CHAPTER 4

AUTOANTIBODIES AGAINST CITRULLINATED HISTONE H3 IN RHEUMATOID ARTHRITIS AND PERIODONTITIS PATIENTS

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Submitted

Abstract

Background

Periodontitis (PD) has been hypothesized to play a role in the initiation of rheumatoid arthritis (RA) via excessive citrullination in the periodontium which could induce autoantibodies directed to citrullinated proteins (ACPAs). Neutrophils are abundantly present in inflamed periodontal tissue and express peptidylarginine deiminase 4 (PAD4), the enzyme involved in citrullination. Histone citrullination is an important step in neutrophil extracellular trap (NET) formation. In this study, presence of citrullinated histones in inflamed periodontal tissue was assessed, and sera from RA- and PD-patients were tested for presence of autoantibodies against citrullinated histones. Moreover, association between presence of these autoantibodies with smoking behavior and periodontal status was investigated in these patients.

Methods

Presence of citrullinated histone H3, PAD4 and CD68 was determined in 15 periodontal tissue biopsies from PD-patients by immunohistochemistry. In addition, sera from 36 healthy controls (HC), 113 PD- and 84 RA-patients were assessed on presence of autoantibodies against citrullinated histones by Western blot and against citrullinated histone H3 by ELISA.

Results

Citrullinated histone H3, PAD4 and CD68 were present in periodontal tissue from, respectively, 9 (60%), 14 (93%) and 13 (87%) PD-patients. Autoantibodies against citrullinated histone H3 were found in 33 (39%) RA-patients compared to 3 (8%) HC and 11 (10%) PD-patients. Anti-citrullinated histone H3 levels were higher in anti-CCP positive compared to anti-CCP negative RA-patients ($p=0.0008$) and correlated moderately with anti-CCP levels ($\rho=0.22$). No associations were found between anti-citrullinated histone H3 levels and periodontal status or smoking behavior of RA-patients.

Conclusion

PD-patients are exposed to citrullinated histone H3 in inflamed periodontal tissue. Citrullinated histone H3 is targeted by autoantibodies which are present in RA sera. This supports a role for periodontitis in the generation of antigens that are targeted by ACPA.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease affecting 0.5-1% of the adult population and is characterized by synovial inflammation and joint destruction (1). The majority of RA patients (50-80%) is seropositive for autoantibodies. The two most common autoantibodies are rheumatoid factor (RF) and autoantibodies against citrullinated proteins (ACPA), with the latter being most specific for RA. Citrullination is a posttranslational modification of peptide-bound arginine which is modified to citrulline by the enzyme peptidyl arginine deiminase (PAD). The number of citrullinated proteins that is known to be targeted by ACPA is limited; citrullinated fibrinogen, vimentin, α-enolase, filaggrin are the major known citrullinated antigens (2). In humans, five different isotypes (PAD1-4, 6) are present of which PAD2 and PAD4 are considered to be involved in the generation of citrullinated autoantigens in RA as these two PAD isotypes have been found in RA synovium (3). PAD2 is mainly present in macrophages whereas PAD4 is expressed by neutrophils. Smoking, the main environmental risk factor for RA, is able to enhance PAD expression in bronchoalveolar lavage (BAL) cells and lung mucosa which in turn may lead to the generation of citrullinated proteins (4).

In recent years, neutrophils have been studied for their role in the pathology of RA, specifically for their possible role in ACPA initiation (5). Neutrophils are important during the innate immune response against microbes via phagocytosis of bacteria, degranulation and formation of neutrophil extracellular traps (NETs). These NETs consist of a chromatin meshwork of which histones are the major component. NETs are able to trap and kill microbes with antimicrobial peptides that are typically present in neutrophil granules (6). Neutrophils from RA-patients show enhanced NETosis compared to neutrophils from healthy controls both in the absence or presence of added stimuli (7). Importantly, citrullination of histones by PAD4 is considered to be an essential step in the formation and stabilization of NETs (8). Recently, citrullinated histones H2A, H2B (9) and H4 (10) have been described as targets of autoantibodies in RA patients. Therefore, NETs can be considered as a potential source of citrullinated proteins which could be targeted by ACPAs (11).

The development of RA has been hypothesized to be associated with periodontitis (PD) by reason of one periodontal pathogen, *Porphyromonas gingivalis* (12), that has its own PAD enzyme (PPAD) (13). In consequence, *P. gingivalis* has the unique capability to citrullinate (human) proteins in periodontal lesions, thereby potentially creating targets for ACPA (14). Earlier work from our group has demonstrated that the inflamed periodontium is indeed a source of citrullinated proteins (15). Neutrophils are abundantly present in inflamed periodontal tissue, and are the first cells to migrate to periodontal lesions upon infection or inflammation (16, 17), this is in

sharp contrast to synovial tissue in which neutrophils are scarce. Also, NETs have been found in the gingival pockets and in the purulent crevicular exudates of patients with chronic periodontitis (18). A study by Neeli et al (19) showed that histone H3 is citrullinated by PAD4 in neutrophils after an inflammatory response.

The current study investigated whether citrullinated histone H3 is present in inflamed periodontal tissue. In addition, this study assessed whether citrullinated histone H3 is a target of autoantibodies in the serum of RA patients. Furthermore, associations between the presence of autoantibodies against citrullinated histone H3 and periodontal status or smoking status in RA patients were studied.

Materials and methods

Study groups

Serum samples were collected from adult patients with established RA (n=84), severe untreated PD (n=113) and healthy controls (HC) (n=36) without systemic disease and without PD.

The periodontal status of established RA patients, all fulfilling the ACR 1987 criteria for RA (20), was assessed according to the Dutch Periodontal Screening Index (DPSI) (21). RA patients were subdivided into having no, moderate, or severe periodontitis.

Patients with untreated generalized severe PD were excluded if they had any other systemic disease or they had antibiotic use <3 months before inclusion (22).

HC were recruited among subjects planned for first consultation at the department for Dentistry of the University Medical Center Groningen. Periodontal health was assessed using the DPSI, with the inclusion criterion defined as DPSI score ≤2 (absence of PD). Exclusion criteria were antibiotic use <3 months before inclusion and presence of any systemic disease (22). Patient characteristics are summarized in Table 1.

Histological staining was performed on inflamed periodontal tissue sections of 15 randomly selected PD patients (DPSI 4), without any other systemic disease, obtained from a former study (15).

All participants provided written informed consent before study enrollment in compliance with the Declaration of Helsinki. The study was conducted with approval of the Medical Ethical Committee of the University Medical Center of Groningen (UMCG 2011/010).

Immunohistochemical detection of citrullinated histone H3, PAD4 and CD68

Human paraffin embedded gingival tissue samples were collected from PD patients. Five µm sections were prepared on glass slides. Before staining, endogenous peroxidase

Table 1. Characteristics of study participants.

	Healthy controls (HC)	Periodontitis patients (PD)	Rheumatoid arthritis patients (RA)
N	36	113	84
Age, yrs, mean (SD)	34 (15)	51 (9)*	56 (11)*
Female (%)	56	59	69
Smoking (former or current), n (%)	8 (22)	88 (78)*	34 (40)
No periodontitis (%)	100	0	31
Moderate periodontitis (%)	0	0	40
Severe periodontitis (%)	0	100	29
DAS28, median, (IQR)	NA	NA	2.2 (1.7-2.7)
CRP (mg/L), median, (IQR)	0.4 (0.3-1.5)	1.0 (0.6-2.4)	1.9 (1.0-6.0)*
IgM RF positive, n (%)	1 (2.8)	8 (7.1)	62 (74)*
Anti-CCP positive, n (%)	0 (0)	1 (0.9)	63 (75)*

DAS28; Disease Activity Score of 28 joints. CRP; C-reactive protein. IgM RF; IgM Rheumatoid Factor (positive score defined as ≥10 IU/ml). Anti-CCP; anti-cyclic citrullinated peptide antibody, measured by Euro Diagnostica anti-CCP2 kit (positive score defined as ≥25 U/ml) *p<0.0001 compared to HC.

activity was inhibited by incubating 0.3% H_2O_2 in methanol and followed by blocking off non-specific antibody binding with 1% BSA and 1% normal goat serum in PBS. Subsequently, tissue samples were stained with rabbit anti-citrullinated histone H3 (ab5103, Abcam, Cambridge, UK) 1:250 in PBS + 1% BSA, rabbit anti-PAD4 (ab3877, Abcam) 1:200 in PBS + 1% BSA or mouse anti-CD68 (clone KP1, Dako, Glostrup, Denmark) 1:100 in PBS + 1% BSA. Next, sections were incubated with goat anti rabbit IgG-HRP (P0448, Dako,) in PBS + 1% BSA or rabbit anti mouse IgG-HRP (P0260, Dako), followed by using a DAB kit (K3467, Dako). Sections were counterstained with hematoxylin and mounted with glycerin. In each periodontal tissues sample, positive cells were counted in 10 adjacent fields with a 40x objective (magnification 400x). Mean cell numbers per tissue sample were calculated for each staining.

Neutrophil isolation and stimulation

Blood was obtained from healthy volunteers. Neutrophils were isolated from buffy coat using Lymphoprep (Axis Shield, Oslo, Norway) centrifugation, followed by lysis of erythrocytes using ice-cold 0.15 M ammonium chloride solution. Neutrophils were rinsed with Hanks Balanced Salt Solution supplemented with 140 mg/mL calcium and 100 mg/mL magnesium (HBSS++). Neutrophils were re-suspended at 1 x 10^6 cells/mL HBSS++. After seeding, neutrophils were stimulated 3h with 1.9 μM calcium ionophore (A23187, Sigma) or left untreated. Then, neutrophils were re-suspended and centrifuged, the resulting pellet containing neutrophils was subsequently used for histone extraction.

Histone extraction

Histones were obtained using an acid extraction method (23). Briefly, pellets of 1 x 10^6 cells/mL stimulated and unstimulated neutrophils cells were incubated overnight in 0.2 M H_2SO_4 at 4°C with agitation. Subsequently, acid extracted proteins were precipitated with 33% trichloroacetic acid (TCA) for 2h at 4°C, followed by two wash steps with 100% acetone and finally re-suspended in dH_2O. Protein concentrations of re-suspended histones were determined using bicinchoninic acid (BCA) Protein Assay (Pierce, Rockford, Illinois, USA).

SDS-Page and immunoblotting

Extracted histones, from unstimulated and stimulated neutrophils, were resolved by 15% polyacrylamide gel electrophoresis (SDS-PAGE), and blotted onto polyvinylidene fluoride (PDVF) membranes (Millipore, the Netherlands). Membranes were blocked with Odyssey blocking buffer (Li-Cor, Lincoln, USA) for 1h followed by incubation overnight at 4°C with human serum diluted 1:100 in PBS, anti-citrullinated histone H3 (ab5103, Abcam) dilution 1:500, or anti-histone H3 (ab1791, Abcam) dilution 1:1000. Detection was performed with secondary antibody goat anti-human IgG labeled with IRD680RD (Li-Cor) or goat anti-rabbit IgG labeled with IRD680RD (Li-Cor). Membranes were scanned and analyzed using the Odyssey infra-red imaging scanner (Li-Cor).

ELISA detecting anti-citrullinated histone H3 in human sera

Costar ELISA plates (Corning, NY, USA) were coated overnight at room temperature (rt) with citrullinated histone H3 (citrulline 2 + 8 + 17) peptides (ab32876, Abcam) (1 µg/ml) or non-citrullinated histone H3 peptides (ab12149, Abcam) (1 µg/ml) in PBS. Subsequent blocking was performed by 2% BSA-PBS for 1h following incubation with human sera diluted 1:100 in 1% BSA in PBS + 0.05% Tween-20 for 1h at rt. After washing, mouse anti human IgG-HRP 1:2000 (9040-05, Southern Biotech, AL, USA) was added to the wells and plates were incubated for 1h at rt. Bound antibodies were visualized by using tetramethylbenzidine and hydrogen peroxide. Reactivity was determined separately by measuring the difference in reactivity against the peptide and background (BSA), with the cut off defined as the difference in optical density (ΔOD) >2SD above the mean of HC.

Statistical analysis

Data were analyzed using GraphPad Prism 5 (Graphpad Software, San Diego, CA, USA). For comparisons between groups, unpaired two-tailed t tests were used for variables with Gaussian distribution and two-tailed Mann-Whitney tests for skewed variables. For group comparisons between three groups, Kruskal–Wallis tests were used with

one-way analysis of variance with Dunn's multiple comparison post-test if overall significance level α was <0.05. The Fisher exact test was used to analyze contingency tables and Spearman ρ was used for correlation between different parameters.

Results

Citrullinated histone H3 is present in inflamed periodontal tissue

Citrullinated histone H3 was detected in 9 out of 15 (60%) PD tissue samples (Figure 1A). PAD4 and CD68 were detected in most of the PD tissue samples (Figures 1 B-C). Periodontal tissues from healthy controls were negative for citrullinated histone H3, PAD4 and CD68 (Figures 1 A-C). Citrullinated histone H3 positive tissue samples had significantly higher positive cell counts for neutrophils (PAD4, p=0.008) and macrophages (CD68, p<0.0001) per visible field compared to citrullinated H3 negative tissue samples (Figure 1D).

RA sera contain autoantibodies that target citrullinated histones

Immunoblotting showed that antibodies in 4 of 13 tested sera from RA patients bound to histones from stimulated neutrophils, while only 1 of 15 PD sera tested positive and whereas none of 9 HC sera had antibodies that bound to histones. This is depicted in Figure 2, where representative immunoblots for HC, PD and RA sera are shown. Of note, the PD patient whose serum reacted to citrullinated histone H3 was anti-CCP positive. Some RA sera also bound to histones from unstimulated neutrophils (data not shown) which suggests that unstimulated neutrophils may have been activated by the isolation procedure that could have induced histone citrullination. Therefore, an ELISA was set up to measure reactivity against a citrullinated peptide from histone H3 to ascertain that the measured reactivity was directed against citrullinated histone H3 and not to any of the other histone proteins. The ELISA system also ensured that a large number of sera could be tested. A cut off was set, which was based on >2SD above the mean of HCs, which resulted in seropositivity for IgG anti-citrullinated histone H3 in 3 (8%) HC, 11 (10%) PD patients and 33 (39%) RA patients, with the latter having significantly increased levels compared to HC and PD patients (p<0.0001, Figure 3A). In RA patients, anti-citrullinated histone H3 levels were higher in anti-CCP positive compared to anti-CCP negative individuals (p=0.0008, Figure 3B), and anti-citrullinated histone H3 levels showed a small, but significant correlation (ρ=0.22, p=0.0462) with anti-CCP levels (Figure 3C). Anti-citrullinated histone H3 levels were not different between RA patients stratified according to their periodontal status (Figure 3D). Additionally, no differences in anti-citrullinated histone H3 levels were found between non-smokers and smokers in PD (Figure 3E) and RA patients (Figure 3F).

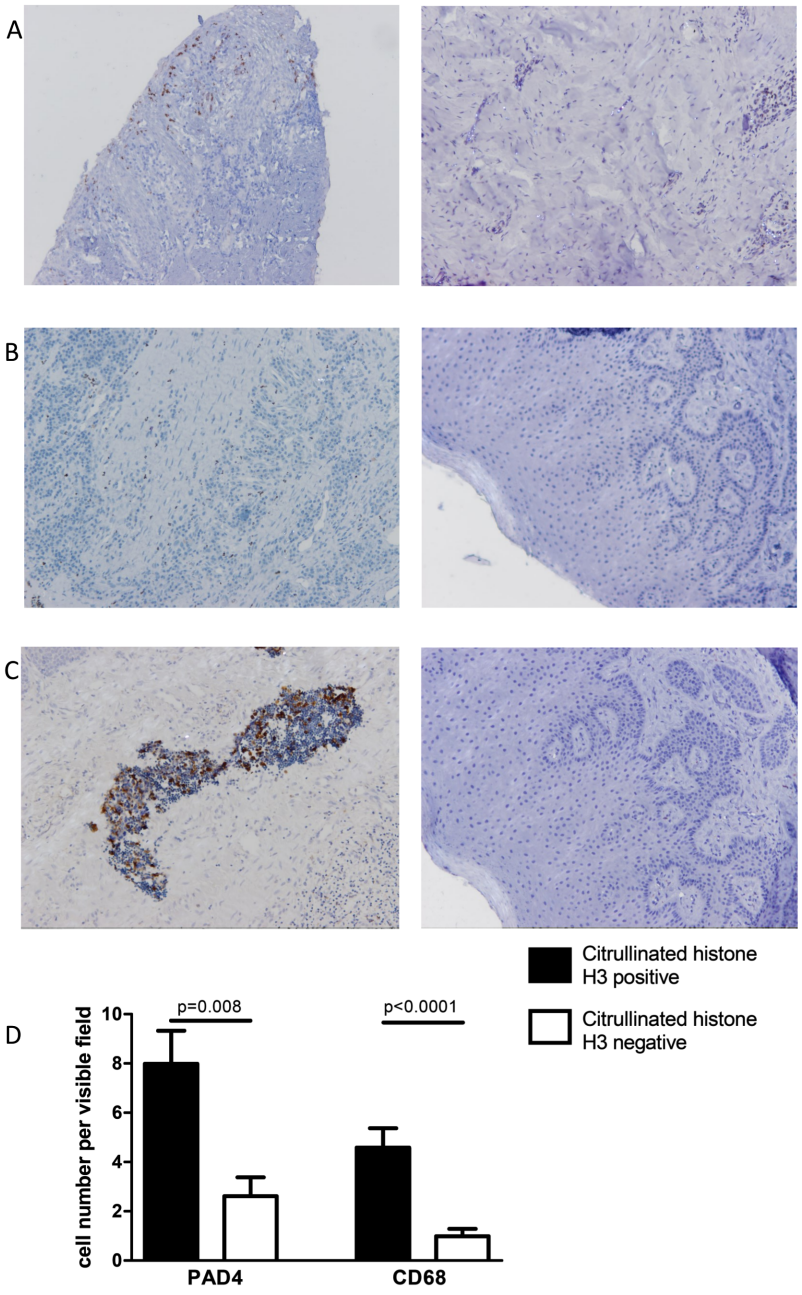

Figure 1. Immunohistological staining in inflamed periodontal tissue from periodontitis patients and healthy controls. *Representative stainings of (A) citrullinated histone H3 (B) PAD4 and (C) CD68 in periodontal tissue from periodontitis patients (left) and healthy controls (right). The brown depicts the presence of stained proteins. Magnification 100x. (D) Mean cell numbers of PAD4 positive cells and CD68 positive cells in citrullinated histone H3 positive and citrullinated histone H3 negative cells. Results represented in mean ± SEM.*

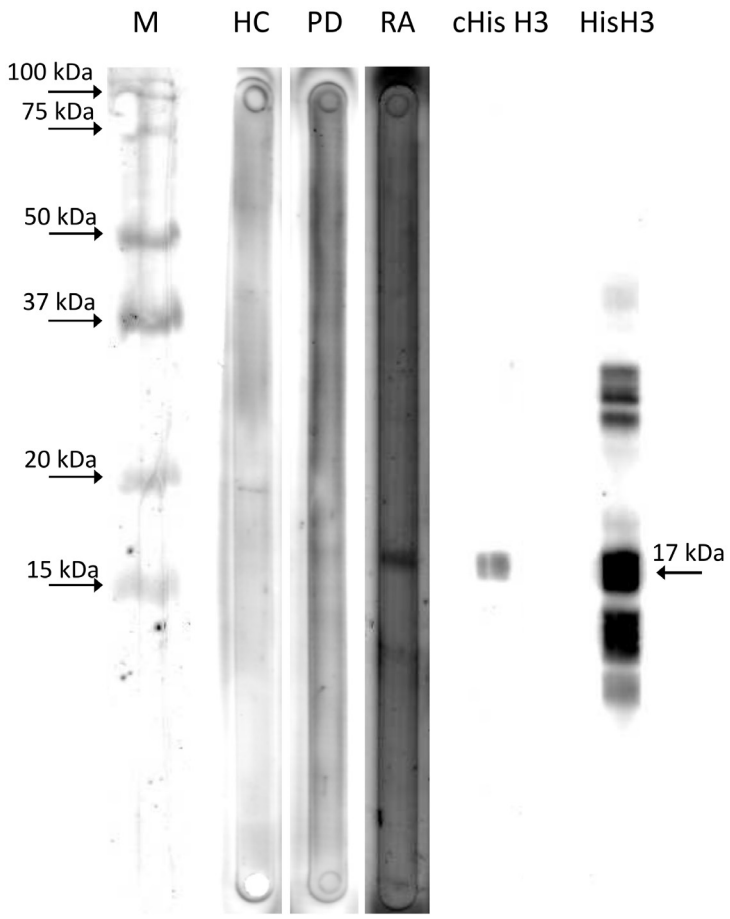

Figure 2. RA sera bind to citrullinated histone H3. *Extracted histones (2 µg/lane) from stimulated neutrophils were subjected to Western blotting and incubated with HC, PD and RA sera and with anti-citrullinated histone H3 (cHis H3, Abcam 5103) and anti-histone H3 (HisH3, abcam 1791) polyclonal antibodies. RA sera and anti-citrullinated histone H3 antibodies recognize a band of the same size.*

Discussion

PD has been hypothesized to be a risk factor for RA development (12), which is supported by the fact that a higher incidence of PD is present amongst RA patients (24). Our study showed presence of citrullinated histone H3 in inflamed periodontal tissue from PD-patients while presence of PAD4 in neutrophils indicates that citrullination is a process that is existent in inflamed periodontal tissue. Besides possessing PAD2 expression which plays a role in citrullination, local activity of macrophages might play a role in ACPA generation by engulfing NET fragments and presenting citrullinated histone H3 to T-cells, leading to an ACPA response in susceptible individuals.

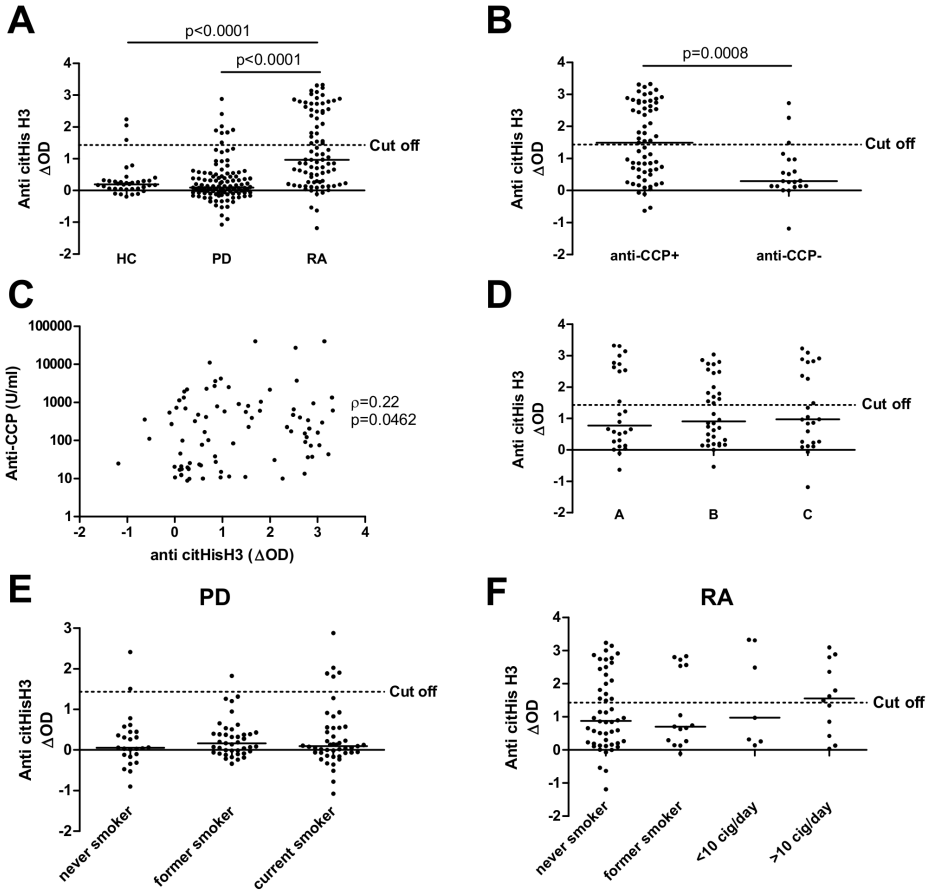

Figure 3. Levels of anti-citrullinated histone H3 antibodies. *(A) in sera from healthy controls (HC), periodontitis (PD) patients and rheumatoid arthritis (RA) patients. (B) In RA-patients, anti-citrullinated histone H3 levels were significantly higher in anti-CCP positive individuals compared to anti-CCP negative individuals. (C) Anti-citrullinated histone H3 levels showed a small but significant correlation with anti-CCP levels in RA-patients. (D) Anti-citrullinated histone H3 levels in RA-patients, stratified according to periodontal status: no periodontitis (a), moderate periodontitis (b) and severe periodontitis (c). No significant differences were observed. (E) Anti-citrullinated histone H3 levels in PD-patients, stratified according to smoking status. No significant differences were observed. (F) Anti-citrullinated histone H3 levels in RA-patients, stratified according to smoking status. No significant differences were observed. Horizontal bars indicate the median values. Anti citHis H3; anti-citrullinated histone H3, ΔOD; delta optical density, Anti-CCP; anti-cyclic citrullinated peptide antibodies, U/ml; Units/ml.*

In this study, we identified citrullinated histone H3 as a target for ACPAs in RA patients. Anti-citrullinated histone H3 levels in PD patients were comparable to HCs which is in agreement with a previous study of our group in which reactivity against specific citrullinated peptides was assessed in PD-patients (22). In recent years, several studies have reported citrullinated histones H2A, H2B (9) and H4 (10) as potential

targets for ACPAs. In addition, we recognized specific binding to citrullinated histone H3. Previously, Dwivedi et al. (25) identified that anti-citrullinated histone H3 in patients with Felty's syndrome, which can be characterized as a form of "super rheumatoid" disease with splenomegaly and neutropenia being present besides joint involvement. The latter study did find a low frequency of anti-citrullinated histone H3 in RA patients, although differences in experimental methods and limited RA patient group size in that study could explain for the different results compared to our study.

ACPA and RF have found to be present years before the onset of RA pre-symptomatic individuals (26), but their role in disease development and pathogenesis is still unclear. Especially individuals seropositive for ACPA are at increased risk for developing RA (27). Whether anti-citrullinated histone H3 autoantibodies also play a role in the initiation of RA is yet unclear and should be assessed in seropositive arthralgia patients.

We found no association between the presence of anti-citrullinated histone H3 and periodontal status and smoking behavior of RA patients. This may indicate that the development of anti-citrullinated histone H3 is more dependent on genetic factors (HLA-DRB1-SE) than exposure to citrullinated antigens via PD or smoking.

The pathogenic role of autoantibodies that recognize citrullinated histones is currently unclear. Autoantibodies directed against histone H2B were found to be arthritogenic in a collagen induced arthritis mouse model (CIA) (28). This is in contrast to a study that recognized anti-citrullinated histone H2A autoantibodies, directed against the N-terminal region of histone H2A, as a possible therapeutic treatment for RA (29). Administration of these therapeutic ACPAs (tACPAs) resulted in reduced inflammation and joint damage in collagen-antibody induced arthritis (CAIA) and CIA mouse models. The therapeutic effect of tACPA is proposed to lie in inhibition of NET formation and clearance of already formed NETs, thereby reducing formation of citrullinated autoantigens. Whether anti-citrullinated histone H3 autoantibodies are pathogenic or therapeutic remains to be determined. Recently, antibodies against citrullinated histone 3 were identified as tACPAs (30). In our study, anti-citrullinated histone H3 levels were not (negatively) correlated with DAS28 scores and CRP levels in RA patients (data not shown). However, the patients were all on treatment and displayed relative low disease activities during the study period. Future studies in newly diagnosed RA patients or seropositive arthralgia patients, should determine whether untreated patients seropositive for anti-citrullinated histone H3 display less disease activity prior to treatment compared to anti-citrullinated histone H3 seronegative patients. Also regarding to whether these ACPAs are pathogenic or not studies should be performed looking at epitope recognition and glycosylation status.

In conclusion, we showed that RA sera contain increased levels of autoantibodies against citrullinated histone H3 compared to sera of PD patients and HCs. Also citrullinated histone H3 is present in inflamed periodontal tissue, which supports a role for periodontitis in the generation of antigens that are targeted by ACPA.

References

1. Willemze A, Trouw LA, Toes RE, Huizinga TW. The influence of ACPA status and characteristics on the course of RA. Nat Rev Rheumatol 2012;8:144-52.
2. Wegner N, Lundberg K, Kinloch A, Fisher B, Malmstrom V, Feldmann M, et al. Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis. Immunol Rev 2010;233:34-54.
3. Foulquier C, Sebbag M, Clavel C, Chapuy-Regaud S, Al Badine R, Mechin MC, et al. Peptidyl arginine deiminase type 2 (PAD-2) and PAD-4 but not PAD-1, PAD-3, and PAD-6 are expressed in rheumatoid arthritis synovium in close association with tissue inflammation. Arthritis Rheum 2007;56:3541-53.
4. Makrygiannakis D, Hermansson M, Ulfgren AK, Nicholas AP, Zendman AJ, Eklund A, et al. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. Ann Rheum Dis 2008;67:1488-92.
5. Wright HL, Moots RJ, Edwards SW. The multifactorial role of neutrophils in rheumatoid arthritis. Nat Rev Rheumatol 2014;10:593-601.
6. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science 2004;303:1532-5.
7. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, Gizinski A, Yalavarthi S, Knight JS, et al. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. Sci Transl Med 2013;5:178ra40.
8. Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. J Exp Med 2010;207:1853-62.
9. Corsiero E, Bombardieri M, Carlotti E, Pratesi F, Robinson W, Migliorini P, et al. Single cell cloning and recombinant monoclonal antibodies generation from RA synovial B cells reveal frequent targeting of citrullinated histones of NETs. Ann Rheum Dis 2015; [Published on 9 Dec 2015] doi:10.1136/annrheumdis-2015-208356.
10. Pratesi F, Dioni I, Tommasi C, Alcaro MC, Paolini I, Barbetti F, et al. Antibodies from patients with rheumatoid arthritis target citrullinated histone 4 contained in neutrophils extracellular traps. Ann Rheum Dis 2014;73:1414-22.
11. Dwivedi N, Radic M. Citrullination of autoantigens implicates NETosis in the induction of autoimmunity. Ann Rheum Dis 2014;73:483-91.
12. Rosenstein ED, Greenwald RA, Kushner LJ, Weissmann G. Hypothesis: the humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis. Inflammation 2004;28:311-8.
13. McGraw WT, Potempa J, Farley D, Travis J. Purification, characterization, and sequence analysis of a potential virulence factor from *Porphyromonas gingivalis*, peptidylarginine deiminase. Infect Immun 1999;67:3248-56.
14. Wegner N, Wait R, Sroka A, Eick S, Nguyen KA, Lundberg K, et al. Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and alpha-enolase: implications for autoimmunity in rheumatoid arthritis. Arthritis Rheum 2010;62:2662-72.
15. Nesse W, Westra J, van der Wal JE, Abbas F, Nicholas AP, Vissink A, et al. The periodontium of periodontitis patients contains citrullinated proteins which may play a role in ACPA (anti-citrullinated protein antibody) formation. J Clin Periodontol 2012;39:599-607.

16. Cooper PR, Palmer LJ, Chapple IL. Neutrophil extracellular traps as a new paradigm in innate immunity: friend or foe?. Periodontol 2000 2013;63:165-97.
17. White PC, Chicca IJ, Cooper PR, Milward MR, Chapple IL. Neutrophil Extracellular Traps in Periodontitis: A Web of Intrigue. J Dent Res 2016;95:26-34.
18. Vitkov L, Klappacher M, Hannig M, Krautgartner WD. Extracellular neutrophil traps in periodontitis. J Periodontal Res 2009;44:664-72.
19. Neeli I, Khan SN, Radic M. Histone deimination as a response to inflammatory stimuli in neutrophils. J Immunol 2008;180:1895-902.
20. 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.
21. Van der Velden U. The Dutch periodontal screening index validation and its application in The Netherlands. J Clin Periodontol 2009;36:1018-24.
22. Janssen KM, de Smit MJ, Brouwer E, de Kok FA, Kraan J, Altenburg J, et al. Rheumatoid arthritis-associated autoantibodies in non-rheumatoid arthritis patients with mucosal inflammation: a case-control study. Arthritis Res Ther 2015;17:174,015-0690-6.
23. Shechter D, Dormann HL, Allis CD, Hake SB. Extraction, purification and analysis of histones. Nat Protoc 2007;2:1445-57.
24. de Smit M, Westra J, Vissink A, Doornbos-van der Meer B, Brouwer E, van Winkelhoff AJ. Periodontitis in established rheumatoid arthritis patients: a cross-sectional clinical, microbiological and serological study. Arthritis Res Ther 2012;14:R222.
25. Dwivedi N, Upadhyay J, Neeli I, Khan S, Pattanaik D, Myers L, et al. Felty's syndrome autoantibodies bind to deiminated histones and neutrophil extracellular chromatin traps. Arthritis Rheum 2012;64:982-92.
26. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004;50:380-6.
27. van de Stadt LA, van der Horst AR, de Koning MH, Bos WH, Wolbink GJ, van de Stadt RJ, et al. The extent of the anti-citrullinated protein antibody repertoire is associated with arthritis development in patients with seropositive arthralgia. Ann Rheum Dis 2011;70:128-33.
28. Sohn DH, Rhodes C, Onuma K, Zhao X, Sharpe O, Gazitt T, et al. Local Joint inflammation and histone citrullination in a murine model of the transition from preclinical autoimmunity to inflammatory arthritis. Arthritis Rheumatol 2015;67:2877-87.
29. Chirivi RG, Jenniskens GJ, Raats JM. Anti-citrullinated protein antibodies as novel therapeutic drugs in rheumatoid arthritis. Clin Cell Immunol 2013;S6:006. doi:10.4172/2155-9899.S6-006
30. Chirivi RGS, van Rosmalen JWG, Schmets G, van Es H, Raats JMH. Mode of Action of Therapeutic Anti-Citrullinated Protein Antibodies Revealed: Inflammation Reduction Due to Inhibition of Netosis [abstract]. Arthritis Rheumatol 2015;67 (suppl 10).