Serum IFI16 and anti-IFI16 antibodies in psoriatic arthritis

Clinical & Experimental Immunology
The Journal of Translational Immunology
British Society for immunology

Serum IFI16 and anti-IFI16 antibodies in psoriatic arthritis: possible correlation with disease activity and response to therapy

Journal:	*Clinical and Experimental Immunology*
Manuscript ID	CEI-2019-7876
Manuscript Type:	Original Article
Date Submitted by the Author:	22-Aug-2019
Complete List of Authors:	De Andrea, Marco; Turin medical school, Department of Public Health and Pediatric Sciences; Novara medical school, Department of Translational Medicine De Santis, Maria; Humanitas clinical and Research Center - IRCCS, Rheumatology and Clinical Immunology Caneparo, Valeria; Turin medical school, Department of Public Health and Pediatric Sciences; Novara medical school, Department of Translational Medicine Generali, Elena; Humanitas clinical and Research Center - IRCCS, Rheumatology and Clinical Immunology Sirotti, Silvia; Humanitas clinical and Research Center - IRCCS, Rheumatology and Clinical Immunology Isailovic, Natasa; Humanitas clinical and Research Center - IRCCS, Rheumatology and Clinical Immunology Guidelli, Giacomo Maria; Humanitas clinical and Research Center - IRCCS, Rheumatology and Clinical Immunology Ceribelli, Angela; Humanitas Research Hospital, Rheumatology and Clinical Immunology, Humanitas Research Hospital Fabbroni, Marta; University of Siena, Rheumatology Simpatico, Antonella; University of Siena, Rheumatology Cantarini, Luca; University of siena, Rheumatology Gisondi, Paolo; University of Verona, Dermatology Idolazzi, Luca; Unity of Rheumatology, University of Verona, Medicine Gariglio, Marisa; Turin medical school, Department of Public Health and Pediatric Sciences; Novara medical school, Department of Translational Medicine Selmi, Carlo; Humanitas clinical and Research Center - IRCCS, Rheumatology and Clinical Immunology; University of Milan, BIOMETRA Department
Key Words:	Autoantibodies, Arthritis (including Rheumatoid Arthritis), Inflammation

Serum IFI16 and anti-IFI16 antibodies in psoriatic arthritis: possible correlation with disease activity and response to therapy

Short title: IFI16 and psoriatic arthritis

Marco De Andrea[1,2,3]*, Maria De Santis[4]*, Valeria Caneparo[1,2,3], Elena Generali[4], Silvia Sirotti[4], Natasa Isailovic[4], Giacomo Maria Guidelli[4], Angela Ceribelli[4], Marta Fabbroni[5], Antonella Simpatico[5], Luca Cantarini[5], Paolo Gisondi[6], Luca Idolazzi[7], Marisa Gariglio[2,3], and Carlo Selmi[4,8]

[1] Department of Public Health and Paediatric Sciences, Turin Medical School; [2] Department of Translational Medicine, Novara Medical School; [3] Intrinsic Immunity Unit, CAAD - Center for Translational Research on Autoimmune and Allergic Diseases, University of Piemonte Orientale, Novara; [4] Rheumatology and Clinical Immunology Unit, Humanitas Research Hospital, Rozzano; [5] Rheumatology, University of Siena; [6] Dermatology, University of Verona; [7] Rheumatology, University of Verona; [8] Department of Biomedical Science and Translational Medicine, University of Milan, Italy.

Corresponding author: Carlo Selmi MD PhD, Division of Rheumatology and Clinical Immunology, Humanitas Research Hospital, via A. Manzoni 56, 20089 Rozzano, Milan, Italy; tel +39-02-8224-5129, fax +39-02-8224-2298, email carlo.selmi@unimi.it

Key words: IFI16; anti-IFI16 antibodies; psoriatic arthritis

List of abbreviations:

interferon-inducible protein 16 (IFI16)

psoriatic arthritis (PsA)

psoriasis (Pso)

C-reactive protein (CRP)

rheumatoid arthritis (RA),

tumor necrosis factor-α (TNF-α)

interleukin(IL)

interferon (IFN)

plasmacytoid dendritic cells (pDC)

immunofluorescence (IIF)

immunoprecipitation (IP)

protein A sepharose (PAS)

sodium dodecyl sulphate- polyacrylamide (SDS-PAGE)

human foreskin fibroblast (HFF)

horseradish peroxidase (HRP)

phosphate buffered saline (PBS)

disease activity score (DAS)

systemic lupus erythematosus (SLE)

SUMMARY

Objective. The nuclear interferon-inducible protein 16 (IFI16) and anti-IFI16 antibodies have been detected in subjects with several rheumatic diseases, often correlating with disease severity, and we herein investigated their prevalence and clinical associations in psoriatic arthritis (PsA) compared to psoriasis (Pso).

Methods. We tested sera and synovial fluids of patients with PsA for IFI16 protein levels by capture ELISA and for anti-IFI16 IgG and IgA by ELISA, protein radio-immunoprecipitation and immunoprecipitation-Western blot of IgG. Sera from patients with Pso and healthy subjects were used as controls, and in a subgroup of patients with PsA we also studied sera after treatment with etanercept.

Results. IFI16 was detectable in the sera of 66% of patients with Pso, 46% of PsA, and 19% of controls. Among PsA cases, 51% of IFI16-positive cases had elevated levels of C-reactive protein (CRP) compared to 31% of patients with undetectable IFI16. Anti-IFI16 of both IgG and IgA isoforms were detected with significantly higher frequency in PsA and Pso compared to healthy controls, with higher IgG titers in patients with elevated CRP ($p=0.015$) and a significant reduction in the majority of patients after 3 month of etanercept treatment. Immunoprecipitation confirmed the presence of anti-IFI16 IgG antibodies and these recognized the HINA epitope predominantly (amino acids 131 to 337). Lastly, IFI16 was detected in 1/7 and anti-IFI16 in 3/7 synovial fluids from patients with PsA.

Conclusion. IFI16 and anti-IFI16 seem to be associated with higher disease activity and with treatment response in PsA. The detectability of these markers also in synovial fluids represents a novel finding with potential clinical implications.

Psoriatic arthritis (PsA) is a chronic inflammatory disease within the broader psoriatic disease encompassing musculoskeletal features (with enthesitis, synovitis and erosion with osteitis of the peripheral joints and/or the axial skeleton), skin and nail manifestations [1], estimated to affect approximately 0.5% of the general population [2]. PsA is found in a variable proportion (15-40%) of the patients with psoriasis (Pso), and in 74% of cases it is preceded by skin manifestations [3].

Different from rheumatoid arthritis (RA), both PsA and Pso lack serum biomarkers for an early diagnosis, while sharing a T cell-mediated response based on the tumor necrosis factor-α (TNF-α)/interleukin(IL)-17 axis [4]. As reported for other immune-mediated diseases [5], an interferon (IFN) signature is observed in Pso as self-DNA-engulfed plasmacytoid dendritic cells (pDC) produce a large amount of IFN-α [6]. Further, an overexpression of IFN-γ and TNF-α-inducible genes is observed in Pso plaques due to T cell and macrophage recruitment and activation [7]. Despite these observations, treatments targeting IFN-α or IFN-γ have proven ineffective in Pso, while TNF-α and IL12/23 or IL17 blockers lead to clear skin in a large proportion of patients [8, 9].

The interferon-inducible protein 16 (IFI16) is a DNA sensor involved in the inflammasome-mediated defense against viral infections [10], that displays also pro-inflammatory and anti-angiogenetic effects [11]. In the case of psoriatic disease, IFI16 is overexpressed in the affected skin [12], in peripheral blood mononuclear cells, and synovial tissues [13, 14]. Previous studies have demonstrated that IFI16 is overexpressed in different connective tissue diseases and in RA [5], and anti-IFI16 antibodies have been detected in systemic lupus erythematosus (SLE) [15], Sjögren's

syndrome [16, 17], systemic sclerosis [18-20], inflammatory bowel diseases [21], and RA [22], possibly associated with disease severity and progression.

We report herein for the first time that IFI16 and anti-IFI16 antibodies are detectable in the sera and synovial fluids of patients with PsA and may represent disease activity biomarkers.

PATIENTS AND METHODS

Patients

We investigated 158 consecutive patients with a diagnosis of PsA based on the CASPAR criteria [23] (**Table 1**), while 182 healthy subjects and 44 patients with skin or nail Pso but no evidence of PsA were used as controls. Serum samples were collected and stored at − 20°C until used; demographic and clinical data (presence or absence of psoriasis, presence or absence of axial involvement and reported blood tests done by the patient within one month) were recorded. DAS28 (disease activity score 28 joint) was available only for 30 PsA patients treated with biologic agents.

In a subgroup of 8 patients with PsA, serum samples were also available at 3 months after starting weekly subcutaneously etanercept 50 mg. Synovial fluid samples from 7 patients with PsA who underwent arthrocentesis for knee effusion and had not received intra-articular medications (e.g., corticosteroids, hyaluronic acid) in the previous 6 months, were also analyzed (blood samples were collected on the same day). Synovial fluids were centrifuged and supernatants stored at − 80°C until use. Clinical and serologic records were collected at the time of enrollment. This study was approved by the local IRB and written informed consents were obtained from patients and controls.

Determination of extracellular IFI16 protein by capture ELISA

A capture ELISA was employed for determination of circulating extracellular IFI16 protein following a procedure described elsewhere [24], and the threshold cut-off value was defined as the 95th percentile of healthy controls as 27 ng/ml.

Determination of antibody titers toward human recombinant IFI16 by ELISA

To determine anti-IFI16 antibody titers of IgG and IgA isotype in sera of patients, we

performed in-house ELISA as previously described [21]. Accordingly, cut-off values were calculated as the 95th percentile of healthy controls and the threshold values were set to 113 U/ml and 9.6 U/ml for IgG and IgA isotype, respectively.

Indirect immunofluorescence assay

The localization of cellular antigens recognized by autoantibodies was tested by indirect immunofluorescence (IIF) on HEp-2 cells (INOVA Diagnostics, San Diego, CA, USA) using a 1:80 dilution of human sera of patients and controls, followed by secondary antibodies marked with fluorochrome (AlexaFluor488 AffiniPure F(ab')$_2$ fragment goat anti-human IgG, Fcγ fragment specific, Jackson Immunoresearch Europe Ltd, Suffolk, UK) as previously described [25]. Samples were acquired on Olympus BX53 Upright fluorescence microscope.

Radioimmunoprecipitation assay

PsA sera were analyzed by protein radio-immunoprecipitation (IP) using marked ^{35}S-HeLa cell extract. IP was used to identify autoantibodies directed against protein self-antigens as described elsewhere [26]; briefly, sera were incubated with Protein A sepharose (PAS) beads and after serial washes samples have been incubated with cell lysate (radioactively marked for protein-IP). After forming immuno-complexes, samples were prepared for 8% sodium dodecyl sulphate- polyacrylamide (SDS-PAGE) electrophoresis for protein-IP. To confirm data obtained with IP we performed IP-Western blotting. In detail, human sera and 50ng of mouse monoclonal anti-human IFI16 as a positive control were cross-linked with PAS beads to isolate human IgG directed against IFI16 [27]. IP was initially performed with cell extract from 5x10^6 HeLa cells/sample, but since human foreskin fibroblast (HFF) express IFI16 at higher

basal levels than HeLa, control experiment was performed with HFF. Proteins were then fractionated by 8% SDS-PAGE and transferred to a nitrocellulose membrane, probed with 1:500 of mouse monoclonal anti-human IFI16 antibody (Novus Biologicals, Littleton, CO, USA) for band 88 kDa identification, followed by horseradish peroxidase (HRP)-goat anti-mouse IgG (1: 10000 dilution; ThermoFisher, Waltham, MA, USA). Development was performed by Immobilon Western Chemiluminescent HRP substrate (Millipore, Darmstadt, Germany) and acquired using ChemiDoc (Bio-Rad, California, USA).

Recombinant IFI16 domains

The coding region of the three IFI16 domains was amplified from full length human IFI16 (isoform b) cDNA using primers containing BamHI and XhoI restriction sites (DAPIN: Fw 5' GGCGGATCCATGGGAAAAAAATACAAGAAC 3'; Rw 5' GCCTCGAGTCATTTTAACTTTTCTTTTTTAAG 3'; HIN A: Fw 5' GGCGGATCCCCGAAAACAGTGGCCAAATG 3'; Rw 5' GCCTCGAGTCATTTCTTTATCTGGATAAAACTA 3'; HIN B: Fw 5' GGCGGATCCGAACCTGAAGAAGTTTCCATA 3'; Rw 5' GCCTCGAGTCAGATGACCTTGATGTGACTATG 3'). The amplified inserts were digested with BamHI and XhoI, and cloned in pET30a expression vector containing an N-terminal histidine tag (Novagen, Madison, WI). Expression and affinity purification of the recombinant proteins were performed according to standard procedures, and purity assessed by 12% SDS-PAGE gel electrophoresis.

IFI16 epitope mapping

IFI16 protein domains were diluted in phosphate buffered saline (PBS) to a concentration of 2 ug/ml. Polystyrene microwell plates (Nunc-Immuno Maxisorp) (Thermo Scientific, Nunc, Roskilde, Denmark) were coated with 100 µl diluted protein per well, and incubated overnight at 4°C. After blocking, sera were added in duplicate. After washing, horseradish peroxidase-conjugated rabbit anti-human IgG (DakoCytomation) was added. Following the addition of the substrate (tetramethylbenzidine, KPL), absorbance was measured at 450 nm using a microplate reader (SpectraCount,Packard BioScience). The background reactivity of the reference mixture was subtracted to calculate the results.

Statistical analysis

All data analyses were performed using Stata for Macintosh 13.1 (StataCorp, College Station, Tx, USA). As detailed throughout, chi-square test, Mann-Whitney U test, Kruskal-Wallis test with Dunn's test for multiple comparison *post hoc*, or Wilcoxon test were employed to compare groups based on the data distribution. Linear regression and Spearman's test were used to correlate IFI16 and anti-IFI16 levels with continuous variables such as disease duration and age. C reactive protein (CRP) levels were reported for patients who had available blood tests within one months, the absolute values were not comparable due to different cut-offs between laboratories, therefore, we used the single cut-off of each center to discriminate high CRP levels.

Multivariate analysis adjusted for demographic and clinical data (age, sex and disease duration) was also performed. All comparisons were two-tailed and P values below 0.05 were considered statistically significant.

RESULTS

Circulating IFI16 in different disease groups.

The levels of IFI16 were significantly higher in PsA and Pso patients compared to healthy controls (PsA median 0, range 0-131 versus healthy controls 0, 0-67.6, p=0.0004; Pso 7.93, 0-223.5 *versus* healthy controls p<0.0001; **figure 1A**). Moreover, IFI16 concentrations were higher in Pso patients compared to PsA (p=0.0006). According to the established 27 ng/ml threshold, 23/158 (15%) of PsA and 8/44 (18%) Pso sera were positive for circulating IFI16, compared to 6/116 (5%) healthy controls (PsA *versus* healthy controls p=0.002; Pso *versus* healthy controls p=0.003), confirming a higher prevalence of free IFI16 circulating protein in PsA and Pso patients in comparison with healthy controls.

Circulating IFI16 and PsA clinical features at baseline.

The clinical features of the PsA cases included disease duration, peripheral or axial involvement, and skin disease were analyzed according to the positivity/negativity for IFI16 protein are illustrated in **Table 1**. We identified a significant association between elevated CRP levels and the presence of the circulating IFI16 protein (p<0.0001). We subsequently evaluated whether IFI16 serum concentration, rather than its presence, could be correlated to any clinical variable. As expected, IFI16 levels were significantly higher in PsA cases with high CRP levels (median 23.5ng/mL, interquartile range (IQR) 10.4-65.4) compared with patients with low CRP levels (2.6 ng/mL, IQR 1.4-10.6; p<0.001). IFI16 levels were also higher in PsA cases with Pso compared with patients without skin disease, albeit not significantly (11.4 ng/ml, IQR 2.6-31.9 *vs.* 6.4 ng/ml, IQR 1.8-67.6). No differences in IFI16 levels were observed with regard to ongoing

therapies. We further performed a multivariate analysis with pre-specified confounders and this failed to identify statistically significant differences after adjustment for age, sex and disease duration.

Circulating IFI16 and PsA response to etanercept.

Samples at 3 months of etanercept treatment were also available for 8 subjects positive for IFI16 at baseline and we could observe a decrease in IFI16 levels in 5/8 patients (baseline median 81.7 ng/ml, IQR 63.2-102.1 *vs.* 31.8 ng/ml, 22.1-85 at 3 months; p=0.16). Similarly, according to DAS28-CRP, disease activity reached a minimal clinically significant reduction in 4/8 IFI16 positive patients, with a median reduction of 1.25 points. Lastly, circulating IFI16 was measured in the synovial fluid of 7 PsA patients and detected in one case at low concentration (data not shown).

Circulating anti-IFI16 IgG and IgA and PsA clinical features at baseline.

Significantly higher levels of anti-IFI16 IgG antibodies were present in PsA and Pso patients in comparison with healthy controls (PsA median 96, IQR 65.3-151.7 *versus* healthy controls 28.9, IQR 17.3-45.3, p<0.001; Pso 94.9, 62.8-126 *versus* healthy controls, p<0.001), while the titers were comparable between PsA and Pso sera. According to the cut-off value of 113 U/ml, 57/158 (36%) PsA and 14/44 (32%) Pso sera were positive for anti-IFI16 IgG, compared to 11/182 (6%) of the healthy controls (p<0,001 *versus* PsA; p<0.001 *versus* Pso; **Figure 1B**).

When the autoantibody status was analyzed according to sex, age, disease duration, skin, axial involvement, or ongoing therapy (**Table 1**), we observed a significant correlation between anti-IFI16 IgG levels and disease duration after adjusting for age

(beta coefficient 0.38; p=0.036). Moreover, by considering anti-IFI16 IgG serum concentrations, anti-IFI16 IgG titers were increased in subjects with high CRP levels compared with subjects with low CRP levels (median 268.1 U/ml, IQR 162.8-562.4 *versus* 162 U/ml, 142.1-199.7; p=0.015). However, there were no differences in the percentage of patients with high CRP and anti-IFI16 positive versus negative subjects (Table 1).

Circulating anti-IFI16 IgG and IgA in different disease groups.

Median anti-IFI16 IgA titers were increased in PsA and Pso sera compared to healthy controls (PsA 4.7, IQR 3.3-7.1 *versus* healthy controls 3.7, 2.5-5.2, p<0.0001; Pso 7.4, 0-16.5 *versus* healthy controls p<0.0001); moreover, anti-IFI16 IgA were higher in Pso compared to PsA sera (p<0.0001). According to the 9.6 U/ml threshold, 25/158 (16%) of PsA and 16/44 (36%) of Pso sera were positive, compared to 7/138 (5%) of healthy controls (p<0.001 *versus* PsA; p<0.001 *versus* Pso; p=0.004 PsA *versus* Pso) (**Figure 1C**). As reported in **Table 1**, anti-IFI16 IgA positive PsA cases were significantly older compared to anti-IFI16 IgA negative (median age 63.5, IQR 49-69 vs 48.5, IQR 42-58; p<0.001).

Circulating anti-IFI16 IgG and IgA and PsA response to etanercept.

Six subjects positive for anti-IFI16 IgG at baseline were tested also during etanercept therapy without significant changes in autoantibody titers after 3 months of therapy (baseline median 197 U/ml, IQR 188-269 *versus* 3months 223 U/ml, 194-269; p=0.9). Of these, 4/6 (67%) did not reach a minimal clinically significant reduction of DAS28-CRP and the median DAS28 change was -0.85 after 3 months of therapy. Four subjects

positive for anti-IFI16 IgA were subsequently tested after 3 months of etanercept therapy without significant changes in antibody titers (basal median 7.56, IQR 5.18, 12.72 *versus* 3 months 6.55, 5.46-12.6; p=0.2), while 3/4 (75%) of these reached a minimal clinically significant reduction of DAS28, with a median DAS28 change of -2.25 (data not shown).

Synovial fluid anti-IFI16 IgG and IgA and PsA clinical features.

Anti-IFI16 IgG and IgA antibodies were measured also in synovial fluids of 7 PsA patients (*data not shown*)**.** Three out of 7 samples were positive for IgG subtype and 2/7 were positive for IgA subtype, and only 1 patient was positive for both Ig subtypes. We failed to detect correlation between antibody titers and any of the clinical parameters analyzed.

Anti-IFI16 confirmation tests

Protein-IP and IP-WB were used to confirm the presence of anti-IFI16 IgG antibodies, as these techniques are considered the gold standard for the identification of autoantibodies thanks to their high sensitivity and specificity. As shown in **Figure 2A,** we performed protein-IP on 19 cases and we identified an IP pattern of PsA sera recognizing a 90 kDa protein (black arrows, **Figure 2A**). This band corresponds to the IFI16 protein as shown by IP-WB (**Figure 2B**). IIF was also performed to localize the IFI16 antigenic target, and we could confirm it is present in the nucleus according the its nuclear function (**Figure 2**C).

Circulating IFI16 and anti-IFI16 IgG and IgA in the study populations.

When IFI16 and anti-IFI16 data were combined, no correlations were found between IFI16 and anti-IFI16 IgG or IgA levels. However, we note that among the 57 PsA patients positive for anti-IFI16 IgG, 13 (23%) were positive also for circulating IFI16. In contrast, 57% of the 23 patients positive for IFI16 were also positive for IgG autoantibodies (**Table 2**). Among the 25 PsA patients positive for anti-IFI16 IgA, 5 (20%) were positive for the circulating protein. A significant correlation between anti-IFI16 IgG and IgA antibody titers was observed in PsA (Spearman's r=0.1802, p=0.0235). Moreover, while only 21% of the 57 PsA patients positive for anti-IFI16 IgG were positive also for the IgA subtype, 48% of 25 IgA positive patients displayed also high titers of anti-IFI16 IgG antibodies.

Anti-IFI16 IgG epitope mapping

To determine whether anti-IFI16 IgG antibodies detected in sera were directed against specific IFI16 domains, we cloned the DAPIN (spanning from aa 1 to 88), HINA (from aa 131 to 337) and HINB (from aa 506 to 705) domains of IFI16 in a pET30a expression vector. The purity and specificity of the recombinant domains were then established by blue Comassie and Western blot analyses, and finally recombinant DAPIN (rDAPIN), rHINA and rHINB were used as immobilized surface in ELISA for testing the sera of the 57 patients positive for anti-IFI16 IgG. As shown in **Figure 3**, the serum samples displayed a strong reactivity against the HIN domains. Regardless of their aminoacid homology (44%), HINA was better recognized in comparison with HINB domain by disease and control sera. In contrast, the DAPIN domain, which is the moiety of the protein mainly involved in inflammatory activity, was poorly detected by

autoantibodies, suggesting that anti-IFI16 IgGs are mainly directed against an epitope outside the N-terminus of the protein.

DISCUSSION

A significant role of DNA sensing peptides in the pathogenesis of Pso has been recently suggested, largely based on data about LL37, a fragment of the anti-microbial protein cathelicidin, capable to activate dendritic cells by complexing self-DNA, and T cells by acting as a self-antigen [28, 29]. Similar to other autoimmune diseases characterized by serum reactivity to intracellular antigens we cannot speculate on the pathogenetic mechanisms linking IFI16 autoantibodies to tissue damage. However, we suggest that the DNA sensor IFI16 is involved as a self-antigen in the humoral response and in the inflammatory response possibly *via* the inflammasome in PsA.

In the present study, we observed that IFI16 and anti-IFI16 seem to be associated with higher disease activity in PsA and possibly with response to anti-TNFalpha treatment, thus possibly being candidate biomarkers of this seronegative condition and representing a different clinical phenotype. Furthermore, these markers are detected also in the synovial fluids for the first time and may thus represent a mechanistic player in the PsA pathogenesis. These findings may be promising in filling the diagnostic gap of PsA where no serum biomarker for the diagnosis or management is available [30].

IFI16 is an interferon-induced protein and a DNA sensor able to start an innate immune response against pathogenic microorganism by activating the inflammasome and the production of type I IFNs [31]. It has been proposed that the large amount of self-DNA secondary to keratinocyte proliferation in Pso plaques can activate pDC *via* LL37/TLR9 to produce INF-α and recruit T cells and, at the same time, can activate the inflammasome with IL-1β production [31, 32]. The overexpression of IFI16 in keratinocytes could be explained by an attempt to control excessive cell proliferation, resulting, however, in a chronic activation of the inflammasome. Furthermore,

extracellular IFI16 can spread inflammation since it has been demonstrated that the protein is able to bind the membrane of endothelial cells and to induce the production of pro-inflammatory cytokines in these cells [24, 33]. The hypothesis based on an accelerated cell turnover seems to be supported by the high IFI16 levels observed in patients with Pso and in PsA with skin PsO and IFI16 overexpression in PsA circulating mononuclear cells and synovial tissue [13]. In fact, in PsA sera IFI16 is significantly higher compared to healthy controls, particularly when associated with an active disease represented by elevated CRP and mechanisms may include changes similar to what observed in systemic lupus erythematosus models [34] or NETosis in a self-perpetuating fashion [35].

Type I IFN inducible genes are elevated in the serum of patients with different systemic autoimmune diseases, albeit with inconclusive evidence and frequently conflicting results in different rheumatic conditions [36]. IFI16 is among the five most important IFN inducible genes being overexpressed in connective tissue diseases and RA, raising the coined "IFN-signature" [5]. Different from PsA in which IFI16 significantly correlates with inflammation, this protein is not a biomarker of disease activity in RA, but is associated with pulmonary involvement and with the presence of rheumatoid factor and anti-cyclic citrullinated peptide antibodies. IFI16 in RA synovial fluid is not associated with joint disease activity, disease duration or erosion [22]. In the case of SLE, IFI16 is associated with disease activity, particularly lupus nephritis, and is found overexpressed in skin lesions [18, 37, 38].

The inflammatory context in which an intracellular protein is released is thought to be one of the mechanisms leading to the breakdown of tolerance and the recognition of self-antigens; this hypothesis could explain the antibody response against IFI16

observed in PsA and Pso, particularly with the intriguing observation of a prevalent IgA response compared to IgG response in PsO *versus* PsA. As previously mentioned and different from PsA (with anti-IFI16 IgG titers slightly increased in subjects with elevated CRP levels), anti-IFI16 antibodies are not correlated to RA activity, but rather with serum autoantibody positivity, while no association between anti-IFI16 and disease activity or erosions has been found [22]. In SLE, anti-IFI16 antibodies were first reported to be associated with anti-double stranded DNA antibodies [17, 18], but this was not confirmed by our group [15]. Anti-IFI16 antibodies positive patients with systemic sclerosis were more likely to have limited cutaneous systemic sclerosis, a longer disease duration and more severe vascular and pulmonary manifestations, i.e. digital ischemia and a low carbon monoxide diffusing capacity [18, 20]. An association with disease severity, as for PsA, has been documented in Sjögren's syndrome, with lower tear and saliva production, higher focus score in minor salivary glands biopsies, germinal center-like structures in the labial salivary gland lymphocytic infiltrates, and higher IgG and antinuclear antibodies levels being associated with anti-IFI16 [16, 17]. The epitope mapping also provided somehow surprising evidence compared to other rheumatic conditions. In systemic sclerosis, anti-IFI16 reacted with either N- or C-terminal fragments or both [18], while in 70% of Sjögren's syndrome anti-IFI16 were directed against an epitope outside the N terminus [17] and in SLE antibodies are directed predominantly against N-terminus [17]. We can hypothesize that in SLE anti-IFI16 blocking the N-terminus allows the formation of self-DNA/IFI16 complexes which favor nucleic acid clearance and are protective in terms of SLE nephritis. Our data in PsA are in agreement with those obtained in Sjögren's syndrome, since the

DAPIN domain was poorly detected by anti-IFI16 IgG. Accordingly, this would have left free the moiety of the protein mainly involved in the inflammatory activity.

We are well aware of the limitations of our study, including the limited clinical data available but we report for the first time that IFI16 and anti-IFI16 are detectable in the sera of patients with PsA and seems to correlate with the degree of inflammation and possibly with treatment response. We cannot exclude a role of this protein and the elicited antibody in the pathogenesis of PsA; moreover, IFI16 and anti-IFI16 maybe represent new candidate biomarkers for PsA diagnosis and monitoring when data confirmed in larger and longitudinal studies.

The authors have no competing interests to declare

REFERENCES

1. Sakkas LI, Bogdanos DP. Are psoriasis and psoriatic arthritis the same disease? The IL-23/IL-17 axis data. Autoimmun Rev 2017; **16**:10-5.

2. Ogdie A, Weiss P. The Epidemiology of Psoriatic Arthritis. Rheum Dis Clin North Am 2015; **41**:545-68.

3. Barnas JL, Ritchlin CT. Etiology and Pathogenesis of Psoriatic Arthritis. Rheum Dis Clin North Am 2015; **41**:643-63.

4. Marinoni B, Ceribelli A, Massarotti MS, Selmi C. The Th17 axis in psoriatic disease: pathogenetic and therapeutic implications. Auto Immun Highlights 2014; **5**:9-19.

5. Higgs BW, Liu Z, White B, Zhu W, White WI, Morehouse C, Brohawn P, Kiener PA, Richman L, Fiorentino D, Greenberg SA, Jallal B, Yao Y. Patients with systemic lupus erythematosus, myositis, rheumatoid arthritis and scleroderma share activation of a common type I interferon pathway. Ann Rheum Dis 2011; **70**:2029-36.

6. Boehncke WH. Etiology and Pathogenesis of Psoriasis. Rheum Dis Clin North Am 2015; **41**:665-75.

7. Yao Y, Richman L, Morehouse C, de los Reyes M, Higgs BW, Boutrin A, White B, Coyle A, Krueger J, Kiener PA, Jallal B. Type I interferon: potential therapeutic target for psoriasis? PLoS One 2008; **3**:e2737.

8. Bissonnette R, Papp K, Maari C, Yao Y, Robbie G, White WI, Le C, White B. A randomized, double-blind, placebo-controlled, phase I study of MEDI-545, an anti-interferon-alfa monoclonal antibody, in subjects with chronic psoriasis. J Am Acad Dermatol 2010; **62**:427-36.

9. Harden JL, Johnson-Huang LM, Chamian MF, Lee E, Pearce T, Leonardi CL, Haider A, Lowes MA, Krueger JG. Humanized anti-IFN-gamma (HuZAF) in the treatment of psoriasis. J Allergy Clin Immunol 2015; **135**:553-6.

10. Dell'Oste V, Gatti D, Giorgio AG, Gariglio M, Landolfo S, De Andrea M. The interferon-inducible DNA-sensor protein IFI16: a key player in the antiviral response. New Microbiol 2015; **38**:5-20.

11. Caposio P, Gugliesi F, Zannetti C, Sponza S, Mondini M, Medico E, Hiscott J, Young HA, Gribaudo G, Gariglio M, Landolfo S. A novel role of the interferon-inducible protein IFI16 as inducer of proinflammatory molecules in endothelial cells. J Biol Chem 2007; **282**:33515-29.

12. Tervaniemi MH, Katayama S, Skoog T, Siitonen HA, Vuola J, Nuutila K, Sormunen R, Johnsson A, Linnarsson S, Suomela S, Kankuri E, Kere J, Elomaa O. NOD-like receptor signaling and inflammasome-related pathways are highlighted in psoriatic epidermis. Sci Rep 2016; **6**:22745.

13. Dolcino M, Ottria A, Barbieri A, Patuzzo G, Tinazzi E, Argentino G, Beri R, Lunardi C, Puccetti A. Gene Expression Profiling in Peripheral Blood Cells and Synovial Membranes of Patients with Psoriatic Arthritis. PLoS One 2015; **10**:e0128262.

14. Belasco J, Louie JS, Gulati N, Wei N, Nograles K, Fuentes-Duculan J, Mitsui H, Suarez-Farinas M, Krueger JG. Comparative genomic profiling of synovium versus skin lesions in psoriatic arthritis. Arthritis Rheumatol 2015; **67**:934-44.

15. Caneparo V, Cena T, De Andrea M, Dell'oste V, Stratta P, Quaglia M, Tincani A, Andreoli L, Ceffa S, Taraborelli M, Magnani C, Landolfo S, Gariglio M. Anti-IFI16 antibodies and their relation to disease characteristics in systemic lupus erythematosus. Lupus 2013; **22**:607-13.

16. Alunno A, Caneparo V, Carubbi F, Bistoni O, Caterbi S, Bartoloni E, Giacomelli R, Gariglio M, Landolfo S, Gerli R. Interferon gamma-inducible protein 16 in primary Sjogren's syndrome: a novel player in disease pathogenesis? Arthritis Res Ther 2015; **17**:208.

17. Baer AN, Petri M, Sohn J, Rosen A, Casciola-Rosen L. Association of Antibodies to Interferon-Inducible Protein-16 With Markers of More Severe Disease in Primary Sjogren's Syndrome. Arthritis Care Res (Hoboken) 2016; **68**:254-60.

18. Mondini M, Vidali M, De Andrea M, Azzimonti B, Airo P, D'Ambrosio R, Riboldi P, Meroni PL, Albano E, Shoenfeld Y, Gariglio M, Landolfo S. A novel autoantigen to differentiate limited cutaneous systemic sclerosis from diffuse cutaneous systemic sclerosis: the interferon-inducible gene IFI16. Arthritis Rheum 2006; **54**:3939-44.

19. Costa S, Mondini M, Caneparo V, Afeltra A, Airo P, Bellisai F, Faggioli P, Gerli R, Lotzniker M, Meroni PL, Morozzi G, Radice A, Riccieri V, Scarsi M, Sebastiani GD, Sinico RA, Tincani A, Gariglio M, Landolfo S. Detection of anti-IFI16 antibodies by ELISA: clinical and serological associations in systemic sclerosis. Rheumatology (Oxford) 2011; **50**:674-81.

20. McMahan ZH, Shah AA, Vaidya D, Wigley FM, Rosen A, Casciola-Rosen L. Anti-Interferon-Inducible Protein 16 Antibodies Associate With Digital Gangrene in Patients With Scleroderma. Arthritis Rheumatol 2016; **68**:1262-71.

21. Caneparo V, Pastorelli L, Pisani LF, Bruni B, Prodam F, Boldorini R, Roggenbuck D, Vecchi M, Landolfo S, Gariglio M, De Andrea M. Distinct Anti-IFI16 and Anti-GP2 Antibodies in Inflammatory Bowel Disease and Their Variation with Infliximab Therapy. Inflamm Bowel Dis 2016; **22**:2977-87.

22. Alunno A, Caneparo V, Bistoni O, Caterbi S, Terenzi R, Gariglio M, Bartoloni E, Manzo A, Landolfo S, Gerli R. Circulating Interferon-Inducible Protein IFI16 Correlates With Clinical and Serological Features in Rheumatoid Arthritis. Arthritis Care Res (Hoboken) 2016; **68**:440-5.

23. Taylor W, Gladman D, Helliwell P, Marchesoni A, Mease P, Mielants H, Group CS. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis Rheum 2006; **54**:2665-73.

24. Gugliesi F, Bawadekar M, De Andrea M, Dell'Oste V, Caneparo V, Tincani A, Gariglio M, Landolfo S. Nuclear DNA sensor IFI16 as circulating protein in autoimmune diseases is a signal of damage that impairs endothelial cells through high-affinity membrane binding. PLoS One 2013; **8**:e63045.

25. Ceribelli A, Fredi M, Taraborelli M, Cavazzana I, Franceschini F, Quinzanini M, Tincani A, Ross SJ, Chan JY, Pauley BA, Chan EK, Satoh M. Anti-MJ/NXP-2 autoantibody specificity in a cohort of adult Italian patients with polymyositis/dermatomyositis. Arthritis Res Ther 2012; **14**:R97.

26. Ceribelli A, Isailovic N, De Santis M, Generali E, Fredi M, Cavazzana I, Franceschini F, Cantarini L, Satoh M, Selmi C. Myositis-specific autoantibodies and their association with malignancy in Italian patients with polymyositis and dermatomyositis. Clin Rheumatol 2017; **36**:469-75.

27. Yamasaki Y, Narain S, Yoshida H, Hernandez L, Barker T, Hahn PC, Sobel ES, Segal MS, Richards HB, Chan EK, Reeves WH, Satoh M. Autoantibodies to RNA helicase A: a new serologic marker of early lupus. Arthritis Rheum 2007; **56**:596-604.

28. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, Cao W, Wang YH, Su B, Nestle FO, Zal T, Mellman I, Schroder JM, Liu YJ, Gilliet M.

Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature 2007; **449**:564-9.

29. Lande R, Botti E, Jandus C, Dojcinovic D, Fanelli G, Conrad C, Chamilos G, Feldmeyer L, Marinari B, Chon S, Vence L, Riccieri V, Guillaume P, Navarini AA, Romero P, Costanzo A, Piccolella E, Gilliet M, Frasca L. The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis. Nat Commun 2014; **5**:5621.

30. McArdle A, Pennington S, FitzGerald O. Clinical Features of Psoriatic Arthritis: a Comprehensive Review of Unmet Clinical Needs. Clin Rev Allergy Immunol 2017.

31. Zhao H, Gonzalezgugel E, Cheng L, Richbourgh B, Nie L, Liu C. The roles of interferon-inducible p200 family members IFI16 and p204 in innate immune responses, cell differentiation and proliferation. Genes Dis 2015; **2**:46-56.

32. Chiliveru S, Rahbek SH, Jensen SK, Jorgensen SE, Nissen SK, Christiansen SH, Mogensen TH, Jakobsen MR, Iversen L, Johansen C, Paludan SR. Inflammatory cytokines break down intrinsic immunological tolerance of human primary keratinocytes to cytosolic DNA. J Immunol 2014; **192**:2395-404.

33. Bawadekar M, De Andrea M, Lo Cigno I, Baldanzi G, Caneparo V, Graziani A, Landolfo S, Gariglio M. The Extracellular IFI16 Protein Propagates Inflammation in Endothelial Cells Via p38 MAPK and NF-kappaB p65 Activation. J Interferon Cytokine Res 2015; **35**:441-53.

34. Shin JI, Lee KH, Joo YH, Lee JM, Jeon J, Jung HJ, Shin M, Cho S, Kim TH, Park S, Jeon BY, Jeong H, Lee K, Kang K, Oh M, Lee H, Lee S, Kwon Y, Oh GH, Kronbichler A. Inflammasomes and autoimmune and rheumatic diseases: A comprehensive review. J Autoimmun 2019:102299.

35. Lee KH, Kronbichler A, Park DD, Park Y, Moon H, Kim H, Choi JH, Choi Y, Shim S, Lyu IS, Yun BH, Han Y, Lee D, Lee SY, Yoo BH, Lee KH, Kim TL, Kim H, Shim JS, Nam W, So H, Choi S, Lee S, Shin JI. Neutrophil extracellular traps (NETs) in autoimmune diseases: A comprehensive review. Autoimmun Rev 2017; **16**:1160-73.

36. Ronnblom L. The importance of the type I interferon system in autoimmunity. Clin Exp Rheumatol 2016; **34**:21-4.

37. Costa S, Borgogna C, Mondini M, De Andrea M, Meroni PL, Berti E, Gariglio M, Landolfo S. Redistribution of the nuclear protein IFI16 into the cytoplasm of ultraviolet B-exposed keratinocytes as a mechanism of autoantigen processing. Br J Dermatol 2011; **164**:282-90.

38. Cao T, Shao S, Li B, Jin L, Lei J, Qiao H, Wang G. Up-regulation of Interferon-inducible protein 16 contributes to psoriasis by modulating chemokine production in keratinocytes. Sci Rep 2016; **6**:25381.

FIGURE LEGENDS.

Figure 1. Serum IFI16 and anti-IFI16 IgG and IgA levels in patients with PsA, Psoriasis and HCs. (A) IFI16 protein levels in patients' and controls' sera were determined using an in-house capture ELISA. Each dot represents the concentration of IFI16 protein (expressed in ng/ml on a linear scale) in each individual subject. The horizontal bar represents the median values. Values over the dotted line indicate the percentage of subjects with IFI16 protein levels above the cut-off value (27 ng/ml). **(B)** Serum IgG and **(C)** IgA specific for IFI16 were quantified by ELISA in HC and patients suffering from psoriatic arthritis and psoriasis. Each dot represents the autoantibody level for each subject sample expressed in arbitrary units on a linear scale. The horizontal bars in each group represent the median values. Values over the dotted line indicate the percentage of subjects with antibody titers above the cutoff value (113 U/mL for anti-IFI16 IgG and 9.6 U/mL for anti-IFI16 IgA, calculated as the 95th percentile of the control population). Statistical significance: ***$p < 0.0001$ (Mann–Whitney tests).

Figure 2. Analysis of protein component of autoantigen IFI16 (8% SDS-PAGE) by radio-immunoprecipitation (IP) (A) and IP-Western Blotting (WB) (B), and indirect immunofluorescence (C). The IP pattern of PsA sera recognizing a 90 kDa protein (black arrows, 2A). IP-WB confirming the identity of the IP bands corresponding to IFI16 (2B). The expected ANA pattern for a representative sample positive for ani-IFI16 IgG (2C).

Figure 3. Epitope mapping for anti-IFI16 IgG. To determine the target of the anti-IFI16 IgG antibodies found in the sera of PsA patients, the DAPIN (spanning from aa 1 to 88), HINA (from aa 131 to 337) and HINB (from aa 506 to 705) domains of IFI16 were purified as recombinant peptides and 2ug/mL each used to perform ELISA. Each dot represents the value of the absorbance at 450nm for the 57 sera tested against the three domains. The horizontal bars in each group represent the median values. Statistical significance: ***$p < 0.0001$ (Mann–Whitney tests). Panel A shows PsA patients and panel B healthy controls.

TABLES.

Table 1. Demographic, clinical and serological characteristics of PsA patients.

		IFI16		Anti-IFI16 IgG		Anti-IFI16 IgA	
	All PsA	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Patients (%)	158 (100)	23 (15)	135 (85)	57 (36)	101 (64)	26 (17)	132 (83)
Women (%)	93 (50.5)	12 (52)	67 (50)	34 (60)	45 (45)	11 (42)	68 (52)
Age, yy (IQR)	50 (43-61)	51 (42-69)	50 (43-60)	49 (43-61)	51 (43-60.5)	**63.5 (49-69)**	**48 (42-58)****
Disease duration, mm (IQR)	60 (16-119)	82 (24-118)	49 (15-120)	72 (18-119.5)	48 (15-118)	60 (18-96)	56 (16-132)
Disease duration <12 mm (%)	33 (29)	**1 (4.3)**	**32 (24)***	8 (14)	10 (10)	2 (8)	16 (12)
Disease duration >60 mm (%)	76 (49)	14 (61)	62 (47)	33 (60)	43 (43)	14 (54)	62 (47)
Psoriasis (%)	127 (80)	17 (74)	109 (81)	45 (79)	82 (81)	24 (92)	102 (77)
Axial involvement, (%)	45 (29)	7 (30)	38 (29)	16 (28)	29 (29)	4 (15)	41 (31)
High CRP (%)	63 (40)	**18 (78)**	**45 (33)***	23 (40)	40 (40)	12 (46)	51 (39)
Ongoing treatments:							
no treatment	64 (40.5)	17 (74)	77 (57)	21 (39)	43 (43)	9 (35)	55 (42)
methotrexate	64 (40.5)	15 (71)	49 (46)	21 (37)	43 (43)	14 (54)	50 (38)
median MTX dose, weekly	15,2 (range 12,5-17,5)	12,7 (range 12,5-15)	15,2 (range 12,5-17,5)	15,2 (range 12,5-17,5)	15,2 (range 12,5-17,5)	12,7 (range 12,5-15)	15,7 (range 15-17,5)
anti-TNFα	30 (19)	2 (9)	28 (21)	15 (26)	15 (15)	3 (12)	27 (20)
etanercept	8	1	7	4	4	1	7
adalimumab	2	0	2	1	1	0	2
infliximab	20	1	19	9	11	2	18

Continuous variables are expressed as median and IQR, binary as numbers and percentages.

PsA: psoriatic arthritis; IFI16: interferon-inducible protein 16; Ig: immunoglobulin; yy: years; mm: months; Ig: immunoglobulin; CRP: C reactive protein; TNF: tumor necrosis factor.

*p<0.01 positive *vs.* negative patients, ** p<0.001 positive *vs.* negative patients

Table 2. Association of IFI16-based biomarkers in the PsA cohort.

PsA n=158	**IFI16 positive n=23 (15%)**	**Anti-IFI16 IgG positive n=57 (36%)**	**Anti-IFI16 IgA positive n=25 (16%)**
IFI16 positive n=23 (15%)	-	13/57 (23%)	5/25 (20%)
Anti-IFI16 IgG positive n=57 (36%)	13/23 (57%)	-	12/25 (48%)
Anti-IFI16 IgA positive n=25 (16%)	5/23 (22%)	12/57 (21%)	-

Variables are expressed as numbers (n) and percentages.

PsA: psoriatic arthritis; IFI16: interferon-inducible protein 16; Ig: immunoglobulin.

*p<0.01, ** p<0.001

Serum IFI16 and anti-IFI16 antibodies in psoriatic arthritis: possible correlation with disease activity and response to therapy

Short title: IFI16 and psoriatic arthritis

Marco De Andrea[1,2,3]*, Maria De Santis[4]*, Valeria Caneparo[1,2,3], Elena Generali[4], Silvia Sirotti[4], Natasa Isailovic[4], Giacomo Maria Guidelli[4], Angela Ceribelli[4], Marta Fabbroni[5], Antonella Simpatico[5], Luca Cantarini[5], Paolo Gisondi[6], Luca Idolazzi[7], Marisa Gariglio[2,3], and Carlo Selmi[4,8]

[1] Department of Public Health and Paediatric Sciences, Turin Medical School; [2] Department of Translational Medicine, Novara Medical School; [3] Intrinsic Immunity Unit, CAAD - Center for Translational Research on Autoimmune and Allergic Diseases, University of Piemonte Orientale, Novara; [4] Rheumatology and Clinical Immunology Unit, Humanitas Research Hospital, Rozzano; [5] Rheumatology, University of Siena; [6] Dermatology, University of Verona; [7] Rheumatology, University of Verona; [8] Department of Biomedical Science and Translational Medicine, University of Milan, Italy.

Corresponding author: Carlo Selmi MD PhD, Division of Rheumatology and Clinical Immunology, Humanitas Research Hospital, via A. Manzoni 56, 20089 Rozzano, Milan, Italy; tel +39-02-8224-5129, fax +39-02-8224-2298, email carlo.selmi@unimi.it

Key words: IFI16; anti-IFI16 antibodies; psoriatic arthritis

List of abbreviations:

interferon-inducible protein 16 (IFI16)

psoriatic arthritis (PsA)

psoriasis (Pso)

C-reactive protein (CRP)

rheumatoid arthritis (RA),

tumor necrosis factor-α (TNF-α)

interleukin(IL)

interferon (IFN)

plasmacytoid dendritic cells (pDC)

immunofluorescence (IIF)

immunoprecipitation (IP)

protein A sepharose (PAS)

sodium dodecyl sulphate- polyacrylamide (SDS-PAGE)

human foreskin fibroblast (HFF)

horseradish peroxidase (HRP)

phosphate buffered saline (PBS)

disease activity score (DAS)

systemic lupus erythematosus (SLE)

SUMMARY

Objective. The nuclear interferon-inducible protein 16 (IFI16) and anti-IFI16 antibodies have been detected in subjects with several rheumatic diseases, often correlating with disease severity, and we herein investigated their prevalence and clinical associations in psoriatic arthritis (PsA) compared to psoriasis (Pso).

Methods. We tested sera and synovial fluids of patients with PsA for IFI16 protein levels by capture ELISA and for anti-IFI16 IgG and IgA by ELISA, protein radio-immunoprecipitation and immunoprecipitation-Western blot of IgG. Sera from patients with Pso and healthy subjects were used as controls, and in a subgroup of patients with PsA we also studied sera after treatment with etanercept.

Results. IFI16 was detectable in the sera of 66% of patients with Pso, 46% of PsA, and 19% of controls. Among PsA cases, 51% of IFI16-positive cases had elevated levels of C-reactive protein (CRP) compared to 31% of patients with undetectable IFI16. Anti-IFI16 of both IgG and IgA isoforms were detected with significantly higher frequency in PsA and Pso compared to healthy controls, with higher IgG titers in patients with elevated CRP ($p=0.015$) and a significant reduction in the majority of patients after 3 month of etanercept treatment. Immunoprecipitation confirmed the presence of anti-IFI16 IgG antibodies and these recognized the HINA epitope predominantly (amino acids 131 to 337). Lastly, IFI16 was detected in 1/7 and anti-IFI16 in 3/7 synovial fluids from patients with PsA.

Conclusion. IFI16 and anti-IFI16 seem to be associated with higher disease activity and with treatment response in PsA. The detectability of these markers also in synovial fluids represents a novel finding with potential clinical implications.

Psoriatic arthritis (PsA) is a chronic inflammatory disease within the broader psoriatic disease encompassing musculoskeletal features (with enthesitis, synovitis and erosion with osteitis of the peripheral joints and/or the axial skeleton), skin and nail manifestations [1], estimated to affect approximately 0.5% of the general population [2]. PsA is found in a variable proportion (15-40%) of the patients with psoriasis (Pso), and in 74% of cases it is preceded by skin manifestations [3].

Different from rheumatoid arthritis (RA), both PsA and Pso lack serum biomarkers for an early diagnosis, while sharing a T cell-mediated response based on the tumor necrosis factor-α (TNF-α)/interleukin(IL)-17 axis [4]. As reported for other immune-mediated diseases [5], an interferon (IFN) signature is observed in Pso as self-DNA-engulfed plasmacytoid dendritic cells (pDC) produce a large amount of IFN-α [6]. Further, an overexpression of IFN-γ and TNF-α-inducible genes is observed in Pso plaques due to T cell and macrophage recruitment and activation [7]. Despite these observations, treatments targeting IFN-α or IFN-γ have proven ineffective in Pso, while TNF-α and IL12/23 or IL17 blockers lead to clear skin in a large proportion of patients [8, 9].

The interferon-inducible protein 16 (IFI16) is a DNA sensor involved in the inflammasome-mediated defense against viral infections [10], that displays also pro-inflammatory and anti-angiogenetic effects [11]. In the case of psoriatic disease, IFI16 is overexpressed in the affected skin [12], in peripheral blood mononuclear cells, and synovial tissues [13, 14]. Previous studies have demonstrated that IFI16 is overexpressed in different connective tissue diseases and in RA [5], and anti-IFI16 antibodies have been detected in systemic lupus erythematosus (SLE) [15], Sjögren's

syndrome [16, 17], systemic sclerosis [18-20], inflammatory bowel diseases [21], and RA [22], possibly associated with disease severity and progression.

We report herein for the first time that IFI16 and anti-IFI16 antibodies are detectable in the sera and synovial fluids of patients with PsA and may represent disease activity biomarkers.

PATIENTS AND METHODS

Patients

We investigated 158 consecutive patients with a diagnosis of PsA based on the CASPAR criteria [23] (**Table 1**), while 182 healthy subjects and 44 patients with skin or nail Pso but no evidence of PsA were used as controls. Serum samples were collected and stored at − 20°C until used; demographic and clinical data (presence or absence of psoriasis, presence or absence of axial involvement and reported blood tests done by the patient within one month) were recorded. DAS28 (disease activity score 28 joint) was available only for 30 PsA patients treated with biologic agents.

In a subgroup of 8 patients with PsA, serum samples were also available at 3 months after starting weekly subcutaneously etanercept 50 mg. Synovial fluid samples from 7 patients with PsA who underwent arthrocentesis for knee effusion and had not received intra-articular medications (e.g., corticosteroids, hyaluronic acid) in the previous 6 months, were also analyzed (blood samples were collected on the same day). Synovial fluids were centrifuged and supernatants stored at − 80°C until use. Clinical and serologic records were collected at the time of enrollment. This study was approved by the local IRB and written informed consents were obtained from patients and controls.

Determination of extracellular IFI16 protein by capture ELISA

A capture ELISA was employed for determination of circulating extracellular IFI16 protein following a procedure described elsewhere [24], and the threshold cut-off value was defined as the 95th percentile of healthy controls as 27 ng/ml.

Determination of antibody titers toward human recombinant IFI16 by ELISA

To determine anti-IFI16 antibody titers of IgG and IgA isotype in sera of patients, we

performed in-house ELISA as previously described [21]. Accordingly, cut-off values were calculated as the 95th percentile of healthy controls and the threshold values were set to 113 U/ml and 9.6 U/ml for IgG and IgA isotype, respectively.

Indirect immunofluorescence assay

The localization of cellular antigens recognized by autoantibodies was tested by indirect immunofluorescence (IIF) on HEp-2 cells (INOVA Diagnostics, San Diego, CA, USA) using a 1:80 dilution of human sera of patients and controls, followed by secondary antibodies marked with fluorochrome (AlexaFluor488 AffiniPure F(ab')$_2$ fragment goat anti-human IgG, Fcγ fragment specific, Jackson Immunoresearch Europe Ltd, Suffolk, UK) as previously described [25]. Samples were acquired on Olympus BX53 Upright fluorescence microscope.

Radioimmunoprecipitation assay

PsA sera were analyzed by protein radio-immunoprecipitation (IP) using marked ^{35}S-HeLa cell extract. IP was used to identify autoantibodies directed against protein self-antigens as described elsewhere [26]; briefly, sera were incubated with Protein A sepharose (PAS) beads and after serial washes samples have been incubated with cell lysate (radioactively marked for protein-IP). After forming immuno-complexes, samples were prepared for 8% sodium dodecyl sulphate- polyacrylamide (SDS-PAGE) electrophoresis for protein-IP. To confirm data obtained with IP we performed IP-Western blotting. In detail, human sera and 50ng of mouse monoclonal anti-human IFI16 as a positive control were cross-linked with PAS beads to isolate human IgG directed against IFI16 [27]. IP was initially performed with cell extract from 5x10^6 HeLa cells/sample, but since human foreskin fibroblast (HFF) express IFI16 at higher

basal levels than HeLa, control experiment was performed with HFF. Proteins were then fractionated by 8% SDS-PAGE and transferred to a nitrocellulose membrane, probed with 1:500 of mouse monoclonal anti-human IFI16 antibody (Novus Biologicals, Littleton, CO, USA) for band 88 kDa identification, followed by horseradish peroxidase (HRP)-goat anti-mouse IgG (1: 10000 dilution; ThermoFisher, Waltham, MA, USA). Development was performed by Immobilon Western Chemiluminescent HRP substrate (Millipore, Darmstadt, Germany) and acquired using ChemiDoc (Bio-Rad, California, USA).

Recombinant IFI16 domains

The coding region of the three IFI16 domains was amplified from full length human IFI16 (isoform b) cDNA using primers containing BamHI and XhoI restriction sites (DAPIN: Fw 5' GGCGGATCCATGGGAAAAAAATACAAGAAC 3'; Rw 5' GCCTCGAGTCATTTTAACTTTTCTTTTTTAAG 3'; HIN A: Fw 5' GGCGGATCCCCGAAAACAGTGGCCAAATG 3'; Rw 5' GCCTCGAGTCATTTCTTTATCTGGATAAAACTA 3'; HIN B: Fw 5' GGCGGATCCGAACCTGAAGAAGTTTCCATA 3'; Rw 5' GCCTCGAGTCAGATGACCTTGATGTGACTATG 3'). The amplified inserts were digested with BamHI and XhoI, and cloned in pET30a expression vector containing an N-terminal histidine tag (Novagen, Madison, WI). Expression and affinity purification of the recombinant proteins were performed according to standard procedures, and purity assessed by 12% SDS-PAGE gel electrophoresis.

IFI16 epitope mapping

IFI16 protein domains were diluted in phosphate buffered saline (PBS) to a concentration of 2 ug/ml. Polystyrene microwell plates (Nunc-Immuno Maxisorp) (Thermo Scientific, Nunc, Roskilde, Denmark) were coated with 100 µl diluted protein per well, and incubated overnight at 4°C. After blocking, sera were added in duplicate. After washing, horseradish peroxidase-conjugated rabbit anti-human IgG (DakoCytomation) was added. Following the addition of the substrate (tetramethylbenzidine, KPL), absorbance was measured at 450 nm using a microplate reader (SpectraCount,Packard BioScience). The background reactivity of the reference mixture was subtracted to calculate the results.

Statistical analysis

All data analyses were performed using Stata for Macintosh 13.1 (StataCorp, College Station, Tx, USA). As detailed throughout, chi-square test, Mann-Whitney U test, Kruskal-Wallis test with Dunn's test for multiple comparison *post hoc*, or Wilcoxon test were employed to compare groups based on the data distribution. Linear regression and Spearman's test were used to correlate IFI16 and anti-IFI16 levels with continuous variables such as disease duration and age. C reactive protein (CRP) levels were reported for patients who had available blood tests within one months, the absolute values were not comparable due to different cut-offs between laboratories, therefore, we used the single cut-off of each center to discriminate high CRP levels.

Multivariate analysis adjusted for demographic and clinical data (age, sex and disease duration) was also performed. All comparisons were two-tailed and P values below 0.05 were considered statistically significant.

RESULTS

Circulating IFI16 in different disease groups.

The levels of IFI16 were significantly higher in PsA and Pso patients compared to healthy controls (PsA median 0, range 0-131 versus healthy controls 0, 0-67.6, p=0.0004; Pso 7.93, 0-223.5 *versus* healthy controls p<0.0001; **figure 1A**). Moreover, IFI16 concentrations were higher in Pso patients compared to PsA (p=0.0006). According to the established 27 ng/ml threshold, 23/158 (15%) of PsA and 8/44 (18%) Pso sera were positive for circulating IFI16, compared to 6/116 (5%) healthy controls (PsA *versus* healthy controls p=0.002; Pso *versus* healthy controls p=0.003), confirming a higher prevalence of free IFI16 circulating protein in PsA and Pso patients in comparison with healthy controls.

Circulating IFI16 and PsA clinical features at baseline.

The clinical features of the PsA cases included disease duration, peripheral or axial involvement, and skin disease were analyzed according to the positivity/negativity for IFI16 protein are illustrated in **Table 1**. We identified a significant association between elevated CRP levels and the presence of the circulating IFI16 protein (p<0.0001). We subsequently evaluated whether IFI16 serum concentration, rather than its presence, could be correlated to any clinical variable. As expected, IFI16 levels were significantly higher in PsA cases with high CRP levels (median 23.5ng/mL, interquartile range (IQR) 10.4-65.4) compared with patients with low CRP levels (2.6 ng/mL, IQR 1.4-10.6; p<0.001). IFI16 levels were also higher in PsA cases with Pso compared with patients without skin disease, albeit not significantly (11.4 ng/ml, IQR 2.6-31.9 *vs.* 6.4 ng/ml, IQR 1.8-67.6). No differences in IFI16 levels were observed with regard to ongoing

therapies. We further performed a multivariate analysis with pre-specified confounders and this failed to identify statistically significant differences after adjustment for age, sex and disease duration.

Circulating IFI16 and PsA response to etanercept.

Samples at 3 months of etanercept treatment were also available for 8 subjects positive for IFI16 at baseline and we could observe a decrease in IFI16 levels in 5/8 patients (baseline median 81.7 ng/ml, IQR 63.2-102.1 *vs.* 31.8 ng/ml, 22.1-85 at 3 months; p=0.16). Similarly, according to DAS28-CRP, disease activity reached a minimal clinically significant reduction in 4/8 IFI16 positive patients, with a median reduction of 1.25 points. Lastly, circulating IFI16 was measured in the synovial fluid of 7 PsA patients and detected in one case at low concentration (data not shown).

Circulating anti-IFI16 IgG and IgA and PsA clinical features at baseline.

Significantly higher levels of anti-IFI16 IgG antibodies were present in PsA and Pso patients in comparison with healthy controls (PsA median 96, IQR 65.3-151.7 *versus* healthy controls 28.9, IQR 17.3-45.3, p<0.001; Pso 94.9, 62.8-126 *versus* healthy controls, p<0.001), while the titers were comparable between PsA and Pso sera. According to the cut-off value of 113 U/ml, 57/158 (36%) PsA and 14/44 (32%) Pso sera were positive for anti-IFI16 IgG, compared to 11/182 (6%) of the healthy controls (p<0,001 *versus* PsA; p<0.001 *versus* Pso; **Figure 1B**).

When the autoantibody status was analyzed according to sex, age, disease duration, skin, axial involvement, or ongoing therapy (**Table 1**), we observed a significant correlation between anti-IFI16 IgG levels and disease duration after adjusting for age

(beta coefficient 0.38; p=0.036). Moreover, by considering anti-IFI16 IgG serum concentrations, anti-IFI16 IgG titers were increased in subjects with high CRP levels compared with subjects with low CRP levels (median 268.1 U/ml, IQR 162.8-562.4 *versus* 162 U/ml, 142.1-199.7; p=0.015). However, there were no differences in the percentage of patients with high CRP and anti-IFI16 positive versus negative subjects (Table 1).

Circulating anti-IFI16 IgG and IgA in different disease groups.

Median anti-IFI16 IgA titers were increased in PsA and Pso sera compared to healthy controls (PsA 4.7, IQR 3.3-7.1 *versus* healthy controls 3.7, 2.5-5.2, p<0.0001; Pso 7.4, 0-16.5 *versus* healthy controls p<0.0001); moreover, anti-IFI16 IgA were higher in Pso compared to PsA sera (p<0.0001). According to the 9.6 U/ml threshold, 25/158 (16%) of PsA and 16/44 (36%) of Pso sera were positive, compared to 7/138 (5%) of healthy controls (p<0.001 *versus* PsA; p<0.001 *versus* Pso; p=0.004 PsA *versus* Pso) (**Figure 1C**). As reported in **Table 1**, anti-IFI16 IgA positive PsA cases were significantly older compared to anti-IFI16 IgA negative (median age 63.5, IQR 49-69 vs 48.5, IQR 42-58; p<0.001).

Circulating anti-IFI16 IgG and IgA and PsA response to etanercept.

Six subjects positive for anti-IFI16 IgG at baseline were tested also during etanercept therapy without significant changes in autoantibody titers after 3 months of therapy (baseline median 197 U/ml, IQR 188-269 *versus* 3months 223 U/ml, 194-269; p=0.9). Of these, 4/6 (67%) did not reach a minimal clinically significant reduction of DAS28-CRP and the median DAS28 change was -0.85 after 3 months of therapy. Four subjects

positive for anti-IFI16 IgA were subsequently tested after 3 months of etanercept therapy without significant changes in antibody titers (basal median 7.56, IQR 5.18, 12.72 *versus* 3 months 6.55, 5.46-12.6; p=0.2), while 3/4 (75%) of these reached a minimal clinically significant reduction of DAS28, with a median DAS28 change of -2.25 (data not shown).

Synovial fluid anti-IFI16 IgG and IgA and PsA clinical features.

Anti-IFI16 IgG and IgA antibodies were measured also in synovial fluids of 7 PsA patients (*data not shown*)**.** Three out of 7 samples were positive for IgG subtype and 2/7 were positive for IgA subtype, and only 1 patient was positive for both Ig subtypes. We failed to detect correlation between antibody titers and any of the clinical parameters analyzed.

Anti-IFI16 confirmation tests

Protein-IP and IP-WB were used to confirm the presence of anti-IFI16 IgG antibodies, as these techniques are considered the gold standard for the identification of autoantibodies thanks to their high sensitivity and specificity. As shown in **Figure 2A,** we performed protein-IP on 19 cases and we identified an IP pattern of PsA sera recognizing a 90 kDa protein (black arrows, **Figure 2A**). This band corresponds to the IFI16 protein as shown by IP-WB (**Figure 2B**). IIF was also performed to localize the IFI16 antigenic target, and we could confirm it is present in the nucleus according the its nuclear function (**Figure 2C**).

Circulating IFI16 and anti-IFI16 IgG and IgA in the study populations.

When IFI16 and anti-IFI16 data were combined, no correlations were found between IFI16 and anti-IFI16 IgG or IgA levels. However, we note that among the 57 PsA patients positive for anti-IFI16 IgG, 13 (23%) were positive also for circulating IFI16. In contrast, 57% of the 23 patients positive for IFI16 were also positive for IgG autoantibodies (**Table 2**). Among the 25 PsA patients positive for anti-IFI16 IgA, 5 (20%) were positive for the circulating protein. A significant correlation between anti-IFI16 IgG and IgA antibody titers was observed in PsA (Spearman's r=0.1802, p=0.0235). Moreover, while only 21% of the 57 PsA patients positive for anti-IFI16 IgG were positive also for the IgA subtype, 48% of 25 IgA positive patients displayed also high titers of anti-IFI16 IgG antibodies.

Anti-IFI16 IgG epitope mapping

To determine whether anti-IFI16 IgG antibodies detected in sera were directed against specific IFI16 domains, we cloned the DAPIN (spanning from aa 1 to 88), HINA (from aa 131 to 337) and HINB (from aa 506 to 705) domains of IFI16 in a pET30a expression vector. The purity and specificity of the recombinant domains were then established by blue Comassie and Western blot analyses, and finally recombinant DAPIN (rDAPIN), rHINA and rHINB were used as immobilized surface in ELISA for testing the sera of the 57 patients positive for anti-IFI16 IgG. As shown in **Figure 3**, the serum samples displayed a strong reactivity against the HIN domains. Regardless of their aminoacid homology (44%), HINA was better recognized in comparison with HINB domain by disease and control sera. In contrast, the DAPIN domain, which is the moiety of the protein mainly involved in inflammatory activity, was poorly detected by

autoantibodies, suggesting that anti-IFI16 IgGs are mainly directed against an epitope outside the N-terminus of the protein.

DISCUSSION

A significant role of DNA sensing peptides in the pathogenesis of Pso has been recently suggested, largely based on data about LL37, a fragment of the anti-microbial protein cathelicidin, capable to activate dendritic cells by complexing self-DNA, and T cells by acting as a self-antigen [28, 29]. Similar to other autoimmune diseases characterized by serum reactivity to intracellular antigens we cannot speculate on the pathogenetic mechanisms linking IFI16 autoantibodies to tissue damage. However, we suggest that the DNA sensor IFI16 is involved as a self-antigen in the humoral response and in the inflammatory response possibly *via* the inflammasome in PsA.

In the present study, we observed that IFI16 and anti-IFI16 seem to be associated with higher disease activity in PsA and possibly with response to anti-TNFalpha treatment, thus possibly being candidate biomarkers of this seronegative condition and representing a different clinical phenotype. Furthermore, these markers are detected also in the synovial fluids for the first time and may thus represent a mechanistic player in the PsA pathogenesis. These findings may be promising in filling the diagnostic gap of PsA where no serum biomarker for the diagnosis or management is available [30].

IFI16 is an interferon-induced protein and a DNA sensor able to start an innate immune response against pathogenic microorganism by activating the inflammasome and the production of type I IFNs [31]. It has been proposed that the large amount of self-DNA secondary to keratinocyte proliferation in Pso plaques can activate pDC *via* LL37/TLR9 to produce INF-α and recruit T cells and, at the same time, can activate the inflammasome with IL-1β production [31, 32]. The overexpression of IFI16 in keratinocytes could be explained by an attempt to control excessive cell proliferation, resulting, however, in a chronic activation of the inflammasome. Furthermore,

extracellular IFI16 can spread inflammation since it has been demonstrated that the protein is able to bind the membrane of endothelial cells and to induce the production of pro-inflammatory cytokines in these cells [24, 33]. The hypothesis based on an accelerated cell turnover seems to be supported by the high IFI16 levels observed in patients with Pso and in PsA with skin PsO and IFI16 overexpression in PsA circulating mononuclear cells and synovial tissue [13]. In fact, in PsA sera IFI16 is significantly higher compared to healthy controls, particularly when associated with an active disease represented by elevated CRP and mechanisms may include changes similar to what observed in systemic lupus erythematosus models [34] or NETosis in a self-perpetuating fashion [35].

Type I IFN inducible genes are elevated in the serum of patients with different systemic autoimmune diseases, albeit with inconclusive evidence and frequently conflicting results in different rheumatic conditions [36]. IFI16 is among the five most important IFN inducible genes being overexpressed in connective tissue diseases and RA, raising the coined "IFN-signature" [5]. Different from PsA in which IFI16 significantly correlates with inflammation, this protein is not a biomarker of disease activity in RA, but is associated with pulmonary involvement and with the presence of rheumatoid factor and anti-cyclic citrullinated peptide antibodies. IFI16 in RA synovial fluid is not associated with joint disease activity, disease duration or erosion [22]. In the case of SLE, IFI16 is associated with disease activity, particularly lupus nephritis, and is found overexpressed in skin lesions [18, 37, 38].

The inflammatory context in which an intracellular protein is released is thought to be one of the mechanisms leading to the breakdown of tolerance and the recognition of self-antigens; this hypothesis could explain the antibody response against IFI16

observed in PsA and Pso, particularly with the intriguing observation of a prevalent IgA response compared to IgG response in PsO *versus* PsA. As previously mentioned and different from PsA (with anti-IFI16 IgG titers slightly increased in subjects with elevated CRP levels), anti-IFI16 antibodies are not correlated to RA activity, but rather with serum autoantibody positivity, while no association between anti-IFI16 and disease activity or erosions has been found [22]. In SLE, anti-IFI16 antibodies were first reported to be associated with anti-double stranded DNA antibodies [17, 18], but this was not confirmed by our group [15]. Anti-IFI16 antibodies positive patients with systemic sclerosis were more likely to have limited cutaneous systemic sclerosis, a longer disease duration and more severe vascular and pulmonary manifestations, i.e. digital ischemia and a low carbon monoxide diffusing capacity [18, 20]. An association with disease severity, as for PsA, has been documented in Sjögren's syndrome, with lower tear and saliva production, higher focus score in minor salivary glands biopsies, germinal center-like structures in the labial salivary gland lymphocytic infiltrates, and higher IgG and antinuclear antibodies levels being associated with anti-IFI16 [16, 17]. The epitope mapping also provided somehow surprising evidence compared to other rheumatic conditions. In systemic sclerosis, anti-IFI16 reacted with either N- or C-terminal fragments or both [18], while in 70% of Sjögren's syndrome anti-IFI16 were directed against an epitope outside the N terminus [17] and in SLE antibodies are directed predominantly against N-terminus [17]. We can hypothesize that in SLE anti-IFI16 blocking the N-terminus allows the formation of self-DNA/IFI16 complexes which favor nucleic acid clearance and are protective in terms of SLE nephritis. Our data in PsA are in agreement with those obtained in Sjögren's syndrome, since the

DAPIN domain was poorly detected by anti-IFI16 IgG. Accordingly, this would have left free the moiety of the protein mainly involved in the inflammatory activity.

We are well aware of the limitations of our study, including the limited clinical data available but we report for the first time that IFI16 and anti-IFI16 are detectable in the sera of patients with PsA and seems to correlate with the degree of inflammation and possibly with treatment response. We cannot exclude a role of this protein and the elicited antibody in the pathogenesis of PsA; moreover, IFI16 and anti-IFI16 maybe represent new candidate biomarkers for PsA diagnosis and monitoring when data confirmed in larger and longitudinal studies.

The authors have no competing interests to declare

REFERENCES

1. Sakkas LI, Bogdanos DP. Are psoriasis and psoriatic arthritis the same disease? The IL-23/IL-17 axis data. Autoimmun Rev 2017; **16**:10-5.

2. Ogdie A, Weiss P. The Epidemiology of Psoriatic Arthritis. Rheum Dis Clin North Am 2015; **41**:545-68.

3. Barnas JL, Ritchlin CT. Etiology and Pathogenesis of Psoriatic Arthritis. Rheum Dis Clin North Am 2015; **41**:643-63.

4. Marinoni B, Ceribelli A, Massarotti MS, Selmi C. The Th17 axis in psoriatic disease: pathogenetic and therapeutic implications. Auto Immun Highlights 2014; **5**:9-19.

5. Higgs BW, Liu Z, White B, Zhu W, White WI, Morehouse C, Brohawn P, Kiener PA, Richman L, Fiorentino D, Greenberg SA, Jallal B, Yao Y. Patients with systemic lupus erythematosus, myositis, rheumatoid arthritis and scleroderma share activation of a common type I interferon pathway. Ann Rheum Dis 2011; **70**:2029-36.

6. Boehncke WH. Etiology and Pathogenesis of Psoriasis. Rheum Dis Clin North Am 2015; **41**:665-75.

7. Yao Y, Richman L, Morehouse C, de los Reyes M, Higgs BW, Boutrin A, White B, Coyle A, Krueger J, Kiener PA, Jallal B. Type I interferon: potential therapeutic target for psoriasis? PLoS One 2008; **3**:e2737.

8. Bissonnette R, Papp K, Maari C, Yao Y, Robbie G, White WI, Le C, White B. A randomized, double-blind, placebo-controlled, phase I study of MEDI-545, an anti-interferon-alfa monoclonal antibody, in subjects with chronic psoriasis. J Am Acad Dermatol 2010; **62**:427-36.

9. Harden JL, Johnson-Huang LM, Chamian MF, Lee E, Pearce T, Leonardi CL, Haider A, Lowes MA, Krueger JG. Humanized anti-IFN-gamma (HuZAF) in the treatment of psoriasis. J Allergy Clin Immunol 2015; **135**:553-6.

10. Dell'Oste V, Gatti D, Giorgio AG, Gariglio M, Landolfo S, De Andrea M. The interferon-inducible DNA-sensor protein IFI16: a key player in the antiviral response. New Microbiol 2015; **38**:5-20.

11. Caposio P, Gugliesi F, Zannetti C, Sponza S, Mondini M, Medico E, Hiscott J, Young HA, Gribaudo G, Gariglio M, Landolfo S. A novel role of the interferon-inducible protein IFI16 as inducer of proinflammatory molecules in endothelial cells. J Biol Chem 2007; **282**:33515-29.

12. Tervaniemi MH, Katayama S, Skoog T, Siitonen HA, Vuola J, Nuutila K, Sormunen R, Johnsson A, Linnarsson S, Suomela S, Kankuri E, Kere J, Elomaa O. NOD-like receptor signaling and inflammasome-related pathways are highlighted in psoriatic epidermis. Sci Rep 2016; **6**:22745.

13. Dolcino M, Ottria A, Barbieri A, Patuzzo G, Tinazzi E, Argentino G, Beri R, Lunardi C, Puccetti A. Gene Expression Profiling in Peripheral Blood Cells and Synovial Membranes of Patients with Psoriatic Arthritis. PLoS One 2015; **10**:e0128262.

14. Belasco J, Louie JS, Gulati N, Wei N, Nograles K, Fuentes-Duculan J, Mitsui H, Suarez-Farinas M, Krueger JG. Comparative genomic profiling of synovium versus skin lesions in psoriatic arthritis. Arthritis Rheumatol 2015; **67**:934-44.

15. Caneparo V, Cena T, De Andrea M, Dell'oste V, Stratta P, Quaglia M, Tincani A, Andreoli L, Ceffa S, Taraborelli M, Magnani C, Landolfo S, Gariglio M. Anti-IFI16 antibodies and their relation to disease characteristics in systemic lupus erythematosus. Lupus 2013; **22**:607-13.

16. Alunno A, Caneparo V, Carubbi F, Bistoni O, Caterbi S, Bartoloni E, Giacomelli R, Gariglio M, Landolfo S, Gerli R. Interferon gamma-inducible protein 16 in primary Sjogren's syndrome: a novel player in disease pathogenesis? Arthritis Res Ther 2015; **17**:208.

17. Baer AN, Petri M, Sohn J, Rosen A, Casciola-Rosen L. Association of Antibodies to Interferon-Inducible Protein-16 With Markers of More Severe Disease in Primary Sjogren's Syndrome. Arthritis Care Res (Hoboken) 2016; **68**:254-60.

18. Mondini M, Vidali M, De Andrea M, Azzimonti B, Airo P, D'Ambrosio R, Riboldi P, Meroni PL, Albano E, Shoenfeld Y, Gariglio M, Landolfo S. A novel autoantigen to differentiate limited cutaneous systemic sclerosis from diffuse cutaneous systemic sclerosis: the interferon-inducible gene IFI16. Arthritis Rheum 2006; **54**:3939-44.

19. Costa S, Mondini M, Caneparo V, Afeltra A, Airo P, Bellisai F, Faggioli P, Gerli R, Lotzniker M, Meroni PL, Morozzi G, Radice A, Riccieri V, Scarsi M, Sebastiani GD, Sinico RA, Tincani A, Gariglio M, Landolfo S. Detection of anti-IFI16 antibodies by ELISA: clinical and serological associations in systemic sclerosis. Rheumatology (Oxford) 2011; **50**:674-81.

20. McMahan ZH, Shah AA, Vaidya D, Wigley FM, Rosen A, Casciola-Rosen L. Anti-Interferon-Inducible Protein 16 Antibodies Associate With Digital Gangrene in Patients With Scleroderma. Arthritis Rheumatol 2016; **68**:1262-71.

21. Caneparo V, Pastorelli L, Pisani LF, Bruni B, Prodam F, Boldorini R, Roggenbuck D, Vecchi M, Landolfo S, Gariglio M, De Andrea M. Distinct Anti-IFI16 and Anti-GP2 Antibodies in Inflammatory Bowel Disease and Their Variation with Infliximab Therapy. Inflamm Bowel Dis 2016; **22**:2977-87.

22. Alunno A, Caneparo V, Bistoni O, Caterbi S, Terenzi R, Gariglio M, Bartoloni E, Manzo A, Landolfo S, Gerli R. Circulating Interferon-Inducible Protein IFI16 Correlates With Clinical and Serological Features in Rheumatoid Arthritis. Arthritis Care Res (Hoboken) 2016; **68**:440-5.

23. Taylor W, Gladman D, Helliwell P, Marchesoni A, Mease P, Mielants H, Group CS. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis Rheum 2006; **54**:2665-73.

24. Gugliesi F, Bawadekar M, De Andrea M, Dell'Oste V, Caneparo V, Tincani A, Gariglio M, Landolfo S. Nuclear DNA sensor IFI16 as circulating protein in autoimmune diseases is a signal of damage that impairs endothelial cells through high-affinity membrane binding. PLoS One 2013; **8**:e63045.

25. Ceribelli A, Fredi M, Taraborelli M, Cavazzana I, Franceschini F, Quinzanini M, Tincani A, Ross SJ, Chan JY, Pauley BA, Chan EK, Satoh M. Anti-MJ/NXP-2 autoantibody specificity in a cohort of adult Italian patients with polymyositis/dermatomyositis. Arthritis Res Ther 2012; **14**:R97.

26. Ceribelli A, Isailovic N, De Santis M, Generali E, Fredi M, Cavazzana I, Franceschini F, Cantarini L, Satoh M, Selmi C. Myositis-specific autoantibodies and their association with malignancy in Italian patients with polymyositis and dermatomyositis. Clin Rheumatol 2017; **36**:469-75.

27. Yamasaki Y, Narain S, Yoshida H, Hernandez L, Barker T, Hahn PC, Sobel ES, Segal MS, Richards HB, Chan EK, Reeves WH, Satoh M. Autoantibodies to RNA helicase A: a new serologic marker of early lupus. Arthritis Rheum 2007; **56**:596-604.

28. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, Cao W, Wang YH, Su B, Nestle FO, Zal T, Mellman I, Schroder JM, Liu YJ, Gilliet M.

Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature 2007; **449**:564-9.

29. Lande R, Botti E, Jandus C, Dojcinovic D, Fanelli G, Conrad C, Chamilos G, Feldmeyer L, Marinari B, Chon S, Vence L, Riccieri V, Guillaume P, Navarini AA, Romero P, Costanzo A, Piccolella E, Gilliet M, Frasca L. The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis. Nat Commun 2014; **5**:5621.

30. McArdle A, Pennington S, FitzGerald O. Clinical Features of Psoriatic Arthritis: a Comprehensive Review of Unmet Clinical Needs. Clin Rev Allergy Immunol 2017.

31. Zhao H, Gonzalezgugel E, Cheng L, Richbourgh B, Nie L, Liu C. The roles of interferon-inducible p200 family members IFI16 and p204 in innate immune responses, cell differentiation and proliferation. Genes Dis 2015; **2**:46-56.

32. Chiliveru S, Rahbek SH, Jensen SK, Jorgensen SE, Nissen SK, Christiansen SH, Mogensen TH, Jakobsen MR, Iversen L, Johansen C, Paludan SR. Inflammatory cytokines break down intrinsic immunological tolerance of human primary keratinocytes to cytosolic DNA. J Immunol 2014; **192**:2395-404.

33. Bawadekar M, De Andrea M, Lo Cigno I, Baldanzi G, Caneparo V, Graziani A, Landolfo S, Gariglio M. The Extracellular IFI16 Protein Propagates Inflammation in Endothelial Cells Via p38 MAPK and NF-kappaB p65 Activation. J Interferon Cytokine Res 2015; **35**:441-53.

34. Shin JI, Lee KH, Joo YH, Lee JM, Jeon J, Jung HJ, Shin M, Cho S, Kim TH, Park S, Jeon BY, Jeong H, Lee K, Kang K, Oh M, Lee H, Lee S, Kwon Y, Oh GH, Kronbichler A. Inflammasomes and autoimmune and rheumatic diseases: A comprehensive review. J Autoimmun 2019:102299.

35. Lee KH, Kronbichler A, Park DD, Park Y, Moon H, Kim H, Choi JH, Choi Y, Shim S, Lyu IS, Yun BH, Han Y, Lee D, Lee SY, Yoo BH, Lee KH, Kim TL, Kim H, Shim JS, Nam W, So H, Choi S, Lee S, Shin JI. Neutrophil extracellular traps (NETs) in autoimmune diseases: A comprehensive review. Autoimmun Rev 2017; **16**:1160-73.

36. Ronnblom L. The importance of the type I interferon system in autoimmunity. Clin Exp Rheumatol 2016; **34**:21-4.

37. Costa S, Borgogna C, Mondini M, De Andrea M, Meroni PL, Berti E, Gariglio M, Landolfo S. Redistribution of the nuclear protein IFI16 into the cytoplasm of ultraviolet B-exposed keratinocytes as a mechanism of autoantigen processing. Br J Dermatol 2011; **164**:282-90.

38. Cao T, Shao S, Li B, Jin L, Lei J, Qiao H, Wang G. Up-regulation of Interferon-inducible protein 16 contributes to psoriasis by modulating chemokine production in keratinocytes. Sci Rep 2016; **6**:25381.

FIGURE LEGENDS.

Figure 1. Serum IFI16 and anti-IFI16 IgG and IgA levels in patients with PsA, Psoriasis and HCs. (A) IFI16 protein levels in patients' and controls' sera were determined using an in-house capture ELISA. Each dot represents the concentration of IFI16 protein (expressed in ng/ml on a linear scale) in each individual subject. The horizontal bar represents the median values. Values over the dotted line indicate the percentage of subjects with IFI16 protein levels above the cut-off value (27 ng/ml). **(B)** Serum IgG and **(C)** IgA specific for IFI16 were quantified by ELISA in HC and patients suffering from psoriatic arthritis and psoriasis. Each dot represents the autoantibody level for each subject sample expressed in arbitrary units on a linear scale. The horizontal bars in each group represent the median values. Values over the dotted line indicate the percentage of subjects with antibody titers above the cutoff value (113 U/mL for anti-IFI16 IgG and 9.6 U/mL for anti-IFI16 IgA, calculated as the 95th percentile of the control population). Statistical significance: ***$p < 0.0001$ (Mann–Whitney tests).

Figure 2. Analysis of protein component of autoantigen IFI16 (8% SDS-PAGE) by radio-immunoprecipitation (IP) (A) and IP-Western Blotting (WB) (B), and indirect immunofluorescence (C). The IP pattern of PsA sera recognizing a 90 kDa protein (black arrows, 2A). IP-WB confirming the identity of the IP bands corresponding to IFI16 (2B). The expected ANA pattern for a representative sample positive for ani-IFI16 IgG (2C).

Figure 3. Epitope mapping for anti-IFI16 IgG. To determine the target of the anti-IFI16 IgG antibodies found in the sera of PsA patients, the DAPIN (spanning from aa 1 to 88), HINA (from aa 131 to 337) and HINB (from aa 506 to 705) domains of IFI16 were purified as recombinant peptides and 2ug/mL each used to perform ELISA. Each dot represents the value of the absorbance at 450nm for the 57 sera tested against the three domains. The horizontal bars in each group represent the median values. Statistical significance: ***$p < 0.0001$ (Mann–Whitney tests). Panel A shows PsA patients and panel B healthy controls.

TABLES.

Table 1. Demographic, clinical and serological characteristics of PsA patients.

		IFI16		Anti-IFI16 IgG		Anti-IFI16 IgA	
	All PsA	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Patients (%)	158 (100)	23 (15)	135 (85)	57 (36)	101 (64)	26 (17)	132 (83)
Women (%)	93 (50.5)	12 (52)	67 (50)	34 (60)	45 (45)	11 (42)	68 (52)
Age, yy (IQR)	50 (43-61)	51 (42-69)	50 (43-60)	49 (43-61)	51 (43-60.5)	**63.5 (49-69)**	**48 (42-58)****
Disease duration, mm (IQR)	60 (16-119)	82 (24-118)	49 (15-120)	72 (18-119.5)	48 (15-118)	60 (18-96)	56 (16-132)
Disease duration <12 mm (%)	33 (29)	**1 (4.3)**	**32 (24)***	8 (14)	10 (10)	2 (8)	16 (12)
Disease duration >60 mm (%)	76 (49)	14 (61)	62 (47)	33 (60)	43 (43)	14 (54)	62 (47)
Psoriasis (%)	127 (80)	17 (74)	109 (81)	45 (79)	82 (81)	24 (92)	102 (77)
Axial involvement, (%)	45 (29)	7 (30)	38 (29)	16 (28)	29 (29)	4 (15)	41 (31)
High CRP (%)	63 (40)	**18 (78)**	**45 (33)***	23 (40)	40 (40)	12 (46)	51 (39)
Ongoing treatments:							
no treatment	64 (40.5)	17 (74)	77 (57)	21 (39)	43 (43)	9 (35)	55 (42)
methotrexate	64 (40.5)	15 (71)	49 (46)	21 (37)	43 (43)	14 (54)	50 (38)
median MTX dose, weekly	15,2 (range 12,5-17,5)	12,7 (range 12,5-15)	15,2 (range 12,5-17,5)	15,2 (range 12,5-17,5)	15,2 (range 12,5-17,5)	12,7 (range 12,5-15)	15,7 (range 15-17,5)
anti-TNFα	30 (19)	2 (9)	28 (21)	15 (26)	15 (15)	3 (12)	27 (20)
etanercept	8	1	7	4	4	1	7
adalimumab	2	0	2	1	1	0	2
infliximab	20	1	19	9	11	2	18

Continuous variables are expressed as median and IQR, binary as numbers and percentages.

PsA: psoriatic arthritis; IFI16: interferon-inducible protein 16; Ig: immunoglobulin; yy: years; mm: months; Ig: immunoglobulin; CRP: C reactive protein; TNF: tumor necrosis factor.

*p<0.01 positive *vs.* negative patients, ** p<0.001 positive *vs.* negative patients

Table 2. Association of IFI16-based biomarkers in the PsA cohort.

PsA n=158	**IFI16 positive n=23 (15%)**	**Anti-IFI16 IgG positive n=57 (36%)**	**Anti-IFI16 IgA positive n=25 (16%)**
IFI16 positive n=23 (15%)	-	13/57 (23%)	5/25 (20%)
Anti-IFI16 IgG positive n=57 (36%)	13/23 (57%)	-	12/25 (48%)
Anti-IFI16 IgA positive n=25 (16%)	5/23 (22%)	12/57 (21%)	-

Variables are expressed as numbers (n) and percentages.

PsA: psoriatic arthritis; IFI16: interferon-inducible protein 16; Ig: immunoglobulin.

*p<0.01, ** p<0.001

Figure 1.

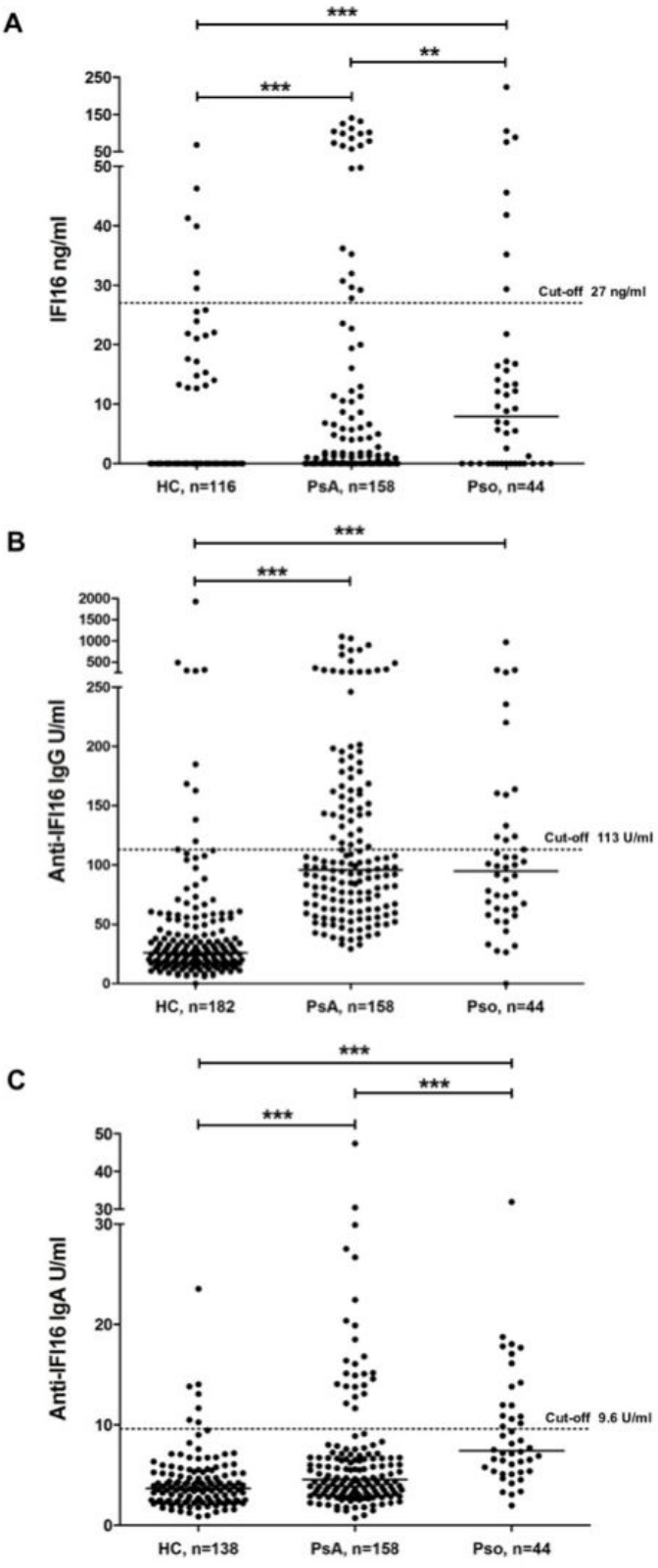
A
***
**
***
IFI16 ng/ml
250
150
50
50
40
30
20
10
0
Cut-off 27 ng/ml
HC, n=116
PsA, n=158
Pso, n=44
B
***
***
Anti-IFI16 IgG U/ml
2000
1500
1000
500
250
200
150
100
50
0
Cut-off 113 U/ml
HC, n=182
PsA, n=158
Pso, n=44
C
***
***
***
Anti-IFI16 IgA U/ml
50
40
30
30
20
10
0
Cut-off 9.6 U/ml
HC, n=138
PsA, n=158
Pso, n=44

Figure 2.

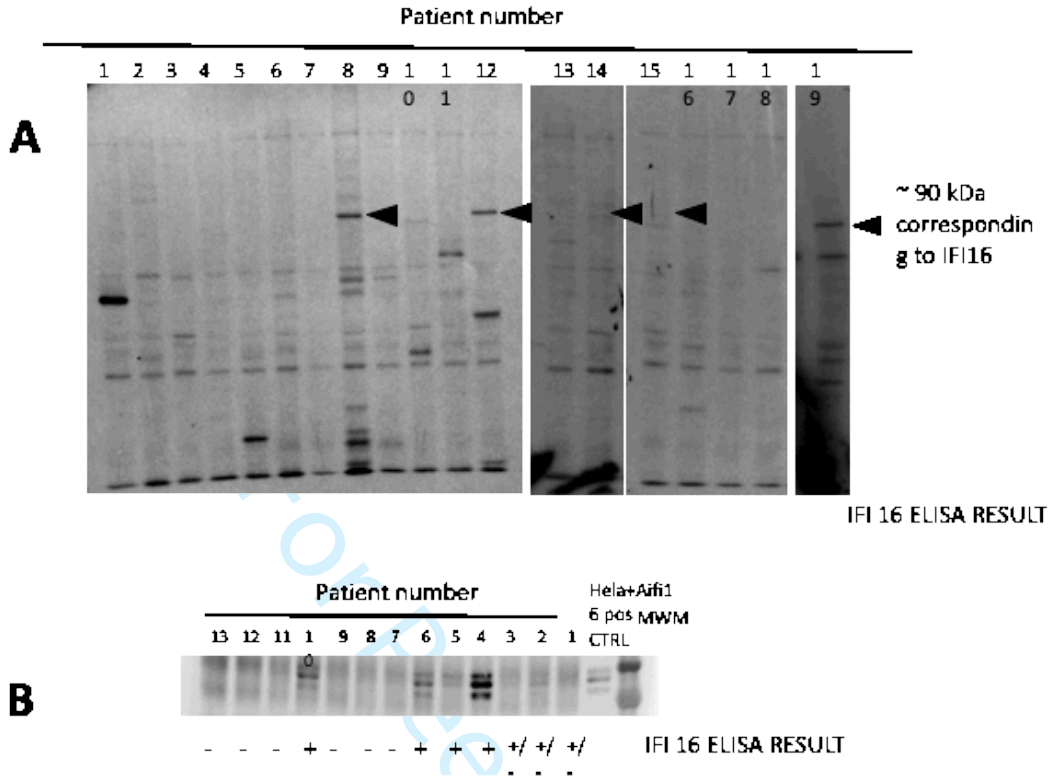
Patient number
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
A
~ 90 kDa correspondin g to IFI16
IFI 16 ELISA RESULT
Patient number
13 12 11 10 9 8 7 6 5 4 3 2 1
Hela+Aifi1 6 pos MWM CTRL
B
- - - + - - - + + + +/- +/- +/-
IFI 16 ELISA RESULT
C

Figure 3.