Exploring seropositive rheumatoid arthritis: from immunological depths to clinical course

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General discussion

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The immune system provides continuous protection from invasive microorganisms, but sometimes an interplay of genetic and environmental factors may lead to reactivity against self-proteins. Autoimmunity can, but does not have to, result in the development of autoimmune disease such as rheumatoid arthritis (RA), a condition causing joint inflammation and eventually joint destruction. Over half of RA patients are positive for antibodies directed against post-translationally modified proteins (AMPA), which are considered to be a reflection of underlying disease pathology. The most clinically important AMPA are anti-citrullinated protein antibodies (ACPA), while anti-carbamylated protein antibodies (anti-CarP) and anti-acetylated protein antibodies (AAPA) can also be found in RA patients (1-3). The processes leading to the break of tolerance against proteins carrying post-translational modifications (PTMs) and the progression from autoimmunity to clinical disease are only partly understood. The current hypothesis is that 'multiple hits' over the course of years in combination with genetic susceptibility can eventually lead to the development of seropositive RA. Better understanding of the mechanisms involved might provide new opportunities for the development of targeted therapies. In this thesis several aspects of the AMPA response are investigated. In **part 1,** the effect of Fc glycosylation on ACPA B-cell receptor function is studied. **Part 2** is focussed on the role of the mucosal immune system in the origin of the AMPA response in RA and in **part 3** the association between the AMPA profile, clinical phenotype and disease outcomes is examined.

Fc glycosylation and ACPA IgG B cell receptor function

Glycans are attached to B-cell receptors (BCRs) and antibodies at specific locations. For example, all IgG contain a conserved N-glycosylation site at position 297 in the CH2 domain of the Fc region (4). Fc glycans have a substantial impact on the structure of IgG antibodies and are essential for the recruitment of antibody effector functions such as complement activation (4, 5). The effect of Fc glycans on ACPA IgG BCR function was investigated in **chapter 2**, using a human germinal center-derived (GC) B-cell line derived from Burkitt lymphoma cells in which the endogenous BCR was knocked out. These cells were transfected with BCR sequences obtained from ACPA IgG B cells in sera of RA patients or with similar sequences containing a mutation at position 297 in the CH2 domain precluding glycan attachment at this site. The absence of the BCR heavy chain glycan did not affect the expression of functional IgG BCRs on the surface of the B cells, despite the previously described involvement of glycans in shaping the 3D structure of BCRs (6). Furthermore, no differences in antigen binding, BCR signalling, and internalization of BCR-antigen complexes were seen in absence of Fc glycans.

Based on the proximity between N(297)-linked glycans and the ITAM-bearing Igα/Igβ signalling complex of the BCR, a functional effect of the Fc glycan on BCR signalling could have been expected. However, the long cytoplasmic tail of the BCR, which amplifies signalling independent of the Igα/Igβ signalling complex (7), might have limited the effect of absent Fc glycans on B-cell activation. It is possible that the impact of constant domain glycans might vary between isotypes and perhaps also between B-cell stages, whereas this study was limited to an IgG GC-derived cell line. Furthermore, in vivo, Fc glycans might interact with lectins expressed on the cell surface or on surrounding immune cells not present in the B cell culture system used in our experiments. Our findings regarding Fc glycans are remarkably different compared to the effects of ACPA IgG BCR variable domain glycans (VDG) in a similar experimental set-up. VDG glycans seem to affect BCR functioning though delayed BCR internalisation, possibly due to differences in spatial BCR organisation (8). Taken together, the results of this study seem to suggest that IgG constant domain glycosylation mainly evolved as a way to modify IgG antibody effector functions and does not play an essential role in IgG BCR functioning. Modulation of Fc glycans is thus essential for the production of antibody-based therapeutics, while unravelling the factors regulating BCR variable domain glycosylation might provide opportunities for development of B cell targeted therapies in RA.

The role of mucosal inflammation in AMPA development

The events leading to the break of tolerance to citrullinated and other post-translational modified proteins and the development of the humoral AMPA response in RA patients remain unclear. One of the current hypotheses, the mucosal origin hypothesis, suggests that AMPA reactivity might originate at mucosal sites. In **chapter 3**, **chapter 4,** and **chapter 5** the potential involvement of mucosal immunity in the AMPA response in RA was investigated using several different methods.

ACPA IgA subclasses

The main antibody isotype present at mucosal surfaces is IgA, of which two subclasses can be produced, IgA1 and IgA2. IgA2 has a shorter hinge region compared to IgA1, making it less susceptible to bacterial proteases (9). This might be one of the reasons why IgA subclass ratios differ throughout the body, with IgA2 being mainly produced in the intestines and to a lesser extent at other mucosal surfaces (10). Furthermore, IgA2 has been reported to have a stronger pro-inflammatory effect on neutrophils and macrophages than IgA1, possibly due to glycosylation differences influencing Fc-receptor binding (11). In **chapter 3**, we found that both total IgA1- and IgA2-levels were increased in seropositive RA patients, compared to seronegative patients and healthy donors. In ACPA IgA seropositive patients, both

circulating ACPA IgA1 and ACPA IgA2 could be detected. Comparable results were found for rheumatoid factor (RF) IgA subclasses. Unfortunately, quantification of ACPA IgA subclass ratios proved challenging, since RF interfered with the ACPA IgA2 ELISA.

The mechanisms behind the elevated IgA (subclass) levels in RA patients are not clear, but there could be a relation with mucosal inflammation. In the bronchial mucosa around 25% of all IgA is of the IgA2 subclass, compared to less than 10% is serum (10). Pulmonary inflammation could thus lead to local production of large quantities of IgA(2), providing a possible explanation for the significantly increased serum levels of total IgA2 and higher RF IgA1 levels in RA patients who smoke. However, research in celiac disease showed that mucosal and serum IgA are clonally related, but do not have the same antibody characteristics and are likely to be produced by different plasma cells (12). Thus, mucosal responses might lead to elevated levels of (antigen-specific) IgA in serum without a typical 'mucosal antibody phenotype' in seropositive RA patients, making it difficult to determine the origin of the elevated serum total IgA(2) levels. Alternatively, elevated IgA subclass levels could be part of a general immunoglobulin hyperproduction in RA patients, for example due to aspecific B-cell hyperreactivity in a proinflammatory context, as IgG and IgM levels can also be increased in RA patients (13-15).

Chapter 3 also explored potential pro-inflammatory effects of IgA2 by examining the association between IgA2 levels and markers of inflammation in RA patients. No association between total IgA2- levels in serum and either CRP or disease activity score were found. Therefore, a substantial contribution of serum IgA2 to systemic inflammation in RA patients does not appear very likely, despite the pro-inflammatory effects described in vitro before. This does not preclude possible effects of IgA2 on local mucosal inflammation in RA patients.

<u>AMPA in mucosal excretions</u>

Several studies have provided evidence of the local production of ACPA at mucosal sites in RA patients. ACPA can be present in both sputum, bronchoalveolar fluid and saliva of RA patients (16-18) and could even be detected in sputum of first-degree relatives of RA patients who did not have detectable ACPA in serum (19). Autoantibody production at the largest mucosal surface, the intestines, was not investigated thus far. There are several indications that the gut is involved in RA pathophysiology. For example, RA patients can be distinguished from healthy controls based on dysbiosis of the gut microbiome (20). Furthermore, circulating plasmablasts in individuals at risk of RA can bind both citrullinated autoantigens and bacteria in faeces (21). The MUCOSA study,

described in **chapter 4,** was designed to investigate whether evidence for local AMPA responses could be found at several different mucosal sites. We assessed not only ACPA, but also anti-CarP, AAPA and RF. To this end, paired serum, saliva and faeces samples of RA patients were collected and tested for autoantibody positivity. The fact that not only citrullinated, but also carbamylated and acetylated proteins can be present at mucosal surfaces (22), and that immunisation with acetylated bacterial proteins could induce a cross-reactive AMPA response in a previous study in mice (23), illustrates the importance of investigating the full AMPA profile.

ACPA, anti-CarP, AAPA IgA antibodies could all be detected in saliva of established seropositive RA patients, although these salivary antibodies were only present in a limited subset of patients. Positivity for a specific AMPA in saliva always coincided with the presence of that specific AMPA in serum, although the detected isotype could differ. This indicates that AMPA can be produced locally at mucosal sites. RF IgA was more abundant in saliva of seropositive patients compared to AMPA and not all saliva RF IgA-positive patients tested positive for RF IgM in serum. No association between saliva ACPA positivity and local inflammatory markers such as saliva total protein content, matrix metalloproteinase 8 (MMP-8) levels and total IgA were observed, although numbers were small. All patients in our study received treatment at the time of sample collection, potentially affecting both local inflammation as well as salivary autoantibody production. Another possible explanation for the low number of saliva AMPA-positive patients is that prior salivary autoantibody responses might have declined over time and titers decreased below the detection limit, similar to the dynamics of anti-pathogen IgA antibodies induced by (upper) airway infections (24).

Although AMPA can be produced and secreted locally in the oral mucosa, no AMPA could be found in faeces samples. Replication in faeces samples of a different Dutch RA cohort and in ileal wash samples of RA patients obtained by colonoscopy in a Swedish study led to similar results. The faecal samples did contain intact antibodies, since total IgA and anti-E. coli IgA antibodies could be readily detected. These data suggest that production of AMPA is limited to certain mucosal sites, with local production of all AMPA taking place in the oral cavity, but not to a substantial degree in the lower intestinal tract (figure 1). However, several other factors might explain the lack of AMPA positivity in faeces, for example strong binding of antibodies to local PTM-containing antigens, degradation of antibodies by digestive enzymes, or the lack of sensitivity of our assays. Unfortunately, the COVID-19 pandemic prohibited the collection of paired sputum samples of RA patients in the MUCOSA study as originally planned.

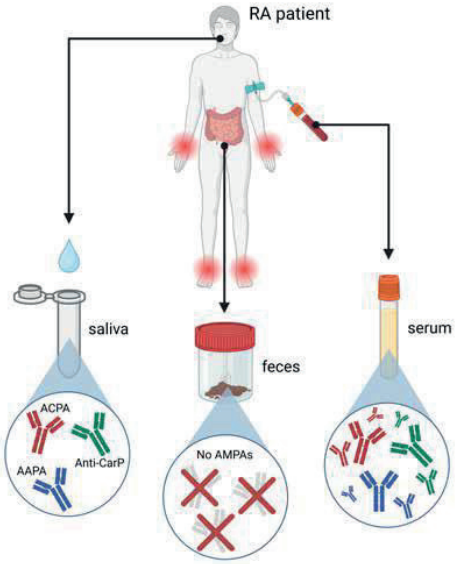

Figure 1: Summary of finding of the MUCOSA study. All AMPA can be found in saliva, but not in faeces of RA patients.

Activated B cells circulate and re-enter to the same tissue where they were activated based on their homing marker expression, although there might be some crossover to other anatomical sites (10). This suggests that plasma cells producing AMPA in the oral mucosa and in the lungs are likely to be derived from B cells activated locally in mucosal associated lymphoid tissue (MALT) or local ectopic lymphoid follicles. Characteristics of the local micro-environment, such as antigen availability and inflammation, might lead to spatial variation in mucosal AMPA production. Neutrophil extracellular traps (NETs), which are increased in saliva and synovial fluid of RA patients (25, 26), might be a potent inducer of ACPA responses, presenting citrullinated proteins in a pro-inflammatory context. In combination with the epidemiological association between RA and both toxic inhalants and periodontitis (27, 28), these data suggest that there is both increased inflammation and a high amount of post-translationally modified proteins present in the oral and pulmonary mucosa. These conditions might be less pronounced in the gut of RA patients, providing a possible explanation for the detection of AMPA in saliva and sputum, but not in faeces of RA patients. Nonetheless, determination of the specific proteins or post-translational modifications driving the AMPA response in patients is difficult, as mice models have shown that initial exposure to a PTM-antigen induces cross-reactive towards other PTMs, a response that can be boosted and skewed upon repeated exposure to different PTM-containing antigens (29).

Onset of RA after COVID-19

Not only bacterial infections, but also inflammation caused by viruses might lead to autoimmunity. The research outlined in this thesis was largely performed in times of the COVID-19 pandemic caused by the Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2), a virus that can lead to severe mucosal inflammation in the lungs. Multiple studies have reported the presence of autoantibodies in patients with severe COVID-19, such as anti-cardiolipin and anti-nuclear antibodies (30). In **chapter 5**, the seroprevalence of ACPA after COVID-19 was investigated among patients visiting the post-COVID outpatient clinic of the Leiden University Medical Center 5 weeks after hospitalization. None of these patients tested positive for ACPA, except two patients previously diagnosed with ACPA-positive RA. Thus, in this limited sample size we could not observe an increase in ACPA-positivity after COVID-19. In addition, five patients who presented in other clinics with polyarthritis compatible with RA after SARS-CoV-2 infection were investigated. Autoantibody measurements in these patients revealed patterns similar to early RA patients presenting before 2019, with two patients being completely seronegative and three patients testing positive for a range of AMPA at presentation. In all post-COVID ACPA-positive RA patients the percentage of ACPA V-domain glycosylation was increased compared to total IgG, similar to ACPA glycosylation in RA patients without preceding SARS-CoV-2 infection. A detailed examination of the specific ACPA IgG V-domain glycan traits in the post-COVID RA patients was performed, as previous research showed inflammatory conditions can induce changes in antibody glycan composition (31). These analyses revealed a significant decrease in bisecting N-Acetylglucosamine-containing moieties, similar to changes observed in total IgG Fc-glycosylation post-COVID. No longitudinal samples were available to investigate whether the glycan profile changed after COVID-infection. The biological consequences of the differences in glycan composition remain unclear, but there is evidence indicating variable domain glycans are able to modulate antibody function (32). Therefore, it is tempting to speculate that changes in antibody glycosylation patterns induced by infections might modulate their pathogenicity.

ACPA responses mature towards RA onset, with rising ACPA IgG levels, epitope spreading and increased variable domain glycosylation, a process which most likely requires multiple events or 'hits'. Given the short time window between COVID-19 and onset of seropositive RA, the similarity in ACPA profile compared to other early RA patients and the lack of ACPA positivity in our post-COVID hospitalisation cohort, it seems more likely that the patients who developed RA shortly after COVID-19 already had quite a mature ACPA response, instead of de novo development of ACPA positivity after infection. In this case, COVID-19 might have been one of the last 'hits' needed to develop clinical disease in these ACPA-positive individuals prone to develop RA, and other (inflammatory) triggers would probably also have provoked disease onset.

The mucosal origin hypothesis

Based on the work presented in this thesis and the research done by others, evidence for a role of mucosal immunity in the development of the AMPA response in seropositive RA accumulates. This model is summarized in figure 2. AAPA IgM can be present in healthy individuals and might thus be part of the physiological immune repertoire, for instance targeting anti-acetylated bacterial proteins (33). Interestingly, germline AMPA IgM can already cross-reactive (34). Environmental risk factors such as smoking and exposure to silica dust might create pro-inflammatory conditions in the oral and airway mucosa and could also increase the amount of post-translationally modified proteins, for example via NETosis (25). Likewise, bacterial dysbiosis and the associated changes in the microbiome metabolome could provide a source of PTMs in combination with inflammatory triggers (20, 35). It is likely that AAPA IgM B cells directed against bacterial components can receive T-cell help, for example from mucosal Th17 T cells. Under the proinflammatory conditions described above, these physiological AAPA response might give rise to a class-switched AMPA clone, reactive to multiple post-translationally modified antigens. This cross-reactive AMPA response could be shaped and matured further over time by repeated expose to different PTM-containing triggers (29), which may vary between individuals. Based on the data described in this thesis, the oral and respiratory mucosa seem the preferred sites of mucosal AMPA production.

It remains unclear how local mucosal B cell responses, whether they target foreign antigens or self-antigens like PTM containing proteins, are related to circulating (auto) antibodies (36, 37). One of the hypotheses is that transient episodes of subclinical bacteraemia might occur, caused by damage to mucosal barriers during infections or by mechanical stress for example during toothbrushing (38). This systemic exposure to either pathogenic bacteria, bystander microorganisms or other pro-inflammatory proteins carrying PTMs, could give rise to a systemic (IgG) AMPA response. It has even been suggested that bacteraemia may lead to translocation of bacteria to the synovial compartment, where they might trigger a local inflammatory reaction. The detection of DNA from Prevotella copri and other bacteria in synovial fluid of RA patients (39, 40) supports this idea. However, mucosal antibody responses might also directly lead to IgG production, as IgG is abundant in the lower respiratory tract (41, 42), where it might be transported to the lumen by binding to the epithelial neonatal Fc receptor (FcRn) (43). ACPA IgG has indeed been detected in sputum of RA patients and individuals at risk for RA (19). Over time, the AMPA response might enter the next stage of development under influence of HLA-shared epitope-restricted T cells (44), although the circumstances provoking this process are currently unclear. These events may lead to broadening of the AMPA profile, including epitope spreading and increased VDG-glycosylation (45, 46), features associated with the transition from autoimmunity to autoimmune disease.

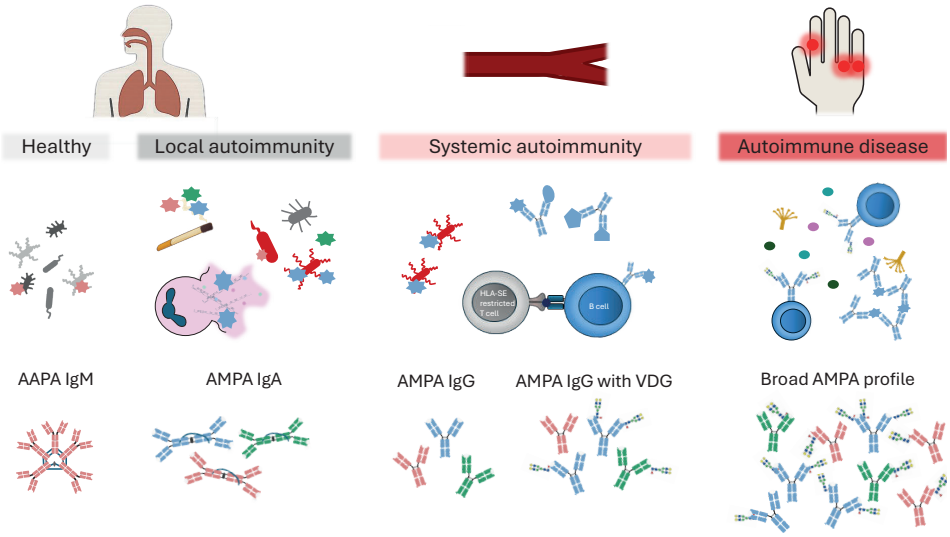

Figure 2: The mucosal origin hypothesis in RA. AAPA IgM, for example targeting acetylated bacterial components seems part of the physiological immune repertoire. Smoke inhalation, microbiome dysbiosis and NETs may lead towards a broader local humoral immune response in the oral and respiratory mucosa. Further 'hits' lead to the development of a systemic AMPA response, for example due to bacteraemia. Over time more epitopes are recognised and VDG glycans are introduced. Eventually arthritis occurs.

Although this model is named the 'mucosal origin hypothesis', the described mucosal immunological processes might be involved in different stages during disease development, such as the introduction of AMPA reactivity, diversification of AMPA response, but a potential role in disease flares should also be considered.

AMPA positivity and clinical disease presentation

The **third part** of this thesis is focused on the AMPA profile in relation to clinical phenotype and disease outcomes in RA. It is known that ACPA-positive patients have worse disease outcomes with more radiological damage over time (47). Whether the AMPA profile is also associated with disease presentation is investigated in **chapter 6**, by analysing the relationship between several clinical features at baseline and the number of autoantibodies detectable in serum. The autoantibodies included in this study were RF IgM, ACPA IgG and anti-CarP IgG, as data on the presence of AAPA in RA patients were first published at the time the manuscript was already drafted (3). In two early RA cohorts, positivity for multiple autoantibodies was associated with younger age of disease onset, smoking, longer symptom duration and higher inflammatory markers at presentation. This effect was not driven by the presence

of a specific individual autoantibody, but rather by the combined positivity for multiple autoantibodies. The observation that patients with multiple autoantibodies develop RA at a younger age might indicate a stronger genetic predisposition in these individuals, accelerating the development of autoimmunity and/or the transition from autoimmunity to autoimmune disease (48). Smoking can potentially increase the amount of protein citrullination, carbamylation and acetylation in the lung (49-51) in pro-inflammatory conditions, providing a possible explanation for the association between smoking and the break of tolerance against multiple PTMs. The AMPA response develops and expands over a long period of time. Less potent last 'hits' might be required to trigger RA onset in individuals with a broad AMPA profile, which could result in a more insidious disease onset in these patients. Moreover, patients who were positive for various autoantibodies also showed higher levels of these autoantibodies, a broader isotype usage and reactivity against more fine specificities. Thus, the number of autoantibodies present can be viewed as a marker for the breath/activity of the humoral autoimmune response which might therefore explain the association between number of autoantibodies present and increased systemic inflammation at disease onset. In conclusion, the extent of the autoantibody response is related to disease presentation in RA patients, which implies a role for the AMPA response in disease development. However, it should be taken into consideration that variations in the AMPA profile could also represent a by-effect of underlying inflammatory processes, given that no direct pathogenic effect of AMPA has been established.

The AMPA profile and treatment response in RA

Whether the autoantibody profile is not only related to clinical presentation, but also to treatment response, was investigated in **chapter 7**. To this end, positivity for RF, ACPA, anti-CarP and AAPA isotypes was measured in serum samples collected over time of patients in the IMPROVED study. Patients who tested positive for a range of autoantibodies at disease presentation showed a larger decrease in disease activity score (DAS) within the first four months after treatment initiation with methotrexate and high-dose prednisone, compared to patients with a more limited autoantibody profile. According to the IMPROVED protocol, medication was tapered in patients with low DAS after initial treatment. The presence of multiple AMPA at baseline was associated with unfavourable outcomes after drug tapering, with a high chance of disease flares within one year after medication was withdrawn. AMPA measurements at the moment of drug tapering did not provide additional prognostic value compared to the baseline autoantibody profile regarding the risk of disease flares after treatment discontinuation. In the subset of patients that did reach drug-free remission for over a year after drug tapering, AMPA-positivity was a risk factor for the relapse of arthritis, but no association between disease flares and the breath of the autoantibody profile was

observed. These data suggest that patients with a broad humoral auto-immune profile respond well to initial treatment with methotrexate and steroids with rapid lowering of disease activity, but that this therapy seems to suppress rather than definitively resolve the inflammation. In most AMPA-positive patients, disease flares at some point after drug tapering, except for a small subset of AMPA-positive patients that reaches sustained drug-free remission. Currently, it is unclear which immunological features are associated with this lasting medication-free remission, the closest approximation of disease cure to date. AMPA levels remain detectable in these patients after all. It is tempting to speculate that the underlying immune response in the patients with sustained drug-free remission has been reverted to a state similar to pre-disease, where there is autoimmunity, but no autoimmune disease. Identification of the autoantibody and B-cell characteristics related to sustained drug-free remission in AMPA-positive patients could provide new insights in the pathogenicity of AMPA in RA.

Long-term disease outcomes in ACPA-positive individuals

Long-term disease outcomes in RA patients are currently not determined by joint destruction, but by the development of potentially life-threatening extra-articular manifestations like interstitial lung disease and premature atherosclerotic disease. The introduction of early intensive treatment in RA has normalised mortality rates in ACPA-negative RA patients, but in ACPA-positive RA excess mortality remains, mainly from cardiac causes (52). An association between ACPA and increased cardiovascular mortality was reported in RA patients (53, 54), although these finding could not be replicated in other studies (55, 56). Therefore, in **chapter 8** the role of ACPA in coronary artery disease (CAD) was investigated in more detail. These analyses were not only performed in RA patients, but also in non-RA patients, as two studies reported the presence of ACPA in approximately 10% of patients with CAD without concomitant RA (57, 58). In these non-RA patients, ACPA was associated with unfavourable CAD outcome (57). However, in the study presented in this thesis, we found no increased prevalence of ACPA in two large CAD non-RA cohorts. Furthermore, presence of ACPA did not lead to worse all-cause survival in these patients.

Given the conflicting results regarding the relationship between ACPA and cardiovascular mortality, we hypothesised that in seropositive RA the inflammatory burden rather than the sole presence of the autoantibodies themself might be associated with increased cardiovascular disease development, as systemic inflammation is a known risk factor for accelerated atherosclerosis (59). Therefore, data from two RA cohorts, in which an association between ACPA and all-cause mortality was found before, were reanalysed using a joint modelling approach to include longitudinal CRP measurements, a marker for inflammation. The hazard ratios for the effect of ACPA on all-cause and cardiovascular

mortality became non-significant after addition of CRP over time to the analyses, while higher CRP levels were significantly associated with mortality. Thus, high inflammation over time seems to be a more decisive factor for cardiovascular disease development and mortality in patients with RA than the presence of ACPA by itself. A direct role of ACPA in cardiovascular disease, for example via binding to citrullinated proteins in the atherosclerotic plague or via FcγRIIa-dependent activation of platelets, may be considered less likely based on these data. Despite the fact that CRP was only measured at intervals of a year or longer and thus only gives a rough estimation of total inflammation, these findings infer that current intensive treatment is insufficient to subdue the continuous inflammatory processes in seropositive RA patients.

In RA patients, the presence of ACPA seems to reflect a disease state in which there is more ongoing inflammation. It is known that ACPA-expressing B cells retain an active and proliferating phenotype during treatment, even in patient in clinical disease remission (60), indicating that clinical remission is not equal to immunological remission. It cannot be excluded that the continuous activation of AMPA B cells could contributes to the chronic systemic inflammation in seropositive RA patients and thus to premature CAD development. Better insights in the processes underlying the sustained inflammatory response in AMPA-positive RA patients might provide new opportunities to improve long-term disease outcomes, as cardiovascular mortality is still a major problem in seropositive RA.

Conclusion and further perspectives

In RA patients, AMPA are related to phenotype at disease presentation and treatment response. Furthermore, ACPA-positive patients seem to have more chronic inflammation, which can affect long-term prognosis. The pathophysiological processes underlying AMPA development and their role in disease onset are unclear. Data presented in this thesis suggest that local mucosal immune responses in the airway and oral cavity may be involved in AMPA production. The higher levels of total IgA1 and IgA2 observed in seropositive RA patients also suggest that mucosal antibody production is increased in these patients. No evidence of de novo AMPA development after COVID-19, a potentially severe mucosal infection, was found, but this viral infection could be one of the final 'hits' leading to RA development in seropositive asymptomatic individuals, as several cases of new-onset RA after COVID-19 have been reported.

To gain further insights in the factors associated with the development of local and eventually systemic autoimmunity in RA, it is key to investigate AMPA responses in mucosal excretions and tissue in more detail. Exploring the relationship between bacterial disbalance, the microbial metabolome and local barrier function in mucosal

tissue, including the respiratory and the female reproductive tract, could provide useful new insights. Moreover, better understanding of the interactions between mucosal AMPA B cells and T cells and the crosstalk between mucosal and systemic immunity in different stages before and after onset of disease, might provide new pieces of the puzzle that is RA pathophysiology. Investigating AMPA B cell homing to mucosal tissue, bone marrow and the synovial compartment, could be one of the first steps in this process.

The influence of current RA treatments, including non-steroidal anti-inflammatory drugs (NSAIDS) known to cause intestinal damage, on mucosal (auto)immune responses in RA also remains an open question. Yet studies into these mechanisms are complex, as collection and usage of mucosal samples is more demanding compared to serum. Furthermore, the mucosal humoral responses involved in the development of AMPA can occur years before onset of symptoms. Fortunately, increasing attention for mucosal immunity in many diseases in combination with novel experimental techniques present new research possibilities. Integration of insights found in RA and other (autoimmune) diseases has the potential to broaden our understanding of general and disease-specific mucosal humoral immune responses.

Meanwhile, we should not hesitate to the translate the insights gained so far on RA pathophysiology into actions to prevent disease and improve quality of life for RA patients. For years, it has been clear that smoking is a major risk factor for RA development and that RA patients who smoke respond worse to treatment while having a higher risk of cardiovascular complications (61). Therefore, both healthcare workers and policy makers should give smoking cessation priority. The power of lifestyle adjustments in RA care was recently further illustrated by the 'Plants for Joints' study, which showed that a 16-week lifestyle program including a whole-food plant-based diet, physical activity and stress management decreased disease activity in RA patients (62). High fibre intake is associated with beneficial alterations in the gut microbiome (63) and might even alter systemic immunity in RA patients (64). These data illustrate the need for larger studies on dietary and microbiome interventions in RA, although funding of these studies could prove more challenging compared to studies on pharmacotherapy. However, the increase in lifestyle-related diseases worldwide justifies prompt implementation of lifestyle education for the whole population, including seropositive RA patients and their relatives.

To conclude, it is an exciting time in translational RA research with new opportunities to study the immunological depths of the mucosal and systemic AMPA response and momentum for novel ideas regarding therapeutic interventions to improve the clinical course for RA patients.

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