Immune response to post-translationally modified proteins in rheumatoid arthritis: what makes it special?

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ABSTRACT

Rheumatoid arthritis (RA) exhibits common characteristics with numerous other autoimmune diseases, including the presence of susceptibility genes and the presence of disease-specific autoantibodies. Anti-citrullinated protein antibodies (ACPA) are the hallmarking autoantibodies in RA and the anti-citrullinated protein immune response has been implicated in disease pathogenesis. Insight into the immunological pathways leading to anti-citrullinated protein immunity will not only aid understanding of RA pathogenesis, but may also contribute to elucidation of similar mechanisms in other autoantibody-positive autoimmune diseases. Similarly, lessons learnt in other human autoimmune diseases might be relevant to understand potential drivers of RA. In this review, we will summarise several novel insights into the biology of the anti-citrullinated protein response and their clinical associations that have been obtained in recent years. These insights include the identification of glycans in the variable domain of ACPA, the realisation that ACPA are polyreactive towards other post-translational modifications on proteins, as well as new awareness of the contributing role of mucosal sites to the development of the ACPA response. These findings will be mirrored to emerging concepts obtained in other human (autoimmune) disease characterised by disease-specific autoantibodies. Together with an updated understanding of genetic and environmental risk factors and fresh perspectives on how the microbiome could contribute to antibody formation, these advancements coalesce to a progressively clearer picture of the B cell reaction to modified antigens in the progression of RA.

INTRODUCTION

A considerable number of human autoimmune diseases (AIDs) exhibit (disease-specific) autoantibodies and show notable responsiveness to B cell-targeted therapies, highlighting the pivotal role of B cells in these conditions. These AIDs include several rheumatic conditions, such as systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody-associated vasculitis and rheumatoid arthritis (RA). Although a clear role for disease-specific autoantibodies has been described in some AIDs, for other diseases such as RA, the mechanistic impact of the (autoreactive) B cell response is less clear and could be multifactorial. The involvement of B cells in these conditions could manifest through various mechanisms, such as the synthesis of harmful antibodies, the release of inflammatory cytokines and/or the presentation of antigens to T cells. In this review, we will focus on RA as prototypic rheumatic AID known to display a defined disease-specific autoreactive B cell response thought to contribute to disease pathogenesis. More specifically, emphasis will be given to the most specific autoantibodies in RA: ACPA, and their close relatives, although several other different autoantibody-reactivities have been described as well, including rheumatoid factors (RF) and anti-peptidyl arginine deiminase (PAD) antibodies.[1] The latter autoantibody reactivities share common features as they are associated with a more severe disease course, and are predominantly, but not exclusively found in RA.[2–5] Furthermore, they have also been hypothesised to contribute to inflammation by enhancing either the inflammatory potential of immune complexes (RF) or the enzymatic activity of PAD, thereby creating a 'feed forward loop' and more citrullinated antigens as target for the anti-citrullinated protein response.[6 7]

Similarly, antibodies to proteins with malondialdehyde/malondialdehyde acetaldehyde residues are found in patients with RA and their presence associates with higher serum C-reactive protein (CRP) levels.[8 9] However, these antibodies are also found in a proportion of healthy individuals as well as in many other clinical conditions.[10–12] If these antibodies contribute to the pathogenesis of these conditions is not known, but it is speculated that they could contribute to more 'inflammatory' disease phenotypes.[9]

As mentioned, the most specific autoantibody response in RA is directed against citrullinated proteins. Immunity to citrullinated proteins is generally believed to either be directly involved in the disease mechanisms underlying RA, or at least to be a distinct marker of the underlying immunopathology. The reason for this is because ACPA, in contrast to the other autoantibodies mentioned above, are also associated with the most prominent genetic susceptibility alleles, the HLA-DRB1 shared epitope (SE) alleles.[13] Similarly, a functional haplotype in the PAD enzyme creating the antigens targeted by anti-citrullinated protein immune responses has also been shown to be associated with susceptibility to RA.[14] For these reasons, although a contribution of other autoimmune responses in RA is certainly not excluded, this review will focus on the immune response to citrullinated proteins.

ANTI-MODIFIED PROTEIN ANTIBODIES AND THEIR TARGETS

ACPA recognise proteins that have undergone a post-translational modification (PTM) in which an

arginine residue is converted into a citrulline. This process is enzymatically mediated by PADs. Although ACPA, by definition, require the presence of citrulline for antigen binding, the recognition of citrullinated proteins can be diverse. On the monoclonal antibody level, it has been shown that some ACPA can be cross-reactive to a wide array of citrullinated proteins and peptides of self-origin and foreign origin.[15 16] These 'promiscuous' antibodies probably do not, or only to a limited extent, interact with the amino acids present in the protein backbone. Others display a more restricted pattern of citrullinated antigen recognition, because they most likely also interact with neighbouring amino acids.[17 18] Although these latter 'private' ACPA are also, in general, cross-reactive to multiple citrullinated antigens, the extent of cross-reactivity is limited.

Citrullination is not the sole PTM of significance in RA. Antibodies can also interact with proteins altered through carbamylation, a non-enzymatic process leading to the transformation of lysine into homocitrulline. Although often regarded as a different family of autoantibodies, the reactivity pattern of anti-carbamylated protein (anti-CarP) antibodies in patient populations resembles (but is not identical to) the reactivity pattern of ACPA.[19 20] This is explained by the promiscuous nature of ACPA as it has been shown that many ACPA monoclonal antibodies are also reactive towards carbamylated proteins. Like ACPA, anti-CarP antibodies can also recognise a plethora of carbamylated antigens and display promiscuity to citrullinated proteins.[15 21]

A third member belonging to the group of anti-modified protein antibodies (AMPA) is antibodies to acetylated proteins (AAPA).[22] Acetylated proteins are formed when acetyltransferases modify lysine's to acetylated lysines. Anti-acetylated proteins antibodies also occur in RA and like ACPA can be cross-reactive to various acetylated proteins as well as to other PTMs (homocitrulline and citrulline). As AAPA have been discovered the last, they are also less well described. At the monoclonal level, it is clear that some ACPA cross-react to acetylated proteins.[15 23] The extent of cross-reactivity, however, is, at present unclear. Nonetheless, this appears more limited as compared with ACPA cross-reactivity to carbamylated proteins as suggested from prevalence studies in different rheumatic populations.[24 25] These data indicate that AAPA belong to a different category of AMPA as also supported by the observation that not all AAPA monoclonal antibodies cross-react to citrullinated proteins.[26]

Together, the evidence indicates that AMPA are a group of antibodies that are promiscuous towards different modified proteins as well as to different types of PTM antigens. This promiscuity is not absolute but does show that many modified autoantigens, as well as many modified non-self-antigens, can be recognised by AMPA.

The B cell response against modified antigens

AMPA are produced by autoreactive B cells that have been activated to become antibody-secreting cells either as short-lived plasmablasts or long-lived plasma cells. For many years, it was difficult to phenotype these cells directly as the tools to visualise them in an autoantigen-specific manner were lacking. Through the development of antigen-specific staining methods, the B cell response against modified antigens can now be visualised and characterised. Studies addressing the phenotype of the modified protein-directed B cell compartment have shown that a large fraction of PTM-directed B cells reacted to multiple modified antigens, indicating that the AMPA response is also promiscuous at the cellular level.[15 27] B cells reacting to citrullinated antigens were most prevalent and more often 'monoreactive' than B cells reacting to carbamylated and acetylated antigens, providing further indications that citrullinated proteins are the dominant antigen in the AMPA response in patients with RA.[27] It is now also clear that these autoreactive B cells are actively proliferating, displaying a recently activated phenotype, even in peripheral blood of patients in clinical remission.[28] These data are intriguing as they indicate that, despite clinical remission, no immunological remission is reached. Subsequent studies have also shown that patients can harbour large fractions of PTM-directed plasmablasts. In fact, the composition of this autoreactive B cell response resembled the composition of the B cell response to the SARS-CoV-2- spike protein at 1 week following mRNA booster vaccination.[27 29] While the number of virus-specific plasmablasts peaked on day 7 post booster, their levels declined markedly and rapidly thereafter. By day 14 after vaccination, virus-specific plasmablasts were nearly absent. The observation that the profile of the PTM-directed B cell response resembles that of B cell responses to viral antigens shortly after vaccination is intriguing as it indicates that the PTM-directed B cell response, unlike antiviral responses, does not transition to a resting state but remains consistently activated, capable of forming plasmablasts. Although the autoreactive B cell compartment was analysed in a cross-sectional manner, these observations nevertheless provide clear evidence pointing to the unceasing activation of autoreactive responses in RA. Next to this sustained 'immunological disease activity', these observations also provide an immunological rationale for disease flares on drug tapering/withdrawal since the disease-specific immune response most likely remains active despite treatment-induced clinical remission. For this reason, it is appealing to pursue interventions aiming at sustained immunological remission as these could, conceivably, also act as the gateway leading to sustained drug-free remissions.

The impact of environmental and genetic risk factors on the temporal development of the AMPA response

The factors underlying the sustained activation of autoreactive B cells described above can best be understood by placing them in the larger context of the temporal development of autoantibodies and RA. This evolution is generally viewed as a multistep process which will first briefly be described in schematic overarching terms before focusing on the specific mechanisms relevant for the AMPA response.[30 31] In the first step, underlying risk factors, both genetic and environmental, form an individual's inherent predisposition to AID. A combination of risk factors and possibly also stochastic events then leads to a break in tolerance of both T and B cells followed by the emergence of signs of systemic autoimmunity in the form of autoantibodies, without the presence of symptoms. Subsequently, risk factors again contribute to the chance whether the autoantibody response matures and arthralgia ensues which, once more depending on the influence of risk factors, can progress to undifferentiated arthritis and finally RA fulfilling the classification criteria. This framework can now be filled in for the AMPA response, based on the insights into its origins and development gleaned in the past years.

Regarding risk factors for disease, it is notable that the vast majority of these factors specifically predisposes to seropositive/ACPA-positive RA. A recent study using the largest study population to date in RA with over 30 000 cases and approximately 1 million controls confirmed this pattern and again found several associations with seropositive RA, but hardly any variants affecting the risk of seronegative RA.[32] Overall, a large number of genetic risk loci have been identified, most of them

being common non-coding variants with small effect sizes which presumably affect gene expression at other loci.[33] The predominant association with seropositive/ACPA-positive RA also holds true for the genetic risk factors associated with the largest effect size: the HLA DRB1 SE alleles and the PTPN22 variant rs2476601-A.[13 34] Considering the cross-reactive nature of AMPA with their binding to different PTMs as described above, it is remarkable that the Human Leucocyte Antigen (HLA) associations for ACPA-positive versus anti-CarP-positive RA differ. Anti-CarP-positive, but ACPA-negative, RA does not seem to associate with the HLA-SE alleles, but rather with an HLA class I allele: HLA B*08.[35] The mechanisms behind this association is as of yet illusive, but the finding itself illustrates that there may be both shared and unique pathways underlying the generation of the different AMPA.

For environmental risk factors, again associations have also been found to be stronger for seropositive compared with seronegative RA. This is particularly the case for tobacco smoking and other inhalant exposures such as silica inhalation or exposure to burn pits.[36–38] For ACPA-positive RA, a genetic–environment interaction has been found for HLA-SE alleles and smoking which has led to the hypothesis that smoking leads to increased citrullination in pulmonary tissue with subsequent presentation of these antigens by the HLA-SE alleles.[39 40] Several reported findings are in line with this hypothesis, including the presence of citrullinated antigens, ACPA-positive B cells and ACPA in bronchoalveolar lavage samples before and after onset of RA.[41–43] Intriguingly, as for the genetic risk factors, the associations with environmental risk factors also differ for different AMPA with smoking not being associated with anti-CarP-positive RA.[20] The absence of this association is even more striking in light of the reported link between anti-CarP antibodies and interstitial lung disease in RA.[44] Whether the pulmonary abnormalities in this case should be considered a risk factor, or might rather be the consequence of a broad AMPA response, remains unknown and requires further investigation.

Besides the findings regarding the lungs, there are also other clues that suggest that the autoimmune response underlying RA starts at mucosal sites; a concept also known as the 'mucosal origins hypothesis'.[45] The periodontium is another possible culprit site with periodontitis being more prevalent in patients with RA.[46] Patients with RA and periodontal disease have been found to experience repeated oral bacteraemias, during which the immune system could be exposed to proteins originating from oral bacteria which may initiate or sustain cross-reactive autoimmune responses.[47] In this respect, it is noteworthy that a bacterium capable of citrullinating proteins itself is *Prophyromonas gingivalis*, which is considered a major pathogen in periodontitis.

A site which has been less explored to date is the human intestinal tract where the microbiome might be a large source of post-translationally modified proteins, with enzymatic activity of microbes having been shown to lead to acetylation of both bacterial and host proteins.[48] A first study specifically looking at the intestinal microbiome in ACPA-positive versus ACPA-negative RA has revealed that intestinal bacteriophage communities indeed diverge based on ACPA status.[49] Furthermore, a recent report charting the variation of the human antibody epitope repertoire also elucidated differences between ACPA-positive versus ACPA-negative healthy individuals, suggesting that previous (possibly infectious) exposures may tip the balance towards developing autoimmune responses to citrullinated proteins.[50] Alternatively, immune response to intestinal bacteria may be involved in RA without specifically targeting PTMs; an intriguing study described immune responses against Subdoligranulum isolates which were sufficient to cause arthritis in germ-free mice.[51]

How do these separate risk factors and observations fit into the overall framework of disease development described above? Based on the phases of disease evolution, it is important to distinguish risk factors that contribute to the initial development of autoantibodies, from those that affect later stages such as symptom onset. Several studies have now shown that smoking is mainly associated with the development of AMPAs.[52 53] This is known to occur many years before the first disease manifestations.[54 55] Several studies have investigated which AMPA/autoantibody in RA might emerge first but there does not appear to be a clear primordial autoantibody, perhaps due to cross-reactivity, perhaps due to different pathophysiological pathways or PTM exposures in different groups of patients.[56–58] Subsequently, the AMPA response matures with rising antibody levels, epitope spreading, isotype switching and the introduction of variable domain glycosylation[56 59] (described in more detail below). Genetic factors and in particular the HLA-SE alleles predominantly exert their action at this point: the maturation of the autoantibody response and the transition to disease.[52 53] Of note: although smoking may particularly affect the first development of autoantibodies, it is yet unclear whether other mucosal/microbial factors could also affect this later stage and influence the maturation of the autoimmune response. After symptom onset, the AMPA response shows no signs of further evolution and may even slightly contract under the influence of therapy.[60] Finally, it is worthwhile to note that this chain of events need not inevitably transpire as described and lead to RA. In a cohort of Indigenous North Americans with a known very high risk of RA, a large proportion of ACPA-positive at-risk relatives did not develop inflammatory arthritis and reverted to an autoantibody-negative state.[61] This raises the tantalising opportunity that inhibiting maturation of the AMPA response (perhaps through interventions aimed at mucosal/microbial exposures) could halt this evolutionary pathway and ultimately prevent RA.

T cells, glycans and AMPA formation

In the last decade, it has become clear that ACPA, and likely also other AMPA,[62] possess an unexpected feature: they harbour additional glycans in the variable antibody domains.[63] Although variable domain or Fab glycans are not unique to ACPA, as they are present on approximately 10% of IgG1 molecules, the level of variable-domain glycans (VDG) in RA is considerably increased to approximately up to 90% of ACPA IgG. Because this upregulation is found in over 95% of patients with RA,[59 64] these data suggest that VDGs play a role in the biology of the ACPA response. The high abundance of VDGs on ACPA from patients with RA is not 'intrinsic' to all ACPA, as the level of ACPA variable-domain glycosylation is much lower on ACPA from ACPA-positive healthy subjects.[65] Before disease onset, the abundance of ACPA VDGs increases over time until the onset of RA after which it stabilises.[59 66 67]

For N-linked glycosylation to occur, proteins must harbour an N-linked glycosylation-site consisting of an asparagine (N) followed by any amino acid except a proline and a serine or threonine. This three amino acid sequence (N-X-S/T) is not encoded by most variable gene segments of antibodies in germline configuration. This means that the glycosylation site must be introduced following, for example, somatic hypermutation (SHM) (figure 1). Indeed, on analysis of the ACPA B cell repertoire, N-linked glycosylation sites were shown to have been introduced

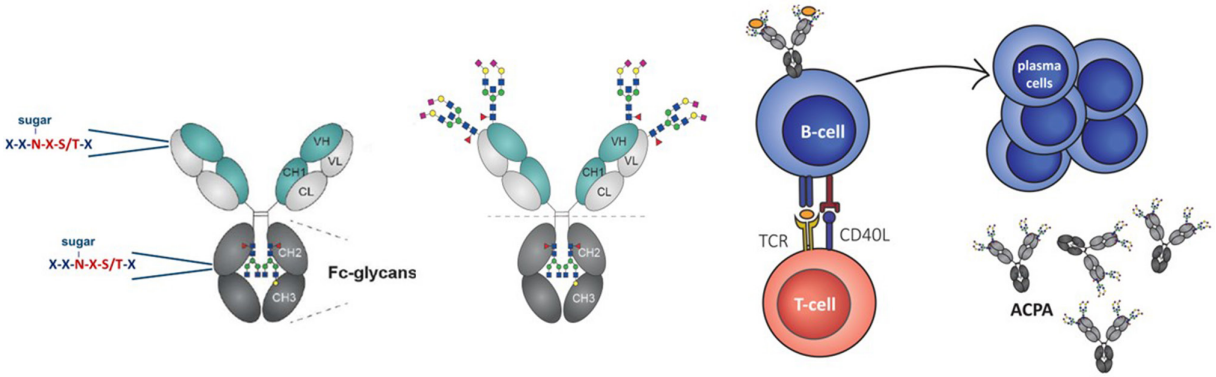

Figure 1 IgG glycosylation. IgG1 antibodies are all glycosylated in their Fc-tail because of the presence of an N-linked glycosylation site at position 297 (left). Most IgG1 antibodies do not contain N-linked glycans in the variable domain (VH/VL) of the antibody, due to the absence of N-linked glycosylation sites. However, these sites can be introduced in the variable domain on somatic hypermutation, a process facilitated by CD4+ T helper cells providing 'help' to B cells (right). Anti-citrullinated protein antibodies (ACPA) present in patients with rheumatoid arthritis are abundantly glycosylated in the variable domain, indicating that ACPA-expressing B cells have obtained a selective advantage after introduction of N-linked glycosylation in the variable domain. TCR, T-cell receptor; VH/VL, variable heavy/variable light.

by SHM.[68] SHM by B cells typically only occurs on provision of T cell help. Immunogenetic data support the notion that T helper activity is crucially involved in variable-domain glycosylation of ACPA as, in the predisease phase when ACPA VDG levels vary, the presence of the most prominent genetic RA-susceptibility factor, the HLA-SE alleles, is specifically associated with the presence of variable-domain glycosylated ACPA and not with the presence of ACPA as such.[66 67] In ACPA-positive RA, the association between ACPA variable-domain glycosylation and the HLA-SE is no longer present since at this stage ACPA have become abundantly glycosylated. The notion that the HLA-SE alleles predispose to the introduction of VDG is also supported by the observation that the HLA-SE alleles do not associate with ACPA IgG in ACPA-positive healthy individuals as shown in large population-based studies.[52 69] In line with this observation, the level of ACPA VDG is low in healthy individuals that are ACPA positive.[65] As the (initial) formation of antigen-specific IgG is also a T-cell driven process, this suggests that at least two different T cell responses are involved in the formation of a fully maturated ACPA response. A first, HLA-SE independent T cell response, is involved in the formation of ACPA, followed by a subsequent, HLA-SE-associated T-cell response that drives the

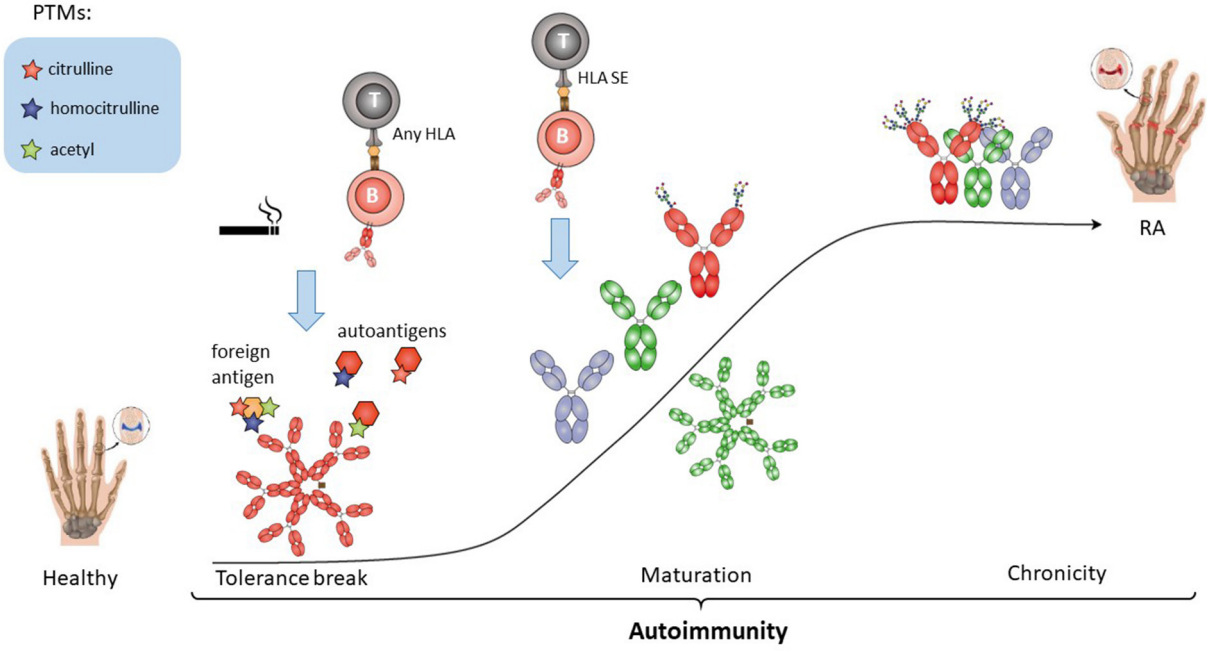

Figure 2 Development and maturation of the anti-modified protein antibodies (AMPA) response. Both T-cell and B-cell tolerance is broken years before disease onset, possibly due to foreign antigens carrying post-translational modifications (PTMs). Smoking is a risk factor for this emergence of rheumatoid arthritis (RA)-associated autoantibodies in otherwise healthy individuals. However, the lack of an Human Leucocyte Antigen (HLA) association indicates that T cells of various HLA restrictions can provide help to autoreactive B cells at this stage. Subsequently, maturation of the response occurs, characterised by rising autoantibody levels, isotype switching, epitope spreading and the emergence of N-glycosylation sites in the variable antibody domains of AMPA. This latter process is associated with HLA-shared epitope (SE) alleles meaning that a second and different T cell response is required for this evolution. Likewise, the HLA-SE alleles are associated with the onset of symptoms and the onset of RA in autoantibody-positive individuals.

ACPA response to further maturation by expediting the introduction of N-linked glycosylation sites by SHM (figure 2). It is tempting to speculate that the latter event contributes to the generation of a pathogenic anti-citrullinated protein response as the presence of VDG on citrullinated antigen-directed B cell receptors enhanced B cell activation and could, thereby, contribute to a breach of control (tolerance) and outgrowth of these autoreactive B cells.[70]

Experiences from other autoantibody-positive immune-mediated diseases

To become activated and produce antibodies, B cells need to recognise antigen. As under homeostatic conditions, most citrullinated antigens are found intracellularly, it is likely that AMPA-expressing B cells are not exposed to these antigens during their development. Hence, B cells will not be deleted to such antigens and therefore AMPA-expressing naïve B cells are conceivably present in both patients and healthy controls. To activate such B cells to become isotype-switched antibody-secreting cells, two additional signals are crucial: 'co-stimulation' and T cell help. Costimulation for B cell activation is generally delivered by innate signals coming from (opsonised) microbes via 'innate signalling receptors' recognising, for example, complement fragments or 'Pattern/Damage associated molecular Patterns'. These receptors include the innate immune receptors that sense microbial DNA or RNA. In this respect, it is remarkable that many rheumatic AIDs are hallmarked by autoantibody responses directed to DNA or DNA/RNA-binding proteins. Examples of such autoantibodies include responses to topoisomerase, centromeres (scleroderma), histidine-tRNA-ligase (anti-Jo1; dermatomyositis) or ribonucleoproteins such as anti-Ro or anti-Sm (systemic lupus erythematosus (SLE)). A common denominator of these antigenic targets is not only that they are linked to molecules able to trigger innate immune receptors, but also that they have 'mimics' in the microbial world. Therefore, it is highly conceivable that in these cases autoimmunity is provoked by foreign antigens that not only deliver 'co-stimulation' (signal 1), but also 'T cell help' (signal 2) to the developing autoimmune B cell response. An example of this scenario is, for example, found in antiphospholipid syndrome. The autoantibody response to β_2-glycoprotein I (β_2GPI) has been shown to cross-react with 'mimotopes' on the bacterial DNA methyltransferase from the commensal *Roseburia intestinalis*.[71] Interestingly, immunisation of mice with this microbe generated autoantibodies to β_2GPI, indicating that, in the context of this 'microbial environment', (DNA-binding) proteins conserved between man and microbes can provide both 'co-stimulatory' and T helper cell-derived triggers allowing the development of B cell-mediated autoimmunity.[71] Such autoimmunity could arise in case commensal microbes breach mucosal barriers as has been shown by the induction of anti-DNA/RNA antibodies by the pathobiont *Enterococcus gallinari* in a mouse model.[72]

Whether similar mechanisms are at play during the induction of AMPA is not known. However, it is intriguing to note that also in the case of AMPA, a link to the microbial world is credible. In case of barrier breach, extracellular microbes will be targeted by neutrophils. These cells will respond to 'danger' via different defence mechanisms, including NETosis, a process in which neutrophils expel their chromatin to form a 'neutrophil extracellular trap' (NET). These NETs will immobilise and disarm microbes but will also expose modified self-proteins, such as acetylated or citrullinated histones, that can be targeted by AMPA. Moreover, the process of NETosis itself will lead to the presence of PADs and acetyl transferases able to modify the extracellular environment. This environment includes proteins derived from microbes and, in this manner, microbe-derived proteins can be modified, even when the microbes themselves do not display PAD or acetyltransferase activity. In this way, all 'signals' required for B cell activation will be available for the induction of an anti-modified protein-directed B cell response; modified antigen, 'co-stimulation' via (opsonized) microbes and T cell help to microbial proteins. As in this scenario, no specific microbes are targeted by the adaptive immune response, an AMPA response, independent of the HLA haplotype of the host, could arise potentially explaining the development of an ACPA-response independent of HLA-SE long before disease onset.

The subsequent events leading to the formation of ACPA carrying VDG and onset of disease are associated with HLA-SE, indicating a second T-cell response driving the further maturation of the ACPA response. The nature of this T-cell response is largely unknown, although it has been speculated that this could be found in the recognition of citrullinated epitopes presented by HLA-SE molecules.[73–75] Nonetheless, it is relevant to note that the ability to present citrullinated epitopes to CD4+ T cells is not restricted to the HLA-SE molecules as many other HLA molecules not associating with RA can do so as well.[76–79] Furthermore, although citrullinated peptide-specific T cell responses have been reported,[73–75 80] this does not necessarily indicate that these T cells can recognise endogenously processed antigens or respond to functionally relevant peptide antigen concentrations.[81] Therefore, it cannot be excluded that also the further maturation of the ACPA response is supported by T cells reacting towards foreign antigens. A prominent example of a human immune-mediated disease in which exogenous antigens drive T cell–B cell interactions leading to autoimmune phenomena is coeliac disease. In coeliac disease, T cells reacting to the foreign antigen of dietary gluten provide help to B cells making autoantibodies to tissue transglutaminase 2. This example also nicely illustrates that foreign antigens can sustain B cell-mediated autoimmune responses that originate from mucosal surfaces.[82]

Thus collectively, given the evidence obtained in RA and other AIDs, it appears likely that the microbiome is intimately involved in the induction of the AMPA response, that through its promiscuous nature, also reacts to modified self-antigens. The nature of the antigens recognised by the different T cells supporting the developing and ongoing AMPA response is not clear, but current evidence indicates that the HLA-SE alleles are instrumental in the introduction of ACPA VDG predisease thereby possibly allowing further maturation of the immune responses underlying disease pathogenesis.

AMPA in association with clinical phenotype

In addition to the insightful association of AMPA characteristics (such as VDG) with risk factors (such as the HLA-SE alleles), the relevance of AMPA for our current understanding of RA is further exemplified by the strong link between autoantibody status and clinical phenotype. Different disease characteristics have all been shown to differ between AMPA-positive versus AMPA-negative individuals, starting with the risk of disease progression to RA in individuals with arthralgia or undifferentiated arthritis which is substantially higher in individuals with AMPA.[83 84] After disease onset, AMPA-positive patients have a more severe disease course than AMPA-negative patients with significantly more joint destruction.[85] AMPA-positive patients are also much less likely to achieve sustained drug-free remission, which illustrates the protracted and relentless nature of AMPA-positive

RA.[86] Extra-articular manifestations such as serositis, vasculitis and pulmonary involvement are other disease features which are more prevalent in patients with AMPA-positive RA.[87]

Considering the stark differences in long-term phenotype, it is remarkable that the presentation of AMPA-positive versus AMPA-negative RA is quite similar. Only on considering the breath of the autoantibody repertoire, do minor differences, such as a slightly younger age in patients with multiple autoantibodies, become apparent.[88] This also applies to treatment response of patients with RA, where some studies dichotomising patients solely based on the presence of ACPA have found similar initial treatment responses, while other studies measuring more and different AMPA reported that a broad autoantibody profile was associated with a better early treatment response.[89 90] This latter finding is more in line with the exciting findings of recent studies investigating treatment responses in patients with pre-RA/arthralgia. In the TREAT EARLIER (TREAT Early Arthralgia to Reverse or Limit Impending Exacerbation to Rheumatoid arthritis) study, arthritis was more successfully postponed in patients with ACPA-positive than in ACPA-negative arthralgia treated with methotrexate, although this effect was not sustained once treatment was discontinued.[91] Similar findings have been reported as preliminary results from another arthralgia study (arthritis prevention in the pre-clinical phase of RA (APIPPRA)) of which publication is eagerly anticipated.[92] How can this difference in the effect of AMPA on treatment response (only minor in established RA, vs more outspoken in patients with arthralgia) be explained? It is tempting to speculate that therapeutic interventions in AMPA-positive patients in the predisease phase may abrogate further maturation of the AMPA response and thereby prevent disease; a hypothesis that remains to be tested. Alternatively, explanations may also lie outside the adaptive immune response and rather reflect differences in the timing and development of inflammation and subsequent reprogramming of innate immune cells or synovial fibroblasts in the context of 'trained immunity' which may be less easily reversed on treatment in AMPA-negative individuals.[93 94]

From a clinical point of view, it is important to note that despite the phenotypical associations with the breath of the AMPA response as described above, these effects are dominated by the immune response to citrullinated proteins over other AMPA. In ACPA-positive patients, the presence of other AMPA does not influence radiographic progression or the chance of achieving sustained drug-free remission.[95] Therefore, the additive value of determining other AMPA in patients with ACPA-positive RA is currently limited. The same holds true for repeated measurements of AMPA after disease onset. Neither changes in autoantibody levels, nor seroconversion from AMPA positive to AMPA negative were found to be associated with favourable long-term outcomes.[96 97] In fact, seroconversion from anti-CCP2 IgG-positive to IgG-negative is known to be very rare: 2% of patients. This indicates that the immunological abnormalities underlying the development of ACPA are deeply ingrained and very difficult, if not impossible, to reverse in established disease.

Are AMPA pathogenic?

Based on the observation that AMPA develop years before symptom onset, one could argue that it is unlikely that AMPA themselves are pathogenic. Historical studies have investigated the effect of plasma transfer from patients with RA to healthy recipients and revealed no evidence of disease development in the latter.[98] Plasma exchange was also found to have no positive effect on RA disease activity, thereby supporting the conclusion that the mere presence of autoantibodies is not pathogenic.[99] On the other hand, one could argue that the distinct microenvironment of the inflamed joint might endow these autoantibodies with pathogenic features that are absent in other surroundings, allowing them to contribute to and sustain inflammation. Along these lines, several studies have investigated and reported possible pathogenic functions of ACPA. Some of these have focused on immune effector functions that can be harnessed by IgG in general. For example, immune complexes consisting of ACPA and citrullinated fibrinogen have been found to be able to stimulate 'tumour necrosis factor secretion via Fcγ-receptor engagement on macrophages.[100] Similarly, ACPA have been shown to be able to recruit complement via both the classical and alternative pathways.[101] Based on the observation that ACPA can target the citrullinated contents of NETs, enhancement of NETosis could also be a mechanism via which ACPA exert a proinflammatory effect.[102] Turning to experiments which more specifically focused on arthritis-related pathology, some studies have reported that ACPA injection in mice can trigger bone loss and pain-like behaviour, in contrast to the lack of effects reported in humans.[103 104] Further insights in this area are needed to understand if these effects are due to binding to citrullinated antigens, and to elucidate the proteins that may be involved.

Recently, novel insights question the concept of pathogenic ACPA and instead suggest that ACPA might rather confer protection from arthritis.[105] In this study, monoclonal ACPA from patients with RA did not induce pain-associated behaviour in mice, contrary to previous findings, but instead protected mice from antibody-induced arthritis. The hypothesis put forward in this publication states that in the context of inflammation, increased citrullination would generate antigens in the joint that can then be bound by specific protective ACPA. These local immune complexes interact with Fcγ-receptor 2B on macrophages and thereby promote IL-10 secretion and reduce osteoclastogenesis. Although the protective effects installed by ACPA have been replicated by several groups,[106–108] it is important to note that these findings stem from murine models which have their limitations regarding the extent to which they mimic RA. It is interesting to consider the possibility that ACPA may be the consequence of an inflammatory reaction, rather than the cause, and may serve as a mechanism to dampen inflammation, but more research will be required to come to a definitive conclusion regarding AMPA function. Overall, it appears possible that different groups of ACPA may have diverging functions, be it pathogenic or protective, perhaps depending on their exact characteristics such as epitope binding and glycosylation.

CONCLUDING REMARKS

In recent years, new insights have been obtained in the nature, evolution and clinical relevance of AMPA and their underlying B cell response. These insights have revealed that the AMPA responses are dynamic, even in patients who are in clinical remission, and that ACPA responses are apparently fuelled by triggers that keep driving their activation. A link to the microbial world is also becoming increasingly clear as well as their promiscuous antigen-recognition abilities. Together, these and other observations have started filling the gaps of a conceptual foundation on how the AMPA response emerges and is linked to clinical phenotype.

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REFERENCES

1 Rheumatoid factor: biology and utility of measurement – UpTodate; Available: https://www.uptodate.com/contents/rheumatoid-factor-biology-and-utility-of-measurement
2 Zhao J, Zhao Y, He J, *et al*. Prevalence and significance of anti-peptidylarginine deiminase 4 antibodies in rheumatoid arthritis. *J Rheumatol* 2008;35:969–74.
3 Zhang G, Xu J, Du D, *et al*. Diagnostic values, association with disease activity and possible risk factors of anti-PAD4 in rheumatoid arthritis: a meta-analysis. *Rheumatology (Oxford)* 2023:kead545.
4 Kim Y, Rebman AW, Johnson TP, *et al*. Peptidylarginine deiminase 2 autoantibodies are linked to less severe disease in multiple sclerosis and post-treatment lyme disease. *Front Neurol* 2022;13:874211.
5 Cappelli LC, Hines D, Wang H, *et al*. Anti-Peptidylarginine deiminase 4 autoantibodies and disease duration as predictors of treatment response in rheumatoid arthritis. *ACR Open Rheumatol* 2023;13:874211.
6 Darrah E, Giles JT, Ols ML, *et al*. Erosive rheumatoid arthritis is associated with antibodies that activate PAD4 by increasing calcium sensitivity. *Sci Transl Med* 2013;5:186ra65.
7 Shi J, Darrah E, Sims GP, *et al*. Affinity maturation shapes the function of agonistic antibodies to peptidylarginine deiminase type 4 in rheumatoid arthritis. *Ann Rheum Dis* 2018;77:141–8.
8 Grönwall C, Amara K, Hardt U, *et al*. Autoreactivity to malondialdehyde-modifications in rheumatoid arthritis is linked to disease activity and synovial pathogenesis. *J Autoimmun* 2017;84:29–45.
9 van den Beukel MD, van Wesemael TJ, Hoogslag ATW, *et al*. Antibodies against advanced glycation end-products and malondialdehyde-acetaldehyde adducts identify a new specific subgroup of hitherto patients with seronegative arthritis with a distinct clinical phenotype and an HLA class II association. *RMD Open* 2023;9:e003480.
10 Hill GE, Miller JA, Baxter BT, *et al*. Association of malondialdehyde-acetaldehyde (MAA) adducted proteins with atherosclerotic-induced vascular inflammatory injury. *Atherosclerosis* 1998;141:107–16.
11 Rolla R, Vay D, Mottaran E, *et al*. Detection of circulating antibodies against malondialdehyde-acetaldehyde adducts in patients with alcohol-induced liver disease. *Hepatology* 2000;31:878–84.
12 Carson JS, Xiong W, Dale M, *et al*. Antibodies against malondialdehyde-acetaldehyde adducts can help identify patients with abdominal aortic aneurysm. *J Vasc Surg* 2016;63:477–84.
13 Huizinga TWJ, Amos CI, van der Helm-van Mil AHM, *et al*. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. *Arthritis Rheum* 2005;52:3433–8.
14 Suzuki A, Yamada R, Chang X, *et al*. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet* 2003;34:395–402.
15 Kissel T, Reijm S, Slot LM, *et al*. Antibodies and B cells recognising citrullinated proteins display a broad cross-reactivity towards other post-translational modifications. *Ann Rheum Dis* 2020;79:472–80.
16 Steen J, Forsström B, Sahlström P, *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;71:196–209.
17 Ge C, Holmdahl R. The structure, specificity and function of anti-citrullinated protein antibodies. *Nat Rev Rheumatol* 2019;15:503–8.
18 Ge C, Xu B, Liang B, *et al*. Structural basis of cross-reactivity of anti-citrullinated protein antibodies. *Arthritis Rheumatol* 2019;71:210–21.
19 Shi J, Knevel R, Suwannalai P, *et al*. Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. *Proc Natl Acad Sci U S A* 2011;108:17372–7.
20 Jiang X, Trouw LA, van Wesemael TJ, *et al*. Anti-CarP antibodies in two large cohorts of patients with rheumatoid arthritis and their relationship to genetic risk factors, cigarette smoking and other autoantibodies. *Ann Rheum Dis* 2014;73:1761–8.
21 Kampstra ASB, Dekkers JS, Volkov M, *et al*. Different classes of anti-modified protein antibodies are induced on exposure to antigens expressing only one type of modification. *Ann Rheum Dis* 2019;78:908–16.
22 Juarez M, Bang H, Hammar F, *et al*. Identification of novel antiacetylated vimentin antibodies in patients with early inflammatory arthritis. *Ann Rheum Dis* 2016;75:1099–107.
23 Sahlström P, Hansson M, Steen J, *et al*. Different Hierarchies of anti-modified protein autoantibody reactivities in rheumatoid arthritis. *Arthritis Rheumatol* 2020;72:1643–57.
24 Studenic P, Alunno A, Sieghart D, *et al*. Presence of anti-acetylated peptide antibodies (AAPA) in inflammatory arthritis and other rheumatic diseases suggests discriminative diagnostic capacity towards early rheumatoid arthritis. *Ther Adv Musculoskelet Dis* 2021;13.
25 van WesemaelTJ, Reijm S, Kawakami A, *et al*. IgM antibodies against acetylated proteins as a possible starting point of the anti-modified protein antibody response in rheumatoid arthritis. *Ann Rheum Dis* 2023. Available: https://ard.bmj.com/content/early/2023/11/01/ard-2023-224553
26 Reijm S, Kissel T, Stoeken-Rijsbergen G, *et al*. Cross-reactivity of IgM anti-modified protein antibodies in rheumatoid arthritis despite limited mutational load. *Arthritis Res Ther* 2021;23:230.
27 Reijm S, Kwekkeboom JC, Blomberg NJ, *et al*. Autoreactive B cells in rheumatoid arthritis include mainly activated CXCR3+ memory B cells and plasmablasts. *JCI Insight* 2023;8:e172006:20.:.
28 Kristyanto H, Blomberg NJ, Slot LM, *et al*. Persistently activated, proliferative memory autoreactive B cells promote inflammation in rheumatoid arthritis. *Sci Transl Med* 2020;12:eaaz5327:570.:.
29 Kardava L, Rachmaninoff N, Lau WW, *et al*. Early human B cell signatures of the primary antibody response to mRNA vaccination. *Proc Natl Acad Sci U S A* 2022;119:e2204607119.
30 Gerlag DM, Raza K, van Baarsen LGM, *et al*. EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid Arthritis. *Ann Rheum Dis* 2012;71:638–41.
31 Klareskog L, Rönnelid J, Lundberg K, *et al*. Immunity to citrullinated proteins in rheumatoid arthritis. *Annu Rev Immunol* 2008;26:651–75.
32 Saevarsdottir S, Stefansdottir L, Sulem P, *et al*. Multiomics analysis of rheumatoid arthritis yields sequence variants that have large effects on risk of the seropositive subset. *Ann Rheum Dis* 2022;81:1085–95.
33 Okada Y, Eyre S, Suzuki A, *et al*. Genetics of rheumatoid arthritis: 2018 status. *Ann Rheum Dis* 2019;78:446–53.
34 Plenge RM, Padyukov L, Remmers EF, *et al*. Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. *Am J Hum Genet* 2005;77:1044–60.
35 Regueiro C, Casares-Marfil D, Lundberg K, *et al*. HLA-B*08 identified as the most prominently associated major histocompatibility complex locus for anti-carbamylated protein antibody-positive/anti-cyclic citrullinated peptide-negative rheumatoid arthritis. *Arthritis Rheumatol* 2021;73:963–9.
36 Deane KD, Demoruelle MK, Kelmenson LB, *et al*. Genetic and environmental risk factors for rheumatoid arthritis. *Best Pract Res Clin Rheumatol* 2017;31:3–18.
37 Ebel AV, Lutt G, Poole JA, *et al*. Association of agricultural, occupational, and military inhalants with autoantibodies and disease features in US veterans with rheumatoid arthritis. *Arthritis Rheumatol* 2021;73:392–400.
38 Stolt P, Källberg H, Lundberg I, *et al*. Silica exposure is associated with increased risk of developing rheumatoid arthritis: results from the Swedish EIRA study. *Ann Rheum Dis* 2005;64:582–6.
39 Kallberg H, Padyukov L, Plenge RM, *et al*. Gene-gene and gene-environment interactions involving HLA-DRB1, PTPN22, and smoking in two subsets of rheumatoid arthritis. *Am J Hum Genet* 2007;80:867–75.
40 Klareskog L, Stolt P, Lundberg K, *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;54:38–46.
41 Makrygiannakis D, Hermansson M, Ulfgren A-K, *et al*. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. *Ann Rheum Dis* 2008;67:1488–92.
42 Joshua V, Loberg Haarhaus M, Hensvold A, *et al*. Rheumatoid arthritis-specific autoimmunity in the lung before and at the onset of disease. *Arthritis Rheumatol* 2023;75:1910–22.
43 Demoruelle MK, Bowers E, Lahey LJ, *et al*. Antibody responses to citrullinated and noncitrullinated antigens in the sputum of subjects with rheumatoid arthritis and subjects at risk for development of rheumatoid arthritis. *Arthritis Rheumatol* 2018;70:516–27.
44 Castellanos-Moreira R, Rodríguez-García SC, Gomara MJ, *et al*. Anti-carbamylated proteins antibody repertoire in rheumatoid arthritis: evidence of a new autoantibody linked to interstitial lung disease. *Ann Rheum Dis* 2020;79:587–94.
45 Holers VM, Demoruelle MK, Kuhn KA, *et al*. Rheumatoid arthritis and the mucosal origins hypothesis: protection turns to destruction. *Nat Rev Rheumatol* 2018;14:542–57.
46 Bingham CO, Moni M. Periodontal disease and rheumatoid arthritis: the evidence accumulates for complex pathobiologic interactions. *Curr Opin Rheumatol* 2013;25:345–53.

47 Brewer RC, Lanz TV, Hale CR, *et al*. Oral mucosal breaks trigger anti-citrullinated bacterial and human protein antibody responses in rheumatoid arthritis. *Sci Transl Med* 2023;15:eabq8476.
48 Zhang X, Ning Z, Mayne J, *et al*. Widespread protein lysine acetylation in gut microbiome and its alterations in patients with Crohn's disease. *Nat Commun* 2020;11:4120:4120.:.
49 Mangalea MR, Paez-Espino D, Kieft K, *et al*. Individuals at risk for rheumatoid arthritis harbor differential intestinal bacteriophage communities with distinct metabolic potential. *Cell Host Microbe* 2021;29:726–39.
50 Andreu-Sánchez S, Bourgonje AR, Vogl T, *et al*. Phage display sequencing reveals that genetic, environmental, and intrinsic factors influence variation of human antibody epitope repertoire. *Immunity* 2023;56:1376–92.
51 Chriswell ME, Lefferts AR, Clay MR, *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:eabn5166:668.:.
52 Hensvold AH, Magnusson PKE, Joshua V, *et al*. Environmental and genetic factors in the development of anticitrullinated protein antibodies (ACPAs) and ACPA-positive rheumatoid arthritis: an epidemiological investigation in twins. *Ann Rheum Dis* 2015;74:375–80.
53 Wouters F, Maurits MP, van Boheemen L, *et al*. Determining in which pre-arthritis stage HLA-shared epitope alleles and smoking exert their effect on the development of rheumatoid arthritis. *Ann Rheum Dis* 2022;81:48–55.
54 Nielen MMJ, van Schaardenburg D, Reesink HW, *et al*. Specific autoantibodies precede the symptoms of rheumatoid arthritis: A study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380–6.
55 Rantapää-Dahlqvist S, de Jong BAW, Berglin E, *et al*. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;48:2741–9.
56 van de Stadt LA, de Koning MHMT, van de Stadt RJ, *et al*. Development of the anti-citrullinated protein antibody repertoire prior to the onset of rheumatoid arthritis. *Arthritis Rheum* 2011;63:3226–33.
57 Brink M, Hansson M, Mathsson-Alm L, *et al*. Rheumatoid factor isotypes in relation to antibodies against citrullinated peptides and carbamylated proteins before the onset of rheumatoid arthritis. *Arthritis Res Ther* 2016;18:43.
58 Kelmenson LB, Wagner BD, McNair BK, *et al*. Timing of elevations of autoantibody isotypes prior to diagnosis of rheumatoid arthritis. *Arthritis Rheumatol* 2020;72:251–61.
59 Kissel T, Hafkenscheid L, Wesemael TJ, *et al*. IgG anti-citrullinated protein antibody variable domain glycosylation increases before the onset of rheumatoid arthritis and stabilizes thereafter: a cross-sectional study encompassing ~1,500 samples. *Arthritis Rheumatol* 2022;74:1147–58.
60 de Moel EC, Derksen V, Trouw LA, *et al*. In rheumatoid arthritis, changes in autoantibody levels reflect intensity of immunosuppression, not subsequent treatment response. *Arthritis Res Ther* 2019;21:28.
61 Tanner S, Dufault B, Smolik I, *et al*. A prospective study of the development of inflammatory arthritis in the family members of indigenous North American people with rheumatoid arthritis. *Arthritis Rheumatol* 2019;71:1494–503.
62 Koers J, Sciarrillo R, Derksen NIL, *et al*. Differences in IgG autoantibody Fab glycosylation across autoimmune diseases. *J Allergy Clin Immunol* 2023;151:1646–54.
63 Rombouts Y, Willemze A, van Beers JJBC, *et al*. Extensive glycosylation of ACPA-IgG variable domains modulates binding to citrullinated antigens in rheumatoid arthritis. *Ann Rheum Dis* 2016;75:578–85.
64 Hafkenscheid L, Bondt A, Scherer HU, *et al*. Structural analysis of variable domain glycosylation of anti-citrullinated protein antibodies in rheumatoid arthritis reveals the presence of highly sialylated glycans. *Mol Cell Proteomics* 2017;16:278–87.
65 Hafkenscheid L, de Moel E, Smolik I, *et al*. N-linked glycans in the variable domain of igg anti-citrullinated protein antibodies predict the development of rheumatoid arthritis. *Arthritis Rheumatol* 2019;71:1626–33.
66 Kissel T, van Schie KA, Hafkenscheid L, *et al*. On the presence of HLA-SE alleles and ACPA-IgG variable domain glycosylation in the phase preceding the development of rheumatoid arthritis. *Ann Rheum Dis* 2019;78:1616–20.
67 Kissel T, van Wesemael TJ, Lundquist A, *et al*. Genetic predisposition (HLA-SE) is associated with ACPA-IgG variable domain glycosylation in the predisease phase of RA. *Ann Rheum Dis* 2022;81:141–3.
68 Vergroesen RD, Slot LM, Hafkenscheid L, *et al*. B-cell receptor sequencing of anti-citrullinated protein antibody (ACPA) IgG-expressing B cells indicates a selective advantage for the introduction of *N*-glycosylation sites during somatic hypermutation. *Ann Rheum Dis* 2018;77:956–8.
69 Terao C, Ohmura K, Ikari K, *et al*. Effects of smoking and shared epitope on the production of anti-citrullinated peptide antibody in a Japanese adult population. *Arthritis Care Res (Hoboken)* 2014;66:1818–27.
70 Kissel T, Ge C, Hafkenscheid L, *et al*. Surface Ig variable domain glycosylation affects autoantigen binding and acts as threshold for human autoreactive B cell activation. *Sci Adv* 2022;8:eabm1759.
71 Ruff WE, Dehner C, Kim WJ, *et al*. Pathogenic autoreactive T and B cells cross-react with mimotopes expressed by a common human gut commensal to trigger autoimmunity. *Cell Host Microbe* 2019;26:100–13.
72 Manfredo Vieira S, Hiltensperger M, Kumar V, *et al*. Translocation of a gut pathobiont drives autoimmunity in mice and humans. *Science* 2018;359:1156–61.
73 Feitsma AL, van der Voort EIH, Franken KLMC, *et al*. Identification of citrullinated vimentin peptides as T cell epitopes in HLA-DR4-positive patients with rheumatoid arthritis. *Arthritis Rheum* 2010;62:117–25.
74 Snir O, Rieck M, Gebe JA, *et al*. Identification and functional characterization of T cells reactive to citrullinated vimentin in HLA-DRB1*0401-positive humanized mice and rheumatoid arthritis patients. *Arthritis Rheum* 2011;63:2873–83.
75 Gerstner C, Turcinov S, Hensvold AH, *et al*. Multi-HLA class II tetramer analyses of citrulline-reactive T cells and early treatment response in rheumatoid arthritis. *BMC Immunol* 2020;21:27.
76 Kampstra ASB, van Heemst J, Moustakas AK, *et al*. The increased ability to present citrullinated peptides is not unique to HLA-SE molecules: arginine-to-citrulline conversion also enhances peptide affinity for HLA-DQ molecules. *Arthritis Res Ther* 2016;18:254:254.:.
77 Symonds P, Marcu A, Cook KW, *et al*. Citrullinated Epitopes Identified on Tumour MHC Class II by Peptide Elution Stimulate Both Regulatory and Th1 Responses and Require Careful Selection for Optimal Anti-Tumour Responses. *Front Immunol* 2021;12.
78 Martín Monreal MT, Hansen BE, Iversen PF, *et al*. Citrullination of myelin basic protein induces a Th17-cell response in healthy individuals and enhances the presentation of MBP85-99 in patients with multiple sclerosis. *Journal of Autoimmunity* 2023;139:103092.
79 Brentville VA, Metheringham RL, Daniels I, *et al*. Combination vaccine based on citrullinated vimentin and enolase peptides induces potent CD4-mediated anti-tumor responses. *J Immunother Cancer* 2020;8:e000560.
80 Song J, Schwenzer A, Wong A, *et al*. Shared recognition of citrullinated tenascin-C peptides by T and B cells in rheumatoid arthritis. *JCI Insight* 2021;6:e145217.
81 Pircher H, Pinschewer DD, Boehm T. MHC I tetramer staining tends to overestimate the number of functionally relevant self-reactive CD8 T cells in the preimmune repertoire. *Eur J Immunol* 2023;53:e2350402.
82 Iversen R, Sollid LM. Autoimmunity provoked by foreign antigens. *Science* 2020;368:132–3.
83 Ten Brinck RM, van Steenbergen HW, van Delft MAM, *et al*. The risk of individual autoantibodies, autoantibody combinations and levels for arthritis development in clinically suspect arthralgia. *Rheumatology (Oxford)* 2017;56:2145–53.
84 van der Helm-van Mil AHM, le Cessie S, van Dongen H, *et al*. A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: how to guide individual treatment decisions. *Arthritis Rheum* 2007;56:433–40.
85 van der Helm-van Mil AHM, Verpoort KN, Breedveld FC, *et al*. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res Ther* 2005;7:R949–58.
86 van der Woude D, Young A, Jayakumar K, *et al*. Prevalence of and predictive factors for sustained disease-modifying antirheumatic drug-free remission in rheumatoid arthritis: results from two large early arthritis cohorts. *Arthritis Rheum* 2009;60:2262–71.
87 Huang S, Kronzer VL, Dellaripa PF, *et al*. Rheumatoid arthritis-associated interstitial lung disease: Current update on prevalence, risk factors, and pharmacologic treatment. *Curr Treatm Opt Rheumatol* 2020;6:337–53.
88 Derksen V, Ajeganova S, Trouw LA, *et al*. Rheumatoid arthritis phenotype at presentation differs depending on the number of autoantibodies present. *Ann Rheum Dis* 2017;76:716–20.
89 Dekkers JS, Bergstra SA, Chopra A, *et al*. Autoantibody status is not associated with early treatment response to first-line methotrexate in patients with early rheumatoid arthritis. *Rheumatology (Oxford)* 2019;58:149–53.
90 de Moel EC, Derksen V, Stoeken G, *et al*. Baseline autoantibody profile in rheumatoid arthritis is associated with early treatment response but not long-term outcomes. *Arthritis Res Ther* 2018;20:33.
91 Krijbolder DI, Verstappen M, van Dijk BT, *et al*. Intervention with methotrexate in patients with arthralgia at risk of rheumatoid arthritis to reduce the development of persistent arthritis and its disease burden (TREAT EARLIER): a randomised, double-blind, placebo-controlled, proof-of-concept trial. *Lancet* 2022;400:283–94.
92 Cope A, Jasenecova M, Vasconcelos J, *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;82:1–86.
93 Jeljeli MM, Adamopoulos IE. Innate immune memory in inflammatory arthritis. *Nat Rev Rheumatol* 2023;19:627–39.
94 Friščić J, Böttcher M, Reinwald C, *et al*. The complement system drives local inflammatory tissue priming by metabolic reprogramming of synovial fibroblasts. *Immunity* 2021;54:1002–21.
95 van Wesemael TJ, Verstappen M, Knevel R, *et al*. Anti-citrullinated protein antibodies dominate the association of long-term outcomes and anti-modified protein antibodies in rheumatoid arthritis. *Lancet Rheumatol* 2022;4:e316–7.
96 Barra L, Bykerk V, Pope JE, *et al*. Anticitrullinated protein antibodies and rheumatoid factor fluctuate in early inflammatory arthritis and do not predict clinical outcomes. *J Rheumatol* 2013;40:1259–67.

97 de Moel EC, Derksen V, Trouw LA, *et al*. In RA, becoming seronegative over the first year of treatment does not translate to better chances of drug-free remission. *Ann Rheum Dis* 2018;77:1836–8.
98 HARRIS J, VAUGHAN JH. Transfusion studies in rheumatoid arthritis. *Arthritis Rheum* 1961;4:47–55.
99 Dwosh IL, Giles AR, Ford PM, *et al*. Plasmapheresis therapy in rheumatoid arthritis. *N Engl J Med* 1983;308:1124–9.
100 Clavel C, Nogueira L, Laurent L, *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;58:678–88.
101 Trouw LA, Haisma EM, Levarht EWN, *et al*. Anti-cyclic citrullinated peptide antibodies from rheumatoid arthritis patients activate complement via both the classical and alternative pathways. *Arthritis Rheum* 2009;60:1923–31.
102 Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, *et al*. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Sci Transl Med* 2013;5:178ra40:178.:.
103 Catrina A, Krishnamurthy A, Rethi B. Current view on the pathogenic role of anti-citrullinated protein antibodies in rheumatoid arthritis. *RMD Open* 2021;7:e001228.
104 Qiu YY, Neogi T, Muhammad LN, *et al*. Anti-cyclic Citrullinated peptide antibody and pain Sensitisation in patients with rheumatoid arthritis: a cross-sectional analysis. *Ann Rheum Dis* 2023;82:1638–40.
105 He Y, Ge C, Moreno-Giró À, *et al*. A subset of antibodies targeting citrullinated proteins confers protection from rheumatoid arthritis. *Nat Commun* 2023;14:691.
106 Raposo B, Afonso M, Israelsson L, *et al*. Divergent and dominant anti-inflammatory effects of patient-derived anticitrullinated protein antibodies (ACPA) in arthritis development. *Ann Rheum Dis* 2023;82:724–6.
107 Chirivi RGS, van Rosmalen JWG, van der Linden M, *et al*. Therapeutic ACPA inhibits NET formation: a potential therapy for neutrophil-mediated inflammatory diseases. *Cell Mol Immunol* 2021;18:1528–44.
108 Gomez AM, Brewer RC, Moon J-S, *et al*. Anti-citrullinated protein antibodies with multiple specificities ameliorate collagen antibody-induced arthritis in a time-dependent manner. *Arthritis Rheumatol* 2024;76:181–91.