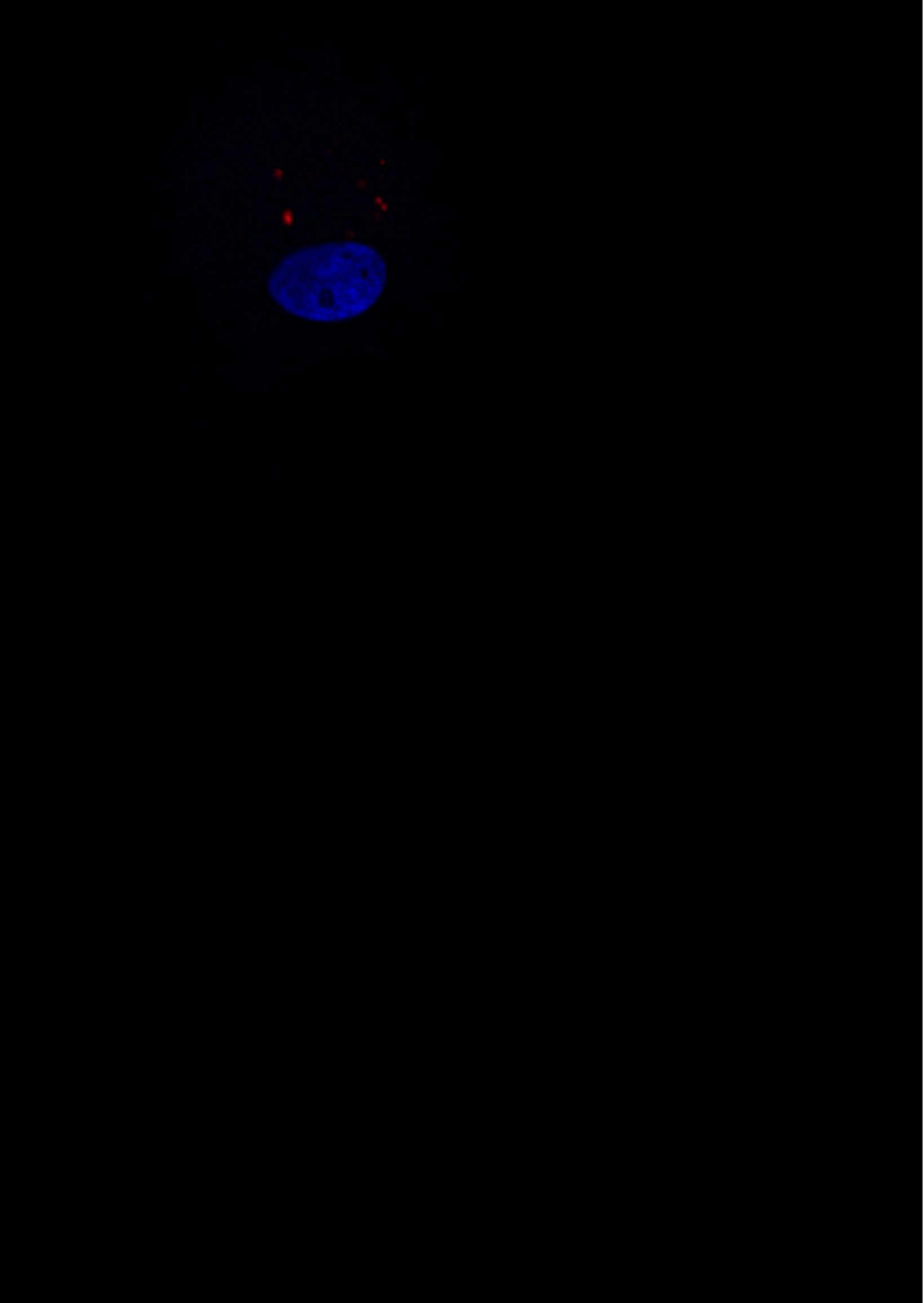
Immunity against post-translationally modified proteins in autoimmune diseases

Beukel, M.D. van den



1

General introduction

Autoimmune diseases

Our immune system defends the body against disease and infection. However, in cases where the immune system falters, it may mistakenly direct an attack on healthy cells, tissues, or organs. Such malfunction can lead to serious disease, resulting in chronic inflammation and autoimmunity that affect various parts of the body. Conditions such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and autoimmune hepatitis (AIH) exemplify the diversity and complexity of autoimmune disorders, each with unique symptoms and challenges.

Rheumatoid arthritis

RA is a chronic autoimmune disorder that primarily affects the joints (Figure 1). Worldwide, in many populations, the prevalence of RA is between 0.5 and 1% and occurs more often in females than in males (1). RA is characterized by an inappropriate immune response that leads to inflammation in the synovium, the lining of the membranes surrounding the joints. This inflammation can cause significant pain, swelling, and stiffness, particularly in the morning or after periods of inactivity. This often leads to a decreased range of motion and functional impairment (2). Currently, RA is classified based on the 2010 rheumatoid arthritis classification criteria (3). A hallmark feature of RA is the presence of specific autoantibodies, including rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) (4). Additionally, an antibody that targets another post-translational modification (PTM), carbamylation, is associated with the development of joint damage (5). Despite the high diagnostic value of ACPA, a group of RA patients is seronegative for RF and ACPA (6). Therefore, novel biomarkers are needed to improve diagnosis of RA. The etiology of rheumatoid arthritis is multifactorial, involving a complex interplay of genetic predisposition, environmental triggers and immune dysregulation. The genetic predisposition to this immune driven disease is exemplified best with the strongest genetic association with genes associated with antigen presentation, e.g. human leukocyte antigen (HLA-)DRB1*01 and HLA-DRB1*04 alleles (7). The environmental factors, impacting on the development of this disease is best exemplified by smoking, an environmental factor that, although not only associated with RA, will chronically stimulate the immune system, triggering the disease in genetically susceptible individuals (8). Despite current sophisticated treatments, the majority of RA patients do not reach complete drug free remission, or cure, for their disease. (9). Drugs used in these patients are called disease-modifying antirheumatic drugs (DMARDs) and include methotrexate or sulfasalazine and leflunomide. A substantial proportion of the patients, however, does not responds sufficiently. If treatment is not effective, methotrexate can be combined with other DMARDs such as TNF inhibitors or the CD20 rituximab, based on Fc-fusion proteins or antibody formats. Additionally, corticosteroids can be provided for short-term management during flare-ups or until DMARDs take effect (9). These

drugs have working mechanisms that are not specific for RA, but aim to achieve systemic immunosuppression.

Systemic lupus erythematosus

SLE is a multifaceted autoimmune disease characterized by the production of autoantibodies and widespread inflammation that can affect multiple organ systems, including the skin, joints, kidneys, heart, and nervous system (Figure 1) (10). The estimated prevalence of SLE ranges from 20 to 70 cases per 10,000 individuals (11). The disease predominantly affects woman, with an average female-to-male ratio of 9:1, which varies with age (12). Currently, the 2019 EULAR/ACR classification criteria for SLE is used for diagnosing SLE (13). One of the hallmark features of SLE is the presence of a diverse range of autoantibodies, including anti-nuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), and anti-Smith (anti-Sm) antibodies (14). These autoantibodies can serve as important diagnostic markers and are often used to monitor disease activity (15). The pathophysiology of SLE involves immune complex formation and deposition in various tissues, leading to inflammation and damage, which can result in serious complications such as nephritis, cardiovascular disease, and lead to increased morbidity and mortality (16, 17). Genetic predisposition, environmental factors, and hormonal influences contribute to the development of SLE, with certain genetic markers, such as those in the complement system and HLA, being associated with increased susceptibility (18). Given the variable manifestations leading to a great spectrum of disease presentations and severity in SLE, also a variety of therapeutic options are available, all suppressing the immune system with the goal to achieve sustained remission (19). Treatment options to achieve this include amongst others corticosteroids, hydroxychloroquine and immunosuppressants/biological agents (12). Recently spectacular results were obtained using anti-CD19 CAR-T cells in small number of patients, reaching B cell depletion and drug-free remission of systemic disease for 12 months even after reconstitution of B cells (20). However, this treatment is not yet available for all patients and substantially more data is needed to understand the full potential of this treatment in autoimmunity. Additionally, feasibility to use CAR-T cell treatment in large patient cohorts needs to be assessed.

Autoimmune liver disease and autoimmune hepatitis

Autoimmune liver disease (AILD) encompasses a group of chronic liver disorders. This category includes AIH, primary biliary cholangitis and primary sclerosing cholangitis. The prevalence of AILD varies widely with AIH being the most frequently diagnosed form. AIH is a chronic autoimmune disease that primarily affects the liver (Figure 1). The prevalence of AIH ranges from 0.1% to 2% in populations in different regions worldwide and is more commonly diagnosed in females compared to males (21). AIH is characterized by an inappropriate immune response that leads to inflammation of the liver, resulting in hepatocellular injury and potentially progressing to cirrhosis, hepatocellular carcinoma,

or death (22). The classification of autoimmune hepatitis is based on clinical, serological, and histological criteria, with the most common types being type 1 and type 2 AIH (23). The etiology of AIH is multifactorial, involving a complex interplay of genetic predisposition, environmental factors, and immune dysregulation. Specific autoantibodies, such as ANA and smooth muscle antibodies (SMA), are often present in affected individuals and can aid in diagnosis (21). Current treatment options for AIH focus on immunosuppression to reduce liver inflammation and prevent disease progression. Corticosteroids, such as prednisone, are commonly used as first-line therapy, often in combination with azathioprine, a disease-modifying immunosuppressive agent (22). While many patients respond well to treatment, some may require additional therapies or adjustments to their treatment regimen to achieve optimal control of the disease (22). Overall, while AIH cannot be cured, effective management strategies can lead to remission and improve long-term outcomes for patients. Regular monitoring and follow-up care are essential to assess liver function and adjust treatment as necessary (23).

Noteworthy is that, in many autoimmune diseases, including RA, SLE and AILD, inflammation occurs in specific anatomical locations, such as the joints, the kidney or the liver, with systemic inflammation contributing to (co)morbidities (2, 24, 25). While several immunopathological processes, may not be disease- or organ-specific, they may contribute to the disease severity in the affected organ and may also contribute to the (chronic) inflammation that is damaging other organ systems. One example is the occurrence of cardiovascular disease in patients with autoimmune diseases like RA and SLE (26).

In several autoimmune diseases autoantibodies are present in the circulation and in the target organs. The success of B-cell targeted therapies in many autoantibody-positive diseases, indicate that B cell-mediated autoimmunity is playing a direct (pathogenic) role (27). Autoantibodies that target PTMs are being included as diagnostic and prognostic biomarkers in RA (5, 28). What triggers the induction of (chronic) inflammation and formation of autoantibody responses is unknown, but if unraveled this will provide the opportunity to design (site-)specific interventions as opposed to the non-specific immune suppression or non-antigen specific depletion of B cells that is currently used.

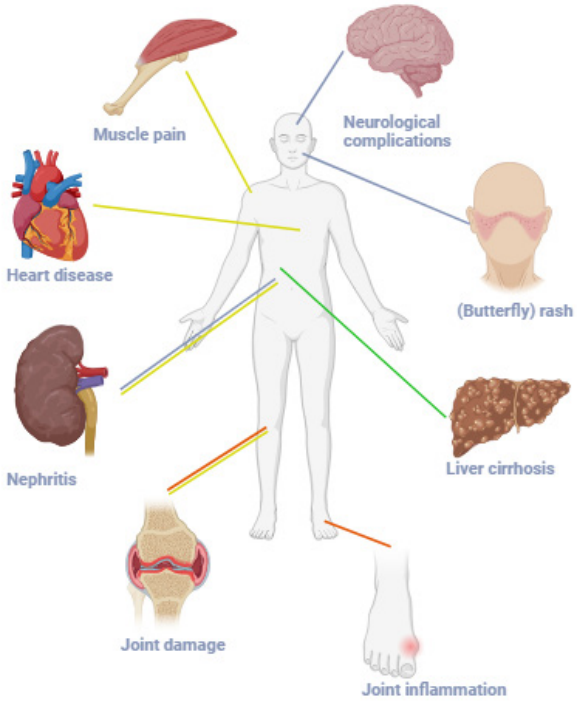


Figure 1: affected sites in RA, AILD and SLE. In some autoimmune diseases specific sites are primarily affected e.g. joints in RA and liver in AILD whereas other diseases display multiple organ involvement such as SLE. However, autoimmune diseases often express similar symptoms, mainly as a consequence of (chronic) inflammation such as coronary artery disease. RA is for instance characterized by inflammation of the joint leading to joint damage (red). Butterfly rash, lupus nephritis and neurological complications are typical manifestations in SLE (blue), as result of systemic inflammation and deposition of immune complexes. In different subtypes of AILD immune-mediated liver injury is playing a central role (green). Additionally, patients with AILD may present with extrahepatic disorders such as arthritis and nephritis (yellow). Higher risk of coronary artery disease is associated with severe systemic inflammation and is observed in RA and SLE patients (yellow). *Image was created using Biorender.com.*

Post-translationally modified proteins

Over the years it has become apparent that the presence of PTMs and/or anti-PTM antibodies is associated with a variety of inflammatory and autoimmune diseases. The role of PTMs and anti-PTMs is however unclear. In this thesis we anticipated that disease associated PTMs, and especially the more readily detectable anti-PTM antibodies, may provide important insight into the disease processes of autoimmune diseases and chronic inflammation. Therefore, we set out to study PTM and anti-PTM reactivities in autoimmunity more closely.

PTM of proteins refers to the changes that occur after the protein has been produced and have a central, mostly physiological, function (29). PTMs occur both inside and outside of the cell and modify many different amino acids. Additionally, both reversible and irreversible PTMs are observed in the body and are commonly employed to regulate protein function. A well-known example is phosphorylation, which is typically a reversible process. Other PTMs are largely irreversible and therefore may persist in the body. It is likely that long-lived proteins, such as laminin and elastin, with half-lives up to 70 years, are more prone to accumulation of PTMs as they are exposed for a longer time period especially during sustained inflammation as compared to for example plasma proteins with a much shorter half-life (29).

Notably, some PTMs are associated with disease (30). Some individuals develop an antibody response against PTM-modified proteins. Interestingly, not every individual that harbors disease-associated PTMs will develop anti-PTM antibodies and only a subset of individuals with PTM and anti-PTM antibodies will develop disease conceivably depending on several factors such as genetic and environmental factors. Noteworthy is that, some individuals develop an anti-PTM antibody response against certain specific PTMs, or combinations of anti-PTM antibody responses, while others develop an anti-PTM antibody response against other PTMs, or develop no anti-PTM antibody response at all (31, 32). In several major human diseases the presence of PTMs or anti-PTM antibodies is associated with disease progression (30-32). Interestingly, in some diseases more emphasis was put on the presence of the PTM proteins e.g. AGEs in diabetes, while in others more emphasis was put on the anti-PTM antibodies, e.g. ACPA in RA. We therefore set out to

study six PTMs; nitration (Nt), citrullination (Cit), carbamylation (Ca), acetylation (Ac), malondialdehyde-acetaldehyde adducts (MAA) and advanced glycation end-products (AGE). We focus on these PTMs as these are observed in a variety of disease including cardiovascular disease, autoimmune disease such as RA, and diabetes type 2. Additionally, some of these PTMs are structurally very different, whereas others are very similar (Figure 2). Notably, the chosen PTMs occur on specific amino acids such as lysine, arginine or tyrosine.

Nitration (Nt)

Protein nitration is an irreversible modification of tyrosine (Figure 2) and may alter the structure and/or diminish the function of proteins (33). Mechanisms of protein nitration include reactions with peroxynitrous acid (ONOOH) or nitrosoperoxycarbonate ($ONOOCO_2$-) (33). peroxynitrous acid (ONOOH) is the protonated form of peroxynitrite (ONOO-) and can directly nitrate free or protein bound tyrosine. Peroxynitrite can also react with CO_2 to form a reactive CO_2 adduct of peroxynitrite, nitrosoperoxycarbonate

($ONOOCO_2$-). Nitrosoperoxycarbonate in turn decomposes to carbonate radical and nitrogen dioxide which can react with tyrosine to form 3-nitrotyrosine.

ONOO- is a result of a reaction of nitric oxide (NO) and superoxide (O_2-). Under physiological conditions, NO is an important small molecule involved in various biochemical processes such as neurotransmission and modulation of blood flow (34-36). Production of excess NO, however, can have detrimental effects and lead to the formation of reactive nitrogen species (RNS) such as ONOO-. During inflammatory processes, cells produce high levels of NO but also superoxide (O_2-) which is a reactive oxygen species (ROS) (37). In case NO and O_2- react, peroxynitrite (ONOO-) formation is inevitably leading to 3-nitrotyrosine formation in protein. Enhanced peroxynitrite formation is associated with a variety of conditions as a consequence of oxidative stress (38). On top of that, 3-nitrotyrosine is strongly associated with cardiovascular disease and diabetes (39, 40). More specifically, immunohistochemistry of atherosclerotic plaques showed 3-nitrotyrosine staining in various studies (41). In some studies, evidence for co-localization with specific extracellular matrix species has been found, including long-lived proteins such as laminin (42) and elastin (43). In cardiovascular disease accumulation of PTMs is suggested to alter for instance viscoelasticity, increasing the risk of coronary artery disease (44).

Citrullination (Cit)

Citrullination is an enzymatic conversion of arginine residues into citrulline residues (Figure 2). This process is mediated by the peptidyl arginine deiminase enzyme (PAD) family, of which PAD1-4 have citrullinating activity (45). PAD activity is regulated by calcium. PADs are activated when calcium homeostasis is lost. PAD activity and the resulting citrullination has been implicated in several physiological processes including apoptosis and terminal differentiation of the epidermis. For example, citrullination may alter the structure of cytoskeletal proteins such as filaggrin and trichohyalin (46, 47).

Under physiological conditions, nuclear substrates such as histones are citrullinated, thereby affecting nucleosome stability. It has been suggested that this process causes the nucleosome to open up, and render DNA more accessible to nucleases (48, 49). Interestingly, granulocytes and more specifically neutrophils, use this histone citrullination, among other things, to form neutrophil extracellular traps (NETs). By histone citrullination, PAD4 will decondense chromatin essential for NET formation. These fibrous chromatin-based NETs therefore contain (citrullinated) histones, DNA and granular proteins (50). During NETosis, NETs are released into the extracellular space to capture and eliminate pathogens or emerge infections. In case of sustained inflammation, PAD enzyme is continuously released in the inflamed site which may lead to localized protein citrullination (49). For example, such sustained inflammation and citrullination of proteins is seen in the joints of rheumatoid arthritis patients. In addition,

it has been shown that expression of PAD2 and 4 is increased in rheumatoid synovial tissue and fluid (51). Importantly, in RA it is well known that autoantibodies are present that bind such citrullinated proteins, called ACPAs.

Carbamylation (Ca)

During carbamylation, the amino acid lysine is converted into the amino acid homocitrulline (Figure 2). The adduct NH_2CO- that is formed is a carbamoyl group, a process nowadays referred to as carbamylation (52). Noteworthy is that citrulline and homocitrulline only differ by one methylene group and are structurally very similar (53) yet occur on a different amino acid residue: citrulline on arginine and homocitrulline on lysine. Protein carbamylation is driven by a chemical reaction with cyanate. Urea is in equilibrium (ratio 1 to 500,000) with ammonium cyanate and thus is a source of cyanate in the body (54). High levels of urea as a result of uremia are observed in case of renal insufficiency (55). Increased levels of cyanate can also be due to the conversion of thiocyanate into cyanate by myeloperoxidase (56). Thiocyanate is therefore another source of cyanate. Both thiocyanate and cyanate itself are observed in smokers (53, 57). Carbamylation dose dependently changes the structure and function of a broad range of proteins and small molecules (32). This may affect cellular functions and trigger systemic disorders. Carbamylation of proteins is shown to be related to chronic kidney disease and (sustained) inflammation, but has also been described in other conditions such as cardiovascular disease (32). Additionally, carbamylated proteins have been detected in synovial tissue and synovial fluid of RA patients (58). In RA patients, next to ACPA also antibodies that bind to carbamylated proteins (anti-CarP) are detected (5). The presence of anti-CarP is associated with joint damage in these patients, especially in the ACPA negative subgroup (5).

Acetylation (Ac)

Acetylation is a reversible enzymatic process during which acetyl groups are added to free amines of lysine residues in the presence of acetyl-CoA (59). Notably, two mechanisms for acetylation of amino groups of proteins have been described: lysine acetylation (Nε-acetylation) and N-terminal protein acetylation Nα-acetylation). Acetylation of histones (Nε-acetylation) is extensively studied in the context of transcription regulation (60-62). Next to histone acetylation also non-histone protein acetylation is described and reviewed by Narita *et al.* (63). Non-histone acetylation is involved in key cellular processes such as gene transcription, signal transduction and protein folding. During lysine acetylation the size of the side chain is increased and the charge neutralized (+1 to 0). Consequently, this may alter protein stability, protein-protein interaction and protein-DNA interactions. Acetylated lysine resembles homocitrulline except for one side-chain terminal amine, which is replaced by a methyl group (Figure 2). Therefore Juarez *et al.* studied whether antibodies recognizing these acetylated proteins are present in sera of RA patients as they harbor antibodies against the structurally very similar citrulline and

homocitrulline (64). Indeed, they noticed that in approximately 40% of the RA patients harbored antibodies against acetylated vimentin. Interestingly, Juarez *et al.* observed that the presence of anti-acetylated vimentin was largely confined to the ACPA-positive subgroup of RA patients. Next to RA, altered protein acetylation is reported in several neurodegenerative diseases, cardiovascular disease and cancer (65, 66).

Malondialdehyde acetaldehyde-adducts (MAA)

Malondialdehyde acetaldehyde-adducts are a highly stable product of oxidative stress and form a ring structure (Figure 2). During inflammation, oxidative stress is induced and reactive oxygen species (ROS) may be formed. Upon exposure of cells to ROS, lipid peroxidation occurs followed by rupture of the cell membrane and membrane lipid oxidation, forming malondialdehyde (MDA) (67). MDA can spontaneously break down and form acetaldehyde (AA) (67). Both MDA and AA are highly reactive aldehydes and together they have shown to modify proteins to produce an MDA-AA protein adduct, termed the malondialdehyde-acetaldehyde adduct (MAA) (68). MAA have been proven to be immunogenic and have pro-inflammatory capacities (69). MDA, AA and MAA, as well as antibodies against these adducts, have been observed in several diseases such as atherosclerosis, alcohol induced liver injury and RA (48, 70-73).

Advanced glycation end-product (AGE)

Advanced glycation end-products are a result of reactive sugar groups that bind to amino acid residues with a free amino group. During this process a reaction occurs between the free amino groups and carbonyl group of reducing sugars such as reducing aldose and reducing ketose. These processes include the Maillard or Polyol pathway (74). Condensation of carbonyl groups of reducing sugars, form reversible reactions yielding the so-called Schiff base. Next, these undergo rearrangement resulting in so-called stable Amadori or Heyns products. These products are considered ''early glycation products''. To become AGEs, the end-compounds (e.g. Schiff bases), must undergo further rearrangements and involve the formation of reactive carbonyls. Reactive carbonyls can undergo further condensation with available amine groups from e.g. lysines. In the end this reaction yield a great variety of final AGEs. Excessive glycation is a response to oxidative stress and inflammation leading to AGEs. AGEs in turn bind to AGE receptors on e.g. macrophages and endothelial cells leading to the perpetuation of inflammation (75). Additionally, dietary intake of AGEs is associated with higher levels of AGEs in plasma (76). AGEs are typically found in patients with diabetes and is associated with cardiovascular disease in these patients (77, 78). Carboxymethyllysine (CML) is a frequently measured AGE and a marker for oxidative stress. CML increases slowly with age and is significantly increased in coronary artery disease and age-related macular degeneration, but is also observed in synovial tissue of RA patients (79-81).

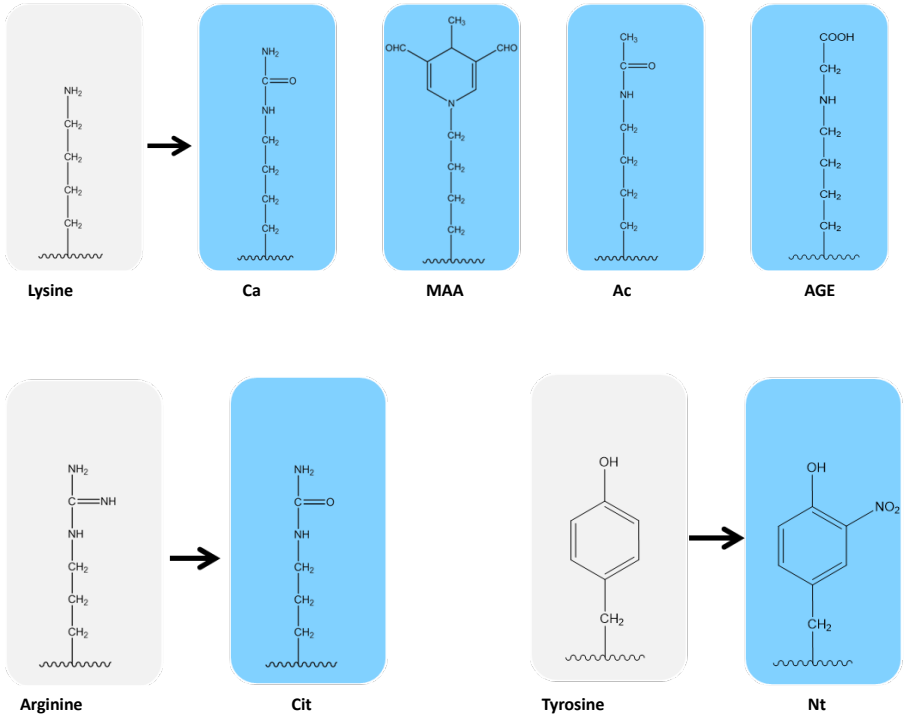


Figure 2: structures of PTMs nitration, citrullination, carbamylation, acetylation, malondialdehyde-acetaldehyde adducts and advanced glycation end-products on different amino acids. *Abbreviations: Ac, Acetylation; AGE, advanced glycation end-products (exemplified by carboxymethyl lysine); Ca, carbamylation; Cit, citrullination; MAA, malondialdehyde-acetaldehyde adducts; Nt, nitration.*

Complement

Since many PTMs are associated with disease but simultaneously are also found in healthy individuals we hypothesized that complement could be a mechanism that could be involved in clearance in health but in breach in tolerance in autoimmunity. If complement is activated directly on PTM-modified proteins, these PTM-modified proteins become opsonized. Complement opsonized PTM-modified proteins, can in turn be recognized by cells expressing complement receptors eliciting further response e.g. clearance by phagocytic cells or triggering autoreactive B-cells. In the following paragraphs basics on the complement system, complement receptors and how this would lead to a breach in tolerance will be introduced.

Research into the origin of the complement system revealed that a primitive form of the complement system dates back more than 500 million years ago (82). The ''modern''

complement system was established upon appearance of the jawed vertebrates. The complement system is activated within seconds after a pathogen has entered (83). Complement is a major component of the innate immune system but is more recently also recognized to play a role in modulating adaptive immunity (84). The complement system consists of over 30 proteins which are involved in opsonization, chemotaxis, activation of leukocytes and cytolysis of target cells through the C5b9-membrane attack complex (85, 86). Additionally, complement is well-known for its role in clearance of apoptotic cells and immune complexes.

Complement can be activated through three pathways: classical (CP), lectin (LP) and alternative pathway (AP) (Figure 3). Each complement pathway has its own initiators and/or activation mechanisms but all form C3 convertases resulting in the formation of the membrane attack complex (MAC, C5b9) at the end.

The CP is initiated by C1q (87). C1q is composed six globular target recognition domains (gC1q) attached to a collagen-like region (CLR) forming a ''bouquet of tulips'' like structure. C1q is able to bind to immune complexes but also serves as a pattern recognition molecule with its ability to sense i.e. eat-me signals on apoptotic. C1q is bound to C1r and C1s in a calcium dependent manner and forms a C1 complex. C1r and C1s from the C1 complex are the first enzymes in this pathway to trigger a series of enzymatic events. After C1q has bound to the target its conformation will change and C1r will be activated. C1r cleaves C1s, forming activated C1s which in turn binds to C4 and enzymatically liberates C4a and C4b. C1s will additionally cleave C2, into C2a and C2b. Together with C2b, C4b will form the C3 convertase C4bC2b formerly referred to as C4b2a (88).

The LP can be activated through binding of collectins (mannan binding lectin (MBL), collectin-10, collectin-11) or ficolins (ficolin-1, ficolin-2, ficolin-3) to sugar groups on the surface of a pathogen in a calcium dependent manner (85). Just like C1q, collectins and ficolins have a N-terminal collagenous region serving as pattern-recognition molecule and form a complex with MBL-associated serine proteases (MASPs). Despite the similarities between the architecture of the C1 an MBL/MASP complexes, the mechanism of activation of the LP is different from the CP. In the CP each C1 complex carries both C1r and C1s, and each complex can therefore activate complement. However, the majority of the MBL molecules are associated with only one homodimer of either MASP-1 or MASP-2 (89). In order to activate the LP both MASP-1 and MASP-2 are required. MASP-1 is able to cleave C2 and is required to activate MASP-2. MASP-2 in turn can cleave both C2 and C4. During activation, MBL or ficolin, associated with either MASP-1 or MASP-2, will bind to its target bringing MASP-1 and MASP-2 into close proximity resulting in cleavage of C2 and C4 (90). After C2 and C4 are cleaved, C2b and C4b will form the C3 convertase C4bC2b.

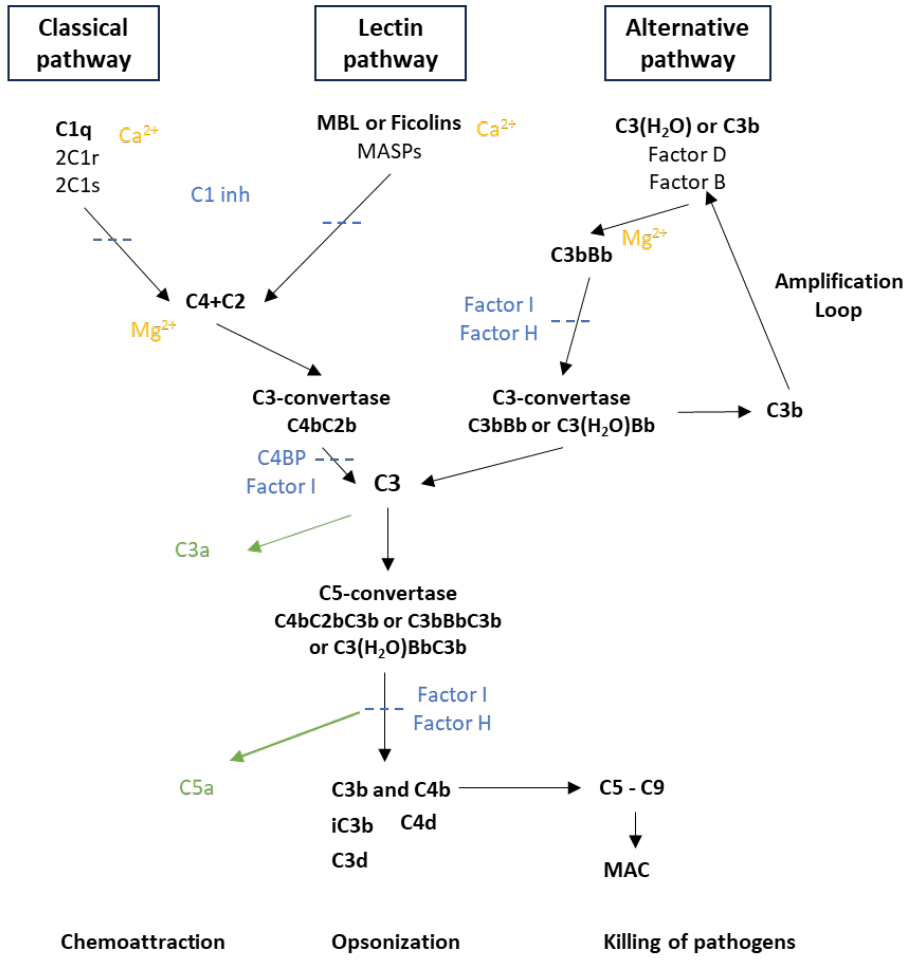


Figure 3: Schematic overview of the activation of the three complement pathways. The complement system consists of three different pathway namely the classical, lectin and alternative pathway. The classical and lectin pathway are calcium-dependent (depicted in orange) whereas the alternative pathway has a magnesium-dependent step (depicted in orange). All pathways come together at the level of C3. Subsequently, C3 is cleaved by C3 convertases formed in the different pathways into C3a (anaphylatoxin depicted in green) and C3b. Prolonged complement activation can result in the addition of C3b to C3 convertases forming C5 convertases. C5 in turn will be cleaved forming C5a (anaphylatoxin depicted in green) and C5b. C5b will subsequently trigger the formation of the membrane attack complex (MAC; C5b-9). Complement is regulated by regulators (C1 inhibitor, Factor I, Factor H, C4BP) at different levels (depicted in blue). C3b can for instance be cleaved by factor I with the help of e.g. factor H, serving as cofactor resulting in iC3b and finally C3d. C3b derivatives can in turn serve as opsonin to elicit further response.

The AP can be activated spontaneously, maintaining a constitutive activation by a process called tick-over (91). During this process a labile thioester bond is hydrolyzed and converts C3 into C3(H_2O). Upon hydrolysis, the thioester domain (TED) of C3 undergoes a structural change exposing a binding site for factor B. Factor B bound to C3(H_2O) is cleaved by factor D allowing formation of the fluid phase C3 convertase C3(H_2O)Bb. This minor form of C3 convertase is responsible for a constant low level of C3a and C3b (tick-over) (92). The formed C3b can bind covalently to the cell surface sugar carbohydrates or immune aggregates (opsonization). In case of a pathogen, factor B associates with C3b in magnesium dependent manner and is cleaved by factor D forming the major form of C3 convertase, namely C3bBb. Properdin, in the AP, serves as a positive regulator recruiting C3b to the surface but also stabilizes C3 convertase. On host cells, bound C3b is rapidly inactivated by surface or fluid phase regulatory complement components maintaining homeostasis.

In summary, for the CP and LP clear recognition molecules have been identified which trigger each pathway only at sites where activation is necessary. These recognition molecules undergo structural changes in order to elicit activation of enzymes which in turn are able to cleave subsequent molecules of the complement cascade. All pathways will form the central enzymatic complexes called C3 convertases (C4bC2b, C3bBb or C3(H_2O)Bb). The AP, lacks a traditional initiator. However, several molecules such as properdin and P-selectin have been described to recruit C3(H_2O) and C3b to the cell surface and serve as a local initiator of the AP.

All three pathways converge at the level of C3 as all pathway specific C3 convertases use C3 as a substrate. C3 convertases cleave C3 into the anaphylatoxin C3a and opsonin C3b. Prolonged complement activation results in addition of one or more molecules of C3b to C3 convertases forming C5 convertases: C4bC2bC3b, C3bBbC3b and C3(H_2O)BbC3b (93, 94). C5 convertases cleaves C5 into the anaphylatoxin C5a, which diffuses away, and C5b which triggers the assembly of the membrane attack complex (MAC; C5b-9). C5b in turn binds C6 which acquires the ability to interact with the lipid bilayer stabilizing the complex (95). Subsequently, C7 and C8 bind to C5b and form the C5b-7 and C5b-8 complexes. Lastly, one molecule of C9 binds to the membrane inserted C8, followed by multiple C9 molecules to complete the MAC complex.

Next to formation of further C3 convertases, C3b undergoes successive proteolytic cleavages leading to inactive C3 products which is mediated by factor I. Factor I requires a cofactor such as factor H, C4BP, CR1 or MCP, in order to bind and inactivate C3b, generating iC3b (inactive form of C3b) and C3f. These iC3b molecules are not able to form new C3 convertases protecting host cells from complement mediated attack. After iC3b is cleaved, factor I will together with CR1 as cofactor cleaves iC3b into C3dg,

and C3c, eventually leading to C3d formation induced by plasma proteases (96). C3b derivatives can in turn bind to complement receptors expressed on a variety of cells and elicit further response.

Complement receptors

Complement receptors (CRs) are critical components of the immune system, facilitating the interaction between immune cells and the complement system, which is a key element of innate immunity and adaptive immunity. These receptors are expressed on various immune cell types, including macrophages, neutrophils, dendritic cells, and B cells, and they play pivotal roles in mediating immune responses such as opsonization, phagocytosis, and the regulation of inflammation. Complement receptors that recognize complement opsonized target include CR1, -2, -3, -4 and CRIg.

CR1 (CD35) is primarily expressed on erythrocytes, macrophages, and dendritic cells, but is also found on B cells and some T cells (97, 98). It binds to C3b and C4b, facilitating the clearance of immune complexes and pathogens. CR1 plays a significant role in regulating complement activation by promoting the decay of C3 convertase and acting as a cofactor for factor I, which inactivates C3b and C4b (99). On B cells, CR1 may function both as a processing molecule, converting C3b to iC3b (100), and as a contrasting signal to CR2 down-modulating B cell responses to C3b-coated antigens (101).

CR2 (CD21) is predominantly found on B cells and follicular dendritic cells. It binds especially to C3d and also to iC3b and C3dg, enhancing B cell activation and signaling through the B cell receptor (97, 102). On follicular dendritic cells within lymphoid tissues the interaction of CR2 and C3 fragment-opsonized antigens is important in trapping and retaining immune complexes (97).

CR3 (CD11b/CD18) is expressed on myeloid cells, including neutrophils and macrophages. But are also found on NK cells and activated B and T cells (103). It binds to iC3b, and is essential for the phagocytosis of opsonized pathogens. Additionally, engagement of CR3 is involved in leukocyte adhesion and migration, and induction of both inflammatory and tolerogenic responses fulfilling an immune modulatory function (104).

Similar to CR3, CR4 (CD11c/CD18) is expressed on myeloid cells and binds to iC3b. It plays a role in phagocytosis and is involved in the activation of antigen-presenting cells, thereby contributing to the initiation of adaptive immune responses. CR4 has also been implicated in the regulation of inflammation (103).

The binding of complement fragments to their respective receptors has several important consequences for immune cell function. It enhances phagocytosis, whereby immune cells engulf and digest opsonized pathogens, which is crucial for effective pathogen clearance. Additionally, the interaction between complement receptors and their ligands triggers intracellular signaling pathways that promote cell activation, leading to the release of pro-inflammatory cytokines and chemokines, and regulation of inflammation. This results in the recruitment of additional immune cells to the site of infection or injury. Next to CR1, -2, -3 and -4, the complement receptor repertoire also encompasses: CRIg and C3aR, C5aR1 and C5aR2. CRIg is a complement receptor of the immunoglobulin family binding C3b and iC3b (105). In contrast to the other C3 fragment receptors (CR1 to CR4), CRIg is found on a constitutive recycling pool of membrane vesicles where it participates in the internalization of C3-opsonized particles from the bloodstream by Kupffer cells and tissue resident macrophages. Notably, functional effect of C3b binding to CRIg is confined to the C3b of the alternative pathway, as it fails to inhibit C3 and C5 convertases of the other pathways. C3aR binds to the anaphylatoxin C3a and C5aR1/2 binds C5a. All anaphylatoxin receptors are G protein-coupled receptors and regulate innate and adaptive immune responses upon ligand binding.

Taken together, cells expressing complement receptors are vital for orchestrating immune responses against pathogens and maintaining homeostasis. Disbalance in immune activation may result in (sustained) inflammation and breach in tolerance.

Breach in tolerance

The breach of immune tolerance is a fundamental mechanism in the development of autoimmune diseases, where the immune system mistakenly targets the body's own tissues. PTMs could trigger the immune system resulting in inflammation or autoimmunity through complement opsonization. Anti-PTM antibodies can form immune complexes on which the complement system will be activated preventing precipitation. In autoimmunity, continued production of antibodies leads to extended formation of immune complexes that can overwhelm the complement system. Consequently, immune complexes will be poorly cleared and may deposit in various tissues, further driving inflammation and tissue damage (106).

One of the primary mechanisms underlying the breach of immune tolerance involves genetic predisposition. Certain genetic factors, such as specific alleles of the HLA complex, have been linked to an increased risk of autoimmune diseases. For instance, the HLA-DRB1 gene is associated with RA, influencing the presentation of autoantigens (107). These genetic variations can alter the immune response, predisposing individuals to a loss of tolerance.

Environmental factors also play a significant role in breaking immune tolerance. Viral and bacterial infections, for instance, can trigger autoimmune responses through mechanisms like molecular mimicry, where pathogens share structural similarities with host tissues. This can lead to cross-reactivity, where the immune system targets both the pathogen and the host tissues (108). Additionally, environmental triggers such as smoking and certain dietary components may result in irreversible post-translational modification on self-proteins (32, 109). The immune system will target the modified proteins and depending on genetic predisposition tolerance will break, further complicating the interplay between genetics and the environment.

In conclusion, the breach of immune tolerance in autoimmunity results from a complex interplay of genetic, environmental, and immunological factors. Understanding these mechanisms is crucial for developing targeted therapies aimed at restoring immune tolerance and mitigating the effects of autoimmune diseases.

Implications for therapeutic interventions

Treatments for autoimmune diseases such as RA, SLE and AILD often include (cortico) steroids and/or biologics. The ultimate goal using these interventions is sustained (drug free) remission, as cure is not often reached for these diseases. Designing an ''one cure fits all'' therapeutic is not possible as patients with these types of autoimmune disease are highly heterogenous. Nonetheless, for many patients B-cell targeted therapy, using for instance rituximab, has proven to be effective (27). B-cell targeted therapy depletes all CD20 positive B cells using monoclonal antibodies (mAb). However, only a subset of B cells produce autoantibodies targeting ''self''. Other ''healthy'' B cells will also be targeted using this type of therapeutics. Over the years, mAb therapeutics have evolved and nowadays bispecific antibodies (bsAbs) are generated and proven to be effective (110). Such bsAbs are able to bind two different antigens as opposed to regular mAb binding only one antigen or epitope. This format would allow to bridge two cell types or to engage two molecules and therefore making treatment more (site-)specifically enhancing therapeutic efficacy (111).

A strategy would be to prevent B cells from becoming autoreactive by targeting the triggers of these B cells. In this thesis we describe that the complement system could be triggered and opsonize PTM-modified proteins. These opsonized PTM-modified proteins could in turn induce (chronic) inflammation and induce autoimmunity under certain circumstances. In patients, blocking the whole complement system systemically, although successful, is unwanted, as the complement is needed to fight infection, heal injury and kill bacteria and viruses (112). BsAbs could in this context also provide

opportunities for interventions. In this case, bsAbs targeting the PTM of interest with one arm and complement inhibitors with the other will provide local complement inhibition (113). Such targeted therapy could inhibit immune reactivity locally, preventing PTM specific B cells to become autoreactive. In RA for example citrullinated and carbamylated proteins could be targeted in the joints, potentially regulating sustained inflammation and dampening of antibody responses. In case of SLE and AIH several PTMs could be targeted and immune regulators brought into close proximity to potentially dampen or prevent anti-PTM antibody responses.

Outline of this thesis

This thesis is mainly focused on PTMs and antibodies targeting these PTMs in the context of autoimmunity. PTMs and anti-PTM responses are described in many (autoimmune) diseases and, in RA patients, antibodies targeting the modifications citrullination and carbamylation are nowadays used as diagnostic and prognostic tools. We focused on six different PTMs namely, Nt, Cit, Ca, Ac, MAA and AGE. With these PTMs we have an broad set of PTMs associating with different diseases, have different structures and/or occur on different amino acids. In the **Chapters 2, 3 and 4** we first measured anti-PTM responses and explored clinical associations in three autoimmune diseases, namely RA, SLE and AILD (including AIH), respectively. In **Chapter 2** anti-MAA and anti-AGE are observed to be significantly increased in RA. These anti-PTM antibodies identify a group of RA patients that are hitherto seronegative (anti-CarP negative and ACPA-negative). These data contribute to closing the serological gap that currently exists in the classification of RA patients. In **Chapter 3** we focused on SLE and SLE patients with neuropsychiatric manifestations (NPSLE). Here we identified three anti-PTM antibodies namely anti-Ca, anti-MAA and anti-AGE that are present more frequently in patients with SLE as compared to healthy controls. Several anti-PTM antibodies (anti-Ca and anti-MAA) were more prevalent in patients with major NPSLE, a disease manifestation currently lacking a suitable biomarker. In addition, all three anti-PTM antibodies also correlated with brain volumes. Next to RA and SLE, we investigated anti-PTM responses in AILD in **Chapter 4**. In these patients the presence of anti-Ca, anti-MAA and anti-AGE correlated with the presence of AIH. In AIH, harboring at least three anti-PTM antibody responses is positively associated with complete biochemical response.

Now that we observed anti-PTM antibody responses in several autoimmune diseases we moved to identifying triggers resulting in these antibody responses. In **Chapter 5** we identified complement components as proteins that increasingly bound to PTM-modified proteins compared to non-modified proteins. We verified complement activation was independent of antibodies, and observed increased binding and/or uptake

of complement opsonized PTM-coupled beads by leukocytes and phagocytic cells (THP-1 macrophages). Furthermore, correlation analysis on complement SNPs from 587 RA patients were performed highlighting a role for complement in driving production of anti-PTM antibodies. Finally, in **Chapter 6** the major findings in this thesis are summarized and the implications of the presented results for future research are discussed.

References

1. Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res. 2002;4 Suppl 3(Suppl 3):S265-72.
2. Straub RH, Cutolo M. Circadian rhythms in rheumatoid arthritis: implications for pathophysiology and therapeutic management. Arthritis Rheum. 2007;56(2):399-408.
3. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569-81.
4. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest. 1998;101(1):273-81.
5. Shi J, Knevel R, Suwannalai P, van der Linden MP, Janssen GM, van Veelen PA, 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(42):17372-7.
6. Trouw LA, Mahler M. Closing the serological gap: promising novel biomarkers for the early diagnosis of rheumatoid arthritis. Autoimmun Rev. 2012;12(2):318-22.
7. Kampstra ASB, Toes REM. HLA class II and rheumatoid arthritis: the bumpy road of revelation. Immunogenetics. 2017;69(8-9):597-603.
8. Kallberg H, Padyukov L, Plenge RM, Ronnelid J, Gregersen PK, van der Helm-van Mil AH, 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(5):867-75.
9. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet. 2016;388(10055):2023-38.
10. Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011;365(22):2110-21.
11. Pons-Estel GJ, Alarcon GS, Scofield L, Reinlib L, Cooper GS. Understanding the epidemiology and progression of systemic lupus erythematosus. Semin Arthritis Rheum. 2010;39(4):257-68.
12. Gergianaki I, Bortoluzzi A, Bertsias G. Update on the epidemiology, risk factors, and disease outcomes of systemic lupus erythematosus. Best Pract Res Clin Rheumatol. 2018;32(2):188-205.
13. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, et al. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. Arthritis Rheumatol. 2019;71(9):1400-12.
14. Hahn B. Epidemiology and pathogenesis of systemic lupus erythematosus. UpToDate, Conner RF (Ed), Wolter Kluwer. (Accessed on August 8, 2024.).
15. Yeo AL, Kandane-Rathnayake R, Koelmeyer R, Golder V, Louthrenoo W, Chen YH, et al. SMART-SLE: serology monitoring and repeat testing in systemic lupus erythematosus-an analysis of anti-double-stranded DNA monitoring. Rheumatology (Oxford). 2024;63(2):525-33.
16. Bertsias GK, Ioannidis JP, Aringer M, Bollen E, Bombardieri S, Bruce IN, et al. EULAR recommendations for the management of systemic lupus erythematosus with neuropsychiatric manifestations: report of a task force of the EULAR standing committee for clinical affairs. Ann Rheum Dis. 2010;69(12):2074-82.
17. Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, et al. Morbidity and mortality in systemic lupus erythematosus during a 5-year period. A multicenter prospective study of 1,000 patients. European Working Party on Systemic Lupus Erythematosus. Medicine (Baltimore). 1999;78(3):167-75.
18. Wakeland EK, Liu K, Graham RR, Behrens TW. Delineating the genetic basis of systemic lupus erythematosus. Immunity. 2001;15(3):397-408.
19. Wilhelm TR, Magder LS, Petri M. Remission in systemic lupus erythematosus: durable remission is rare. Ann Rheum Dis. 2017;76(3):547-53.
20. Mackensen A, Muller F, Mougiakakos D, Boltz S, Wilhelm A, Aigner M, et al. Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus. Nat Med. 2022;28(10):2124-32.
21. Manns MP, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, et al. Diagnosis and management of autoimmune hepatitis. Hepatology. 2010;51(6):2193-213.
22. Wang Q, Yang F, Miao Q, Krawitt EL, Gershwin ME, Ma X. The clinical phenotypes of autoimmune hepatitis: A comprehensive review. J Autoimmun. 2016;66:98-107.
23. Heneghan MA. Autoimmune hepatitis. The Lancet, 2018.
24. Santodomingo-Garzon T, Swain MG. Role of NKT cells in autoimmune liver disease. Autoimmun Rev. 2011;10(12):793-800.
25. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD. Manifestations of systemic lupus erythematosus. Maedica (Bucur). 2011;6(4):330-6.
26. Myasoedova E, Crowson CS, Kremers HM, Roger VL, Fitz-Gibbon PD, Therneau TM, et al. Lipid paradox in rheumatoid arthritis: the impact of serum lipid measures and systemic inflammation on the risk of cardiovascular disease. Ann Rheum Dis. 2011;70(3):482-7.
27. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, et al. Efficacy of B-cell-

targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med. 2004;350(25):2572-81.
28. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum. 2000;43(1):155-63.
29. Pejaver V, Hsu WL, Xin F, Dunker AK, Uversky VN, Radivojac P. The structural and functional signatures of proteins that undergo multiple events of post-translational modification. Protein Sci. 2014;23(8):1077-93.
30. Karve TM, Cheema AK. Small changes huge impact: the role of protein posttranslational modifications in cellular homeostasis and disease. J Amino Acids. 2011;2011:207691.
31. Baka Z, Gyorgy B, Geher P, Buzas EI, Falus A, Nagy G. Citrullination under physiological and pathological conditions. Joint Bone Spine. 2012;79(5):431-6.
32. Shi J, van Veelen PA, Mahler M, Janssen GM, Drijfhout JW, Huizinga TW, et al. Carbamylation and antibodies against carbamylated proteins in autoimmunity and other pathologies. Autoimmun Rev. 2014;13(3):225-30.
33. Gunaydin H, Houk KN. Mechanisms of peroxynitrite-mediated nitration of tyrosine. Chem Res Toxicol. 2009;22(5):894-8.
34. Peluffo G, Radi R. Biochemistry of protein tyrosine nitration in cardiovascular pathology. Cardiovasc Res. 2007;75(2):291-302.
35. Bult H, Boeckxstaens GE, Pelckmans PA, Jordaens FH, Van Maercke YM, Herman AG. Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. Nature. 1990;345(6273):346-7.
36. Pawloski JR, Hess DT, Stamler JS. Export by red blood cells of nitric oxide bioactivity. Nature. 2001;409(6820):622-6.
37. Radi R, Peluffo G, Alvarez MN, Naviliat M, Cayota A. Unraveling peroxynitrite formation in biological systems. Free Radic Biol Med. 2001;30(5):463-88.
38. MacMillan-Crow LA, Crow JP, Kerby JD, Beckman JS, Thompson JA. Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts. Proc Natl Acad Sci U S A. 1996;93(21):11853-8.
39. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007;87(1):315-424.
40. Turko IV, Murad F. Protein nitration in cardiovascular diseases. Pharmacol Rev. 2002;54(4):619-34.
41. Beckmann JS, Ye YZ, Anderson PG, Chen J, Accavitti MA, Tarpey MM, et al. Extensive nitration of protein tyrosines in human atherosclerosis detected by immunohistochemistry. Biol Chem Hoppe Seyler. 1994;375(2):81-8.
42. Degendorfer G, Chuang CY, Hammer A, Malle E, Davies MJ. Peroxynitrous acid induces structural and functional modifications to basement membranes and its key component, laminin. Free Radic Biol Med. 2015;89:721-33.
43. Degendorfer G, Chuang CY, Mariotti M, Hammer A, Hoefler G, Hagglund P, et al. Exposure of tropoelastin to peroxynitrous acid gives high yields of nitrated tyrosine residues, di-tyrosine cross-links and altered protein structure and function. Free Radic Biol Med. 2018;115:219-31.
44. Vadseth C, Souza JM, Thomson L, Seagraves A, Nagaswami C, Scheiner T, et al. Pro-thrombotic state induced by post-translational modification of fibrinogen by reactive nitrogen species. J Biol Chem. 2004;279(10):8820-6.
45. Raijmakers R, Zendman AJ, Egberts WV, Vossenaar ER, Raats J, Soede-Huijbregts C, et al. Methylation of arginine residues interferes with citrullination by peptidylarginine deiminases in vitro. J Mol Biol. 2007;367(4):1118-29.
46. Pearton DJ, Dale BA, Presland RB. Functional analysis of the profilaggrin N-terminal peptide: identification of domains that regulate nuclear and cytoplasmic distribution. J Invest Dermatol. 2002;119(3):661-9.
47. Tarcsa E, Marekov LN, Andreoli J, Idler WW, Candi E, Chung SI, et al. The fate of trichohyalin. Sequential post-translational modifications by peptidyl-arginine deiminase and transglutaminases. J Biol Chem. 1997;272(44):27893-901.
48. Nakashima K, Hagiwara T, Yamada M. Nuclear localization of peptidylarginine deiminase V and histone deimination in granulocytes. J Biol Chem. 2002;277(51):49562-8.
49. Vossenaar ER, Zendman AJ, van Venrooij WJ, Pruijn GJ. PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. Bioessays. 2003;25(11):1106-18.
50. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. PLoS Pathog. 2009;5(10):e1000639.
51. Foulquier C, Sebbag M, Clavel C, Chapuy-Regaud S, Al Badine R, Mechin MC, et al. Peptidyl arginine deiminase type 2 (PAD-2) and PAD-4 but not PAD-1, PAD-3, and PAD-6 are expressed in rheumatoid arthritis synovium in close association with tissue inflammation. Arthritis Rheum. 2007;56(11):3541-53.
52. Jelkmann W. 'O', erythropoietin carbamoylation versus carbamylation. Nephrol Dial Transplant. 2008;23(9):3033; author reply -4.
53. Verheul MK, van Veelen PA, van Delft MAM, de Ru A, Janssen GMC, Rispens T, et al. Pitfalls in the detection of citrullination and carbamylation. Autoimmun Rev. 2018;17(2):136-41.

54. Hagel P, Gerding JJ, Fieggen W, Bloemendal H. Cyanate formation in solutions of urea. I. Calculation of cyanate concentrations at different temperature and pH. Biochim Biophys Acta. 1971;243(3):366-73.
55. Kraus LM, Kraus AP, Jr. Carbamoylation of amino acids and proteins in uremia. Kidney Int Suppl. 2001;78:S102-7.
56. Holzer M, Zangger K, El-Gamal D, Binder V, Curcic S, Konya V, et al. Myeloperoxidase-derived chlorinating species induce protein carbamylation through decomposition of thiocyanate and urea: novel pathways generating dysfunctional high-density lipoprotein. Antioxid Redox Signal. 2012;17(8):1043-52.
57. Wang Z, Nicholls SJ, Rodriguez ER, Kummu O, Horkko S, Barnard J, et al. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. Nat Med. 2007;13(10):1176-84.
58. Verheul MK, Janssen GMC, de Ru A, Stoeken-Rijsbergen G, Levarht EWN, Kwekkeboom JC, et al. Mass-spectrometric identification of carbamylated proteins present in the joints of rheumatoid arthritis patients and controls. Clin Exp Rheumatol. 2021;39(3):570-7.
59. Hosp F, Lassowskat I, Santoro V, De Vleesschauwer D, Fliegner D, Redestig H, et al. Lysine acetylation in mitochondria: From inventory to function. Mitochondrion. 2017;33:58-71.
60. Sabari BR, Zhang D, Allis CD, Zhao Y. Metabolic regulation of gene expression through histone acylations. Nat Rev Mol Cell Biol. 2017;18(2):90-101.
61. Phillips DM. The presence of acetyl groups of histones. Biochem J. 1963;87(2):258-63.
62. Hebbes TR, Thorne AW, Crane-Robinson C. A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J. 1988;7(5):1395-402.
63. Narita T, Weinert BT, Choudhary C. Functions and mechanisms of non-histone protein acetylation. Nat Rev Mol Cell Biol. 2019;20(3):156-74.
64. Juarez M, Bang H, Hammar F, Reimer U, Dyke B, Sahbudin I, et al. Identification of novel antiacetylated vimentin antibodies in patients with early inflammatory arthritis. Ann Rheum Dis. 2016;75(6):1099-107.
65. Kabir F, Atkinson R, Cook AL, Phipps AJ, King AE. The role of altered protein acetylation in neurodegenerative disease. Front Aging Neurosci. 2022;14:1025473.
66. Jiang N, Li W, Jiang S, Xie M, Liu R. Acetylation in pathogenesis: Revealing emerging mechanisms and therapeutic prospects. Biomed Pharmacother. 2023;167:115519.
67. Uchida K. Lipofuscin-like fluorophores originated from malondialdehyde. Free Radic Res. 2006;40(12):1335-8.
68. Tuma DJ, Thiele GM, Xu D, Klassen LW, Sorrell MF. Acetaldehyde and malondialdehyde react together to generate distinct protein adducts in the liver during long-term ethanol administration. Hepatology. 1996;23(4):872-80.
69. Horkko S, Binder CJ, Shaw PX, Chang MK, Silverman G, Palinski W, et al. Immunological responses to oxidized LDL. Free Radic Biol Med. 2000;28(12):1771-9.
70. Tuma DJ. Role of malondialdehyde-acetaldehyde adducts in liver injury. Free Radic Biol Med. 2002;32(4):303-8.
71. Duryee MJ, Klassen LW, Schaffert CS, Tuma DJ, Hunter CD, Garvin RP, et al. Malondialdehyde-acetaldehyde adduct is the dominant epitope after MDA modification of proteins in atherosclerosis. Free Radic Biol Med. 2010;49(10):1480-6.
72. Rolla R, Vay D, Mottaran E, Parodi M, Traverso N, Arico S, et al. Detection of circulating antibodies against malondialdehyde-acetaldehyde adducts in patients with alcohol-induced liver disease. Hepatology. 2000;31(4):878-84.
73. Thiele GM, Duryee MJ, Anderson DR, Klassen LW, Mohring SM, Young KA, et al. Malondialdehyde-acetaldehyde adducts and anti-malondialdehyde-acetaldehyde antibodies in rheumatoid arthritis. Arthritis Rheumatol. 2015;67(3):645-55.
74. Heyns K, Beilfuss W. [Ketosylamine rearrangement of D-threo-pentulose (D-xylulose) with alpha-amino acids]. Chem Ber. 1970;103(9):2873-6.
75. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. J Clin Invest. 2001;108(7):949-55.
76. Scheijen J, Hanssen NMJ, van Greevenbroek MM, Van der Kallen CJ, Feskens EJM, Stehouwer CDA, et al. Dietary intake of advanced glycation endproducts is associated with higher levels of advanced glycation endproducts in plasma and urine: The CODAM study. Clin Nutr. 2018;37(3):919-25.
77. Negrean M, Stirban A, Stratmann B, Gawlowski T, Horstmann T, Gotting C, et al. Effects of low- and high-advanced glycation endproduct meals on macro- and microvascular endothelial function and oxidative stress in patients with type 2 diabetes mellitus. Am J Clin Nutr. 2007;85(5):1236-43.
78. Luevano-Contreras C, Gomez-Ojeda A, Macias-Cervantes MH, Garay-Sevilla ME. Dietary Advanced Glycation End Products and Cardiometabolic Risk. Curr Diab Rep. 2017;17(8):63.
79. Stanislovaitiene D, Zaliuniene D, Steponaviciute R, Zemaitiene R, Gustiene O, Zaliunas R. N-carboxymethyllysine as a biomarker for coronary artery disease and age-related macular degeneration. Medicina (Kaunas). 2016;52(2):99-103.
80. Kralev S, Zimmerer E, Brueckmann M, Lang S, Kalsch T, Rippert A, et al. Elevation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in patients presenting with acute myocardial infarction. Clin Chem

Lab Med. 2009;47(4):446-51.
81. Drinda S, Franke S, Canet CC, Petrow P, Brauer R, Huttich C, et al. Identification of the advanced glycation end products N(epsilon)-carboxymethyllysine in the synovial tissue of patients with rheumatoid arthritis. Ann Rheum Dis. 2002;61(6):488-92.
82. Nonaka M. Evolution of the complement system. Subcell Biochem. 2014;80:31-43.
83. Elvington M, Liszewski MK, Atkinson JP. Evolution of the complement system: from defense of the single cell to guardian of the intravascular space. Immunol Rev. 2016;274(1):9-15.
84. Rus H, Cudrici C, Niculescu F. The role of the complement system in innate immunity. Immunol Res. 2005;33(2):103-12.
85. Merle NS, Church SE, Fremeaux-Bacchi V, Roumenina LT. Complement System Part I - Molecular Mechanisms of Activation and Regulation. Front Immunol. 2015;6:262.
86. Walport MJ. Complement. First of two parts. N Engl J Med. 2001;344(14):1058-66.
87. Gaboriaud C, Thielens NM, Gregory LA, Rossi V, Fontecilla-Camps JC, Arlaud GJ. Structure and activation of the C1 complex of complement: unraveling the puzzle. Trends Immunol. 2004;25(7):368-73.
88. Bohlson SS, Garred P, Kemper C, Tenner AJ. Complement Nomenclature-Deconvoluted. Front Immunol. 2019;10:1308.
89. Heja D, Kocsis A, Dobo J, Szilagyi K, Szasz R, Zavodszky P, et al. Revised mechanism of complement lectin-pathway activation revealing the role of serine protease MASP-1 as the exclusive activator of MASP-2. Proc Natl Acad Sci U S A. 2012;109(26):10498-503.
90. Kidmose RT, Laursen NS, Dobo J, Kjaer TR, Sirotkina S, Yatime L, et al. Structural basis for activation of the complement system by component C4 cleavage. Proc Natl Acad Sci U S A. 2012;109(38):15425-30.
91. Lachmann PJ, Halbwachs L. The influence of C3b inactivator (KAF) concentration on the ability of serum to support complement activation. Clin Exp Immunol. 1975;21(1):109-14.
92. Nilsson B, Nilsson Ekdahl K. The tick-over theory revisited: is C3 a contact-activated protein? Immunobiology. 2012;217(11):1106-10.
93. Rawal N, Pangburn M. Formation of high-affinity C5 convertases of the alternative pathway of complement. J Immunol. 2001;166(4):2635-42.
94. Rawal N, Pangburn MK. Formation of high affinity C5 convertase of the classical pathway of complement. J Biol Chem. 2003;278(40):38476-83.
95. Tegla CA, Cudrici C, Patel S, Trippe R, 3rd, Rus V, Niculescu F, et al. Membrane attack by complement: the assembly and biology of terminal complement complexes. Immunol Res. 2011;51(1):45-60.
96. Morgan HP, Schmidt CQ, Guariento M, Blaum BS, Gillespie D, Herbert AP, et al. Structural basis for engagement by complement factor H of C3b on a self surface. Nat Struct Mol Biol. 2011;18(4):463-70.
97. Roozendaal R, Carroll MC. Complement receptors CD21 and CD35 in humoral immunity. Immunol Rev. 2007;219:157-66.
98. Torok K, Dezso B, Bencsik A, Uzonyi B, Erdei A. Complement receptor type 1 (CR1/CD35) expressed on activated human CD4+ T cells contributes to generation of regulatory T cells. Immunol Lett. 2015;164(2):117-24.
99. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat Immunol. 2010;11(9):785-97.
100. Tuveson DA, Ahearn JM, Matsumoto AK, Fearon DT. Molecular interactions of complement receptors on B lymphocytes: a CR1/CR2 complex distinct from the CR2/CD19 complex. J Exp Med. 1991;173(5):1083-9.
101. Jozsi M, Prechl J, Bajtay Z, Erdei A. Complement receptor type 1 (CD35) mediates inhibitory signals in human B lymphocytes. J Immunol. 2002;168(6):2782-8.
102. Carroll MC. The role of complement in B cell activation and tolerance. Adv Immunol. 2000;74:61-88.
103. Vorup-Jensen T, Jensen RK. Structural Immunology of Complement Receptors 3 and 4. Front Immunol. 2018;9:2716.
104. Lamers C, Pluss CJ, Ricklin D. The Promiscuous Profile of Complement Receptor 3 in Ligand Binding, Immune Modulation, and Pathophysiology. Front Immunol. 2021;12:662164.
105. Santos-Lopez J, de la Paz K, Fernandez FJ, Vega MC. Structural biology of complement receptors. Front Immunol. 2023;14:1239146.
106. Wollina U. Immune complexes--pathogenetic factors of autoimmune systemic lupus erythematosus. Allerg Immunol (Leipz). 1984;30(1):3-13.
107. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum. 1987;30(11):1205-13.
108. Rojas M, Restrepo-Jimenez P, Monsalve DM, Pacheco Y, Acosta-Ampudia Y, Ramirez-Santana C, et al. Molecular mimicry and autoimmunity. J Autoimmun. 2018;95:100-23.
109. Klareskog L, Stolt P, Lundberg K, Kallberg H, Bengtsson C, Grunewald J, et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum. 2006;54(1):38-46.
110. Hagen M, Bucci L, Boltz S, Nothling DM, Rothe T, Anoshkin K, et al. BCMA-Targeted T-Cell-Engager Therapy for Autoimmune Disease. N Engl J Med. 2024;391(9):867-9.

111. Labrijn AF, Janmaat ML, Reichert JM, Parren P. Bispecific antibodies: a mechanistic review of the pipeline. Nat Rev Drug Discov. 2019;18(8):585-608.
112. Harris CL. Expanding horizons in complement drug discovery: challenges and emerging strategies. Semin Immunopathol. 2018;40(1):125-40.
113. Wang H, van de Bovenkamp FS, Dijkstra DJ, Abendstein L, Borggreven NV, Pool J, et al. Targeted complement inhibition using bispecific antibodies that bind local antigens and endogenous complement regulators. Front Immunol. 2024;15:1288597.