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FROM ORAL INFECTION TO AUTOIMMUNITY: STUDIES OF ANTIBODIES AND B CELLS ON THE PATH TOWARDS RHEUMATOID ARTHRITIS

Charlotte de Vries

Karolinska Institutet

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From oral infection to autoimmunity: studies of antibodies and B cells on the path towards rheumatoid arthritis

Thesis for Doctoral Degree (Ph.D.)

By

Charlotte de Vries

The thesis will be defended in public at Center for Molecular Medicine (CMM) Lecture hall, L8:00, Karolinska University Hospital, Solna. Friday January 12, 2024, at 9:00

Principal Supervisor:
Associate Professor Karin Lundberg
Karolinska Institutet
Department of Medicine
Division of Rheumatology

Co-supervisors:
Professor Vivianne Malmström
Karolinska Institutet
Department of Medicine
Division of Rheumatology

Dr Aase Hensvold
Karolinska Institutet
Department of Medicine
Division of Rheumatology

Opponent:
Associate Professor Kulveer Mankia
University of Leeds
Leeds Institute of Rheumatic and Musculoskeletal Medicine

Examination Board:
Professor Meliha Kapetanovic
Lund University
Department of Clinical Sciences

Professor Karin Loré
Karolinska Institutet
Department of Medicine, Solna

Associate Professor Kåre Buhlin
Karolinska Institutet
Department of Dental Medicine

Voor papa

Popular science summary of the thesis

This thesis is about the autoimmune disease rheumatoid arthritis, in short RA. RA patients have pain in their joints, and although there are treatments, it is not possible to cure patients with RA, and so is the case for many autoimmune diseases. Autoimmune diseases are characterised by a dysregulated immune system. In a healthy body, the immune system fights invaders, like viruses and bacteria. Important players in this process are antibodies, which can specifically bind to invaders and clear them out. Antibodies are produced by B cells. In the case of RA, the B cells produce antibodies that are not directed to any virus or bacteria, so we can say that the immune system is overactive: it produces antibodies that are not necessary and can even be destructive. These antibodies are called **auto**antibodies because they attack the body itself. Remarkably, autoantibodies can already be detected in the blood years before a patient develops RA.

Another facet is the daily exposure of the body to many microbes like viruses and bacteria. For example, we have a lot of bacteria living on our skin, but also in the mouth and the gut. Interestingly, our body does not attack those bacteria, because we need them. But, when the bacterial composition is out of balance, or there are bacterial species present that should not grow there, we get an immune response. My thesis focuses on the bacteria in the oral cavity. A disbalance of the bacteria in the mouth can lead to periodontitis, a very common disease.

Periodontitis and RA are linked: patients with RA more often have periodontitis and vice versa. However, it is not yet known how exactly these two diseases are linked and therefore I have studied their relationship. This was done by investigating the antibody response against one of the key bacteria in the development of periodontitis. I measured these antibodies in healthy controls, patients with periodontitis, patients with RA, and in people at increased risk for developing RA. I specifically examined the anti-bacterial antibody response in relation to autoantibodies. By doing this, we now know that antibody levels to this oral bacterium are higher in patients with very severe periodontitis and associate with presence of autoantibodies. Moreover, we can confirm that this anti-bacterial antibody response is increased in RA patients compared to controls, and in at-risk (of RA) individuals. These findings suggest that the link between periodontitis and RA is already established many years before people develop symptoms of RA. This link may be explained by oral bacteria and the generation of autoantibodies.

Apart from studying antibodies, I have also studied B cells. B cells are the producers of the autoantibodies and are therefore important in RA. What this thesis work shows, is that in the gingival tissue of a patient with RA, there are B cells that can produce autoantibodies. Finding these cells in the gingiva suggests that the dysregulated immune response is triggered in the oral cavity. We also found that B cells in the blood of the at-risk individuals have started to change and look more like B cells we find in patients with RA than those found in healthy individuals. This means that the immune system is already to some extent dysregulated before arthritis starts, and the fact that we can detect these changes is indicating that the B cells and antibodies are important players. This opens a window of opportunity for interventions before

RA onset, with an aim to stop pathogenic immune processes that are linked to RA. This thesis work has brought us one step closer to understanding the relationship between oral pathogens, periodontitis and RA, and the dysregulated immune response.

Abstract

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by inflammation of the synovial joints, which can lead to irreversible joint destruction and disability if not treated properly. The majority of patients are seropositive, defined by presence of autoantibodies, *i.e.*, rheumatoid factor (RF) and/or anti-citrullinated protein antibodies (ACPA). My thesis work focuses on ACPA+ RA, which is known for its more severe disease course. ACPA can be detected in the blood years before clinical signs of RA and were for a long time suggested to contribute to pathology, an hypothesis which is currently under debate. Still, the presence of ACPA, and the successful use of B-cell depleting therapies in RA, points to an important role for autoantibodies and B cells in the development of RA.

Notably, most known risk factors for RA, in particular smoking and *HLA-DRB1* shared epitope (SE) alleles, are specifically linked to onset of ACPA+ RA. My studies have investigated another potential risk factor for RA, namely the oral pathogen *Porphyromonas gingivalis (Pg)*, one of the main drivers of periodontitis (PD). PD is a common disease that is driven by dysbiosis in the oral cavity triggering gingivitis and eventually leads to destruction of the jawbone and tooth-supporting surrounding soft tissues. PD has a higher prevalence in RA than in the general population. Interestingly, *Pg* has the unique characteristic to express its own citrullinating enzyme and has therefore been suggested to contribute to the generation of RA autoantigens, break of tolerance and systemic ACPA production.

The overall aims of my thesis were: 1) to determine if presence of antibodies against the *Pg* virulence factor RgpB could serve as biomarker to identify patients with PD at increased risk for systemic autoimmunity and onset of RA; 2) to explore the gingiva as a site for ACPA production, and *Pg* as a driver of the ACPA response; and 3) to phenotypically characterise peripheral blood B cells in the risk-RA phase, to understand B-cell dysregulation prior to RA onset.

Investigating the anti-Rgp IgG response in two PD cohorts showed that this antibody response could only poorly discriminate PD from controls. However, elevated anti-Rgp IgG levels defined a subset of PD patients with active gingivitis and advanced marginal jawbone loss. We also showed a higher prevalence of ACPA+ individuals in PD versus controls, and higher anti-Rgp IgG levels in ACPA+ versus ACPA- individuals. Moreover, in a prospective study of ACPA+ individuals at increased risk for RA we found significantly higher anti-Rgp IgG levels compared to controls, but antibody levels did not differ between those who progressed to arthritis and those who remained arthritis free.

Generation of monoclonal antibodies derived from RA gingival tissue B cells demonstrated the presence of citrulline-reactive clones binding epitopes on both *Pg* and self-proteins, and this cross-reactivity was also shown for an RA peripheral blood-derived ACPA+ clone. Investigating the serum polyclonal response, 11% of patients with early RA were positive for antibodies targeting a citrullinated *Pg* peptide.

When assessing peripheral blood B cells in ACPA+ Risk-RA individuals, we detected dysregulation of B-cell subsets already before clinical onset, specifically showing loss of CD27 on class-switched IgG+ memory B cells, a feature previously described in autoimmunity.

Collectively, these studies can link anti-*Pg* antibodies – as a proxy for *Pg* infection – to severe forms of PD and to the ACPA response, but not to arthritis onset. Thus, suggesting *Pg*-infection could be an early event in the natural history of RA, possibly triggering ACPA production in the gum mucosa by mechanisms of molecular mimicry. Moreover, the detection of B-cell changes already in the at-risk phase, supports the use of immune monitoring to capture individuals at risk of RA onset, that may benefit the most from pre-clinical intervention.

List of scientific papers

I. **Antibodies to *Porphyromonas gingivalis* Are Increased in Patients with Severe Periodontitis, and Associate with Presence of Specific Autoantibodies and Myocardial Infarction.** de Vries C[*], Ruacho G[*], Kindstedt E, Potempa BA, Potempa J, Klinge B, Lundberg P, Svenungsson E, Lundberg K.
J Clin Med. 2022 Feb 15;11(4):1008

II. **Antibodies to *Porphyromonas gingivalis* gingipains associate with gingival inflammation and advanced marginal jawbone loss – data from the PerioGene North case-control study.** Kindstedt E, de Vries C, Wänman M, Potempa BA, Potempa J, Lindquist S, Esberg A, Lundberg K, Lundberg P.
Manuscript

III. ***Porphyromonas gingivalis* associates with presence of anti-citrullinated protein antibodies, but not with onset of arthritis – studies in an at-risk population.**
de Vries C, Cîrciumaru A, Potempa BA, Potempa J, Lundberg K[#], Hensvold A[#].
Manuscript

IV. **Antibodies to a Citrullinated *Porphyromonas gingivalis* Epitope Are Increased in Early Rheumatoid Arthritis, and Can Be Produced by Gingival Tissue B Cells: Implications for a Bacterial Origin in RA Etiology.** Sherina N, de Vries C, Kharlamova N, Sippl N, Jiang X, Brynedahl B, Kindstedt E, Hansson M, Mathsson-Alm L, Israelsson L, Stålesen R, Saevarsdottir S, Holmdahl R, Hensvold A, Johannsen G, Eriksson K, Sallusto F, Catrina AI, Rönnelid J, Grönwall C, Yucel-Lindberg T, Alfredsson L, Klareskog L, Piccoli L, Malmström V, Amara K, Lundberg K.
Front Immunol, 2022 Apr 20;13:804822

V. **B-cell changes in the at-risk phase leading up to ACPA-positive rheumatoid arthritis.** de Vries C, Huang W, Sharma RK, Turcinov S, Argyriou A, Horuluoglu B, Wangriatisak K, Cîrciumaru A, Rönnblom L, Grönwall C, Malmström V[#], Hensvold A[#], Lundberg K[#].
Manuscript

*These authors contributed equally and share first authorship
[#]These authors contributed equally and share senior authorship

Scientific papers not included in the thesis

I. **Anti-Citrullinated Protein Antibody Reactivity towards Neutrophil-Derived Antigens: Clonal Diversity and Inter-Individual Variation.** Cîrciumaru A, Gomes Afonso M, Wähämaa H, Krishnamurthy A, Hansson M, Mathsson-Alm L, Keszei M, Stålesen R, Ottosson L, de Vries C, Shelef MA, Malmström V, Klareskog L, Catrina AI[†], Grönwall C, Hensvold A, Réthi B.
*Biomolecules (*2023), 13(4), 630

Contents

List of abbreviations

Aa	*Aggregatibacter actinomycetemcomitans*
ACPA	anti-citrullinated protein antibody
ACR	American College of Rheumatology
ADCC	antibody-dependent cell-mediated cytotoxicity
AMPA	anti-modified protein antibody
ANOVA	analysis of variance
ANCA	anti-neutrophil cytoplasmic antibody
AU	arbitrary units
AUROC	area under ROC
BAFF	B cell activating factor
BAL	bronchoalveolar lavage
BCR	B cell receptor
BM	bone marrow
BOP	bleeding on probing
BSA	bovine serum albumin
CAR	chimeric antigen receptor
CCP2	cyclic citrullinated peptide
CII	collagen type II
COVID-19	coronavirus disease 2019
CPP3	citrullinated peptide derived from *Pg* PAD
CRP	C-reactive protein
CSR	class switch recombination
CV	coefficient of variation
DMARD	disease modifying anti-rheumatic drug
DN	double negative

dsDNA	double stranded DNA
EIRA	Epidemiological investigation of RA
ELISA	enzyme linked immunosorbent assay
ESR	erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
Fab	fragment antigen-binding
FACS	fluorescence-activated cell sorting
Fc	fragment crystallizable
GC	germinal center
GT	gingival tissue
HC	healthy control
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
hnRNP	heterogenous nuclear ribonucleoprotein
Ig	immunoglobulin
IL	interleukin
JAK	Janus kinase
Kgp	lysine gingipain
LPS	lipopolysaccharide
MAA	malondialdehyde aceetaldehyde
mAb	monoclonal antibody
MDA	malondialdehyde
MHC	major histocompatibility complex
MI	myocardial infarction
NET	neutrophil extracellular trap
NF-kB	nuclear factor kappa B
NSAID	non-steroidal anti-inflammatory drug

PAD	peptidylarginine deiminase
PAMP	pathogen associated molecular pattern
PAROKRANK	Periodontitis and Its Relation to Coronary Artery Disease
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PD	periodontitis
Pg	*Porphyromonas gingivalis*
PGN	PerioGene North
PTPN22	protein tyrosine phosphatase non-receptor type 22
PPD	pocket probing depth
RA	rheumatoid arthritis
RAG	recombination activating genes
RANK	receptor activator of NF-kB
RF	rheumatoid factor
Rgp	arginine gingipain
ROC	receiver operating characteristic
scRNA-seq	single cell RNA sequencing
SDS-PAGE	sodium dodecyl-sulphate polyacrylamide gel electrophoresis
SE	shared epitope
SHM	somatic hypermutation
SLE	systemic lupus erythematosus
SNP	single nucleotide polymorphism
TCR	T cell receptor
Tfh	T follicular helper cell
TLR	toll like receptor
TNF	tumour necrosis factor
Tph	T peripheral helper cell

1 Introduction

The human immune system is a diverse and potent system that has been protecting us against pathogens throughout evolution. One of the most important features is that it can distinguish between self and non-self. Failure of the immune system can lead to severe diseases of different kinds, varying from absence of an immune response to self-destruction as a result of an overreactive immune system.

Throughout history, humankind has faced different types of challenges when looking from an immunological perspective. Before the introduction of vaccines, the mortality rate among children was about 50%, mostly due to infections(1). Furthermore, pandemics have had large consequences for societies, with the black plague (caused by the bacterium Yersinia pestis), being considered the most fatal pandemic recorded in human history(2). Since Edward Jenner developed the first form of vaccination, humankind has become only better and better at understanding, preventing, and treating infectious diseases. The fact that we can help and train our immune system has prevented the death of many people worldwide. However, the assumption that since the discovery of vaccines, the threat coming from infectious diseases is less relevant in our current society could not be further from the truth. The coronavirus disease 2019 (COVID-19) pandemic taught us that in our globalized and urbanized world it is hard to contain infectious diseases and most likely new infectious threats will arise due to climate change(3).

As another consequence of climate change the biodiversity is decreasing, in its turn leading to a change in our commensal microbiome. The microbiome plays a vital role in training and shaping our immunological tolerance and changes in the microbiome can alter our general antigen exposure, challenging the tolerance of our immune system(4). Whereas the worldwide morbidity and mortality due to infectious diseases has declined, the incidence of autoimmune diseases has been steadily increasing(5). Declining deaths due to infection increases life expectancy and thus it is not surprising that in the ageing population the incidence of autoimmune diseases is rising(5). Taken together, increasing age, altered antigen exposure, and potential new infectious threats will be challenging our immune system in the coming decades(6, 7).

With this in mind, the importance of understanding our evolving immune system in health and disease is and will be of great relevance. In this literature review, I will describe the basic principles of immunity and tolerance, with a focus on the autoimmune disease rheumatoid arthritis and the role of B cells and antibodies.

2 Literature review

2.1 Immunity in health and disease

The human immune system is a vital part of the human body, with its main function to protect the body from invaders such as bacteria, viruses, fungi, and parasites. The initial line of defence against infections are the epithelial barriers, followed by the players of the innate immune system, like neutrophils, monocytes, macrophages, and natural killer cells. In contrast to the innate immune system, which responds fast but is mostly nonspecific, the adaptive immune response mediated by B- and T- lymphocytes targets antigens in a specific, adapted manner and over time builds up a memory to previous antigen exposures. One of the most important features of the immune system is the ability to distinguish self from non-self. This discrimination allows protection from invaders while maintaining tolerance to the body´s own tissues. Another critical feature of the immune system is that immune cell activation and deactivation is tightly regulated. Failure of this system could lead to under- or overactivity, with the possibility to induce a wide range of diseases.

2.1.1 The innate immune system

A fast response is the key feature of the innate immune system. The first line of defence is formed by the skin and mucosal tissues, which physically separate the outside world from the inside of the body. Next, innate immune cells are ready to act fast when pathogens break through the barriers. The innate, also called “nonspecific”, immune system is able to respond quickly due to its nonspecific way of action. Immune cells can detect common structures shared by invaders, the so-called pathogen associated molecular patterns (PAMPs), for example molecules that are parts of bacterial cell walls. Detection of PAMPs occurs via pathogen recognition receptors, for example toll-like receptors (TLR), which are mainly expressed on the surface of antigen presenting cells like macrophages and dendritic cells but also by monocytes, neutrophils, and epithelial cells. Furthermore, the complement system, first described as a system “complementing” the antibody response, plays a vital role in opsonization, activation of inflammation and direct killing of microbes. The complement system consists of small proteins in the plasma and acts through three main routes: the classical pathway, the mannan-binding lectin pathway, or the alternative pathway. Each of the pathways consists of a cascade of protein bindings, eventually all contributing to clearance of the pathogen. During the time the innate immune system deals with the invading pathogen, the adaptive immune system has time to establish a more specific immune response.

Communication between different (immune) cells of both the innate and adaptive immune system occurs via secretion and recognition of chemokines and cytokines. Cytokines function as messengers between different cells and can have either pro- or anti-inflammatory properties. Chemokines act as inducers of cell migration towards sites of inflammation or lymphoid structures, and act mostly on leukocytes.

2.1.2 The adaptive immune system

The adaptive immune system gives rise to specific cell-mediated immune responses aimed at eradicating infections. In brief, activation of the adaptive immune response occurs when peptide antigens from pathogens are presented to T cells – in the form of short amino acid sequences bound to major histocompatibility complex (MHC) proteins – by antigen-presenting cells like B cells, dendritic cells, and macrophages. Interaction between activated antigen-specific T cells and antigen-specific B cells then leads to activation of B cells and eventually to the production of antibodies. Another essential element of adaptive immunity is the ability to generate a sustained immunological memory. Circulating memory B and T lymphocytes allow the immune system to respond quickly upon re-infection. In addition, long-lived plasma cells, residing in the bone marrow (BM) and producing high-affinity antibodies, are the main source of antibodies in the circulation(8). To achieve an effective and specific response, generation and maturation of B and T cells, the key players of the adaptive immune system, are tightly regulated. Immunologic tolerance, defined by unresponsiveness to self-antigens, is heavily dependent on central and peripheral selection mechanisms.

2.1.3 B cells

B cells are fundamental to the function of the adaptive immune system, with a critical part being their potential to be both highly specific as well as very diverse. To obtain a diverse repertoire, B-cell maturation and differentiation is a tightly regulated process. In the BM, hematopoietic stem cells develop into pro-B cells expressing CD19, and upon upregulation of recombination activating genes (RAG) turn into pre-B cells, which give rise to immature B cells. An essential part of this process is the rearrangement of the B-cell receptor (BCR). Expression of RAG induces recombination of the variable (V), diversity (D) and joining (J) domains(9), that together form the variable region of the heavy chain of the BCR. Next, the recombination of kappa or lambda light chain follows, consisting of only a V and J domain. Together, the heavy and light chains will form a surface BCR in the form of immunoglobulin M (IgM), that characterizes the state of an immature B cell(10). VDJ recombination is an iterative process that results either in a productive rearrangement, or in an unproductive rearrangement. In the latter scenario, the light chain rearrangement will be repeated using the second chromosome and lead to a productive combination or the B cells will go into apoptosis(11). This process of receptor editing will result in a lower chance of a potentially harmful autoreactive B cell leaving the BM. If an autoreactive B cell did not go into apoptosis or undergo receptor editing, it can become an anergic B cell, being characterized by reduced receptor expression and becoming unresponsive (anergic).

Within the BM compartment, many autoreactive B cells will be deleted through central tolerance mechanisms, like BCR editing, clonal deletion and positive selection(12-14). After going through the central tolerance mechanisms as described above, still about 20% of the naïve B cells retain low levels of autoreactivity(15). A second selection step takes place in the periphery when B cells become mature. In the circulation we find both naïve and antigen-experienced B cells. When a B cell recognizes its antigen, it will migrate to the germinal center (GC) where it goes through multiple rounds of GC reactions. Here, class switch recombination

(CSR) takes place; the isotype of the BCR changes. In the dark zone of the GC, the BCR undergoes the process of somatic hypermutation (SHM), increasing the affinity for the antigen, resulting in increased survival chances. In the light zone, B cells interact with follicular helper T cells (Tfh) and undergo selection for having a high-affinity BCR(16). Signals from T cells are essential for the survival of a B cell in the GC. When a mature B cell recognizes self-antigen, it will not receive T cell help, due to T-cell tolerance, and thereby the B cell becomes either anergic, apoptotic, or its activation is inhibited. However, when the B cell receives T-cell help, the antigen-specific B cell can migrate to the circulation as a plasmablast, being able to secrete antibodies, or as a memory B cell, ready to act upon re-infection. Plasmablasts tend to migrate to the bone marrow or to mucosal sites where they can live for years as long-lived plasma cells.

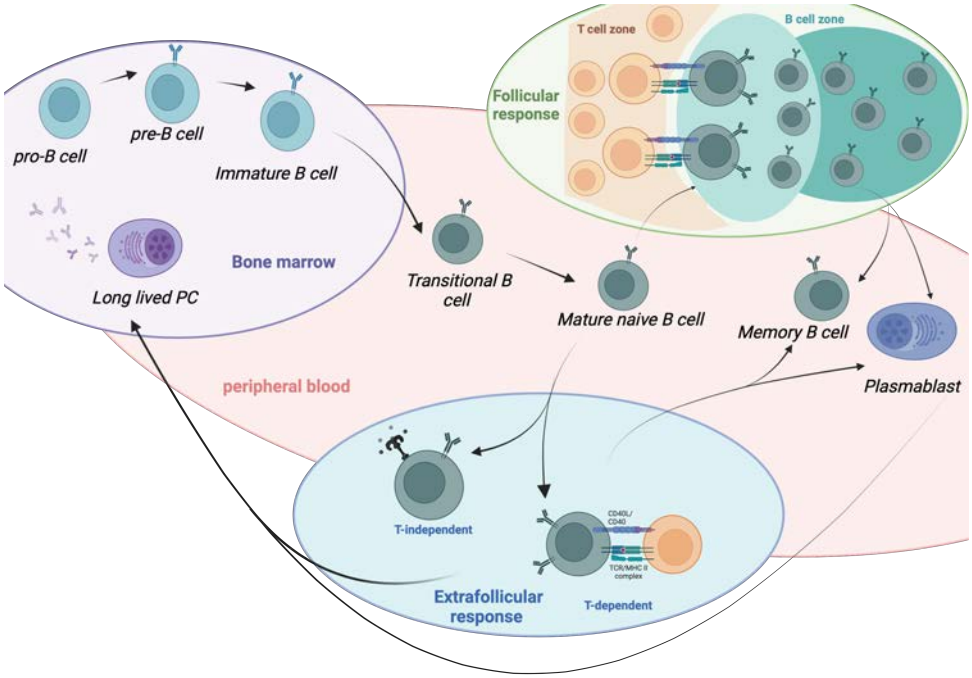

Figure 1. **B-cell maturation and differentiation.** B cells are generated in the bone marrow (top left) and once they transition to the peripheral blood, they will mature and become naive B cells. Mature naive B cells can then upon antigen recognition either migrate into the follicle (top right) or go into an extrafollicular response (bottom). Whereas the extrafollicular response can be T-cell dependent or T-cell independent, the follicular response is always T-cell dependent. After one of these responses, B cells either enter the circulation as memory B cell or become a plasmablast. Finally, B cells can become long-lived plasma cells and migrate back to the bone marrow where they can reside for many years.

2.1.4 T cells

T lymphocytes were firstly described to be the helpers of B cells. They are crucial in mediating antibody responses and activation of other immune cells. Two large categories of T cells are the CD4 T cells, also called T-helper cells, which recognize extracellular peptides presented on MHC class II by the so-called professional antigen-presenting cells, and CD8 T cells – the cytotoxic T cells involved in anti-viral responses, cancer and other (chronic) infections – that recognize intracellular peptides presented on MHC class I, expressed by all nucleated cells. T-cell progenitors arising from the bone marrow home to the thymus where they undergo T-cell receptor (TCR) rearrangement. Based on the affinity of the TCR for self-peptide-MHC complexes, T cells will either die by neglect, differentiate into CD4 or CD8 T cells or go through selection mechanisms to ensure tolerance, that include clonal deletion, receptor editing and anergy(17). Self-reactive T cells that have escaped central tolerance mechanisms will, in a healthy individual, be regulated through peripheral tolerance mechanisms.

2.1.5 Immunoglobulins

About 70% of the serum immunoglobulins (Ig) consists of IgG, followed by IgA (15-20%), and IgM being the smallest fraction making up only a few percent. IgG are subdivided into four different subclasses, IgG1-IgG4, having differences in their constant regions(18). Differences occur mostly in the hinge-region which is responsible for the flexibility of the antibody and thus affects the antigen-binding properties as well as antibody effector functions(18). Immunoglobulins have different effector functions, divided into four modes of action: neutralization, opsonization, complement activation and antibody-dependent cell-mediated cytotoxicity (ADCC). Firstly, antibodies can directly neutralize toxins, microorganisms, and their virulence factors. Second, they have activating functions, as they can activate neutrophils and macrophages for the process of opsonization, and activation of the complement system to induce phagocytic clearance or generation of chemoattractants. ADCC is the process of cytotoxicity, as a result of antibody crosslinking with the fragment crystallizable region (Fc) receptor, resulting in cell death(19).

While IgG is the most abundant isotype in serum, different compartments of the body have different isotype compositions. Mucosal surfaces for example have IgA as the dominant isotype. The secretory IgA, that is an IgA dimer secreted at the mucosal tissues, plays an important role in microbiota homeostasis(20). The monomeric serum IgA has pro-inflammatory properties and is suggested to play a role in different autoimmune diseases, including RA(21, 22). IgA appears in two different subclasses, and whereas IgA1 is the most abundant in serum, IgA1 and IgA2 abundance at mucosal surfaces is more equal. The IgM antibody occurs predominantly in a pentameric form and is in a typical humoral immune response the antibody that is produced first.

Antigen binding and antibody structure are important features for the effectiveness of immunoglobulins and can be modulated by glycosylation. Glycosylation is a process where carbohydrates are covalently bound to the target molecule, in this case the antibody. The glycan can be attached to an asparagine residue (N-linked glycosylation) or to serine or

threonine (O-linked glycosylation)(23). Whereas all IgG molecules carry N-linked glycans in their Fc region, about 15-25% of human IgG also carry glycans on the fragment antigen-binding (Fab) region(24). N-linked glycosylation sites in the Fab region are almost always introduced during somatic hypermutation. Importantly, increased levels of N-linked Fab glycosylation have been shown to be present on autoantibodies that appear in different autoimmune rheumatic diseases like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis(25-27). Glycosylation of the variable domain can affect antibody binding to its cognate antigen. It has been shown that depending on the location and the number of N-linked glycosylations, the binding can be enhanced or decreased(28). Besides interfering with the antigen-antibody binding, glycosylation can also alter complement activation. Altogether, this shows that when studying (monoclonal) antibodies and their binding, it is highly relevant to take glycosylation sites into consideration(29).

2.2 Rheumatoid arthritis

RA is a systemic autoimmune disease characterised by joint pain and morning stiffness. RA is a clinical diagnosis, but a combination of different classification criteria is frequently used for research purposes and can serve as help in daily clinical practice. The criteria, defined by the European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR) were last updated in 2010(30) with the goal of identifying RA at an earlier stage, whereas the 1987 criteria were more focused on symptoms of established disease. The criteria are a combination of swollen/tender joint counts, symptom duration, presence and levels of autoantibodies, and levels of the acute phase reactants C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Based on presence or absence of anti-citrullinated protein antibodies (ACPA) and/or rheumatoid factor (RF), we distinguish between seronegative and seropositive RA. About two-thirds of RA patients are seropositive, having a positive ACPA and/or RF antibody test(31, 32).

RA is a common autoimmune disease with a prevalence of about 0.5-1%(33, 34), and occurs two to three times more often in woman than men. When left untreated, the disease will lead to severe joint damage and can eventually cause disability. Treatment is often a combination of pain reduction through non-steroidal anti-inflammatory drugs (NSAID) and glucocorticoids, and disease modifying anti-rheumatic drugs (DMARDs). Methotrexate is recommended to be the first treatment option, and close monitoring is advised. When the treatment goal, clinical remission, is not reached, changing, or adding other drugs is recommended. Since inhibitors of the tumour necrosis factor (TNF) entered the market, more and more biological DMARDS (bDMARDS) are used for treatment of RA, among others interleukin 6 (IL-6) receptor inhibitors, B-cell depletion, and T-cell co-stimulation inhibitors. More recently, Janus kinase (JAK) inhibitors, which are small molecule drugs, are also used in treating RA(35). Even though there is a variety of treatment options available, none of the treatments can sustainably cure the disease as of today. Importantly, early detection and early treatment are important for clinical outcome(36, 37), and efforts are being made today to stop the pathogenic process before arthritis becomes manifest. Both rituximab (B-cell depletion) and abatacept (inhibition of T-cell

activation) treatment in the preclinical phase have been shown to delay, although not prevent, RA onset(38, 39).

2.2.1 Risk factors

Our understanding of the pathogenesis of RA is growing, but the aetiological mechanisms are not yet elucidated. We know that both environmental and genetic factors contribute to the risk of getting disease. The best-known genetic risk factors for RA are certain *human leukocyte antigen (HLA)* class II alleles, encoding for the MHC molecules, followed by a single nucleotide polymorphism (SNP) in the *protein tyrosine phosphatase non-receptor type 22 (PTPN22)* gene.

The HLA molecule is highly important for binding of antigen to the TCR, since the TCR responsiveness is dependent on the binding of the MHC-peptide complex. Certain *HLA-DRB1* alleles contain a five amino acid sequence motif in position 70-74 of the fourth antigen-peptide binding pocket(40, 41), which are referred to as the shared epitope (SE). Presence of *HLA-DRB1* SE alleles is specifically associated with risk for ACPA+ RA(42, 43), and some of these DRB1 molecules can bind citrullinated peptides better than non-SE MHC molecules, and/or citrullinated peptides bound to MHC II interact better with the TCR. Today, there are several studies supporting this theory(44-46). Apart from these well-known and well-studied genetic risk factors, the introduction of genomic wide association studies made it possible to discover >150 other loci of genetic variation that are relevant for the risk of RA, mainly ACPA+ RA(47). Hence, RA is a multigenic disorder, where each polymorphism confers a small increased risk. Notable is also that many of these SNPs are common gene variants, for example around 50% of the healthy population in Sweden carries *HLA-DRB1* SE alleles(48).

Environmental risk factors for RA include smoking, exposure to occupational inhalable agents, and air pollution(49-51), which are all affecting the lungs in some way. Besides, smoking is known to have multiple negative systemic effects. Importantly, the risk to develop RA is drastically increased in case the smoking individual also carries *HLA*-SE alleles, demonstrating a statistical interactive effect between SE genes and smoking(52, 53), suggesting a biological interaction, which will be discussed further below.

Unhealthy eating patterns, Vitamin D deficiency and obesity have also been linked to increased risk for RA, while a Mediterranean diet, fatty fish and omega-3 intake, Vitamin D supplement as well as physical activity have shown protective effects, although these data are not as strong as the data for smoking(54).

Viral infections have been extensively studied in relation to autoimmune diseases including RA, in particular Epstein-Barr virus, human cytomegalovirus and human parvovirus B19, but due to contradicting study results, a causative link to RA has been difficult to prove(55, 56). Recently, there has been a growing interest for alterations in the oral, lung and gut microbiome that seem to be linked to onset of RA. These findings will be further discussed below.

2.2.2 Pathology

One of the current hypotheses for the development of RA, and more specifically ACPA+ RA, is that the path towards systemic autoimmunity starts long before the onset of arthritis. This hypothesis suggests that genetically predisposed individuals who are exposed to environmental risk factors undergo a break of immune tolerance, leading to the production of autoantibodies years before clinical onset(57, 58). Since presence of autoantibodies in itself does not cause arthritis(31), a so-called "second hit" (*e.g.*, transient infection, microtrauma, or other) has been proposed for progression towards synovial inflammation.

RA affects mainly the synovial joints. In healthy joints, the synovium is a thin lining, consisting of only one to two cell-layers. It produces synovial fluid to keep the cartilage healthy and to lubricate the surfaces for optimal function. Inflammation in the synovium leads to proliferation of the synoviocytes, and infiltration of different types of inflammatory cells, including lymphocytes, neutrophils, and macrophages, forming a pannus which can cover the joint's bone and cartilage. Synovial fibroblasts in arthritic joints are affected by this distortion of the synovial lining, resulting in the upregulation of the activity and expression of TLRs. *In vitro* studies show that stimulation of these TLRs leads to production of inflammatory cytokines, and upregulation of vascular endothelial growth factor(59). Thus, synovial fibroblasts act as immunomodulators by releasing chemokines and pro-inflammatory cytokines like IL-6, IL-8 and TNF-α, as well as secretion of matrix metalloproteinases, which enhance joint destruction through cartilage erosion. The above-mentioned cytokines have been shown to have a stimulating effect on the process of osteoclastogenesis(60, 61). Since the discovery that signalling of nuclear factor-kappa B (NF-kB), and its receptor activator (RANK), are required for osteoclast formation(62), the role of osteoclasts in RA started to become much better understood. RANK ligand is expressed by activated T cells. T cells are known to infiltrate the RA joint, and a direct effect of activated T cells on osteoclasts (mediated by RANK ligand) has been demonstrated *in vitro*(63, 64). Since osteoclasts derive from monocytes and macrophages, the high prevalence of resident monocytes and macrophages in the lining and sublining layers of the joint contribute to the accumulation of osteoclasts. Very recently, evidence has come out that synovial inflammation starts with the activation of lining macrophages. Upon stimulation by immune complexes, these macrophages start to release cytokines/chemokines, leading to the recruitment of neutrophils(65). Taken together, monocytes and macrophages in the arthritic joint receive signals from activated T cells or synovial fibroblasts which promote osteoclastogenesis, thus contributing to bone loss in RA.

2.2.3 B cells in RA

The fact that B-cell depletion is shown to be an effective treatment strategy for certain RA patients, together with the presence of autoantibodies in about two thirds of RA patients, point towards a contribution of B cells in RA pathogenesis(66). However, heterogeneity in both clinical and immunopathological presentation also suggest that the disease can be driven in various ways(67).

Using tetramer technology, with biotinylated antigens coupled to fluorophores in flow cytometry, or single B-cell sorting, followed by BCR cloning and expression of monoclonal antibodies, have greatly advanced our knowledge regarding ACPA+ B cells. First of all, studying various tissues has shown that ACPA+ B cells are not only found in the blood and joints (synovial fluid and synovial tissue), but also in the bronchoalveolar lavage (BAL) fluid, bone marrow and as shown in my thesis work, the gingival tissue(68-72). Furthermore, the BCR on ACPA+ B cells appear to recognize small linear epitopes, so called citrulline-motifs, where citrulline and just a few flanking amino acids are essential for binding. Examining monoclonal antibodies also show that ACPA+ B cells can be multireactive, demonstrated by the presence of BCRs that recognize multiple citrullinated peptides and even carbamylated and acetylated antigens(73-75). Moreover, the BCR of ACPA+ B cells generally have a very high number of SHM and N-linked glycosylation sites, suggesting continuous antigen-triggering and T-cell driven affinity maturation. ACPA+ B cells have also been described to be in an activated and proliferative state, able to secrete the chemoattractant cytokine IL-8, and to often be of the memory B cell or plasmablast phenotype(73, 76).

Importantly, it is not only the ACPA+ B cells that seem to be altered in RA. When investigating the total B-cell population in peripheral blood, we have learned that in general more autoreactive B cells escape central and peripheral tolerance mechanisms in patients with autoimmune diseases(15). In addition, the total B-cell repertoire of RA patients is less mutated compared to healthy individuals, which is potentially explained by increased extrafollicular responses(77, 78). The availability of single-cell technologies has advanced our knowledge about B-cell populations in peripheral blood, but importantly also at the site of inflammation; the synovial tissue and synovial fluid. Comparing peripheral blood and synovial tissue, class-switched memory B cells and plasmablasts were relatively more abundant in the synovial tissue of both ACPA+ and ACPA- patients. However, when looking at the total immune profile of CD45+ cells, the composition of the abnormally distributed inflammatory cells was significantly different between ACPA+ and ACPA- RA. More specifically, an enrichment of plasma cell genes indicates a more lymphoid pathotype in ACPA+ RA patients, highlighting the importance of the adaptive immune system in this subset(79). Another study focusing on B cells in paired blood and synovial tissue highlights the importance of formation of tertiary lymphoid structures. Dissecting these so-called ectopic lymphoid structures with the potential to mimic GC features, suggests B-cell activation, class-switching, SHM and importantly, chronic antigen-stimulation, to happen inside the joint(80, 81). T peripheral helper (Tph) cells have also been identified in the RA synovium and are able to recruit and interact with B cells(82, 83). These findings support the important role of T-B cell interaction at the site of inflammation.

Whereas B-cell depletion gives good results for some patients, others do not respond to this type of therapy. The best-known B-cell depletion therapy is rituximab, a chimeric monoclonal antibody against CD20. Apart from rituximab, there are different versions of monoclonal antibodies on the market, targeting CD20, CD19 or B-cell activating factor (BAFF)(84). More recently, another B-cell depletion therapy has been introduced in autoimmune diseases, namely the anti-CD19 chimeric antigen receptor (CAR) T cell therapy. This therapy has not been

used to treat RA patients yet, but has shown incredible results in the treatment of severe lupus(85).

2.2.4 Autoantibodies

The first autoantibody discovered in RA was RF, which is directed against the Fc part of IgG. Already in 1956, this antibody was part of the criteria for RA. Many years later, ACPA, the antibodies against citrullinated proteins, were discovered(86). Citrullination is a post-translational modification, where the amino acid arginine is replaced by a citrulline in a calcium dependent enzymatic reaction performed by the peptidyl arginine deiminase (PAD) enzyme(87). This process is coupled to terminal differentiation of cells as well as to different types of cell death. Citrullination is part of normal physiology, for example citrullination of filaggrin in the skin keratinocytes, citrullination of myelin basic protein, making up the myelin sheets surrounding nerve fibres, or citrullination of histones in the nucleus involved in gene regulation. In addition, citrullination occurs in many different inflammatory settings. Hence, citrullination is not considered specific for RA(88), while the ACPA response is. *In vitro*, ACPA target a wide range of citrullinated proteins and peptides, including citrullinated vimentin, fibrinogen, α-enolase, collagen type II, and histones, proteins which have also been detected in the inflamed synovial joint(89). Still, the main *in vivo* antigen target(s) remain to be identified.

RF is an autoantibody that occurs in a relatively high frequency in the healthy population (about 5%), mainly in older individuals and in smokers, and in association with certain infections, and can also occur in other rheumatic diseases, like SLE and Sjögren´s syndrome(90, 91). Whereas the dominant isotype among ACPA is IgG, RF occurs mainly as IgM, but is also detected as IgA and IgG. Compared to RF, the ACPA response is more specific for RA, with the cyclic citrullinated peptide (CCP2) test having a specificity of 98%. Whereas ACPA and RF are the only two autoantibodies included in the classification criteria of RA, there are many other RA-related autoantibodies discovered to be present in subsets of RA patients. Within the spectrum of anti-modified protein antibodies (AMPA), there are antibodies against acetylated and carbamylated proteins, which ACPA show cross-reactivity with(74). Antibodies to the malondialdehyde (MDA) and MDA-acetaldehyde (MAA) modified proteins show no cross-reactivity with ACPA(92). Then there are also autoantibodies against native self-proteins, like the anti-collagen type II (CII) antibodies, and antibodies targeting heterogeneous nuclear ribonucleoprotein (hnRNP)A2/B1 (RA33)(93, 94).

It is important to note that autoantibodies can be detected in the blood of RA patients many years before arthritis onset(32). When focusing on the ACPA, multiple studies have shown a maturation of the antibody response, with an increase in ACPA levels and the number of ACPA isotypes and subclasses over time(95), as well as epitope-spreading, with an increase in the number of fine-specificities, *i.e.* citrulline-containing epitopes/peptides recognized by the ACPA(96, 97), supporting the idea that break of tolerance to citrullinated proteins takes place before onset of clinical symptoms. Furthermore, in the Karolinska Risk-RA prospective cohort, we see that having any ACPA fine-specificity, in addition to a positive anti-CCP2 test, is predictive for progression to arthritis and an RA diagnosis(98). Studying affinity-purified polyclonal ACPA has demonstrated that numbers of N-linked glycosylation sites in the Fab

region are not only higher in RA patients, but also in ACPA+ first-degree relatives, especially in those who progressed to RA(99, 100).

2.2.5 ACPA: pathogenic or protective?

Due to the fact that presence of ACPA is predictive of RA onset, and that ACPA+ RA patients develop a more severe and destructive disease course than ACPA- patients(32, 101), it was for a long time hypothesized that ACPA are pathogenic. Many scientists in the research field have tried to understand the role of ACPA by studying their effects in *in vitro* and *in vivo* models(57).

The effect of polyclonal and monoclonal ACPA on innate immune cells is broadly studied. Macrophages are widely abundant in the RA synovium(102) and play a role in the regulation of synovial inflammation in RA through the release of proinflammatory mediators, most notably TNF. Polyclonal ACPA can form immune complexes and thereby activate macrophages in an Fcγ-dependent manner, where this stimulation is more potent when using citrullinated instead of native antigen(103, 104). Moreover, RA patients are known to have increased release of neutrophil extracellular traps (NET), and IgG from ACPA+ RA patients seem to enhance this effect(105, 106). Furthermore, both purified polyclonal and monoclonal ACPA can bind citrullinated antigens, like histones that are released during NETosis(107), and human monoclonal bone marrow derived ACPA, known to bind citrullinated histones, were recently shown to bind activated neutrophils using flow cytometry(72). The formation of ACPA immune complexes, which may potentially also contain RF, can trigger the activation of complement(108), and may further enhance NETosis and cytokine release from macrophages by interactions with Fc receptors. Purified polyclonal ACPA from RA synovial fluid binds antigens present in RA joints(109). Importantly though, different monoclonal ACPA have different binding-patterns *in vitro*, and thus most likely also *in vivo*, and we have recently shown that there is a high variability between different ACPA in their interaction with neutrophils and NETs(110). This also implies that different ACPAs might be functionally different.

When looking at other cell types involved in RA pathology, the osteoclasts and fibroblasts are often studied. In a healthy situation, bone generation by osteoblasts and bone degeneration by osteoclasts are in balance, maintaining the shape and function of the bone(111). In RA on the other hand, osteoclasts are overrepresented, induced upon RANK-L stimulation(112). Interestingly, both polyclonal and monoclonal ACPA can enhance macrophage-derived osteoclast differentiation *in vitro*(68), and ACPA-stimulated osteoclasts showed high production of IL-8, which is known to be a neutrophil chemoattractant in inflammatory environments(113). Another study used an *in vivo* model to show that antibodies against citrullinated vimentin can enhance periarticular bone loss and local osteoclastogenesis in mice(114). Sensitization of fibroblasts with ACPA combined with IL-8 resulted in the upregulation of PAD expression by the fibroblasts, leading to increased protein citrullination and cell migration(115). However, it must be mentioned again that there is a difference in potency between different monoclonal ACPA(68, 116)

In vivo models show ACPA-induced tenosynovitis, pain, and bone loss in a PAD-4 dependent manner(117). In addition to ACPA, monoclonal anti-MDA/MAA antibodies originating from

synovial B cells from an RA patient have also been shown to promote osteoclastogenesis *in vitro*(118, 119), and contrary to the ACPA response, anti-MDA/MAA antibody levels correlate with RA disease activity(118). Furthermore, animal studies have taught us that monoclonal and polyclonal ACPA can induce pain(120, 121), which together with bone loss constitute early symptoms of RA. However, it is important to note that pain in itself does not lead to inflammation or arthritis, and that patient-derived ACPA by themselves do not induce arthritis in mice. Very recent animal studies have shed a different light on the potential pathogenicity of ACPA, by demonstrating protective effects of patient-derived monoclonal ACPA(122, 123), where one study suggests that protective ACPA immune complexes bind inhibitory Fc receptors, triggering macrophage release of IL-10. A study from 2021 also report on a therapeutic ACPA, demonstrating that this ACPA could ameliorate disease in multiple inflammatory disease models, including a chronic inflammatory arthritis model. The suggested mechanism of action was inhibition of NETosis and enhanced NET uptake by macrophages(124).

Besides investigating the antibodies, we should also consider the potential pathogenicity of the antigen. An early study could demonstrate that citrullination of CII enhanced both the immunogenicity and arthritogenicity of the antigen in a rat model of CII-induced arthritis(125). Moreover, although the event of citrullination is not restricted to ACPA+ individuals, studies in humanized *HLA-DR4* transgenic mice suggest that some but not all citrullinated antigens have direct arthritogenic potential(126, 127).

The recent findings of protective ACPAs have raised questions about the heterogeneity of the ACPA response. Hence, the role played by different ACPAs, as well as the PAD enzymes and specific citrullinated proteins, in RA pathogenesis require further investigations.

2.2.6 The mucosal origins hypothesis

Imbalance in the microbiome, known as dysbiosis, has been widely suggested to play a role in autoimmune conditions(128). The interaction between the human immune system and the microbes from “outside” takes place at epithelial and mucosal sites such as the skin, the oral cavity, the lungs, and the gut. One of the dominant hypotheses linking dysbiosis to onset of autoimmunity is the molecular mimicry hypothesis(129). Structural similarities between microbial proteins and host proteins are suggested to be able to activate the adaptive immune system. Furthermore, it has been demonstrated that first-degree relatives of RA patients are more often positive for ACPA IgA, while lacking ACPA IgG(130), whereas at disease onset it has been shown that all IgA ACPA+ patients were also IgG ACPA+(131). This observation is supported by the finding that IgA positive plasmablasts are elevated in at-risk individuals compared to established RA(132). The potential shift from IgA-immunity to IgG could imply that an initial autoimmune response takes place at mucosal sites.

The lungs have for a long time been considered as a site of inflammation preceding onset of RA(52, 133). First and foremost, smoking is one of the most important risk factors for RA. Smoking exposes lung epithelium to continuous damage, triggering inflammation and cell death. An increase in PAD expression, citrullination and lymphocyte infiltration has been

reported in the lungs of newly diagnosed RA patients(134, 135). In addition, alterations in the lung tissue, depicted by high resolution computer tomography, have been shown in ACPA+ RA patients at and before onset of disease(136). ACPA have been detected in BAL and sputum of at-risk and newly diagnosed RA, and recently it has been shown that monoclonal ACPA could be generated from single B cells isolated from the BAL fluid of ACPA+ at-risk and new onset RA individuals(137). The fact that these autoreactive B cells can be found in the lung itself, strengthens the hypothesis of break of tolerance to citrullinated proteins taking place in the lung.

In the gut, immune homeostasis plays a large role for maintaining a healthy state. Multiple studies have highlighted increased relative abundance of *Prevotella copri* in stool of RA patients as well as enrichment of this species in pre-clinical RA(138, 139). In addition, *Prevotella copri* derived from RA patients appeared to have a different genomic composition compared to *Prevotella* from healthy individuals(140). The RA-derived *Prevotella* has been shown to be more competent in inducing arthritis in mice. Another study using animal models demonstrated that the expression of IL-10, a key mediator for regulation of gut homeostasis, was downregulated in the gut of arthritic mice, proposing that arthritic inflammation affects the immune resolution in the intestine(141). A recent study also shows that monoclonal IgA and IgG, derived from blood plasmablasts of ACPA+ at-risk individuals bind gut bacteria in the *Lachnospiraceae/Ruminococcaceae* family. Colonisation of mice with a clinical isolate of this species *(Subdoligranulum)* induced autoantibodies and joint swelling, suggesting that an immune response to gut bacteria may lead to development of pathogenic autoantibodies by mechanisms of molecular mimicry(142). Besides the gut and the lungs, the oral mucosa has been described as a site for potential break of tolerance in the context of RA, which will be described more in depth below.

2.3 Periodontitis

Periodontitis, in this thesis referred to as PD, is defined by gingival inflammation and destruction of the tooth-supporting tissues, including jawbone. When left untreated, periodontitis can eventually lead to tooth loss. The incidence of PD varies between populations and between studies(143). Nonetheless, it it is a very common disease, especially in older populations. It has been estimated that up to 50% of people 50-64 years in the United States are affected by PD(143). Other studies report that 11% are affected by severe forms of PD(144). Furthermore, PD is linked to many different systemic diseases like cardiovascular diseases(145), rheumatic diseases(146), diabetes(147), and Alzheimer's disease(148).

The oral cavity is one of the body´s primary barrier sites and is constantly exposed to the outer world. The oral mucosa forms a physical as well as an immunological barrier. The composition of the salivary proteins, together with the commensal bacteria, protect against the airborne and food-derived antigens and substances that the oral cavity is daily exposed to. When this composition is altered, a dysbiosis can occur and this can eventually lead to gingivitis and PD. Gingivitis, preceding PD, is a reversible state in which only the gingival tissue and ligaments,

but not the bone, are involved. However, when left untreated, gingivitis can eventually lead to PD. Risk factors for PD include lifestyle factors as well as genetic risk factors, with the most important ones being older age and smoking(149). Clinically, PD is described as the detachment of the gingival tissue from the tooth, assessed by presence of (deep) periodontal pockets, bleeding on probing and loss of the alveolar bone(150). Whereas a healthy periodontium is a relatively leukocyte-poor environment, chronic PD is characterized by the infiltration of many different types of immune cells(151). A central role for neutrophils has been described in both healthy and diseased gingival tissue. Whereas in health, neutrophils reside in the gingival tissue and gingival crevicular fluid in order to maintain homeostasis, in PD neutrophils are constantly activated due to interaction with pathogens, which can lead to tissue damage(152). In addition, B cells, T cells and plasma cells are found to be infiltrated in periodontitis lesions with a dominating presence of plasma cells in advanced lesions(153).

Based on the earlier 1999 classification, PD diagnosis was divided into chronic and aggressive PD. In 2017, periodontologists agreed on a new, worldwide diagnostic consensus(154). As noted by Tonetti and colleagues(155), the diagnosis of PD should be based on clinical attachment loss. Using only radiographic bone loss could lead to misinterpretation, the diagnosis of mild or moderate PD could be missed, as already described in 1977(156). According to the new classification, periodontitis is staged I-IV based on disease severity and graded A-C based on risk for disease progression. The newest guidelines in Sweden were written based on the new classification criteria and were implemented in the beginning of 2021.

Treatment of PD is now recommended to be based on the different stages of disease. Treatment always includes motivating and educating patients about the importance of daily dental care routines. Next, mechanical removal of biofilm and plaque is done by scaling and root planing. When the disease is more advanced, surgical treatment might be necessary(157).

2.3.1 The oral microbiome and dysbiosis

The microbiome in the oral cavity consists of over 200 species, whereas in total over 700 different oral species have been identified as of today, collected in the Human Oral Microbiome Database, which is available online at http://www.homd.org/. The oral microbiome is determined by different factors and can be altered by for example smoking, diet, systemic diseases, medication etc., and differs in populations around the world. The commensal microbes act as a natural protection against opportunistic invaders and contribute to metabolising food. The different niches within the oral cavity vary greatly from each other resulting in the fact that different conditions give rise to the growth of different species and different bacterial compositions(158, 159). This means that in healthy individuals the difference in species varies more within the mouth than between two individuals when sampling at the same site. Bacteria that play a key role in the promotion of dysbiosis are often referred to as the "red complex bacteria". These include *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*(160). In addition, *Aggregatibacter actinomycetemcomitans (Aa)* and some other bacteria correlate with presence of PD(161). However, it must be mentioned that PD cannot be attributed to presence of one or two single species, but rather

to a shift in microbial composition, and inter-microbial as well as, importantly, host-microbial, interactions. Dysbiosis can be induced by (a combination of) different factors, such as tissue injury, use of antibiotics, genetic predisposition, and poor oral hygiene(162). Dysbiosis will lead to tissue damage, due to the direct destruction by virulence factors, and as a result of the host immune response. This means that the host contributes to PD pathogenesis, and more specifically to alveolar bone resorption, because of increased concentrations of inflammatory mediators(163).

2.3.2 *Porphyromonas gingivalis*

Porphyromonas gingivalis (Pg) is a gram-negative opportunistic anaerobic bacterium with strong proteolytic properties, that can cause degradation of complement and antibodies, resulting in an impaired host defence response. This impaired response does not only affect the growth of pathogenic *Pg*, but also allows other strains to colonize, resulting in dysbiotic overgrowth of pathobionts(164). However, *Pg* can also occur as a commensal and does not always contribute to PD.

The virulence factors of *Pg* have appeared to be highly potent, acting through different mechanisms, causing biofilm formation and coaggregation with other bacteria(164). Not only does *Pg* support its own growth, but it also creates a microenvironment that is beneficial for other pathogens. Lipopolysaccharide (LPS) is a cell wall component of gram-negative bacteria but is also known for its potency of causing inflammation. *Pg* LPS, recognized as a PAMP, acts on TLR´s, thereby inducing cytokine secretion(165). Moreover, *Pg* fimbriae have pathogenic properties through the formation of biofilm. The fimbriae are able to attach to tissues while giving colonizing bacteria the opportunity to co-aggregate. Gingipains (gp), another type of *Pg* virulence factor, can directly support the formation of biofilm through its co-aggregative activities. Gingipains can either cleave after arginine (R) residues (Rgp) or lysine (K) residues (Kgp). RgpA and Kgp both possess a hemagglutinin domain, which is responsible for the coaggregation. In addition, the proteolytic gingipains RgpA and RgpB contribute to the production of short fibriae A. Furthermore, gingipains play an important role in the degradation of different proteins in the tissue, like collagens and adhesion molecules, but also parts of the immune system, *e.g.*, antibodies and complement(166, 167). On top of that, gingipains modulate the immune system in different ways, for example; *Pg* gingipain can reduce the CD14 expression on macrophages, resulting in a decreased anti-bacterial response(168) and gingipains can induce NET formation while at the same time preventing those NETs from killing other bacteria, thereby promoting their pathogenicity(169). Another *Pg* virulence factor co-localizes (in the outer membrane and in secreted outer membrane vesicles) and cooperates with gingipains, namely *P.*PAD. This *Pg*-derived PAD enzyme citrullinates peptidyl arginine, in particular C-terminal arginine which becomes more freely available after cleavage by RgpA or RgpB(170). The unique property of *Pg* – among known microbes – to express a PAD enzyme, led Rosenstein *et al.* to propose the hypothesis that the ACPA response in RA is triggered by *Pg* infection(171).

2.3.3 Linking PD, *Pg* & RA

The idea that PD causes RA dates back to the classical period around 400 years BCE, when Hippocrates claimed arthritis could be treated by pulling out bad teeth, and a publication from 1918 presents a case report, where a patient with RA was cured by ionization of periodontal membrane(172). Today, many studies can confirm an epidemiological association between PD and RA and between *Pg* infection and RA(146, 173, 174). However, the mechanistic link has not yet been elucidated, and different hypotheses have been suggested. Whereas certain reports attribute the epidemiological link between PD and RA to *Pg* infection, others suggest that microbial dysbiosis in general, or shared genetics and inflammatory pathways, might explain the connection.

Several studies report increased prevalence of antibodies against different *Pg* virulence factors in RA patients compared to healthy controls(175, 176). Furthermore, increased levels of anti-*Pg* antibodies have been detected in the blood of RA patients years before onset of disease(176, 177). The mechanism by which *Pg* infection is linked to RA has been topic of extensive debate. The hypothesis first put forward by Rosenstein and colleagues, is that *Pg* is linked to ACPA+ RA because of an increase in citrullination, induced by *P.*PAD, leading to break of tolerance to citrullinated proteins (in the PAMP-rich milieu) and subsequently ACPA production. It has been shown that *P.*PAD is able to citrullinate human proteins like fibrinogen and enolase(178), as well as bacterial antigens(179, 180). Furthermore, ACPA cross-reactivity between citrullinated human and *Pg* enolase has been shown, suggesting molecular mimicry to play a role in the onset of systemic autoimmunity to citrullinated proteins(181, 182). Moreover, like the human PAD enzymes, also *P.*PAD has been shown to autocitrullinate, and RA patients have antibodies to citrullinated *P.*PAD, including the citrullinated *P.*PAD peptide 3 (CPP3) epitope(183). Taken together, these data suggest a "two-hit" model, starting with an immune response against citrullinated antigens generated by *P.*PAD in the gums, followed by epitope-spreading to citrullinated proteins in the joints. However, this theory has been debated(184). There are contradictory reports regarding whether *P.*PAD can autocitrullinate *in vivo*, whether *P.*PAD can generate endocitrullinated epitopes or only C-terminal epitopes, and whether ACPA target epitopes with C-terminal citrulline(180, 184-187).

Apart from focusing on the link with *P.*PAD citrullination, there are also other mechanisms whereby oral microbes could be linked to ACPA+ RA. As mentioned earlier, *Pg* can affect the efficacy of NETs(169). NETosis is an important process that is studied in both PD and RA and can lead to the extrusion of intracellular post-translationally modified (auto)antigens and thereby potentially contribute to the onset of autoimmunity. Importantly, the gingival tissue has been shown to be an extra-synovial source of citrullinated proteins, including citrullinated histones(188, 189). Both RA and PD are known to have neutrophil infiltrates in diseased lesions(190). Recent work proposes a mechanism by which NETs mediate inflammation of tissues in the oral cavity during PD. Kim and colleagues show that pathogenicity of NETs is dependent on citrullination and PAD4 in animal models. In addition, they show involvement of Th17 cells in the mucosal inflammatory response, and Th17 involvement in periodontal bone loss, a relevant T-cell subset also in RA pathogenesis(191). Their animal models also show that knockout of PAD4 protects from periodontal bone loss. In conclusion, Kim *et al*. suggest a

pathogenic role for NETs by mediating bone loss, possibly linking RA and PD by this shared mechanism(192).

Besides *Pg*, another oral bacterium has been implicated in the aetiology of RA. The oral bacterium *Aa* has also been suggested to enhance protein citrullination in the gums, through the activation of endogenous host PAD enzymes. *Aa* expresses the virulence factor Leukotoxin A, which is a pore-forming protein, inducing permeabilization of plasma membranes and thereby altering the calcium influx, subsequently leading to a process called hypercitrullination(193, 194).

When investigating the oral microbiome as a whole, several reports show an alteration in the oral microbiome of CCP2+ at-risk individuals(195, 196), though there is no clear consensus of the potential mechanism by which dysbiosis could lead to the onset of arthritis, other than the suggestion that dysbiosis can lead to break of tolerance. Whereas the molecular mimicry hypothesis focuses mainly on the role of the adaptive immune system(171), there are also potential links between PD and RA that could be explained by the role of the innate immune system. For example, a recent report shows that genes upregulated during RA flares are different between RA patients with or without PD(197). The pathways upregulated in PD patients with RA flare were enriched for antibody effector functions against microbes leading the authors to suggest that repeated oral bacteremia could contribute to flares.

One of the complicating factors when studying PD and RA in the same individuals, is the effect of treatment. It is hard to adjust for medications that can strongly affect (inflammatory) parameters of both diseases, for example cytokine blockers. Whereas reports about the influence of treating PD on the disease activity in RA are contradicting(198, 199), RA-treatments like cytokine blockers seem to affect PD in a positive way(200, 201). Furthermore, a recent report suggests that RA patients with high levels of antibodies against *Pg* and *Aa* respond less well to RA-treatment(202). However, this study was small and needs to be replicated to get a better understanding of the outcome.

Apart from studying antibodies in the serum, different researchers have studied the added value of measuring presence of antibodies in the saliva of patients. Recently, saliva and serum samples from RA patients and controls were investigated for presence of antibodies against Rgp(203) This study showed increased levels of salivary anti-Rgp IgA in RA patients, compared to controls, and that these IgA levels correlated with disease activity. However, this study was small, and results need to be repeated to better understand the presence of anti-Rgp IgA antibodies in RA patients. Interestingly, investigation of ACPA IgA in sera of individuals at risk for RA, show that positivity for ACPA IgA1 gives higher risk of developing RA, and presence of ACPA IgA in saliva has been shown to associate with higher RA disease activity, suggesting that also IgA, not only IgG, is a relevant isotope in ACPA+ RA(204, 205). Further investigation is needed to understand the link between anti-*Pg* and ACPA responses of both IgA and IgG isotypes in serum and saliva.

2.4 Aetiological hypothesis

The onset of autoimmunity that characterises ACPA+ RA likely finds its origin in the combination of genetic and environmental factors. At mucosal sites, exposure to external triggers, for example cigarette smoke and microbes, could activate a local immune response, causing inflammation, cell death, activation of PAD enzymes, and increased citrullination, giving rise to neo-epitopes on bacterial proteins and on self-proteins. At this point, an infection with *Pg*, known to be able to modulate the immune system, can elicit a more extensive immune response. We hypothesize that the adaptive immune system now comes into play, and citrulline-reactive B cells drive the low-affinity ACPA production, targeting both bacterial and human proteins.

The next step is maturation of the ACPA response. At this stage, genetic factors like *HLA-DRB1* SE and *PTPN22* are important, affecting antigen-presentation as well as T- and B-cell signalling. This phase of the autoimmune response includes the interaction between autoreactive B- and T-cells, epitope-spreading, and generation of ACPA-secreting plasmablasts and plasma cells. ACPA can now be detected systemically, and clinically, these individuals can develop early signs of arthralgia, characterised by joint pain. At this point, we can define these individuals as being at risk of developing RA. Taken together, we hypothesize that break of tolerance against citrullinated proteins takes place at the oral mucosal tissue, and that colonization of *Pg* plays a significant role in this process.

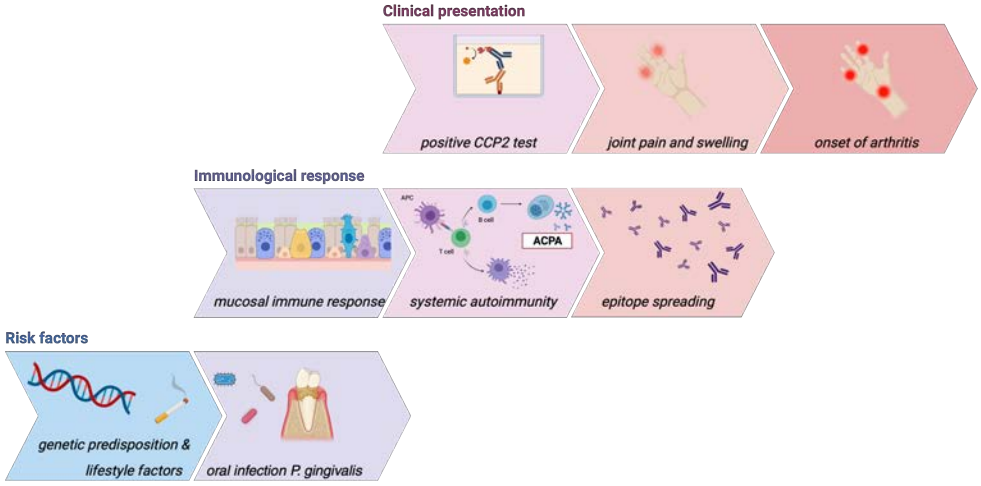

Figure 2. Steps towards the onset of ACPA+ rheumatoid arthritis.

3 Research aims

The overall aim of this thesis was to investigate the role of B cells and antibodies in the context of *Porphyromonas gingivalis* infection, and PD as an aetiological link to the development of ACPA autoimmunity and rheumatoid arthritis.

Specific aims:

- In **study I-III**, we set out to investigate the relationship between anti-*Pg* antibodies and ACPA in three large cohorts, and the potential clinical application of using anti-Rgp IgG as a proxy for PD and as a biomarker for developing ACPA+ RA.
- The aim of **study IV** was to examine the gingiva as a site for ACPA production and *Pg* as a driver of the ACPA response, by analysing ACPA targeting *Pg* in early RA patients, and by generating human monoclonal antibodies from gingival tissue B cells and study their binding to citrullinated bacterial and human proteins.
- The aim of **Study V** was to explore alterations of B-cell phenotypes in the at-risk phase of RA, to increase our understanding of B-cell dysregulation leading up to arthritis onset.

4 Materials and methods

4.1 Ethical considerations

The aim of this work was to clarify the aetiological background of RA, as a basis for novel treatment and prevention strategies, to ultimately reduce pain and other symptoms for patients suffering from RA. Protocols for these studies, including the use of personal data and human samples, were in compliance with the Declaration of Helsinki(206) and were approved by the regional ethics review board Stockholm or the regional ethics review board in Umeå. Individuals included in the different studies have given consent to use their clinical data, personal information, and different types of samples for research purposes. Samples were collected by experienced medical staff and did not involve significant risk for participants. The gingival biopsies, used in **study IV**, were only taken when periodontal surgery was needed.

Handling of personal data was done according to GDPR guidelines and privacy was protected at all times. Data was stored only at secured servers.

4.2 Patient material

4.2.1 Gingival tissue biopsies

In **study IV**, we have used biopsies from gingival tissue (GT), obtained during surgery that was part of the treatment strategy in individuals suffering from severe PD (n=7) or gingivitis (n=1). Four of the patients with PD had an RA diagnosis. Collection of gingival tissues took place at the Dental Department at Karolinska University Hospital and at Folktandvården, Mörby Centrum, Stockholm. In total, nine GT biopsies were collected, of which four were directly processed for single-cell sorting and five were snap-frozen in liquid nitrogen and stored at -80°C for 12 to 184 days before processed.

4.2.2 Peripheral blood mononuclear cells

In **study V**, we investigated peripheral blood mononuclear cells (PBMC) from RA patients, Risk-RA individuals, and healthy donors. RA patients fulfilling the ACR 1987 and/or ACR/EULAR 2010 classification criteria for RA were selected based on having high anti-CCP2 IgG levels. The Risk-RA samples were obtained from the Karolinska Risk-RA prospective cohort. Individuals were included in this cohort based on a positive anti-CCP2 test and having musculoskeletal complaints, without being diagnosed with a rheumatic disease, and without clinical- or ultrasound-detected arthritis. For this specific study, we selected Risk-RA individuals carrying at least one *HLA*-SE allele. Follow-up time was minimum three years, or until onset of arthritis. A total of 37 PBMC samples from 18 Risk-RA individuals, of which nine developed arthritis

within the timeframe of this study, were included. Healthy control samples were obtained from blood donors in the Uppsala Bioresource sample collection.

4.2.3 Serum samples

In **study I-IV** we have used serum samples from different patient cohorts. In **study I** we used the case-control study Periodontitis and its Relation to Coronary Artery Disease (PAROKRANK), which was initially set up to investigate the association between PD and having a first myocardial infarction (MI)(145). Individuals with (n=779) or without (n=719) a first MI were matched based on sex, age, and residential area; n=557 had PD. Presence of 19 autoantibodies related to rheumatic diseases were measured previously. We also used serum samples from two additional cohorts in **study I**: the PerioGene North (PGN) case-control study(207) and the Karolinska SLE case-control study(208). PerioGene North, which was set up in the north of Sweden and designed to study genetic risk, biomarkers, and other inflammatory conditions in PD, comprises 526 PD patients and 532 periodontally healthy controls. In **study I**, we included 39 PD and 41 controls from PGN, while in **study II**, we included the full cohort. The SLE samples used in **study I** are also a part of a larger cohort; here we included 101 SLE patients and 100 controls. Presence of autoantibodies was measured previously.

In **study III** we used serum samples from the Karolinska Risk-RA prospective cohort, described above. In total, 507 samples from 260 Risk-RA individuals were included, 260 baseline and 247 follow-up samples. Data on genetics, smoking and anti-CCP2 IgG was generated previously(98). We also included serum samples from 126 healthy controls in this study.

In **study IV** we have made use of the Epidemiological Investigation of Rheumatoid Arthritis (EIRA), a population-based case-control study from Sweden(50), comprising DMARD-naive RA patients who were included within one year from being diagnosed with RA. Diagnosis was set by a rheumatologist, and all patients fulfilled the ACR 1987 and/or ACR/EULAR 2010 classification criteria. Data on genetics, smoking, anti-CCP2 IgG, and 5-year clinical data (retrieved trough linkage with the Swedish Rheumatology Quality Register (SRQ)) was generated previously. Serum samples from 2,859 RA patients and 372 population controls, matched to cases on age, sex and smoking, were included.

4.3 Clinical examinations and diagnoses

Dental clinical examinations were performed in **study I and II**. The definition of PD was set based on different criteria in the two cohorts. In the PAROKRANK study, collected between 2010 and 2014, PD diagnosis was defined based on mean bone loss measured by panoramic X-ray, with the following classification: no PD (> 80% remaining bone), mild to moderate PD (66-79% remaining bone), and severe PD (< 66% remaining bone). In addition, bleeding on probing (BOP) index, measured at four sites per tooth, and pocket probing depth (PPD) (defined as high: ≥ 6 mm or low < 6 mm) were measured during dental examination. Since PAROKRANK was initially designed to investigate MI in relation to PD, controls were matched to MI cases, not to PD cases. In PGN, which was collected between 2007 and 2019, PD was

defined as ≥ 1 tooth in each quadrant with bone loss > 1/3 of the root length and total number of teeth ≥ 15. During full periodontal examination, BOP was measured at six sites per tooth and reported as the percentage of sites with gingival bleeding divided by the total number of sites. PPD was also assessed; a tooth with PPD < 4 mm was scored as 1, 4-6 mm was scored as 2, and > 6 mm was scored as 3. A total PPD score was calculated based on the total number of teeth. Levels of bone loss were assessed by dental X-rays where marginal bone loss < 1/3 of the root length was scored as 1; bone loss between 1/3 and 2/3 of the root length was scored as 2; and bone loss > 2/3 of the root length was scored 3. A total score was calculated and divided by number of teeth. Scores for both PPD and marginal bone loss were defined as low (scores 1.01-1.49), moderate (scores 1.50-1.99), or high (scores >2.0). Controls in PGN, matched to cases on sex, had > 24 teeth, and no signs of clinical attachment loss, determined by the distance between the gingival margin and marginal jawbone (≤ 3 mm), and the PPD (< 4 mm).

In **study III, IV and V**, clinical examinations were performed by rheumatologists. In **study III and V**, Risk-RA individuals were included based on having musculoskeletal complaints and being anti-CCP2 IgG positive. Individuals were not included in the study if synovitis was detected clinically or by ultrasound. Follow-up was done yearly, or when symptoms worsened. Progression was defined as clinical onset of arthritis. In **study IV**, newly diagnosed RA patients were included, fulfilling the 1987 ACR criteria and/or the 2010 ACR/EULAR criteria(209). In **study V**, we included patients with established RA, selected to have high levels of anti-CCP2 IgG.

4.4 Immunoassays

4.4.1 ELISA

Enzyme linked immunosorbent assays (ELISA) were used in **study I-IV**. Both commercially available and different in-house ELISAs were used to measure antibody binding. In brief, the antigen of interest was coated on a 96-well plate, followed by a blocking-step to minimise unspecific binding, and incubation with the serum samples, purified antibodies, or monoclonal antibody of interest. Detection of bound antibodies was done with a specific secondary antibody, conjugated to horse radish peroxidase, an enzyme subsequently activated by adding a substrate. The intensity of the emitted light was then measured as optical density (OD) in a spectrophotometer.

In **study I-III** we used an in-house ELISA to measure the antibody response against the *Pg* virulence factor RgpB. Coating was done using the full-length C-terminal hexahistidine-tagged RgpB protein (purified from cultures of a genetically modified *Pg* strain by affinity chromatography on Ni-Sepharose). A standard curve using serial dilutions of pooled sera from PD patients with high anti-Rgp reactivity was included on each plate to compare antibody levels presented in arbitrary units (AU) between different plates. All sera were analysed in duplicates and re-analysed when the coefficient of variation (CV) exceeded a value of 10. In **study IV** we used other in-house ELISAs according to the same procedure as described for the Rgp IgG ELISA

to analyse reactivity of monoclonal antibodies (at 5µg/ml). Here, we either coated with citrullinated peptides and, in parallel, with the corresponding arginine-containing peptides to detect ACPA, or with the soluble membrane protein fraction of Hek293 cells to assess polyreactivity, as previously described(210). In **study I-V** we have assessed presence of ACPA using second generation anti-cyclic citrullinated peptide assays.

4.4.2 Multiplex array

The custom-made multiplex peptide array (ThermoFisher Scientific, ImmunoDiagnostics, Uppsala, Sweden) that has been used to screen RA and Risk-RA sera for reactivity against citrullinated peptides and their non-citrullinated counterparts in **study II and IV**, with small adjustments between versions, is based on the same principle as the ELISA assay, but with the advantage that it allows to measure binding to many different antigens at the same time, using small sample volumes(211). Citrullinated peptide antigens that were included on the array (and in the ACPA fine-specificity ELISA) were previously described as potential autoantigens in RA. However, one needs to consider that here we evaluated reactivity against linear peptides, some biotinylated and some with added N- and C-terminal cysteines to allow for more conformational epitopes, but not to full-length proteins as expressed *in vivo*.

4.5 Multiparameter flow cytometry and single cell sorting

Flow cytometry allows studying cellular phenotypes on a single cell level based on expression of cell surface antigens, intracellular antigens, cell adherence and cell viability. Over the past years, the technique has drastically evolved, especially due to the introduction of full spectrum flow cytometry. Compared to conventional flow cytometry, spectral flow cytometry has the advantage that the full emission spectrum of the fluorescent molecule is captured, which allows a more precise distinction between fluorochromes and thus allows for the use of more fluorochromes when using the same number of lasers(212).

In **study IV** we have used fluorescence activated cell sorting (FACS) using a BD Influx cell sorter. Cells were index-sorted, allowing for further analysis of their phenotype. In **study V**, we had the opportunity to use spectral flow cytometry, allowing to study up to 21 different markers at the same time by using a 4-laser or 5-laser Cytek® Aurora instrument. Flow cytometry data analysis was performed using FlowJo, including the plugins Downsample, FlowSOM, UMAP, and T-REX(213-215).

4.6 Production of monoclonal antibodies

Production of monoclonal antibodies (mAbs) in **study IV** was done according to the protocol of Amara and colleagues(216). In brief, after single cell sorting the CD19+ B cells derived from gingival tissue, paired Ig heavy and light chains were amplified by polymerase chain reaction (PCR) and sequenced. The number of SHM was evaluated using IgBLAST(217) and IMGT/V-QUEST(218) and N-linked glycosylation sites were identified using NetNGlyc1.0 server (www.cbs.dtu.dk/services/NetNGlyc/). Heavy and light-chain Ig-transcripts were ligated into their respective expression vectors expressing a certain constant region (Igγ1 for heavy chains

and Igκ and Igλ for the light chains), followed by DH5α bacteria transformation. Plasmids were co-transfected in Expi293 cells for the recombinant expression of monoclonal IgG1. Purification was done using protein-G Sepharose beads followed by buffer exchange. Quality control steps included sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and polyreactivity ELISA using bovine serum albumin (BSA), LPS, insulin and double stranded deoxyribonucleic acid (dsDNA) as well as Hek293 membrane protein lysates, as described above(210, 216).

4.7 Re-analysis of a published 10X single cell RNA-sequencing dataset

In **study V**, we have used a previously published 10X single cell RNA-sequencing (scRNA-seq) dataset to validate findings from the flow cytometry datasets. The dataset comprised cells from four seropositive RA patients(72). Due to a disbalance of cell number between individuals, we have downsampled the cell number from one individual before performing new clustering to the median cell number of the other samples. Filtering for optimal quality was done based on number of transcripts, number of genes and percentage of mitochondrial genes. Analysis was focused on B cells, and thus T cells, monocytes, NK cells and platelets were filtered out, based on the expression of CD3D, CD3E, CD3G, CD247, CD14, CD300E, NKG7, FCGR3A1 and PPBP. Data was analysed in R Studio (version 4.3.1) using the Seurat package (version 4.1.4)(219). Gene expression was normalised using NormalizeData function, and principal component analysis was performed. Batch variation was removed using Harmony package (version 1.0.1). The function FindClusters (Seurat package) was used for finding clusters and FindAllMarkers (Seurat package) function was used to calculate the differential gene expression per cluster.

4.8 Statistical analyses

Statistical analyses throughout **study I-V** were mainly performed using GraphPad Prism and R. In addition, Statistical Package for Social Sciences (SPSS) was used for analysis in **study I** and **II**, JMP was used in **study II** and SAS was used in **study IV**. Based on normality distribution, continuous data were compared using (paired) Student T test, Mann-Whitney U test or Analysis of Variance (ANOVA) for paired and unpaired data. Categorical data was assessed using Chi-square or Fisher´s exact test. Spearman and Pearson correlations were calculated. Different types of regression analysis were performed. We have used linear regression in **study II**, Cox proportional hazard regression model in **study III**, and logistic regression in **study IV**, including adjusting for age, sex, residential area and smoking where needed. In **study I, II and III,** we used the area under the receiver operating characteristic (AUROC) together with Youden´s J statistic to set a cut-off for anti-Rgp IgG and calculate sensitivity and specificity.

5 Results and discussion

5.1 The anti-Rgp IgG response in PAROKRANK, PerioGene North and Risk-RA

In **study I, II and III**, we have measured the anti-Rgp IgG response as a proxy for *Pg* infection in different cohorts. Based on previous findings from our group, demonstrating an increased anti-Rgp IgG response in RA patients, especially in ACPA+ RA, as well as in samples collected before symptom onset of RA(175, 176), we here aimed to investigate whether anti-Rgp can function as a biomarker for PD, and whether anti-Rgp IgG can serve as a marker to identify individuals at high risk for developing ACPA-positivity and/or onset of ACPA+ RA. We have studied two cohorts consisting of individuals with and without PD: PAROKRANK **(study I)** and PerioGene North **(study I and II)**, and one cohort with ACPA+ individuals at risk of developing RA (Risk-RA, **study III**). Anti-CCP2 IgG data was available/measured for all cohorts.

Anti-Rgp IgG in relation to dental clinical parameters

Anti-Rgp IgG levels were increased in PD patients compared to controls, both in PAROKRANK and PGN **(study I & II)**, confirming previous findings(175, 220). When investigating the anti-Rgp IgG response in relation to clinical dental parameters, we reported both in PAROKRANK and PGN increased levels of anti-Rgp IgG in individuals with higher levels of bone loss and in patients with high degree of BOP **(figure 3)**. Interestingly, deep pocket depth (in PAROKRANK defined as ≥ 6 mm) was related to higher anti-Rgp IgG levels, whereas in PGN (pocket probing depth defined as low (< 4 mm), medium (4-6 mm) or high > 6 mm)), anti-Rgp IgG levels were not different between the different categories of pocket depth. An important difference when analysing dental parameters is that for PGN **(figures 3 A1, B1, C1)**, only the PD cases are included, whereas for PAROKRANK **(figures 3 A2, B2, C2)**, we included all individuals with or without a PD diagnosis. The reason for doing so, is that part of the individuals who were considered periodontally healthy in PAROKRANK would have PD according to the definition in PGN. In PAROKRANK, diagnosis was set based only on bone loss and thus controls could have deep pockets and gingival bleeding, whereas presence of pockets ≥ 4mm was an exclusion criterion for controls in PGN. Therefore, also individuals who did not have a PD diagnosis were included in **figure 3 (A2, B2 and C2)** in order to assess anti-Rgp IgG in relation to the clinical parameters themselves, without being affected by different diagnosis criteria.

Clinical variables like bleeding on probing and PPD have been shown to correlate with prevalence and numbers of "red complex" bacteria, and specifically with *Pg*(160, 221). Even though we cannot make a one to one comparison of bacterial counts measured in the subgingival plaque and antibody levels in the blood(222), our anti-Rgp IgG data support the association between *Pg* and PD severity.

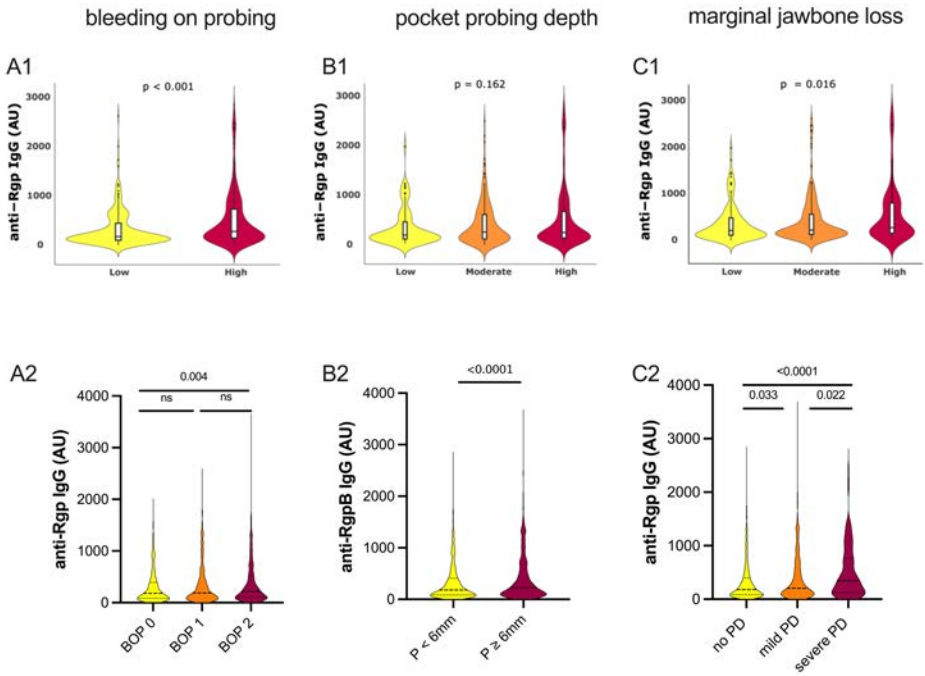

Figure 3. Comparison of anti-Rgp IgG levels in PerioGene North and PAROKRANK, based on different dental parameters. The first row **(A1, B1, C1)** show data from cases in PGN and the second row **(A2, B2, C2)** show data from all PAROKRANK individuals, divided based on different dental parameters; with panel A showing bleeding on probing (BOP), defined as low (≤20%) or high (>20%) in PGN **(A1)** and defined as grade 0 (<10%), 1 (10-29.9%) or 2 (≥30%) in PAROKRANK **(A2)**; panel B: pocket depth, defined as low (<4 mm), moderate (4-6 mm) or high (>6 mm) in PGN **(B1)**, and pocket probing depth (P) <6 mm or P≥6 mm in PAROKRANK **(B2)**; panel C showing bone loss defined as low, moderate or high, based on the average score of the root length (described in detail in Methods) in PGN **(C1)** and no PD (≥80% remaining bone), mild PD (66-79% remaining bone) or severe PD (<66% remaining bone) in PAROKRANK **(C2)**.

ROC curve analysis based on anti-Rgp IgG levels

When assessing the discriminatory capacity of anti-Rgp IgG in the different cohorts, we observed a generally low power of anti-Rgp IgG **(figure 4)**. Starting with measuring anti-Rgp IgG in PAROKRANK **(study I)**, we found an AUROC of 0.56 (CI:0.53-0.59). In order to investigate whether this low AUROC value could be explained by the way PD was defined, we measured anti-Rgp IgG in a pilot of PGN, selecting severe PD cases and healthy individuals. Indeed, when we selected 41 severe PD cases and 39 periodontally healthy controls from the PGN study **(study I)**, we could demonstrate an AUROC of 0.79 (CI: 0.69-0.89) with a sensitivity of 58% and specificity of 89%, showing that the extreme sides of the spectrum can be separated by measuring anti-Rgp IgG. However, when we next measured anti-Rgp IgG in the whole PGN study, we found an AUROC of 0.63 (CI: 0.6-0.67). Thus, even though in PGN the healthy controls were more strictly defined as not having any signs of PD, which was not only assessed by bone loss but also based on PPD, an anti-Rgp IgG response can be observed among controls. This thorough investigation of anti-Rgp IgG suggests that these antibodies are not strictly limited to

individuals suffering from PD, hence cannot be used as a biomarker for PD *per se*. A potential explanation could be a previous *Pg* infection. Even though some studies show decreased anti-Pg antibody levels after treatment(223-225), other studies show stable levels despite clinical improvement(221). Alternatively, since (less invasive) *Pg* strains are part of the commensal microflora(226), the anti-Rgp IgG response that we see in healthy could be antibodies "regulating" *Pg*, protecting against extensive colonisation(227). The association between high anti-Rgp IgG levels and PD-severity on the other hand, may point to a role for these antibodies as biomarkers for individuals who will suffer from severe periodontal damage, who will require close monitoring and early intervention. Prospective studies are needed to confirm anti-Rgp IgG as a predictive biomarker for this subset of PD.

The AUROC of the separation between Risk-RA individuals and rheumatologically healthy controls (regardless of periodontal status), had a value of 0.64 (CI: 0.58-0.70), similar to that of PGN **(figure 4 C and D)**. Hence, on a group level the anti-Rgp IgG response in Risk-RA individuals looks like that seen in periodontitis patients compared to periodontally healthy controls.

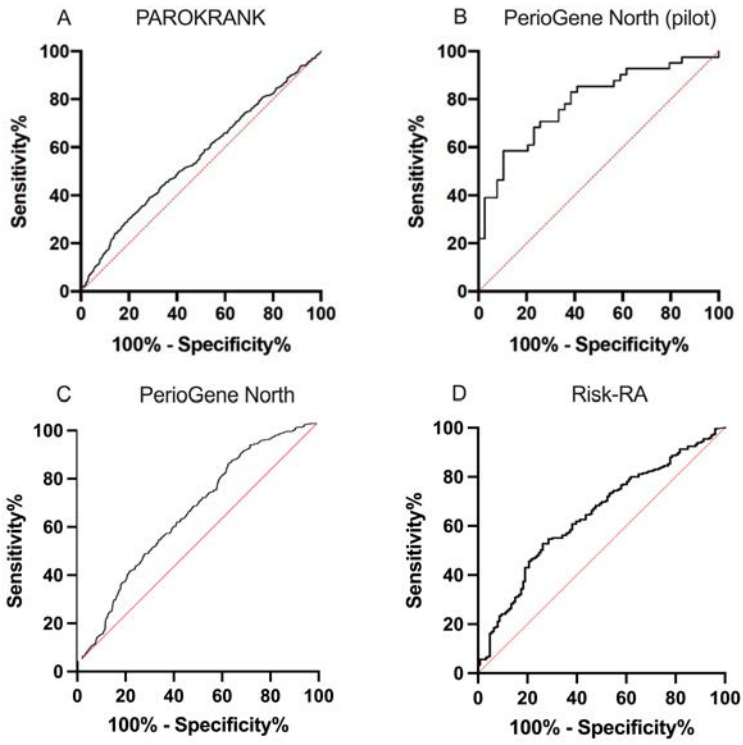

Figure 4. ROC curve analysis based on anti-Rgp IgG in different cohorts. ROC curves representing **A)** the PAROKRANK cohort separating PD cases from controls; **B)** a pilot of PGN, separating 41 severe PD cases from 39 periodontally healthy individuals; **C)** the whole PGN case-control study, separating PD cases from periodontally healthy controls, and **D)** the Risk-RA prospective cohorts showing separation of Risk-RA individuals from (rheumatologically) healthy controls.

A limitation here is the lack of periodontal examination in Risk-RA individuals and controls. Importantly though, higher prevalence of periodontitis has been described in RA patients compared to arthritis-free controls(146), and in ACPA+ at-risk individuals compared to healthy controls(177), hence we assume that this is the case also for our Risk-RA individuals versus controls. Moreover, with the presence of anti-Rgp IgG among periodontitis-free controls, which we observed in PAROKRANK and PGN, our data may suggest that *Pg* itself, rather than PD, is associated with the ACPA-positive Risk-RA phase.

Anti-Rgp IgG in relation to ACPA and arthritis

In PAROKRANK and PGN, we have investigated ACPA status in relation to anti-Rgp IgG levels. However, in these two cohorts we did not have access to diagnoses of rheumatic diseases, and thus ACPA positivity should not be interpreted as RA. In PAROKRANK, 52 individuals were ACPA+ (3,5%) whereas in PGN 21 individuals were positive (2,1%). The frequency of ACPA-positivity was higher among PD patients in both PAROKRANK ($p = 0.003$) and PGN ($p = 0.046$) when doing unadjusted analysis. However, after adjusting for age and smoking, this finding lost significance (PGN). In addition, ACPA+ individuals in PAROKRANK, regardless of periodontal status, showed higher anti-Rgp IgG levels compared to ACPA- individuals, and significance remained after performing matched analysis based on age, sex and smoking. The fact that anti-Rpg IgG is higher among ACPA+ individuals is in line with previous findings of higher anti-Rgp IgG levels among ACPA+ RA cases compared to ACPA- RA cases(175). Importantly, comparing ACPA+ Risk-RA progressors with ACPA+ non-progressors did not show difference in levels, nor was significant in Cox regression models.

These data suggest that the anti-*Pg* antibody response – as a proxy for *Pg* infection – is linked to ACPA-positivity rather than to arthritis onset. Hence, we propose that *Pg* triggers loss of tolerance and ACPA production, while additional risk factors are needed for maturation of the ACPA response and development of arthritis. With that said, additional analyses of anti-Rgp IgG in relation to arthritis onset should be performed at later timepoints. Notably, ACPA levels were significantly lower in ACPA+ non-progressors than in progressors, suggesting maturation of the ACPA response has not yet occurred in those who remained arthritis free during the study period. Time will tell if this process is yet to come, and whether some individuals will develop RA later.

Moreover, to separate the influence of *Pg* itself from that of periodontitis, on the ACPA response itself versus arthritis, we would need to study larger numbers of ACPA-positive at-risk individuals over a longer period and perform dental examinations together with measurements of *Pg* infection, including analyses of *Pg* strains and virulence. The risk-RA phase opens a window of opportunity for prevention of disease. In order to understand the role of oral health in progression towards RA, periodontal interventions need to be studied in this phase, an intervention that is presumably acceptable for individuals at risk for developing RA(228). In support of such approach, smaller studies suggest periodontal treatment may have beneficial effects on RA disease activity, and a case report could even demonstrate remission in early RA after periodontal treatment(229, 230).

5.2 The polyclonal and monoclonal anti-CPP3 response

In **study IV**, we aimed to dissect the ACPA response to *P. gingivalis* and investigate the gum mucosa as a site for ACPA production. This was done by analysing the antibody response against citrullinated *Pg* PAD(183) in the large EIRA RA case-control study, using a citrullinated *P.*PAD peptide, CPP3, and by generating mAbs from the gum mucosa to evaluate their reactivity with human and *Pg* derived citrullinated antigens.

As outlined in **figure 5,** we here investigated the polyclonal CPP3 response in 2,859 RA patients and 372 controls, by screening for anti-CPP3 IgG. We detected higher levels of anti-CPP3 IgG in RA compared to controls **(figure 5A)**. A cut-off based on the 98th percentile among controls was applied, and 11% of the RA patients were positive for ACPA binding to the citrullinated peptide, with no significant reactivity to the arginine-containing control peptide (RPP3). The majority of CPP3+ RA patients were also positive for CCP2 (91%) **(figure 5B)**, in line with many other ACPA fine-specificities(186). In addition, affinity-purified polyclonal anti-CCP2 IgG(109) showed binding to CPP3, but not to RPP3 **(figure 5C)**, showing cross-reactivity between CCP2 and the bacterial CPP3 peptide.

Next, we investigated presence of CPP3-reactive B cells in the gingival tissue of PD patients. Four fresh and four frozen GT biopsies were taken from seven PD patients, of whom four also had ACPA+ RA. After tissue digestion, single B cells were sorted based on CD19-positivity **(figure 5)**. From two individuals, GT01 (PD and APCA+ RA, fresh biopsy) and GT06 (PD only, frozen biopsy), we were able to recover paired heavy and light chain sequences. For GT01, we randomly selected 70 out of the total of 94 paired VH and VL sequences for mAb expression. For GT06, selection was based on the previously described ACPA-characteristics (having a high number of SHM and N-linked glycosylation sites(68, 70, 231)). Sixteen paired VH and VL sequences were selected for expression, having > 15 SHM and/or presence of N-glycosylation sites. Anti-CPP3 IgG ELISA showed reactivity of 10 mAbs, derived from the ACPA+ RA/PD patient (n=5 IgG, n = 3 IgA), and from the PD patient (n = 2 IgG). Out of these 10 CPP3+ mAbs, four also reacted to the non-citrullinated RPP3 peptide and six were positive in the polyreactivity test. Still three of the CPP3+ clones were not polyreactive or RPP3+. All three clones were derived from the ACPA+ RA/PD patient. Although these antibodies were negative in the CCP2 ELISA, two of them cross-reacted with peptides derived from human proteins, citrullinated filaggrin and/or citrullinated histone. In addition to the GT-derived mAbs, we screened previously generated mAbs derived from RA synovial fluid and peripheral blood for CPP3 reactivity and identified one CPP3+ clone, denoted BVCA1, which is also CCP2-reactive and shows multireactivity against citrullinated vimentin, filaggrin and histone peptides.

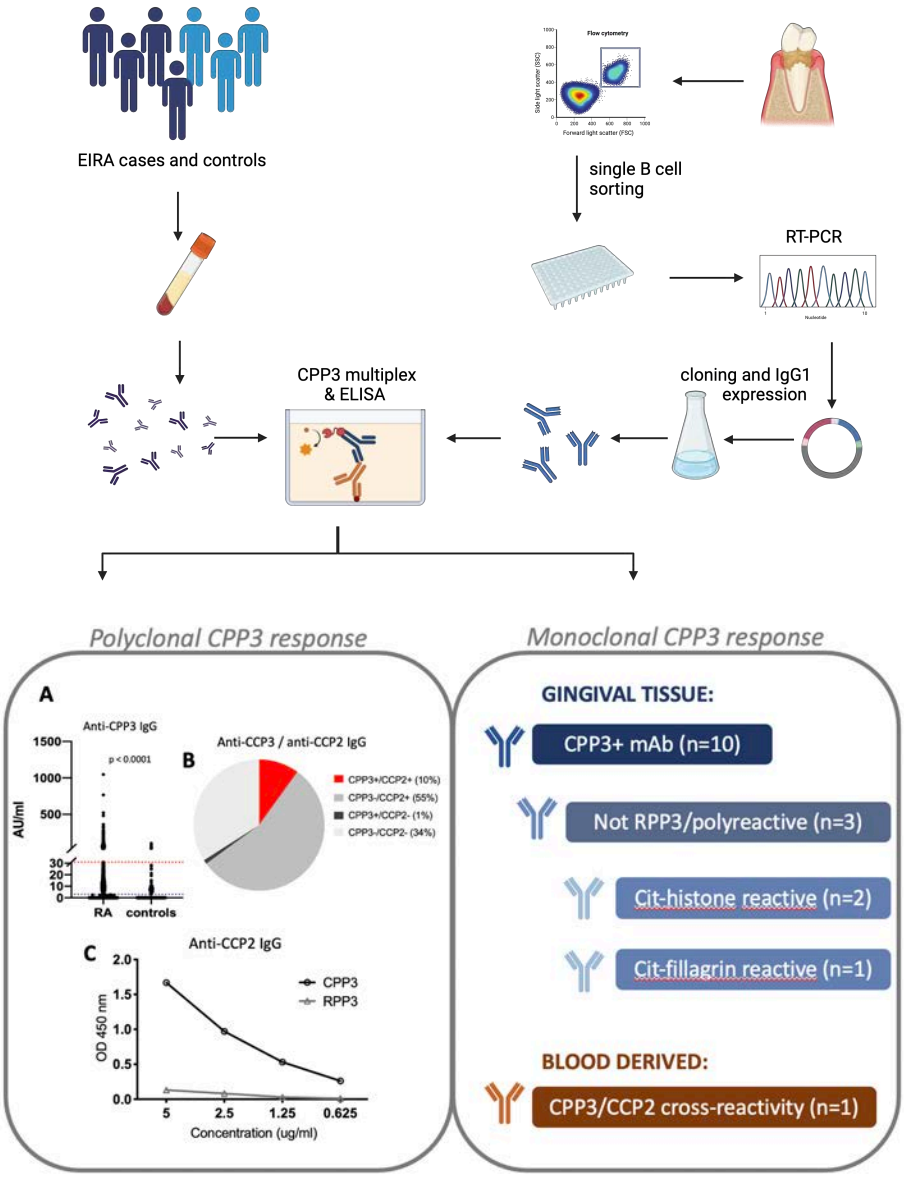

Figure 5. Investigation of polyclonal and monoclonal anti-CPP3 IgG. Outline of the study, where we investigated anti-CPP3 IgG in EIRA RA cases and controls (top left) using multiplex, and generated mAbs from single sorted B cells after BCR sequencing, cloning and expression of mAbs (top right) followed by CPP3 ELISA. From the polyclonal CPP3 response (bottom left) we show: **A)** anti-CPP3 IgG levels in RA patients and controls; **B)** a pie chart illustrating the frequency of CPP3+/CPP3- RA patients in relation to anti-CCP2 IgG; **C)** reactivity to CPP3 and RPP3 of a pool of affinity-purified anti-CCP2 IgG in serial dilutions. From the monoclonal CPP3 response (bottom right) we show CPP3+ clones identified in gingival tissue or blood.

Here, we show a monoclonal and polyclonal antibody response to a citrullinated peptide derived from *Pg* PAD in RA patients, with binding also to other well-studied citrullinated peptides derived from human proteins, using both mAbs and affinity-purified ACPA. These findings show cross-reactivity between bacterial and human citrullinated epitopes, and the fact that these cross-reactive B cells reside in the gums, supports the hypothesis that break of tolerance can take place in the oral mucosa, possibly by mechanisms of molecular mimicry.

It is particularly interesting that the CPP3 cross-reactive mAbs targeted citrullinated filaggrin and histone peptides. The buccal mucosa is rich in citrullinated filaggrin, and the first ever described ACPA test, the antiperinuclear factor, used indirect immunofluorescence to stain buccal mucosa with RA sera(232, 233), and the first citrulline-containing peptide ELISA is also based on citrullinated filaggrin(234). Notably, filaggrin is not expressed in the joint. Additionally, NETosis is a common feature of the inflammatory process in the gums, and citrullinated histones released by NETs have been detected in the inflamed oral mucosa(235, 236). Moreover, ACPA binding citrullinated histones have been described in PD(237). Collectively, these data support the hypothesis that the ACPA response could be triggered in the inflamed oral cavity, where citrullination and danger signals are widespread.

With the absence of CCP2+ clones, but presence of multiple polyreactive CPP3+ clones also targeting RPP3, we hypothesise that the ACPA response in the gums is "immature", in the sense that antibody-binding is rather weak and polyreactive in its nature, more like natural antibodies involved in maintaining immune homeostasis, and described to target both bacterial and self-proteins(238, 239). The *bona fide* systemic autoimmune ACPA response on the other hand, is stronger and citrulline-specific.

In line with these findings and speculations, we identified one peripheral blood memory B cell clone with strong reactivity to CCP2, Cit-vim$_{60\text{-}75}$ and CPP3, but no arginine-reactivity and no polyreactivity. Cit-vim$_{60\text{-}75}$ and CPP3 share a similar "citrulline motif", which could explain the cross-reactivity, and we speculate that the initial ACPA response would have targeted *Pg* CPP3, with epitope-spreading to Cit-vim$_{60\text{-}75}$ (and CCP2) occurring during affinity maturation. Data from another group give some support for this hypothesis, as they could demonstrate loss of CCP2 reactivity but maintained *Pg* reactivity when reverting a CCP2/*Pg* cross-reactive plasmablast clone to its germline sequence(240). However, we need to mention the small number of patients in our study. We have been able to recover GT mAbs from two individuals. In addition, the labour-intensive nature of producing mAbs has limited us in the number of clones that were expressed(241). Nonetheless, the fact that we can find citrulline-reactive B cells in the gums, although in low numbers, supports the need for further investigation of these cells in the context of RA and PD.

5.3 B-cell phenotyping in the Risk-RA phase

In **study V**, we aimed to characterize B cells in Risk-RA individuals to assess if changes in B-cell phenotype occur before onset of RA and whether these changes can predict progression to arthritis. We applied multicolour spectral flow cytometry followed by manual gating strategies and unbiased analysis approaches on flow cytometry data from healthy controls (HC), longitudinal samples of Risk-RA individuals and RA patients.

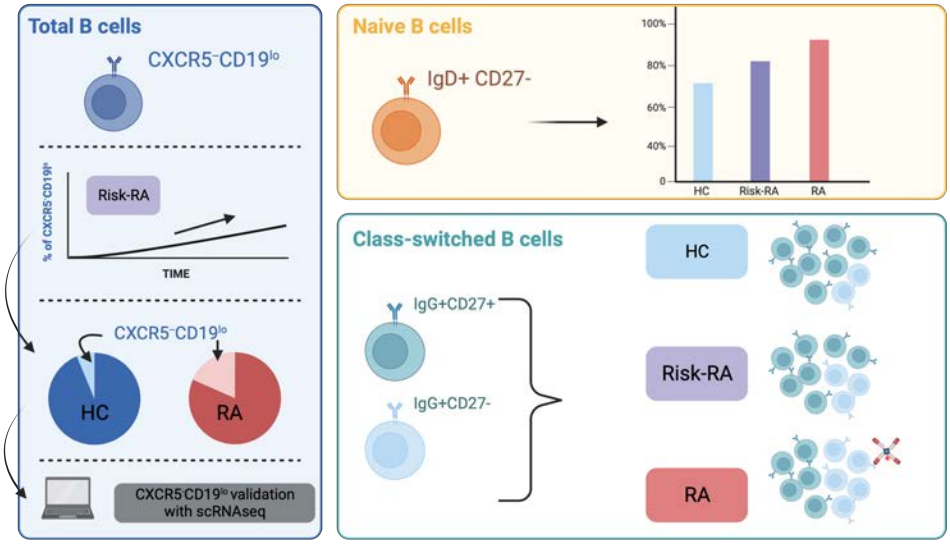

Figure 6. Overview of B-cell changes in the total B-cell population, and among naïve B cells and class-switched B cells. The blue box illustrates the identification of CXCR5^{-}CD19^{lo} B cells in longitudinal Risk-RA samples, identification of CXCR5^{-}CD19^{lo} B cells at a higher frequency in RA compared to HC, and validation of the presence of these cells in a separate scRNA-seq dataset comprising four RA patients. The yellow box shows significantly increased frequencies of naive (IgD^{+}CD27^{-}) B cells in Risk-RA and RA, compared to HC. The green box illustrates CD27 expression in class-switched IgG^{+} B cells; increased levels of CD27^{-} B cells were detected in Risk-RA and RA versus HC, and in addition a large proportion of the ACPA+ B cells in RA were CD27-negative.

Investigation of total CD19^{+} B cells in longitudinal Risk-RA samples using the unbiased algorithm T-REX revealed CXCR5^{-}CD19^{lo} B cells to increase over time. The increase was most pronounced in Risk-RA progressors, but also a number of non-progressors showed an increase of CXCR5^{-}CD19^{lo} B cells over time. This finding led us to investigate this population further. CXCR5^{-}CD19^{lo} B cells could indeed also be found at increased frequencies among RA patients compared to healthy controls. Importantly, we did not only confirm presence of the CXCR5^{-}CD19^{lo} population by flow cytometry, but also using a published 10X scRNA-seq dataset, where we were able to validate presence of this population among B cells in four RA patients using an RNA-based method(72). We found CXCR5^{-}CD19^{lo} B cells both in the naive (IgD^{+}) and in the double negative (IgD^{-}CD27^{-}) compartment.

Applying manual gating based on CD27 and IgD showed an increase of naive and a decrease of unswitched B cells among RA patients as well as Risk-RA individuals at time of inclusion when

compared to healthy controls. A decrease of memory B cells was detected in RA patients, but not in Risk-RA, compared to controls. However, when assessing the time of arthritis onset in Risk-RA progressors, a significant reduction in memory and unswitched memory B cells was observed compared to controls. Comparing Risk-RA progressors with non-progressors at the time of inclusion, showed no statistical differences for naïve, unswitched or memory or DN B cells.

Investigation of ACPA+ B cells using fluorophore-coupled citrullinated peptide antigens (tetramers) in RA patients showed presence of class-switched ACPA+ B cells. The majority of the IgG^+ $ACPA^+$ B cells had no expression of the classical memory B-cell marker CD27, in contrast to what has previously been reported for ACPA+ B cells(73, 76). This finding led us to further explore the total IgG^+ B cells in RA patients, Risk-RA individuals, and controls. A significantly higher proportion of IgG^+ B cells derived from RA patients were negative for CD27 (median: 45%, IQR:28-59%) compared to controls (median: 22%, IQR 20-39%), p=0.035. Comparing progressors with non-progressors, we could observe a similar trend, with a higher proportion of $CD27^-IgG^+$ B cells among progressors (median 37%, IQR:28-55%) compared to non-progressors (median 29%, IQR:19-30%), p=0.022. These cells, being IgD^- and $CD27^-$, are referred to as double negative (DN), and have been described to be expanded in multiple autoimmune diseases, including RA. However, we did not see a significant increase of DN B cells among total B cells in Risk-RA or RA, compared to controls, but within the IgG^+ population, a larger fraction was indeed $CD27^-$. Besides reduction of CD27, these cells also expressed lower levels of CD21 and CD24.

Together, the three main findings of **study V** are: *i)* the increase of naive B cells in Risk-RA and RA, *ii)* the increase of a $CXCR5^-CD19^{lo}$ population over time in Risk-RA and in RA compared to HC, and, *iii)* the increase of $CD27^-$ B cells among IgG class-switched B cells in Risk-RA and RA. Importantly, the increase of $CD27^-$ B cells among IgG^+ B cells was even found to be significantly different between progressors and non-progressors at baseline, and might thus point towards an early feature of immune-dysregulation.

The increase of naive B cells in the risk-RA phase, going hand in hand with a decrease of memory B cells, has been described previously in individuals with arthralgia, being at risk for RA(242, 243). Increased $CXCR5^-CD19^{lo}$ B cells have been shown in RA and SLE patients compared to controls(244). The fact that we can show an increase of $CXCR5^-CD19^{lo}$ B cells over time in the risk phase, although not significant, points towards a potential contribution to onset of RA. CXCR5 is a chemokine receptor binding to its ligand CXCL13, that is secreted in lymphoid tissues. The CXCR5 and CXCL13 axis mediates the homing of B cells and T follicular helper cells. The downregulation of CXCR5 has been described in SLE, Sjögren's syndrome, and human immunodeficiency virus (HIV) infection(245-247). Interestingly, in Sjögen's syndrome, lower numbers of $CXCR5^+$ peripheral blood B cells was detected while higher numbers of $CXCR5^+$ B cells were found in infiltrates in the salivary glands, suggesting that lower CXCR5 expression in the periphery could be explained by increased homing into follicular structures.

A strength of our data is the longitudinal nature of the Risk-RA samples. On the other hand, a limiting factor of the longitudinal data were the small numbers. Whereas we had baseline samples from 18 Risk-RA individuals, we only had 14 individuals (six progressors and eight non-progressors) where multiple samples were available. Further research is needed to be able to validate an increase in $CXCR5^{-}CD19^{lo}$ B cells over time in Risk-RA individuals, and to expand our understanding of this $CXCR5^{-}CD19^{lo}$ population in the at-risk phase.

Loss of CD27 on IgG^{+} B cells, which not only increased in the risk phase, but also marked a difference between progressors and non-progressors at baseline, has previously been shown in the context of autoimmunity and specifically RA, where this feature is linked to hypomutated BCR sequences, a characteristic of autoimmunity(78, 248, 249). In line with findings in other diseases like HIV, the $CD27^{-}$ cells also showed reduced expression of CD21, and these cells were suggested to be anergic or exhausted(250, 251). In addition, synovial $CD27^{-}IgG^{+}$ B cells derived from RA patients showed higher TNF secretion upon stimulation compared to $CD27^{+}IgG^{+}$ B cells. Further understanding of the role of these cells in the risk-RA phase is needed, and the potential predictive role of this feature on development of RA needs to be elucidated.

Multiple studies have described alterations in the T- and B cell populations in the risk phase of ACPA+ RA using mass cytometry(252), flow cytometry(243) or methylation analysis of B and T cells(253). Generally, these studies show altered lymphocyte profiles in Risk-RA compared to healthy controls but also compared to RA patients, indicating that these individuals are immunologically in an “in-between” state. This supports the idea of a window of opportunity for intervention aimed at delaying or ideally preventing disease onset. To this date, only one of the studies has implemented lymphocyte subset alterations in a model for predicting onset of RA(243). Although in this study, B-cell phenotypes had relatively low predictive capacity on their own, while combined lymphocyte subset data together with clinical, serological and genetic data resulted in good prediction models. Our findings of B-cell subsets in Risk-RA need to be validated in larger cohorts and their contribution in prediction models need to be investigated.

Synovial tissue cellular signatures have been investigated in the context of predicting response to therapy in RA(254). However, since synovial biopsies cannot be collected from individuals without arthritis, monitoring of B cells and other PBMCs should be considered in prediction of treatment response in Risk-RA individuals. Importantly, interventions in the risk phase, specifically using abatacept, which blocks T-cell activation(39, 255, 256), but also using rituximab, which depletes CD20+ B cells, have shown effectiveness(38). With the identification of B-cell subsets changing during the risk phase, like the ones described in this work, we should also consider targeting these cells specifically to prevent onset of RA. But first, their contribution to driving RA must be investigated in more detail, and confirmed.

6 Conclusions and future perspectives

In conclusion, the findings in my thesis support the link between oral infection and autoimmunity on the path towards rheumatoid arthritis.

The studies on anti-Rgp IgG show that:

- anti-Rgp IgG cannot discriminate well between PD patients and periodontally healthy individuals
- anti-Rgp IgG identifiies a subgroup of patients with severe periodontal disease, characterised by advanced jawbone loss and active gingivitis
- anti-Rgp IgG levels are increased in ACPA+ at-risk individuals compared to ACPA- controls
- anti-Rgp IgG cannot predict onset of arthritis in ACPA+ at-risk individuals with musculoskeletal complaints

These findings lead to the conclusion that anti-Rgp IgG is not a good biomarker for periodontitis *per se* but has potential as a biomarker for individuals who will suffer from severe forms of PD. Moreover, this subset of PD patients may be at increased risk of developing systemic autoimmunity and eventually ACPA+ RA. The fact that anti-Rgp IgG associates with the risk phase, but does not predict progression to RA, suggests that *Pg* infection is an early event in RA aetiology, possibly causatively involved in triggering the initial ACPA response, rather than contributing directly to arthritis onset.

Investigating the antibody response to another *Pg* epitope, CPP3, taught us that:

- anti-CPP3 antibodies are part of the ACPA immune response in RA patients
- anti-CPP3 antibodies show cross-reactivity between human and bacterial sequences on a monoclonal and polyclonal level
- CPP3+ B cells reside in the gums of PD patients (with or without ACPA+ RA)
- Many CPP3+ gingival tissue B cell clones are polyreactive

From these findings, we conclude that the ACPA response might start but does not mature in the oral mucosa, and that *Pg* may play an important role in driving the ACPA response through mechanisms of molecular mimicry.

The B-cell study in the risk-RA phase reveals that:

- B-cell populations in the peripheral blood are altered already before onset of RA, with increased levels of naïve B cells
- The percentage of $CXCR5^{-}CD19^{lo}$ B cells increases over time in the risk-RA phase

- $CD27^{-}IgG^{+}$ B cells are increased in RA patients compared to controls and also elevated in Risk-RA indidivuals, with a higher proportion of $CD27^{-}IgG^{+}$ B cells in progressors compared to non-progressors at baseline.

Here, we conclude that the observed B-cell alterations pinpointing specific cell types – that have previously been linked to autoimmunity – could potentially play a role in driving RA. Moreover, the increased proportion of $CD27^{-}IgG^{+}$ B cells among progressors could possibly contribute to predicting the onset of arthritis in Risk-RA individuals.

→ From our data on anti-Rgp IgG, together with the cross-reactivity between citrullinated human and bacterial epitopes that we have demonstrated on a monoclonal level, we propose an aetiological model where *Pg* infection contributes to the first "hit" on the path towards RA, *i.e.,* the break of (B cell) tolerance and a systemic ACPA response, triggered by *Pg* in the gum mucosa. Further research is needed to dissect the mechanistic link and to understand the role of oral health and specific bacteria in the at-risk phase.

Future research should clarify the following points:

- The role of the anti-*Pg* antibody response in long-term prediction of RA onset. Progression to RA needs to be re-evaluated in the Risk-RA cohort at a later timepoint, with repeated analysis of anti-Rgp IgG levels in relation to arthritis onset.
- The role of periodontitis versus *Pg* in the risk phase. Periodontal assessment as well as analyses of the oral microbiome should be performed in Risk-RA individuals to gain better insight into the oral health during the at-risk phase of RA, and to determine whether periodontitis itself, or other oral bacteria, rather than *Pg* infection, can predict onset of arthritis.
- The impact of periodontal treatment on progression to RA. Importantly, presence/absence of certain oral bacteria, including *Pg*, should be studied in this context. While periodontal treatment of Risk-RA individuals with PD will tell us weather RA can be prevented, more subtle effects, including delayed onset and milder disease course, will be more difficult to assess, given that confirmed PD should always be treated, hence from an ethical point of view there could never be an untreated control group.

On a cellular level, the following points need to be addressed:

- The role of B cells in the gum mucosa during the risk phase of RA. Studying gingival tissue biopsies and single sorted gingival B cells derived from ACPA+ Risk-RA individuals with severe PD can contribute to our understanding of break of tolerance in the gum mucosa.

- The characteristics of circulating ACPA+ B cells in at-risk individuals over time. Citrullinated peptide tetramers should be used (in combination with an extensive B-cell panel) to monitor ACPA+ B cells in at-risk individuals, and in severe PD patients.
- The role of $CXCR5^{-}CD19^{lo}$ B cells and $CD27^{-}IgG^{+}$ B cells in progression towards RA. Functional assays are needed to understand the potential pathogenic properties and mechanisms.
- Whether B-cell signatures can be used to predict response to preclinical interventions. RA therapies need to be further investigated in the context of preventing/delaying RA onset, and phenotypic B-cell changes should be studied in such settings to determine if they can help identify individuals at risk that will benefit the most from treatment (and which treatment).

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8 References

1. Greenwood B. The contribution of vaccination to global health: past, present and future. Philos Trans R Soc Lond B Biol Sci. 2014;369(1645):20130433.

2. Spyrou MA, Musralina L, Gnecchi Ruscone GA, Kocher A, Borbone P-G, Khartanovich VI, et al. The source of the Black Death in fourteenth-century central Eurasia. Nature. 2022;606(7915):718-24.

3. Baker RE, Mahmud AS, Miller IF, Rajeev M, Rasambainarivo F, Rice BL, et al. Infectious disease in an era of global change. Nature Reviews Microbiology. 2022;20(4):193-205.

4. Ray C, Ming X. Climate Change and Human Health: A Review of Allergies, Autoimmunity and the Microbiome. Int J Environ Res Public Health. 2020;17(13).

5. Bach J-Fo. The Effect of Infections on Susceptibility to Autoimmune and Allergic Diseases. New England Journal of Medicine. 2002;347(12):911-20.

6. Lee AS, Aguilera J, Efobi JA, Jung YS, Seastedt H, Shah MM, et al. Climate change and public health. EMBO reports. 2023;24(4):e56821.

7. Giovanni A, Marco P, Giorgio C, Maurizio R, Ombretta V, Giovanni O, et al. Association between long-term exposure to air pollution and immune-mediated diseases: a population-based cohort study. RMD Open. 2022;8(1):e002055.

8. Manz RA, Thiel A, Radbruch A. Lifetime of plasma cells in the bone marrow. Nature. 1997;388(6638):133-4.

9. Menetski JP, Gellert M. V(D)J recombination activity in lymphoid cell lines is increased by agents that elevate cAMP. Proc Natl Acad Sci U S A. 1990;87(23):9324-8.

10. Nemazee D. Mechanisms of central tolerance for B cells. Nat Rev Immunol. 2017;17(5):281-94.

11. Abbas AK, Lichtman AH. Basic Immunology: Functions and Disorders of the Immune System: Saunders; 2010.

12. Tiegs SL, Russell DM, Nemazee D. Receptor editing in self-reactive bone marrow B cells. J Exp Med. 1993;177(4):1009-20.

13. Goodnow CC, Crosbie J, Adelstein S, Lavoie TB, Smith-Gill SJ, Brink RA, et al. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nature. 1988;334(6184):676-82.

14. Benschop RJ, Aviszus K, Zhang X, Manser T, Cambier JC, Wysocki LJ. Activation and anergy in bone marrow B cells of a novel immunoglobulin transgenic mouse that is both hapten specific and autoreactive. Immunity. 2001;14(1):33-43.

15. Wardemann H, Nussenzweig MC. B-Cell Self-Tolerance in Humans. Advances in Immunology. 95: Academic Press; 2007. p. 83-110.

16. Crotty S. T follicular helper cell differentiation, function, and roles in disease. Immunity. 2014;41(4):529-42.

17. Xing Y, Hogquist KA. T-cell tolerance: central and peripheral. Cold Spring Harb Perspect Biol. 2012;4(6).

18. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol. 2014;5:520.

19. Lu LL, Suscovich TJ, Fortune SM, Alter G. Beyond binding: antibody effector functions in infectious diseases. Nature Reviews Immunology. 2018;18(1):46-61.

20. Bunker JJ, Bendelac A. IgA Responses to Microbiota. Immunity. 2018;49(2):211-24.

21. He Y, Zha Q, Liu D, Lu A. Relations between serum IgA level and cartilage erosion in 436 cases of rheumatoid arthritis. Immunol Invest. 2007;36(3):285-91.

22. Eliçabe RJ, Silva JE, Dave MN, Lacoste MG, Tamashiro H, Blas R, et al. Association between IL-17 and IgA in the joints of patients with inflammatory arthropathies. BMC Immunology. 2017;18(1):8.

23. Zielinska DF, Gnad F, Wiśniewski JR, Mann M. Precision mapping of an in vivo N-glycoproteome reveals rigid topological and sequence constraints. Cell. 2010;141(5):897-907.

24. Bondt A, Rombouts Y, Selman MHJ, Hensbergen PJ, Reiding KR, Hazes JMW, et al. Immunoglobulin G (IgG) Fab Glycosylation Analysis Using a New Mass Spectrometric High-throughput Profiling Method Reveals Pregnancy-associated Changes. Molecular & Cellular Proteomics. 2014;13(11):3029-39.

25. Holland M, Takada K, Okumoto T, Takahashi N, Kato K, Adu D, et al. Hypogalactosylation of serum IgG in patients with ANCA-associated systemic vasculitis. Clin Exp Immunol. 2002;129(1):183-90.

26. Watson M, Rudd PM, Bland M, Dwek RA, Axford JS. Sugar printing rheumatic diseases: a potential method for disease differentiation using immunoglobulin G oligosaccharides. Arthritis Rheum. 1999;42(8):1682-90.

27. Vučković F, Krištić J, Gudelj I, Teruel M, Keser T, Pezer M, et al. Association of Systemic Lupus Erythematosus With Decreased Immunosuppressive Potential of the IgG Glycome. Arthritis Rheumatol. 2015;67(11):2978-89.

28. Leibiger H, Wüstner D, Stigler RD, Marx U. Variable domain-linked oligosaccharides of a human monoclonal IgG: structure and influence on antigen binding. Biochem J. 1999;338 529-38.

29. Kissel T, Toes REM, Huizinga TWJ, Wuhrer M. Glycobiology of rheumatic diseases. Nature Reviews Rheumatology. 2023;19(1):28-43.

30. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2010;69(9):1580-8.

31. Hensvold AH, Frisell T, Magnusson PK, Holmdahl R, Askling J, Catrina AI. How well do ACPA discriminate and predict RA in the general population: a study based on 12 590 population-representative Swedish twins. Ann Rheum Dis. 2017;76(1):119-25.

32. Rantapää-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum. 2003;48(10):2741-9.

33. Englund M, Joud A, Geborek P, Felson DT, Jacobsson LT, Petersson IF. Prevalence and incidence of rheumatoid arthritis in southern Sweden 2008 and their relation to prescribed biologics. Rheumatology (Oxford). 2010;49(8):1563-9.

34. Safiri S, Kolahi AA, Hoy D, Smith E, Bettampadi D, Mansournia MA, et al. Global, regional and national burden of rheumatoid arthritis 1990-2017: a systematic analysis of the Global Burden of Disease study 2017. Ann Rheum Dis. 2019;78(11):1463-71.

35. Emery P, Pope JE, Kruger K, Lippe R, DeMasi R, Lula S, et al. Efficacy of Monotherapy with Biologics and JAK Inhibitors for the Treatment of Rheumatoid Arthritis: A Systematic Review. Adv Ther. 2018;35(10):1535-63.

36. van der Linden MP, le Cessie S, Raza K, van der Woude D, Knevel R, Huizinga TW, et al. Long-term impact of delay in assessment of patients with early arthritis. Arthritis Rheum. 2010;62(12):3537-46.

37. Finckh A, Liang MH, van Herckenrode CM, de Pablo P. Long-term impact of early treatment on radiographic progression in rheumatoid arthritis: A meta-analysis. Arthritis Rheum. 2006;55(6):864-72.

38. Gerlag DM, Safy M, Maijer KI, Tang MW, Tas SW, Starmans-Kool MJF, et al. Effects of B-cell directed therapy on the preclinical stage of rheumatoid arthritis: the PRAIRI study. Ann Rheum Dis. 2019;78(2):179.

39. Rech J, Østergaard M, Tascilar K, Hagen M, Mendez LV, Kleyer A, et al. Abatacept reverses subclinical arthritis in patients with high-risk to develop rheumatoid arthritis-results from the randomized, placebo-controlled ARIAA Study in RA-at Risk Patients. Arthritis and Rheumatology. 2021;73(S9):939-41.

40. Raychaudhuri S, Sandor C, Stahl EA, Freudenberg J, Lee HS, Jia X, et al. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. Nat Genet. 2012;44(3):291-6.

41. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum. 1987;30(11):1205-13.

42. Padyukov L, Seielstad M, Ong RT, Ding B, Rönnelid J, Seddighzadeh M, et al. A genome-wide association study suggests contrasting associations in ACPA-positive versus ACPA-negative rheumatoid arthritis. Ann Rheum Dis. 2011;70(2):259-65.

43. van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Huizinga TW, Toes RE, de Vries RR. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. Arthritis Rheum. 2006;54(4):1117-21.

44. Mobbs JI, Illing PT, Dudek NL, Brooks AG, Baker DG, Purcell AW, et al. The molecular basis for peptide repertoire selection in the human leucocyte antigen (HLA) C*06:02 molecule. J Biol Chem. 2017;292(42):17203-15.

45. Scally SW, Petersen J, Law SC, Dudek NL, Nel HJ, Loh KL, et al. A molecular basis for the association of the HLA-DRB1 locus, citrullination, and rheumatoid arthritis. Journal of Experimental Medicine. 2013;210(12):2569-82.

46. Lim JJ, Jones CM, Loh TJ, Ting YT, Zareie P, Loh KL, et al. The shared susceptibility epitope of HLA-DR4 binds citrullinated self-antigens and the TCR. Science Immunology. 2021;6(58):eabe0896.

47. Padyukov L. Genetics of rheumatoid arthritis. Semin Immunopathol. 2022;44(1):47-62.

48. Lundberg K, Bengtsson C, Kharlamova N, Reed E, Jiang X, Kallberg H, et al. Genetic and environmental determinants for disease risk in subsets of rheumatoid arthritis defined by the anticitrullinated protein/peptide antibody fine specificity profile. Ann Rheum Dis. 2013;72(5):652-8.

49. Tang B, Liu Q, Ilar A, Wiebert P, Hägg S, Padyukov L, et al. Occupational inhalable agents constitute major risk factors for rheumatoid arthritis, particularly in the context of genetic predisposition and smoking. Ann Rheum Dis. 2023;82(3):316-23.

50. Stolt P, Bengtsson C, Nordmark B, Lindblad S, Lundberg I, Klareskog L, et al. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. Ann Rheum Dis. 2003;62(9):835-41.

51. Silman AJ, Newman J, MacGregor AJ. Cigarette smoking increases the risk of rheumatoid arthritis. Results from a nationwide study of disease-discordant twins. Arthritis Rheum. 1996;39(5):732-5.

52. Klareskog L, Stolt P, Lundberg K, Källberg H, Bengtsson C, Grunewald J, et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum. 2006;54(1):38-46.

53. Linn-Rasker SP, van der Helm-van Mil AHM, van Gaalen FA, Kloppenburg M, de Vries RRP, le Cessie S, et al. Smoking is a risk factor for anti-CCP antibodies only in rheumatoid arthritis patients who carry HLA-DRB1 shared epitope alleles. Ann Rheum Dis. 2006;65(3):366-71.

54. Maisha JA, El-Gabalawy HS, O'Neil LJ. Modifiable risk factors linked to the development of rheumatoid arthritis: evidence, immunological mechanisms and prevention. Front Immunol. 2023;14:1221125.

55. Kudaeva FM, Speechley MR, Pope JE. A systematic review of viral exposures as a risk for rheumatoid arthritis. Seminars in Arthritis and Rheumatism. 2019;48(4):587-96.

56. Sherina N, Hreggvidsdottir HS, Bengtsson C, Hansson M, Israelsson L, Alfredsson L, et al. Low levels of antibodies against common viruses associate with anti-citrullinated protein antibody-positive rheumatoid arthritis; implications for disease aetiology. Arthritis Res Ther. 2017;19(1):219.

57. Catrina A, Krishnamurthy A, Rethi B. Current view on the pathogenic role of anti-citrullinated protein antibodies in rheumatoid arthritis. RMD Open. 2021;7(1):e001228.

58. Firestein GS, McInnes IB. Immunopathogenesis of Rheumatoid Arthritis. Immunity. 2017;46(2):183-96.

59. Hu F, Li Y, Zheng L, Shi L, Liu H, Zhang X, et al. Toll-like receptors expressed by synovial fibroblasts perpetuate Th1 and th17 cell responses in rheumatoid arthritis. PloS one. 2014;9(6):e100266-e.

60. Kopesky P, Tiedemann K, Alkekhia D, Zechner C, Millard B, Schoeberl B, et al. Autocrine signaling is a key regulatory element during osteoclastogenesis. Biology Open. 2014;3(8):767-76.

61. Kleyer A, Schett G. Arthritis and bone loss: a hen and egg story. Current Opinion in Rheumatology. 2014;26(1).

62. Iotsova V, Caamaño J, Loy J, Yang Y, Lewin A, Bravo R. Osteopetrosis in mice lacking NF-κB1 and NF-κB2. Nature Medicine. 1997;3(11):1285-9.

63. Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature. 1999;402(6759):304-9.

64. Horwood NJ, Kartsogiannis V, Quinn JM, Romas E, Martin TJ, Gillespie MT. Activated T lymphocytes support osteoclast formation in vitro. Biochem Biophys Res Commun. 1999;265(1):144-50.

65. Zec K, Schonfeldova B, Ai Z, Van Grinsven E, Pirgova G, Eames HL, et al. Macrophages in the synovial lining niche initiate neutrophil recruitment and articular inflammation. Journal of Experimental Medicine. 2023;220(8):e20220595.

66. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, et al. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med. 2004;350(25):2572-81.

67. Lewis MJ, Barnes MR, Blighe K, Goldmann K, Rana S, Hackney JA, et al. Molecular Portraits of Early Rheumatoid Arthritis Identify Clinical and Treatment Response Phenotypes. Cell Rep. 2019;28(9):2455-70.e5.

68. Steen J, Forsström B, Sahlström P, Odowd V, Israelsson L, Krishnamurthy A, et al. Recognition of Amino Acid Motifs, Rather Than Specific Proteins, by Human Plasma Cell–Derived Monoclonal Antibodies to Posttranslationally Modified Proteins in Rheumatoid Arthritis. Arthritis Rheumatol. 2019;71(2):196-209.

69. Joshua V, Loberg Haarhaus M, Hensvold A, Wähämaa H, Gerstner C, Hansson M, et al. Rheumatoid Arthritis-Specific Autoimmunity in the Lung Before and at the Onset of Disease. Arthritis Rheumatol. 2023.

70. Titcombe PJ, Wigerblad G, Sippl N, Zhang N, Shmagel AK, Sahlström P, et al. Pathogenic Citrulline-Multispecific B Cell Receptor Clades in Rheumatoid Arthritis. Arthritis Rheumatol. 2018;70(12):1933-45.

71. Sherina N, de Vries C, Kharlamova N, Sippl N, Jiang X, Brynedal B, et al. Antibodies to a Citrullinated Porphyromonas gingivalis Epitope Are Increased in Early Rheumatoid Arthritis, and Can Be Produced by Gingival Tissue B Cells: Implications for a Bacterial Origin in RA Etiology. Front Immunol. 2022;13:804822.

72. Hensvold A, Horuluoglu B, Sahlström P, Thyagarajan R, Diaz Boada JS, Hansson M, et al. The human bone marrow plasma cell compartment in rheumatoid arthritis - Clonal relationships and anti-citrulline autoantibody producing cells. J Autoimmun. 2023;136:103022.

73. Reijm S, Kwekkeboom JC, Blomberg NJ, Suurmond J, Van der Woude D, Toes REM, et al. Autoreactive B cells in rheumatoid arthritis include mainly activated CXCR3+ memory B cells and plasmablasts. JCI Insight. 2023.

74. Sahlström P, Hansson M, Steen J, Amara K, Titcombe PJ, Forsström B, et al. Different Hierarchies of Anti-Modified Protein Autoantibody Reactivities in Rheumatoid Arthritis. Arthritis Rheumatol. 2020;72(10):1643-57.

75. Kissel T, Reijm S, Slot LM, Cavallari M, Wortel CM, Vergroesen RD, et al. Antibodies and B cells recognising citrullinated proteins display a broad cross-reactivity towards other post-translational modifications. Ann Rheum Dis. 2020;79(4):472.

76. Kristyanto H, Blomberg NJ, Slot LM, van der Voort EIH, Kerkman PF, Bakker A, et al. Persistently activated, proliferative memory autoreactive B cells promote inflammation in rheumatoid arthritis. Sci Transl Med. 2020;12(570).

77. Wang Y, Lloyd KA, Melas I, Zhou D, Thyagarajan R, Lindqvist J, et al. Rheumatoid arthritis patients display B-cell dysregulation already in the naïve repertoire consistent with defects in B-cell tolerance. Scientific reports. 2019;9(1):19995-.

78. Cowan GJM, Miles K, Capitani L, Giguere SSB, Johnsson H, Goodyear C, et al. In Human Autoimmunity, a Substantial Component of the B Cell Repertoire Consists of Polyclonal, Barely Mutated IgG+ve B Cells. Front Immunol. 2020;11.

79. Wu X, Liu Y, Jin S, Wang M, Jiao Y, Yang B, et al. Single-cell sequencing of immune cells from anticitrullinated peptide antibody positive and negative rheumatoid arthritis. Nature Communications. 2021;12(1):4977.

80. Manzo A, Bombardieri M, Humby F, Pitzalis C. Secondary and ectopic lymphoid tissue responses in rheumatoid arthritis: from inflammation to autoimmunity and tissue damage/remodeling. Immunol Rev. 2010;233(1):267-85.

81. Meednu N, Rangel-Moreno J, Zhang F, Escalera-Rivera K, Corsiero E, Prediletto E, et al. Dynamic spectrum of ectopic lymphoid B cell activation and hypermutation in the RA synovium characterized by NR4A nuclear receptor expression. Cell Reports. 2022;39(5):110766.

82. Rao DA, Gurish MF, Marshall JL, Slowikowski K, Fonseka CY, Liu Y, et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. Nature. 2017;542(7639):110-4.

83. Kobayashi S, Murata K, Shibuya H, Morita M, Ishikawa M, Furu M, et al. A distinct human CD4+ T cell subset that secretes CXCL13 in rheumatoid synovium. Arthritis Rheum. 2013;65(12):3063-72.

84. Lee DSW, Rojas OL, Gommerman JL. B cell depletion therapies in autoimmune disease: advances and mechanistic insights. Nature Reviews Drug Discovery. 2021;20(3):179-99.

85. Mackensen A, Müller F, Mougiakakos D, Böltz S, Wilhelm A, Aigner M, et al. Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus. Nature Medicine. 2022;28(10):2124-32.

86. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum. 2000;43(1):155-63.

87. Rogers GE, Harding HW, Llewellyn-Smith IJ. The origin of citrulline-containing proteins in the hair follicle and the chemical nature of trichohyalin, an intracellular precursor. Biochim Biophys Acta. 1977;495(1):159-75.

88. Curran AM, Naik P, Giles JT, Darrah E. PAD enzymes in rheumatoid arthritis: pathogenic effectors and autoimmune targets. Nature Reviews Rheumatology. 2020;16(6):301-15.

89. Kinloch A, Lundberg K, Wait R, Wegner N, Lim NH, Zendman AJ, et al. Synovial fluid is a site of citrullination of autoantigens in inflammatory arthritis. Arthritis Rheum. 2008;58(8):2287-95.

90. van Schaardenburg D, Lagaay AM, Otten HG, Breedveld FC. The relation between class-specific serum rheumatoid factors and age in the general population. Br J Rheumatol. 1993;32(7):546-9.

91. Husby G, Gran JT, Johannessen A. Epidemiological and genetic aspects of IgM rheumatoid factors. Scand J Rheumatol Suppl. 1988;75:213-8.

92. Sahlström P, Joshua V, Valkovskaia V, Biese C, Stålesen R, Israelsson L, et al. Autoreactive B cells against malondialdehyde-induced protein cross-links are present in the joint, lung, and bone marrow of rheumatoid arthritis patients. J Biol Chem. 2023:105320.

93. Burkhardt H, Sehnert B, Bockermann R, Engström A, Kalden JR, Holmdahl R. Humoral immune response to citrullinated collagen type II determinants in early rheumatoid arthritis. Eur J Immunol. 2005;35(5):1643-52.

94. Hassfeld W, Steiner G, Hartmuth K, Kolarz G, Scherak O, Graninger W, et al. Demonstration of a new antinuclear antibody (anti-RA33) that is highly specific for rheumatoid arthritis. Arthritis Rheum. 1989;32(12):1515-20.

95. Verpoort KN, Jol-van der Zijde CM, Papendrecht-van der Voort EAM, Ioan-Facsinay A, Drijfhout JW, van Tol MJD, et al. Isotype distribution of ANTI–CYCLIC citrullinated peptide antibodies in undifferentiated arthritis and rheumatoid arthritis reflects an ongoing immune response. Arthritis & Rheumatism. 2006;54(12):3799-808.

96. van der Woude D, Rantapää-Dahlqvist S, Ioan-Facsinay A, Onnekink C, Schwarte CM, Verpoort KN, et al. Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis. Ann Rheum Dis. 2010;69(8):1554-61.

97. Ioan-Facsinay A, Willemze A, Robinson DB, Peschken CA, Markland J, van der Woude D, et al. Marked differences in fine specificity and isotype usage of the anti-citrullinated protein antibody in health and disease. Arthritis Rheum. 2008;58(10):3000-8.

98. Cîrciumaru A, Kisten Y, Hansson M, Mathsson-Alm L, Joshua V, Wähämaa H, et al. Identification of early risk factors for anti-citrullinated-protein-antibody positive

rheumatoid arthritis - a prospective cohort study. Rheumatology (Oxford), under revision. 2023.

99. Lloyd KA, Steen J, Amara K, Titcombe PJ, Israelsson L, Lundström SL, et al. Variable domain N-linked glycosylation and negative surface charge are key features of monoclonal ACPA: Implications for B-cell selection. Eur J Immunol. 2018;48(6):1030-45.

100. Hafkenscheid L, de Moel E, Smolik I, Tanner S, Meng X, Jansen BC, et al. N-Linked Glycans in the Variable Domain of IgG Anti-Citrullinated Protein Antibodies Predict the Development of Rheumatoid Arthritis. Arthritis Rheumatol. 2019;71(10):1626-33.

101. Forslind K, Ahlmén M, Eberhardt K, Hafström I, Svensson B. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). Ann Rheum Dis. 2004;63(9):1090-5.

102. Orr C, Vieira-Sousa E, Boyle DL, Buch MH, Buckley CD, Cañete JD, et al. Synovial tissue research: a state-of-the-art review. Nature Reviews Rheumatology. 2017;13(8):463-75.

103. Sokolove J, Zhao X, Chandra PE, Robinson WH. Immune complexes containing citrullinated fibrinogen costimulate macrophages via Toll-like receptor 4 and Fcγ receptor. Arthritis Rheum. 2011;63(1):53-62.

104. Clavel C, Nogueira L, Laurent L, Iobagiu C, Vincent C, Sebbag M, et al. Induction of macrophage secretion of tumor necrosis factor alpha through Fcgamma receptor IIa engagement by rheumatoid arthritis-specific autoantibodies to citrullinated proteins complexed with fibrinogen. Arthritis Rheum. 2008;58(3):678-88.

105. Sur Chowdhury C, Giaglis S, Walker UA, Buser A, Hahn S, Hasler P. Enhanced neutrophil extracellular trap generation in rheumatoid arthritis: analysis of underlying signal transduction pathways and potential diagnostic utility. Arthritis Res Ther. 2014;16(3):R122.

106. 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. Science Translational Medicine. 2013;5(178):178ra40-ra40.

107. 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. 2016;75(10):1866-75.

108. Trouw LA, Haisma EM, Levarht EW, van der Woude D, Ioan-Facsinay A, Daha MR, et al. Anti-cyclic citrullinated peptide antibodies from rheumatoid arthritis patients activate complement via both the classical and alternative pathways. Arthritis Rheum. 2009;60(7):1923-31.

109. Ossipova E, Cerqueira CF, Reed E, Kharlamova N, Israelsson L, Holmdahl R, et al. Affinity purified anti-citrullinated protein/peptide antibodies target antigens expressed in the rheumatoid joint. Arthritis Res Ther. 2014;16(4):R167.

110. Cîrciumaru A, Afonso MG, Wähämaa H, Krishnamurthy A, Hansson M, Mathsson-Alm L, et al. Anti-Citrullinated Protein Antibody Reactivity towards Neutrophil-Derived Antigens: Clonal Diversity and Inter-Individual Variation. Biomolecules [Internet]. 2023; 13(4).

111. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2003;423(6937):337-42.

112. Rivollier A, Mazzorana M, Tebib J, Piperno M, Aitsiselmi T, Rabourdin-Combe C, et al. Immature dendritic cell transdifferentiation into osteoclasts: a novel pathway sustained by the rheumatoid arthritis microenvironment. Blood. 2004;104(13):4029-37.

113. Krishnamurthy A, Joshua V, Haj Hensvold A, Jin T, Sun M, Vivar N, et al. Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss. Ann Rheum Dis. 2016;75(4):721-9.

114. Engdahl C, Bang H, Dietel K, Lang SC, Harre U, Schett G. Periarticular Bone Loss in Arthritis Is Induced by Autoantibodies Against Citrullinated Vimentin. J Bone Miner Res. 2017;32(8):1681-91.

115. Sun M, Rethi B, Krishnamurthy A, Joshua V, Circiumaru A, Hensvold AH, et al. Anticitrullinated protein antibodies facilitate migration of synovial tissue-derived fibroblasts. Ann Rheum Dis. 2019;78(12):1621-31.

116. Raposo B, Grönwall C. The diversity of anti-citrulline reactivity and ACPA-associated phenotypes. Joint Bone Spine. 2023;90(6):105617.

117. Krishnamurthy A, Circiumaru A, Sun J, Kisten Y, Damberg P, Sakuraba K, et al. Combination of two monoclonal ACPAs induced tenosynovitis, pain and bone loss in mice in a Peptidyl Arginine DeiminaseΓÇÉ4 dependent manner. Arthritis Rheumatol. 2022;75.

118. Grönwall C, Amara K, Hardt U, Krishnamurthy A, Steen J, Engström M, et al. Autoreactivity to malondialdehyde-modifications in rheumatoid arthritis is linked to disease activity and synovial pathogenesis. Journal of Autoimmunity. 2017;84:29-45.

119. Sakuraba K, Krishnamurthy A, Sun J, Zheng X, Xu C, Peng B, et al. Autoantibodies targeting malondialdehyde-modifications in rheumatoid arthritis regulate osteoclasts via inducing glycolysis and lipid biosynthesis. J Autoimmun. 2022;133:102903.

120. Wigerblad G, Bas DB, Fernades-Cerqueira C, Krishnamurthy A, Nandakumar KS, Rogoz K, et al. Autoantibodies to citrullinated proteins induce joint pain independent of inflammation via a chemokine-dependent mechanism. Ann Rheum Dis. 2016;75(4):730-8.

121. Jurczak A, Delay L, Barbier J, Simon N, Krock E, Sandor K, et al. Antibody-induced pain-like behavior and bone erosion: links to subclinical inflammation, osteoclast activity, and acid-sensing ion channel 3-dependent sensitization. Pain. 2021.

122. Raposo B, Afonso M, Israelsson L, Wähämaa H, Stålesen R, Wermeling F, et al. Divergent and dominant anti-inflammatory effects of patient-derived anticitrullinated protein antibodies (ACPA) in arthritis development. Ann Rheum Dis. 2023:ard-2022-223417.

123. He Y, Ge C, Moreno-Giró À, Xu B, Beusch CM, Sandor K, et al. A subset of antibodies targeting citrullinated proteins confers protection from rheumatoid arthritis. Nature Communications. 2023;14(1):691.

124. Chirivi RGS, van Rosmalen JWG, van der Linden M, Euler M, Schmets G, Bogatkevich G, et al. Therapeutic ACPA inhibits NET formation: a potential therapy for neutrophil-mediated inflammatory diseases. Cell Mol Immunol. 2021;18(6):1528-44.

125. Lundberg K, Nijenhuis S, Vossenaar ER, Palmblad K, van Venrooij WJ, Klareskog L, et al. Citrullinated proteins have increased immunogenicity and arthritogenicity and their presence in arthritic joints correlates with disease severity. Arthritis Res Ther. 2005;7(3):R458-67.

126. Hill JA, Bell DA, Brintnell W, Yue D, Wehrli B, Jevnikar AM, et al. Arthritis induced by posttranslationally modified (citrullinated) fibrinogen in DR4-IE transgenic mice. Journal of Experimental Medicine. 2008;205(4):967-79.

127. McElwee MK, Dileepan T, Mahmud SA, Jenkins MK. The CD4+ T cell repertoire specific for citrullinated peptides shows evidence of immune tolerance. Journal of Experimental Medicine. 2023;220(12):e20230209.

128. Li B, Selmi C, Tang R, Gershwin ME, Ma X. The microbiome and autoimmunity: a paradigm from the gut–liver axis. Cellular & Molecular Immunology. 2018;15(6):595-609.

129. Holers VM, Demoruelle MK, Kuhn KA, Buckner JH, Robinson WH, Okamoto Y, et al. Rheumatoid arthritis and the mucosal origins hypothesis: protection turns to destruction. Nat Rev Rheumatol. 2018;14(9):542-57.

130. Barra L, Scinocca M, Saunders S, Bhayana R, Rohekar S, Racapé M, et al. Anti-Citrullinated Protein Antibodies in Unaffected First-Degree Relatives of Rheumatoid Arthritis Patients. Arthritis & Rheumatism. 2013;65(6):1439-47.

131. Svärd A, Kastbom A, Reckner-Olsson A, Skogh T. Presence and utility of IgA-class antibodies to cyclic citrullinated peptides in early rheumatoid arthritis: the Swedish TIRA project. Arthritis Res Ther. 2008;10(4):R75.

132. Kinslow JD, Blum LK, Deane KD, Demoruelle MK, Okamoto Y, Parish MC, et al. Elevated IgA Plasmablast Levels in Subjects at Risk of Developing Rheumatoid Arthritis. Arthritis Rheumatol. 2016;68(10):2372-83.

133. Malmström V, Catrina AI, Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. Nature Reviews Immunology. 2017;17(1):60-75.

134. Reynisdottir G, Olsen H, Joshua V, Engström M, Forsslund H, Karimi R, et al. Signs of immune activation and local inflammation are present in the bronchial tissue of patients with untreated early rheumatoid arthritis. Ann Rheum Dis. 2016;75(9):1722-7.

135. 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(10):1488-92.

136. Reynisdottir G, Karimi R, Joshua V, Olsen H, Hensvold AH, Harju A, et al. Structural changes and antibody enrichment in the lungs are early features of anti-citrullinated protein antibody-positive rheumatoid arthritis. Arthritis Rheumatol. 2014;66(1):31-9.

137. Joshua V, Loberg Haarhaus M, Hensvold A, Wähämaa H, Gerstner C, Hansson M, et al. Rheumatoid arthritis specific autoimmunity in the lung before and at the onset of disease. Arthritis & Rheumatology. 2023;n/a(n/a).

138. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, et al. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. Elife. 2013;2:e01202.

139. Alpizar-Rodriguez D, Lesker TR, Gronow A, Gilbert B, Raemy E, Lamacchia C, et al. Prevotella copri in individuals at risk for rheumatoid arthritis. Ann Rheum Dis. 2019;78(5):590-3.

140. Takuro N, Yuichi M, Daisuke M, Mariko N, Yuki M, Takao O, et al. Genomic repertoires linked with pathogenic potency of arthritogenic Prevotella copri isolated from the gut of patients with rheumatoid arthritis. Ann Rheum Dis. 2023;82(5):621.

141. Flak MB, Colas RA, Muñoz-Atienza E, Curtis MA, Dalli J, Pitzalis C. Inflammatory arthritis disrupts gut resolution mechanisms, promoting barrier breakdown by Porphyromonas gingivalis. JCI Insight. 2019;4(13).

142. Chriswell ME, Lefferts AR, Clay MR, Hsu AR, Seifert J, Feser ML, et al. Clonal IgA and IgG autoantibodies from individuals at risk for rheumatoid arthritis identify an arthritogenic strain of Subdoligranulum. Sci Transl Med. 2022;14(668):eabn5166.

143. Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Borgnakke WS, et al. Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 to 2012. Journal of Periodontology. 2015;86(5):611-22.

144. Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. J Dent Res. 2014;93(11):1045-53.

145. Rydén L, Buhlin K, Ekstrand E, Faire Ud, Gustafsson A, Holmer J, et al. Periodontitis Increases the Risk of a First Myocardial Infarction. Circulation. 2016;133(6):576-83.

146. Fuggle NR, Smith TO, Kaul A, Sofat N. Hand to Mouth: A Systematic Review and Meta-Analysis of the Association between Rheumatoid Arthritis and Periodontitis. Front Immunol. 2016;7:80.

147. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, et al. Periodontitis and diabetes: a two-way relationship. Diabetologia. 2012;55(1):21-31.

148. Abbayya K, Puthanakar NY, Naduwinmani S, Chidambar YS. Association between Periodontitis and Alzheimer's Disease. N Am J Med Sci. 2015;7(6):241-6.

149. Genco RJ, Borgnakke WS. Risk factors for periodontal disease. Periodontol 2000. 2013;62(1):59-94.

150. Armitage GC. Periodontal diagnoses and classification of periodontal diseases. Periodontol 2000. 2004;34:9-21.

151. Hajishengallis G, Korostoff JM. Revisiting the Page & Schroeder model: the good, the bad and the unknowns in the periodontal host response 40 years later. Periodontol 2000. 2017;75(1):116-51.

152. Uriarte SM, Edmisson JS, Jimenez-Flores E. Human neutrophils and oral microbiota: a constant tug-of-war between a harmonious and a discordant coexistence. Immunol Rev. 2016;273(1):282-98.

153. Thorbert-Mros S, Larsson L, Berglundh T. Cellular composition of long-standing gingivitis and periodontitis lesions. Journal of Periodontal Research. 2015;50(4):535-43.

154. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. 2018;89 Suppl 1:S173-s82.

155. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. Journal of Periodontology. 2018;89(S1):S159-S72.

156. Lang NP, Hill RW. Radiographs in periodontics. J Clin Periodontol. 1977;4(1):16-28.

157. Sanz M, Herrera D, Kebschull M, Chapple I, Jepsen S, Beglundh T, et al. Treatment of stage I-III periodontitis-The EFP S3 level clinical practice guideline. J Clin Periodontol. 2020;47 Suppl 22(Suppl 22):4-60.

158. Caselli E, Fabbri C, D'Accolti M, Soffritti I, Bassi C, Mazzacane S, et al. Defining the oral microbiome by whole-genome sequencing and resistome analysis: the complexity of the healthy picture. BMC Microbiology. 2020;20(1):120.

159. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science. 2009;326(5960):1694-7.

160. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL, Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134-44.

161. Curtis MA, Zenobia C, Darveau RP. The relationship of the oral microbiotia to periodontal health and disease. Cell Host Microbe. 2011;10(4):302-6.

162. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. Nat Rev Immunol. 2017;17(4):219-32.

163. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontol 2000. 1997;14:216-48.

164. Xu W, Zhou W, Wang H, Liang S. Chapter Two - Roles of Porphyromonas gingivalis and its virulence factors in periodontitis. In: Donev R, editor. Advances in Protein Chemistry and Structural Biology. 120: Academic Press; 2020. p. 45-84.

165. Hajishengallis G, Tapping RI, Harokopakis E, Nishiyama S-i, Ratti P, Schifferle RE, et al. Differential interactions of fimbriae and lipopolysaccharide from Porphyromonas gingivalis with the Toll-like receptor 2-centred pattern recognition apparatus. Cellular Microbiology. 2006;8(10):1557-70.

166. Hočevar K, Potempa J, Turk B. Host cell-surface proteins as substrates of gingipains, the main proteases of Porphyromonas gingivalis. Biological Chemistry. 2018;399(12):1353-61.

167. Guo Y, Nguyen K-A, Potempa J. Dichotomy of gingipains action as virulence factors: from cleaving substrates with the precision of a surgeon's knife to a meat chopper-like brutal degradation of proteins. Periodontol 2000. 2010;54(1):15-44.

168. Wilensky A, Tzach-Nahman R, Potempa J, Shapira L, Nussbaum G. Porphyromonas gingivalis Gingipains Selectively Reduce CD14 Expression, Leading to Macrophage Hyporesponsiveness to Bacterial Infection. Journal of Innate Immunity. 2014;7(2):127-35.

169. Bryzek D, Ciaston I, Dobosz E, Gasiorek A, Makarska A, Sarna M, et al. Triggering NETosis via protease-activated receptor (PAR)-2 signaling as a mechanism of hijacking neutrophils function for pathogen benefits. PLoS pathogens. 2019;15(5):e1007773-e.

170. 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(7):3248-56.

171. 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. Inflamm. 2004;28(6):311-8.

172. Sturridge E. Case of Rheumatoid Arthritis treated by Ionization of Periodontal Membrane. Proc R Soc Med. 1918;11(Odontol Sect):112-4.

173. Bender P, Bürgin WB, Sculean A, Eick S. Serum antibody levels against Porphyromonas gingivalis in patients with and without rheumatoid arthritis - a systematic review and meta-analysis. Clin Oral Investig. 2017;21(1):33-42.

174. Potempa J, Mydel P, Koziel J. The case for periodontitis in the pathogenesis of rheumatoid arthritis. Nat Rev Rheumatol. 2017;13(10):606-20.

175. Kharlamova N, Jiang X, Sherina N, Potempa B, Israelsson L, Quirke AM, et al. Antibodies to Porphyromonas gingivalis Indicate Interaction Between Oral Infection, Smoking, and Risk Genes in Rheumatoid Arthritis Etiology. Arthritis Rheumatol. 2016;68(3):604-13.

176. Johansson L, Sherina N, Kharlamova N, Potempa B, Larsson B, Israelsson L, et al. Concentration of antibodies against Porphyromonas gingivalis is increased before the onset of symptoms of rheumatoid arthritis. Arthritis Res Ther. 2016;18(1):201.

177. Mankia K, Cheng Z, Do T, Hunt L, Meade J, Kang J, et al. Prevalence of Periodontal Disease and Periodontopathic Bacteria in Anti-Cyclic Citrullinated Protein Antibody-Positive At-Risk Adults Without Arthritis. JAMA Netw Open. 2019;2(6):e195394.

178. Wegner N, Wait R, Sroka A, Eick S, Nguyen KA, Lundberg K, et al. Peptidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and α-enolase: implications for autoimmunity in rheumatoid arthritis. Arthritis Rheumatol. 2010;62(9):2662-72.

179. Larsen DN, Mikkelsen CE, Kierkegaard M, Bereta GP, Nowakowska Z, Kaczmarek JZ, et al. Citrullinome of Porphyromonas gingivalis Outer Membrane Vesicles: Confident Identification of Citrullinated Peptides. Mol Cell Proteomics. 2020;19(1):167-80.

180. Stobernack T, Glasner C, Junker S, Gabarrini G, de Smit M, de Jong A, et al. Extracellular Proteome and Citrullinome of the Oral Pathogen Porphyromonas gingivalis. J Proteome Res. 2016;15(12):4532-43.

181. Kinloch A, Tatzer V, Wait R, Peston D, Lundberg K, Donatien P, et al. Identification of citrullinated alpha-enolase as a candidate autoantigen in rheumatoid arthritis. Arthritis Res Ther. 2005;7(6):R1421.

182. Lundberg K, Kinloch A, Fisher BA, Wegner N, Wait R, Charles P, et al. Antibodies to citrullinated alpha-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. Arthritis Rheum. 2008;58(10):3009-19.

183. Quirke AM, Lugli EB, Wegner N, Hamilton BC, Charles P, Chowdhury M, et al. Heightened immune response to autocitrullinated Porphyromonas gingivalis peptidylarginine deiminase: a potential mechanism for breaching immunologic tolerance in rheumatoid arthritis. Ann Rheum Dis. 2014;73(1):263-9.

184. Konig MF, Paracha AS, Moni M, Bingham CO, 3rd, Andrade F. Defining the role of Porphyromonas gingivalis peptidylarginine deiminase (PPAD) in rheumatoid arthritis through the study of PPAD biology. Ann Rheum Dis. 2015;74(11):2054-61.

185. Jenning M, Marklein B, Ytterberg J, Zubarev RA, Joshua V, van Schaardenburg D, et al. Bacterial citrullinated epitopes generated by Porphyromonas gingivalis infection-a missing link for ACPA production. Ann Rheum Dis. 2020;79(9):1194-202.

186. Reed E, Hedström AK, Hansson M, Mathsson-Alm L, Brynedal B, Saevarsdottir S, et al. Presence of autoantibodies in “seronegative” rheumatoid arthritis associates with classical risk factors and high disease activity. Arthritis Res Ther. 2020;22(1):170.

187. Yu X, Hester S, Harnden K, Chowdhury R, Duquenne L, Letton R, et al. POS0442 ENDOCITRULLINATION OF RA AUTOANTIGENS IS MORE ABUNDANT THAN C-TERMINAL CITRULLINATION IN THE GINGIVAL CREVICULAR FLUID OF ANTI-CCP POSITIVE AT-RISK INDIVIDUALS WITH PERIODONTAL DISEASE BUT NO SYNOVITIS. Ann Rheum Dis. 2023;82(Suppl 1):477.

188. Bright R, Thiele GM, Manavis J, Mikuls TR, Payne JB, Bartold PM. Gingival tissue, an extrasynovial source of malondialdehyde-acetaldehyde adducts, citrullinated and carbamylated proteins. J Periodontal Res. 2018;53(1):139-43.

189. Janssen KMJ, de Smit MJ, Withaar C, Brouwer E, van Winkelhoff AJ, Vissink A, et al. Autoantibodies against citrullinated histone H3 in rheumatoid arthritis and periodontitis patients. Journal of Clinical Periodontology. 2017;44(6):577-84.

190. Casc ├úo R, Ros ├írio HS, Souto-Carneiro MM, Fonseca JE. Neutrophils in rheumatoid arthritis: More than simple final effectors. Autoimmunity Reviews. 2010;9(8):531-5.

191. Leipe J, Grunke M, Dechant C, Reindl C, Kerzendorf U, Schulze-Koops H, et al. Role of Th17 cells in human autoimmune arthritis. Arthritis Rheum. 2010;62(10):2876-85.

192. Kim TS, Silva LM, Theofilou VI, Greenwell-Wild T, Li L, Williams DW, et al. Neutrophil extracellular traps and extracellular histones potentiate IL-17 inflammation in periodontitis. Journal of Experimental Medicine. 2023;220(9):e20221751.

193. Konig MF, Abusleme L, Reinholdt J, Palmer RJ, Teles RP, Sampson K, et al. Aggregatibacter actinomycetemcomitansΓÇôinduced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. Science Translational Medicine. 2016;8(369):369ra176-369ra176.

194. Romero V, Fert-Bober J, Nigrovic PA, Darrah E, Haque UJ, Lee DM, et al. Immune-mediated pore-forming pathways induce cellular hypercitrullination and generate citrullinated autoantigens in rheumatoid arthritis. Sci Transl Med. 2013;5(209):209ra150.

195. Cheng Z, Do T, Mankia K, Meade J, Hunt L, Clerehugh V, et al. Dysbiosis in the oral microbiomes of anti-CCP positive individuals at risk of developing rheumatoid arthritis. Ann Rheum Dis. 2020:annrheumdis-2020-216972.

196. Tong Y, Zheng L, Qing P, Zhao H, Li Y, Su L, et al. Oral Microbiota Perturbations Are Linked to High Risk for Rheumatoid Arthritis. Frontiers in Cellular and Infection Microbiology. 2020;9.

197. Brewer RC, Lanz TV, Hale CR, Sepich-Poore GD, Martino C, Swafford AD, et al. Oral mucosal breaks trigger anti-citrullinated bacterial and human protein antibody responses in rheumatoid arthritis. Sci Transl Med. 2023;15(684):eabq8476.

198. Al-Katma MK, Bissada NF, Bordeaux JM, Sue J, Askari AD. Control of periodontal infection reduces the severity of active rheumatoid arthritis. J Clin Rheumatol. 2007;13(3):134-7.

199. Monsarrat P, Fernandez de Grado G, Constantin A, Willmann C, Nabet C, Sixou M, et al. The effect of periodontal treatment on patients with rheumatoid arthritis: The ESPERA randomised controlled trial. Joint Bone Spine. 2019;86(5):600-9.

200. Zhang X, Zhang D, Jia H, Feng Q, Wang D, Liang D, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med. 2015;21(8):895-905.

201. Kobayashi T, Yoshie H. Host Responses in the Link Between Periodontitis and Rheumatoid Arthritis. Curr Oral Health Rep. 2015;2(1):1-8.

202. Takeuchi-Hatanaka K, Koyama Y, Okamoto K, Sakaida K, Yamamoto T, Takashiba S. Treatment resistance of rheumatoid arthritis relates to infection of periodontal pathogenic bacteria: a caseΓÇôcontrol cross-sectional study. Scientific Reports. 2022;12(1):12353.

203. Svärd A, Kastbom A, Ljungberg KR, Potempa B, Potempa J, Persson GR, et al. Antibodies against Porphyromonas gingivalis in serum and saliva and their association with rheumatoid arthritis and periodontitis. Data from two rheumatoid arthritis cohorts in Sweden. Front Immunol. 2023;14.

204. Maria VS, Fabian H, Daniela S, Holger B, Guenter S, Arnd K, et al. Antibodies against citrullinated proteins of IgA isotype are associated with progression to rheumatoid arthritis in individuals at-risk. RMD Open. 2023;9(1):e002705.

205. Roos Ljungberg K, Börjesson E, Martinsson K, Wetterö J, Kastbom A, Svärd A. Presence of salivary IgA anti-citrullinated protein antibodies associate with higher disease activity in patients with rheumatoid arthritis. Arthritis Res Ther. 2020;22(1):274.

206. Association WM. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA. 2013;310(20):2191-4.

207. Boström EA, Kindstedt E, Sulniute R, Palmqvist P, Majster M, Holm CK, et al. Increased Eotaxin and MCP-1 Levels in Serum from Individuals with Periodontitis and in Human Gingival Fibroblasts Exposed to Pro-Inflammatory Cytokines. PloS one. 2015;10(8):e0134608-e.

208. Gustafsson JT, Herlitz Lindberg M, Gunnarsson I, Pettersson S, Elvin K, Öhrvik J, et al. Excess atherosclerosis in systemic lupus erythematosus,-A matter of renal involvement: Case control study of 281 SLE patients and 281 individually matched population controls. PloS one. 2017;12(4):e0174572-e.

209. Berglin E, Dahlqvist SR. Comparison of the 1987 ACR and 2010 ACR/EULAR classification criteria for rheumatoid arthritis in clinical practice: a prospective cohort study. (1502-7732 (Electronic)).

210. Jardine JG, Sok D, Julien JP, Briney B, Sarkar A, Liang CH, et al. Minimally Mutated HIV-1 Broadly Neutralizing Antibodies to Guide Reductionist Vaccine Design. PLoS Pathog. 2016;12(8):e1005815.

211. Hansson M, Mathsson L, Schlederer T, Israelsson L, Matsson P, Nogueira L, et al. Validation of a multiplex chip-based assay for the detection of autoantibodies against citrullinated peptides. Arthritis Res Ther. 2012;14(5):R201.

212. Nolan JP, Condello D. Spectral flow cytometry. Curr Protoc Cytom. 2013;Chapter 1:1.27.1-1..13.

213. Barone SM, Paul AG, Muehling LM, Lannigan JA, Kwok WW, Turner RB, et al. Unsupervised machine learning reveals key immune cell subsets in COVID-19, rhinovirus infection, and cancer therapy. Elife. 2021;10.

214. McInnes L, Healy, J., Melville, J. UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction. arXiv:1802.03426; 2018 February 9th, 2018.

215. Van Gassen S, Callebaut B, Van Helden MJ, Lambrecht BN, Demeester P, Dhaene T, et al. FlowSOM: Using self-organizing maps for visualization and interpretation of cytometry data. Cytometry Part A. 2015;87(7):636-45.

216. amara k, Israelsson L, Stålesen R, Sahlström P, Steen J, Malmström V, et al. A Refined Protocol for Identifying Citrulline-specific Monoclonal Antibodies from Single Human B Cells from Rheumatoid Arthritis Patient Material. Bio-protocol. 2019;9(17):e3347.

217. Ye J, Ma N, Madden TL, Ostell JM. IgBLAST: an immunoglobulin variable domain sequence analysis tool. Nucleic Acids Res. 2013;41(Web Server issue):W34-40.

218. Brochet X, Lefranc MP, Giudicelli V. IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis. Nucleic Acids Res. 2008;36(Web Server issue):W503-8.

219. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, 3rd, Zheng S, Butler A, et al. Integrated analysis of multimodal single-cell data. Cell. 2021;184(13):3573-87.e29.

220. Hirai K, Yamaguchi-Tomikawa T, Eguchi T, Maeda H, Takashiba S. Identification and Modification of Porphyromonas gingivalis Cysteine Protease, Gingipain, Ideal for Screening Periodontitis. Front Immunol. 2020;11(1017).

221. Buhlin K, Holmer J, Gustafsson A, Hörkkö S, Pockley AG, Johansson A, et al. Association of periodontitis with persistent, pro-atherogenic antibody responses. Journal of Clinical Periodontology. 2015;42(11):1006-14.

222. Papapanou PN, Neiderud A-M, Papadimitriou A, Sandros J, Dahlén G. "Checkerboard" Assessments of Periodontal Microbiota and Serum Antibody Responses: A Case-Control Study. Journal of Periodontology. 2000;71(6):885-97.

223. Nakayama Y, Ogata Y, Hiromatsu Y, Imamura K, Suzuki E, Saito A, et al. Clinical Usefulness of Novel Immunochromatographic Detection Device for Porphyromonas gingivalis in Evaluating Effects of Scaling and Root Planing and Local Antimicrobial Therapy. J Periodontol. 2016;87(10):1238-47.

224. Morozumi T, Yashima A, Gomi K, Ujiie Y, Izumi Y, Akizuki T, et al. Increased systemic levels of inflammatory mediators following one-stage full-mouth scaling and root planing. J Periodontal Res. 2018;53(4):536-44.

225. Kudo C, Naruishi K, Maeda H, Abiko Y, Hino T, Iwata M, et al. Assessment of the plasma/serum IgG test to screen for periodontitis. J Dent Res. 2012;91(12):1190-5.

226. Jandik KA, Bélanger M, Low SL, Dorn BR, Yang MCK, Progulske-Fox A. Invasive differences among Porphyromonas gingivalis strains from healthy and diseased periodontal sites. Journal of Periodontal Research. 2008;43(5):524-30.

227. Takahashi J, Saito A, Nakagawa T, Yamada S, Ishihara K, Okuda K. Dynamics of Serum Immunoglobulin G Avidity for Porphyromonas gingivalis in Adult Periodontitis. Journal of Periodontology. 1998;69(3):367-73.

228. Chapman L, Vinall-Collier K, Siddle H, Mustufvi Z, Mankia K, Serban S. 'It surprised me a lot that there is a link': a qualitative study of the acceptability of periodontal treatment for individuals at risk of rheumatoid arthritis. RMD Open. 2023;9(2):e003099.

229. Möller B, Bender P, Eick S, Kuchen S, Maldonado A, Potempa J, et al. Treatment of severe periodontitis may improve clinical disease activity in otherwise treatment-refractory rheumatoid arthritis patients. Rheumatology (Oxford). 2020;59(1):243-5.

230. Salemi S, Biondo MI, Fiorentino C, Argento G, Paolantonio M, Di Murro C, et al. Could early rheumatoid arthritis resolve after periodontitis treatment only?: case report and review of the literature. Medicine (Baltimore). 2014;93(27):e195.

231. Germar K, Fehres CM, Scherer HU, van Uden N, Pollastro S, Yeremenko N, et al. Generation and Characterization of Anti-Citrullinated Protein Antibody-Producing B Cell Clones From Rheumatoid Arthritis Patients. Arthritis Rheumatol. 2019;71(3):340-50.

232. Nienhuis RL, Mandema E. A NEW SERUM FACTOR IN PATIENTS WITH RHEUMATOID ARTHRITIS; THE ANTIPERINUCLEAR FACTOR. Ann Rheum Dis. 1964;23(4):302-5.

233. Sebbag M, Simon M, Vincent C, Masson-Bessière C, Girbal E, Durieux JJ, et al. The antiperinuclear factor and the so-called antikeratin antibodies are the same rheumatoid arthritis-specific autoantibodies. J Clin Invest. 1995;95(6):2672-9.

234. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest. 1998;101(1):273-81.

235. Magán-Fernández A, Rasheed Al-Bakri SM, O'Valle F, Benavides-Reyes C, Abadía-Molina F, Mesa F. Neutrophil Extracellular Traps in Periodontitis. Cells. 2020;9(6).

236. Vitkov L, Hartl D, Minnich B, Hannig M. Janus-Faced Neutrophil Extracellular Traps in Periodontitis. Front Immunol. 2017;8:1404.

237. Janssen KMJ, de Smit MJ, Withaar C, Brouwer E, van Winkelhoff AJ, Vissink A, et al. Autoantibodies against citrullinated histone H3 in rheumatoid arthritis and periodontitis patients. J Clin Periodontol. 2017;44(6):577-84.

238. Grönwall C, Vas J, Silverman G. Protective Roles of Natural IgM Antibodies. Front Immunol. 2012;3:66.

239. Haynes BF, Fleming J, St Clair EW, Katinger H, Stiegler G, Kunert R, et al. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science. 2005;308(5730):1906-8.

240. Li S, Yu Y, Yue Y, Liao H, Xie W, Thai J, et al. Autoantibodies From Single Circulating Plasmablasts React With Citrullinated Antigens and Porphyromonas gingivalis in Rheumatoid Arthritis. Arthritis Rheumatol. 2016;68(3):614-26.

241. Cashman KS, Jenks SA, Woodruff MC, Tomar D, Tipton CM, Scharer CD, et al. Understanding and measuring human B-cell tolerance and its breakdown in autoimmune disease. Immunol Rev. 2019;292(1):76-89.

242. Lübbers J, van Beers-Tas MH, Vosslamber S, Turk SA, de Ridder S, Mantel E, et al. Changes in peripheral blood lymphocyte subsets during arthritis development in arthralgia patients. Arthritis Res Ther. 2016;18(1):205.

243. Anioke I, Duquenne L, Parmar R, Mankia K, Shuweihdi F, Emery P, et al. Lymphocyte subset phenotyping for the prediction of progression to inflammatory arthritis in anti-citrullinated-peptide antibody-positive at-risk individuals. Rheumatology. 2023:kead466.

244. Szelinski F, Stefanski AL, Schrezenmeier E, Rincon-Arevalo H, Wiedemann A, Reiter K, et al. Plasmablast-like Phenotype Among Antigen-Experienced CXCR5–CD19low B Cells in Systemic Lupus Erythematosus. Arthritis Rheumatol. 2022;74(9):1556-68.

245. Aqrawi LA, Ivanchenko M, Björk A, Ramírez Sepúlveda JI, Imgenberg-Kreuz J, Kvarnström M, et al. Diminished CXCR5 expression in peripheral blood of patients with Sjögren's syndrome may relate to both genotype and salivary gland homing. Clin Exp Immunol. 2018;192(3):259-70.

246. Cagigi A, Mowafi F, Phuong Dang LV, Tenner-Racz K, Atlas A, Grutzmeier S, et al. Altered expression of the receptor-ligand pair CXCR5/CXCL13 in B cells during chronic HIV-1 infection. Blood. 2008;112(12):4401-10.

247. Jenks SA, Cashman KS, Zumaquero E, Marigorta UM, Patel AV, Wang X, et al. Distinct Effector B Cells Induced by Unregulated Toll-like Receptor 7 Contribute to Pathogenic Responses in Systemic Lupus Erythematosus. Immunity. 2018;49(4):725-39.e6.

248. Fecteau JF, Côté G, Néron S. A new memory CD27-IgG+ B cell population in peripheral blood expressing VH genes with low frequency of somatic mutation. J Immunol. 2006;177(6):3728-36.

249. Samuels J, Ng Y-S, Coupillaud C, Paget D, Meffre E. Impaired early B cell tolerance in patients with rheumatoid arthritis. The Journal of experimental medicine. 2005;201(10):1659-67.

250. Moir S, Ho J, Malaspina A, Wang W, DiPoto AC, O'Shea MA, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. Journal of Experimental Medicine. 2008;205(8):1797-805.

251. Courey-Ghaouzi A-D, Kleberg L, Sundling C. Alternative B Cell Differentiation During Infection and Inflammation. Front Immunol. 2022;13.

252. Inamo J, Keegan J, Griffith A, Ghosh T, Horisberger A, Howard K, et al. Deep immunophenotyping reveals circulating activated lymphocytes in individuals at risk for rheumatoid arthritis. bioRxiv. 2023.

253. James EA, Holers VM, Iyer R, Prideaux EB, Rao NL, Rims C, et al. Multifaceted immune dysregulation characterizes individuals at-risk for rheumatoid arthritis. Nature Communications. 2023;14(1):7637.

254. Lliso-Ribera G, Humby F, Lewis M, Nerviani A, Mauro D, Rivellese F, et al. Synovial tissue signatures enhance clinical classification and prognostic/treatment response algorithms in early inflammatory arthritis and predict requirement for subsequent biological therapy: results from the pathobiology of early arthritis cohort (PEAC). Ann Rheum Dis. 2019;78(12):1642-52.

255. Cope A, Jasenecova M, Vasconcelos J, Filer A, Raza K, Qureshi S, et al. OP0130 ABATACEPT IN INDIVIDUALS AT RISK OF DEVELOPING RHEUMATOID ARTHRITIS: RESULTS FROM THE ARTHRITIS PREVENTION IN THE PRE-CLINICAL PHASE OF RA WITH ABATACEPT (APIPPRA) TRIAL. Ann Rheum Dis. 2023;82(Suppl 1):86.

256. van Steenbergen HW, Cope AP, van der Helm-van Mil AHM. Rheumatoid arthritis prevention in arthralgia: fantasy or reality? Nature Reviews Rheumatology. 2023;19(12):767-77.

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