Background Periodontitis (PD) is the most common chronic inflammatory disease caused by bacterial infection resulting in alveolar bone resorption and tooth loss.1,2 A main causative agent of PD is Porphyromonas gingivalis (Pg).3,4 This periodontopathogen produces peptidylarginine deiminase (PPAD) catalysing conversion of arginine to citrulline.5,6 PD shares common mechanism and risk factors with rheumatoid arthritis (RA). Due to the presence of pathogenic autoantibodies reactive with citrullinated proteins in RA, citrullination of host and bacterial proteins by PPAD was proposed as a mechanistic link between PD and RA.7,9 The aim of this study was to investigate the nature of a novel polymorphism identified in the PPAD gene on bacterial virulence and PD clinical status.

Materials and methods Gingival crevicular fluid (GCF) from 20 patients with PD was plated on blood agar and Pg colonies re-cultured to isolate individual strains of the bacterium. From each strain the PPAD gene was cloned, sequenced and analysed. The native PPAD gene in the reference strain was supplemented with the polymorphic gene. The phenotype of clinical isolates harbouring polymorphism, the mutated and native ATCC33277 strains was examined. Further, periodontal clinical parameters were compared amongst patients infected by Pg expressing PPAD with and w/o polymorphism.

Results A three amino acid polymorphism (G231N, E232T, N235D) was identified in the vicinity of the PPAD catalytic His residue in Pg isolates obtained from 30% of PD patients. The PPAD activity of clinical strains with polymorphism and the ATCC33277 mutant was 2-fold higher in comparison to the reference strain. Gingival fibroblasts infection with strains carrying polymorphic PPAD caused significantly higher upregulation of cyclooxygenase 2 (COX-2) than infection with native ATCC33277. Probing pocket depth (PPD) and clinical attachment (CAL) assessment revealed that patients infected with Pg expressing polymorphic PPAD suffered from more severe disease than those carrying ‘classical’ Pg w/o polymorphism.

Conclusions A three amino acid polymorphism of PPAD augments the virulence of Pg and severity of periodontitis apparently due to higher PPAD activity. The increased citrullination of bacterial and/or host proteins by Pg with the characterised PPAD genotype can trigger autoimmune response in RA.

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antibodies were detected in 41% (n=32) of patients with RA but not in SLE (n=16) and control sera (n=15). In a RA follow-up study (n=30), we detected nearly similar antibody-sensitivities for citrullinated mPPAD before and after onset of RA (13/20%). Only a minority (7%) of RA patients show higher mPPAD antibody levels after RA diagnosis. In the CAIA RA mouse model mPPAD containing Pg. vesicles when injected IP showed a TLR2-dependent protective anti-inflammatory effect like Pg. LPS and Lipomannan. Conclusions Pg. infection and RA disease diagnosis occurs at different time points and Pg. infection induces a TLR2-dependent protective anti-inflammatory effect. We show the first time that mPPAD can citrullinate major human autoantigens internally and their immunologically and diagnostic relevance in RA.

NLRP1 MUTATIONS CAUSE AUTOINFLAMMATORY DISEASES IN HUMAN: IMPLICATION OF THE NLRP1 INFLAMMASOME?

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Background Inflammation is a vital and complex process in response to diverse tissue damaging stimuli such as trauma, injury and pathogen. NLRP1, NLRP3 and NLRC4 belonging to the intracellular proteins Nod like receptor family, are capable of sensing the inflammatory inducers and trigger the assembly of a large complex called the inflammasome. By inducing the caspase-1 activation, inflammasome plays a crucial role in the release of IL-1β and IL-18, two critical cytokines of the initial steps of inflammatory responses.

Whereas mutations in NLRP3 and NLRC4 have been linked to two rare monogenic systemic autoinflammatory diseases (SAIDs), several polymorphisms in the NLRP1 gene have been associated extensively to an increased risk of autoimmune disorders (e.g. vitiligo, psoriasis, type 1 diabetes, and rheumatoid arthritis). We identified for the first time two distinct NLRP1 mutations in patients displaying a novel SAID combining autoinflammation and autoimmunity. We named this disease NAIAD, for NLRP1-associated autoinflammation arthritis and dyskeratosis.

The aim of our study was to unravel how mutation in NLRP1 impaired its function and triggered autoinflammation. Materials and methods Peripheral blood mononuclear cells from patients were analysed to identify the immunologic components involved in these novel diseases, using flow cytometry. The pathogenic effect of the NLRP1 mutations in inflammation was investigated using in vitro functional assays in transfected HEK293T.

Results The level of caspase-1, IL-18 and IL-1β in serum samples from patients was increased as compared to controls and asymptomatic parents. Moreover, patient’s cells displayed constitutive production of IL-1β. Functional studies in HEK293T revealed that the NLRP1 mutations resulted in a constitutive activation of the NLRP1 inflammasome.

Conclusions We demonstrated that two mutations in the NLRP1 gene are involved in autoinflammation in human. This novel disease could be a novel inflammasomopathy combining autoinflammatory and autoimmune features. Our data, combined with that in the literature, highlight the pleomorphic role of NLRP1 in inflammation and immunity.

THE MUTATED RNA SPLICING PROTEIN HNRNP-A3 IS A NOVEL AUTOANTIGEN IN SYSTEMIC RHEUMATIC DISEASES A LINK TO WARBURG EFFECT IN RA

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Objective Novel mutated hnRNP-A3 (MA3) was cloned out of RA synovial tissue involved in alternative splicing of PK2 linking it directly to Warburg effect and lactate production in RA. Methods After immunoblotting and 2D-gel-electrophoresis out of a semipurified hnRNP fraction two protein spots were sequenced and identified to be highly similar to hnRN-A3. hnRNP-A3 variants were cloned from RA synovial-tissue. 3700 RA sera were screened for the presence of mutated anti-hnRNP-A3 autoantibodies using recombinant proteins and mutated citrullinated A3 peptides (MCA3) thereof. Identification of RNA and antibody binding sites to hnRNP A3 (MA3). Expression of hnRNP-A3 in synovial tissue was analysed by immunohistochemistry.

Results Autoantibodies to MA3 protein were detected in 13% of RA (n=215) patients, in 9% SLE (n=154), in 27% of MCTD patients (n=44/10) and in less than 5% of 129 patients with other rheumatic disorders but not at all in healthy controls on immunoblot. When using native MA3-ELISA 22% of early RA patients (n=130) were detected and 87% of these patients had erosive arthritis. Identical modification on MA3 as in cancer cells were identified in synovial tissue and verified by MS and DNA sequencing. Using 2–3 citrullinated MCA3 peptides up to 81% of patients (n=150) with established and 67% (n=2926) of patients with an early RA with a specificity of 97% were detected. In early RA 27% and 25% in established RA of CCP2 negative and 93% of CCP2 positive patients were identified.

By combining with the already established CCP2 and the new MCA3, 72% of early patients are positive. MCA3 autoantibodies predominantly occur (p<0.001) in an erosive, severe course of disease. MRL Lpr/lpr sera were hnRNPA3 reactive and the antibody generation is Toll 7 and 9 dependent. Anti-hnRNPA3 antibodies are directed to conformational RNA binding epitopes. Expression of hnRNP-A3 revealed the antigen is overexpressed in RA synovial tissue. Conclusion Mutated hnRNP-A3 is as a novel Toll7/9 dependent autoantigen in systemic rheumatic diseases. These mutated proteins are components of RNA and DNA containing alternative splicing complexes leading to the Warburg effect and predominantly occurring in an erosive and severe courses of RA.