the ArrayExpress database (online at https://www.ebi.ac.uk/arrayexpress; accession no. E-MTAB-6141).

Histological assessment

Synovial biopsies were analysed by immunohistochemistry and semiquantitatively scored (0–4) for the presence of B cell aggregates (cluster of differentiation [CD]20 +), plasma cells (CD138 +), T cells (CD3 +), and monocytes or macrophages (CD68 +) in the synovial lining or sublining layers. Based on histology scores, synovial samples were codified as lympho-myeloid (CD20 B cell aggregate rich), diffuse-myeloid (CD68 rich in the lining or sublining layer but poor in B cells), or fibroid (paucity of immune-inflammatory cell infiltrate), as previously performed [21, 22]. This evaluation was performed on all assessed patients who were enrolled in PEAC cohort (RA/T2D n = 16, RA patients without T2D n = 16). After that, the synovial expression of ubiquitin in macrophages and lining FLSs was assessed by immunohistochemistry/immunofluorescence and correlated with synovial pathotypes comparing RA/T2D patients (n = 15) with RA patients without T2D (n = 11), due to the availability of further samples for histological evaluation. Immunohistochemical staining for ubiquitin (Ubiquitin (A-5): sc-166553; 1:200 dilution) was performed on sequential 3-µm cut slides, which were acquired using the NanoZoomer S60 Digital slide scanner (Hamamatsu) and analysed using a pixel-based digital image analysis (QuPath) to compare the percentage of ubiquitine positive cells (per mm^2 of tissue) in RA/T2D patients and patients without T2D. Immunofluorescence staining was performed on 3-µm, formalin-fixed, paraffin embedded human sections obtained from synovial tissues of 4 patients with RA, as previously described [23]. Briefly, following deparaffination and antigen retrieval (pH 6.0; Dako, no. S1699), peroxidase and biotin activity blocking with peroxidase (Dako, no. S2023) and protein block (Dako, no. X0909), slides were stained with primary antibodies (mouse anti-human Ubiquitin A-5 sc-166553, mouse antihuman CD68 Dako clone KP1, rabbit anti-human CD55 Ab133684) followed by secondary antibodies (HRP-polymer DAKO envision reagent K4002) and fluorophore-conjugated tyramide reagent (Invitrogen Alx488 for CD68, Invitrogen Alx555 for CD55 and Invitrogen Alx647 for ubiquitin), and DAPI (Thermofisher) nuclear counterstaining.

Slides were then mounted with ProLong Gold Antifade reagent (Thermofisher) and images were captured using a NanoZoomer S60 Digital slide scanner (Hamamatsu, no. C13210-01). Image analysis was performed using NDP. view 2 Software (Hamamatsu Photonics, no. U12388-01).

In vitro experiments

RA-FLSs from RA patients without T2D (n = 5) were isolated immediately after biopsy by digestion of the synovial tissues with collagenase type I (0,5 mg/mL, Thermo Fisher) at 37 °C overnight. In this experiment, we used RA-FLSs from RA without T2D to evaluate the effect of high concentration of insulin and glucose in vitro on ubiquitinated proteins without the possible confounding effect of the concomitant glucose derangement. After washing, the cells were grown in a Dulbecco's modification of Eagle medium (DMEM) supplemented with 10% FBS, 50 IU/ml penicillin/streptomycin, 2 mM glutamine, and 10 mM HEPES. The baseline concentration of glucose in DMEM was 100 mg/dL. For in vitro experiments, RA-FLSs, used between passages 4 and 8, were plated on 6-well plates (5 × 105 cells/well) and treated with human insulin (200 and 500 nM; Lonza) and with high

concentration of glucose (40 mM, Sigma). Dose–response experiments were preliminarily performed on FLSs isolated from patients with osteoarthritis (data not shown). After 24 h of culture, RA-FLS were collected for analysis of ubiquitinated proteins by western blot as described below. In addition, RA-FLSs treated or not with insulin were lysed in RIPA buffer (Cell Signalling), and whole cell lysates (30ug) were separated by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto a polyvinylidene difluoride membrane by Trans-Blot Turbo Transfer System (Biorad). Western blot was performed using anti-ubiquitin antibody (1:1000, Santa Cruz Biotechnology) and anti-β-actin antibody (1:5000, Proteintech). Peroxidase-conjugated goat anti-rabbit IgG and anti-mouse IgG antibodies (Life Technologies) were used as secondary antibodies and the reaction was developed using the LightWave Max Substrate (GVS). Acquisition and quantification of proteins expression was performed by using ChemiDoc System (Biorad).

Results

RA/T2D synovial tissues show a consistent reduction gene expression of ubiquitin proteasome system

RA/T2D patients and age- and sex-matched RA patients without T2D were selected among those included in PEAC cohort (Table 1). In these patients, already assessed synovial samples for RNA sequencing were newly evaluated for the purposes of the present study without performing a new processing.

Synovial RNA-sequencing analysis showed that one third of IL-1 pathway genes (41/138) were significantly different in RA/T2D patients compared to RA patients without T2D. Assessing IL-6, TNF, and insulin pathway genes, some differences were also retrieved at individual gene level. Synovial tissues of early treatment-naïve RA/T2D patients were characterised by a consistent reduced expression of ubiquitin–proteasome genes. More specifically, ubiquitin genes (*UBB, UBC,* and *UBA52*) were significantly lower in T2D/RA patients than RA patients. Furthermore, some genes codifying proteasome subunits were significantly lower in RA/T2D patients (*PSMA2, PSMA6, PSMA7, PSMB1, PSMB3, PSMB4, PSMB6, PSMB8, PSMB9, PSMB10, PSMC1, PSMD9, PSME1,* and *PSME2*). Additionally, some positive gene regulators of the ubiquitin–proteasome system (*RACK1, RBX1, RPS27A, SEM1, S100A12, S100B,* and *SAA1*) were significantly downregulated in RA/T2D patients than others. Some additional genes resulted also to be downregulated in RA/T2D patients in comparison with RA patients (*CILP, CASP1, FGF23, IL31RA,* and *IL1B*). On the contrary, some genes regulating fibroblast functions were upregulated (*FGF7, FGF10, FRS2, FGFR3,* and *SOS1*). Furthermore, *APP, IRAK2,* and *OSMR* were significantly increased in RA/T2D patients than others. Similarly, RA/T2D patients showed an enhanced synovial expression of IL6ST IL18R1, and LIF. These findings are summarised in Fig. 1.

Ubiquitin-positive cells are reduced in synovial tissues of RA/T2D patients

Based on histology scores, synovial patient samples were codified as lympho-myeloid [RA: 7 (43.7%) vs RA/T2D 7 (43.7%), $p=0.999$)], diffuse-myeloid [RA: 4 (25.0%) vs RA/T2D 6 (43.7%), $p=0.704$)], fibroid [RA: 4 (25.0%) vs RA/T2D 1 (6.2%), $p=0.333$)], or ungraded [RA: 1 (6.2%) vs RA/T2D 2 (12.5%), $p=0.426$)]. Analysing the synovitis score, no difference was also retrieved comparing RA patients with and without T2D [RA: 4.0 ± 2.7 vs RA/T2D 4.0 ± 2.1, $p=0.999$)].

Immunohistochemistry showed a significant reduction of the percentage of ubiquitin-positive cells in synovial tissues of early treatment-naïve RA/T2D patients (RA: 47.9% vs RA/T2D: 29.0%, $p=0.01$, representative examples of the staining in Fig. 2A – RA, and B – RA/T2D, and summary in Fig. 2D). Accordingly, a significantly lower number of ubiquitin-positive cells per mm^2 of tissue was observed in RA/T2D patients [RA: 4070.9 (2040.4–5960.1) vs RA/T2D: 1621.1 (1028.2–2743.3), $p=0.01$] (Fig. 2E). Furthermore, a higher percentage of ubiquitin positive cells was observed in lympho-myeloid group when compared with both pauci-immune and diffuse-myeloid pathotypes [lympho-myeloid: 50.97 (35.7–73.2), pauci-immune: 8.9 (5.4–24.4), diffuse myeloid: 28.08 (17.2–31.8) respectively, $p=0.003$] (Fig. 2F). These data may suggest a reduced expression of ubiquitin in synovial tissues of RA/T2D patients than RA patients without this comorbidity. Although, as expected, ubiquitin showed a widespread expression in synovia, by immunofluorescence, we observed the colocalization of ubiquitin with synovial macrophages and lining RA-FLSs in overall assessed patients without clear distinctions in RA patients with and without T2D (Fig. 2C).

Ubiquitin is reduced in RA-FLS following the stimulation with insulin

Considering the findings derived from both RNA sequencing and histological assessment, we evaluated the effects of high concentrations of both insulin and glucose on RA-FLSs derived from RA patients without T2D following a 24 h-stimulation. Dose–response experiments were preliminarily performed on FLSs isolated from patients with osteoarthritis (data not shown). RA-FLSs were cultured in the presence of insulin (200 and 500 nM) and glucose (40 mM). RA-FLSs stimulated with insulin at concentration of 500 nM showed a significant reduction of total amount of

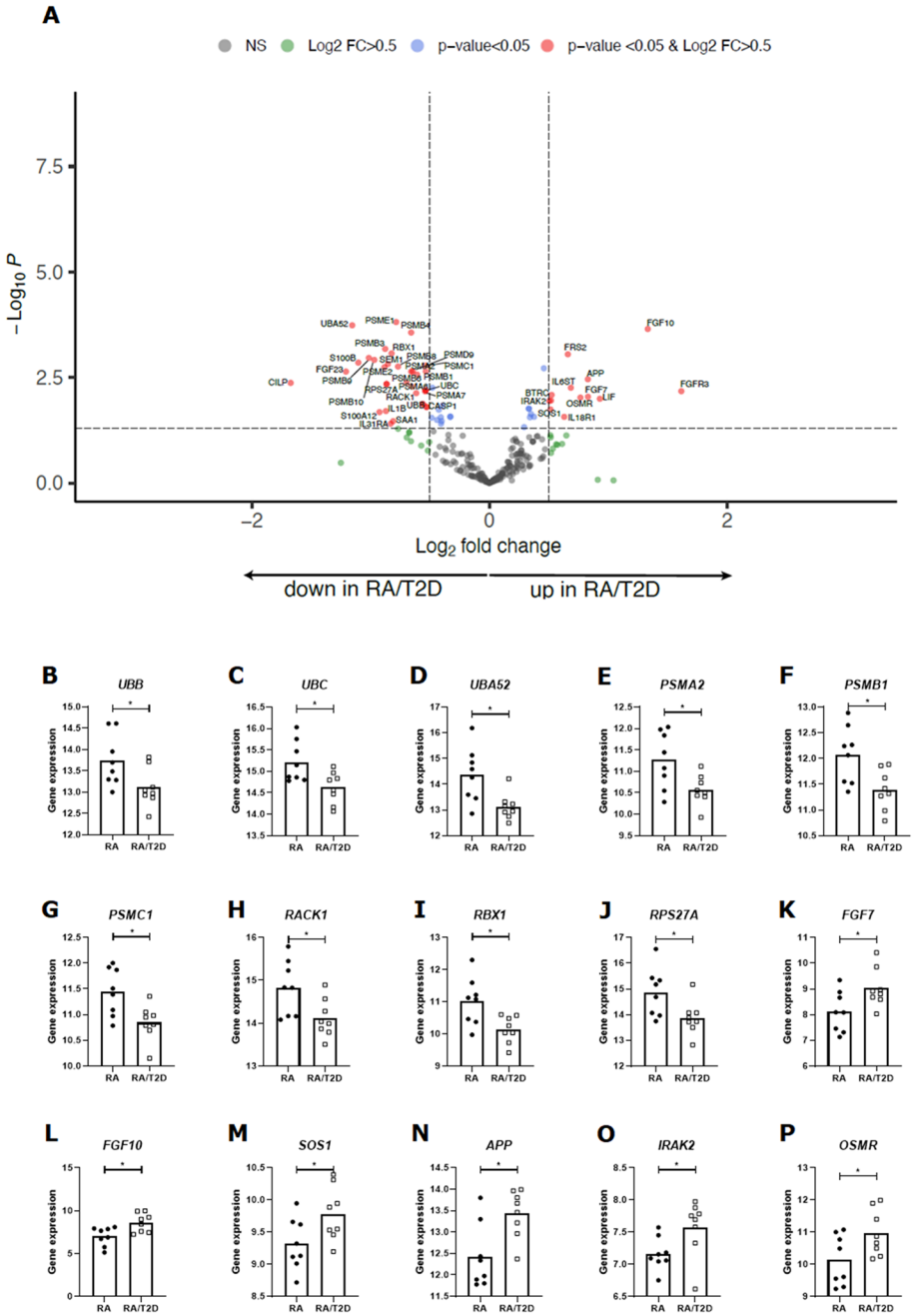

Fig. 1 RNA sequencing assessment of RA patients with and without T2D. **A** Volcano plot is shown reporting differential expressed genes analysis between RA/T2D and RA patients using nominal p values. Ubiquitin genes (*UBB, UBC,* and *UBA52*) were significantly lower in T2D/RA patients than RA patients. Furthermore, some genes codifying proteasome subunits were significantly lower in RA/T2D patients (*PSMA2, PSMA6, PSMA7, PSMB1, PSMB3, PSMB4, PSMB6, PSMB8, PSMB9, PSMB10, PSMC1, PSMD9, PSME1,* and *PSME2*). Additionally, Additionally, some positive gene regulators of the ubiquitin–proteasome system (*RACK1, RBX1, RPS27A, SEM1, S100A12, S100B,* and *SAA1*) were significantly downregulated in RA/T2D patients than others. In addition, some additional genes resulted to be downregulated in RA/T2D patients in comparison with RA patients (*CILP, CASP1, FGF23, IL31RA,* and *IL1B*). On the contrary, some genes regulating fibroblast functions resulted to be upregulated (*FGF7, FGF10, FRS2, FGFR3,* and *SOS1*). Furthermore, *APP, IRAK2,* and *OSMR* were upregulated in RA/T2D patients than others. Similarly, RA/T2D patients showed an increased synovial expression of *IL6ST IL18R1,* and *LIF*. **B**, **C**, and **D** Individual differences in ubiquitin genes (*UBB, UBC,* and *UBA52*) are reported between RA and RA/T2D. **E**, **F**, **G**, **H**, **I**, and **J** Representative individual differences of genes for proteasome subunits (*PSMA2, PSMB1,* and *PSMC1*) and positive gene regulators of the ubiquitin proteasome system (*RACK1, RBX1,* and *RPS27A*) are shown between RA and RA/T2D. **K**, **L**, and **M** Representative individual differences of genes regulating fibroblast functions (*FGF7, FGF10,* and *SOS1*) are described. **N**, **O**, and **P** Individual genes differences for *APP, IRAK2,* and *OSMR* are reported between RA and RA/T2D

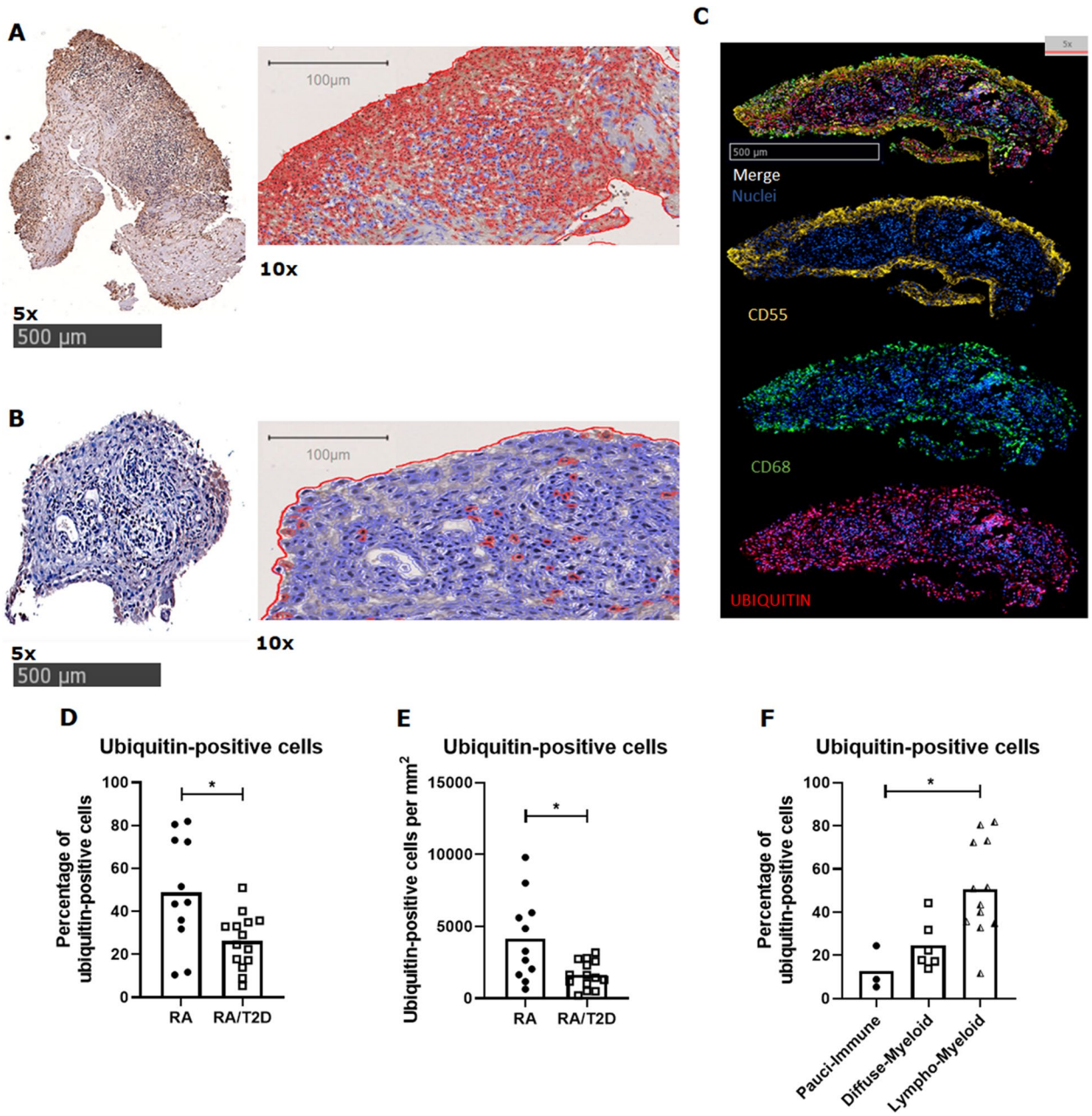

Fig. 2 Histologic assessment of synovial tissues of RA patients with or without T2D. **A** and **B** Representative images are reported of immunohistochemistry for ubiquitin on synovial tissues of patients with early treatment naïve RA patient without T2D (out of a total of n = 11) (A) and with T2D (out of a total of n = 15) (B); BROWN: DAB positive staining for ubiquitin. In the 10 × magnification: VIOLET circles show the nuclei detected by QuPath, RED circles the positive cell detection for ubiquitin by QuPath. **C** Representative image is shown of Immunofluorescence on synovial tissue (out of n = 4 stainings); DAPI-blue for nuclei, CD55-yellow for lining synovial fibroblasts, CD68-green for macrophages, ubiquitin-red. **D**, **E**, and **F** Summary of differences are shown in the assessment of ubiquitin-positive cells between RA and RA/T2D, considering percentage of cells (**D**) or number per mm.^{2} (**E**), and according to different pathotype (**F**)

ubiquitin compared to untreated condition ($p = 0.02$). These results are reported in Fig. 3. Differently, no changes were detected in ubiquitin levels after stimulation with high concentration of glucose.

Discussion

In this study, we observed that synovial tissues of patients with early treatment-naïve RA and concomitant T2D were characterised by a different expression of IL-1

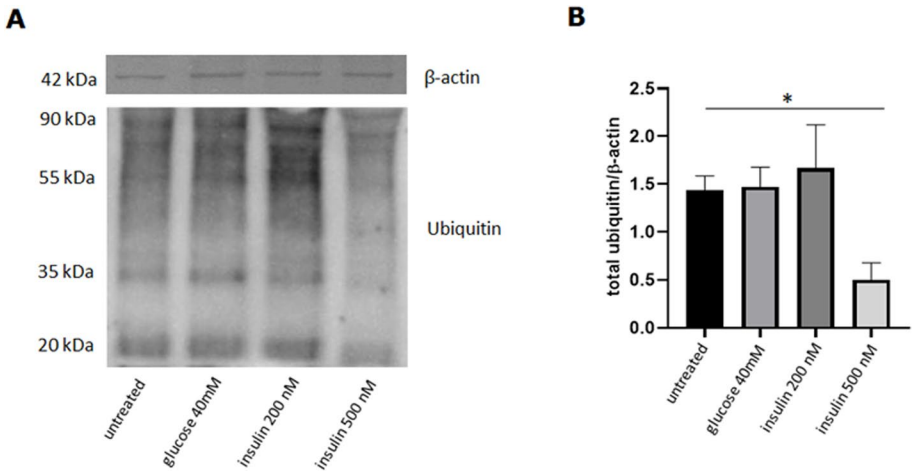

Fig. 3 The effect of insulin and glucose on RA-FLSs. Western blot analysis of total ubiquitin levels of RA-FLS treated with insulin at concentrations of 200 and 500 nM and glucose for 24 h. **A** Blot shown is representative of three independent experiments performed using RA-FLS samples from different patients. **B** Densitometry analysis of total ubiquitin levels relative to β-actin is also reported. Values are expressed as mean ± sd. * $p = 0.02$

pathway genes compared to age- and sex-matched RA patients without T2D, with the differences mostly linked to a decreased expression of genes of the ubiquitin–proteasome system. Accordingly, we observed a decreased percentage of ubiquitin-positive cells in RA/T2D patients, which colocalized with synovial macrophages and lining RA-FLSs. In addition, the stimulation of RA-FLSs with insulin reduced the expression of ubiquitin, suggesting the impact of the metabolic T2D burden on RA synovial tissues mediated by the ubiquitin pathway.

In the present study, we assessed patients enrolled in PEAC; this is a unique cohort of treatment-naïve early RA patients [14–16]. The latter may provide the exclusive opportunity to gain relevant pathogenic insights before the therapeutic modification of the disease pathology due to the treatment. Furthermore, in PEAC study, small joints were assessed, which are less probably influenced by additional features as degenerative disorders. All things considered, a detailed assessment of synovial tissues in treatment-naïve early RA patients may provide crucial information in dissecting the clinical heterogeneity of this disease and, consequently, a more tailored treatment [24, 25].

The reduced synovial expression of genes for ubiquitin (*UBB, UBC,* and *UBA52*), for proteasome (*PSMA2, PSMA6, PSMA7, PSMB1, PSMB3, PSMB4, PSMB6, PSMB8, PSMB9, PSMB10, PSMC1, PSMD9, PSME1,* and *PSME2*), and for positive regulators of this system (*RACK1, RBX1, RPS27A, SEM1, S100A12, S100B,* and *SAA1*) in early treatment-naïve RA/T2D patients may possibly underpin a hyper-activity of IL-1 pathway, despite the apparent lower expression of *IL1B*. In fact, IL-1β is rapidly turned over by ubiquitylation and proteasomal targeting [26]. After its decoration with ubiquitin, precursor IL-1β may become inaccessible to caspase-1 cleavage, limiting the activation of this cytokine [26].

In parallel, the lack of ubiquitin may enhance the levels of precursor IL-1β and the production of bioactive IL-1β, enhancing its pro-inflammatory activity [26, 27]. Thus, ubiquitin-mediated post-translational control and proteasomal targeting of IL-1β may critically regulate its inflammatory capacity. Our study also showed that increased levels of insulin were associated with a reduction of the expression of ubiquitin in FLSs derived from RA patients without T2D according to in vitro experiments. Thus, in addition to stimulating the activation of the immune cells [20], the hyperinsulinemia could possibly modulate the expression of ubiquitin in metabolically contributing to the pro-inflammatory burden of RA/T2D patients. Along with RA-FLSs, we observed that ubiquitin was expressed by synovial macrophages. This finding may furtherly suggest the central role of these cells in the cardiometabolic burden of RA, since they are associated with obesity and are activated by increased concentrations of glucose [28, 29]. Furthermore, *APP, IRAK2,* and *OSMR* resulted to be upregulated in RA/T2D patients than others suggesting an IL-1 pathway hyper-activity. All these genes are implicated in the amplification of IL-1β activity in inflammatory cascade, in RA-FLSs, and in diabetic islets [4, 18, 30, 31]. Altogether, our findings may provide a mechanistic explanation of the observed clinical benefits of IL-1 inhibition in RA/T2D patients [7–9]. In addition, further mechanisms may enhance the expression

of IL-1β in diabetic islets [18]. In fact, free fatty acids (FFAs) may induce the production of IL-1β as well as the induction of IL-1β-dependent pro-inflammatory molecules in experimental models of diabetic islets [32, 33]. Interestingly, a combined stimulation of glucose and FFAs may lead to higher production of IL-1β in respect to the stimulation with FFAs alone [34]. On these bases, future studies are needed to evaluate the possible pathogenic role of FFAs in the context of RA/T2D in providing further insights about the influence of the metabolic burden in rheumatoid inflammatory *milieu* and activity of RA-FLSs.

Furthermore, the upregulation of *IL6ST* and the reduction of *IL31RA* may also suggest the involvement of IL-6 pathway. In fact, IL-6 is overexpressed in IR and impairs insulin action in liver and adipose tissue, although its inhibition produced a less marked reduction of glycaemic burden in RA/T2D patients [17, 35]. Our results also suggested the involvement of additional genes regulating fibroblast functions in RA/T2D patients (*FGF7, FGF10, FRS2, FGFR3,* and *SOS1*), thus paving the way of further studies targeting RA-FLSs [36].

Although providing pathogenic insights in the context of RA and comorbid T2D, our study has some limitations. The relatively small sample size may advocate a cautious interpretation of the data and may limit the generalization of the results. Therefore, the "hypothesis-generating" nature of our findings should be recognized. In fact, we exploratory assessed RA/T2D patients enrolled in PEAC cohort, which was not originally designed to these study purposes. The derived preliminary data may thus provide the rationale to perform additional specific studies to elucidate these findings. In fact, our hypothesis-driven approach was devoted to evaluating individual genes of interest, which could be flattened by the adjustment for multiple testing. In addition, the ubiquitin–proteasome system is one of the main pathways for protein turnover, which is essential for maintaining the cell homeostasis [37]. Consequently, the ubiquitination is tightly regulated at multiple levels and implicated in other critical cellular processes such as autophagy, mitophagy, cell-cycle control, metabolic pathways, DNA stability, repair, and replication [38, 39]. Therefore, additional possible mechanisms, to be fully explored, may be implicated in reducing the expression of the ubiquitin–proteasome system which we observed in RA/T2D patients. Moreover, the metabolic burden of RA/T2D could also lead to an increased degradation of ubiquitin which should be assessed in specific designed studies. Finally, considering the potential role of ubiquitin–proteasome in this context, its potential role as therapeutic target could be also hypothesized and tested [40].

Conclusions

In conclusion, a different IL-1 pathway gene expression was observed in the synovial tissues of early treatment-naïve RA/T2D patients, possibly linked to decreased expression of the ubiquitin–proteasome system. These findings may provide a potential mechanistic explanation of the observed clinical benefits of IL-1 inhibition in patients with RA and concomitant T2D, as the reduction of the ubiquitin–proteasome system may enhance the levels of precursor IL-1β and the production of bioactive IL-1β, making this cytokine a suitable target in RA/T2D patients. Taking together our "hypothesis-generating" findings, a basis may be provided for further confirmatory studies in fully evaluating the pathogenic steps involving both ubiquitin–proteasome system and IL-1β in RA/T2D patients.

Abbreviations

RA	Rheumatoid arthritis
bDMARDs	Biologic disease-modifying antirheumatic drugs
IR	Insulin resistance
T2D	Type 2 diabetes
IL-1β	Interleukin-1β
TNF	Tumour necrosis factor
HbA1c%	Glycated haemoglobin
RA-FLSs	RA-fibroblast-like synoviocytes
PEAC	Pathobiology of Early Arthritis Cohort
CD	Cluster of differentiation
DMEM	Dulbecco's modification of Eagle medium
SDS-PAGE	SDS polyacrylamide gel electrophoresis
FFAs	Free fatty acids

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-024-03392-9.

Supplementary Material 1.

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None

Authors' contributions

All authors made substantial contributions to the conception or design of the work, the acquisition and interpretation of data. PR and FR prepared Fig. 1. PR and DC prepared Fig. 2. MV prepared Fig. 3. PR wrote the initial draft of the manuscript. All authors contributed to the critical review and revision of the manuscript and approved the final version. All the authors agreed to be accountable for all aspects of the work.

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Availability of data and materials

All data relevant to the study are included in the article.

Data Availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All patients provided written informed consent, and the study received local ethics approval (PEAC LREC: 05/Q0703/198).

Consent for publication
Not applicable, all the patients' data are de-identified.

Competing interests
The authors declare no competing interests.

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