Review Article

The Inflammatory Process During Childhood and the Risk of Developing Atheromatous Disease in Adult Life: The Role of C-Reactive Protein

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C-reactive protein (CRP) is nowadays considered to be an independent risk factor for acute cardiac events.[1,2] Recently, there has been discussion about the possible pro-atherogenic action of CRP in causing endothelial dysfunction and promotion of early atherosclerotic lesions.[2] Since atherosclerosis is an inflammatory process[3,4] that starts in childhood,[5] researchers have turned their attention towards a possible relationship between CRP levels in children and cardiac risk in adult life.[6] Studies in children have shown that there is indeed a relationship between CRP levels and the early phase of atherogenesis.[6-8]

This review begins with a brief description of the structure, properties and role of CRP in atherosclerosis, and then explores the relationship between levels of this protein in childhood and the future appearance of cardiovascular disease in adult life.

Chemical structure – biosynthesis – properties

CRP belongs to the so-called "acute phase" proteins. It was discovered by Tillet and Francis in 1930. It is a pentraxin composed of 5 equal subgroups, each with 206 amino acids, that are combined in a three-dimensional structure. It has a molecular weight of 115085 Daltons.[9] According to the literature, its biologically active form, which has proinflammatory effects, arises from modification of its pentamer to the formation of monomer subgroups.[10]

CRP is produced in the liver at a steady rate that accelerates up to 100 times when there is a stimulus.[6] It has also been found to be produced in the smooth muscle cells of the coronary arteries.[11] The main inflammatory cytokines that promote its secretion by the liver are tumour necrosis factor-a and interleukin-6.[12] Its normal levels range from 0.2-0.5 mg/dl, with upper bound 1 mg/dl. Recent studies have shown the existence of a correlation between age and peak CRP levels.[1]

An increase in serum CRP levels, in response to any stimulus, starts within 4 hours, accelerates at 8 hours, reaches its peak at 48-72 hours, and thereafter the levels start to decrease rapidly.[12] In the case of chronic inflammation CRP may remain elevated for a long period of time.[12] Both the magnitude and the duration of its elevation are a function of the intensity of the stimu-

lus: the larger the lesion, the higher the level of CRP and the longer the increase persists.[11,12] As a prognostic index of cardiovascular risk it is considered particularly useful and reliable, since its levels do not show a circadian rhythm, it has a long half life (18-20 hours) and it does not require fasting before blood sampling.[13]

The form of CRP that is considered most specific for atherosclerosis is highly sensitive CRP (hsCRP), and it is that form that is mainly measured today.[14]

There are certain conditions that are associated with elevated or reduced CRP levels (Table 1).[10]

Actions – biological role

CRP has the property of binding to exogenous pathogens and/or to destroyed cells of the same organism, via its chemical adhesiveness to a large number of substances, such as phospholipids and nucleic acids.[9] It is also able to activate the process of cell and micro-organism sequestration by binding to constituents of the blood.[7,9]

Its main actions are as follows:[9]

- as an immune complex, namely activation of the complement, via the conventional route, and binding with its C3 and C4 constituent in CRP-destroyed molecule complexes;[9]
- binding with polymorphonuclear and mononuclear blood cells and acceleration of phagocytosis;[4]
- in platelet adhesion, via triggering of platelet activation factor;[9]
- increase in the endothelial production and expression of monocyte chemotactic protein-1 and of adhesion molecules, including intercellular adhesion molecule-1;[16,17]
- reduction in the expression of endothelial NO synthetase (eNOS).[13,17,18]
- increase in monocyte chemotaxis;[15]
- induction of the activation and secretion of cytokines and tissue factor by macrophages;[19]
- mediation of low density lipoprotein (LDL) phagocytosis by macrophages;[19]
- increase in the reactant production of free oxygen radicals by macrophages, leading to LDL oxidation in the subendothelial region.[17]

Table 1. Conditions associated with high or low levels of C-reactive protein (CRP).

High CRP:	Low CRP:
• High blood pressure	• Alcoholism
• High body mass index	• Exercise
• Smoking	• Weight loss
• Metabolic syndrome	• Medication (statins, fibrates, niacin)
• Hyperglycaemia	
• Low high density lipoprotein / elevated triglycerides	
• Oestrogen or progesterone administration	
• Chronic infection	
• Rheumatoid arthritis	

CRP as a risk factor for atherosclerosis

In recent years a large number of studies have examined the possibility that hsCRP, apart from being a simple index of inflammation, might also be a risk factor for atherosclerosis via its proinflammatory and pro-atherogenic action. These studies have been related to both primary and secondary prevention.

Studies of primary prevention have shown that hsCRP has high prognostic value for a first cardiovascular event in all population groups (healthy men, women, the elderly, smokers, diabetics).[20] Individuals with high CRP levels and without other risk factors have a threefold higher risk of future myocardial infarction and a twofold higher risk of cerebrovascular stroke, compared with individuals who have lower CRP levels.[21-28] CRP measurement is a powerful prognostic index of future myocardial infarction or stroke in healthy individuals with no or few conventional risk factors.[20] People with CRP >3 mg/L have 4-6 times greater risk of developing type 2 diabetes mellitus than do those with lower levels.[29] Finally, a recent study showed that CRP measurement in combination with determination of LDL-cholesterol was a better prognostic index for a future cardiovascular event than lipid measurement alone.[30]

Studies of secondary prevention showed that mean CRP levels are significantly higher in individuals who have had an acute myocardial infarction or stroke than in those who have suffered no cardiovascular disease.[2,26,30] In a post mortem study of 302 individuals with atherosclerosis it was found that mean CRP levels were higher in acute rupture of atherosclerotic plaque than in stable plaques or in controls.[32] In acute coronary syn-

Table 2. C-reactive protein (CRP) and cardiovascular risk.

CRP levels (mg/dL)	Risk estimate
0.1 – 0.6	Small
0.7 – 1.1	Low
1.2 – 1.9	Moderate
2.0 – 3.8	High
>3.8	Very high

dromes CRP levels appear to be a powerful prognostic index of greater mortality.[2,32] The persistence of high CRP levels after thrombolytic therapy suggests an obstructed artery.[33] After coronary angioplasty a large increase in CRP during the first six months is an unfavourable prognostic index for restenosis.[34] In patients with unstable angina, CRP levels >1 mg/L on admission are associated with an unfavourable outcome (death, acute myocardial infarction, emergency reperfusion) with 100% sensitivity.[32] Also, CRP levels >1.5 mg/L on discharge from hospital are related with a poor prognosis.[32] Finally, in individuals with stable angina a strong correlation has been found between initial CRP levels and the subsequent occurrence of cardiovascular events.[35]

Table 2 shows the risk stratification for the occurrence of cardiovascular events in relation to CRP levels.[13]

The role of CRP in the onset and progression of atherosclerosis

Atherosclerosis is nowadays viewed as a progressive, multifactorial, inflammatory disease that is brought about by the interaction of all the conventional and newer risk factors with genetic, molecular, or acquired environmental factors.[3,4]

The process of atheromatosis involves three stages. The first stage, which is the formation of fatty streaks, occurs during childhood and is the only one that is reversible.[3,4] Fatty streaks, or striations, are known to appear as early as infancy or childhood, while recent studies have shown that hypercholesterolaemia in the pregnant mother is associated with an increased risk of foetal atheromatosis.[5,36,37] Fatty streaks are clearly inflammatory lesions and consist of macrophages arising from monocytes and T-lymphocytes.[3,4]

The onset of atherogenesis occurs as a result of dysfunction of the vascular endothelium, which leads to an increase in the adhesion molecules on the cellular surface and the production of a large number of cytokines.[3,4] Among these are interleukin-6, which is the main cytokine involved in the acute phase response and which in its turn triggers the production of fibrinogen and CRP by the liver.[38,39]

As soon as the CRP is produced it causes an increase in the endothelial production and expression at the surface of the endothelium of monocyte chemotactic protein-1 and adhesion molecules, including intercellular adhesion molecule-1 and E-selectin, as well as vascular adhesion molecule-1, which specialises in monocyte chemotaxis.[19]

Once it passes to the intima, the monocyte undergoes differentiation into a special type of macrophage, the "lipid laden" foam cell. CRP causes further activation of macrophages so that they secrete cytokines and tissue factors.[19]

The entry of LDL into the subendothelial region leads to its oxidation and to the formation of oxidised forms of lipids that are in a position to cause inflammation. By increasing the reactant production of free oxygen radicals, CRP promotes LDL oxidation in the subendothelial region.[15,18]

The oxidised LDL in the subendothelial region is taken up by the macrophage cells via special scavenger receptors and converted to foam cells. In this phase CRP acts as a mediator of LDL phagocytosis by macrophages.[19]

The leukocyte adhesion, cell chemotaxis and activation occurring in early atheroma represent the steps in a typical inflammatory response. As described above, CRP plays an active role in all stages of the creation of lipoid-rich foam cells, which is the first stage in the creation of atheromatous plaque.

If the inflammatory stimulus persists, the fatty striations may progress to more complex forms of atherosclerosis, such as the creation of a fibrous cap.[4]

Maintaining inflammation at the fatty striation stage triggers a series of mechanisms that ultimately result in the creation of the fibrous cap (second stage atherosclerosis) and permanent atherosclerotic lesions. From this point on, the arterial wall cannot continue its compensatory remodelling and so the atheromatous lesion protrudes into the lumen, altering blood flow and causing the acute complications of atherosclerosis, such as unstable angina or, in the case of plaque rupture, acute myocardial infarction (third stage).[4]

CRP also plays a basic role in both the second and third stages of atherosclerosis. CRP accumulates within the atheromatous plaque, where it exerts a chemotactic effect on the monocytes and, via receptors, mediates the deposition of monocytes on the arterial wall, in this way contributing to the second stage of atherosclerosis.[4] By reducing the activation of eNOS transcription and destabilising the eNOS –m RNA, CRP has been found to cause a reduction in NO production, resulting in the inhibition of vascular relaxation, facilitation of endothelial cell apoptosis, and the prevention of angiogenesis.[17] In smooth muscle cells it increases the activation of type 1 angiotensin receptors and stimulates migration, proliferation of smooth muscle fibres, and the formation of new arterial intima. Additionally, in chronic ischaemia, by causing inhibition of progenitor endothelial cells by the bone marrow, CRP also leads to a reduction in neovascularisation.[18] Finally, as an acute phase protein, it also plays an important role in the rupture of atherosclerotic plaque.[4]

CRP, atherosclerosis, and childhood

Since the first stage of the development of atherosclerosis occurs during childhood, and given that CRP is an important protein for both the onset and the development of atheromatosis, many researchers have tried to correlate CRP levels in childhood with the development and progression of atheromatous lesions.[6,8]

As stated above, production of CRP in the liver is affected mainly by the action of interleukin-6, which is the central stimulus for the acute phase response.[15] Haddy et al, in the STANISLAS study,[40] determined in healthy families that interleukin-6 levels were influenced by age, sex, body mass index, white cell count, and smoking, while the relationship between interleukin-6 and CRP appeared to be independent of other parameters of inflammation (white cells, haptoglobins, etc.). In that study it was found that when children had elevated serum CRP levels, this was associated with an increased body mass index, a fact that has been attributed to the increased production of tumour necrosis factor-a by fat cells.[28] Tumour necrosis factor-a stimulates interleukin-6 synthesis, which in turn stimulates CRP production in the liver. In the same study it was found that in parents interleukin-6 was associated with intercellular adhesion molecule-1 and L-selectin, while in children it was associated with E-selectin.[40]

Recently, CRP has been shown to stimulate the expression of intercellular adhesion molecule-1 by endothelial cells, and high concentrations of intercellular adhesion molecule-1 are associated with elevated CRP levels, which are considered to be an index of endothelial activation in children with a positive familial history of coronary artery disease.[41]

Jarvisalo et al studied CRP in children in relation to the brachial artery flow-mediated dilatation (FMD) and the carotid artery intima-media thickness (IMT).[6] These parameters were measured using ultrasound and the study population included 79 children aged 10.5 ± 1.1 years. In comparison with children who had undetectable CRP levels (<0.1 mg/L), those with higher levels (0.1-0.7 mg/L and >0.7 mg/L) had smaller FMD and greater IMT. CRP levels remained a significant, independent prognostic index of FMD and IMT under various statistical methods. These data show that CRP affects the arteries of healthy children by causing endothelial dysfunction and thickening of the vascular intima and media.[6]

In a study carried out in Greece[8] that included 55 apparently healthy children, 30 with a positive family history of early coronary artery disease, and 25 with no such history, it appeared that the children in the former group had higher levels of the proinflammatory cytokines interleukin-1a, interleukin-6 and tumour necrosis factor-a in the supernatant fluid of cultured peripheral blood monocytes, as well as higher CRP levels, than the children with no family history of coronary artery disease. These findings suggest that the inflammatory atherosclerotic process has already begun in childhood in individuals who have a genetic predisposition.[8]

The conclusions of all the above studies reinforce the hypothesis that CRP plays an important role in the pathogenesis of early atherosclerosis. It is likely that in the future, measurement of hsCRP in healthy children will be used to identify groups at high risk for the development of atherosclerosis in adult life. Of course, before definite conclusions can be drawn, multi-centre prospective studies must be carried out in children, including measurement of both inflammatory and conventional risk factors, followed by monitoring for signs of atheromatous disease in adult life.

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