

REVIEW

CD14 and toll-like receptor 4: a link between infection and acute coronary events?

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The CD14 receptor is a pattern recognition molecule in the innate immune response against microorganisms and other exogenous and endogenous stress factors. The most important CD14 signalling co-receptor is toll-like receptor 4 (TLR4), which activates, among others, the nuclear factor κ B (NF- κ B) inflammatory pathway. Besides its role in innate immunity and host defence, the proinflammatory cytokines expressed upon TLR4/NF- κ B pathway activation exert proatherogenic effects. The CD14 C(-260)T promoter and TLR4 Asp299Gly functional polymorphisms have been recently implicated in the development of cardiovascular events, suggesting that the genetically determined inflammatory response against pathogens or their antigens may have a major role in atherogenesis and subsequent acute events. Is the association of these polymorphisms with cardiovascular disease more evidence for the implication of infection, especially by Gram negative bacteria, in the development of acute coronary events? This article reviews the molecular basis, biological functions, and clinical implications of the CD14/TLR4 polymorphisms in the development of cardiovascular events.

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MOLECULAR BASIS

The CD14 receptor is a 356 amino acid membrane glycoprotein¹² where the C-terminal leader sequence of 28–30 amino acids is replaced by a glycosyl phosphatidylinositol (GPI) anchor after translation.¹³ Thus, CD14 is not a transmembrane protein but is anchored to the cellular membrane through GPI linkage. The membrane expressed CD14 (mCD14) is present on the surface of mature myeloid cells¹⁴ and differentiation of monocytes into macrophages within different tissues is accompanied by a change in mCD14 receptor number.^{15–16} GPI anchored proteins, such as mCD14, are clustered in membrane microdomains called “lipid rafts”,^{17–18} which are implicated in a wide array of cellular processes including transcytosis,¹⁹ potocytosis,²⁰ and transmembrane signalling.²¹ By localising all of the components of specific signalling pathways within a membrane compartment, lipid rafts enable efficient and specific signalling in response to stimuli.¹⁷ CD14 also exists as a soluble molecule (sCD14) that can be found with two different molecular weights in serum.²² Various stimuli induce shedding of the GPI anchored mCD14, probably mediated by serine proteases such as leucocyte elastase,²³ resulting in sCD14 with a molecular mass of 48–49 kDa. Some CD14 molecules stored intracellularly escape GPI anchor attachment and keep the C-terminal leader sequence, resulting in sCD14 with a molecular weight of 55–56 kDa.²² The sCD14s have an important role in LPS mediated activation of CD14 negative cells (epithelial and smooth muscle cells)^{16–24} but the biological differences between these two forms are unknown.

Although CD14 receptor per se binds LPS, the presence of serum LPS binding protein (LBP), a 60 kDa acute phase response glycoprotein synthesised mainly in the liver and lung, enhances this interaction 100–1000 times.²⁵ LBP is essential for the rapid induction of an inflammatory response by small amounts of

It has been suggested that chronic infection by Gram negative microorganisms may contribute to the inflammatory component of atherosclerosis.¹ Endotoxin or lipopolysaccharide (LPS) generated by Gram negative bacteria exerts proatherogenic effects by contributing to low density lipoprotein oxidation, foam cell formation, and thrombogenesis.² The main receptor for LPS is the CD14 receptor, a pattern recognition molecule of the innate immune system.³ CD14 has been implicated in monocyte activation,⁴ leucocyte–endothelial cell interactions,⁵ and regulation of programmed cell death (apoptosis) in both monocytes and endothelial cells,^{6–7} pivotal processes in the development of atherosclerosis and its complications. The most important CD14 co-receptor, toll-like receptor 4 (TLR4), is responsible for activating intracellular signalling pathways. Functional polymorphisms recently identified in both CD14 promoter and TLR4 genes^{8–9} have been associated with acute coronary events,^{10–11} suggesting that the intensity of the genetically determined inflammatory response against pathogens or their antigens may have a major role in determining the magnitude of atherogenesis and subsequent

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Abbreviations: ACS, acute coronary syndromes; Cp-HSP, *Chlamydia pneumoniae* heat shock protein; GPI, glycosyl phosphatidylinositol; hu-HSP, human heat shock protein; IL-1R, interleukin 1 receptor; LBP, lipopolysaccharide binding protein; LPS, lipopolysaccharide; mCD14, membrane expressed CD14; MI, myocardial infarction; NF- κ B, nuclear factor κ B; sCD14, soluble CD14; TLR4, toll-like receptor 4

LPS or Gram negative bacteria, as well as for survival upon bacterial infection.²⁶ LBP binds LPS aggregates and catalytically transfers several hundred LPS monomers per LBP molecule, forming a ternary complex with the CD14 receptor.²⁷ The LPS binding domain on CD14 has been located near the amino terminal region of the molecule. Recent single point mutations and monoclonal antibody epitope mapping studies suggest that CD14 can bind multiple microbial ligands in a non-specific fashion by presenting an array of charged residues on one surface of the protein close to each other.²⁸ This tactic is similar to that previously reported for the macrophage scavenger protein.

A CD14 co-receptor is needed to activate the intracellular signalling pathways because of the lack of a cytoplasmic domain and the inability of GPI to activate signalling pathways directly.²⁹ The most important of the CD14 co-receptors is TLR4.³⁰ TLRs are members of the interleukin 1 receptor (IL-1R) family,^{31, 32} an evolutionary conserved signalling system that is a critical determinant of the innate immune and inflammatory responses against invading pathogens. The IL-1R family contains several common structural features that include leucine-rich repeats in the ectodomain and a toll/IL-1R cytoplasmic domain. The TLRs share the toll/IL-1R cytoplasmic signalling cascade but are distinguished by their extracellular leucine-rich repeat structure. Two of the human TLRs (TLR4 and TLR2) have been reported to be associated with CD14 receptor and to mediate recognition of cell wall components from Gram negative and Gram positive bacteria, respectively.³³ TLR4 activates several intracellular signalling pathways, the most important one being the TLR4/nuclear factor κ B (NF- κ B) pathway,³⁴ leading to the synthesis and release of antimicrobial peptides, inflammatory cytokines and chemokines, and other costimulatory molecules that provide a link to adaptive immunity (fig 1).³⁵

Although TLR4 alone can activate the intracellular NF- κ B signalling pathway, co-expression with MD-2 enhances TLR4 dependent activation of NF- κ B and is required to activate or augment the mitogen activated protein kinase pathways and Elk-1 stimulation.³⁶ MD-2, which can also function as a soluble receptor for cells that do not otherwise express it,³⁷ is physically associated with TLR4, and directly influences its specificity. TLR4/MD-2 seems to discriminate LPS from other phospholipids such as those implicated in the recognition of apoptotic bodies.³⁸ Thus, the LPS receptor is a multiprotein complex that consists of at least three proteins, CD14, TLR4, and MD-2 (fig 1).³⁹ Both mCD14 and sCD14, together with LBP, are first line screeners of microbial antigens and present them to the more pathogen specific signalling receptor TLR4-MD-2.¹⁶

BIOLOGICAL FUNCTIONS OF CD14/TLR4 AND ATHEROSCLEROSIS

The CD14 receptor is a pattern recognition molecule that has a central role in innate immunity, as it can interact with several ligands, including LPS from Gram negative bacteria and components from Gram positive bacteria and fungi.⁴⁰⁻⁴³ Human heat shock protein 60 (hu-HSP60) and hu-HSP70, intracellular proteins thought to be involved in protective functions against cellular stress and infection, and *Chlamydia pneumoniae* HSP60 (Cp-HSP60) have been reported to activate endothelial cells and monocyte derived macrophages through the CD14 receptor,⁴⁴⁻⁴⁶ although these interactions have been recently questioned.⁴⁷

The CD14 receptor has been implicated in several biological functions associated with atherosclerosis and its complications. Among these are monocyte activation,⁵ leucocyte-endothelial cell interactions,⁶ and regulation of apoptosis.^{6, 7} Monocyte activation by the CD14 receptor induces several

intracellular changes that enhance the affinity of monocyte β_2 integrins (CD11/CD18) for their ligand, intercellular adhesion molecule 1, on endothelial cells.⁴⁸⁻⁵⁰ This promotes monocyte-endothelium adhesion, which is one of the first and most crucial steps in the development of atherosclerotic lesions. The CD14 receptor has been implicated in the regulation of programmed cell death in both endothelial cells and monocytes. LPS has been shown to trigger apoptosis in endothelial cells through an sCD14 dependent mechanism.⁷ On the other hand, LPS induced increase in CD14 expression promotes survival of monocytes, whereas down-regulated CD14 expression evokes apoptosis.⁷ Protection against apoptosis induced by CD14 dependent NF- κ B activation is due to an induction of antiapoptotic factors, such as mitochondrial antiapoptotic factor Bcl-2,⁵¹ inhibitor of apoptosis protein 1,⁵² or X linked inhibitor of apoptosis protein,⁵³ which inhibit several of the caspase enzymes involved in the cell death programme. Resistance of macrophages to apoptotic triggers may be beneficial for inflammatory processes where macrophages are needed as phagocytes for removal of moribund cells and apoptotic bodies, processes in which the CD14 receptor has also been implicated.⁵⁴ Apoptotic cells are recognised by different cellular systems, such as the phosphatidylserine receptor, the CD36/ $\alpha_v\beta_3$ integrin/thrombospondin system, and the lectin and CD14 receptor systems.^{55, 56} However, apoptotic cells, unlike LPS, do not provoke the release of proinflammatory cytokines from macrophages.⁵⁵ Therefore, the multifunctional CD14 receptor is a surface molecule of monocytes that can promote survival and antagonise apoptosis and a recognition receptor of macrophages that enables interaction with apoptotic cells.⁵⁷

CD14 PROMOTER POLYMORPHISM AND ATHEROMATOUS PLAQUE INSTABILITY

The CD14 receptor is considered to be a monocyte activation marker,^{58, 59} and both increased density of mCD14 and serum concentrations of sCD14^{58, 59} have been reported in patients with acute coronary syndromes (ACS). However, it has been shown that the CD14 receptor is not merely a monocyte activation marker, as it can synergise with C reactive protein in the activation of endothelium,⁶⁰ considered to be the first step in atherogenesis and coronary events. Moreover, increased monocytic CD14 expression during ACS was associated with a 2.4-fold higher secretion of tumour necrosis factor α by infectious stimuli (LPS).⁵⁹ Thus, patients with increased monocyte CD14 expression may have an enhanced inflammatory response to LPS or other Gram negative bacteria products (that is, Cp-HSP60) and this may contribute to the development of ACS.

A recently identified single nucleotide polymorphism (C \rightarrow T) in position -260 of the CD14 promoter has been shown to increase transcriptional activity by lowering the affinity of the GC box for Sp3,⁸ a factor known to inhibit the activity of a number of promoters. This enhanced transcriptional activity has been associated with higher concentrations of sCD14,⁶¹ enhanced expression of mCD14⁶² on monocytes, and with the risk of myocardial infarction (MI) (table 1).^{10, 62-70}

Unkelbach *et al*¹⁰ found an increased risk of MI among homozygous carriers of the T allele with a low atherosclerotic risk profile. Three other studies involving Czech⁶² and Japanese^{63, 64} populations showed an increased risk of MI associated with the T allele and the T/T genotype of the CD14 promoter polymorphism. We have found that the T/T genotype was also associated with a higher prevalence of ACS among patients with coronary artery disease.⁶⁵ Thus, the CD14 receptor seems to have a role in atheromatous plaque vulnerability and may be considered a genetically determined risk factor for ACS. In fact, it has been recently reported that the T/T genotype was prevalent among patients with MI with

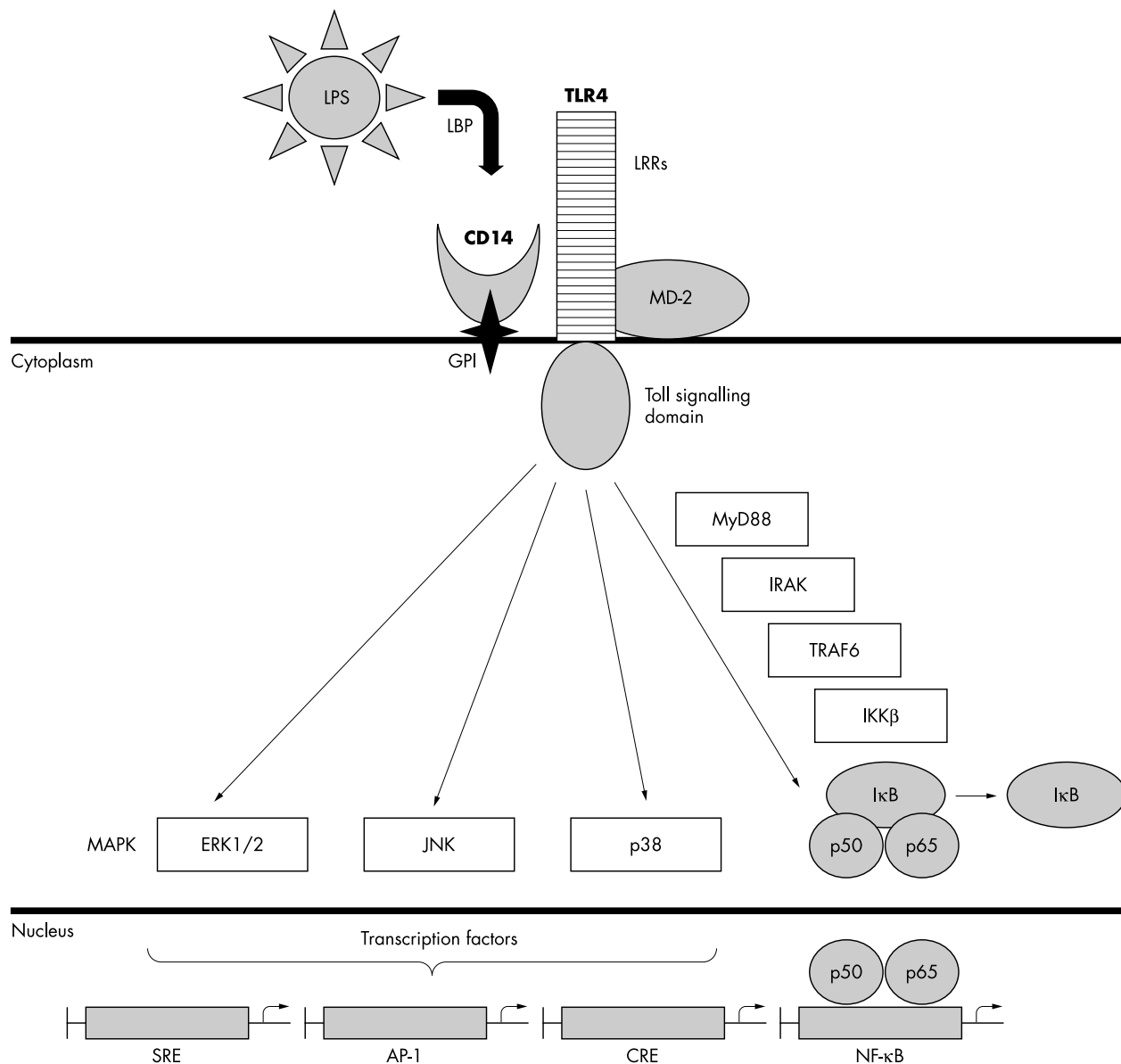


Figure 1 CD14/toll-like receptor 4 (TLR4) intracellular signalling complex: lipopolysaccharide (LPS) binds to serum LPS binding protein (LBP) and is transferred to the glycosyl phosphatidylinositol (GPI) linked CD14 receptor, which interacts with the signalling receptor TLR4 and the accessory protein MD-2. Leucine-rich repeats (LRRs) in the extracellular domain of toll family proteins have been implicated in the recognition of pathogens. LPS stimulates the activation of various mitogen activated protein kinase (MAPK) pathways, such as extracellular signal regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38. These pathways activate various transcription factors, including Elk-1, c-Jun, c-Fos, activating transcription factor 1 (ATF-1), ATF-2, serum response factor, and cAMP response element binding protein. In addition, LPS activates the nuclear factor κ B (NF- κ B) pathway through myeloid differentiation protein 88 (MyD88), interleukin 1 (IL-1) receptor associated kinase (IRAK), and tumour necrosis factor (TNF) receptor associated factor 6 (TRAF6). Activated inhibitory κ B kinase β (IKK β) phosphorylates inhibitory κ B (I κ B) and permits nuclear translocation of NF- κ B/Rel complexes, such as p50/p65. LPS stimulation of monocytes/macrophages induces many inflammatory mediators, such as cytokines (TNF- α , IL-1, IL-2, IL-6, and granulocyte, macrophage, and granulocyte-macrophage colony stimulation factors), adhesion molecules (E selectin, intercellular adhesion molecule 1, vascular cell adhesion molecule 1), chemokines (IL-8, macrophage chemotactic protein 1), receptors (tissue factor, IL-2R α), and inflammatory enzymes (inducible nitric oxide synthase and cyclo-oxygenase 2, etc). AP-1, activating protein 1; CRE, cAMP response element; SRE, serum response element.

insignificant coronary artery stenosis at the culprit lesion and that peripheral blood mononuclear cells from T/T homozygotes release a large amount of tumour necrosis factor α when challenged with LPS, more than C/T or C/C genotypes.⁶⁶ Taking these data together and given that the density of the CD14 receptor has been reported to be higher in T/T homozygotes than in the other two genotypes,⁶² coronary plaques of patients carrying the T/T genotype have a tendency to rupture because of the existence of activation prone monocytes/macrophages, even when there is only a small amount of coronary atheroma.⁶⁶ This supports the role of

CD14 promoter polymorphism in atheromatous plaque vulnerability. However, findings of an association between the T allele of the CD14 promoter polymorphism and the risk of MI are not universal (table 1). Recent studies found no association between allele and genotype distributions of CD14 promoter polymorphism and the risk of MI.⁶⁷⁻⁷⁰ The discrepancies between these negative studies and those where an association has been found may be explained by a different ethnic background of the study population and the use of subgroup analysis. Moreover, most of the CD14 studies were cross sectional and not prospective studies.

Table 1 Results from studies investigating the potential association between risk of myocardial infarction and the C(-260)T polymorphism in the promoter of the CD14 receptor gene

Author and reference	n	Cases		Controls		Association	Comments
		Allele T	Genotype T/T	Allele T	Genotype T/T		
Unkelbach <i>et al</i> ¹⁰	2228*	48%	23%	46%	21%	No	Association in low risk patients, especially >62 years
Hubacek <i>et al</i> ⁶²	313*	49%	28%	35%	16%	Yes	OR 1.8, 95% CI 1.3 to 2.5, p<0.05†
Shimada <i>et al</i> ⁶³	164*	65%	52%	49%	23%	Yes	OR 3.8, 95% CI 1.5 to 9.4, p<0.05†
Zee <i>et al</i> ⁶⁸	774‡	47%	19%	47%	22%	No	Association in >60 years and BMI >25 kg/m ²
Nauck <i>et al</i> ⁶⁹	2062*	48%	24%	47%	21%	No	None
Koch <i>et al</i> ⁷⁰	1895*	46%	22%	48%	22%	No	None
Hohda <i>et al</i> ⁶⁴	1029*	57%	33%	52%	25%	Yes	OR 1.4, 95% CI 1.1 to 1.9, p<0.05†
Arroyo-Espliguero <i>et al</i> ⁶⁵	428*	55%	33%	45%	22%	Yes	OR 3.1, 95% CI 1.3 to 7.4, p<0.05†
Longobardo <i>et al</i> ⁷⁰	430*	56%	33%	51%	27%	No	None
Kondo <i>et al</i> ⁶⁵	333*	NA	NA	NA	NA	No	Association in patients with myocardial infarction with insignificant coronary artery stenosis (OR 3.32, 95% CI 1.38 to 8.12)

*Case-control studies; †odds ratios of genotype T/T; ‡nested case-control study. BMI, body mass index; CI, confidence interval; NA, not available; OR, odds ratio.

Therefore, the reported association between CD14 promoter polymorphism and the risk of MI may reflect a real susceptibility of patients who carry the T allele but it may also reflect either linkage disequilibrium or be a chance finding. Thus, prospective studies are needed to further clarify this issue.

Despite the discrepancies, the weight of the evidence points to a higher vascular risk among patients with proinflammatory gene polymorphisms,⁷¹ and a potential role of the CD14 promoter polymorphism in the risk of ACS can be proposed. Chronic infection and recent reinfection by *C pneumoniae* have been associated with the development of acute coronary events.⁷² Chlamydial LPS antigen and Cp-HSP60 released from damaged tissues of distant sites of infection or inflammation may trigger proinflammatory responses through the CD14 receptor in atheromatous plaque infiltrating monocytes/macrophages. The signalling receptor for LPS and for both Cp-HSP60 and hu-HSP60 is the CD14 co-receptor TLR4.³⁰ Recently, it has been shown that TLR4 is preferentially expressed by macrophages within lipid rich atherosclerotic lesions and is upregulated by oxidised low density lipoprotein.⁷³ Normal arteries have only minimal or no TLR4 expression. Thus, the enhanced expression of CD14 and TLR4 on monocytes/macrophages, associated with the C(-260)T polymorphism and by oxidised low density lipoprotein of lipid rich atherosclerotic plaque, respectively, may fuel the inflammatory response of monocytes to infection by *C pneumoniae* or its components and therefore promote plaque vulnerability in patients with coronary artery disease. The exaggerated immune response to *C pneumoniae* or its products, as reflected by the increased monocyte responsiveness to LPS stimulation in patients with recurrent unstable angina,⁷⁴ may be mediated by these two mechanisms and implicated in atheromatous plaque instability. Moreover, seropositivity for Cp-HSP60 appears to be a sensitive and specific marker of ACS, unrelated to *C pneumoniae* IgG antibody titres or C reactive protein concentrations.⁷⁵ These findings suggest an enhanced anti-self immune response related to antigenic mimicry between Cp-HSP60 and hu-HSP60 (and not directly related to *C pneumoniae* infection), possibly also CD14/TLR4 mediated.

TLR4 POLYMORPHISM AND CARDIOVASCULAR EVENTS

A few genetic variants of TLR4 that affect composition, structure, and function of the receptor have been identified.⁹ The most frequent polymorphisms, TLR4 Asp299Gly and

Thr399Ile, both affect the extracellular domain of the receptor.⁹ The Asp299Gly variant is biologically important, as it attenuates TLR4 mediated LPS signalling and is associated with a diminished airway response to inhaled LPS in healthy volunteers.⁹ Recent results from the prospective population based Brunek study showed that patients with the Asp299Gly TLR4 polymorphism had a significantly lower risk of early plaque development in the carotid arteries.⁷⁶ This study also showed that the cumulative burden of cardiovascular disease was reduced by more than half in patients with both 399Ile and 299Gly TLR4 polymorphisms, as compared with the background population. Moreover, a decreased risk of acute coronary events has also been reported among carriers of the 299Gly TLR4 polymorphism.¹¹ Further indirect support for a protective role of the Asp299Gly TLR4 polymorphism in human atherogenesis derives from the association of this genetic variant with low concentrations of C reactive protein, adhesion molecules, and other acute phase reactant and inflammatory molecules.⁷⁶

These data, and those for the CD14 promoter polymorphism, support the concept that the intensity of the genetically determined individual inflammatory response may have a major role in determining the magnitude of atherogenesis and subsequent clinical outcome. As underlined in a recent review,⁷⁷ the selection of genes with an enhanced inflammatory response against infection from earlier times may have switched to a maladaptive response in our modern environment. This genetic variance in the innate immune system may be associated with clinical events related to atheromatous plaque rupture. Taking all this information together, it is tempting to speculate that a CD14/TLR4 mediated link exists between infection and the development of acute coronary events.

CONCLUSION

CD14 and the TLR4/NF- κ B pathway have been associated with various biological functions implicated in the development of atherosclerosis and acute coronary events. The C(-260)T polymorphism in the promoter of the CD14 receptor gene is associated with enhanced transcriptional activity,⁸ increased CD14 expression,⁶¹ and acute coronary events.¹⁰ The 299Gly TLR4 polymorphism, which is associated with attenuated receptor signalling, is related to the risk of developing acute severe infections but has been associated with low concentrations of circulating mediators of inflammation and a decreased risk of atherogenesis and ACS.¹¹ The presence of gene polymorphisms with

proinflammatory associations may fuel the inflammatory response of monocytes/macrophages to infection by *C pneumoniae* or its components and therefore promote atheromatous plaque vulnerability. Further well designed prospective trials are needed to understand fully the role of genetic polymorphisms in atherogenesis and the development of cardiovascular events.

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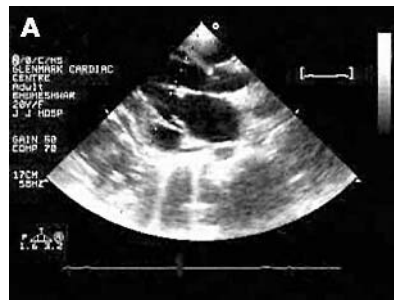
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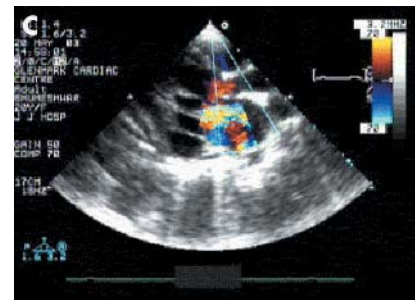
Submitral aneurysm

A 12 year old Indian boy presented with a history of progressively worsening exertional dyspnoea. Clinical examination revealed left heart failure and mitral regurgitation. Echocardiography showed a large, submitral aneurysm arising immediately below the posterior mitral leaflet. Colour Doppler flow imaging demonstrated systolic flow into the aneurysm and mitral regurgitation. The mechanism for the latter was interference of the aneurysm with posterior mitral leaflet function.

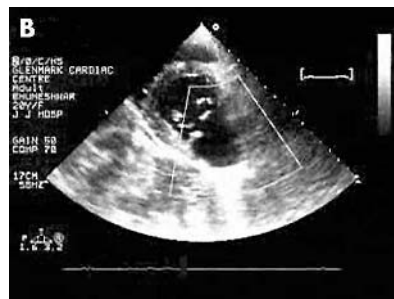
Submitral left ventricular aneurysm is a cardiac pathology widely recognised, but relatively unknown, occurring almost exclusively in African black patients. Although this idea of racial prevalence still exists, cases have been described in patients of all the races including, black, white, and yellow races and also in Brazilian Indians. It was described in 1812 by Corvisart and, since this time, around 100-120 cases of these aneurysms have been reported. They are thought to be aneurysms caused by a congenital defect in the posterior portion of the mitral annulus, and may produce symptoms through diastolic overload (by virtue of their volume or by causing mitral regurgitation), thromboembolism, arrhythmias, or compression of the left circumflex artery leading to ischaemic manifestations.



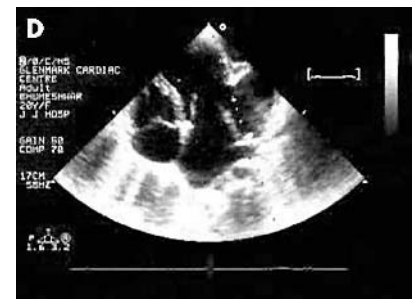
Parasternal long axis view showing the aneurysm just beneath the posterior mitral leaflet.



Colour mitral Doppler showing significant mitral regurgitation.



Short axis view showing the aneurysm.



Apical four chamber view showing the aneurysm.

This particular patient had a successful surgical repair of the aneurysm along with mitral valve replacement.

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