Pathogenic implications for autoantibodies against C-reactive protein and other acute phase proteins

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Title: Pathogenic implications for autoantibodies against C-reactive protein

Running head: Pathogenic implications for anti-CRP

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Abbreviations

ANA, antinuclear antibody; APR, acute-phase reaction; CRP, C-reactive protein; Fc γ R, Fc gamma receptor; FH, factor H; ICs, immune complexes; IL, interleukin; MBL, mannose-binding lectin; mCRP, monomeric CRP; OD, optical density; PC, phosphorylcholine; PTX3, pentraxin 3; RA, rheumatoid arthritis; SAA, serum amyloid A; SAP, serum amyloid P component; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; SS, primary Sjögren's syndrome

Abstract

Systemic lupus erythematosus (SLE) is a systemic rheumatic disease characterized clinically by multiorgan involvement and serologically by the occurrence of antinuclear antibodies. SLE patients may present with multiple autoantibodies to cytoplasmic and cell surface antigens as well as to circulating plasma proteins. Another feature of SLE is that serum levels of C-reactive protein (CRP) often remain low despite high disease activity and despite high levels of other acute phase proteins and interleukin-6, *i.e.* the main CRP inducing cytokine. Apart from its important role as a laboratory marker of inflammation, CRP attracts increasing interest due to its many intriguing biological functions, one of which is a role as an opsonin contributing to the elimination of apoptotic cell debris, *e.g.* nucleosomes, thereby preventing immunization against autoantigens. Recently, autoantibodies against CRP and other acute phase proteins have been reported in certain rheumatic conditions, including SLE. Although the presence of anti-CRP autoantibodies does not explain the failed CRP response in SLE, antibodies directed against acute phase proteins have several implications of pathogenetic interest. This paper thus highlights the biological and clinical aspects of native and monomeric CRP and anti-CRP, as well as autoantibodies against mannose-binding lectin, serum amyloid A and serum amyloid P component.

1. The acute-phase response and CRP

The acute-phase reaction (APR) is an early set of inflammatory reactions composed of non-specific biochemical and biophysical responses of endothermic animals and is initiated by microbes/microbial constituents and tissue degradation [1]. The APR is characterized by changes in the concentrations of many plasma proteins, known as the acute-phase proteins, but also a large number of behavioral, physiologic, biochemical, and nutritional changes. In humans, the APR comprises release of leukocytes and platelets from the bone marrow to the circulation and increased hepatic production and release of acute-phase proteins, such as C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin, α_1 -acid glycoprotein (orosomucoid), ferritin and α_1 -antitrypsin; while other proteins are reduced, for instance albumin and transferrin [1, 2].

CRP was discovered more than 75 years ago at the Rockefeller University. Investigating blood from patients with acute febrile illness, William S Tillett and Thomas Francis Jr demonstrated the precipitation of a non-antibody serum component with a soluble extract of *Streptococcus pneumoniae* [3]. This serum reaction was present during the acute phase of the disease and diminished as the patients recovered. Identification of C-polysaccharide as the serum constituent gave rise to its designation C-reactive protein [4]. In parallel with the discovery that minor CRP elevation is a useful risk marker in cardiovascular disease, substantial progress has been made over the last decade concerning the biological properties and physiological importance of CRP in health and disease.

1:1 The pentraxin family

The pentraxins are evolutionarily conserved pentameric molecules, which are expressed during infection, systemic inflammation or tissue damage and participate in the acute-phase response in many species. The family includes long pentraxins, *e.g.* pentraxin 3 (PTX3)

produced by mononuclear cells in response to lipopolysaccharide, the neuronal pentraxins (NP1, NP2 and neuronal pentraxin receptor) and the liver-derived short pentraxins, *i.e.* CRP and serum amyloid P component (SAP). Pentraxins have been found in all vertebrate species as well as in some invertebrates, for instance the phylogenetically ancient arthropod *Limulus polyphemus* [5].

The pentameric structure of CRP is composed of five identical non-covalently bound subunits with 206 amino acids (~23-kDa) arranged in cyclic symmetry around a central pore [5, 6].

The subunits are synthesized as non-glycosylated monomers consisting of two anti-parallel β -sheets with flattened jellyroll topology (Figure 1). Each subunit has a single phosphorylcholine (PC) binding site and two bound calcium ions adjacent to a hydrophobic pocket. All five binding sites are located on the same face of the pentamer [7]. The binding of complement factor C1q occurs at the opposite face of the pentamer where binding to cellular IgG receptors (Fc γ Rs) is also presumed to take place [6, 8].

Apart from the liver, CRP synthesis has been reported to occur in neurons [9], lymphocytes [10], smooth muscle cells [11], alveolar macrophages [12] and tubular epithelial cells in the kidney [13]. The mechanisms by which synthesis is regulated at these sites are not known.

Although extrahepatic CRP may mediate local effects, it is unlikely that it substantially affects the plasma levels. The CRP synthesis is mainly regulated at the transcriptional level through IL-6 and IL-1 β directed induction of the CRP gene, located on the short arm of chromosome 1 by activation of NF- κ B and transcriptional factor NF-IL-6/CAAT-enhancer binding protein (C/EBP) family members C/EBP β and C/EBP δ [6, 14, 15]. Tumor necrosis factor alpha (TNF α) may also indirectly enhance the production, while transforming growth factor beta (TGF β) displays an inhibitory influence [5, 16]. Single nucleotide polymorphisms in the CRP promoter gene are associated with differences in baseline levels of CRP [17, 18].

Recently, the impact of stress and hormones on the regulation of CRP production has been discussed [19, 20].

1:1 Monomeric CRP

Under conditions of altered pH, high urea or low calcium concentration, native CRP dissociates irreversibly into monomers [21], which undergo conformational rearrangement resulting in expression of a distinct isomer (also recognized as ‘modified CRP’ or ‘neo-CRP’ in the literature) with distinct antigenic and physiochemical characteristics [22]. There is strong evidence that CRP dissociation occurs under physiological relevant conditions after binding to plasma membranes [23]. Monomeric CRP (mCRP) has a lower isoelectric point than the pentameric form and is considered to be a tissue and/or cell based form of the acute phase protein [23–26].

1:2 Biological CRP effects

Many biological functions of native CRP have been recognized, whereas less is known about the properties and biological effects of mCRP, but this attracts increasing interest [27]. Like many cytokines, native CRP has pleiotropic actions, for instance pro- as well as anti-inflammatory effects. It inhibits many functions of neutrophil granulocytes, including the chemotactic response to interleukin 8 (IL-8) [28] and the production of reactive oxygen species and degranulation [29], possibly via alteration of actin polymerization by increasing F-actin and decreasing G-actin [30]. By contrast, mCRP up-regulates complement receptor 3 (CD11b/CD18) [31] and activates neutrophils, monocytes and platelets [21]. While mCRP enhances attachment of neutrophils to endothelial cells and thereby promotes transmigration [31], native CRP exerts modulatory effects on monocytes both by activating and limiting the early stages of diapedesis [32].

In peripheral blood mononuclear cells, native CRP was shown to stimulate the production of IL-1 receptor antagonist (IL-1ra) to a greater extent than it stimulates the generation of IL-1 β [33]. In mice with experimental allergic encephalomyelitis (EAE), native CRP increases the release of IL-10 while decreasing the secretion of TNF α and interferon gamma (IFN γ) [34]. CRP has also been linked to enhanced expression and activity of plasminogen activator inhibitor-1 (PAI-1) in human monocytes [35] and decreased release of prostacyclin by endothelial cells [36]. On the contrary, megakaryocyte proliferation and platelet generation is stimulated by mCRP [37], which also exerts anti-apoptotic actions on human neutrophils [38] and inhibitory effects on the growth of mammary adenocarcinoma in mice [39]. On the other hand, elastase-digested CRP products (but not native CRP) were recently suggested to instead promote neutrophil apoptosis [40]. In apolipoprotein E knockout mice, mCRP reduced atherosclerosis whereas the opposite was seen for native CRP [41].

1:3 CRP receptors and ligands

Although previously questioned [42, 43], there is now compelling evidence that CRP interacts with Fc γ Rs in man and mouse, eliciting a response from phagocytic cells [44–46]. Ligation to phagocytic Fc γ Rs is believed to account for the opsonizing properties of CRP; pentameric CRP primarily binds to the low-affinity Fc γ RIIa (CD32) and to some extent to the high-affinity Fc γ RI (CD64), whereas mCRP binds to the low-affinity Fc γ RIII (CD16) [6, 31, 44–50]. Ligand recognition by CRP may thus contribute to a range of metabolic, scavenging and host-defense functions.

Native CRP plays several important roles by calcium-dependent binding to specific ligands, such as PC in oxidized phospholipids on damaged cell membranes [6] or LDL [51], fibronectin [52, 53] and protein A [54]. The ability to bind nuclear structures at physiological ionic strength, both nucleosome core particles and extrachromosomal constituents such as

snRNPs, is also well documented for native CRP [6, 55]. Interestingly, many of the nuclear antigens to which CRP binds are the same as those targeted by antinuclear antibodies (ANA) seen in sera from patients with systemic lupus erythematosus (SLE) and other systemic inflammatory rheumatic diseases [56, 57]. It is conceivable that CRP, by Fc γ R-mediated uptake in phagocytes, facilitates the clearance of circulating nucleosomes and apoptotic blebs on which nuclear antigens are exposed [55, 58, 59], thereby limiting the contact of these autoantigens with the adaptive immune system. In an inflammatory microenvironment with acidic conditions, native CRP dissociates into CRP monomers, which bind to IgG-containing immune complexes (ICs) [60] and to Fc γ RIII [47].

1:4 CRP interactions with complement

Activation of the complement cascade is regarded as one of the main physiological functions of CRP. When a ligand binds to a CRP subunit, it induces a conformational change revealing a cleft on the opposite side of the subunit. C1q binds avidly in this cleft, thereby inducing complement activation via formation of the classical C3 convertase, which in turn leads to decoration of the ligand surface with opsonizing complement fragments [8, 61]. The ability to induce the complement cascade has been reported to be a unique feature of the pentameric CRP form [21, 62] and the activation is progressively increased by the presence of apoptotic cells with immobilized cell surfaces [63]. However, a recent report by Ji *et al* indicates that mCRP may also have a regulatory role of the classical complement pathway, depending on whether the interaction with C1q appears in fluid-phase or surface-bound state [64]. Our own findings support the notion that mCRP may activate the classical pathway with kinetics similar to that of native CRP. In addition, we found that substantially elevated levels of native CRP (>150 mg/L) down-regulate complement activation efficiently through a mechanism dependent on fluid-phase interaction between C1q and CRP [Sjöwall *et al*, to be published].

In contrast to IgM- and IgG-mediated complement activation, CRP-mediated activation appears to be essentially limited to the initial stage involving C1-C4 with less formation of the membrane attack complex (MAC) [65]. This is presumably due to a direct interaction between CRP and factor H (FH), leading to inhibition of the alternative complement pathway C3 and C5 convertases [61, 66–68]. Another maneuver by which CRP may regulate complement activation is by increasing the expression of complement-inhibitory proteins, such as decay-accelerating factor (DAF; CD55), membrane cofactor protein (MCP; CD46) and protectin (CD59; MAC inhibitor) [69]. Thus, CRP participates in host defense at the same time as it restricts potentially harmful side effects of inflammation (Figure 2).

1:6 CRP in clinical practice

Since hepatic CRP can rise rapidly with the plasma concentration increasing from less than 1.0 to more than 500 mg/L within 24 to 72 hours, it has been extensively used in clinical practice as a measurement of the APR, *e.g.* in order to evaluate the response to anti-microbial pharmacotherapy and to distinguish bacterial from viral infections [5, 6]. In healthy blood donors, the median CRP concentration is 0.8 mg/L. The median baseline value in the ostensibly healthy population is slightly higher and tends to increase with age. Females have negligibly higher CRP levels than men. The plasma half-life of CRP is about 19 hours, surprisingly unaffected by simultaneous disease [70].

Minor CRP elevations have been shown to reflect a low-grade vascular inflammation.

Numerous studies have established the high-sensitivity CRP test with levels ≥ 3.0 mg/L as the most powerful independent biochemical marker in the prediction of future coronary vascular events and survival in patients with angina pectoris as well as in apparently healthy subjects [71–73]. The molecular mechanisms that link CRP to atherogenesis are incompletely

understood, but recent investigations have revealed that CRP directly interacts with several major components in the process of atherosclerosis [74, 75].

2. SLE and the waste disposal hypothesis

One important exception to the generalization that CRP concentrations correlate with the extent and severity of inflammation is SLE. Many patients with active SLE, particularly if they present without serositis, do not have elevated CRP (or serum amyloid A) concentrations but do have marked increases during bacterial infections [76–81]. This may be of etiopathogenic importance concerning both immunoregulation and induction of autoimmunity [6, 73]. Regarding the capacity of CRP to bind nuclear antigens/apoptotic cells and to interact with Fc γ Rs, it has been proposed that the modest CRP response in SLE contributes to the deficient handling of apoptotic material, thereby increasing the risk of abnormal immunization to autoantigens [63].

In mice, deletion of the SAP gene leads to the development of a lupus resembling illness [82]. In analogy, CRP supplementation to lupus-prone (NZB x NZW) F₁ mice delays the onset of nephritis, decreases autoantibody levels, leads to less autoimmune manifestations and prolongs the survival through an Fc γ R and IL-10 dependent mechanism [83, 84]. When the human CRP gene was transferred to the same lupus prone mouse model, similar results were achieved, with the exception that anti-DNA antibody levels were not lowered [85].

In humans, it has recently been shown that a polymorphism at the CRP locus influences the basal CRP expression and predispose to SLE [86]. Modest CRP reactions during active disease are also common in ulcerative colitis. Patients with either of these conditions share the intact capability of a ‘normal’ CRP response in intercurrent infections [87–91]. Application of this knowledge to the differential diagnosis of fever in patients with SLE has been somewhat limited by the finding that CRP levels are also high in patients with active lupus serositis or chronic synovitis [87, 88]. This is in agreement with the findings of a normal CRP turnover in SLE and resembles other inflammatory disorders [92]. However, the remarkably low CRP levels seen in patients with hypocomplementemic disease involving skin and kidneys, often in

contrast to raised levels of other acute-phase reactants, could hypothetically be due to CRP consumption by ICs [93–97] and indeed CRP has been identified as a component in isolated ICs from SLE patients [98].

Raised circulating levels of cytokines, such as TNF α , IFN α and IL-6, and their specific anti-cytokine autoantibodies are seen in SLE, in some instances paralleling disease activity [99–102]. In SLE, however, IL-6 levels do not correlate with circulating CRP levels, as is the case in RA [103]. Genetic polymorphisms of CRP-inducing cytokines and their concomitant receptors have been found in association with SLE and might predispose to distinct clinical and immunological features [104–107].

Apoptosis is essential in the normal function of multicellular organisms, and is implicated in developmental and homeostatic mechanisms. It is a complex and firmly regulated process aiding to prevent intracellular material from being recognized by the immune system. During apoptosis, caspase activity leads to fragmentation of the nucleus and redistribution of nuclear fragments on the cell surface. Some of these blebs are shed as apoptotic bodies on which nuclear autoantigens (*e.g.* nucleosomes, Ro/SS-A, La/SS-B and Sm) are exposed and may in turn become available to professional antigen-presenting cells [58, 108]. Interestingly, monocytoïd dendritic cells but not macrophages efficiently present antigens derived from apoptotic cells to cytotoxic T cells [109]. In addition, during apoptosis the autoantigens that are composed of complex particles often become modified, leading to increased immunogenicity [110].

Rapid removal of apoptotic cells or cellular debris by phagocytosis is critical to ensure safe elimination of potentially pro-inflammatory or immunogenic material from the circulation [108]. Under normal circumstances, any material that primarily escapes clearance by endocytosis can be rapidly cleared from the circulation via the reticuloendothelial system after a number of additional mechanisms, *e.g.* complex formation and opsonization by

proteins such as CRP, mannose-binding lectin (MBL), C1q and/or antibodies [111, 112].

There is considerable evidence for dysfunction in several of these key events in human SLE.

Supported also by results from lupus animal models, apoptosis of lymphocytes, monocytes as well as neutrophils have been reported to be accelerated in SLE [113–115]. In addition, several groups have reported defective processing, and Fc γ R-dependent clearance, of ICs and apoptotic cells in lupus patients [116–118]. Further indications of deficient removal of apoptotic material in SLE patients include the associations between relative or absolute deficiencies of certain components of the classical complement pathway (C1q, C1r, C1s, C4 or C2) and the occurrence of SLE [119, 120].

Taking it all together, it is conceivable to regard SLE as a disease with dysregulated apoptosis and/or defective clearance of apoptotic material, leading to increased levels of circulating autoantigens, an autoantigen overload yielding a ‘mission impossible’ for the body’s waste disposal system. Structurally altered autoantigens on apoptotic blebs may ultimately be presented to T lymphocytes leading to B cell activation and formation of autoantibodies. This, together with subsequent IC-formation/deposition, promotes inflammatory tissue destruction as well as apoptosis and constitutes a pathogenic vicious circle [121].

3. Apoptosis-related autoantibodies

A characteristic feature of SLE is the multitude of autoantibodies targeting nuclear antigens expressed on apoptic structures, *e.g.* dsDNA, histones, DNA–histone complexes (nucleosomes) and extra-chromosomal nuclear antigens such as Ro/SS-A, La/SS-B, Sm and snRNP [57]. IgG class autoantibodies are most common in SLE, but IgM class autoantibodies also occur [122]. Interestingly, the appearance of autoantibodies in lupus patients tends to follow a predictable course with a progressive accumulation of certain autoantibodies before clinical disease onset [123]. Apart from ANA, autoantibodies in SLE are frequently directed against cytoplasmic constituents (*e.g.* ribosomal phosphoprotein and phospholipids) and extracellular antigens, for instance plasma proteins such as β_2 -glycoprotein I, annexin V, C1q and IgG (rheumatoid factor) [56, 57, 124–126] and CRP [127–130].

3:1 Autoantibodies against CRP

In 1985, Frank A Robey and coworkers described autoantibodies against CRP in one out of eight SLE patients and reported a depressed ability of CRP to solubilize chromatin in some SLE individuals [131]. Later, Susanne A Bell demonstrated a high frequency of IgG antibodies to cryptic epitopes of CRP (anti-CRP) in patients suffering from the ‘autoimmune like’ toxic oil syndrome [127]. Bell and colleagues also reported high frequencies of autoantibodies to mCRP in SLE (78 percent) and lower prevalences in subacute cutaneous lupus erythematosus and primary biliary cirrhosis [128]. Rosenau and Schur demonstrated antibodies against CRP in sera from patients with different rheumatologic conditions, including SLE, where they observed a frequency of 23 percent [132].

Our studies indicate an approximately 40 percent overall prevalence rate of anti-CRP antibodies in SLE, with clear-cut positive correlation between antibody occurrence/concentration and disease activity. Thus, in our first study on anti-CRP [129], we

found that some SLE patients were positive on one occasion but negative on another. In proceeding investigations we analyzed antibody levels in consecutive samples from 10 well-characterized SLE patients and demonstrated that anti-CRP paralleled the clinical disease activity, usually with high levels at the time of flare [130]. Seventy percent of the patients were positive on at least one occasion. The correlation between anti-CRP level and SLE disease activity index (SLEDAI) is illustrated in Figure 3. All patients with active lupus nephritis tested positive for anti-CRP autoantibodies during disease flare, strong inverse relationship was noted between anti-CRP autoantibody levels and complement levels and lymphocyte count, while anti-CRP autoantibody levels correlated positively to anti-dsDNA levels [130].

Recently, a study on anti-CRP antibodies in a larger patient material confirmed several of our findings [133]. Figueredo *et al* investigated 137 patients with SLE and 127 with persistent anti-phospholipid syndrome. Presence of anti-CRP antibodies was found in 51 percent of patients with SLE and in 54 percent of patients with primary antiphospholipid syndrome. No correlation between anti-CRP reactivity and CRP levels was recorded. Anti-CRP positive SLE patients had lower C3 levels and were more likely to have anti-dsDNA and cardiolipin antibodies as compared to anti-CRP antibody negative individuals. In addition, the frequency of nephritis was higher in anti-CRP antibody positive SLE patients [133].

Analyses of antigen specificity of the anti-CRP assay have clearly revealed that autoantibodies to CRP in SLE are directed to mCRP (Figure 4) and that ICs isolated from SLE sera do not induce positive anti-CRP tests [Mathsson *et al*, to be published]. In our hands, sera from patients with RA or inflammatory bowel disease have consistently turned out negative in the anti-CRP assay, whereas a few patients with primary SS have tested positive [129].

3:2 Autoantibodies against MBL

Mannose-binding lectin (MBL) is an acute phase reactant in humans and its production is enhanced by inflammatory stimuli. In addition, MBL binds both dimeric and polymeric IgA and activates complement [134]; it binds agalactosyl IgG and IgM, including IgM rheumatoid factor complexes from RA patients [135]. Occurrence of autoantibodies against MBL (anti-MBL) has been reported by several groups [136–139]. Seelen and coworkers demonstrated significant higher levels of anti-MBL in SLE patients as compared to healthy subjects, but with no correlation with disease activity or specific organ involvement [136]. In the Japanese study by Takahashi and colleagues, elevated anti-MBL antibody levels were only found in sera from 9 of 111 SLE patients as compared to 2 of 113 healthy controls. No significant correlation between anti-MBL antibody levels and serum MBL or any specific lupus feature was found [137]. Mok *et al* [138] found anti-MBL antibodies in 24 percent among 135 SLE patients. A smaller percentage of the SLE patients were also found to have IgM class anti-MBL antibodies. IgG anti-MBL antibody levels correlated positively to circulating MBL, but not with levels of complement, anti-DNA or disease activity measures.

Recently, the first study on anti-MBL autoantibodies in RA was presented [139]. Gupta *et al* investigated sera from 107 patients with established RA of which 65 were anti-MBL antibody positive, and 121 healthy controls of which only 2 were anti-MBL antibody positive. In comparison with both IgM and IgG isotypes of rheumatoid factor, anti-MBL autoantibodies were found more often in the RA patient sera and could therefore have a diagnostic value for RA as suggested by the authors. Anti-MBL autoantibodies were also found in synovial fluid from several RA patients [139].

3:3 Autoantibodies against SAA and SAP

In 2004, Rosenau and Schur described the occurrence of autoantibodies against SAA and that a positive test significantly associated with different cardiovascular conditions, such as aortic stenosis, deep vein thrombosis and atrial fibrillation, but also with seizures and SLE [140].

Only one out of 62 blood donors was found to be anti-SAA antibody positive.

Contrasting to CRP, PTX3, MBL and SAA, SAP is in fact not an acute phase protein in humans, but rather a constitutive serum protein [5]. However, considering SAP's important role in the opsonization of (late) apoptotic cells [82], the finding of autoantibodies against SAP is highly interesting. Zandman-Goddard *et al* recently showed presence of anti-SAP in 44 percent of patients with SLE [141]. This frequency is comparable with our findings of anti-CRP in SLE. Anti-SAP antibody levels associated positively with disease activity (SLEDAI) and decreased with improvement. The authors' suggestion of anti-SAP as an additional prognostic marker in SLE is interesting, but their findings await independent confirmation.

4. Development and pathogenicity of antibodies against acute-phase proteins

The current view on the pathogenesis of SLE is that autoantigens from dying cells are abnormally exposed to the immune system as a consequence of dysregulated apoptosis and/or deficient elimination of apoptotic material via the reticuloendothelial system [108, 142].

Hence, successful removal of apoptotic cells or cellular debris is critical to avoid undesired immune reactions. The players of the innate immune system have central roles in this process. CRP, MBL, SAP and C1q have direct and/or indirect opsonic potentials, but they also form a bridge with the adaptive immune system by interactions with antibodies and FcγRs [6]. On the other hand, imbalances in these systems may promote the induction of autoimmunity [59, 86, 125, 143–145].

Autoantibodies against acute phase proteins might be generated by various mechanisms such as molecular mimicry, or these immunoglobulins might just be innocent bystanders. The presence of IgG class autoantibodies to C1q in lupus was first reported in 1984 [146]. Further investigations revealed that the majority of IgG binding to C1q in solid phase assays was attributable to autoantibodies reacting with an epitope only exposed in structurally modified C1q [147]. Such a change in structure, to reveal a ‘neo-epitope’, may follow proteolytic cleavage, a conformational change following activation or following binding to another protein. Thus, it seems likely that anti-C1q antibodies develop as a part of an autoantibody response to structurally altered forms of C1q, possibly evolving from binding to cells, apoptotic structures, proteins or ICs. Exposure of hidden epitopes on conformationally changed antigens or the appearance of neo-epitopes on post-translationally modified autoantigens (*e.g.* glycosylation or citrullination) may result in the production of various autoantibodies [119, 124, 148–150]. Increased immunogenicity of modified autoantigens is also supported by data from experiments in mice [110].

The binding of CRP to cellular FcγRs is believed to account for its opsonizing properties [6].

It is conceivable that mCRP exposed on cellular surfaces may be a target for anti-CRP autoantibodies. In this connection, and regarding earlier findings of mCRP expression on human peripheral blood lymphocytes [151, 152] and accelerated apoptosis of lymphocytes from SLE patients [113], the inverse relation between high anti-CRP antibody levels and lymphopenia, which we demonstrated [130], is intriguing. Hypothetically, this correlation may result from opsonization of lymphocytes expressing mCRP on their cell surfaces, leading to increased elimination of circulating lymphocytes via the reticuloendothelial system. CRP facilitates the clearance of apoptotic debris by FcγR-mediated uptake in phagocytes [153] and when the tissue microenvironment becomes acidic due to inflammation CRP is dissociated to mCRP, which further enhances the binding of ICs to FcγRs [60]. Speculatively, anti-CRP autoantibodies could interfere with the physiological mCRP-mediated removal of ICs and/or nuclear constituents [58–60, 111, 153]. In addition, via C1q-binding CRP has complement activating properties, which also promote IC clearance [61, 154].

It is not likely that the presence of anti-CRP antibodies explains the relative failure of CRP response in patients with active SLE. Instead, the possibility of post-translational modification of the CRP molecule by glycosylation could be relevant both with regard to clearance of circulating CRP and the induction of anti-CRP autoantibodies. In fact, it has been demonstrated that CRP molecules in different disease states, including SLE, differ both in their carbohydrate content and their amino acid sequences [155, 156]. Interestingly, CRP purified from pooled sera of SLE patients showed a single band by SDS/PAGE, suggesting an identical abnormal glycosylated variant [155].

Three research groups have reported the occurrence of autoantibodies to MBL in SLE [136–138]. Seelen and coworkers also presented data indicating that reduced functional activity of MBL leads to enhanced production of autoantibodies against cardiolipin and C1q [157].

When CRP dissociates into its neo-CRP subunits and deposits on tissue surfaces, it is conceivable that it may result in immunization in a way similar to that of C1q and MBL. Anti-CRP may also have other pathogenic implications, for instance by reacting with surface-bound CRP on cells and tissue surfaces. Hypothetically, mCRP exposed on surfaces of apoptotic bodies, for instance in the renal glomeruli [158, 159], could constitute a target for circulating anti-CRP antibodies *in situ*, which may subsequently initiate or amplify inflammation in the target organs [63, 160]. In analogy, both anti-C1q antibodies and presence of C1q-containing ICs in glomerulus are needed to induce renal exacerbation in lupus-prone mouse models [161] (Figure 5).

5. Conclusion

During the last few years, new interest and knowledge has emerged regarding the highly conserved proteins of the innate immune system – the acute phase proteins – in relation to autoimmunity. CRP, MBL, C1q and SAP all display important biological functions with implications for the etiopathogenesis of many autoimmune diseases. In this context, several research groups have reported the occurrence of autoantibodies directed against native or structurally altered forms of acute phase proteins. In some cases, the levels of such antibodies seem to correlate with disease activity or certain disease manifestations. For instance, levels of anti-CRP antibodies have proved to be a useful tool to assess disease activity in SLE [130, 133]. However, extensive and elaborate studies on well-characterized patient materials aiming at defining the potential clinical benefits of measuring this ‘new’ group of autoantibodies are warranted.

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Figure legends

Figure 1: The pentameric CRP molecule displayed as a ribbon diagram.

Figure 2: Schematic illustration of CRP interactions with the human complement system.

Figure 3: Significant correlation between anti-CRP antibody levels and disease activity in SLE (SLEDAI) [130].

Figure 4: IgG-binding to monomeric CRP (mCRP) but not to pentameric CRP (pCRP) in anti-CRP-positive SLE serum ($P < 0.0001$ for both dilutions).

Figure 5: Hypothetic roles and consequences of CRP $\pm$ complement and anti-CRP antibodies in the handling of apoptotic material:

1. A normal hepatocyte formation and release of CRP in response to IL-6 stimulation leads to sufficiently high levels of circulating CRP, which binds to nuclear antigens from apoptotic cells. CRP also binds complement factor C1q. Surface-bound CRP activates the classical complement pathway and mediates clearance of the particles via Fc γ - and complement receptors on non-parenchymal liver cells.
2. If circulating CRP levels are insufficient, apoptotic material will escape the reticuloendothelial system, leading to production of autoantibodies, *e.g.* antinuclear antibodies (ANA).
3. Circulating nuclear antigens and/or CRP may also be deposited in tissues, *e.g.* kidneys. Circulating ANA and/or CRP may then be adsorbed to their corresponding tissue-bound antigens, eventually leading to local complement activation and recruitment of leukocytes, which in turn leads to local tissue inflammation, *e.g.* glomerulonephritis.

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Figure 1

Figure 1: The pentameric CRP molecule displayed as a ribbon diagram.

Figure 2: Schematic illustration of CRP interactions with the human complement system.

Figure 3

Figure 3: Significant correlation between anti-CRP antibody levels and disease activity in SLE (SLEDAI) [130].

Figure 4

Figure 4: IgG-binding to monomeric CRP (mCRP) but not to pentameric CRP (pCRP) in anti-CRP-positive SLE serum ($P < 0.0001$ for both dilutions).

Figure 5

Figure 5: Hypothetic roles and consequences of CRP $\pm$ complement and anti-CRP

antibodies in the handling of apoptotic material:

1. A normal hepatocyte formation and release of CRP in response to IL-6 stimulation leads to sufficiently high levels of circulating CRP, which binds to nuclear antigens from apoptotic cells. CRP also binds complement factor C1q. Surface-bound CRP activates the classical complement pathway and mediates clearance of the particles via Fc γ - and complement receptors on non-parenchymal liver cells.
2. If circulating CRP levels are insufficient, apoptotic material will escape the reticuloendothelial system, leading to production of autoantibodies, *e.g.* antinuclear antibodies (ANA).
3. Circulating nuclear antigens and/or CRP may also be deposited in tissues, *e.g.* kidneys. Circulating ANA and/or CRP may then be adsorbed to their corresponding tissue-bound antigens, eventually leading to local complement activation and recruitment of leukocytes, which in turn leads to local tissue inflammation, *e.g.* glomerulonephritis.