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Monomeric C-reactive protein: a link between chronic inflammation and neurodegeneration?

Nicoleta Arnaut, Ylenia Pastorello, Mark Slevin*

Pre-diabetic insulin resistance is associated with sub-clinical inflammation and concomitant increase in systemic C-reactive protein (CRP) levels. Type 2 diabetes mellitus (T2DM) patients register even higher chronic levels of inflammation, with excess circulating CRP originating from both typical hepatic synthesis, and also visceral white adipose tissue. In addition, infiltration of pro-inflammatory macrophages into the expanding fat, as obesity becomes morbid, contributes further to dysregulation and symptomatic disease (Stanimirovic et al., 2022). Most importantly, in diabetic individuals, a sustained and chronic inflammation is perpetuated in the presence of non-healing wounds and ulcers, with associated further increase in CRP (Dangwal et al., 2015).

Recent evidence suggests that CRP can initiate or maintain pathogenic inflammatory changes, also within the central nervous system (neuroinflammation), eventually resulting in neurodegeneration, cognitive decline, and Alzheimer's disease (AD) (Ehtewish et al., 2022). The aim of this perspective is to analyze and provide an opinion on the association between the inflammatory effects of CRP, particularly monomeric CRP (mCRP; its biologically active form), impaired diabetic wound healing, and the contribution of this chronic process to neurodegeneration.

mCRP contributes to aberrant wound healing found in diabetes through immuno-vascular activation: CRP is a non-specific acute phase reactant that increases in inflammatory states, as in diabetes or in infectious processes. It has a native form (nCRP), composed of five identical subunits, which is produced by hepatocytes, as a response to increased levels of interleukin (IL)-6, IL-1, and tumor necrosis factor α (TNF-α). Its major role is to activate the C1q molecule in the complement cascade, in this way inducing the opsonization of pathogens. After binding to the cell membrane, within damaged tissues and blood vessels, this homopentameric form irreversibly dissociates into the active form, mCRP. These free monomers can bind to the endothelial and inflammatory cells, causing vascular dysfunction through deregulated intracellular signal transduction, and increased cytokine release (Pastorello et al., 2023).

Vascular effects of mCRP: Khreiss et al. (2004) first demonstrated that mCRP at physiological concentrations (1 μg/mL) activated the P38 mitogen-activated protein kinase-dependent pathway in human coronary artery endothelial cells, leading to increased gene expression of monocyte chemoattractant protein-1 (MCP-1), the major promoter of monocyte recruitment. IL-8, the neutrophil chemoattractant and degranulation stimulator, was also increased, providing the first mechanistic evidence for an important role of mCRP in modulation of micro-environmental focalized inflammation. Since this finding, numerous other studies have implicated mCRP in both endothelial cell activation and macrophage pro-inflammatory phenotypic switch, which together result in their abnormal feed-sideways paracrine interactions.

Glycation is the permanent addition of one or more glucose molecules to susceptible cell proteins by the Amadori reaction, causing their impaired function (measured by the HbA1c assay). The interaction between receptor for advanced glycation end-products (RAGE) and advanced glycation end products (AGE) contributes to chronic, impaired diabetic wound healing in several ways: by blocking the hypoxic stimulus to angiogenesis, so diminishing the blood flow to the wound; by reducing the response to infectious pathogens, and inhibiting pro-apoptotic signaling. Johnson et al. (2023) revealed that the parenteral administration of a RAGE-blocking antibody hastened the healing of dorsal wounds in Alloxan-induced diabetic pigs, compared with a non-immune IgG. Quantitative immunohistology indicated increased staining for collagen and lower expression of Il-6 and RAGE, with fewer identifiable macrophages in the lesion.

Increasing systemic concentration of CRP is correlated with the extent of systemic glycation, a process therefore implicating mCRP as a risk factor in exacerbation of multifactorial aberrations. Zhong et al. (2015) showed that mCRP stimulated RAGE gene and protein expression in human coronary artery endothelial cells, concomitant with reactive oxygen species generation, activation of extracellular signal-regulated kinase/nuclear factor kappa-light-chain-enhancer of activated B cells signaling pathways and secretion of MCP-1 pro-inflammatory cytokine amongst others. Hence, the negative synergistic interaction between aberrant signaling and vascular dysfunction in diabetes, together with the impact of glucose on RAGE and IR/IRS-1 is magnified in the presence of perpetually produced mCRP associated with the hypoxic refractory inflammatory phase, creating a cycle that maintains increased vascular permeability.

mCRP promotes macrophage M1 phenotypical shift, impairing diabetic wound repair: Macrophage polarization is closely interconnected with these processes. Initially, in normal wound healing, depending on the stimuli from the local microenvironment, macrophages differentiate into subtype M1, which has a pro-inflammatory role. Later they polarize into M2 anti-inflammatory phenotype, which promotes angiogenesis and collagen deposition. In abnormal diabetic wounds, the primary phase is extended, sometimes chronically, and here, M1 macrophages are excessively numerous. Later, during the subsequent proliferative and regenerative stages, successful wound closure is hampered by a lack of M2 macrophages (Wu et al., 2022).

In a study performed by Devaraj et al. (2011), human peripheral blood mononuclear cells, isolated from the blood of healthy volunteers, were incubated with pleural fluid-derived, pro-inflammatory CRP (50 μg/mL) and differentiated into macrophages for 7 days. Phenotypic characterization showed that CRP-treated monocytes secreted high levels of TNF-α, IL-6, IL-1β, and MCP-1, and consequently, they differentiated in the M1 subtype. Also, M2 macrophages incubated with CRP-secreted pro-inflammatory molecules, respectively TNF-α, MCP-1, and IL-1β, concomitant with conversion by CRP from M2 to M1 phenotype. These studies indicate a probable role for mCRP in hyper-and chronically induced aberrant inflammatory responses seen in non-healing diabetic and complex wounds.

No other studies to our knowledge have examined the role of mCRP in misrepair of diabetic wounds. In other models of wound healing, the impact of mCRP has been identified. For example, using a left anterior descending coronary artery, murine model of myocardial infarction, Zha et al. (2021) demonstrated significantly increased disruptive inflammation, M1 macrophage polarization, and increased ultimate scar size and fibrosis in mCRP injected animals. Therefore, in spite of limited evidence, mCRP is highly likely to be a key protagonist of the aberrant diabetic wound response, maintaining the pro-inflammatory micro-environment within complex diabetic wounds and providing a mechanism for the transfer of mCRP systemically into the central nervous system.

mCRP, diabetes, and neurodegeneration: It is established that there is an association between diabetes and cognitive dysfunctions, such as mild cognitive impairment (MCI), AD, and different forms of dementia. In preclinical studies, the most intensively studied mechanisms of this connection include abnormal insulin signaling, neuronal insulin resistance, systemic inflammation, mitochondrial dysfunction, and vascular pathogenic changes.

In addition to the known risks of dementia in people with T2DM, such as regular hypoglycemic intervals and dyslipidemia, there are also retinal microangiopathy and neurodegeneration. As the retina originates ontogenically from brain tissue, Ciudin et al. (2017) concluded that determining its sensitivity by Macular Integrity Assessment microperimetry could be an appropriate noninvasive method to identify patients with T2DM who are more susceptible to developing AD. A factor that could be involved in promoting neuroinflammation and subsequent neurovascular unit dysfunction is the presence of chronic, complex diabetic lesions, which could maintain a systemic inflammation with high levels of CRP, IL-6, TNF-α, and other pro-inflammatory mediators. These cytokines can interact with the central nervous system by penetrating the blood-brain barrier, directly through afferent vagal nerve transmission, carried through the blood-brain barrier within cell-excreted exosomes, or indirectly through the cerebral endothelium. The increase of serum pro-inflammatory markers, especially CRP, in patients with T2DM and MCI, has been confirmed by several studies. One of them, conducted on 90 elderly subjects (aged 60 years old or more) showed that serum levels of high sensitivity CRP in diabetic elderly patients with MCI were significantly higher than those of diabetic elderly patients without cognitive impairment and control (Hosny et al., 2019). mCRP has been directly shown to stimulate inflammation, promote M1 macrophage polarization, and inside the brain, activate microglia inducing pro-inflammatory cytokine secretion. mCRP may enter the brain directly attached to the membrane of immune cells and monocytes or within their secreted extracellular vesicles, e.g., originating from monocytes or even endothelial cells.

When there is damage to the brain, leaking vessels also allow mCRP penetration into the parenchyma and mCRP has been found directly to act upon protein, including Tau, causing hyperphosphorylation. So our hypothesis and perspective is that the systemic impact of chronic inflammation correlates with an increased risk of development of MCI and dementia. Since in diabetes, we have additional RAGE and IR/IRS-1, and we know that mCRP stimulates their overactivity associated with later neurodegenerative consequences, the impact of chronic inflammation in diabetic wounds and ulcers on MCI and AD will likely be exacerbated (Slevin et al., 2015; Pastorello et al., 2023; **Figure 1**).

Conclusion and future directions: Taken together, the CRP and especially mCRP immunomodulating effects, mediated by activating different signaling pathways in endothelial cells, monocytes/macrophages, and other cells, include macrophage M1 polarization, complement C1q fixation, uncontrolled chronic inflammation, an increase of the number of RAGE, phosphorylation of IRS-1. All of these create a pro-inflammatory immune microenvironment, with a prolonged local hyperglycemia, which perpetuates tissue damage and delays the healing of the diabetic wound. Further work should investigate the significance of chronically raised plasma mCRP in patients with diabetes to establish if this is a critical factor associated with later neurodegenerative risk. Since the few studies that have been carried out have indicated no relationship between native CRP systemic concentration and that of mCRP, a reliable method is urgently required to quantify blood concentrations of this protein.

The manipulation of these pro-inflammatory pathways, and indeed abrogation of mCRP membrane attachment and signal propagation, may serve as a potential therapeutic target to ameliorate the healing process of diabetic wounds. Reducing systemic inflammation could offer considerable advantages in terms of preventing MCI among elderly individuals with type 2 diabetes.

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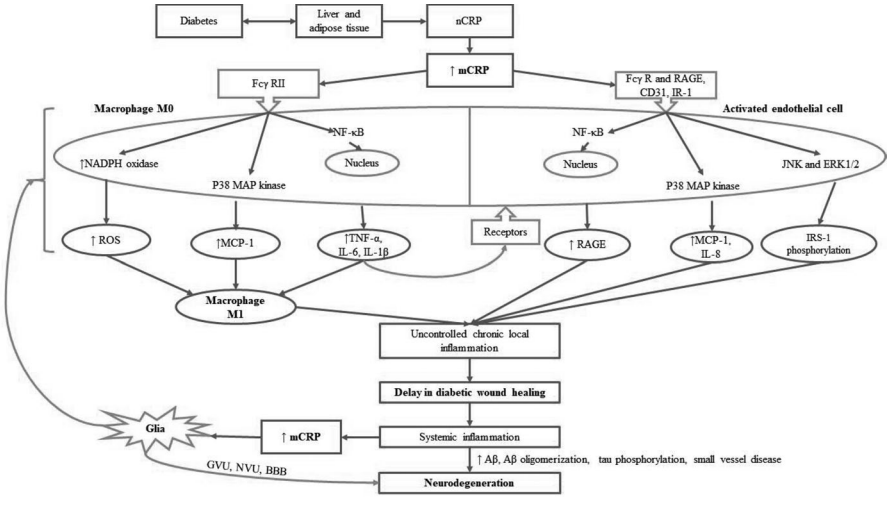

Figure 1 | **The effects of mCRP on diabetic wound healing and neurodegeneration.**
In damaged tissue, nCRP binds to the cell membrane and dissociates into mCRP. mCRP interacts with membrane receptors, such as those of macrophages and endothelial cells, and through different intracellular signaling pathways leads to the release of multiple factors, which promote monocytes differentiation into pro-inflammatory M1 macrophages phenotype, and block conversion of M1 to anti-inflammatory M2 macrophages. This causes further activation of the endothelial cells (including stimulation of RAGE and IR/IRS-1), with a subsequent pro-inflammatory, abnormal secretion. All of these factors perpetuate an uncontrolled chronic local inflammation, which leads to a delay in diabetic wound healing. Complex diabetic lesions maintain a systemic inflammation that may induce neuroinflammation and neurodegeneration. Systemic inflammation can also activate glia within the brain with mCRP having the direct capacity to disrupt the NVU, and the BBB, and promote the build-up of hyperphosphorylated tau and Aβ. Created with Microsoft PowerPoint. Aβ: Amyloid-β; BBB: blood-brain barrier: ERK 1/2: extracellular signal-regulated kinase 1/2; Fcγ R: Fc-gamma receptor; GVU: glial vascular unit; IL: interleukin; IR1: insulin receptor 1; IRS-1: insulin receptor substrate 1; JNK: c-Jun N-terminal kinase; MCP-1: monocyte chemoattractant protein-1; mCRP: monomeric C reactive protein; NADPH oxidase: nicotinamide adenine dinucleotide; nCRP: native C reactive protein; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NVU: neurovascular unit; P38 MAP kinase: p38 mitogen-activated protein kinases phosphate oxidase; RAGE: receptor for advanced glycation end-products; ROS: reactive oxygen species; TNF-α: tumor necrosis factor alpha.

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