Periodontitis and rheumatoid arthritis

de Smit, Menke

rijksuniversiteit groningen

PERIODONTITIS AND RHEUMATOID ARTHRITIS

A search for causality and role of *Porphyromonas gingivalis*

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Menke Janke de Smit

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Promotores	Prof. dr. A.J. van Winkelhoff Prof. dr. A. Vissink
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Paranimfen	Ing. B. Doornbos-van der Meer Dr. R.Z. Thomas

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INTRODUCTION TO THE THESIS

Periodontitis

Periodontitis is a chronic inflammatory disease that leads to destruction of the soft and hard tissues supporting the teeth (the periodontium). If left untreated, advanced periodontitis may ultimately result in loss of teeth. The essential role of dental plaque in the etiology of periodontitis has been well established by studies that have shown that removal of supra- and subgingival dental biofilm normally results in disease resolution. Extensive microbial composition analyses have identified oral bacteria, such as *Porphyromonas gingivalis,* as strong markers of disease status.
Periodontal health requires a controlled immuno-inflammatory state that can maintain host–microorganism homeostasis in the periodontium [1]. However, in periodontitis, the host immune response is deregulated - either because it is subverted by the microbial community or because of host immunoregulatory defects- and is therefore ineffective at restraining bacterial outgrowth and overt pathogenicity [2].

Periodontitis is not a local phenomenon

Periodontitis is not only a cause of tooth loss but has also been shown to affect systemic health [3]. It is linked to the initiation, progression and/or disease activity of systemic autoimmune or inflammatory diseases including diabetes, cardiovascular disease, inflammatory bowel disease and rheumatoid arthritis [4, 5]. However, it is yet difficult to determine whether periodontitis is a cause or a consequence of these complex and multifactorial diseases. Before periodontitis can be admitted into the causal chain of a disease, the evidence of the association of periodontitis with a particular disease has to be extremely high. In addition, the relationship can be bidirectional because of common environmental and genetic risk factors and parallel pathogenic pathways [6].

Association with rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease that is characterized by chronic destructive polyarthritis of synovial joints. Immune dysfunction causes accumulation of destructive pro-inflammatory mediators in the synovial membrane leading to synovitis and destruction of cartilage and bone tissue of the joint.
In the past decade, the interest in the epidemiological and pathological relationships between periodontitis RA has been rising, driven in part by interest in the role of citrullination and attendant autoantibody responses as a disease-defining feature of RA, and the recognition that oral bacteria and inflammation may play important roles [7]. Citrullination is a post-translational modification catalyzed by a family of enzymes called peptidylarginine deiminases (PAD) [8]. In this reaction, an arginine residue within a protein is converted into the non-coded amino acid citrulline. This modification leads to a loss of positive charge, reduction in hydrogen-bonding ability and subsequently in conformational and functional changes of the protein. Citrulline is not a natural amino acid in proteins and may therefore induce an immune response. Citrullination is involved in several physiological processes including terminal differentiation of the epidermis, apoptosis, and gene expression regulation, but it has also been implicated in pathological processes and autoimmunity like in RA [9].

Autoimmunity in periodontitis

Autoimmune reactions to native and post-translationally modified self-antigens may play a role in the pathogenesis of aggressive periodontitis [10]. Overproduction of reactive oxygen species (ROS) within the inflamed lesion, as a result of influx of oxygen consuming inflammatory cells, leads to post-translational modification by ROS [11]. Furthermore, break of tolerance may be initiated by enzymatic post-translational modification, for example, cleavage of extracellular proteins by matrix metalloproteases [12], bacterial proteases [13] or citrullination by PAD enzymes [14]. The exact etiology of autoimmune reactivity in periodontitis is not known, but may be linked to the inflammatory process resulting from infection with *P. gingivalis*, which expresses both arginine-specific proteases (gingipains) and PAD [10, 15].

Inflammation and auto-immunity

Chronic inflammation as a result of infection may play a role in the initiation, progression, and perpetuation of chronic autoimmune diseases. Although many other factors are necessary to develop autoimmunity, a number of mechanisms have been postulated by which infection can trigger autoimmune disease. Infectious agents might induce T- or B-cell responses that can cross-react with self antigens, due to similarities between microbial and self-proteins or peptides that are sufficient to result in the activation of auto-reactive T- and B- cells (molecular mimicry). In addition, microbial processing of self peptides, such as posttranslational modification, can result in 'foreign' antigens or antigens that activate cross-reactive T- or B- cells (cryptic antigens). Activation of the cross-reactive T- and B- cells results in the release of cytokines and chemokines that recruit and activate monocytes and macrophages, which mediate self-tissue damage, cell apoptosis and/or necrosis (bystander activation) and enhanced processing and presentation of self-antigens (epitope spreading) (see Fig. 1, page 12, reused from [16]).

Self-reactive T-cells in RA

The human leukocyte antigen (HLA) system is the locus of genes that encodes for proteins on the surface of cells (the major histocompatibility complex, MHC), and is responsible for regulation of the immune system in humans. The major function of MHC is to bind to peptide fragments derived from pathogens and display them on the cell surface of an antigen-presenting cell (APC) for recognition by the appropriate T-cells via the T-cell receptor (TCR). In RA, HLA is considered to be the major genetic factor determining disease susceptibility because MHC has to interact with citrullinated self-peptides with a certain affinity in order to elicit an antigen-specific immune response. In 1987 a 'shared epitope hypothesis' was postulated for the association of particular MHC-II molecules and RA [17]. The shared epitope is located in one of the substrate binding sites (the P4 pocket) of the MHC class II molecule in the HLA-DR β-chain. Individuals carrying positively charged P4 pockets (with shared epitope of HLADRB*0101, *0401 or *0404) can mount an immune response to citrullinated peptides and are susceptible to RA [18]. In contrast, those who express negatively charged P4 pockets (HLA-DRB*0402) might be protected from the disease [19].

AIM AND OUTLINE OF THE THESIS

A search for causality and role of Porphyromonas gingivalis

The aim of the thesis was to assess possible causality in the association between periodontitis and RA, with the focus on the role of *P. gingivalis*. Causality was analyzed with assistance of the Bradford Hill criteria [20]. These criteria have been designed to be considered before deciding that the most likely interpretation of an association between two diseases is causation. The criteria to be considered involve: strength and consistency of the association, biological plausibility, temporal relationship, specificity regarding to *P. gingivalis*, dose-response relationship, experimental evidence and coherence of clinical findings, and analogy.

In **chapter 1**, we started our research by summarizing at the time present available knowledge and hypotheses regarding the disease association between periodontitis and RA.
In **chapter 2**, epidemiological data are presented from a population of RA patients of the northern part of the Netherlands. In this study several Bradford Hill criteria were considered, including strength and consistency of the association between RA, periodontitis, and *P. gingivalis*, and dose-response relationship, i.e., whether severity of periodontitis is linked to severity of RA.
In **chapter 3**, temporal relationship, i.e., cause (infection with *P. gingivalis*) precedes consequence (RA), was investigated by assessment of the antibody response against *P. gingivalis* in a cohort of patients at risk for RA and who were prospectively followed for RA development.

Because RA auto-antibodies often precede clinical signs of RA and because of the assumption that mucosal inflammation, such as periodontitis, can precede RA, in **chapter 4**, presence of RA auto-antibodies was assessed in patients without RA but with oral (periodontitis) or lung mucosal inflammation.
Objective of the study in In **chapter 5** was assessment of *P. gingivalis* PAD gene expression and citrullination patterns in representative samples of *P. gingivalis* isolates from patients with and without RA and in related microbes of the *Porphyromonas* genus. In **chapter 6**, available evidence for possible causality between periodontitis, *P. gingivalis* and RA, according to the Bradford Hill criteria, is summarized.

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Figures

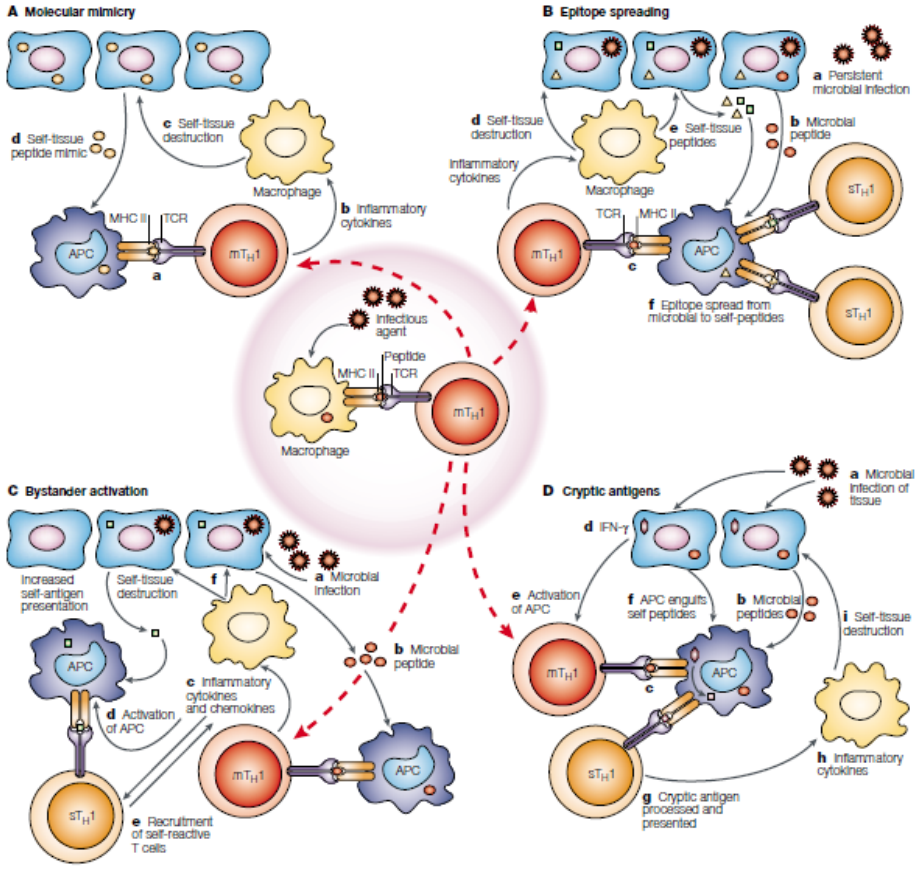

Fig. 1 Mechanisms of infection-induced autoimmunity via self-reactive T-cells. Reused from [16].

A. Molecular mimicry displayed as the activation of cross-reactive TH1 cells that recognize both the microbial epitope (mTH1) and the self epitope (sTH1) (a). Activation of the cross-reactive T-cells results in the release of cytokines and chemokines (b) that recruit and activate monocytes and macrophages, which mediate self-tissue damage (c). The subsequent release of self-tissue antigens and their uptake by APCs perpetuates the autoimmune disease (eptitope spreading, see also figure B)

B. Epitope spreading involves a persistent microbial infection (a) that causes the activation of microorganism-specific TH1 cells (b, c) which mediate self-tissue damage (d). This results in the release of self peptides (e), which are engulfed by APCs and presented to self-reactive TH1 cells (f). Continuous damage and release of self peptides results in the spread of the self-reactive immune response to multiple self-epitopes (f).

C. Bystander activation is the nonspecific activation of self-reactive TH1 cells. Activation of microorganism-specific TH1 cells (a, b) leads to inflammation (c, d) and results in the increased infiltration of T-cells at the site of infection and the activation of self-reactive TH1 cells by TCR-dependent and -independent mechanisms (e) Self-reactive T-cells activated in this manner mediate self-tissue damage and perpetuate the autoimmune response (f).

D. Cryptic antigen model describing the initiation of autoimmunity by processing of self peptides, such as post-translation modification. Following microbial infection (a) pro-inflammatory cytokines are secreted by both activated microbe-specific TH1 cells (b, c) and microbe-infected tissue cells (d). This activates APCs (e) and can lead to APC engulfing self-antigens (f). Cytokine activation of APCs can induce increased protease production and different processing of captured self-antigens, resulting in presentation of cryptic epitopes. The presentation of these cryptic epitopes can activate self-reactive TH1 cells (g), leading to self-tissue destruction (h, i).

TH1: T helper 1 cell that recognize the microbial epitope (mTH1), the self epitope (sTH1) or both (cross-reactive TH1), APC: antigen-presenting cell, MHC II: major histocompatibility complex class II, TCR: T-cell receptor.