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Role of Protein Phosphatase Magnesium-Dependent 1A and Anti–Protein Phosphatase Magnesium-Dependent 1A Autoantibodies in Ankylosing Spondylitis

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Objective. **Although ankylosing spondylitis (AS) is driven by immune-mediated processes, little is known about the presence and role of autoantibodies in this disease. This study was undertaken to investigate whether autoantibodies occur in and are involved in AS.**

Methods. **We performed human protein microarray analysis of sera derived from patients with AS or other autoimmune disorders to identify autoantibodies associated specifically with AS, and identified autoantibody targeting of protein phosphatase magnesium-dependent 1A (PPM1A) in AS. We performed enzyme-linked immunosorbent assay (ELISA) analysis of sera from 2 independent AS cohorts to confirm autoantibody targeting of PPM1A, and to assess associations between levels of anti-PPM1A antibodies and AS disease severity or response to anti–tumor necrosis factor (anti-TNF) therapy (as measured by Bath AS Disease Activity Index [BASDAI] score). Levels of anti-PPM1A antibodies were also evaluated in sera from rats transgenic for HLA–B27 and human β_2-microglobulin. The expression of PPM1A was assessed by immunohistochemistry in synovial tissue samples from patients with AS, rheumatoid arthritis, or osteoarthritis. The role of PPM1A in osteoblast differentiation was investigated by gene knockdown and overexpression.**

Results. **AS was associated with autoantibody targeting of PPM1A, and levels of anti-PPM1A autoantibodies were significantly higher in patients with more advanced sacroiliitis and correlated positively with BASDAI score after treatment with anti-TNF agents. The levels of anti-PPM1A autoantibodies were also higher in the sera of transgenic rats that are prone to develop spondyloarthritis than in those that are not. PPM1A was expressed in AS synovial tissue, and PPM1A overexpression promoted osteoblast differentiation, whereas PPM1A knockdown suppressed it.**

Conclusion. **Anti-PPM1A autoantibodies are present in AS, and our findings suggest that PPM1A may contribute to the pathogenic bone ankylosis characteristic of AS.**

Ankylosing spondylitis (AS), the prototype of a group of interrelated diseases known collectively as spondyloarthritis (SpA), is a chronic inflammatory arthritis that affects the spine, sacroiliac joints, and peripheral joints. It has a prevalence of 0.2–0.5% in the US and frequently results in functional disability (1,2). The diagnosis of AS is typically delayed, being made on the basis of radiographic features, such as joint erosion and

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subchondral bone erosion, that are observed at late stages of the disease (3). Although the more recent use of magnetic resonance imaging (MRI) enables detection of inflammatory lesions that develop at early stages of the disease, the usefulness of such MRI assessment in predicting subsequent structural damage remains to be established (4). In addition, disease activity and treatment response in AS are assessed using the Bath AS Disease Activity Index (BASDAI) (5) or Assessment of SpondyloArthritis international Society improvement criteria (6), both of which are complex and comprise several subjective parameters. Thus, there is great need for biomarkers that can aid in early diagnosis or in assessment of disease activity in AS.

Although its pathogenesis is incompletely understood, AS is considered an immune-mediated disease: 80–90% of individuals with AS carry the HLA–B27 allele, suggesting involvement of HLA–B27 in the pathogenesis of the disease. Compared to other rheumatic autoimmune diseases, little is known about a possible role of autoantibodies in AS. A recent screen, however, identified the presence of several autoantibodies targeting connective, skeletal, and muscular tissue autoantigens in the blood of individuals with AS (7).

Besides aberrant activation of the immune system (8), ankylosis is a hallmark of AS. Ankylosis is the result of bony apposition occurring along periosteal sites and leading to new bone formation, a process that requires differentiation of osteoblasts. Differentiation of osteoblasts from mesenchymal cells in turn requires a series of signals, including prostaglandin E_2, parathyroid hormone, bone morphogenetic proteins (BMPs), and Wingless proteins (Wnt) (9). The process is regulated by activation of genes such as runt-related transcription factor 2 (RUNX-2), osterix, osteocalcin, and bone sialoprotein (BSP), depending on the stage of differentiation (9). Repair mechanisms activated in response to local joint destruction have been proposed to trigger the activation of osteoblasts in AS, resulting in syndesmophyte formation and in ankylosis of the affected joint (10). Limiting abnormal osteoblast activation might slow radiographic progression in AS. For example, levels of BMPs are increased in AS serum (11,12), and systemic transfer of the BMP antagonist Noggin prevented radiographic progression in a mouse model of AS (13). Levels of sclerostin, a natural inhibitor of Wnt, are lower in the skeleton of individuals with AS than in that of individuals with rheumatoid arthritis (RA) (14), and dickkopf-1, another inhibitor of Wnt, was proposed as a predictor of radiographic progression in AS (15).

In a screen to identify autoantibodies associated with AS, we found that serum levels of autoantibodies against protein phosphatase magnesium-dependent 1A (PPM1A)—a Ser/Thr protein phosphatase that regulates BMP and Wnt signaling (16)—are higher in AS than in other autoimmune diseases. Whether PPM1A activation positively or negatively affects osteoblast differentiation is controversial. Overexpression of PPM1A dephosphorylates and thereby blocks the nuclear translocation of BMP-2–induced Smad1, a transcription factor that promotes skeletal and osteogenic development (17). However, another report demonstrated that PPM1A is a positive regulator of Wnt signaling, which induces osteoblastogenesis (18).

In this study, using serum samples from 2 independent cohorts of AS patients and from HLA–B27–transgenic rats, we show that AS is associated with the presence of anti-PPM1A autoantibodies. Moreover, we show that serum levels of anti-PPM1A autoantibodies correlate positively with the degree of sacroiliitis in AS and with the change in disease activity in response to anti–tumor necrosis factor (anti-TNF) therapy. In addition, we found that PPM1A protein is highly expressed in synovial tissue from AS patients and drives osteoblast differentiation, suggesting that PPM1A itself may contribute to the pathogenesis of AS.

PATIENTS AND METHODS

Human samples. All biologic samples from patients with AS or other rheumatic diseases, and from healthy individuals, were studied according to Stanford University Institutional Review Board (IRB)–approved protocols. Informed consent was obtained from all subjects. Plasma and sera derived from AS patients and control patients came from the Multiple Autoimmune Disease Genetics Consortium (MADGC) (19), the Stanford Arthritis Center, the University of Texas Health Sciences Center, and the rheumatology clinic of Asan Medical Center. Synovial tissue from patients with AS, RA, or osteoarthritis (OA) were collected in accordance with human subject protocols approved by the Stanford University IRB and with the patients' informed consent, as previously described (20). Joint fluids from patients with AS, RA, or OA were collected at Asan Medical Center and at Stanford University Medical Center. The diagnosis was limited to cases that met the modified New York criteria for AS (21), American College of Rheumatology 1987 revised criteria for RA (22), or criteria for OA (23).

For serum samples collected from AS patients at Asan Medical Center, blood was drawn at the time of diagnosis, and clinical information (age, sex, BASDAI score, radiographic findings, and laboratory indices, including erythrocyte sedimentation rate [ESR] and C-reactive protein [CRP], etc.) was extracted from an electronic clinical database. The severity of sacroiliitis was graded according to the modified New York

criteria (21) by a musculoskeletal radiologist. AS disease activity was determined according to the BASDAI index (5).

Rat sera. The HLA–B27/human β_2-microglobulin (hβ_2m)–transgenic rat lines (inbred Lewis background) used in this study have been described previously (24,25), and non-transgenic rats or *Dazl*-deficient B27/hβ_2m–transgenic rats were used as negative controls as previously described (26). All animal studies were performed under Institutional Animal Care and Use Committee–approved protocols at the University of Texas Southwestern. Rats were examined for signs of arthritis at the time of blood sampling as previously described (25,26), and serum samples from these rats and from nontransgenic Lewis rats were analyzed.

Protein microarray. ProtoArrays V4 (Invitrogen) were used to profile the autoantibodies present in AS and control sera, and were run using the manufacturer's protocols. Arrays were scanned using a GenePix4000B Scanner (Molecular Devices). Median pixel intensities of features and background were determined using GenePix Pro software version 5.0 (Axon Instruments).

Enzyme-linked immunosorbent assay (ELISA). Levels of anti-PPM1A, anti-PTPN6, and anti-influenza antibodies were measured by ELISA. ELISA plates were coated with recombinant human PPM1A (rhPPM1A; Creative Biomart), rhPTPN6 (Creative Biomart), or influenza virus vaccine (Fluzone; Sanofi Pasteur). Human PPM1A protein is 99% identical to rat PPM1A and 98.2% identical to mouse PPM1A. Secondary antibodies were horseradish peroxidase (HRP)–conjugated goat anti-human IgG (Jackson ImmunoResearch) or HRP-conjugated goat anti-rat IgG (Millipore). Optical density was measured at 450-nm absorbance. Serum concentrations of PPM1A were measured with commercially available ELISA kits (USCN Life Science).

Cells, antibodies, and plasmids. Mouse preosteoblastic MC3T3-E1 cells (subclone 4 and 14) were purchased from ATCC and maintained in α-minimum essential medium supplemented with 10% fetal bovine serum, 100 units/ml of penicillin, and 100 μg/ml of streptomycin (Gibco) in 5% CO_2 at 37°C. Antibodies used were against PPM1A (p6c7 for immunoblotting and EP1684Y for immunohistochemistry [both from Abcam]), p-Smad1/5/8 (Cell Signaling Technology), active β-catenin (Millipore), and β-actin (Sigma-Aldrich). Human complementary DNA (cDNA) for PPM1A was purchased from Open Biosystems and subcloned into p3XFLAG-CMV-10 vector (Sigma-Aldrich) for expression in mammalian cells.

RNA interference and plasmid transfection. On-Target Plus SMARTpool small interfering RNA (siRNA) targeting mouse PPM1A (Dharmacon L-040052-00-0005) and non-targeting control siRNA (Dharmacon: D-001810-10-05) were used for RNA interference. The siRNA sequences for mouse PPM1A are as follows: 5′-GCAAGCGGAAUGUAAUUGA-3′, 5′-ACACGGCUGUGAUCGGUUU-3′, 5′-UCACCAAUAACCAGGAUUU-3′, and 5′-ACAAUAGACUGAACCCUUA-3′. MC3T3-E1 cells (1×10^6) were plated into 100-mm culture dishes and 24 hours later transfected with a 60 n*M* solution containing siRNAs by using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. For plasmid transfection, cells were transfected with 12 μg of FLAG-PPM1A expression plasmid or p3XFLAG-CMV-10 empty vector by using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. After 24 hours of incubation, cells were trypsinized, plated on 24-well plates, and allowed to differentiate. To determine alkaline phosphatase (AP) activity, we used 1×10^5 cells/well (siRNA-transfected cells) or 2×10^5 cells/well (plasmid-transfected cells) in 1 ml of medium. For alizarin red S staining, we used 2×10^5 cells/well (siRNA-transfected cells) or 3×10^5 cells/well (plasmid-transfected cells).

Differentiation of osteoblasts from MC3T3-E1 cells. Cells were incubated with osteogenic differentiation media containing 50 μg/ml of ascorbic acid (Sigma-Aldrich), 10 m*M* of β-glycerophosphate (Sigma-Aldrich), and 50 ng/ml of BMP-2 (Sigma-Aldrich) for 7 or 14 days, with differentiation media refreshed every 3 days. On day 7, AP activity was determined. Cells were washed with phosphate buffered saline (PBS), fixed by air drying for 30 minutes, and stained with nitroblue tetrazolium/BCIP (Thermo Scientific) for 5–10 minutes in the dark at room temperature. Cells were washed with PBS and air dried for 30 minutes, and images were taken with an Olympus BX51 microscope outfitted with an Olympus DP72 digital camera. To quantify the AP enzyme activity, we used an Alkaline Phosphatase Assay Kit (Colorimetric; Abcam). On day 14, we performed alizarin red S (Sigma-Aldrich) staining to confirm calcium deposition on the cultured cells. Cells were washed with PBS and fixed with 70% ethanol for 1 hour. The fixed cells were rinsed with distilled water and stained with 40 m*M* of alizarin red S solution (pH 4.2) for 10 minutes. The stained cells were briefly washed with distilled water 4 times, washed with PBS for 15 minutes, and images were obtained. To quantify the degree of mineralization, we incubated cells with 400 μl of 10% acetic acid for 30 minutes at room temperature with rocking, detached the cells from the culture plates by scraping, and then transferred the cells and acetic acid to a 1.5-ml microcentrifuge tube. The samples were vortexed vigorously for 30 seconds, heated to 85°C for 10 minutes, and centrifuged at 20,000*g* for 15 minutes. The supernatants were neutralized with 25 μl of 10% ammonium hydroxide, and the concentration of alizarin red S was quantified by measuring the absorbance at 405 nm on a microplate reader with an alizarin red S standard curve in the same solution.

Quantitative polymerase chain reaction (PCR). Total RNA was isolated from cells by using an RNeasy kit (Qiagen) and reverse transcribed into cDNA by using a qScript cDNA synthesis kit (Quanta Bioscience). Real-time quantitative PCR was performed using PerfeCTa SYBR Green SuperMix ROX (Quanta Biosciences) with an Applied Biosystems 7900HT Fast Real-Time PCR system, and messenger RNA levels were normalized according to levels of the housekeeping gene hypoxanthine guanine phosphoribosyltransferase 1 and β-actin. Primer sequences (Integrated DNA Technologies) were as follows: for hypoxanthine guanine phosphoribosyltransferase 1, forward 5′-TGTTGTTGGATATGCCCTTG-3′ and reverse 5′-TGGCAACATCAACAGGACTC-3′; for β-actin, forward 5′-TTCTTTGCAGCTCCTTCGTT-3′ and reverse 5′-ATGGAGGGGAATACAGCCC-3′; for Col1a1, forward 5′-ACATGTTCAGCTTTGTGGACC-3′ and reverse 5′-TAGGCCATTGTGTATGCAGC-3′; for BSP-2, forward 5′-GTCTTTAAGTACCGGCCACG-3′ and reverse 5′-TGAAGAGTCACTGCCTCCCT-3′; for osteocalcin, forward 5′-GCGCTCTGTCTCTCTGACCT-3′ and reverse 5′-GCCGGA-

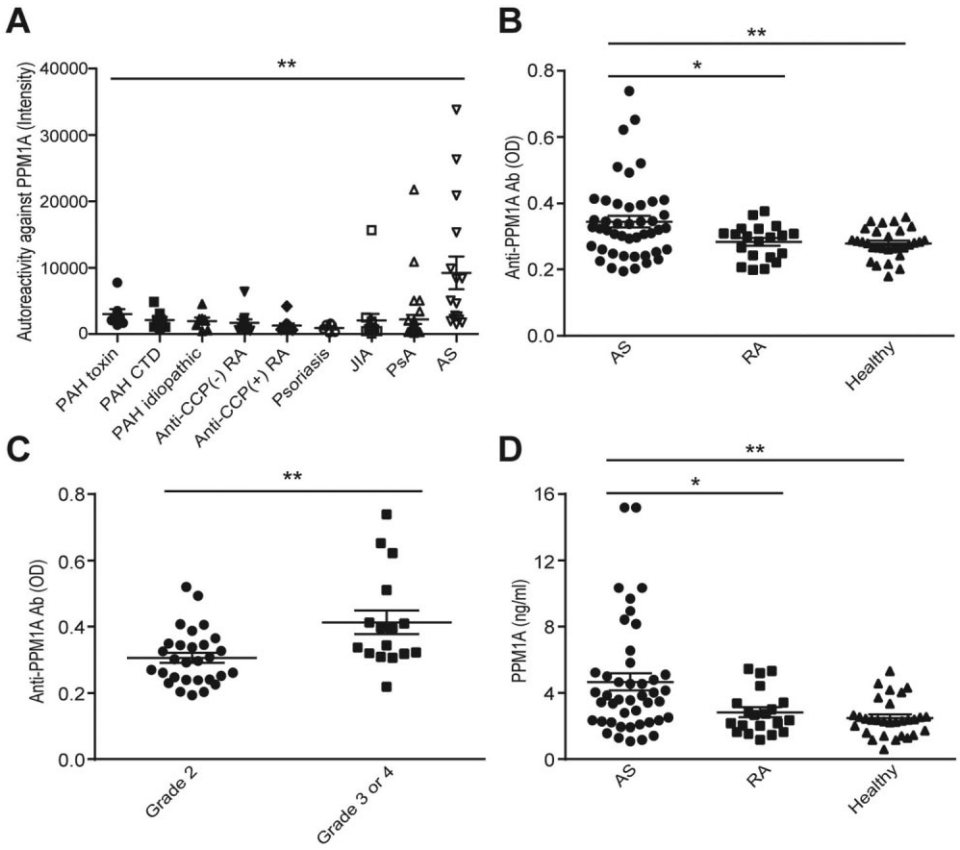

Figure 1. Association of ankylosing spondylitis (AS) with an increase in serum levels of anti–protein phosphatase magnesium-dependent 1A (anti-PPM1A) autoantibodies. **A,** Protein microarray analysis of autoreactivity to PPM1A in sera from patients with toxin-induced pulmonary artery hypertension (PAH toxin; n = 8), PAH associated with connective tissue disease (PAH CTD; n = 8), idiopathic PAH (n = 7), anti–cyclic citrullinated peptide (anti-CCP)–negative rheumatoid arthritis (RA) (n = 11), anti-CCP–positive RA (n = 10), psoriasis (n = 6), juvenile idiopathic arthritis (JIA; n = 15), psoriatic arthritis (PsA; n = 34), or AS (n = 16) in the Multiple Autoimmune Disease Genetics Consortium cohort. **B,** Enzyme-linked immunosorbent assay (ELISA) analysis of anti-PPM1A autoantibody (Ab) levels in sera from patients with AS, patients with RA, and healthy individuals in a Korean cohort. **C,** Levels of anti-PPM1A autoantibodies in sera from Korean AS patients with high-grade (grade 3 or 4) or low-grade (grade 2) radiographic sacroiliitis. **D,** ELISA analysis of PPM1A protein levels in sera from patients with AS, patients with RA, and healthy individuals in a Korean cohort. Each symbol represents an individual patient; horizontal lines and error bars show the mean ± SEM. $* = P < 0.05$; $** = P < 0.01$, by one-way analysis of variance or Student's *t*-test.

GTCTGTTCACTACC-3′; for osterix, forward 5′-CAACCTG-CTAGAGATCTGAG-3′ and reverse 5′-TGCAATAGGAGA-GAGCGA-3′; for RUNX-2, forward 5′-ACACCGTGT-CAGCAAAGC-3′ and reverse 5′-GCTCACGTCGCTCAT-CTTG-3′.

Immunohistochemistry. Synovial tissue specimens embedded in OCT compound were cut on a cryostat (Thermo Scientific) and fixed in 4% paraformaldehyde for 10 minutes at 4°C. The tissue sections were then washed twice (for 5 minutes each) with PBS, permeabilized with 0.1% Triton X-100 for 3 minutes at room temperature, and washed again with PBS. Endogenous peroxidase activity was quenched with 0.3% H_2O_2 solution. The tissue sections were blocked with 5% normal goat serum for 20 minutes and then incubated with 3 μg/ml of PPM1A antibodies (Abcam) or control rabbit IgG (Cell Signaling Technology) for 1 hour at room temperature. They were washed twice with PBS and incubated with biotinylated secondary antibody (Vectastain Elite ABC kit; Vector) at a 1:250 dilution for 30 minutes. The tissue sections were washed with PBS, incubated with complexed avidin–biotin–HRP reagent (Vector) for 30 minutes, and exposed to 3,3′-diaminobenzidine substrate according to the manufacturer's instructions (Vector). The nuclei were subsequently counterstained with hematoxylin (Vector). Tissue samples were examined and photographed with an Olympus BX51 microscope outfitted with an Olympus DP72 digital camera.

Immunoblotting. Cells were lysed with cell lysis buffer (M-PER; Thermo Scientific) containing a Halt protease and phosphatase inhibitor cocktail (Thermo Scientific). Lysates were separated on 4–12% Bis-Tris Gels (Bio-Rad), transferred to PVDF membranes (Millipore), blocked with 5% (weight/volume) milk, and probed with primary and secondary anti-

bodies in milk. Signal was detected with SuperSignal West Femto chemiluminescent substrate (Thermo Scientific).

Statistical analysis. Differences between 2 groups were calculated using the Mann-Whitney U test or Student's unpaired *t*-test, and differences between 3 or more groups were analyzed by one-way analysis of variance and Bartlett's test for equal variances. Relationships between parameters were tested using Spearman's rank correlation coefficient. *P* values less than 0.05 were considered significant.

RESULTS

Detection of anti-PPM1A antibodies in AS sera from a US-based cohort. We used high-density protein microarrays, containing 8,087 human proteins, to test antibody reactivity in sera derived from individuals with pulmonary artery hypertension (n = 23), RA (n = 21), juvenile idiopathic arthritis (n = 15), psoriatic arthritis (PsA; n = 34), psoriasis (n = 6), or AS (n = 16). We found that reactivity to PPM1A was higher in sera from patients with AS than in that from patients with other autoimmune diseases in the US-based MADGC cohort (Figure 1A) (additional results are available online at http://aml.amc.seoul.kr/amc_proof/ygkim_suppl.pdf) as well as in a second US-based cohort at the University of Texas Health Science Center at Houston (data not shown). Because transforming growth factor β (TGFβ) causes sacroiliitis at the time of bone proliferation in advanced AS (27), we focused our investigation on antibodies against PPM1A, known as an inhibitor of TGFβ signaling (28). ELISA analysis confirmed that serum levels of anti-PPM1A autoantibodies are significantly higher in patients with AS than in healthy individuals or individuals with anti–cyclic citrullinated peptide–negative RA or PsA in the Stanford Arthritis Center (results are available online at http://aml.amc.seoul.kr/amc_proof/ygkim_suppl.pdf).

Levels of anti-PPM1A autoantibodies and PPM1A in AS sera from a Korean cohort. To confirm our findings in an independent cohort of treatment-naive AS patients (n = 45), RA patients (n = 20), and healthy individuals (n = 30), we evaluated levels of anti-PPM1A autoantibodies in a cohort of Korean patients. Levels of anti-PPM1A autoantibodies were significantly higher in the AS patients than in the RA patients or the healthy controls (Figure 1B), whereas levels of anti-PTPN6 antibodies (anti–tyrosine phosphatase antibodies), used as a negative control, were not significantly different between groups (data are available online at http://aml.amc.seoul.kr/amc_proof/ygkim_suppl.pdf).

When levels of anti-PPM1A antibodies >2 SD above control were considered positive, the sensitivity and specificity were 66.67% and 73.33%, respectively, for AS. Interestingly, levels of anti-PPM1A autoantibodies were higher in AS patients with high-grade radiographic sacroiliitis (grade 3 or 4) than in those with low-grade radiographic sacroiliitis (grade 2) (Figure 1C). Clinical parameters, including age, sex, disease duration, HLA–B27 allele, BASDAI score, and levels of inflammatory markers (ESR and CRP), were not found to be associated with the level of anti-PPM1A antibodies (data are available online at http://aml.amc.seoul.kr/amc_proof/ygkim_suppl.pdf). Given that PPM1A is an intracellular protein, we performed ELISAs on sera to determine whether it might be released into extracellular compartments or into the blood. Indeed, we found that serum concentrations of PPM1A protein were

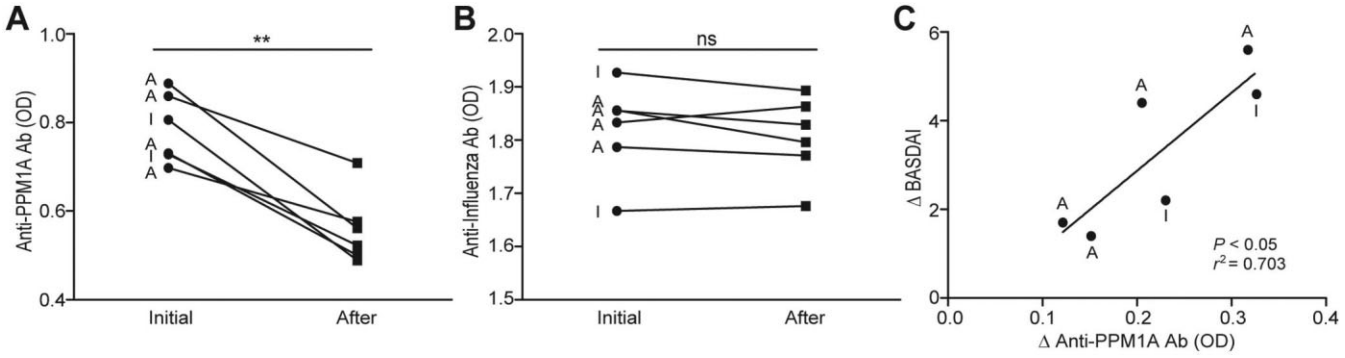

Figure 2. Serum levels of anti-PPM1A autoantibodies decrease after anti–tumor necrosis factor (anti-TNF) therapy and correlate with change in disease activity in AS. **A** and **B,** Serum levels of anti-PPM1A autoantibodies (**A**) and anti-influenza antibodies (**B**) in AS patients at baseline and after 3 months' treatment with anti-TNF agents. **C,** Correlation between the change in serum levels of anti-PPM1A autoantibodies and the change in Bath AS Disease Activity Index (BASDAI) score after 3 months' treatment with anti-TNF agents. Symbols represent individual patients. ** = $P < 0.01$ by Mann-Whitney U test. NS = not significant; A = adalimumab; I = infliximab (see Figure 1 for other definitions).

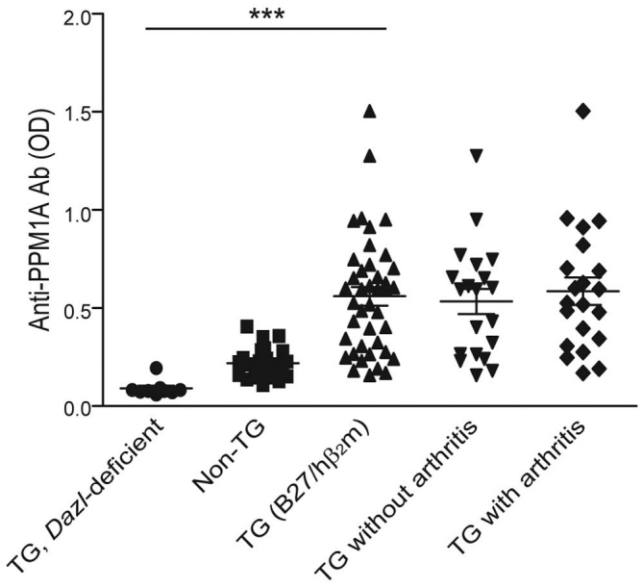

Figure 3. Elevated serum levels of anti-PPM1A autoantibodies in disease-prone rats irrespective of evident arthritis. Levels of anti-PPM1A autoantibodies in sera from *Dazl*-deficient HLA–B27/human β_2-microglobulin (hβ_2m)–transgenic (TG) rats (n = 9), nontransgenic rats (n = 26), and B27/hβ_2m–transgenic rats (n = 41) were determined by ELISA. *Dazl*-deficient B27/hβ_2m transgenic rats were used as negative controls because deficiency of the *Dazl* gene in these rats prevents both epididymoorchitis and arthritis (26). Each symbol represents a single rat; horizontal lines and error bars show the mean ± SEM. *** = $P < 0.001$ by one-way analysis of variance. See Figure 1 for other definitions.

significantly higher in sera derived from AS patients than in sera from RA patients or healthy controls (Figure 1D).

Decreased serum levels of anti-PPM1A autoantibodies after anti-TNF therapy. To determine whether levels of anti-PPM1A autoantibodies change in response to anti-TNF treatment of AS, we measured anti-PPM1A autoantibodies in 6 patients with AS (4 treated with adalimumab and 2 with infliximab) before and 3 months after initiation of treatment with a TNF inhibitor. Levels of anti-PPM1A autoantibodies decreased significantly after treatment (Figure 2A), whereas levels of anti-influenza antibodies did not (Figure 2B). The BASDAI score, an indicator of disease activity and clinical response (5), also decreased significantly after treatment (data are available online at http://aml.amc.seoul.kr/amc_proof/ygkim_suppl.pdf). Notably, a drop in BASDAI score was positively correlated with a decrease in the levels of anti-PPM1A autoantibodies ($r^2 = 0.703$, $P < 0.05$), suggesting that serum levels of anti-PPM1A autoantibodies might be able to serve as a pharmacodynamic biomarker of response to anti-TNF therapy in AS (Figure 2C).

Levels of anti-PPM1A autoantibodies in disease-prone transgenic rats compared to healthy controls. Rats transgenic for B27/hβ_2m develop SpA (24–26). Among 41 double-transgenic rats, 21 (age 122–349 days at blood draw) exhibited peripheral arthritis and 20 (age

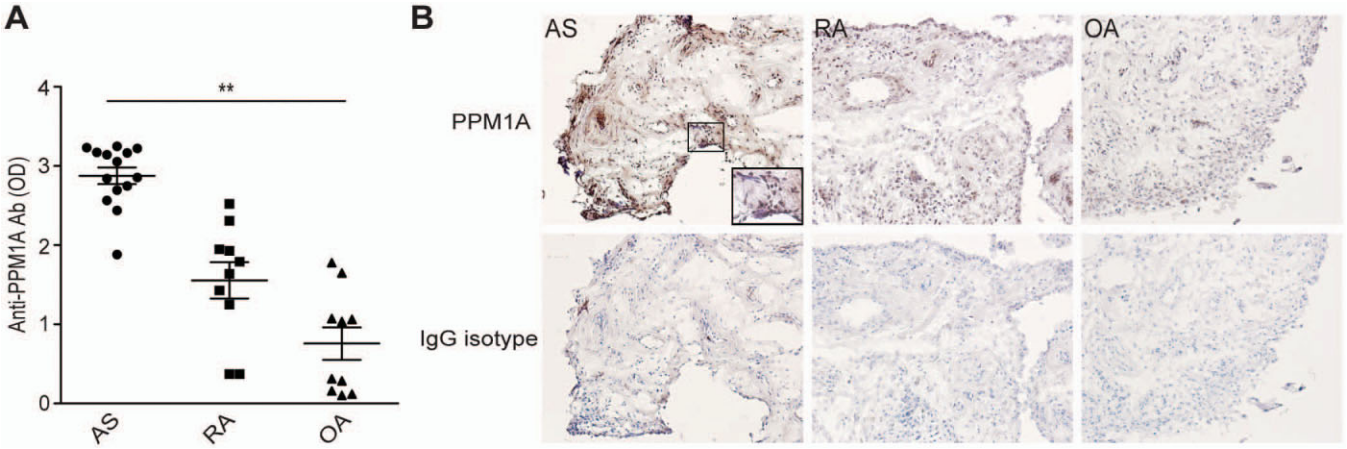

Figure 4. Strong expression of PPM1A in synovial tissue from individuals with AS. **A,** ELISA analysis of levels of anti-PPM1A autoantibodies in synovial fluid from patients with AS (n = 14), RA (n = 10), or osteoarthritis (OA; n = 10). Symbols represent individual patients; horizontal lines and error bars show the mean ± SEM. ** = $P < 0.01$ by one-way analysis of variance. **B,** Immunohistochemical images of synovial tissue specimens from a patient with AS, a patient with RA, and a patient with OA, stained with anti-PPM1A antibodies or IgG isotype controls. **Inset** shows a higher-magnification view of the boxed area in the center of the panel. Results are representative of 3 independent experiments. Original magnification × 200. See Figure 1 for other definitions.

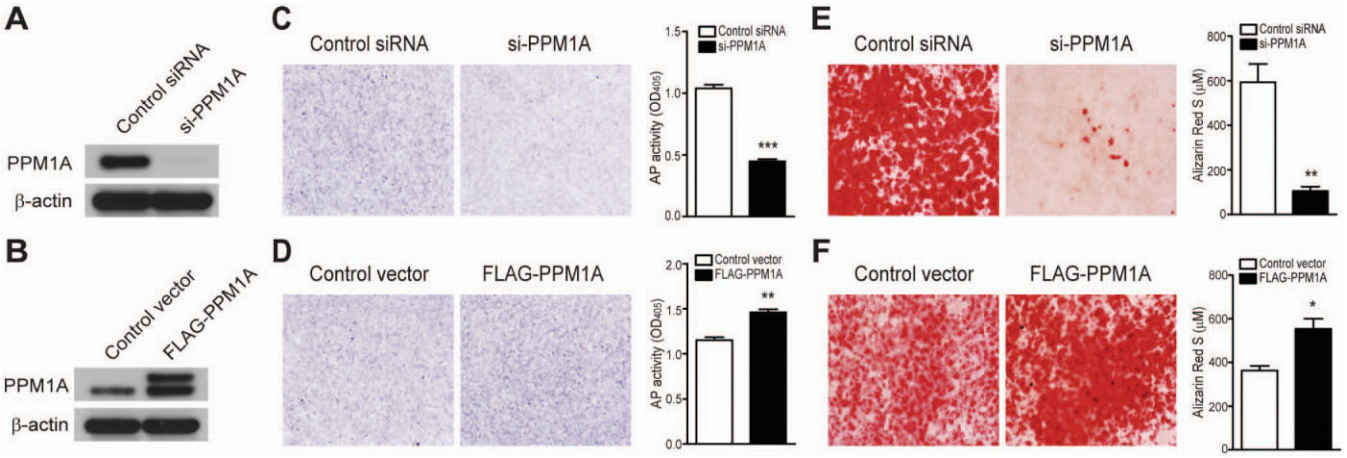

Figure 5. Protein phosphatase magnesium-dependent 1A (PPM1A) drives osteoblast differentiation. Preosteoblastic MC3T3-E1 cells were transiently transfected with small interfering RNA (siRNA) against PPM1A (si-PPM1A) or with a non-targeting control siRNA, or with a PPM1A expression plasmid (FLAG-PPM1A) or control vector, and cultured in osteogenic media for 7–14 days. **A,** Knockdown of PPM1A protein in si-PPM1A–transfected cells. **B,** Overexpression of PPM1A in FLAG-PPM1A–transfected cells. **C** and **E,** Alkaline phosphatase (AP) activity (**C**) and nodule formation (**E**) in cells transfected with si-PPM1A or control siRNA. **D** and **F,** Alkaline phosphatase activity (**D**) and nodule formation (**F**) in cells transfected with FLAG-PPM1A or control vector. Original magnification × 100. Bars show the mean ± SEM of triplicate determinations. Results are representative of 3 independent experiments. $* = P < 0.05$; $** = P < 0.01$; $*** = P < 0.001$, versus control. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.38763/abstract.

93–218 days) did not. Levels of anti-PPM1A autoantibodies were significantly higher in these SpA-prone transgenic rats, irrespective of clinically evident arthritis, compared to the controls (nontransgenic rats or hypospermatogenic *Dazl*-deficient B27/hβ_2m–transgenic rats) ($P < 0.001$) (Figure 3).

Elevated expression of PPM1A in AS synovium. Because we found that levels of anti-PPM1A autoantibodies are higher in AS synovial fluid (Figure 4A) and sera (Figure 1B) than in synovial fluid from patients with other diseases, we sought to determine whether expression of PPM1A protein is also higher in synovial tissue derived from AS patients than in synovial tissue from patients with other diseases. Immunohistochemical analysis revealed that PPM1A expression was higher in AS synovial tissue than in RA or OA synovial tissue (Figure 4B). PPM1A localized to the nuclei of AS synoviocytes (Figure 4B, inset).

PPM1A promotes osteoblast differentiation in vitro. To evaluate the role of PPM1A in osteoblastogenesis, we modulated the expression of PPM1A by knockdown or overexpression in the mouse preosteoblast cell line MC3T3-E1 and assessed differentiation of these cells into osteoblasts after 1–2 weeks of incubation in osteogenic media. Knockdown of PPM1A significantly decreased AP activity and nodule formation (assessed by alizarin red S staining) (Figures 5A, C, and E). Conversely, overexpression of PPM1A increased AP activity and nodule formation (Figures 5B, D, and F).

Furthermore, PPM1A knockdown significantly decreased, and PPM1A overexpression increased, expression of type I collagen, BSP-2, and osteocalcin during differentiation (Figures 6A and B). Osteocalcin expression was the most prominently affected. We found that PPM1A overexpression did not activate Smad1/5/8 or β-catenin, suggesting that PPM1A promotes osteoblast differentiation independently of BMP and Wnt signaling (Figure 6C).

DISCUSSION

In this study, we demonstrated that serum levels of anti-PPM1A autoantibodies were higher in sera derived from individuals with AS than from individuals with other autoimmune diseases. Further, HLA–B27–transgenic rats, which spontaneously develop AS-like arthritis, also had anti-PPM1A autoantibodies. Anti-PPM1A autoantibody levels were higher in AS patients with high-grade sacroiliitis than in those with low-grade sacroiliitis. Moreover, serum levels of anti-PPM1A autoantibodies decreased in AS patients treated with anti-TNF agents, and the change in levels of anti-PPM1A autoantibodies correlated positively with the change in disease activity. Finally, we found that PPM1A protein is

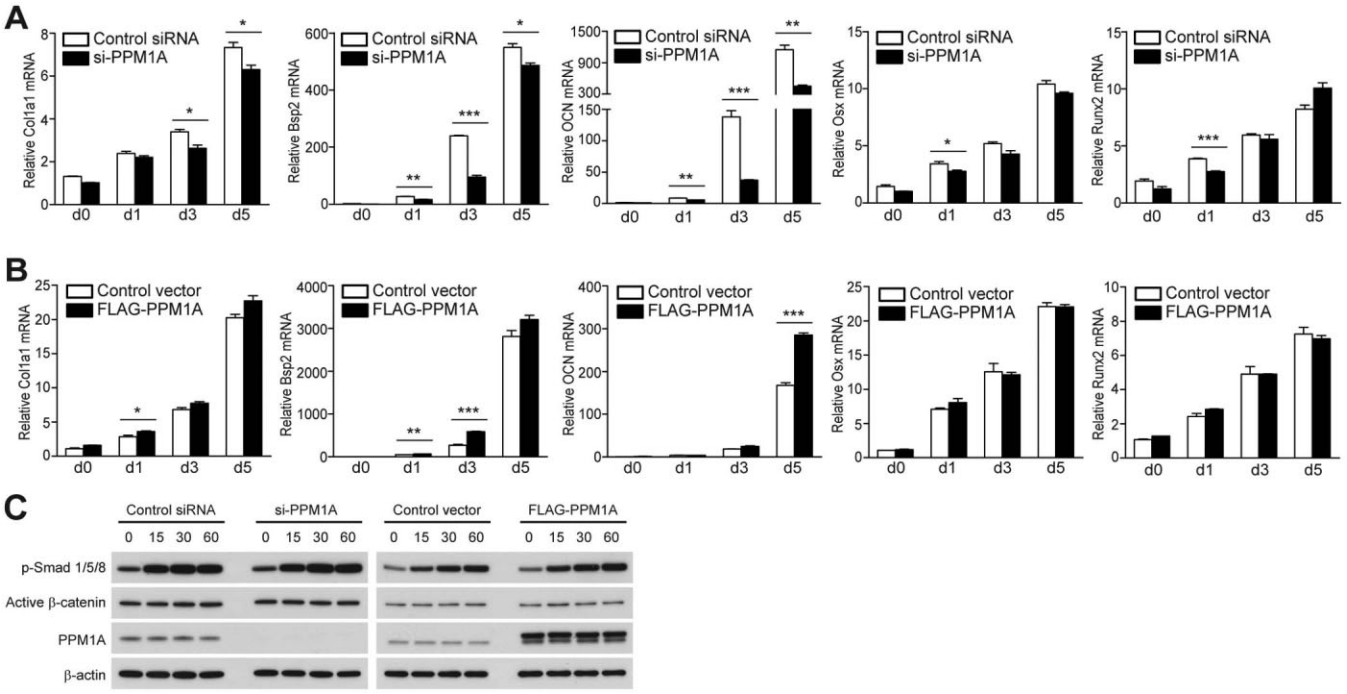

Figure 6. Gene and protein expression associated with osteoblast differentiation. **A** and **B,** Quantitative real-time polymerase chain reaction analysis of gene expression associated with osteoblast differentiation in cells transfected with small interfering RNA (siRNA) against protein phosphatase magnesium-dependent 1A (si-PPM1A) (**A**) or FLAG-PPM1A (**B**) and stimulated with osteogenic media. Bars show the mean ± SEM of triplicate determinations. Results are representative of 3 independent experiments. $* = P < 0.05$; $** = P < 0.01$; $*** = P < 0.001$. **C,** Immunoblot analysis of phospho-Smad1/5/8 and active β-catenin in si-PPM1A–transfected or FLAG-PPM1A–transfected cells stimulated with osteogenic media for 15, 30, or 60 minutes. Col1a1 = type I collagen; BSP-2 = bone sialoprotein 2; OCN = osteocalcin; OSX = osterix; RUNX-2 = runt-related transcription factor 2.

highly expressed in synovial tissue from AS patients and drives osteoblast differentiation, suggesting that PPM1A itself contributes to the pathogenesis of AS.

Although autoantibodies are an immunologic hallmark of many autoimmune diseases (29), AS has been largely regarded as a seronegative disease to date because of a lack of reliable autoantibody biomarkers. Nevertheless, some groups have reported the presence of autoantibodies to collagen in the blood of individuals with AS (30), and others have reported the presence of circulating plasma cells and an increase in immunoglobulin levels, comparable to that observed in patients with RA (31,32). Levels of autoantibodies to mutated citrullinated vimentin, which are considered a specific serologic biomarker of RA (33), were also shown to be elevated in AS sera (34), although anti–citrullinated protein antibodies are not generally detected in patients with AS. Recently, autoantibodies to multiple autoantigens were detected in patients with AS (compared to patients with RA or healthy controls) by using nucleic acid programmable protein arrays (7), demonstrating that 44% of AS patients possess autoantibodies to multiple autoantigens and that 60% of these autoantibodies are present in AS patients but not RA patients. In this study, we profiled the autoantibodies present in the blood of AS patients by using a protein array containing >8,000 human proteins and sera acquired from patients with AS or other autoimmune diseases. We detected the presence of antibody reactivity to PPM1A in AS serum samples, and validated this finding in an independent cohort of AS patients.

Interestingly, anti-PPM1A antibodies were detected irrespective of evident arthritis in the SpA-prone B27/hβ_2m–transgenic rats. In this particular model, only males develop disease, and they developed epididymoorchitis beginning around 1–2 months of age, well before the onset of arthritis, which typically appears at 4–6 months. Hypospermatogenic *Dazl*-deficient B27/hβ_2m–transgenic rats (used as a negative control in this study) are protected against both epididymoorchitis and subsequent arthritis, and persistent testicular inflammation and/or antigenic stimulation thus seem to be essen-

tial prerequisites for subsequent arthritis or spondylitis (26). Whether the anti-PPM1A antibodies in the rats without overt arthritis resulted from the epididymoorchitis, from subclinical arthritis or spondylitis, or from some other aspect of the disease is not yet clear. Subclinical spondylitis almost certainly is common in these rats (35).

Levels of anti-PPM1A antibodies were higher in the disease-prone B27/hβ_2m–transgenic rats than in the *Dazl*-deficient B27/hβ_2m–transgenic rats, suggesting that the expression of B27 is insufficient to induce these antibodies, independent of inflammatory disease. However, TNF as well as TGFβ activation is characteristic of AS (27), and PPM1A overexpression might be influenced by these conditions. Reuter et al (36) reported that PPM1A, induced by TNF stimulation in K562 cells, was suppressed by pretreatment with an NF-κB inhibitor, suggesting that PPM1A is a downstream gene activated by TNF/NF-κB signaling. Furthermore, PPM1A is a known inhibitor of TGFβ signaling, and ectopic expression of PPM1A in C2C12 cells abolished the TGFβ-induced antiproliferative response (28). Taken together, these findings suggest that PPM1A might be induced by TNF and/or TGFβ.

In AS patients who had started anti-TNF therapy, there was a positive correlation between the change in anti-PPM1A antibody levels and the change in BASDAI scores. It is possible that this observation arises from suppression of TNF-induced PPM1A activation (36) by anti-TNF therapy. Moreover, considering the efficacy of anti-TNF therapy in controlling disease activity in AS (37,38), it is possible that the increase in baseline levels of anti-PPM1A autoantibodies is caused by an increase in exposure of (normally intracellular) PPM1A to the immune system, with a drop in the levels of anti-PPM1A autoantibodies after anti-TNF therapy reflecting a decrease in exposure of PPM1A following the control of synovial and/or entheseal inflammation. Although these findings remain to be validated in an independent cohort, they suggest that baseline levels of anti-PPM1A antibodies may serve as a serologic biomarker of AS, and that the decrease in anti-PPM1A autoantibody levels after anti-TNF therapy may serve as a pharmacodynamic biomarker. Whether anti-PPM1A autoantibodies contribute to the pathogenesis of AS, or are simply secondary to an underlying disease process in AS, remains to be determined. Further studies of the role of anti-PPM1A autoantibodies and/or PPM1A-containing immune complexes in AS and its rat model are needed to further define the biologic role of anti-PPM1A autoantibodies in the pathogenesis of AS.

In addition to the extracellular release of PPM1A and its targeting by autoantibodies in AS, we identified a role for endogenous PPM1A in osteoblast differentiation, a process that contributes to the AS phenotype (9). That AS is dominated by anabolic processes after the onset of chronic inflammation and is characterized by bony overgrowth and ankylosis (10) further supports a role for osteoblasts, and hence PPM1A, in the pathogenesis of AS. The molecular mechanisms of bone formation in AS involve BMP and Wnt signaling (39), and PPM1A can inhibit BMP signaling by decreasing protein levels of Smad1, Smad5, and Smad8 in C2C12 mouse myoblasts (40). However, we show that PPM1A enhances osteoblast differentiation independently of BMP and Wnt in an in vitro setting. Our experiments were designed to identify the direct role of PPM1A in osteoblast differentiation, by using two different strains of mouse MC3T3-E1, a monopotential cell line that can only differentiate into osteoblasts when cultured in osteogenic media (41,42). We demonstrated that preosteoblasts overexpressing PPM1A differentiated into mature osteoblasts, and that this differentiation was accompanied by an increase in the expression of markers of osteoblast formation, but not by an increase in the activation of Smad1/5/8 and β-catenin, key molecules of BMPs and the Wnt signaling pathway. We propose that overexpression of PPM1A in AS promotes the differentiation and activation of osteoblasts by activating several target genes, especially osteocalcin.

In conclusion, we showed that high levels of anti-PPM1A autoantibodies are present in AS patients and are associated with greater radiographic severity of AS and with greater disease activity before anti-TNF therapy. We also demonstrated increased expression of PPM1A in AS synovium and identified intracellular PPM1A as a potential enhancer of osteoblastogenesis. Thus, our data suggest that levels of anti-PPM1A autoantibodies in the blood of AS patients may serve as a diagnostic biomarker, as a biomarker of disease severity, and as a biomarker of response to anti-TNF therapy. These findings could not only give rise to an actionable mechanistic biomarker (43) for the management of AS, but also identify PPM1A as a novel therapeutic target in attenuating bony ankylosis and thus radiographic progression in AS.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Kim had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Kim, Sohn, Zhao, Yoo, Lee, Reveille, Taurog, Robinson.

Acquisition of data. Kim, Sohn, Zhao, Yoo, Lee, Reveille, Taurog, Robinson.

Analysis and interpretation of data. Kim, Sohn, Zhao, Sokolove, Lindstrom, Yoo, Lee, Reveille, Taurog, Robinson.

REFERENCES

1. Maurer K. Basic data on arthritis knee, hip, and sacroiliac joints in adults ages 25-74 years. Vital Health Stat 1979;11:1–31.
2. Sieper J, Braun J, Rudwaleit M, Boonen A, Zink A. Ankylosing spondylitis: an overview. Ann Rheum Dis 2002;61 Suppl 3:iii8–18.
3. Braun J, Baraliakos X. Imaging of axial spondyloarthritis including ankylosing spondylitis. Ann Rheum Dis 2011;70 Suppl 1:i97–103.
4. Maksymowych WP. MRI in ankylosing spondylitis. Curr Opin Rheumatol 2009;21:313–7.
5. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 1994;21:2286–91.
6. Van Tubergen A, van der Heijde D, Anderson J, Landewe R, Dougados M, Braun J, et al. Comparison of statistically derived ASAS improvement criteria for ankylosing spondylitis with clinically relevant improvement according to an expert panel. Ann Rheum Dis 2003;62:215–21.
7. Wright C, Sibani S, Trudgian D, Fischer R, Kessler B, LaBaer J, et al. Detection of multiple autoantibodies in patients with ankylosing spondylitis using nucleic acid programmable protein arrays. Mol Cell Proteomics 2012;11:M9.00384.
8. Colbert RA. The immunobiology of HLA-B27: variations on a theme. Curr Mol Med 2004;4:21–30.
9. Schett G. Bone formation versus bone resorption in ankylosing spondylitis. Adv Exp Med Biol 2009;649:114–21.
10. Lories R. The balance of tissue repair and remodeling in chronic arthritis. Nat Rev Rheumatol 2011;7:700–7.
11. Chen HA, Chen CH, Lin YJ, Chen PC, Chen WS, Lu CL, et al. Association of bone morphogenetic proteins with spinal fusion in ankylosing spondylitis. J Rheumatol 2010;37:2126–32.
12. Park MC, Park YB, Lee SK. Relationship of bone morphogenetic proteins to disease activity and radiographic damage in patients with ankylosing spondylitis. Scand J Rheumatol 2008;37:200–4.
13. Lories RJ, Derese I, Luyten FP. Modulation of bone morphogenetic protein signaling inhibits the onset and progression of ankylosing enthesitis. J Clin Invest 2005;115:1571–9.
14. Appel H, Ruiz-Heiland G, Listing J, Zwerina J, Herrmann M, Mueller R, et al. Altered skeletal expression of sclerostin and its link to radiographic progression in ankylosing spondylitis. Arthritis Rheum 2009;60:3257–62.
15. Heiland GR, Appel H, Poddubnyy D, Zwerina J, Hueber A, Haibel H, et al. High level of functional dickkopf-1 predicts protection from syndesmophyte formation in patients with ankylosing spondylitis. Ann Rheum Dis 2012;71:572–4.
16. Zolnierowicz S. Type 2A protein phosphatase, the complex regulator of numerous signaling pathways. Biochem Pharmacol 2000; 60:1225–35.
17. Duan X, Liang YY, Feng XH, Lin X. Protein serine/threonine phosphatase PPM1A dephosphorylates Smad1 in the bone morphogenetic protein signaling pathway. J Biol Chem 2006;281: 36526–32.
18. Strovel ET, Wu D, Sussman DJ. Protein phosphatase 2Cα dephosphorylates axin and activates LEF-1-dependent transcription. J Biol Chem 2000;275:2399–403.
19. Criswell LA, Pfeiffer KA, Lum RF, Gonzales B, Novitzke J, Kern M, et al. Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. Am J Hum Genet 2005;76:561–71.
20. Paniagua RT, Chang A, Mariano MM, Stein EA, Wang Q, Lindstrom TM, et al. c-Fms-mediated differentiation and priming of monocyte lineage cells play a central role in autoimmune arthritis. Arthritis Res Ther 2010;12:R32.
21. Van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis: a proposal for modification of the New York criteria. Arthritis Rheum 1984;27:361–8.
22. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
23. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. Arthritis Rheum 1986;29:1039–49.
24. Hammer RE, Maika SD, Richardson JA, Tang JP, Taurog JD. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human β_2m: an animal model of HLA-B27-associated human disorders. Cell 1990;63:1099–112.
25. Tran TM, Dorris ML, Satumtira N, Richardson JA, Hammer RE, Shang J, et al. Additional human β_2-microglobulin curbs HLA–B27 misfolding and promotes arthritis and spondylitis without colitis in male HLA–B27–transgenic rats. Arthritis Rheum 2006;54:1317–27.
26. Taurog JD, Rival C, van Duivenvoorde LM, Satumtira N, Dorris ML, Sun M, et al. Autoimmune epididymoorchitis is essential to the pathogenesis of male-specific spondylarthritis in HLA–B27–transgenic rats. Arthritis Rheum 2012;64:2518–28.
27. Francois RJ, Neure L, Sieper J, Braun J. Immunohistological examination of open sacroiliac biopsies of patients with ankylosing spondylitis: detection of tumour necrosis factor α in two patients with early disease and transforming growth factor β in three more advanced cases. Ann Rheum Dis 2006;65:713–20.
28. Lin X, Duan X, Liang YY, Su Y, Wrighton KH, Long J, et al. PPM1A functions as a Smad phosphatase to terminate TGFβ signaling. Cell 2006;125:915–28.
29. Ambarus C, Yeremenko N, Tak PP, Baeten D. Pathogenesis of spondyloarthritis: autoimmune or autoinflammatory? Curr Opin Rheumatol 2012;24:351–8.
30. Tani Y, Sato H, Hukuda S. Autoantibodies to collagens in Japanese patients with ankylosing spondylitis. Clin Exp Rheumatol 1997;15:295–7.
31. Eghtedari AA, Davis P, Bacon PA. Immunological reactivity in ankylosing spondylitis: circulating immunoblasts, autoantibodies, and immunoglobulins. Ann Rheum Dis 1976;35:155–7.
32. Veys EM, van Leare M. Serum IgG, IgM, and IgA levels in ankylosing spondylitis. Ann Rheum Dis 1973;32:493–6.
33. Liu X, Jia R, Zhao J, Li Z. The role of anti-mutated citrullinated vimentin antibodies in the diagnosis of early rheumatoid arthritis. J Rheumatol 2009;36:1136–42.
34. Bodnar N, Szekanecz Z, Prohaszka Z, Kemeny-Beke A, Nemethne-Gyurcsik Z, Gulyas K, et al. Anti-mutated citrullinated vimentin (anti-MCV) and anti-65 kDa heat shock protein (anti-hsp65): new biomarkers in ankylosing spondylitis. Joint Bone Spine 2012;79:63–6.
35. Van Duivenvoorde LM, Dorris ML, Satumtira N, van Tok MN, Redlich K, Tak PP, et al. Relationship between inflammation, bone destruction, and osteoproliferation in the HLA–B27/human

β_2-microglobulin–transgenic rat model of spondylarthritis. Arthritis Rheum 2012;64:3210–9.
36. Reuter S, Charlet J, Juncker T, Teiten MH, Dicato M, Diederich M. Effect of curcumin on nuclear factor κB signaling pathways in human chronic myelogenous K562 leukemia cells. Ann N Y Acad Sci 2009;1171:436–47.
37. Van der Heijde D, Kivitz A, Schiff MH, Sieper J, Dijkmans BA, Braun J, et al, for the ATLAS Study Group. Efficacy and safety of adalimumab in patients with ankylosing spondylitis: results of a multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2006;54:2136–46.
38. Braun J, Deodhar A, Dijkmans B, Geusens P, Sieper J, Williamson P, et al, and the Ankylosing Spondylitis Study for the Evaluation of Recombinant Infliximab Therapy Study Group. Efficacy and safety of infliximab in patients with ankylosing spondylitis over a two-year period. Arthritis Rheum 2008;59:1270–8.
39. Schett G, Rudwaleit M. Can we stop progression of ankylosing spondylitis? Best Pract Res Clin Rheumatol 2010;24:363–71.
40. Kokabu S, Nojima J, Kanomata K, Ohte S, Yoda T, Fukuda T, et al. Protein phosphatase magnesium-dependent 1A-mediated inhibition of BMP signaling is independent of Smad dephosphorylation. J Bone Miner Res 2010;25:653–60.
41. Wang D, Christensen K, Chawla K, Xiao G, Krebsbach PH, Franceschi RT. Isolation and characterization of MC3T3-E1 preosteoblast subclones with distinct in vitro and in vivo differentiation/mineralization potential. J Bone Miner Res 1999;14:893–903.
42. Yamaguchi A, Komori T, Suda T. Regulation of osteoblast differentiation mediated by bone morphogenetic proteins, hedgehogs, and Cbfa1. Endocr Rev 2000;21:393–411.
43. Robinson WH, Lindstrom TM, Cheung RK, Sokolove J. Mechanistic biomarkers for clinical decision making in rheumatic diseases. Nat Rev Rheumatol 2013;9:267–76.

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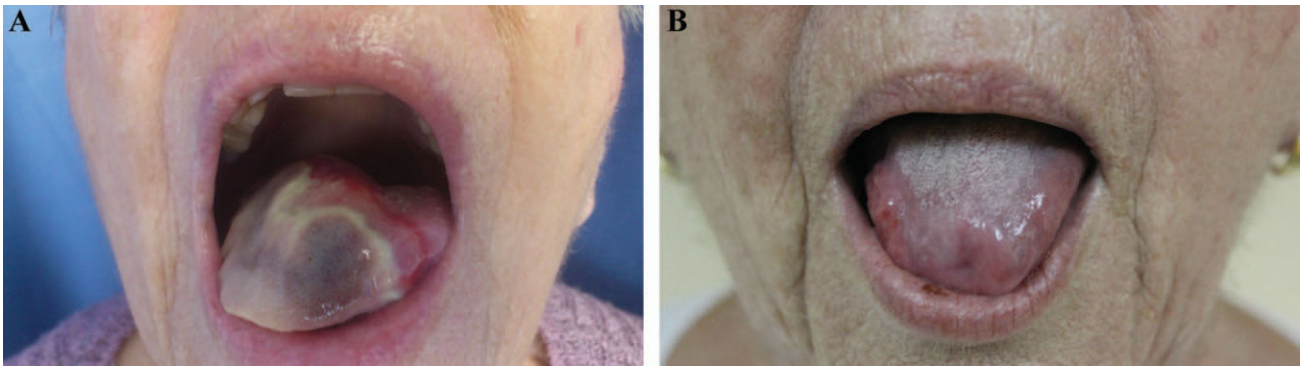
Clinical Images: Tongue necrosis: an unusual clinical presentation of giant cell arteritis



The patient, a 74-year-old woman, presented to the emergency room with tongue pain. She had a 4-week history of headache, with ptosis and blurred vision in her left eye. She also reported fatigue, anorexia, and jaw pain. She was prescribed low-dose systemic corticosteroids for suspected giant cell arteritis (GCA) and was discharged. Her condition initially improved but 2 weeks later it worsened, with an inability to masticate due to jaw and tongue pain and development of an erythematous and necrotic plaque on the right side of the tongue (**A**). Laboratory studies revealed anemia, a high platelet count, and an elevated C-reactive protein level and erythrocyte sedimentation rate. With the suspicion of tongue necrosis due to GCA, an intravenous bolus of methylprednisolone was administered, followed by a tapering regimen of oral prednisone starting at 1 mg/kg/day. The tongue healed rapidly over the subsequent 2 weeks (**B**), and the pain on mastication disappeared. Right temporal artery biopsy and histologic assessment revealed a narrowed arterial lumen with a granulomatous pattern in the media and destruction of the internal elastic lamina, consistent with a diagnosis of GCA. Tongue necrosis is a rare complication of GCA that represents an advanced stage of ischemia secondary to stenosis of the lingual arteries.

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