ORIENTAÇÃO DE RECURSOS

BANCA: PSU-GO

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QUESTÃO: 100

Prezada banca,

Venho, respeitosamente, por meio deste solicitar recurso na questão de número 100 na prova de acesso direto tipo 2.

A questão versa sobre a proteína C-reativa (PCR) e procura a alternativa que traz uma característica acerca deste reagente de fase aguda. O gabarito fornecido foi a letra B, que traz "ser sintetizada exclusivamente no fígado, à semelhança de outras proteínas de fase aguda". No entanto, esta alternativa não está correta.

Segundo Sproston NR et al no artigo "Role of C-Reactive Protein at Sites of Inflammation and Infection" publicado em 2018 no periódico Frontiers in Immunology, temos: "CRP is synthesized primarily in liver hepatocytes but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes." Em tradução livre, "PCR é produzida, primariamente, pelos hepatócitos, mas também por células musculares lisas, macrófagos, células endoteliais, linfócitos e adipócitos.

Segundo Jialal I et al no artigo "C-Reactive Protein: Risk Marker or Mediator in Atherothrombosis?" publicado em 2004 no periódico Hypertension (American Heart Association), temos: "Much recent data challenge the dogma that CRP is exclusively produced by the liver. Indeed, cogent data suggest that it is produced in the atherosclerotic lesion (especially by smooth muscle cells and macrophages), the kidney, neurons, and alveolar macrophages.". Em tradução livre, "Dados mais recentes desafiam o dogma de que a PCR é exclusivamente produzida pelo fígado. De fato, dados convincentes sugerem que também é produzida por lesões ateroscleróticas (especialmente por células musculares lisas e macrófagos), rim, neurônios e macrófagos alveolares".

Segundo Black S et al no artigo "C-reactive Protein" publicado em 2004 no Journal of Biological Chemistry, temos: "Extrahepatic synthesis of CRP has also been reported in neurons, atherosclerotic plaques, monocytes, and lymphocytes." Em tradução livre, "Produção extrahepática da PCR também já foi relatada em neurônios, placas ateroscleróticas, monócitos e linfócitos".

Pelo que foi exposto anteriormente, solicito gentilmente a anulação da questão.

Att,

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REVIEW
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Role of C-Reactive Protein at Sites of Inflammation and Infection

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C-reactive protein (CRP) is an acute inflammatory protein that increases up to 1,000-fold at sites of infection or inflammation. CRP is produced as a homopentameric protein, termed native CRP (nCRP), which can irreversibly dissociate at sites of inflammation and infection into five separate monomers, termed monomeric CRP (mCRP). CRP is synthesized primarily in liver hepatocytes but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes. Evidence suggests that estrogen in the form of hormone replacement therapy influences CRP levels in the elderly. Having been traditionally utilized as a marker of infection and cardiovascular events, there is now growing evidence that CRP plays important roles in inflammatory processes and host responses to infection including the complement pathway, apoptosis, phagocytosis, nitric oxide (NO) release, and the production of cytokines, particularly interleukin-6 and tumor necrosis factor-α. Unlike more recent publications, the findings of early work on CRP can seem somewhat unclear and at times conflicting since it was often not specified which particular CRP isoform was measured or utilized in experiments and whether responses attributed to nCRP were in fact possibly due to dissociation into mCRP or lipopolysaccharide contamination. In addition, since antibodies for mCRP are not commercially available, few laboratories are able to conduct studies investigating the mCRP isoform. Despite these issues and the fact that most CRP research to date has focused on vascular disorders, there is mounting evidence that CRP isoforms have distinct biological properties, with nCRP often exhibiting more anti-inflammatory activities compared to mCRP. The nCRP isoform activates the classical complement pathway, induces phagocytosis, and promotes apoptosis. On the other hand, mCRP promotes the chemotaxis and recruitment of circulating leukocytes to areas of inflammation and can delay apoptosis. The nCRP and mCRP isoforms work in opposing directions to inhibit and induce NO production, respectively. In terms of pro-inflammatory cytokine production, mCRP increases interleukin-8 and monocyte chemoattractant protein-1 production, whereas nCRP has no detectable effect on their levels. Further studies are needed to expand on these emerging findings and to fully characterize the differential roles that each CRP isoform plays at sites of local inflammation and infection.

Keywords: C-reactive protein, native C-reactive protein, monomeric C-reactive protein, inflammation, infection

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C-REACTIVE PROTEIN (CRP)

C-reactive protein is a homopentameric acute-phase inflammatory protein, a highly conserved plasma protein that was initially discovered in 1930 by Tillet and Francis while investigating the sera of patients suffering from the acute stage of *Pneumococcus* infection and was named for its

reaction with the capsular (C)-polysaccharide of *Pneumococcus* (1). In the presence of calcium, CRP binds to polysaccharides such as phosphocholine (PCh) on microorganisms and triggers the classical complement pathway of innate immunity by activating C1q (2). CRP has many homologs in vertebrates and some invertebrates (3) and is a member of the pentraxin family, which includes other structurally related molecules such as serum amyloid A (4). Transcriptional induction of the *CRP* gene mainly occurs in hepatocytes in the liver in response to increased levels of inflammatory cytokines, especially interleukin-6 (IL-6) (5).

C-reactive protein exhibits elevated expression during inflammatory conditions such as rheumatoid arthritis, some cardiovascular diseases, and infection (6). As an acute-phase protein, the plasma concentration of CRP deviates by at least 25% during inflammatory disorders (7). The highest concentrations of CRP are found in serum, with some bacterial infections increasing levels up to 1,000-fold (8). However, when the stimuli ends, CRP values decrease exponentially over 18–20 h, close to the half-life of CRP (9). CRP plasma levels increase from around 1 μg/mL to over 500 μg/mL within 24–72 h of severe tissue damage such as trauma and progressive cancer (10). IL-6 is reported to be the main inducer of *CRP* gene expression, with IL-1 enhancing the effect (11). However, although IL-6 is necessary for *CRP* gene induction, it is not sufficient to achieve this alone (12).

There are many factors that can alter baseline CRP levels including age, gender, smoking status, weight, lipid levels, and blood pressure (13). The average levels of CRP in serum in a healthy Caucasian is around 0.8 mg/L, but this baseline can vary greatly in individuals due to other factors, including polymorphisms in the *CRP* gene (14). The human *CRP* gene can be found at 1q23.2 on the long arm of chromosome 1, and to date, there have been no allelic variations or genetic deficiencies discovered for this gene although some polymorphisms have been identified (13). For example, up to 50% of baseline variance in CRP is associated with the number of dinucleotide repeats found in an intronic region of the gene (15).

There is no significant seasonal variation in baseline CRP concentration; however, twin studies show a significant heritable component in baseline CRP values that is independent of age and body mass index (16). Pankow et al. (17) found evidence that interindividual variation in blood CRP levels is 35–40% heritable. Increased CRP levels are typically associated with disease, but liver failure is one condition observed to impair CRP production. Very few drugs reduce elevated CRP levels unless they treat the underlying pathology that is causing the acute-phase stimulus (16).

There is emerging research that oral hormone replacement therapy (HRT) causes background levels of circulating CRP to increase in postmenopausal women, increasing the risk of thrombotic events such as clots (18). Corcoran et al. (19) found that a combination of estrogen and oxidized low-density lipoproteins (oxLDLs) increased CRP expression in a model of coronary heart disease in both older men and postmenopausal women, but no effect on CRP expression was seen when estrogen supplementation was replaced with testosterone. Ridker et al. (20) found that healthy postmenopausal women had nearly twofold increased levels of circulating CRP when they were taking oral HRT and that CRP was the most affected inflammatory marker. Numerous studies have confirmed that CRP is a predictive marker for cardiovascular disease and that HRT use in postmenopausal women increases the risk of stroke and blood clots (20–23).

Interestingly the mode of HRT delivery appears to influence the effect on circulating CRP levels. Vongpatanasin et al. (23) found that estrogen administered orally increases circulating CRP levels twofold, whereas estrogen administered transdermally had no effect on circulating CRP levels. Similarly, patients taking oral HRT containing estrogens combined with progestogens had an increase in circulating CRP levels in the first 12 months of therapy compared to those using transdermal therapy who demonstrated no change in circulating CRP levels (22). In contrast, several other studies have instead shown that circulating CRP levels are reduced in humans treated with transdermal estrogen (24, 25). A reduction in CRP levels following peripheral estrogen administration supports the findings of Ashcroft et al. (26) demonstrating that estrogen reduces the inflammatory response during wound healing. The effect of transdermal administration of estrogen on local CRP levels in peripheral tissues such as skin has not yet been elucidated, with previous studies measuring only circulating levels of CRP.

ISOFORMS OF CRP

The pentameric protein, termed native CRP (nCRP), is characterized by a discoid configuration of five identical non-covalently bound subunits, each 206 amino acids long with a molecular mass of about 23 kDa. These five subunits lie in the same orientation around a central pore and arranged in a characteristic "lectin fold" with a two-layered beta sheet (15). Each subunit lies with the PCh binding site facing the "recognition" face of the nCRP molecule (27). The molecule has a ligand-binding face that has a characteristic feature of having two calcium ions per protomer. The calcium ions are important for the stability and binding of ligands. The "opposite" face interacts with the C1q aspect of the complement pathway as well as interacting with Fc receptors (6).

The pentameric protein is synthesized primarily in liver hepatocytes but has also been reported to be synthesized in other cell types such as smooth muscle cells (28), macrophages (29), endothelial cells (30), lymphocytes, and adipocytes (31). CRP is first synthesized as monomers and then assembled into the pentamer in the endoplasmic reticulum of the source cell. In hepatocytes, the pentameric protein is retained in the endoplasmic reticulum by binding to two carboxylesterases, gp60a and gp50b (32). While in a resting (non-inflammatory) state, CRP is released slowly from the endoplasmic reticulum, but following an increase in inflammatory cytokine levels, the binding CRP to the carboxylesterases decreases and CRP is secreted rapidly (6). The stimulation of CRP synthesis mainly occurs in response to pro-inflammatory cytokines, most notably IL-6 and to a lesser degree IL-1 and tumor necrosis alpha (TNF-α) (33).

Pentameric CRP can be irreversibly dissociated, with the resultant free subunits termed monomeric (or modified) CRP (mCRP). The dissociation of nCRP into free subunits has been observed at either high concentrations of urea (34) or high temperatures in the absence of calcium (35). The mCRP molecules are distinguished from nCRP by their different antigenic, biological,

and electrophoretic activities (36) and by the fact that they express different neoepitopes (37). The two isoforms of CRP have been shown to have distinct biological functions in the inflammatory process. For example, Khreiss et al. (37) provided evidence that nCRP suppresses the adherence of platelets to neutrophils, whereas mCRP enhances these interactions. This difference in function can be explained by the two isoforms binding to differing types of Fcgamma (Fcγ)-receptor involved in the signaling process. The mCRP isoform utilizes the low-affinity immune complex binding immunoglobulin G (IgG) receptor called FcγRIIIb (CD16b) on neutrophils and FcγRIIIa (CD16a) on monocytes, while nCRP binds to the low-affinity IgG receptor FcγRIIa (CD32) (38).

Evidence is emerging of new structural intermediates of CRP with biological function. Ji et al. (39) found that the native protein first dissociates into subunits while retaining some of the native conformation before fully dissociating into mCRP. This intermediate, termed $mCRP_m$, is formed when the nCRP is bound to cell membranes and then dissociates, allowing the subunits to retain some of the conformation before fully dissociating into mCRP subunits on detachment from the membrane. It is suggested that this transitional process allows for more effective regulation of CRP function, with $mCRP_m$ allowing for the enhanced activation of the classical complement pathway (39). Further work needs to be conducted to determine the biological functions of the $mCRP_m$ intermediate, but initial findings suggest that it behaves in a similar manner to mCRP, typically promoting pro-inflammatory activity.

CRP IN DISEASE PATHOLOGY

The majority of CRP research has focused on the role of CRP and its isoforms on cardiovascular disease and stroke. CRP is used as a clinical marker of inflammation, with elevated serum levels being a strong independent predictor of cardiovascular disease in asymptomatic individuals (40). CRP levels have been linked to prognosis in patients with atherosclerotic disease, congestive heart failure, atrial fibrillation, myocarditis, aortic valve disease, and heart transplantation, suggesting that it has an active role in the pathophysiology of cardiovascular disease (41). High-sensitivity assays, such as nephelometric assays, are used to detect baseline levels of CRP and patients who are at risk of cardiovascular disease. An individual with a CRP level higher than 3 mg/L has an increased risk of coronary heart disease (42), and this risk increases in those with type 2 diabetes (43).

Increased levels of CRP have been found in patients with appendicitis, cholecystitis, pancreatitis, and meningitis (44). In patients suffering possible symptoms of appendicitis, acute appendicitis can be excluded in those with CRP levels lower than 25 mg/L in blood taken 12 h after the onset of symptoms (45). When clinical symptoms of cholecystitis occur concurrently with CRP levels of over 30 mg/L, an accurate diagnosis of cholecystitis can be obtained with 78% sensitivity, suggesting that CRP is a more sensitive marker than erythrocyte sedimentation rate and white cell count in supporting cholecystitis diagnosis (46). In terms of acute pancreatitis, CRP levels of more than 210 mg/L were able to discriminate between mild and severe cases, with 83% sensitivity and 85% specificity (47). Serum CRP is elevated in bacterial meningitis, and resolution of symptoms following treatment with antibiotics is slow in those with the highest CRP levels (48). Measurement of CRP in cerebrospinal fluid has a sensitivity of 100% and a specificity of 94% for differentiating between patients with bacterial meningitis, viral meningitis, and no infection (49).

Although studies have shown that CRP levels increase during infections and inflammatory diseases, the precise role of CRP isoforms in their development and progression remains largely unknown. Thus, urgent investigations are required to determine the effects of each CRP isoform on specific cellular processes during disease development. Evidence shows that in general nCRP tends to exhibit more anti-inflammatory activities relative to the mCRP isoform, possibly because nCRP limits the generation of the membrane attack complex (MAC) and C5a, thus inhibiting the alternative complement activation (50). In contrast, mCRP can have marked pro-inflammatory properties both *in vitro* and *in vivo* by promoting monocyte chemotaxis and the recruitment of circulating leukocytes to areas of inflammation *via* Fcy-RI and Fcy-RIIa signaling (50). Thus, in addition to therapeutic strategies to inhibit CRP activity (51), more targeted therapies have been proposed for the treatment of CRP-mediated pathologies, including inhibiting mCRP activity (52) or preventing the dissociation of nCRP into mCRP (53).

CRP AND INFLAMMATION

C-reactive protein levels are known to increase dramatically in response to injury, infection, and inflammation (**Figure 1**). CRP is mainly classed as an acute marker of inflammation, but research is starting to indicate important roles that CRP plays in inflammation. CRP is the principal downstream mediator of the acute-phase response following an inflammatory event and is primarily synthesized by IL-6-dependent hepatic biosynthesis (54, 55). The main role of CRP in inflammation tends to focus around the activation of the C1q molecule in the complement pathway leading to the opsonization of pathogens. Although CRP can initiate the fluid phase pathways of the host defense by activating the complement pathway, it can also initiate cell-mediated pathways by activating complement as well as to binding to Fc receptors of IgG (54). CRP binds to Fc receptors with the resulting interaction leading to the release of pro-inflammatory cytokines (56). CRP also has the ability to recognize self and foreign molecules based on the pattern recognition, something that other activators of complement such as IgG cannot achieve because these molecules only recognize distinct antigenic epitopes (56).

Evidence suggests that CRP is not only just a marker of inflammation but also plays an active role in the inflammatory process. However, most early research in the literature only refers to CRP and does not distinguish between the two isoforms. Thus, unlike more recent publications, the findings of early work on CRP can seem somewhat unclear and at times conflicting since it was often not specified which CRP isoform was measured or utilized in experiments, whether responses attributed to nCRP were in fact possibly due to partial/full dissociation into mCRP or if lipopolysaccharide (LPS) contamination could be present. More recent studies generally distinguish between the differential

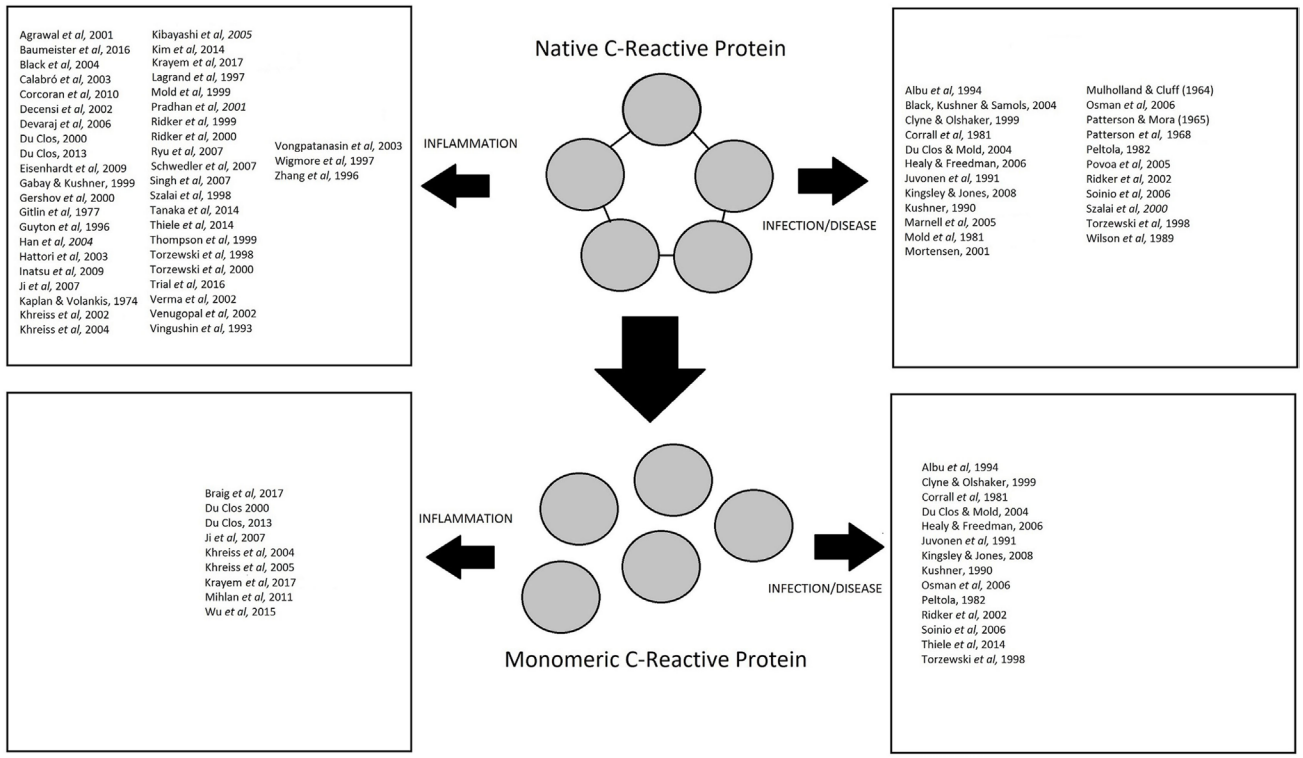

FIGURE 1 | Summary of studies investigating the role of native C-reactive protein (CRP) and monomeric CRP in inflammation, infection, and disease.

effects of each CRP isoform on inflammatory processes, but since antibodies for mCRP are not commercially available to date, few laboratories are able to conduct studies investigating the mCRP isoform.

There is increasing evidence that CRP has a functional role in the inflammatory process. It is well established that CRP is an acute marker of inflammation and that its concentration increases in circulation during inflammatory events. CRP is deposited at sites of inflammation and tissue damage in both naturally occurring and experimental conditions (57). However, there is a raft of published data investigating CRP that does not consider its two different isoforms. Understandably, when some of these studies were conducted, the existence of two CRP isoforms was not well established and available antibodies would have been raised against the pentameric nCRP alone. Another issue with published data is that CRP localization is often investigated in only a narrow range of inflammatory conditions and tissue types. Although the mCRP isoform has been shown to be insoluble in plasma, it becomes localized in inflamed tissues and amplifies a pro-inflammatory response by a positive feedback loop (58).

The literature suggests that CRP binds to damaged cell membranes and contributes to the inflammatory response (59), with CRP molecules becoming associated with terminal complement complexes, especially in atherosclerotic lesions (60). Lagrand et al. (61) provided evidence that CRP localizes to infarcted heart tissue and promotes local complement activation, triggering further damage to the heart tissue. Gitlin et al. (62) concluded that CRP was localized to the nuclei of cells within the synovium of rheumatoid arthritis patients, but the cell type was not identified at the time. However, other studies indicate no significant localization of CRP in a number of pathologies, suggesting that CRP is found predominantly in the fluid phase rather than becoming deposited in tissues at sites of inflammation or injury (63). There has been little research conducted on the localization of CRP in inflammatory cells to date. There is a correlation between the localization of CRP in neutrophil infiltrates, especially in lesions of vasculitis and allergic encephalomyelitis (64, 65).

CRP AND INFECTION

C-reactive protein is a marker for inflammation, and its levels increase during bacterial infection (66). Kingsley and Jones (67) stated that CRP increases during infection in response to monocytic mediators such as IL-1 and IL-6 and that it has a stable decay rate. It is thought that most of the interaction between CRP and the immune response to pathogens involves the binding of CRP to PCh and the activation of the classical complement pathway (68). Mold et al. (69) showed that CRP provides mice with protection against infection by the gram-positive pathogen *Streptococcus pneumoniae* by binding to a PCh determinant of the pathogen cell wall and activating the complement pathway. Mice pretreated with 200 μg CRP before being infected showed an increase in percentage survival across all pathogen doses tested. The study concluded that the ability of CRP to protect against infection lies

in its ability to bind to pneumococcal polysaccharide C in the bacterial cell wall (69).

Szalai et al. (70) showed that CRP can confer protective benefits against *Salmonella enterica* serovar Typhimurium, a gram-negative pathogen that provides a model of typhoid fever in mice. By using transgenic mice expressing human CRP, the study found that CRP offered protection against a low dose of Typhimurium and increased resistance to a fatal infection with a low dose of Typhimurium. Szalai et al. (70) concluded that CRP increases the early clearance of intravenously injected bacteria from the blood and reduces dissemination of bacteria to the liver and spleen during the initial stages of infection, thus allowing the mice to survive infection.

Marnell et al. (71) reviewed the protective role CRP against *Haemophilus influenza* infection in both transgenic and wild-type mice treated by passive inoculation. CRP was shown to bind the pneumococcal C-polysaccharide of bacteria and opsonize them for phagocytosis. This process did not require the use of the Fcγ receptors, suggesting that CRP is not primarily protective by direct opsonization but more likely through activation of complement and subsequent opsonophagocytosis.

Kingsley and Jones (67) tested whether CRP could be used to distinguish different types of infections. They discovered that mean CRP levels in a spreading infection were higher than those in other colonized, critically colonized, and locally infected groups. All cases of infection showed an increase in CRP levels compared to non-infected controls, but CRP levels could not distinguish between the infection types, showing that it is infection in general that causes CRP levels to increase, rather than the type of infection. This was also noted by Healy and Freedman (66) who showed that CRP levels can be used only as a method of detecting infection, rather than distinguishing it.

C-reactive protein can mediate host responses to *Staphylococcus aureus* including some protective function against infection and an increase in phagocytosis of this pathogen. Povoa et al. (72) stated that the normal CRP level for the healthy population is about 0.08 mg/dL, and this increases to more than 8.7 mg/dL during chronic *S. aureus* infection. Thus, CRP can be used as an indicator of infection, alongside a body temperature of more than 38.2°C. Patterson and Mora (73) observed that enhanced resistance to intraarticular infection with *S. aureus* in chickens was associated with an increase in serum CRP and that isolated preparations of the protein produced antibacterial activity. Mulholland and Cluff (74) discovered that endotoxin-induced changes in resistance to local infection with *S. aureus* in rabbits were correlated with the circulating levels of leukocytes in the blood. The study showed that induced resistance was paralleled by an increase in CRP and leukocytes. This was collaborated by Patterson et al. (75) who found an association between CRP and non-specific resistance to infection, including *S. aureus* and showed that CRP was acting upon the polysaccharide bacterial cell wall. Black et al. (3) stated that CRP enhances the *in vitro* phagocytosis of many microorganisms (including *S. aureus*) by leukocytes. Their work confirmed this finding even in the absence of complement, suggesting that the enhancement of phagocytosis by CRP is due to the interactions with Fcγ receptors.

In summary, evidence shows that CRP is not only a marker of infection and inflammation but that CRP also has a protective role against bacterial infections (**Figure 1**), principally through the activation of complement and subsequent opsonization of pathogens.

CRP AND COMPLEMENT

Complement is one of the major defenses of the human immune system that is involved in the clearance of foreign particles and organisms after recognition by antibody. The complement pathway is made up of 35 plasma or membrane proteins that is an important system in immunity and the defense of the host against microbial infection. The components of the complement pathway can be activated in three different pathways to trigger a cascade of proteins, which are used to help bind microbial surfaces for the immune system to recognize and activate phagocytosis (76, 77). The classical pathway is triggered by a target bound antibody, whereas the lectin pathway is triggered by microbial repetitive polysaccharide structures and the alternative pathway is triggered by recognition of other foreign surface structures. Even though the triggers are different, the three pathways merge at a pivotal activation of the C3 and C5 convertases. A majority of the components are synthesized in the liver, C1 in the intestinal epithelium, and factor D in the adipose tissue (76).

The role of CRP in activating the complement pathway has been extensively investigated. In 1974, Kaplan and Volanakis first described the ability of CRP to activate the classical complement pathway using C-polysaccharide and phospholipid ligands (59). The activation of complement by CRP is considered a crucial step since when complement was depleted, and the effects of CRP were abrogated (50).

The opposite face of the CRP molecule, which is typically complexed with polyvalent ligand or chemically cross-linked, binds to C1q and activates the classical complement pathway (56). C1q is a large 460-kDa molecule made up of six identical subunits, each made up of three structurally similar but distinct polypeptide chains (78). This process requires the use of calcium ions for the stable formation of the C1 complex (79). CRP is most effective during the early classical pathway activation of C1, C4, and C2 (80). This is because the ligand-bound interaction with C1q leads to the formation of C3 convertase, triggering the complement activation of C1–C4 but with little activation of the late complement proteins C5–C9 (15).

Activation of complement by CRP varies from activation by antibody in that CRP has selective activation of early components without the need to form the MAC. In addition to activating the classical complement pathway, CRP can inhibit the alternative complement pathway by decreasing C3 and C5 convertase activities and inhibiting the complement amplification loop. This is achieved by the recruitment of factor H to the cell surface and by preventing C5 convertase cleaving C5 to recruit neutrophils and prevent the formation of the MAC (71). As the levels of CRP increase, this causes decreased binding of C3b and C5b-9 to liposomes, possibly also explaining the lack of C5–C9 consumption by CRP during classical pathway activation (80).

Both the initiator (C1q) and the inhibitor (C4bp) of the classic complement pathway compete for mCRP binding, with the competition controlling the local balance of activation and

inhibition of the pathway in tissues (58). Interestingly, mCRP but not nCRP binds the C4bp inhibitor, suggesting that mCRP rather than nCRP is able to provide a high degree of control over the classic complement pathway (58).

CRP AND APOPTOSIS

There has been little research conducted into the effect of CRP on the proliferation process. However, there is evidence that CRP has a major role in the apoptosis process. Devaraj et al. (81) showed that CRP stimulates the production of pro-apoptotic cytokines and inflammatory mediators *via* the activation of Fc-γ receptors. The pro-apoptotic cytokines and inflammatory mediators induced by CRP include interleukin-1β (IL-1β), tumor necrosis factor-α (TNFα), and reactive oxygen species (82, 83).

C-reactive protein induces the upregulation of p53 in monocytes and affects cell cycle kinetics of monocytes through CD32 (FcγRII), inducing apoptosis by G_2/M arrest in the cell cycle (84). CD32 receptors have been shown to trigger apoptotic signals and are expressed in a subset of monocytes that polarize to pro-inflammatory macrophages, suggesting that CRP may dampen macrophage-driven pro-inflammatory responses by inducing apoptosis (85).

C-reactive protein is elevated in cardiovascular disorders and is a mediator of atherosclerosis. CRP localizes directly in the atherosclerotic plaques where it induces the expression of genes that are directly involved in the adhesion of monocytes and the recruitment of intracellular molecules such as E-selectin and monocyte chemoattractant protein-1 (MCP-1). CRP has also been shown to play a role in mediating low-density lipoprotein uptake in macrophages and activating the complement system, which is implicated in atherogenesis (86). Apoptosis occurs in atherosclerotic plaques and the number of apoptotic cells increase as lesions become more advanced. As cells become apoptotic, they start to cause plaque disruption, leading to the expression of growth arrest- and DNA damage-inducible gene 153 (*GADD153*). *GADD153* upregulation has been shown to induce G_1 arrest or apoptosis in some cancer cell lines (87). Blaschke et al. (88) found that CRP can induce the apoptosis of human coronary vascular smooth muscle cells through a caspase-mediated mechanism, especially through increased caspase-3 activity. CRP was co-localized to the *GADD153* gene product in atherosclerotic lesions suggesting that CRP is triggering the caspase cascade and apoptosis by inducing the expression of the *GADD153* gene.

There is little research on how the two isoforms of CRP interact with the apoptosis process. It is suggested that CRP can exert anti-apoptotic activity but only when the cyclic pentameric structure is lost. This would suggest that the apoptotic activity of CRP is induced through the native isoform. Native CRP (nCRP) can bind to low-affinity IgG FcγRIIa (CD32) and IgG FcγRI (CD64), leading to depressed functional activities, degranulation, and the generation of superoxide by inducible respiratory burst. On the other hand, mCRP binds to low-affinity IgG FcγRIIIb (CD16) that can delay apoptosis by triggering the cell survival pathway in neutrophils, even at low concentrations (89).

The nCRP isoform has the ability to opsonize apoptotic cells and induce the phagocytosis of damaged cells. Removal of nCRP-bound apoptotic monocytes and macrophages may be *via* FcγR-mediated phagocytosis (84). CRP binds to apoptotic cells, inhibits the assembly of terminal complement components, and promotes the opsonization of apoptotic cells (89, 90).

CRP AND NITRIC OXIDE (NO)

C-reactive protein has the ability to attenuate NO production with a marked reduction in *in vitro* angiogenesis, cell migration, and capillary-like tube formation by CRP at concentrations known to cause cardiovascular risk (91). Eisenhardt et al. (15) showed that CRP upregulated the expression of adhesion molecules and inhibited endothelial nitric oxide synthase (eNOS) expression, indicating a role for CRP in the production of NO. Several studies have revealed that CRP inhibits NO production *via* downregulation of eNOS in cardiovascular endothelial cells, thereby inhibiting angiogenesis *in vitro* and promoting the pathogenesis of atherosclerotic vascular disease through vasoconstriction, leukocyte adherence, and inflammation (14, 91–93). Another study found that it was in fact the nCRP isoform that downregulated eNOS and thus impaired endothelial function in ApoE knockout mice, *via* a mechanism thought to involve iNOS (94). Eisenhardt et al. (15) provided evidence that nCRP suppresses endothelium-dependent NO-mediated dilation by activating the p38 mitogen-activated protein kinase (MAP kinase) pathway and NADPH oxidase, suggesting that multiple pathways could be interacting with this process.

In contrast, mCRP has the opposite effect, enhancing NO production in neutrophils *via* upregulation of eNOS (95) with reverse transcription polymerase chain reaction showing an amplification of eNOS mRNA, but not iNOS or nNOS mRNA. This study highlighted that mCRP initiates calcium (Ca^{2+}) mobilization and activation of calmodulin and PI3 kinase to induce NO formation in neutrophils (95). The effect of CRP isoforms on other inflammatory cells, such as monocytes or macrophages, has not been investigated to date.

CRP ISOFORMS AND INFLAMMATORY CYTOKINES

There has been increasing evidence of a relationship between CRP and several pro-inflammatory cytokines.

IL-6 and CRP

Interleukin-6 is a pro-inflammatory cytokine secreted by various cells including inflammatory cells, keratinocytes, fibroblasts, and endothelial cells. It regulates the acute-phase response, and its main role involves the host response to infection (96). Even though it is predominantly a pro-inflammatory cytokine, in some cells, IL-6 can have regenerative and anti-inflammatory effects through the activation of membrane-bound IL-6 receptor signaling (97).

Interleukin-6 is synthesized in the initial stages of inflammation and induces a number of acute-phase proteins, including CRP (98). IL-6 can also reduce the production of fibronectin, albumin, and transferrin as well as the promotion of $CD4^+$ T

helper cells, which initiates the linking of innate and acquired immunity (98). There is a correlation between increasing levels of IL-6 during inflammation and increasing levels of CRP (11), with IL-6 inducing the *CRP* gene (12). However, most investigations of CRP production by IL-6 generally fail to indicate which isoforms of CRP are generated. In some cases, the antibodies used suggest that nCRP is present, but given IL-6 occurs at the sites of inflammation, the pentameric CRP may be dissociating into mCRP.

When CRP levels become elevated in atheroma, this leads to the induction of IL-6 by macrophages indicating that CRP may have a direct effect on IL-6 release (99). Krayem et al. (100) found that a combination of mCRP, nCRP, and oxLDL decreases IL-6 production in a model of atherosclerosis. This triple combination suggests that nCRP might downregulate the IL-6 release by macrophages that have been stimulated by both mCRP and oxLDL.

Interleukin-8 (IL-8) and CRP

Interleukin-8 is a cytokine produced by numerous cell types including inflammatory cells, keratinocytes, fibroblasts, and endothelial cells. IL-8 acts as a potent chemoattractant of neutrophils (101) and is overexpressed in chronic inflammatory diseases and during septic shock (102). IL-8 stimulates the release of granules from neutrophils by a process called degranulation. These granules contain a range of antimicrobial effectors that can help combat infection (103). Neutrophils are the first inflammatory cells to arrive at the site of inflammation, and they carry out the phagocytosis of bacteria and release chemotactic mediators that recruit other leukocytes to the affected tissue (103).

Kibayashi et al. (104) indicated that CRP plays a role in atherosclerosis *via* enhanced IL-8 production and increased expression of IL-8 mRNA in a CRP dose-dependent manner. They showed that CRP promotes IL-8 production *via* the activation of the ERK, p38 MAPK, and JNK pathways. Conversely, Wigmore et al. (105) indicated that IL-8 induces CRP production in hepatocytes, providing a potential feedback loop. The effect of the different CRP isoforms on IL-8 production has been investigated. Khreiss et al. (37) showed that nCRP had no detectable effect on the production of IL-8, whereas mCRP increased IL-8 production and IL-8 gene expression, promoting pro-inflammatory activity through a p38 MAPK-dependent mechanism. When treated with anti-CD16, there was inhibition of mCRP-stimulated NO formation and IL-8 release.

MCP-1 and CRP

Monocyte chemoattractant protein-1 is a cytokine that plays a role in the regulation of migration and infiltration of monocytes and macrophages (106). It is released by a number of cell types in response to events such as oxidative stress, cytokine release, and growth factor release (107). Human MCP-1 is known to bind to at least two receptors, and its production can be induced by interleukin-4 (IL-4), IL-1, TNF-α, bacterial LPS, and IFN-γ (107). There is increasing evidence that MCP-1 influences T-cell immunity by enhancing the secretion of IL-4 by T cells, as well as having a role in the migration of leukocytes (106). This in turn has a regulatory function on monocytes and macrophages, which are the major source of MCP-1 (107). MCP-1 is known to recruit monocytes to the vessel wall (99) and cause the arrest of rolling monocytes on endothelial monolayers that express E-selectin (108).

Evidence suggests that CRP stimulates endothelial cells to express MCP-1 (99) in addition to being a direct chemoattractant of monocytes itself (109). CRP can promote monocyte chemotactic activity in response to MCP-1 *via* upregulation of the monocyte chemotaxis receptor CCR2, with elevated CRP levels promoting the accumulation of monocytes in the atherogenic arterial wall (99). When vascular smooth muscle cells are exposed to increasing levels of CRP, MCP-1 mRNA substantially increased within 2 h and remained elevated for at least 24 h (110). Incubation with mCRP increases the secretion of MCP-1, leading to pro-inflammatory activity through a p38 MAPK-dependent mechanism, whereas nCRP had no detectable effect (37).

TNF-α and CRP

Tumor necrosis factor-α is a component of the acute-phase response and is mainly produced by monocytes and macrophages but can be produced by numerous other immune cells such as neutrophils, natural killer cells, and eosinophils. TNF-α is not usually detectable in a healthy host, but levels become elevated in a number of inflammatory and infectious conditions (111). The main stimulant of TNF-α production is LPS, but many other pathological conditions such as trauma infection, impaired wound healing, and heart failure also induce its production (111, 112). TNF-α mediates various processes such as cell proliferation, differentiation, and apoptosis.

Studies have shown a correlation between TNF-α production and the concentration of CRP. TNF-α induces a dose-dependent secretion of CRP in hepatocytes, which corresponds to an increase in CRP mRNA (28). Conversely, elevated CRP levels in atheroma leads to the induction of IL-1β, IL-6, and TNF-α production by macrophages (99). Research shows a close relationship between TNF-α and IL-6 levels in inflammation (113), with both TNF-α and IL-6 inducing the transcription of CRP (33). However, there is some contradictory evidence showing a potential inhibitory effect of CRP on TNF-α production, suggesting that there could be a negative feedback mechanism whereby elevated levels of CRP inhibit further stimulation of CRP by reducing the TNF-α production (114). A combination of mCRP, nCRP, and oxLDL also causes a decrease in both TNF-α and IL-6 production in a macrophage model of atherosclerosis (100). This triple combination suggests that nCRP might downregulate TNF-α and IL-6 production by macrophages stimulated by both mCRP and oxLDL.

CONCLUSION

C-reactive protein is a homopentameric acute-phase inflammatory protein that exhibits elevated expression during inflammatory conditions such as rheumatoid arthritis, some cardiovascular diseases, and infection. Evidence suggests that CRP is an important regulator of inflammatory processes and not just a marker of inflammation or infection. Key areas of inflammation and host responses to infection mediated by CRP include the complement pathway, apoptosis, phagocytosis, NO release, and cytokine

production. However, most research to date has investigated the role of CRP in the vascular tissues, highlighting the need to conduct further work to determine the precise role of CRP in peripheral tissues.

C-reactive protein is synthesized primarily in liver hepatocytes but also other cell types such as smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes. Evidence also suggests that the sex steroid hormone estrogen can influence CRP levels, with HRT having a profound influence on CRP levels in the elderly. Administration of oral HRT increases background levels of CRP in circulation, whereas evidence suggests that transdermal estrogen supplementation either reduces or has little effect on circulating CRP levels. A reduction in CRP levels following local administration of estrogen supports findings showing that estrogen reduces the inflammatory response in peripheral tissues such as skin.

There are two distinct isoforms of CRP, nCRP and mCRP, and the nCRP isoform can irreversibly dissociate at sites of inflammation, tissue damage, and infection into five mCRP subunits. Evidence indicates that nCRP often tends to exhibit more anti-inflammatory activities compared to mCRP. The nCRP isoform activates the classical complement pathway, induces phagocytosis, and promotes apoptosis. On the other hand, mCRP promotes the chemotaxis and recruitment of circulating leukocytes to areas of inflammation and can delay apoptosis. The nCRP and mCRP isoforms inhibit and induce NO production *via* downregulation and upregulation of eNOS, respectively. In terms of pro-inflammatory cytokine production, mCRP increases IL-8 and MCP-1 production, whereas nCRP has no detectable effect on their levels. CRP can also induce IL-6 and TNF-α production at sites of inflammation, again suggesting probable involvement of mCRP from the dissociation of nCRP. Further studies are needed to expand on these emerging findings and to fully characterize the differential roles that each CRP isoform play at sites of local inflammation and infection.

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Case Report

Nonconvulsive status epilepticus secondary to acute porphyria crisis☆

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ABSTRACT

Both variegate and acute intermittent porphyria can manifest with various neurological symptoms. Although acute symptomatic seizures have been previously described, they are typically tonic–clonic and focal impaired awareness seizures. Convulsive status epilepticus and epilepsia partialis continua are rare and have been described on a case report basis. To our knowledge, there are no previously reported cases describing non-convulsive status epilepticus (NCSE) with electroencephalogram (EEG) documentation in the setting of acute porphyria crisis. We report a unique presentation of NCSE, which resolved after administering levetiracetam in a patient with variegate porphyria, without a known seizure disorder.

1. Introduction

Variegate porphyria (VP) is an autosomal dominant disorder wherein heme biosynthesis is disrupted due to deficiency of protoporphyrinogen oxidase (PPOX) enzyme. The clinical presentation for VP is similar to acute intermittent porphyria (AIP), which typically presents with altered mentation and unexplained abdominal pain, chest, and back pain.

Neurological complications include peripheral neuropathy, acute encephalopathy, and acute symptomatic seizures. Seizures can affect 10%–20% of patients with acute porphyria; the most commonly reported are tonic–clonic and focal seizures with impaired awareness [1–4]. Although rare, convulsive status epilepticus and epilepsia partialis continua have been described in case reports [1,4]. There are no cases previously describing non-convulsive status epilepticus (NCSE) with electroencephalogram (EEG) documentation to our knowledge. We report a unique presentation of NCSE, which resolved after administration of levetiracetam in a patient with nearly 50 years of VP and without a known, prior seizure disorder who presented in an acute porphyria crisis.

2. Methods

We reviewed a case of nonconvulsive status epilepticus secondary to variegate porphyria crisis presenting to a tertiary referral medical center in the Southwest United States. A literature review was performed using PubMed.

3. Results

A 71-year-old woman, with a 50 year history of hereditary variegate porphyria and a history of end-stage renal disease on hemodialysis and coronary artery disease, presented to the emergency department with generalized weakness, lethargy, and confusion.

Upon admission, she was combative, moving all of her extremities, fluent speech, but intermittently confused. Infectious work-up was unrevealing. A chest radiograph, urine toxicology screen, urinalysis, comprehensive blood count, ammonia, and a head computed tomography (CT) were within normal limits. Her comprehensive metabolic profile showed evidence of her known chronic kidney disease. Her cerebrospinal fluid (CSF) analysis was within normal range excluding any infectious causative agent in the central nervous system. Her urine and blood cultures, HBsAg, thyroid stimulating hormone (TSH) and thiamine were unremarkable. Her urine porphobilinogen was elevated, 4.4 μmol/L (reference value is ≤1.3 μmol/L), which substantiated her acute porphyria crisis presentation. Interestingly, she had three previous admissions with no prior EEGs for unexplained encephalopathy, which were attributed to her acute porphyria crisis.

The following day her neurologic exam changed and she became stuporous, responding only to noxious stimuli. A neurology consultation was placed. Her physical examination demonstrated no spontaneous movement of her extremities as well as a lack of deep tendon reflexes.

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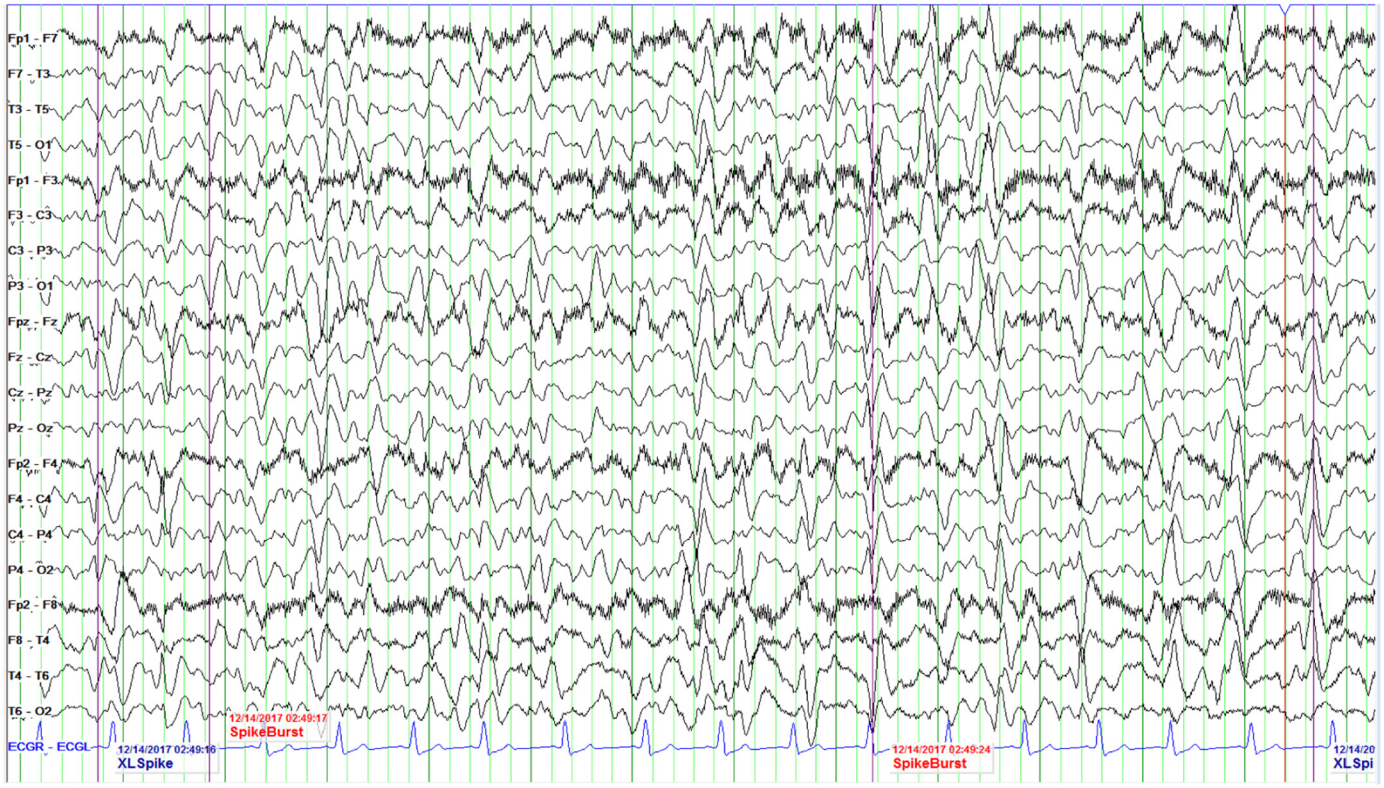

Fig. 1. Trend EEG revealed generalized 5–6 Hz theta with admixed 2–3 Hz delta frequency slowing. There was evidence of multifocal, independently occurring, sharp waves in the bilateral cerebral hemispheres occurring most prominently with stimulation. With stimulation, discharges occur with 1–2 Hz periodicity. Additionally, frequent generalized triphasic waves were seen. Triphasic waves were seen in addition to generalized slowing of the background activity. These collective findings in the setting of underlying diffuse cerebral dysfunction suggested the potential for seizures to arise.

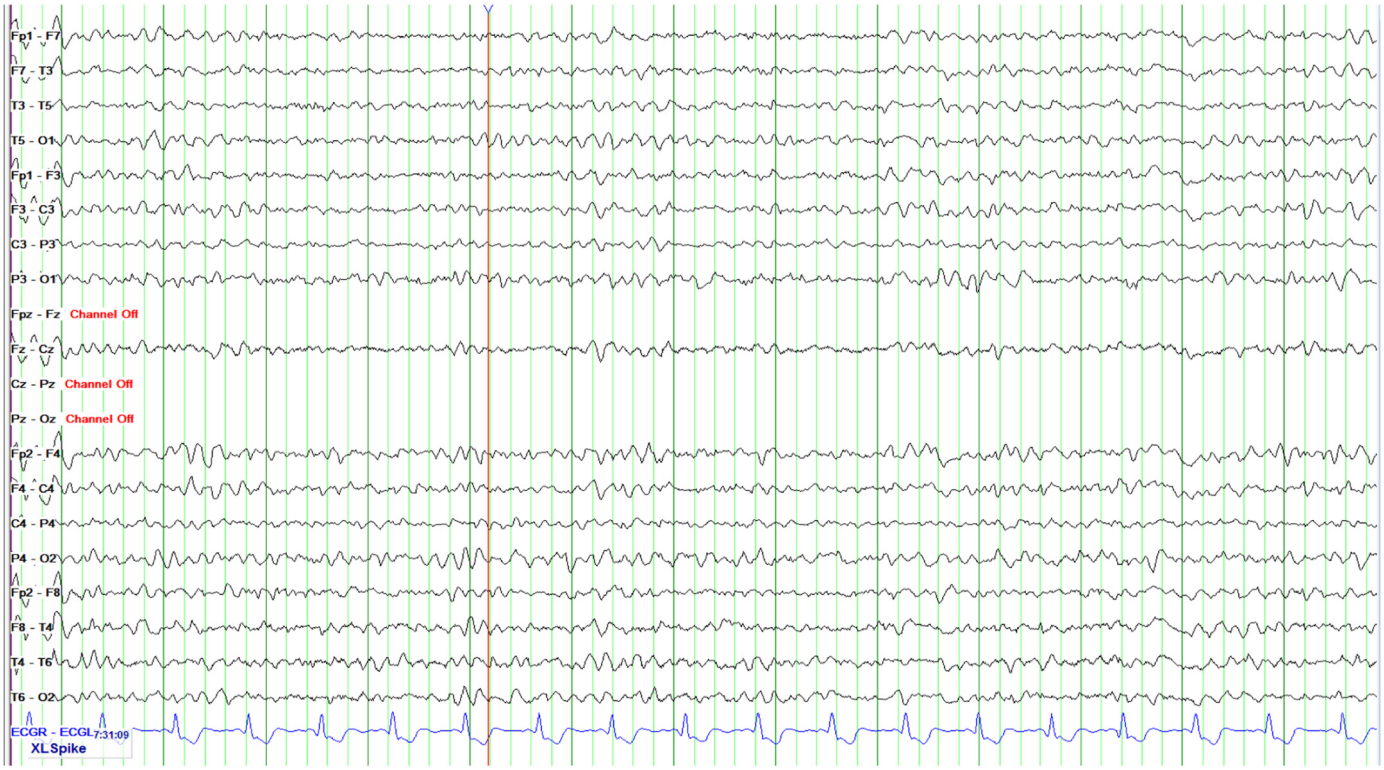

Fig. 2. Trend EEG shows dramatic improvement after levetiracetam administration.

She was nonverbal, and did not respond to verbal cues or follow commands. Her Glasgow Coma Scale was 9 (E3V2M4). An EEG was ordered which showed generalized triphasic waves and independent multifocal sharp waves, concerning for nonconvulsive status epilepticus (Fig. 1).

Her EEG pattern improved significantly upon a 2-gram loading dose of intravenous levetiracetam. She continued to receive maintenance levetiracetam doses and her mental status continued to improve (Fig. 2). Brain magnetic resonance imaging (MRI) was unremarkable (Fig. 3). Hemin was administered in the treatment of her acute porphyria. Ultimately, she was discharged home following return to her baseline.

Two months later, she followed up in epilepsy clinic with plans to repeat her EEG and continue levetiracetam in the interim. Her mental status and neurological exam were within normal limits.

4. Discussion

Porphyrias are a group of rare metabolic disorders caused by disruption in the heme synthesis pathway. There are eight enzymes involved in this pathway and VP is a type of porphyria caused by mutation in the PPOX gene. The estimated incidence of VP is 1 in 100,000 individuals in the general population of European decent [5].

Neurologic complications include peripheral neuropathy, acute encephalopathy, and acute symptomatic seizures. Although the data on lifetime prevalence of status epilepticus in VP patients is unknown, seven cases of status epilepticus associated with porphyria disorders have been reported [4]. Our patient presented with non-specific symptoms of porphyria crisis upon admission, but later had a distinct deterioration of her mental status, raising the clinical concern for nonconvulsive status epilepticus.

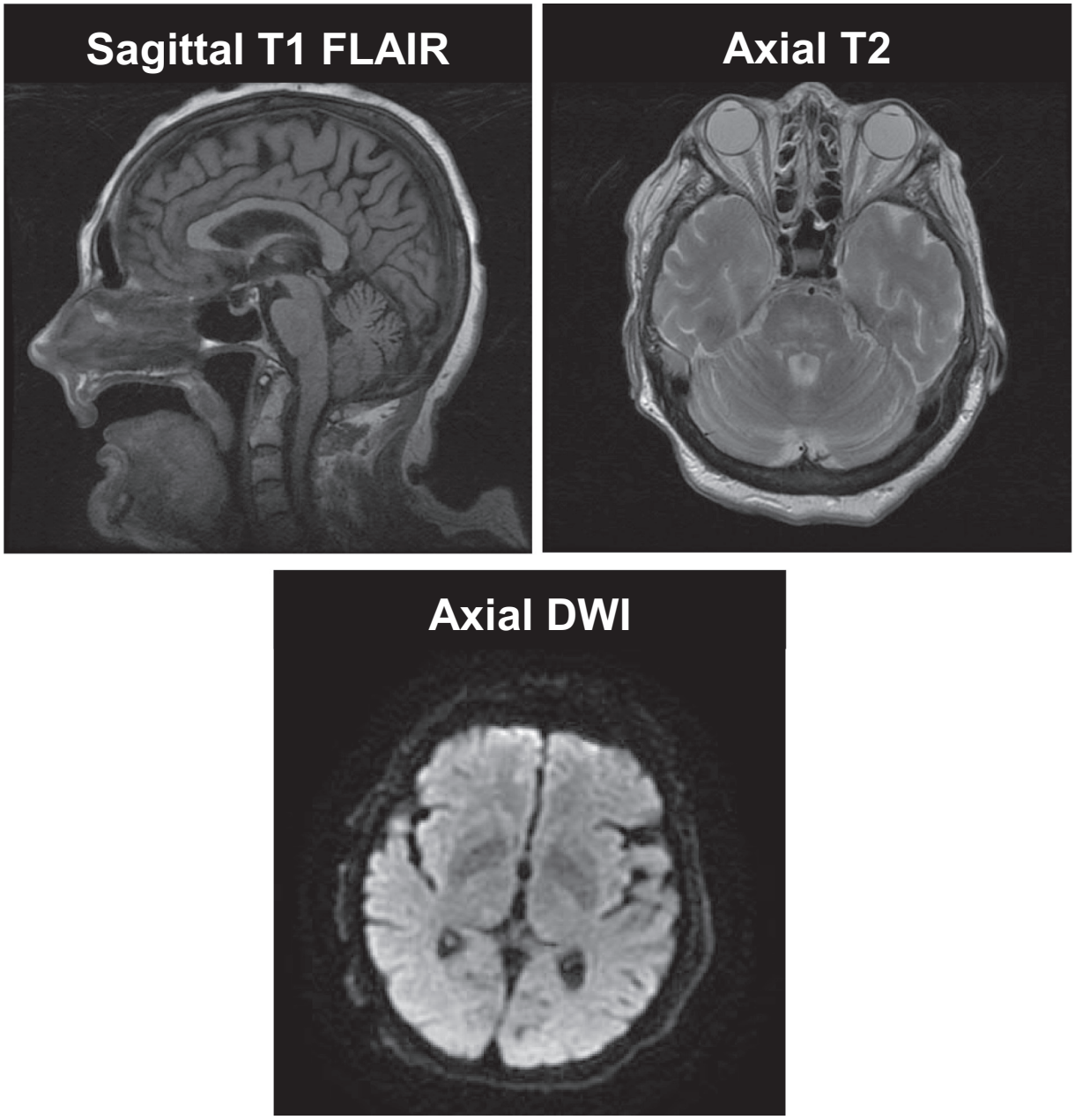

Fig. 3. Representative images of patient's brain MRI. There was no evidence of hemorrhage, infarct, or other acute intracranial abnormalities.

Table 1
Assessment of antiepileptic drug porphyrogenicity.

Non-porphyrinogenic antiepileptic drugs	Porphyrinogenic antiepileptic drugs
Clobazam	Carbamazepine
Clonazepam	Ethosuximide
Gabapentin	Felbamate
Lacosamide	Oxcarbazepine
Levetiracetam	Phenobarbital
	Phenytoin
	Lamotrigine
	Clonazepam
	Primidone
	Tiagabine
	Topiramate
	Valproate

Note: Rufinamide, eslicarbazepine, and perampanel are of uncertain porphyrogenicity. These agents are not yet classified and possibly porphyrinogenic [1].

Her EEG revealed generalized triphasic and independent multifocal sharp waves which improved dramatically after levetiracetam treatment, suggesting NCSE as the culprit. She underwent initial and repeat serological and neurodiagnostic studies to rule out seizures and further evaluate her acute encephalopathy. Fortunately, her clinical status continued to improve with maintenance levetiracetam as well as administration of hemin in the treatment of her acute porphyria crisis.

Identification and treatment of seizures in patients with porphyrias can be challenging. Treatment of patients with porphyrias can be difficult due to the porphyrogenicity of many anti-seizure medications (Table 1). First line treatment options for seizures and status epilepticus include porphyria precipitating anti-seizure drugs such as phenytoin, valproate, benzodiazepines, and lacosamide [1,6,7]. These anti-seizure drugs are metabolized by heme containing cytochrome P450 enzymes in the liver, thereby leading to increased liver heme biosynthesis and deposition of porphyrin complexes [5,8]. Therefore, if an inappropriate anti-seizure medication is selected, a patient's acute porphyria crisis can be exacerbated. This is an important clinical distinction in treating patients with VP who develop epilepsy and present with drug-resistant seizures while in hospital.

Levetiracetam was effective in the treatment of this patient's NCSE. It has high oral bioavailability and reaches peak plasma concentration in approximately 1 h. More importantly, levetiracetam does not induce hepatic cytochrome P450 enzymes and, therefore, does not increase heme synthesis in VP patients. Approximately 66% of the drug is excreted unchanged in the urine and the remaining metabolites are excreted independent of hepatic enzymes [9].

There have been various mechanisms described; however, the underlying pathophysiology of seizures in patients with porphyria has not been fully elucidated. In one study, Tracy and colleagues report that patients with porphyria can also present with syndrome of inappropriate antidiuretic hormone (SIADH) which can be a possible contributing factor in seizures [11]. Solinas and colleagues report the trigger of these seizures is probably related to metabolic imbalance and to the intrinsic epileptogenic role of some porphyrins [10]. Additional studies have reported that neural damage can follow a porphyric attack which may predispose to brain lesions that may be epileptogenic [10,11]. Furthermore, porphyria attacks are also exacerbated by environmental factors such as alcohol, drugs, hormonal, and dietary changes. These factors amplify the deficiency of PPOX enzyme which leads to deposition of porphyrin complexes [5]. We speculate that there could also be a role for protoporphyrinogen IX to have an epileptogenic role given its dysfunction in the role of VP.

5. Conclusions

This case highlights the importance of consideration of nonconvulsive status epilepticus in patients with known variegate porphyria and acute encephalopathy. Additionally, 10%–20% of patients with acute intermittent porphyria in relapse have been noted to manifest acute symptomatic seizures which warrant added caution while treating patients with anti-seizure medication in porphyria crisis [1]. Recognition of seizures is critical so that appropriate anti-seizure medications are selected so as to avoid exacerbation of porphyria. Levetiracetam could be a preferred choice of treatment for NCSE in patients with variegate porphyria. Hemin treatment should be started urgently to treat porphyria crisis and help reduce porphyrin complex deposition.

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C-Reactive Protein: Risk Marker or Mediator in Atherothrombosis?

Ishwarlal Jialal, Sridevi Devaraj, Senthil K. Venugopal

Abstract—Inflammation appears to be pivotal in all phases of atherosclerosis from the fatty streak lesion to acute coronary syndromes. An important downstream marker of inflammation is C-reactive protein (CRP). Numerous studies have shown that CRP levels predict cardiovascular disease in apparently healthy individuals. This has resulted in a position statement recommending cutoff levels of CRP <1.0, 1.0 to 3.0, and >3.0 mg/L equating to low, average, and high risk for subsequent cardiovascular disease. More interestingly, much in vitro data have now emerged in support of a role for CRP in atherogenesis. To date, studies largely in endothelial cells, but also in monocyte-macrophages and vascular smooth muscle cells, support a role for CRP in atherogenesis. The proinflammatory, proatherogenic effects of CRP that have been documented in endothelial cells include the following: decreased nitric oxide and prostacyclin and increased endothelin-1, cell adhesion molecules, monocyte chemoattractant protein-1 and interleukin-8, and increased plasminogen activator inhibitor-1. In monocyte-macrophages, CRP induces tissue factor secretion, increases reactive oxygen species and proinflammatory cytokine release, promotes monocyte chemotaxis and adhesion, and increases oxidized low-density lipoprotein uptake. Also, CRP has been shown in vascular smooth muscle cells to increase inducible nitric oxide production, increase NFκb and mitogen-activated protein kinase activities, and, most importantly, upregulate angiotensin type-1 receptor resulting in increased reactive oxygen species and vascular smooth muscle cell proliferation. Future studies should be directed at delineating the molecular mechanisms for these important in vitro observations. Also, studies should be directed at confirming these findings in animal models and other systems as proof of concept. In conclusion, CRP is a risk marker for cardiovascular disease and, based on future studies, could emerge as a mediator in atherogenesis. **(*Hypertension*. 2004;44:6-11.)**

Key Words: endothelium ■ macrophages ■ atherosclerosis

Much evidence supports a pivotal role for inflammation in all phases of atherosclerosis from the initiation of the fatty streak to the culmination in acute coronary syndromes (plaque rupture).[1,2] The earliest event in atherogenesis appears to be endothelial cell (EC) dysfunction. Various noxious insults including hypertension, diabetes, smoking, dyslipidemia, hyperhomocystinemia, etc, can result in EC dysfunction that manifests primarily as deficiency of nitric oxide (NO) and prostacyclin and an increase in endothelin-1 (ET-1), angiotensin II (Ang II), and plasminogen activator inhibitor-1 (PAI-1), among other aberrations. After EC dysfunction, mononuclear cells such as monocytes and T lymphocytes attach to the endothelium initially loosely and thereafter adhere firmly to the endothelium and then diapedese into the subendothelial space. The rolling and tethering of leukocytes on the endothelium is orchestrated by adhesion molecules such as selectins (E-selectin, P-selectin), cell adhesion molecules (intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1]), and integrins. Chemotaxis and entry of monocytes into the subendothelial space is promoted by monocyte chemoattractant protein-1, interleukin-8 (IL-8), and a newly reported chemokine, fractalkine. Thereafter, macrophage colony-stimulating factor promotes the differentiation of monocytes into macrophages. Macrophages incorporate lipids from oxidized low-density lipoprotein via the scavenger receptor pathway (CD36, scavenger receptor-A), becoming foam cells, the hallmark of the early fatty streak lesion. After the fatty streak lesion, smooth muscle cells migrate into the intima, proliferate, and form the fibrous cap. It is currently believed that lipid-laden macrophages, during the process of necrosis and apoptosis, release matrix metalloproteinases, which cause a rent in the endothelium. Because the lipid-laden macrophage is enriched in tissue factor, this is released from the macrophage and comes in contact with the circulating platelets, resulting in thrombus formation and acute coronary syndromes (unstable angina and myocardial infarction). Various knockouts and transgenic experiments have

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underscored the importance of the various cytokines, chemokines, and adhesion molecules in atherogenesis, emphasizing the importance of the inflammatory component.[1,2]

There are numerous inflammatory markers that have been shown in various studies to predict cardiovascular events. These include cell adhesion molecules, cytokines, chemokines, acute phase reactants such as fibrinogen, serum amyloid A, and C-reactive protein (CRP). In this brief review, we focus on the inflammatory marker, CRP, because the largest amount of published data support a role for CRP as a risk marker for cardiovascular disease (CVD). Furthermore, data are reviewed to suggest that in addition to being a risk marker, CRP may indeed be a participant and culprit in atherogenesis.

C-Reactive Protein

Biochemistry and Biology

CRP is a member of the pentraxin family. It comprises 5 noncovalently associated protomers arranged symmetrically around a central pore and has a molecular weight of 118 000 Da.[3] It is a nonglycosylated protein in humans and the gene has been mapped to chromosome 1. Twin studies have shown a highly heritable component to baseline levels of CRP. With regard to its production, the general consensus is that the production is predominantly under the control of IL-6. However, IL-1 and tumor necrosis factor may also contribute to hepatic synthesis and secretion of CRP. CRP has a half-life of ≈19 hours and this appears to be constant in health and disease. Thus, the sole determinant of CRP levels is the synthetic rate. Its ligand-binding site contains 2 ligated calcium ions and it binds phosphocholine and small ribonucleoproteins. To date in phagocytes, it has been shown to bind Fc-γ receptors I and II and its function appears to clear apoptotic and necrotic cells.

Much recent data challenge the dogma that CRP is exclusively produced by the liver. Indeed, cogent data suggest that it is produced in the atherosclerotic lesion (especially by smooth muscle cells and macrophages), the kidney, neurons, and alveolar macrophages.[4–9] Also, there is evidence to suggest that the stimulus for the production of CRP might be lipid peroxidation and infection such as cytomegalovirus that triggers a proinflammatory cytokine cascade resulting in CRP release. In this regard, it is interesting that adipose tissue, previously thought to be an inert triglyceride depot, has been shown to produce cytokines such as tumor necrosis factor-α and IL-6, which also could contribute to production of CRP.[10]

Hs-CRP and Cardiovascular Risk

Numerous studies from various parts of the world have clearly established that CRP predicts future risk for CVD in apparently healthy persons, independent of established risk factors in the majority of studies. In the studies to date, CRP has been shown to predict myocardial infarction, coronary artery disease (CAD) death, stroke, peripheral arterial disease, sudden death, etc.[11] In the Women's Health Study, Ridker et al have shown that CRP is additive to low-density lipoprotein (LDL) cholesterol and the Framingham 10-year risk score in predicting future CVD in healthy American women.[12] Thus, based on these data, the American Heart Association and Centers for Disease Control and Prevention have issued a statement recommending that CRP be used as a risk marker for CVD in individuals with a Framingham risk score between 10% and 20%.[13] In their recommendations, CRP levels <1 mg/L were considered low-risk, 1 to 3 mg/L as average risk, and >3 mg/L as high-risk for CVD (Table). With regard to risk assessment, if the value on 2 occasions 1 month apart is in the same category, ie, <1, 1 to 3, and 3 to 10 mg/L, this can be taken as reliable evidence with regard to low, average, and high risk for subsequent CVD. However, if the CRP level is >10 mg/L, then CRP cannot be used to assess cardiovascular risk and other active inflammatory processes (eg, trauma, infection, etc) should be excluded. Thus, when using CRP to assess cardiovascular risk in primary prevention, one needs to adopt the high sensitive (hs) CRP assay, and the patient should be free from any kind of acute inflammation such as infection, trauma, etc, for at least 2 weeks.

Hs-CRP and Cardiovascular Risk

Framingham Risk Score (10%–20%)	Risk
<1.0 mg/L	Low
1.0–3.0 mg/L	Average
>3.0 mg/L	High

Conditions that have been associated with increased levels of CRP include adiposity, chronic inflammation, metabolic syndrome, type 2 diabetes, hypertension, and sleep apnea.[14,15] It is also important to note that in patients with metabolic syndrome who already have an increased risk of CVD,[14] CRP levels >3 mg/L predict a greater risk for CVD than in patients with metabolic syndrome with CRP levels <3 mg/L.[16] To date, modalities that have been shown to lower Hs-CRP levels include weight loss in obese individuals and certain medications such as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), peroxisome proliferator-activated receptor-α agonists (fibrates), peroxisome proliferator-activated receptor-γ agonists (glitazones), aspirin in patients with CAD, and high doses of RRR-α tocopherol.[14,17] There is no doubt that this list of therapeutic modalities that modulate CRP will increase as new data are generated.

Thus, it is clearly accepted that CRP is a risk marker for CVD. The more important question that arises, because CRP has now been demonstrated in the vessel wall by numerous investigators, is whether it is an innocent bystander or culprit in atherogenesis.

CRP: A Mediator in Atherogenesis?

In this regard, it should be emphasized that several investigators have now clearly demonstrated the presence of CRP mRNA and protein in human atherosclerotic lesion and vascular cells.[4–9] Data that support the contention that CRP contributes to atherogenesis derive largely from in vitro observations.

CRP and ECs

The initial data from Yeh's group[18,19] showed that incubation of human umbilical vein ECs and human coronary artery ECs

with CRP induces increased expression of ICAM, VCAM, and E-selectin and the chemokine, monocyte chemoattractant protein-1. They also showed that this increase in adhesion molecule and chemokine expression translated into a biological effect, ie, increased adhesion of U937 cells to human umbilical vein ECs.[18,19]

Also, at least 3 groups have shown that CRP levels correlate inversely with endothelial vasoreactivity.[20–22] This prompted our group to examine the effect of CRP on a critical enzyme in ECs, endothelial nitric oxide synthase (eNOS). In human aortic endothelial cells (HAEC), we showed that CRP resulted in significant reduction in mRNA and protein for eNOS.[23] Furthermore, we showed that eNOS activity (ie, conversion of L-arginine to L-citrulline) and bioactivity (secretion of cyclic guanosine 5′-monophosphate [cGMP]) was decreased in aortic ECs. This effect of CRP appeared to be via decreasing the stability of eNOS mRNA. These findings were also confirmed by another group in venous endothelium.[24] By virtue of inhibiting eNOS expression and NO release, CRP blocks NO-dependent processes such as angiogenesis. Through inhibiting NO production, CRP facilitates EC apoptosis, uncovering yet another proatherogenic and proinflammatory phenotype. Future studies should now focus on the mechanisms for the reduction in eNOS mRNA stability, the effect of CRP on eNOS phosphorylation, and interaction with heat shock protein-90 and caveolin-1.

Another important product of ECs is prostacyclin, a potent vasodilator, inhibitor of platelet aggregation, and inhibitor of smooth muscle cell proliferation. In our studies, we showed that CRP in doses as low as 10 μg/mL resulted in a decrease in the release of the stable metabolite of prostacyclin, prostaglandin F-1α (PGF-1α), in both HAEC and human coronary artery ECs.[25] We also showed that CRP stimulated superoxide anion (O_{2-}) release and because it inhibits eNOS, we investigated another mechanism by which CRP could decrease prostacyclin synthase (PGIS) activity. Ullrich's group previously showed that PGIS has a great susceptibility to nitration.[26] Because an increase in inducible nitric oxide synthase (iNOS) could result in increased formation of peroxynitrite, we examined the effects of CRP on iNOS. We showed that CRP increased iNOS activity, resulting in increased nitration of PGIS. This inhibition of PGIS activity was reversed by the peroxynitrite scavengers, urate, and ascorbate. The schema depicting this mechanistic pathway is shown in Figure 1. For proof of concept, studies need to be undertaken in vitro in aortic rings and with CRP administration in animal models such as rats and rabbits to show that CRP induces impairment in vasoreactivity in vivo. In accordance with the Yeh group, we also showed in aortic ECs that CRP augments both ICAM and VCAM expression and monocyte adhesion to endothelium; however, we were not able to confirm an increase in expression of E-selectin in HAEC.[23]

PAI-1 is a member of the serine protease inhibitors. It appears that PAI-1 is synthesized in the liver, adipose tissue, EC, vascular smooth muscle cells (VSMCs), and macrophages. PAI-1 is clearly a marker of impaired fibrinolysis and atherothrombosis and is increased in CAD patients. Increased PAI-1 gene expression is present in human atherosclerotic

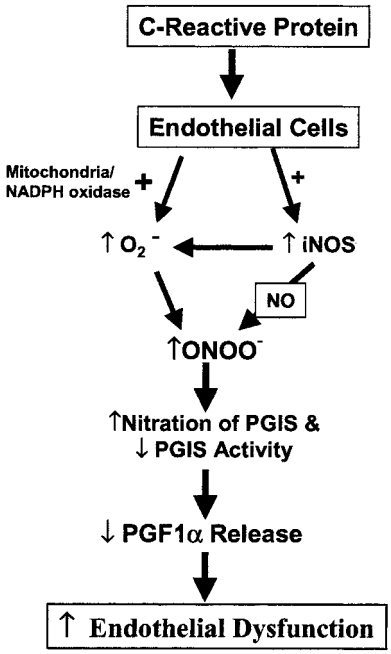

Figure 1. Schema depicting the mechanism of inhibition of prostacyclin synthase by CRP.

arteries and correlates with the degree of atherosclerosis, and PAI-1 deficiency protects against atherosclerotic progression in the mouse carotid artery. Transgenic mice that express a stable form of the human PAI-1 gene develop coronary artery thrombosis.[27] We have shown that CRP induces PAI-1 mRNA, antigen, and activity in HAEC.[28] These findings suggest that CRP may be an atherothrombotic agent. This was recently confirmed in the CRP transgenic mice by Danenberg et al, who showed that compared with wild-type mice in which CRP levels are undetectable, in the CRP transgenic mice, CRP levels increased to 18.6 mg/L. After injury to the femoral artery, there was complete thrombotic occlusion in the femoral artery in 75% of the human CRP transgenic mice when compared with 17% of the wild-type mice at 28 days ($P<0.05$).[29] Furthermore, arterial photochemical injury to the carotids shortened clot formation time in the human CRP transgenic mice compared with the wild-type. Thus, this in vivo finding strongly supports the notion that CRP may function as a procoagulant, based on its effects documented in vitro, ie, reduction in eNOS, prostacyclin, increased PAI-1, and tissue factor.

CRP has been shown in venous endothelium to promote the release of the potent endothelial-derived contracting factor, ET-1.[30] ET-1 not only is a potent vasoconstrictor but also appears to be a mediator of CRP-induced upregulation of adhesion molecules and monocyte chemoattractant protein-1 in venous EC.

An important chemokine is IL-8, which is a powerful trigger of adhesion of monocytes to endothelium. The mouse homolog of IL-8 triggers arrest of monocytes in carotid arteries of ApoE$^{-/-}$ mice. IL-8 is also an angiogenic factor and inhibits tissue inhibitor of metalloproteinase. Knockout of the homolog of IL-8 receptor, CXCR2, decreases intimal accumulation of macrophages and decreases progression of

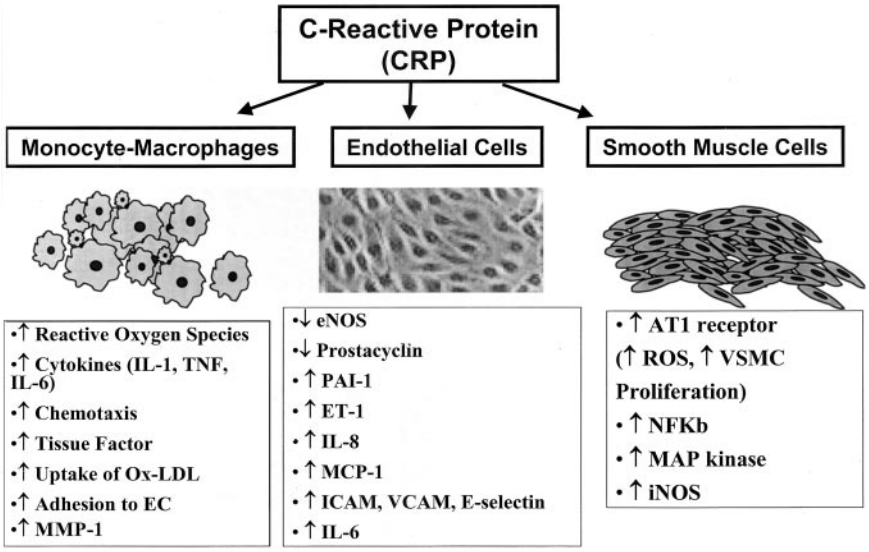

Figure 2. Potential atherothrombotic effects of CRP on vascular cells.

atherosclerotic lesions.[31] We have recently shown that CRP induces IL-8 expression in HAEC and human coronary artery ECs.[32] This was evidenced by both secreted IL-8 and also intracellular IL-8 by flow cytometry. The increase in IL-8 was caused by increased mRNA for IL-8. Also, transcription of IL-8 was increased. Thus, we examined the effect of CRP on NFκB activity and showed that CRP increases NFκB activity, as evidenced by increase in nuclear p65 and cytosolic IκB kinase. Inhibitors of NFκB including SN50, parthenolide, and Bay-11 reduced intracellular and secreted IL-8 from HAEC. The increased adhesion of monocytes to endothelium in the presence of CRP was reduced by ≈30% by pre-incubating the cells with IL-8 antibodies, suggesting that other factors such as ICAM and VCAM may be more important with regard to CRP's augmentation of monocyte EC adhesion. In all these experiments conducted in our laboratory, we were careful in purifying CRP to an endotoxin level that is not sufficient to induce inflammatory molecules, ie, $<$12.5 pg/mL. We also showed that addition of polymixin B did not abrogate the effects of CRP, and trypsinizing or boiling CRP abolishes its effects on EC.

CRP and Monocyte-Macrophages

The initial data derive from Cermak et al,[33] who showed that CRP induced monocyte tissue factor secretion. In this study, they showed that CRP induced tissue factor antigen and procoagulant activity. However, no studies were undertaken to elucidate the mechanism.

In monocyte macrophages, CRP, after internalization and degradation, has been shown to induce production of hydrogen peroxide at concentrations $>$10 μg/mL.[34] Ballou et al[35] conducted a study in which they incubated human monocytes with CRP at different doses for 16 hours and were able to demonstrate significantly increased levels of IL-1β, tumor necrosis factor-α, and IL-6 at concentrations of CRP $>$5 μg/mL. This induction of cytokine release was unaffected by polymixin B but was completely abrogated by boiling of CRP, confirming that this effect of CRP was not caused by lipopolysaccharide contamination. A single report has shown increased CD11b expression on monocytes incubated with CRP, and this resulted in increased adhesion of these monocytes to lipopolysaccharide-activated human umbilical vein ECs.[36] CRP has been shown to activate complement and stimulate human monocyte chemotaxis.[37] There has been a report that CRP promotes uptake of native LDL. However, this has been brought into question by the Witztum group, who showed recently in an elegant study that CRP promotes the uptake of oxidized but not native LDL because of certain unexposed phosphocholine epitopes on oxidized low-density lipoprotein.[38] A more recent article that further supports the role of CRP in later stages of atherosclerosis is by Williams et al, who showed that CRP stimulated matrix metalloproteinase-1 mRNA, protein, and collagenase activity in human monocyte/macrophages.[39] This appeared to be orchestrated via Fc-γ receptor II, and the signal pathway appeared to be via extracellular signal regulating kinase. CRP had no effect on tissue inhibitor of metalloproteinase-1. Thus, it is clear that CRP is proatherogenic in monocyte-macrophages because it increases tissue factor expression, promotes monocyte chemotaxis and adhesion to EC, reactive oxygen species release, and matrix metalloproteinase-1, and promotes oxidized low-density lipoprotein uptake, thus leading to increased foam cell formation. Furthermore, CRP is present in foam cells in the atherosclerotic lesion and activates complement.

CRP and Smooth Muscle Cells

The angiotensin type-1 receptor (AT_1R) is a key atherosclerotic switch facilitating Ang II-induced ROS production, VSMC migration, proliferation, and remodeling. Given the central importance of AT_1R in the development and clinical course of atherosclerosis,[40] the demonstration by the Verma laboratory that CRP upregulates AT_1R mRNA and protein in VSMC and increases the number of AT_1R-binding sites in VSMC could have major implications with regard to atherogenesis.[41] CRP also augmented Ang II-induced VSMC migration and proliferation, further supporting a functional relationship between CRP and Ang II in mediating vascular

smooth muscle cellular pathology. In an in vivo model of carotid balloon angioplasty, CRP exposure facilitated AT1R expression, with resultant increases in neointimal formation, VSMC migration, and proliferation, and promoted collagen and elastin production, which are key matrix proteins in the vessel wall. These effects were attenuated by angiotensin receptor blockade. Therefore, CRP exerts direct proatherosclerotic effects at the level of VSMC. Also, in VSMC, CRP has been previously shown to upregulate iNOS, certain cell signal transduction pathways including mitogen-activated protein kinase pathway and NFκb.[42]

Conclusions

In summary, CRP is clearly a risk marker for CVD and is recommended for use in primary prevention for this purpose. In addition, CRP appears to also contribute to atherogenesis (Figure 2). However, much further research is needed, especially in appropriate animal models, to confirm that CRP is a mediator of the proinflammatory, prothrombotic phenotype and does contribute to atherothrombosis. In this regard, it is important to note in Wistar rats after coronary ligation that human CRP enhanced infarct size by 40%.[43] Because CRP levels can be modulated by weight loss and certain pharmacological interventions including statin therapy, this further underscores the importance of establishing the role of CRP in atherothrombosis. Thus, in addition to being an adjunct to lipid screening in individuals at high risk for CAD, it is clearly a better method to target therapies in the setting of primary prevention. Potential prognostic value in acute coronary syndrome needs to be confirmed in future studies. It is clear that inflammation is now a new target for both prevention and treatment of CVD. Future research efforts should be directed at investigating the effect of CRP on other atherogenic mediators and elucidating the molecular mechanisms of the procoagulant/proatherogenic effects documented to date.

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C-Reactive Protein: Risk Marker or Mediator in Atherothrombosis?

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