Exploring the landscape of rheumatoid arthritis: piecing together risk factors and autoantibodies

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Summary of findings and general discussion

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent joint inflammation and progressive tissue destruction. Although a general pathophysiological model has been established, many aspects of RA development remain insufficiently understood. In particular, the expanding spectrum of RA-related autoantibodies—including anti-citrullinated protein antibodies (ACPA), anti-carbamylated protein antibodies (anti-CarP) and anti-acetylated protein antibodies (AAPA)—as well as newly recognized features such as the extensive glycosylation of ACPA, highlight the complexity of early autoimmune processes. It is still unclear whether risk factors and clinical outcomes differ among the various anti-modified protein antibody (AMPA) families, and most previous work has focused primarily on AMPA-IgG. Far less is known about AMPA-IgA and AMPA-IgM, despite their potential to illuminate key mechanisms: IgM may reflect the earliest stages of autoantibody formation, while IgA could offer insights into the mucosal origin hypothesis. Understanding the full isotype profile of RA-related autoantibodies may therefore shed light on the relation between genetic predisposition, environmental triggers, and clinical disease expression.

Therefore, this thesis aims to clarify the risk factors and clinical implications associated with different AMPA isotypes, ultimately contributing to a deeper understanding of RA pathophysiology. The work is organized into four parts: genetic risk factors, environmental risk factors, autoantibody isotypes, and clinical outcomes in relation to autoantibodies.

PATHOGENESIS OF RHEUMATOID ARTHRITIS: RISK FACTORS AND AUTOANTIBODIES

Genetic predisposition

Rheumatic diseases often exhibit numerous overlapping features, making it challenging to distinguish between shared and distinct immunopathological mechanisms.[1] Fortunately, advancements in research techniques have provided a wealth of information regarding the genetic foundations of these conditions. In **Chapter 2** we endeavored to provide a comprehensive overview of the immunological background of various rheumatic diseases based on this wealth of genetic information and autoantibodies, to clarify both the commonalities and unique aspects of the pathophysiology associated with rheumatic diseases. Based on genetics and autoantibodies, three categories of rheumatic diseases were found: the first cluster comprises diseases in which dysregulation of the adaptive immune system plays a central role, the second cluster includes diseases that are associated with HLA class 1 alleles and the third cluster is characterized by an aberrant innate immune response. Each of these categories is associated with a distinct set of targeted therapies,

suggesting that underlying genetic and immunological features may help explain therapeutic responses. Indeed, the insights gained from genetic research have already begun to inform the development of novel therapeutic strategies for rheumatic diseases. For instance, the most prominent genetic risk factor for RA are the HLA-DRB1 shared epitope alleles (SE) which play an important role in T cell activation.[2] This finding has let to exploration of biologic therapies such as abatacept, which acts as a co-stimulation modulator preventing activation of T cells.[3] Similarly, in systemic lupus erythematosus (SLE), numerous genetic alterations in B cell signaling pathways have been identified, leading to the development of targeted therapies directed against B-cells such as rituximab and belimumab.[4-6] There are numerous additional examples of therapies derived from genetic findings in rheumatic diseases, including tumor necrosis factor (TNF) inhibitors and interleukin (IL) inhibitors targeting IL-1, IL-6, IL-17, and IL-12/23. These examples underscore the potential of genetic findings to develop tailored treatments that address the distinct pathophysiological mechanisms present in various rheumatic diseases, highlighting the importance of research in genetic preposition of rheumatic diseases. While this classification based on genetic risk factors and autoantibody profiles provides valuable insights into underlying disease mechanisms, it is not without limitations. It does not fully capture the complexity and heterogeneity of immune responses, and alternative classifications—such as those based on functional measurements of innate or adaptive immune cell activity in peripheral blood, transcription factor activation patterns, or cytokine expression profiles—may offer additional perspectives. However, we have chosen to focus on genetic risk factors and autoantibodies as they represent well-characterized and readily measurable indicators of underlying pathophysiological processes.

Rheumatoid arthritis (RA) is the most frequent autoimmune rheumatic disease and exhibits a complex and multifaceted etiology, with several elements still remaining unidentified. Both genetic and environmental risk factors play a key role in the development of the disease. A paradigm shift in the field of risk factor research in RA followed the discovery of ACPA several years ago.[7] ACPA-positive and ACPA-negative forms of the disease not only differ in clinical phenotypes, but they are also associated with distinct genetic and environmental risk factors.[8] The insight that most of the genetic risk factors for RA, including the SE and single nucleotide polymorphism (SNP) Protein tyrosine phosphatase non-receptor type 22 (PTPN22) rs2476601, specifically predisposes individuals to ACPA-positive disease has been pivotal in shaping the current understanding of the pathophysiological mechanisms underlying RA.[2, 9]

In recent years, other autoantibodies to post-translationally modified proteins, together called AMPA, have been identified. Anti-CarP are not

associated with SE or with PTPN22 rs2476601).[10] The most recently described AMPA family member are AAPA.[11] The association between AAPA and genetic risk factors has scarcely been studied. Therefore, we aimed to further investigate the association of genetic risk factors in rheumatoid arthritis with the presence of these autoantibodies. The association of several genetic risk factors with AMPA-isotypes in the Leiden Early Arthritis Clinic (EAC) was investigated in **Chapter 7**. HLA SE alleles, other HLA-DRB1 alleles and PTPN22 rs2476601 were all solely associated with ACPA-IgG and not with other ACPA isotypes or with AAPA isotypes. The specific association of ACPA-IgG with SE, as opposed to other AMPAs, underscores the concept that AMPAs, despite the cross-reactivity of these autoantibodies, are distinct antibody responses with unique underlying predisposing factors.[12-15] This observation is consistent with earlier findings that HLA-B alleles are specifically associated with anti-CarP antibodies, while HLA-DR alleles are associated with ACPA-IgG.[16]

In the light of the exclusive association of SE with ACPA, we studied the association of SE with a unique feature of ACPA, the presence of ACPA-IgG variable domain glycans (VDGs) in **Chapter 3**. Previous studies have shown that the presence of VDGs on ACPA is associated with disease progression and may enhance the pathogenic potential of these autoantibodies.[17] However, it remained unclear whether genetic risk factors such as SE influence the glycosylation process leading to VDG acquisition. The objective of this study was to investigate at what timepoint, from pre-symptomatic disease to the onset of RA, SE exerts it's influence on glycosylation of ACPA. ACPA-IgG VDG was measured in healthy individuals from Japan, pre symptomatic individuals from Sweden and individuals with arthralgia from the Netherlands, and the association between SE and ACPA VDG was calculated. The results revealed that SE was associated with the presence of ACPA-IgG carrying VDGs in individuals with arthralgia, suggesting that the influence of SE on ACPA glycosylation begins in the early immunological phase preceding RA onset. In contrast, no such association was observed in healthy ACPA-positive individuals from Japan, which is consistent with previous studies reporting a lack of association between SE and ACPA in healthy individuals.[18, 19] These findings support a model in which ACPA in healthy individuals develop in an SE independent manner, while the transition from asymptomatic to the symptomatic phase involves SE-restricted T cells that most likely provide help to ACPA-expressing B cells leading to BCR somatic hypermutation and the introduction of N-linked glycosylation sites. Since the association of SE with ACPA-positive individuals is ultimately based on its link to VDGs, and no association has been found between SE and other AMPAs, these mechanisms lead to the hypothesis that other AMPAs are not as heavily glycosylated as ACPA-IgG. In anti-Carp IgG VDG is present, however in lower percentages

compared to ACPA-IgG.[20] For AAPA VD-glycosylation has not been studied so far, however this is an interesting topic for future research, since it could further support or argue against the hypothesis that SE exerts it influence on VDG glycosylation and is therefore primarily associated with ACPA-IgG and not with other AMPA.

Environmental risk factors

Smoking

Smoking is the most prominent environmental risk factor for RA, with an attributable risk of approximately 20% for its development.[21] It is particularly associated with ACPA-IgG-positive RA.[21] Pathophysiologically, it is hypothesized that smoking increases citrullination in the lungs, potentially facilitating the presentation of citrullinated antigens via the SE alleles, thereby promoting the formation of ACPA. Supporting this, elevated citrullination levels have been detected in the bronchoalveolar lavage fluid of smokers compared to non-smokers[22] Additionally, ACPA-positive B cells and ACPA have been identified in the sputum of RA patients and individuals at risk of developing RA, suggesting local production of ACPA within the respiratory tract.[23, 24] However, more recently an association between anti-CarP and smoking was also described, as well as an association between smoking and rheumatoid factor (RF) in healthy Japanese individuals.[10, 18] Therefore, in **Chapter 4**, the association between smoking and the presence of several autoantibodies in RA was studied. Our findings revealed that smoking was associated with the presence of multiple RA-related autoantibodies (RF, ACPA, anti-CarP) rather than specifically with ACPA. Interestingly, the total amount of antibodies (total IgG) produced was not raised in smokers compared to non-smokers, indicating that smoking appears to contribute to the specific formation of an autoimmune response against various RA-associated autoantigens.

Previous studies, as well as the above-mentioned studies, have mostly focused on the association between smoking and ACPA-IgG as well as RF-IgM. However, since smoking primarily affects the lung mucosa, where IgA is the predominant immunoglobulin isotype, we chose to focus in IgA in **Chapter 7** in which we investigated the association between smoking and IgA isotype autoantibodies, specifically RF and AMPA. The results indicated that smoking was primarily associated with AMPA-IgA (ACPA and AAPA), particularly with current smoking. These findings align with previous studies that described an association between smoking and ACPA-IgA. Furthermore, our data now also establish a similar association for AAPA-IgA.[25, 26] The finding that smoking is primarily associated with AMPA-IgA is in line with the mucosal origin hypothesis. The mucosal origin hypothesis postulates that immune dysregulation and the generation of autoantibodies, such as AMPA, may initially occur at mucosal

sites like the lungs or gut, due to environmental triggers. These antibodies may subsequently cross-react with self-antigens in the joints, contributing to the onset of the disease. The observation described in this chapter, that RA patients who test positive for AMPA-IgA showed a significant correlation with active smoking is consistent with the notion of a mucosal origin. Given that the serum half-life of IgA is typically between four and seven days, it seems reasonable to suggest that continuous mucosal stimulation—such as that provided by ongoing smoking—is necessary to maintain IgA production from mucosal tissues. The precise mechanisms that connect smoking to AMPA-IgA, and subsequently to potential mucosal immune responses, remain to be fully elucidated. Beyond its influence on the local availability of post-translational modification (PTM) antigens in the mucosa, smoking may also have additional effects on the immune system. A recent study has indicated that smoking enhances the adaptive immune response in both current and former smokers through epigenetic modifications, while also amplifying the innate inflammatory response following bacterial exposure.[29]

No significant association was found between anti-CarP-IgA and smoking; however, the sample size in our research was limited. Previous investigations have shown that smoking leads to carbamylation in the lungs, indicating an increased antigen load in this area.[27] However, the relationship between smoking and anti-CarP, specifically with IgA, warrants further exploration. In contrast to the findings presented in **Chapter 4** and other studies, **Chapter 7** exclusively identified a link between smoking and AMPA-IgA, without an association to RF itself.[28-30] This discrepancy may stem from the fact that previous studies, including Chapter 4, did not account for AMPA-IgA, which might explain the differences observed between the findings in Chapter 4 and those in Chapter 7. In Chapter 7, an association was also observed between smoking and RF-IgA as well as RF-IgM; however, after adjusting for ACPA-IgA levels, the connection with RF was no longer significant.

In conclusion, smoking is primarily associated with AMPA-IgA. This finding supports the mucosal origin hypothesis for the development of autoantibodies in RA. It would be interesting to further study whether other stimuli, alongside smoking, may contribute to the onset of AMPA production in the mucosa. Environmental factors such as silica exposure and air pollution, both known risk factors for RA, could also facilitate AMPA formation through the pulmonary mucosa.[31] Another potential site for AMPA production is the oral cavity, where ACPA has been detected in the saliva of ACPA-positive RA patients.[32] Furthermore, studies have linked periodontitis, specifically with Porphyromonas gingivalis, to the development of RA.[33] Lastly, the intestinal microbiome may play a significant role in RA, as research indicates dysbiosis in patients with this condition.[34]

Sodium

Next to smoking, several environmental risk factors for rheumatoid arthritis (RA) have been identified, including obesity, specific infections, and hormonal differences.[35] In recent years, dietary factors have gained significant attention as potentially modifiable risk factors. One such factor is sodium intake, which has been implicated as a risk factor for ACPA-positive RA and other autoimmune diseases.[36-38] Interestingly, sodium also exhibits pro-inflammatory effects in the skin, both in the context of infections and in autoimmune conditions such as systemic sclerosis.[39, 40] However, the role of sodium in arthritis has never been studied. In **Chapter 5**, the role of sodium both as a risk factor and as a pro-inflammatory agent in arthritis was investigated. The presence of sodium in the synovial fluid of the knee and in tissues known for their sodium storage (skin and muscle) was studied using advanced non-invasive 7-Tesla ^{23}Na MRI techniques in patients with autoimmune arthritis. A significantly elevated total sodium concentration was found in the knee joint (synovium, SF, and cartilage) in patients with autoimmune joint disease compared to healthy controls. Upon further investigation into whether bound or unbound sodium compounds were elevated in knee arthritis, we discovered that primarily bound sodium was increased, suggesting that this sodium is likely not osmotically active. Consequently, unlike in skin infections or high-osmolality in vitro models, this bound sodium is unlikely to activate macrophages or T-helper 17 cells, making a pro-inflammatory role for sodium in arthritis less probable.[41, 42] Instead, sodium appears to be an innocent bystander, bound to glycosaminoglycans (GAGs) and other macromolecules released from cartilage during inflammation. Interestingly, knee sodium concentrations showed an inverse correlation with both local and systemic markers of inflammation, leading to the hypothesis that mainly damage leads to an increase of sodium in synovial fluid due to the release of GAGs from cartilage and bone. This hypothesis is supported by the finding that GAG concentration increase with the severity of osteoarthritis.[43] However, future research is needed to provide further support for this hypothesis.

Sodium concentrations in skin and muscle were also measured, as these are known sodium storage organs.[44, 45] Elevated sodium levels in these tissues in arthritis patients would suggest increased sodium intake, potentially supporting the notion that sodium is a risk factor for RA. However, no significant differences were observed in skin and muscle sodium concentrations between arthritis patients and healthy controls.

The discrepancy between our finding, and the description of sodium as a risk factor for RA could be explained by several factors. First, our study included patients with various types of arthritis (RA, spondylarthritis (SpA) and psoriatic arthritis (PsA)), not just RA. It is unknown if sodium is a risk factor for SpA and PsA, and if this is not the case this could have possibly diluted

the association between sodium and RA. Additionally, the lack of significance could be attributed to the sample size, as higher sodium concentrations were observed in the muscle and skin of arthritis patients compared to controls, but these were not significantly higher.

Concluding, our findings suggest that the elevated sodium concentrations observed in the joints of patients with autoimmune arthritis are primarily due to osmotically inactive sodium, which is likely associated with increased levels of proteoglycans. This points to a minimal role for sodium in the inflammatory processes within the joints. Given these results, a sodium-restricted diet does not seem to be a promising intervention for rheumatoid arthritis or other types of arthritis at this stage. Further studies with larger sample sizes and RA-specific cohorts are warranted to clarify these findings.

Interaction between environmental and genetic risk factors

In their effects on biology or pathophysiology, genetic and environmental factors can interact, with genetic predispositions modifying the effects of environmental exposures, and vice versa. A gene-environment interaction in RA has been found between SE and smoking in ACPA-IgG positive patients.[29, 46] Since smoking is specifically associated with AMPA-IgA, we investigated the interaction between SE and smoking for different AMPA isotypes in **Chapter 7**, and we hypothesized that both IgA (associated with smoking) and IgG (associated with SE) needed to be present for the interaction to occur. Indeed, the interaction between SE & smoking was only present in patients that were both positive for ACPA-IgG and ACPA-IgA.

When integrating the data presented in this chapter with existing literature to construct a timeline of risk factors influencing the onset of RA, smoking emerges as an early contributor. Wouters et al. demonstrated that smoking is associated with the early onset of autoantibodies, such as ACPA, during the pre-symptomatic phase, which is a key marker for the development of RA.[47] Similarly, Hensvold et al. highlighted that smoking acts as an early risk factor by promoting the production of autoantibodies before clinical symptoms appear.[19] These findings are in line with our research: the association of smoking with AMPA-IgA supports the mucosal origin hypothesis, indicating that smoking plays an early role in the development of RA by promoting the onset of autoantibodies during the pre-symptomatic phase.The involvement of HLA-SE alleles occurs later, only affecting disease onset in ACPA-positive individuals and specifically influencing the introduction of VDG.[47] This progression is likely to be driven by SE-restricted T-cells that promote the introduction of N-linked glycosylation sites during somatic hypermutation in ACPA-expressing B cells. Notably, the interaction between smoking and SE is observed only in ACPA-

positive patients who harbor both IgA and IgG ACPA. Figure 1 provides an overview of the associations between risk factors and AMPA.

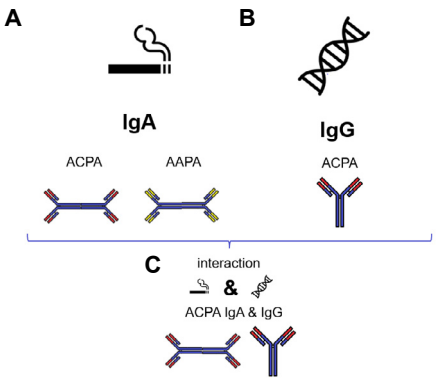

Figure 1. Association of smoking and shared epitope with anti-modified protein antibodies isotypes in rheumatoid arthritis

Starting point of AMPA

In the previous paragraphs, risk factors that may contribute to the development of AMPA were discussed. However, the precise mechanisms underlying the formation of AMPA remain unclear. In **Chapter 6** we aimed to find clues for the starting point of the AMPA response and focused on AMPA-IgM since IgM is the first isotype to be produced. AMPA-IgG and IgM were measured in an asymptomatic Japanese cohort and in two groups of patients from the Leiden Early arthritis cohort: RA patients, and patients with arthritis due to other causes. We demonstrated that the AAPA-IgM response differs from the ACPA- or Anti-CarP-IgM response: unlike ACPA- and anti-CarP-IgM, high levels of AAPA-IgM were found in ACPA-positive and ACPA-negative healthy donors, non-RA arthritis patients, and ACPA-negative RA patients. These results suggest that the AAPA-IgM response may be part of the normal immune response, potentially induced by viral or bacterial infections. It is known that bacteria can acetylate self-proteins as well as host proteins,[48] and in a bacterial infection this could lead to an antibody response directed to an acetylated protein. Moreover, in a mice model AMPA production was induced upon immunizing mice with chemically acetylated proteins from E. coli lysates,[49] indicating that acetylated bacterial proteins can induce AMPA responses. Hypothetically, in the years before onset of RA, an ongoing (auto)antibody response after an infection with isotype switching and epitope spreading to other post translationally modified proteins could lead the RA-associated AMPA response via molecular mimicry. However, which triggers induce this response in some individuals and not in others remains unclear. The only risk factor for the development of AAPA thus

far, as described in **Chapter 7**, is smoking. Perhaps smoking could sustain an ongoing autoimmune response by leading to post-translational modification of protein. Alternatively, a trigger with another PTM might skew the autoantibody response in the direction of ACPA or anti-CarP since AMPA are highly cross-reactive.[12, 13, 15] This hypothesis is further supported by the finding that AMPA responses are dynamic overtime, with observations in mice showing that cross-reactive responses induced by immunization with PTM-proteins, can be skewed towards another PTM-reactivity upon boosting with an antigen of another PTM type.[50]

AUTOANTIBODIES AND CLINICAL OUTCOME

AMPA as prognostic biomarkers

Besides the pathophysiology of RA, we have also devoted attention to the clinical disease course, as autoantibodies in RA are also associated with poorer clinical outcomes. Understanding the role of these antibodies in disease progression and remission is crucial, as they may serve as biomarkers for predicting long-term disease trajectories and guiding treatment strategies. ACPA have been associated with radiological progression and a reduced likelihood of achieving medication-free remission.[51, 52] Similarly, anti-CarP are associated with increased radiological progression in ACPA-negative RA patients.[53] Additionally, the presence of multiple AMPA is associated with enhanced radiological progression. However, the association of AAPA with radiological progression or remission has not yet been investigated.[54] Studying the association of AAPA with clinical outcomes is important because if AAPA is associated with either of these clinical outcomes, its determination could prove clinically useful in assessing RA prognosis. Therefore, in **Chapter 8** we investigated the impact of the different AMPA (ACPA, anti-CarP and AAPA) and number of AMPA on radiological progression as measured by the Sharp van der Heijde score (SHS) and sustained DMARD-free remission (SDFR) in RA. For SDFR, only an association with ACPA-positivity was found, while other AMPA did not influence the chance of obtaining SDFR. Interestingly, anti-CarP-single positivity was associated with a higher rate of radiological progression measured bij SHS, as was shown before,[53] but not with SDFR. A pathophysiological mechanism underlying the association of anti-CarP with bone erosions could be considered, since carbamylated proteins are found in the synovium and anti-CarP antibodies can bind to these proteins.[55] Therefore, it can be hypothesized that immune complexes containing anti-CarP antibodies could lead to inflammation, which in turn may contribute to cartilage and bone damage. Another explanation is the difference in type of outcome, with SDFR being a dichotomized outcome and SHS a continues outcome and therefor SHS could be more sensitive. Further research is needed to investigate these

hypothesis. Since AAPA is almost exclusively present in ACPA positive patients, this analysis was not possible to perform for AAPA, however in ACPA-positive patients no association of AAPA or anti-CarP was found with SHS. These results indicate that testing other AMPA in ACPA-positive patients does not seem to provide additional predictive value for clinical outcomes.

Autoantibodies and the risk of autoimmune related adverse events in treatment with anti-PD1

In the final chapter of this dissertation, we examine autoantibodies in a different context: in immunotherapy for cancer treatment. Over the past few decades, immunotherapy has emerged as a groundbreaking approach in oncology, harnessing the body's immune system to fight cancer cells. These therapies, including immune checkpoint inhibitors, have revolutionized the treatment landscape for various cancers, offering promising outcomes for patients who previously had limited treatment options.[56] One of the most significant advances in immunotherapy is the development of immune checkpoint inhibitors, particularly those targeting cytotoxic T-lymphocyte associated protein-4 (CTLA-4) and those targeting the Programmed cell death protein (PD) 1 axis. PD-1 is an inhibitory receptor expressed on T cells, which, when engaged by its ligands PD-L1 or PD-L2, inhibits T cell activation, thereby allowing tumors to evade immune surveillance. By blocking the interaction between PD-1 and its ligands, anti-PD-1 antibodies, such as nivolumab and pembrolizumab, effectively reinvigorate the immune system, enabling it to recognize and destroy cancer cells.[56] These agents have shown remarkable efficacy in a variety of malignancies, including melanoma, non-small cell lung cancer, and head and neck cancer, amongst others.

However, despite the therapeutic successes of anti-PD-1 therapy, a significant challenge remains in the form of immune-related adverse events (irAEs).[57] These adverse effects arise due to the hyperactivation of the immune system, which, in some cases, leads to immune-mediated damage to healthy tissues. Autoimmune-related adverse events can affect nearly every organ system, including the skin, gastrointestinal tract, liver, endocrine glands, and lungs. These side effects are typically managed with immunosuppressive treatments such as corticosteroids, but they can significantly impact patient quality of life and may require the discontinuation of therapy in severe cases.

The occurrence of irAEs has prompted increased interest in understanding the underlying mechanisms that drive these immune-mediated reactions. One potential factor contributing to the development of irAEs is the presence of specific autoantibodies. For CTLA-4 inhibitors, an association has been observed with the development of autoantibodies targeting the thyroid and with hypothyroidism.[58] In **Chapter 9**, we explored the relationship between

autoantibody profiles and the development of immune-related adverse events in patients receiving anti-PD-1 therapy for advanced malignant melanoma. Autoantibody positivity prior to anti-PD-1 therapy was associated with the development of irAEs in these patients. While we did not observe an association between anti-CCP2, RF and/or ANA (ENA) with respectively arthritis, dermatitis, sicca or colitis, a very strong association between anti-thyroid antibodies and thyroid dysfunction was seen both at baseline as well as after three months of treatment, especially in female patients. In our patient cohort, autoantibody positivity was not associated with cancer recurrence or treatment response. These findings therefore indicate that measuring anti-thyroid antibodies at baseline and after three months of treatment is a very potent biomarker for predicting the development of thyroid dysfunction especially in women. This conclusion is supported by evidence from an immune-related thyroiditis mouse model, which showed that mice with pre-existing anti-thyroglobulin antibodies were at high risk of developing anti-PD-1-induced thyroid dysfunction. Furthermore, it was demonstrated that this condition could be prevented by the depletion of CD4+ T cells, and not, or only partially, by depleting CD8+ T cells and B cells.[59] Moreover, destructive anti-PD-1-induced thyroiditis was observed exclusively in mice with prior immunization against Tg, suggesting that pre-existing autoimmunity against the thyroid gland, together with unleashing of CD4 T cells by anti-PD1-treatment, is critical for the development of immune-related thyroid dysfunction. If we translate these findings to patients receiving anti-PD-1 therapy, the onset of overt thyroiditis may be triggered by anti-PD-1 therapy in those with pre-existing thyroid antibodies. This is likely because autoreactive T cells directed against the thyroid gland, which would otherwise remain quiescent, can be activated by this therapy, leading to thyroid inflammation and destruction.

Together, the chapters of this dissertation underscore the significant value of studying autoantibodies and their associated risk factors in both rheumatic diseases and other autoimmune phenomena, such as those observed after checkpoint inhibitor therapy. This research provides vital insights into underlying disease mechanisms and highlights the clinical relevance of autoantibodies in disease outcome and prognosis. Key findings include the association of AMPA in rheumatoid arthritis (RA), with genetic factors—particularly the HLA-DRB1 shared epitope (SE)—linked to ACPA-IgG, which influences disease onset through ACPA glycosylation. In contrast, smoking is associated with the production of AMPA-IgA, supporting the mucosal origin hypothesis for RA. Clinically, ACPA serves as a critical biomarker for both radiological progression and the likelihood of achieving sustained drug-free remission (SDFR), while anti-CarP is associated with radiological progression in ACPA-negative patients, and no additional value was found for AAPA positivity

in either radiological progression or remission. In melanoma patients treated with PD-1 inhibitors, an association was found between thyroiditis and pre-existing thyroid autoantibodies, likely triggered by the activation of autoreactive T cells targeting the thyroid gland. This highlights the importance of monitoring thyroid autoantibodies as potential biomarkers for predicting thyroid-related immune adverse events during PD-1 therapy. Collectively, these findings not only enhance our understanding of autoimmune mechanisms but also point to the clinical potential of autoantibodies in improving patient management and informing therapeutic strategies.

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