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Interferon-α coincides with suppressed levels of pentraxin-3 (PTX3) in systemic lupus erythematosus and regulates leucocyte PTX3 *in vitro*

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Summary

Dysfunctional elimination of cell debris, and the role of opsonins such as pentraxins, is of interest regarding systemic lupus erythematosus (SLE) pathogenesis. Interferon (IFN)-α is typically elevated during SLE flares, and inhibits hepatocyte production of the pentraxin 'C-reactive protein' (CRP), partly explaining the poor correlation between CRP levels and SLE disease activity. The extrahepatically produced 'pentraxin 3' (PTX3) shares waste disposal functions with CRP, but has not been studied extensively in SLE. We analysed serum PTX3 in SLE, and assessed its interference with IFN-α *in vitro*. Serum samples from 243 patients with SLE and 100 blood donors were analysed regarding PTX3. Patient sera were analysed for IFN-α, and genotyped for three PTX3 single nucleotide polymorphisms reported previously to associate with PTX3 levels. Stimulated PTX3 release was assessed in the presence or absence of IFN-α in blood donor neutrophils and peripheral blood mononuclear cells (PBMC). Serum PTX3 was 44% lower in patients with SLE compared to blood donors ($P < 0{\cdot}0001$) and correlated with leucocyte variables. Patients with undetectable IFN-α had 29% higher median PTX3 level than patients with detectable IFN-α ($P = 0{\cdot}01$). PTX3 production by PBMC was inhibited by IFN-α, whereas neutrophil degranulation of PTX3 was increased. No differences in PTX3 levels were observed between the SNPs. In conclusion, median serum PTX3 is lower in SLE (especially when IFN-α is detectable) compared to blood donors. In addition to its potential consumption during waste disposal, it is plausible that IFN-α also attenuates PTX3 by inhibiting synthesis by PBMC and/or exhausting PTX3 storage in neutrophil granules.

Keywords: biomarkers, interferon-α, leucocytes, pentraxin, systemic lupus erythematosus

Introduction

The type I interferon (IFN) system is important in the pathogenesis of systemic lupus erythematosus (SLE) [1]. Many patients with SLE present elevated circulating levels of IFN-α and/or express IFN-inducible genes, i.e. 'the type I IFN signature' during periods with raised disease activity. The main IFN-α-producing cells are the plasmacytoid dendritic cells (pDC) [2], which respond to viral nucleic acids via endosomal Toll-like receptors (TLR)-7 and −9 by massive IFN-α production. The IFN-α/β receptor (IFNAR) is expressed by almost all cell types, and binding of IFN-α to its receptor has consequences such as B cell proliferation, plasma cell differentiation and antibody secretion [3].

Based on results from animal models, it has been hypothesized that the short pentraxin C-reactive protein (CRP) has a protective role in SLE [4,5]. CRP is used widely as a biomarker of inflammation in bacterial infections and chronic inflammatory diseases such as rheumatoid arthritis, whereas it is an unreliable marker of inflammation in SLE [6–8] and viral infections [9]. We have shown previously that IFN-α inhibits interleukin (IL)-6-induced CRP production by human hepatocytes *in vitro* [10] and that serum IFN-α levels, as well as CRP genotype *rs*1205, affects the CRP response in patients with SLE [11]. The lack of correlation between CRP levels and disease activity is thus probably explained largely by CRP

gene polymorphisms and activation of the type I IFN system.

The 340 kDa protein pentraxin 3 (PTX3) is a long pentraxin that is related structurally and functionally to CRP, but its production differs both with regard to its non-hepatic cell origin as well as to inducing stimuli [12,13]. PTX3 is composed of eight identical protomers associated through disulphide bonds [14]. Monocyte- and macrophage-derived production is induced by lipopolysaccharide (LPS) and IL-1β [15,16], and the release of stored PTX3 from neutrophils is triggered by LPS and tumour necrosis factor (TNF) [15,17]. PTX3 can also be produced by myeloid DC, but not by pDC [18]. A number of single nucleotide polymorphisms (SNPs) in the PTX3 gene have been found [19]. Some SNPs have been reported to associate with different blood levels of PTX3 when comparing patients with acute myocardial infarction with controls [20]. Variants of the SNP *rs*2305619 have been associated with differences in PTX3 plasma levels both at baseline and 24 h after lung transplantation [21].

Similar to CRP, PTX3 has a role in humoral innate immunity. It is involved in waste disposal of material released from dying cells as well as in the elimination of pathogens via complement protein C1q and classical complement activation [19]. Another feature of PTX3 is its ability to bind to apoptotic cells and inhibit recognition by DC in order to maintain peripheral immune tolerance [19,22]. PTX3 can also induce macrophage secretion of immunosuppressive cytokines, such as IL-10 and transforming growth factor (TGF)-β [19], and has been suggested to be important during tissue repair and remodelling [23,24], as well as in female fertility [25]. Considering the waste-handling functions of PTX3, and that a dysfunctional elimination process of cell debris is believed to be a key feature of SLE pathogenesis, together with observations that monocyte PTX3 production is affected by IFN-γ [16,26], it is highly relevant to investigate PTX3 in SLE. The scarce previous reports have pointed both at elevated [27–29] and lowered [30–32] PTX3 levels in patients with SLE compared to control subjects.

The aims of this study were to: (i) analyse PTX3 levels in clinically well-characterized cases with SLE; and (ii) determine whether PTX3 is influenced by IFN-α both *in vitro* and *in vivo*.

Materials and methods

Patients and control subjects

A total of 243 patients (211 women, 32 men, Table 1) diagnosed with SLE were included in the study. All patients took part in the prospective, structured follow-up programme 'KLURING' (Swedish acronym for Clinical LUpus Register In Northeastern Gothia) [33] at the rheumatology out-patient clinic, Linköping University Hospital, Sweden.

Table 1. Characteristics of the SLE patients, $n = 243$

	Mean (range) or %
Age (years)	49 (18–88)
Females	86·8%
Caucasian ethnicity	90·1%
Disease duration (years)	10·3 (0–45)
Prednisolone dosage (mg/day)	5 (0–60)
SLICC/ACR damage index (score)	1·1 (0–9)
SLEDAI-2K (score)	2·8 (0–24)
PGA (score)	0·5 (0–4)
Patients meeting SLICC-12 (%)	239 (98·4)
Fulfilled ACR-82 criteria (n)	4.7 (3–9)
ACR-82 criteria	***n* (%)**
1. Malar rash	106 (43·6)
2. Discoid rash	39 (16·0)
3. Photosensitivity	124 (51·0)
4. Oral ulcers	28 (11·5)
5. Arthritis	184 (75·7)
6. Serositis	92 (37·9)
7. Renal disorder	62 (25·5)
8. Neurological disorder	13 (5·3)
9. Haematological disorder	139 (57·2)
10. Immunological disorder	122 (50·2)
11. IF-ANA	240 (98·8)

SLEDAI-2K = systemic lupus erythematosus disease activity index 2000; SLICC = Systemic Lupus International Collaborating Clinics; ACR = American College of Rheumatology; PGA = physician's global assessment; IF-ANA = immunofluorescence microscopy antinuclear antibodies.

Of the 243 patients, 205 (84%) met at least four of the 1982 American College of Rheumatology classification criteria (ACR-82) [34]. Another 38 patients (16%) fulfilled solely the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria [35]; 201 patients (83%) met both ACR-82 and SLICC-12. The patients were recruited consecutively. Most were prevalent cases (200 patients, 82%), but 43 patients (18%) had recent-onset disease at the time of sampling. The mean disease duration was 10 years (range = 0–45 years). The SLE Disease Activity Index 2000 (SLEDAI-2K) [36] and the physician's global assessment (PGA; 0–4) [37] were recorded at each visit and acquired organ damage according to the SLICC/ACR damage index (SDI) score [38] was registered prospectively after inclusion in KLURING. One hundred blood donors (50 women, 50 men; mean age = 46 years; range = 22–70 years) served as healthy controls for the PTX3 analyses.

At all patient visits, routine laboratory analyses [leucocytes, erythrocytes, platelets, urinalysis, CRP and erythrocyte sedimentation rate (ESR)] were performed at the clinical chemistry department, Linköping University Hospital.

Peripheral venous blood was drawn from each individual at baseline. Serum was prepared and stored at −70°C until analysed.

In addition, 15 of the included patients who all met ACR-82 were selected for consecutive analyses (two to 13 visits per patient), due to their fluctuations in disease activity (i.e. SLEDAI-2K peak score of at least 4) over time.

Leucocyte isolation and stimulation

Polymorphonuclear neutrophil granulocytes (neutrophils) and peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation from heparinized healthy donor blood. The blood was layered on top of gradient media consisting of Lymphoprep® (Axis-Shield/Alere Technologies AS, Oslo, Norway) prelayered on top of Polymorphprep® (Axis-Shield/Alere), and centrifuged for 30 min (480 ***g*** at room temperature). Neutrophils and PBMC, respectively, were collected and washed with phosphate-buffered saline (PBS), pH 7·4. Trace amounts of erythrocytes contaminating the neutrophil fraction were lysed by two brief (35 s) exposures to 4°C ultra-pure water. After additional washing in PBS, neutrophils were incubated in RPMI-1640 supplemented with 2% fetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin and 20 mM HEPES (ThermoFisher Scientific, Waltham, MA, USA). PBMCs were cultured in macrophage serum-free medium (ThermoFisher Scientific) supplemented with 20 mM HEPES, 100 IU/ml penicillin and 100 μg/ml streptomycin.

During the experiments, neutrophils and PBMC were incubated at a concentration of 2×10^6 and 4×10^6 cells/ml, respectively, at 37°C with 5% CO_2. IL-1β, LPS *Escherichia coli* (serotype O26:B6), TNF and mouse immunoglobulin (Ig)G2A isotype control were obtained from R&D Systems (Abingdon, UK), IFN-α2b (IntronA®) was from Schering-Plough (Kenilworth, NJ, USA) and neutralizing mouse anti-human IFN-α receptor (IFNAR) chain 2 (clone MMHAR-2) from PBL InterferonSource (Piscataway, NJ, USA). In receptor-blocking experiments, the cells were pre-incubated with blocking monoclonal antibody or isotype control, respectively, 2 h prior to cytokine addition. Cell culture supernatants were collected, centrifuged and stored for a short time at −20°C prior to analysis.

Immunoassays

An enzyme-linked immunosorbent assay (ELISA) kit was used to analyse PTX3 levels in SLE and control sera (Quantikine®; R&D Systems, Minneapolis, MN, USA). This plasma-validated kit showed excellent correlation between plasma and serum ($r = 0{\cdot}972$, $P = 0{\cdot}001$, $n = 6$). For analysis of PTX3 in cell culture supernatants, a DuoSet ELISA was used (R&D Systems). Assays were performed according to the manufacturers' instructions. Briefly, Costar (Corning, NY, USA) half-area plates were coated with 2 μg/ml of mouse anti-human PTX3 and incubated overnight. Plates were blocked by 1% bovine serum albumin in PBS for 1 h and incubated thereafter with samples and standards for 2 h. Biotinylated goat anti-human PTX3 (360 ng/ml) was added and incubated for 2 h, followed by addition of streptavidin horseradish peroxidase (R&D Systems) diluted 1 : 200 and another 20 min of incubation. Plates were developed with tetramethylbenzidine substrate and the reaction was stopped by adding 1 M H_2SO_4. All incubations were performed at room temperature. Plate reader Sunrise (Tecan, Männedorf, Switzerland) and software Magellan version 7.1 (Tecan) were used.

IFN-α levels were measured in sera from patients with SLE by a dissociation-enhanced lanthanide fluoroimmunoassay (detection limit 1 unit/ml) at Uppsala University, described elsewhere [39].

IL-1β and TNF were analysed by a high-sensitivity multiplex magnetic bead assay (Milliplex, Millipore, Solna, Sweden).

Cell viability

A tetrazolium-based assay was used to determine the relative number of viable cells (CellTiter 96 Aqueous One Solution Cell Proliferation Assay; Promega, Madison, WI, USA). Optical density was measured at 490 nm (Sunrise, Tecan) and the number of viable cells was expressed as the percentage of unstimulated control cells.

Genotyping

Genomic DNA was obtained from whole blood samples using a QIAamp DNA Blood Midi kit (Qiagen, Hilden, Germany). The SNPs *rs*3816527, *rs*3845978 and *rs*2305619, selected based on their associations with PTX3 blood levels [19,20,40], were genotyped using the Infinium ImmunoChip (Illumina Inc., San Diego, CA, USA). Genotyping was performed at the SNP&SEQ Technology Platform at the National Genomics Infrastructure (NGI) hosted by the Science for Life Laboratory in Uppsala, Sweden.

Statistics

Wilcoxon's matched-pairs signed-rank test was used to evaluate differences in the neutrophil and PBMC experiments. The Mann–Whitney *U*-test was used to evaluate differences in PTX3 levels between patients and controls and between SLE cases with and without detectable IFN-α. Spearman's correlation was used to determine the association between PTX3 and disease variables. The Kruskal–Wallis test was used to evaluate differences in PTX3 levels between the genetic variants of the SNPs. Two-tailed *P*-values $< 0{\cdot}05$ were considered significant. Statistical analyses were performed with SPSS statistics version 22 (IBM, Armonk, NY, USA) or GraphPad Prism version 5.04 (GraphPad Software, La Jolla, CA, USA).

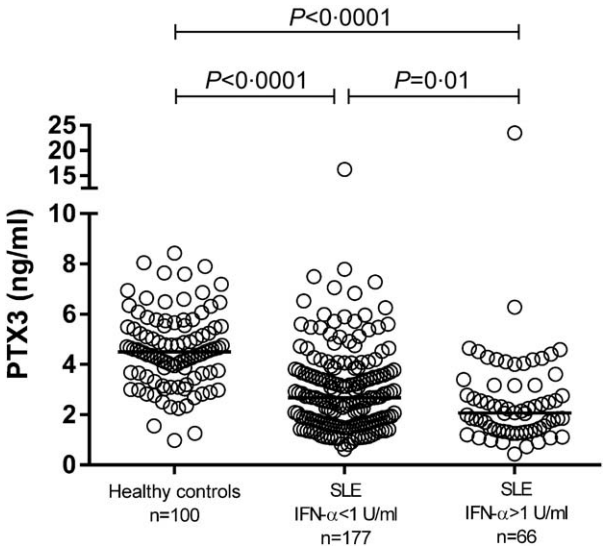

Fig. 1. Serum pentraxin 3 (PTX3) levels determined by enzyme-linked immunosorbent assay (ELISA) in healthy controls and patients with systemic lupus erythematosus (SLE). Serum levels of PTX3 were significantly lower in the patients with SLE (median 2·5 ng/ml) compared to the healthy controls (median 4·5 ng/ml). Patients without detectable interferon (IFN)-α (< 1 U/ml) showed significantly higher levels (median 2·7 ng/ml) of PTX3 compared with patients with detectable IFN-α (> 1 U/ml) (median 2·1 ng/ml). Solid lines represent median values. Note axis break.

Ethics

Oral and written informed consent was obtained from all subjects. The study protocol was approved by the Regional Ethics Review Board in Linköping (Dnr: M75-08/2008).

Results

PTX3 levels in SLE and healthy controls

Levels of PTX3 were 44% lower ($P < 0{\cdot}0001$) among the patients with SLE (median 2·5 ng/ml) compared to the healthy controls (median 4·5 ng/ml) (Fig. 1). A less pronounced, but statistically significant, inverse correlation was found between IFN-α and PTX3 ($r = -0{\cdot}154$, $P = 0{\cdot}017$) for all patients. Hence, we compared patients with and without detectable IFN-α levels. Patients without detectable IFN-α (IFN-α < 1 U/ml) showed a 29% higher median serum level (2·7 ng/ml) of PTX3 compared to patients with detectable IFN-α (IFN-α > 1 U/ml) (median 2·1 ng/ml), $P = 0.01$ (Fig. 1). There were no significant differences in PTX3 levels between men and women, either among the controls or among the patients. We found no significant correlation between PTX3 and the inducing cytokines IL-1β and TNF in patients with SLE, nor between PTX3 and CRP (data not shown).

Correlation analyses between PTX3 and different disease activity variables revealed weak significant correlations with leucocyte-associated variables; leucocyte count ($r = 0{\cdot}293$, $P < 0{\cdot}0001$), monocytes ($r = 0{\cdot}143$, $P = 0{\cdot}027$),

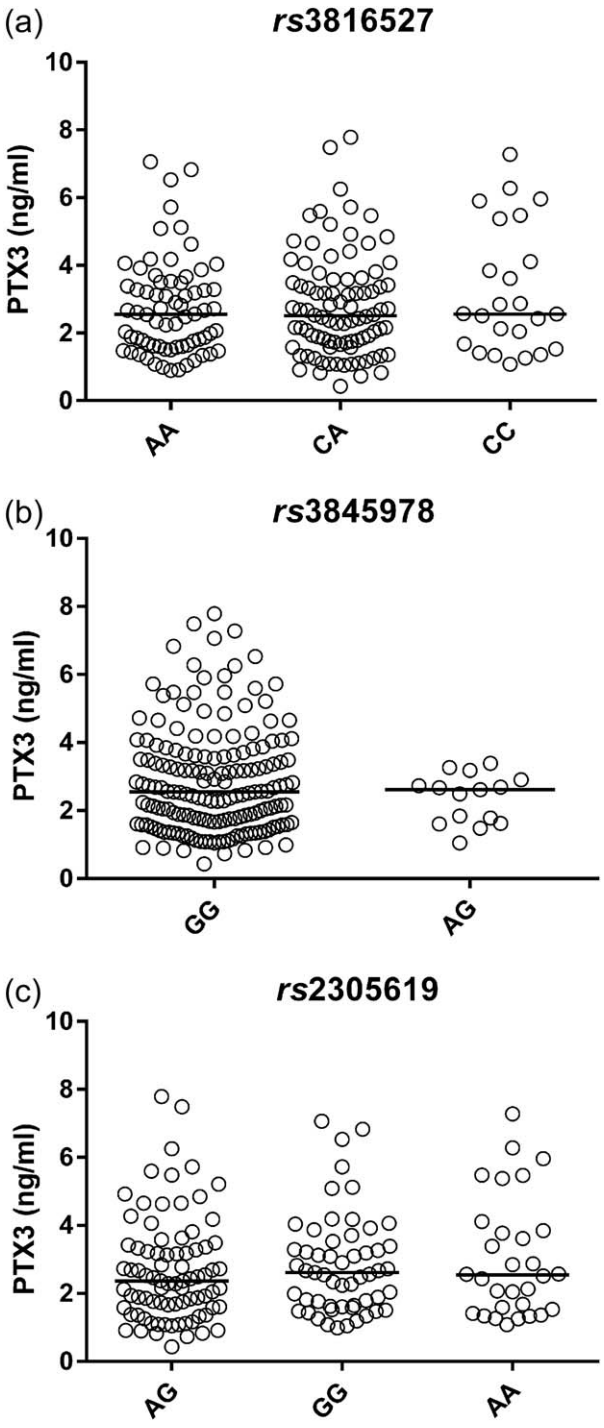

Fig. 2. Influence of pentraxin 3 (PTX3) genetic variants on PTX3 serum levels. Patients with systemic lupus erythematosus (SLE) were genotyped for three PTX3 single nucleotide polymorphisms (SNPs), *rs*3816527, *rs*3845978 and *rs*2305619. No significant differences were observed based on gene variants.

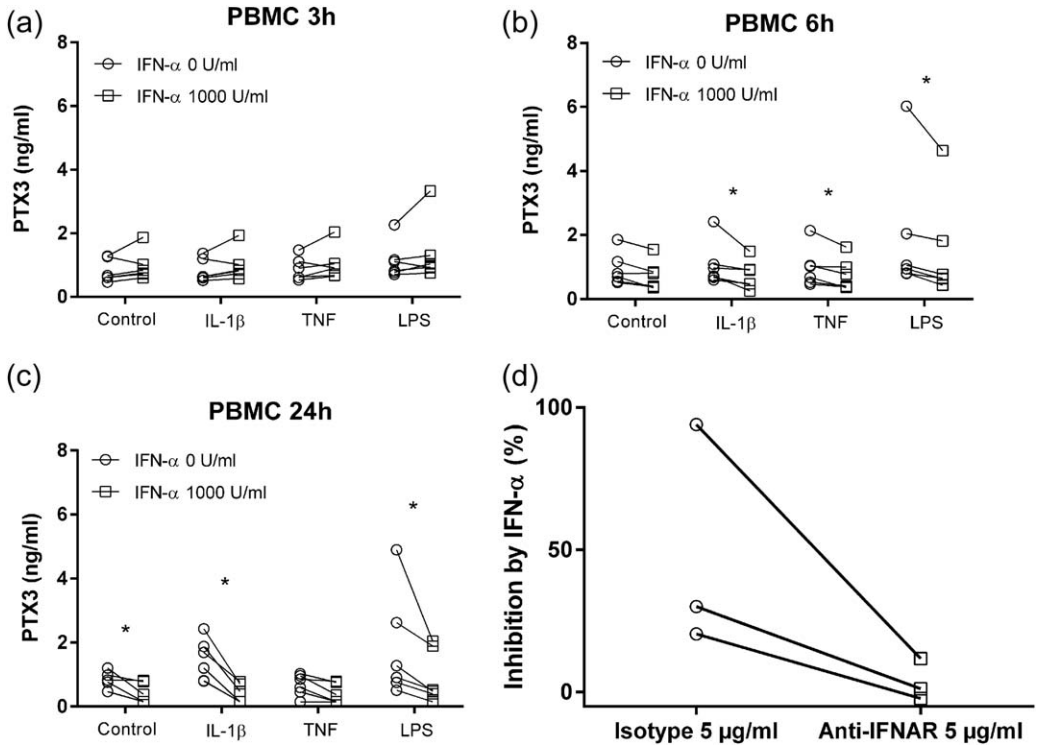

Fig. 3. Pentraxin 3 (PTX3) production from peripheral blood mononuclear cells (PBMC). The effect of interferon (IFN)-α on PTX3 production induced by interleukin (IL)-1β (20 ng/ml), tumour necrosis factor (TNF) (25 ng/ml) or lipopolysaccharide (LPS) (10 ng/ml) in PBMC stimulated for (a) 3 h, (b) 6 h and (c) 24 h ($n = 6$). (d) Percentage inhibition of PTX3 by IFN-α. Effect of a neutralizing antibody to type I interferon receptor (IFNAR). PBMC were preincubated with 5 µg of antibody for 2 h and then stimulated with IFN-α and IL-1β, $n = 3$. *$P < 0{\cdot}05$.

neutrophils ($r = 0{\cdot}262$, $P < 0{\cdot}0001$) and urinary leucocytes ($r = -0{\cdot}242$, $P = 0{\cdot}045$). No association was found between PTX3 and disease activity defined as SLEDAI-2K (data not shown).

PTX3 levels were also analysed in the consecutive samples from 15 cases (Supporting information, Fig. S1 shows longitudinal data of each patient). PTX3 levels at the highest and lowest disease activity (defined by SLEDAI-2K and PGA) were compared, but no significant differences were found (data not shown).

No influence of PTX3 genetic variants on PTX3 serum levels

Patients with SLE were genotyped for three PTX3 SNPs, *rs*3816527, *rs*3845978 and *rs*2305619, and the effects on PTX3 serum levels was examined. No significant differences in PTX3 serum levels were observed between the genetic variants (Fig. 2).

Effects of IFN-α on PTX3 release in neutrophils and PBMC

In order to investigate a possible mechanistic connection to the inverse correlation between IFN-α and PTX3, the influence of IFN-α on PTX3 release from neutrophils and PBMC was analysed. Production of PTX3 in PBMC was induced by IL-1β and LPS and to some extent by TNF (Fig. 3a–c). After 3 h of stimulation, no statistically significant differences were seen in PMBC production of PTX3 (Fig. 3a). IL-1β-induced PTX3 production in PBMC was inhibited significantly by IFN-α with a 17% median decrease at 6 h and a 78% median decrease at 24 h (Fig. 3b,c). The PTX3 production induced by LPS was inhibited significantly with a 29% decrease at 6 h and a 54% decrease at 24 h (Fig. 3b,c). TNF-induced PTX3 production was inhibited significantly with a 30% decrease at 6 h (Fig. 3b).

We observed no reduced cell viability due to IFN-α exposure in the cell viability assay ($n = 3$, data not shown). To ensure that the IFN-α-mediated suppression of PBMC PTX3 was mediated by receptor-dependent signalling, we used a neutralizing antibody to the type I IFN receptor (IFNAR). PBMC were preincubated with 5 µg of antibody for 2 h and then stimulated with IFN-α and IL-1β. Presence of this receptor-blocking antibody reversed the IFN-α-dependent inhibition (Fig. 3d).

Neutrophil release of PTX3 was induced by TNF and LPS, but not by IL-1β (Fig. 4a–d), and the stimulated PTX3 release was similar at all sampling time-points. LPS-induced neutrophil release of PTX3 was increased

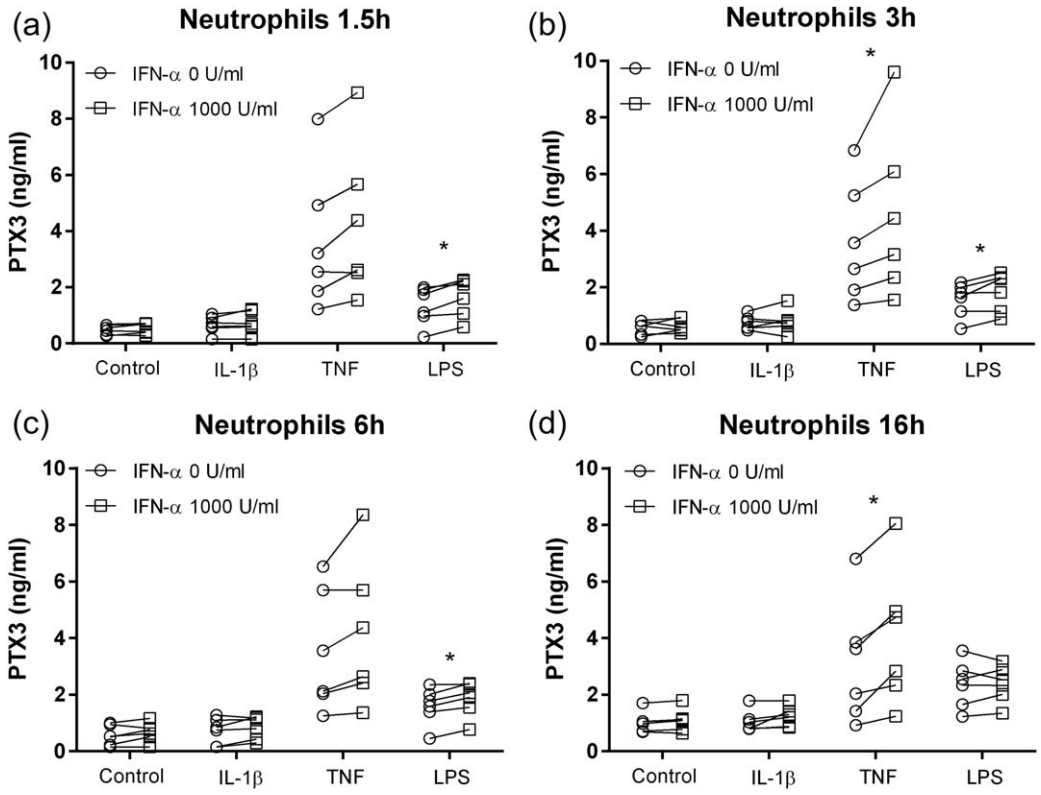

Fig. 4. Pentraxin 3 (PTX3) release from neutrophils. The effect of interferon (IFN)-α on PTX3 release induced by interleukin (IL)-1β (20 ng/ml), tumour necrosis factor (TNF) (25 ng/ml) or lipopolysaccharide (LPS) (10 ng/ml) in neutrophils stimulated for (a) 1·5 h, (b) 3 h, (c) 6 h and (d) 16 h ($n = 6$).

significantly by IFN-α with a 30% median increase at 1·5 h, 19% at 3 h and 18% at 6 h (Fig. 4a–c). Furthermore, TNF-induced release of PTX3 was amplified significantly by IFN-α at 3 and 16 h, with a median increase of 22 and 34%, respectively (Fig. 4b,d).

Discussion

Accumulation of cellular debris due to insufficient elimination is considered a key feature of lupus pathogenesis. Pentraxins such as CRP and PTX3 have biological properties that contribute to clearance of dying cells, and low levels of these proteins could thus result in the accumulation of cell debris and subsequent inflammation and autoimmunity [41,42]. Similarly, CRP supplementation to lupus model mice leads to decreased levels of autoantibodies, fewer autoimmune manifestations and enhanced survival [43]. Conversely, a recent study demonstrated that immunization with PTX3 in a murine model led to anti-PTX3 antibody production which delayed lupus-like nephritis and prolonged survival [44]. Together with our findings, this points towards a complex biological regulation and role of PTX3.

In the present study we found that serum levels of PTX3 were markedly lower among patients with SLE compared to healthy controls, due perhaps to circulating IFN-α. Based on results from our *in-vitro* experiments, we draw the conclusion that circulating IFN-α cause reduction in PTX3 production from PBMC. This theory is strengthened by the fact that patients with detectable IFN-α had lower PTX3 levels than patients without detectable IFN-α. Consequently, it is likely that IFN-α, together with the potential PTX3 consumption during waste disposal of dying cells, is a major cause of lowered systemic levels of PTX3 in SLE.

The biological roles of PTX3 in SLE are far from proved, but results from animal models of lupus suggest a protective role of pentraxins in SLE [4,5]. As both CRP and PTX3 contribute to the clearance of apoptotic cells and inhibits self-recognition by DC, a hampered production, or other exhaustion, of CRP and PTX3 could enhance further the problems of deficient waste disposal in SLE. Conversely, both circulating and tissue levels of PTX3 were reported recently to associate with lupus nephritis, and PTX3 was suggested as a biomarker of tubulointerstitial injury [45]. Moreover, PTX3 plays part in angiogenesis and remodelling of the extracellular matrix [24,46].

Circulating PTX3 has been reported previously to be both elevated [27,28] and lowered [30–32] in SLE. The reasons to the discrepancies between the studies remain unknown, but may be due to differences in study design,

e.g. selection of study population (sex and age may influence PTX3 levels [47]), and definition of disease activity, ethnicity, detection methodologies and genetics. For the latter, some PTX3 gene variants have been associated with differences in PTX3 levels [20,21]. However, genotyping of three PTX3 SNPs in the present study revealed no significant differences in PTX3 serum levels based on genetic variants. To our knowledge, the influence of SNPs on PTX3 blood levels in SLE has not been investigated previously. Differences in absolute PTX3 levels between studies may be related to the use of serum *versus* plasma.

To pursue the inverse relation between IFN-α and PTX3 mechanistically, *in vitro* studies on PBMC and neutrophils were performed. PTX3 production by PBMC increased with time, especially the IL-1β induced production, and IFN-α inhibited both IL-1β- and LPS-stimulated PTX3 production at 6 and 24 h of incubation. Furthermore, PTX3 was inhibited in control PBMC at 24 h, implying that IFN-α also inhibits the baseline synthesis. Doni *et al.* examined the effect of IFN-α on PTX3 production [26], albeit in purified myeloid DC. However, in their study IFN-α had no suppressive effect, but rather amplified PTX3 production in response to LPS. PTX3 is stored in the specific granules of neutrophils, and becomes exocytosed upon stimulation [17,46], e.g. following IFN-α activation [48]. Accordingly, in the present study, stimulated release of neutrophil PTX3 was increased significantly by IFN-α in LPS-stimulated cells. Furthermore, the TNF-induced neutrophil release of PTX3 was increased by IFN-α. Given that PTX3 levels were relatively stable in cell culture supernatants over time, our results support that it is rather a quick release/degranulation, but not *de-novo* synthesis of the protein by the neutrophils. In accordance, neutrophils have been described as a reservoir of 'ready-to-use' PTX3 [17]. Neutrophils exposed to IFN-α are primed to become activated by immune complexes and subsequent induction of neutrophil extracellular trap (NET) formation [49], allowing co-localization of PTX3 [17]. Speculatively, raised IFN-α may provoke tissue-recruited neutrophils to release and deposit PTX3 by degranulation. Moreover, neutrophil degranulation has been suggested as a major source of local elevation of PTX3 in rheumatoid arthritis [50].

In addition to IFN-α-dependent inhibition of PTX3 production in PBMCs, autoantibodies directed towards PTX3 [51] and tissue deposition of PTX3 could possibly explain low circulating levels in patients in general [45]. Although we found no significant association between PTX3 and CRP levels, it is interesting to compare with our previous finding of circulating anti-CRP antibodies in SLE [52] as well as co-localization of glomerular IgG-, CRP- and C1q-deposits in lupus nephritis [53]. The typically low levels of circulating CRP in SLE are not explained by circulating anti-CRP antibodies. It would be interesting to investigate if this also holds true for anti-PTX3 in the present study group. Furthermore, many patients presenting with increased expression of IFN inducible genes (the type I IFN signature) lack measurable IFN-α in serum [54,55], indicating that IFN-α can exert its biological effects locally and/or in concentrations that could not be measured properly. Such effects could explain why patients without detectable IFN-α have lower levels compared to healthy controls.

In conclusion, we have shown that IFN-α exerts diverse effects on neutrophils and PBMCs, leading to release of PTX3 from neutrophils and attenuated synthesis of PTX3 by PBMCs *in vitro*. The net effect is probably reduced circulating levels of PTX3, as lupus patients with raised circulating IFN-α have reduced levels of circulating PTX3. Given the important role of PTX3 in the clearance of dying cells and the prominent activation of the type I IFN system in SLE, our results suggest that the suppressed PTX3 levels in SLE contribute to the autoimmune disease process by providing autoantigens to B cells and endogenous IFN inducers to pDC, all of which further sustain the disease. This has implications regarding waste disposal and peripheral immune tolerance in SLE.

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Disclosure

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's web-site

Fig. S1. Individual systemic lupus erythematosus (SLE) manifestations and longitudinal variations of pentraxin 3 (PTX3) and interferon (IFN)-α. The graphs illustrate individual variations in PTX3 and IFN-α over time for the 15 patients who were followed consecutively (A–O). Observe that axes and scales are different in the graphs.