

Genomics in cardiovascular diseases: analysis of the importance of the toll-like receptor signaling pathway

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Abstract: The development of techniques for genomics study makes it possible for us to further our knowledge about the physiopathology of various immunological or infectious diseases. These techniques improve our understanding of the development and evolution of such diseases, including those of cardiovascular origin, whilst they help to bring about the design of new therapeutic strategies. We are reviewing the genetic alterations of immunity in said field, and focusing on the signaling pathway of toll-like receptors because not only does this play a decisive role in response to microorganisms, it is also heavily involved in modulating the inflammatory response to tissue damage, a side effect of numerous cardiovascular diseases. These alterations in tissue homeostasis are present under a wide range of circumstances, such as reperfusion ischemia (myocardial infarction) phenomena, arteriosclerosis, or valvulopathy.

Keywords: genome-wide association study, single-nucleotide polymorphism, innate immune system, ischemic/reperfusion, myocardial infarction

Introduction

Recent advances in molecular biology have increased our knowledge of how genes control physiological functions involved in the modulation of specific phenotypes. The ability to interrogate the entire genome was made possible by two key advances: the Human Genome Project, with a draft sequence in 2001, and a nearly-complete sequence in 2003, and the International HapMap project.¹⁻⁴

The human genome consists of approximately 25,000 genes codified by approximately 3.1 billion base pairs, distributed in 23 pairs of chromosomes.⁵ When DNA is sequenced, an inter-individual variability in nucleotide sequences can be detected. Differences in single bases are by far the most common example of genetic variation, known as single-nucleotide polymorphisms (SNPs). A SNP is a single nucleotide DNA sequence variant in the genome that differs between members of the same species or a pair of chromosomes in an individual. SNPs occur on average every 300 base pairs. Hundreds of thousands of SNPs have been discovered and are available in public databases.⁶ Comparing DNA sequences of two unrelated individuals has shown the existence of, on average, approximately one difference at every 1,200 bases.

After years in which there were reports of only isolated data and occasional associations of genes with specific pathologies, we have come to a point where high-throughput technologies (which genotype more than 500,000 genetic markers known as SNPs) and novel statistical tools have led to a virtual explosion of novel genetic markers associated with complex human diseases.

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SNP assay can become a method of analysis that makes it possible to explain the genesis of specific pathologies, and to define the underlying patterns of inheriting genetic variation. The inheritance pattern is quantified by the linkage disequilibrium, which represents the likelihood that alleles of nearby SNPs will stay together and retain their linear arrangement on a haplotype during meiosis. This likelihood depends on the mechanisms of recombination as well as their frequency, it being more frequent among alleles less separated in the genome. Once these mechanisms are known, researchers should analyze subsets of SNPs (called tag SNPs) to design genotyping arrays, and be able to perform association studies. Genetic variants that are not directly genotyped can then be imputed from the genotyped tag-SNP subset. Imputation presumes the SNP allele at a different location, inferred by its degree of linkage disequilibrium with an allele at a directly genotyped variant.⁷

Knowledge about genes and SNP functions is essential for establishing the scientific basis for how genes interact with the environment and produce a specific phenotype. However, while analyzing genetic variations and their association with diseases is relatively simple, final confirmation of their functional importance is normally more complex. Precisely for that reason, one of the groups of biomarkers being intensely studied, is that of the genetic variants.⁸

Our review attempts to update, and show the evolution that the development of knowledge about the genetic alterations that affect the immune system has undergone in the last years. We are specifically going to analyse the role of the signaling pathway for toll-like receptors (TLRs).^{9,10} These receptors and their alteration have been implicated in the genesis of some the most currently relevant cardiovascular pathologies, given their function as immune-regulators that regulate determined signaling pathways. Their regulation is extremely complex. Understanding the regulation of signaling pathways is of great importance since it permits us a better understanding of the physiopathology of the diseases, and will allow for a better therapeutical targeting of disease in the future via blockage of specific signaling pathways.¹¹

Genome-wide association studies

Recently, genome-wide association studies (GWAS) have made it possible to analyze new loci associated with various diseases such as, rheumatoid arthritis, Crohn's disease, Type 2 diabetes, bipolar disorder, and other pathologies included in GWAS catalog.¹² These studies are based on the genetic analysis of very large case samples and controls, by genotyping thousands of SNPs using DNA microchips.

The results of the HapMap project on genotype frequencies and haplotype structure make it possible to select the minimum SNPs required in GWAS genotyping, in order to make the detection of the majority of the shared genetic variability existing in the human genome possible.¹³

The advantages of GWAS over genetic studies of cases and controls, lie in the availability of greater sample sizes. Automated genotyping of the SNPs in the entire genome (consequently, not restricted to candidate genes) improves the quality of the analysis. In addition, GWAS perform a SNP quality control, given that they exclude SNPs from the analysis when the Hardy–Weinberg equilibrium is not met, when an allele frequency is < 1%, or when the genotyping success rate is < 80%–90%. The SNPs are selected on the basis of ability to reproduce the results in other populations in order for them to be considered significant.^{13,14}

Limitations of these studies are that: (1) it is difficult to detect loci of small effect; (2) there is preferential selection of SNPs so that other polymorphisms, such as copy number variation and microsatellites, are not analyzed; (3) the contribution of low-frequency SNPs is not evaluated; and (4) they are generally performed using caucasian individuals and other population groups have not been analyzed.

Innate immune system

The innate immune system is the first line of defense against external damaging agents. It is also known to intervene in inflammatory-induced phenomena, such as those produced by the activation that tissue damage or ischemia-reperfusion (I/R) injury generates in tissue.¹⁵

Within the immune system, TLRs are a crucial link between the pathogen-associated molecular patterns and membrane-bound CD14, causing intracellular signaling with the translocation of the regulatory transcription nuclear factor kappa-B (NF- κ B) into the nucleus. NF- κ B participates in enhancing the expression of cytokines and other immunoregulatory mediators.^{16,17} This response is also present against endogenous stimuli (Figure 1).

In response to pattern detection, TLRs are involved in the activation of complement, coagulation, phagocytosis, and apoptosis functions. Alterations in the regulation of each of these processes can give rise to various pathological phenomena.^{18,19}

Toll like receptors

The TLRs, members of the interleukin 1R superfamily, are transmembrane receptors with extracellular leucine-rich repeats and an intracellular signaling domain, and are found

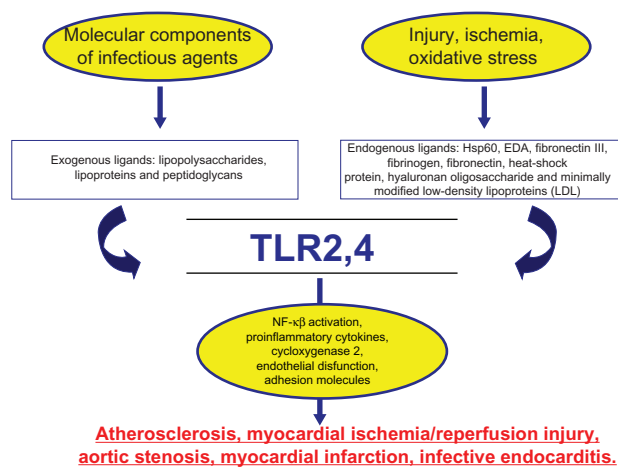


Figure 1 Diagram representing the possible endogenous and exogenous TLR activation pathways and the accompanying response.

Abbreviation: TLR, toll-like receptor.

in monocytes, macrophages and neutrophils. TLRs recognize microbial products (lipopolysaccharide [LPS], lipoproteins and peptidoglycans) and induce a signal in the affected cell through the p38 mitogen-activated protein kinase and NF- κ B.^{20–22} To date, 11 TLRs have been identified, which recognize distinct pathogen-associated molecular patterns that have been evolutionarily conserved in specific classes of microbes (Table 1). These antigens include cell-wall components of gram-positive (bacterial lipoproteins and lipoteichoic acids, detected by TLR2) and gram-negative (LPS, detected by TLR4) bacteria. Recognition of LPS involves an LPS receptor complex, of which CD14 and the TLR4 are important components.²³

Data suggests that the ability of certain individuals to respond properly to TLR ligands may be impaired by SNPs within TLR genes, resulting in an altered susceptibility to infection or inflammation.²⁴ Polymorphisms of the TLR2 R753Q gene have been linked to variations in responses to Staphylococcal infection²⁵ and are associated with a significantly increased risk of infective endocarditis,²⁶ while polymorphisms of R677W have been associated with an increased susceptibility to leprosy and tuberculosis in Asian populations.²⁷ Two common mutations in the TLR4 gene have been described, TLR4 D299G and T399I. These mutations are reportedly linked to an increased risk of gram-negative bacterial infections and septic shock.^{23,28–30}

TLR signaling pathway

The stimulation of the TLRs through intracellular signaling pathways activates various transcription factors that cause the production of inflammatory mediators. In addition to

Table 1 Different endogenous and exogenous components that recognize TLRs

TLRs	Components
TLR2 (partnered with TLR6)	Lipoproteins Lipoteichoic acid Modulin Lipopeptides MALP-2 Glycolipids GPI anchors Peptidoglycan Zymosan Lipoarabinomannan Viral porins Viral hemagglutinin Heat-shock proteins 60 and 70
TLR4	LPS Lipoteichoic acid Mannan Taxol Heat-shock proteins 60 and 70 Fibrinogen Hyaluronan Fibronectin EDA domain Respiratory syncytial virus fusion protein Glycosylphospholipids

Abbreviations: TLR, toll-like receptor; LPS, lipopolysaccharide.

their pivotal role in host immune defense against invading pathogens, emerging evidence over the past ten years has demonstrated that TLRs appear capable of responding to stress, and modulating inflammation and tissue damage following noninfectious insults such as hypoxia, and ischemia in various tissues,³¹ including the lung,³² liver³³ or heart^{34–38} (Figure 1).

After joining their specific ligand, TLRs recruit and activate various downstream kinases such as interleukin receptor-associated kinase (IRAK)-1,4, tumor necrosis factor (TNF) receptor-associated factor (TRAF)-family member-associated NF- κ B activator-binding kinase 1 (TBK1) through a specific set of adaptors. TLR signaling can be divided into two general pathways, namely, MyD88- and Trif-dependent (or MyD88-independent) (Figure 2).

In the MyD88-dependent pathway, activated TLR4 recruits downstream IRAKs through the adapter proteins Mal and MyD88. Following a cascade of kinase activation this pathway ultimately leads to the activation of NF- κ B and the production of proinflammatory cytokine.³⁹

In the Trif-dependent pathway, TLR4 signals through TRAM-Trif, resulting in TBK1 activation and a downstream stimulation of interferon regulatory factor 3 and production of interferon.⁴⁰

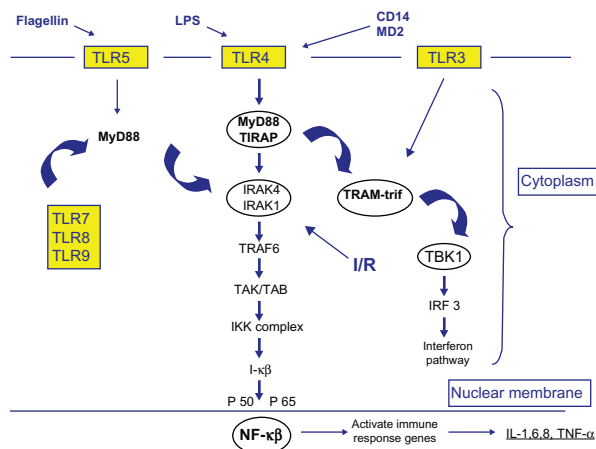


Figure 2 TLR4 signaling pathway.

Note: Activation sequence of each of the mediators that is involved and the molecules produced as a consequence of their activation and expression.⁶⁸

Abbreviations: TLR, toll-like receptor; I/R, ischemic/reperfusion; NF-κB, nuclear factor κB; IL, interleukin; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; TRAM, TRIF-related adaptor molecule; TBK1, activator-binding kinase 1; IRAK, interleukin receptor-associated kinase; TNF-α, tumour necrosis factor-α; TRAF, TNF receptor-associated factor.

In TLR2 signaling, TLR2 dimerizes with either TLR1 or TLR6. The heterodimers recruit and activate IRAK 4,1 via a Mal/MyD88-dependent mechanism and ultimately lead to the induction of cytokines. In the case of TLR2, this signaling pathway does not induce interferon production. All TLRs, with the exception of TLR3, signal through MyD88-dependent pathways.⁴¹

In normal conditions TLRs are highly concentrated in the endothelium and heart, suggesting that TLRs are of functional importance in the cardiovascular system.^{42–44} Activation of most Toll-like receptors leads to the recruitment of the interleukin 1 receptor-associated kinase, and nuclear translocation of the latent cytoplasmic transcriptional regulator nuclear factor-kappa B (NF-κB). Underscoring its central role in regulating the immune response, NF-κB is a critical mediator of several inflammatory pathways in addition to the Toll-like receptors. TLR2 and TLR4 bind bacterial cell wall components. TLR2 and TLR4 also respond to the endogenous ligands heat shock protein 60 (HSP60), HSP70, and Gp96, which may be released during necrotic cell death (Table 1). TLR3 recognizes polyI:C and doublestranded viral RNA, TLR5 binds gram-negative bacterial flagellin, TLR7 recognizes viral single-stranded RNA while TLR9 recognizes unmethylated CpG bacterial DNA (Table 2).

Following Toll-like receptor ligation, immune cells signal through NF-κB to produce the diverse cytokines and chemokines required for leukocyte activation and chemotaxis. Specialized tissues such as skeletal muscle, initially thought

Table 2 Different endogenous and exogenous components that recognize TLRs

TLRs	Components
TLR1 (partnered with TLR2)	<ul style="list-style-type: none"> Bacterial triacyl lipopeptides Certain proteins in parasites
TLR3	<ul style="list-style-type: none"> Viral double-stranded RNA
TLR5	<ul style="list-style-type: none"> Flagellin
TLR7/8	<ul style="list-style-type: none"> Viral single-stranded RNA
TLR9	<ul style="list-style-type: none"> Unmethylated CpG containing DNA Hemozoin Unknown
TLR10	<ul style="list-style-type: none"> Unknown
TLR11	<ul style="list-style-type: none"> Profilin, a protein from <i>Toxoplasmosis gondii</i> Not determined; present in uropathogenic bacteria

Abbreviations: TLR, toll-like receptor; CpG, DNA oligonucleotides containing dinucleotide cytosine guanine.

to be bystanders in the immune response to pathogens, have recently been found to be active participants in response to Toll-like receptor ligation. In cardiomyocytes this response can give rise to inflammatory processes and intervene by diminishing contractility.

In mice, the occurrence of at least six of these receptors, namely TLR2, TLR3, TLR4, TLR5, TLR7 and TLR9, has been observed at cardiac level.⁴⁵ It has been shown that a pathogenic ligand stimulation of TLR2, TLR4, TLR5 and TLR9 can lead to the activation of the NF-κB pathway and cardiomyocyte contractile dysfunction.^{45–47} The two receptors most studied at cardiac level are TLR2 and TLR4.^{47–52}

In reference to their occurrence at cellular level, TLRs 2, 3, 4, 5 and 9 occur in arteries and in endothelial-origin cells,^{53,54} while arterial smooth muscle cells express mRNA for TLRs 3, 4, 5 and 9.^{54,55} Macrophages, the principal cell type within atheroma, express all of the TLRs and are responsive to all TLR ligands.⁵⁴ Smooth muscle cells are responsive to TLR3, TLR4 and TLR9 ligands, whereas venous endothelial cells are responsive to TLRs 3, 4, 5 and 9 ligands.^{53,54,56}

Myocardial ischemic/reperfusion injury

The innate immune system plays a fundamental role in the physiopathology of myocardial ischemic/reperfusion (I/R) injury.⁵⁷ Furthermore, the physiological mechanisms that attempt to limit the inflammatory response, to promote survival of the myocardium, and to maintain homeostasis following cardiac I/R remain unclear.

Ischemia is associated with hypoxia. Apoptosis has also been connected with this process, and therefore an increase

in cell apoptosis is found in the ischemic-reperfused area,⁵⁸ located mainly on the borders of necrotic areas. LPS seems to induce apoptosis in endothelial cells,⁵⁹ while it has an anti-apoptotic effect in monocytes,⁶⁰ macrophages,⁶¹ neutrophils,⁶² and cardiomyocytes.⁶³ This complements the explanation of certain processes that take place in I/R.

The TLR4-mediated NF- κ B activation pathway plays an important role in myocardial I/R injury. Modulation of the TLR4-mediated signaling pathway or TLR4 deficiency is known to result in the protection against myocardial I/R.^{34,36,64} TLR2 is also significant to these same processes of immune inflammatory response regulation. However, the mechanisms that regulate this response are not well known. Studies in mice with the inactive mutant TLR4, or genetically deficient for TLR4,^{36,65} TLR2⁶⁶ or MyD88,³⁵ or pretreated with a TLR4 antagonist (eritoran) exhibited reduced myocardial infarction (MI) sizes compared with wild type or vehicle-treated animals, respectively.⁶⁷ This suggests that signaling through TLR2 and TLR4 modulates ischemic response at cardiac level.

LPS is known to induce cardiac dysfunction during endotoxemia in the adult mammalian heart. In wild type mice, at doses of 5 mg/kg and 25 mg/kg, LPS induces significant left ventricle contractile dysfunction (% LV fractional shortening and myocardial velocity of LV circumferential shortening (Vcf)) through a TLR4-dependent mechanism. In contrast, there was no significant change in these parameters in the TLR4-deficient mice when compared with diluent-treated control mice.⁵¹ Animal studies have demonstrated that administering sublethal doses of LPS confers a “preconditioning-like” effect.⁶⁸ This effect is similar to that of ischemic preconditioning, or that of anesthesia-induced methods (inhaled anesthetics), protecting the heart from I/R injury. This effect is cancelled out by cycloheximide, a protein synthesis inhibitor that blocks translation elongation.⁶⁹

Administering LPS provokes inducible nitric oxide synthase (iNOS) induction at cardiac level^{63,70} mediated by TLR4.^{51,63} Likewise, as with ischemic preconditioning, LPS-induced cardioprotection seems to be mediated by iNOS.^{70,71}

The data also seem to agree with *in vitro* observations that Akt, among other survival pathways, mediates a TLR4-mediated antiapoptotic benefit.^{63,72} In a study in mice, Wang et al⁷³ showed that TLR4-MyD88 signaling confers potent cardiac protection against I/R injury via iNOS- and sGC-dependent mechanisms. These data suggest that MyD88, but not Trif, signaling mediates the LPS-induced cardioprotection against I/R injury. Nevertheless, iNOS inhibition by 1400W significantly lowers the cardioprotective

effect of TLR4. Deficient TLR2 or TLR4 signaling in mice prevents adverse cardiac remodelling, resulting in preserved cardiac function and geometry after MI.^{37,38} Inhibition of TLRs may provide new therapeutic options after MI, a view which is supported by recent observations in TLR-knockout mice.⁷⁴

In reference to TLR2, administration of TLR2 ligands protects the myocardium against I/R injury, and improves cardiac function and hemodynamics after I/R. However, this protection is lost in TLR2-deficient mice. It has been observed that blocking the PI3K/Akt signaling pathway impaired the TLR2 ligand-induced and PGN-induced cardioprotection. Modulation of TLR2-induced cardioprotection is known to be mediated through a PI3K/Akt-dependent mechanism. Treating mice with the TLR2 ligand significantly attenuated I/R-induced MI.^{11,75} Ischemic preconditioning has also been studied on other levels, such as at the cerebral level,⁷⁶ where it was again found that the TLR2-specific ligand, Pam3CSK4, decreased I/R injury.

Another important fact is that NF- κ B activation is inhibited in human mononuclear THP-1 cells pretreated with OPN-301 after P3C stimulation (selective ligand for TLR2). However, NF- κ B activation does occur after lipopolysaccharide stimulation pretreated with OPN-301. OPN-301 selectively inhibits TLR2 signaling.

Atherosclerosis and coronary artery disease

The TLR signaling pathway plays a crucial role in the innate immune response, just as in the case of I/R. Previous studies⁷⁷⁻⁷⁹ have shown how elevated circulating markers of systemic inflammatory status, such as TNF- α , C-reactive protein, IL-6, IL-8, and soluble vascular adhesion molecules (all related to the TLR signaling pathway) were also correlated with cardiovascular risk. Immunohistochemical studies have revealed that inflammatory processes are active within the developing plaque, with the demonstration that inflammatory gene products, such as IL-8, intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, E-selectin, and TNF- α were upregulated in atheroma plaque, as compared with healthy arterial areas or even with the control.

Evidence from diverse sources has suggested that TLRs can affect atherosclerosis in multiple ways, which might include both endogenous (MM-LDL, HSP60, EDA) and exogenous (that is, originating from pathogens) molecules among their cognate ligands. TLRs are capable of recognizing endogenous “danger signals”, released during cell death.⁸⁰ TLRs exert their inflammatory response through

NF- κ B translocation to the nucleus; the signaling pathway is superimposed on that, mediated in response to an external infectious agent.

It has been observed in mice, that specific deletion of either TLR2 or TLR4 also led to a significant reduction in the atherosclerosis burden.^{81–84} In arterial tissue obtained from Apo E-deficient mice, the levels of both TLR-2 and TLR-4 mRNA increased with time, and TLR-2 and TLR-4 expression on circulating monocytes increased after 40 weeks in Apo E-deficient mice compared with controls.⁸⁵ A TLR-2 antagonist, Pam3CSK4, also enhanced lesion development in Apo E-deficient mice.⁸⁶

TLR4 expression has also been seen to increase significantly in macrophages and endothelial cells in patients with arteriosclerosis.⁸⁷ TLR4 ligation activates NF- κ B, resulting in the expression of several inflammatory genes, and the proliferation of vascular smooth muscle cells. Kiechl et al⁸⁸ observed that the TLR4 polymorphism Asp299Gly was associated with a decrease in atherosclerosis risk at femoral and carotid levels. The Bruneck study showed that allele asp 299-carrier patients had less progression in development of carotid atherosclerotic plaque.⁸⁸

With respect to coronary pathology, a few months after the first large GWAS was published in early 2007, three independent GWAS for coronary artery disease were published from the Ottawa Heart Study,⁸⁹ deCODE genetics,⁹⁰ and from the Wellcome Trust Case-Control Consortium.⁹¹ The three studies concurred in locating the same significant loci on chromosome 9p21. Until that time, no other study had reported this association. Nevertheless, the SNPs identified were not associated with any of the traditional cardiovascular risk factors. Consequently, it appears that the genetic mechanism underlying the association signal is operating through a novel pathway. These results emphasise how difficult it is to link some of these genetic alterations with pathogenic mechanisms.

Other findings on coronary pathology, published by Ameziane et al,⁹² have been in agreement with those obtained in the Bruneck study. These authors observed that the allele Gly299 was associated with a reduction in the risk of acute coronary events, independently of the basal risk factors. Certain humeral mediators such as fibrinogen plasma and soluble VCAM-1 also showed lower concentrations in this patient group, just as pro-inflammatory cytokines, soluble adhesion molecules, and acute phase reactants.⁹² Similar findings have been reported by other authors such as Holloway et al⁹³ and Kolek et al.⁹⁴ The results, found in nonacute coronary pathology, showed that the presence of

the allele Gly299 was not associated with a reduced time of plaque evolution. This differs from what has been observed in arteriopathy at the peripheral level, where plaque evolution was indeed found to slow down.^{95,96}

These alterations are related not only with the possibility of pathology development, but also with response to certain drugs. Damani et al⁹⁷ showed the significance and clinical repercussions of the genomic component in the response to certain antiaggregants, such as clopidogrel. In a recent study, it was observed how patients who were Gly299 allele carriers benefited from pravastatin treatment significantly more, in comparison with other groups.⁹⁵

Valvular heart disease

As we have indicated, the TLR pathway modulates the inflammatory response, and these mechanisms are related to the development of arteriosclerosis.^{81,82} Pathological changes similar to those observed in arteriosclerosis are known to occur in the genesis of aortic stenosis (AS). AS is the most frequent valve pathology in the western world; although its occurrence was previously thought to be determined by passive mechanisms, it is now known to be an active process that can be considered an inflammatory process.^{98–101}

The human aortic valve interstitial cell (HAVIC) has been implicated in the pathogenesis of AS. In response to proinflammatory stimulation via TLR2 and TLR4, the HAVIC phenotype changes from that of a myofibroblast to that of a bone-forming-like cell.¹⁰² Characteristics of this osteogenic phenotype include increased concentrations of the potent bone-forming protein, the osteogenic transcription factor, bone morphogenetic protein-2 (BMP-2), and Runx2, as well as increased concentration and activity of alkaline phosphatase.^{103–106} TLR4 generates cytoplasm signaling, leading to the phosphorylation of the IKK complex and mitogen-activated protein kinases, including p38 and JNK. Phosphorylation of IKK liberates bound NF- κ B from the cytoplasm, and it then facilitates the production of proinflammatory mediators.

Meng et al¹⁰³ showed that HAVICs express TLR2 and TLR4, and that PGN- or LPS-induced HAVIC stimulation leads to proinflammatory mediator expression, and production of factors related to the upregulation of osteogenesis. We can say that TLR2 and TLR4 are crucial in inflammatory phenomena at the level of the aortic valve, and in the development of stenosis.¹⁰⁴

Song et al, in a study on native aortic valves, found that TLR4 mediated the ICAM-1 response to LPS in HAVICs. These results coincide with those observed by other authors,

who found that ICAM-1 played an important role in leukocyte infiltration during inflammation.^{107,108} Microfilaments are associated with ICAM-1 after LPS stimulation, and also play an important role in regulating ICAM-1 expression and the influence of microfilaments on the cell surface. When the cells were treated with a TLR4-neutralising antibody or TLR4 siRNA, ICAM-1 induction by LPS was significantly attenuated. This reinforces the idea that TLR4 are crucially involved in mediating the ICAM-1 response to LPS in HAVICs. In valve pathology, it has been observed that cell exposure to viral as well as gram-negative bacteria patterns promotes TLR-mediated sustained inflammatory and pro-osteogenic responses, which could be relevant to the pathophysiology of degenerative AS.

In another area within valve pathology, our work group observed that the presence of TLR2 R753Q polymorphism was associated with a significantly increased risk of infective endocarditis, specifically with gram-positive bacterial infection.²⁶

Applications of genomics in cardiovascular disease

Genomics has made it possible to further our knowledge of the mechanisms regulating the development of specific pathologies. In the cardiovascular sphere, there has been an emphasis on genomic studies for the analysis of risk markers, and experts are attempting to incorporate results into the classic risk scales, in order to stratify patients for patient management.^{77,109,110}

For a long time researchers have been working on designing molecules which allow them to modify the responses mediated by a damaging stimulus in which the immune response is involved. Said response is often exaggerated, and thereby causing an injury. It is known that these mechanisms are ultimately coded genetically and that, as indicated above, their alteration will lead to modification of the susceptibility to developing certain pathologies or the evolution of disease, as protein coding and its functionality will be affected.

Since the discovery of TLRs decades ago, much progress has been made in our understanding of the complex TLR signaling. The role of TLR agonists/antagonists is now being analyzed, in the hopes of decreasing the inflammatory response mediated by said signaling pathway.¹¹¹ One example is OPN-301; this molecule was shown to inhibit TLR2 and reduce myocardial I/R injury, as well as preserve cardiac function and geometry *in vivo*.⁸⁷ The same is true of specific TLR2 ligands such as PGN and Pam3CSK4, which

improved cardiac function in mice after I/R injury, and therefore lowered its effects.⁷⁵

Given the role of TLR2 in mediating ischemia–reperfusion injury, this molecule is attractive for testing in phase I clinical trials of patients with ischemic cardiomyopathy. In one study, AP177, a DNA aptamer identified by a SELEX (systematic evolution of ligands by exponential enrichment) screen, was found to bind to TLR and competitively antagonize TLR2 ligand binding, thereby inhibiting NF- κ B activity and proinflammatory cytokine production.^{111,112}

There are a number of strategies that have been undertaken to inhibit TLR4 activation. Eritoran (E5564), which reduces the binding of lipid-A (the biologically active part of the lipopolysaccharide molecule), reduced mortality by 6.4% compared with the placebo group in one phase II sepsis trial, and is currently undergoing evaluation in phase III sepsis trials (NCT00334828). Given that the pharmacodynamic profile of eritoran requires administration as a continuous infusion or by repeated intravenous injections, this TLR4 antagonist may not be practical in the treatment of chronic heart failure. However, it may be useful during myocardial inflammatory states or in the setting of acute coronary syndromes that lead to the development of heart failure. Alternative approaches have been to develop variations of lipid-A that bind TLR4 but these have reduced agonist activity (eg, CRX-527, lipid-IVa).¹¹³ TAK-242 also targeted TLR4-dependent signaling, although the precise target is not known.¹¹⁴ Development of this compound was discontinued during a phase III sepsis clinical trial because the drug's profile did not meet the criteria required to support continued development, and not because of drug safety issues (NCT00633477).¹¹⁵ Ibudilast (AV411) is another TLR4 antagonist that suppresses proinflammatory cytokines such as TNF and IL-6, and may induce the anti-inflammatory cytokine IL-10; Ibudilast is undergoing phase II trials for opioid dependence (NCT00723177). Finally, OPN-401 is a viral protein-derived peptide that inhibits TLR4-dependent signaling and is also in preclinical development.

Limitations

It is important to remember that in genomics, the studies of cases and controls present significant limitations in terms of establishing associations, and their use in risk scales should consequently be accompanied by further, in-depth analysis. In addition, the results obtained in many cases present important variability with respect to the type of sample studied. In this sense, the GWAS represent an attempt to improve the results that have been already obtained. Genotyping of

large data sets, and robust estimates that combine the results of different studies, are needed. The SNPs in the currently identified loci do not represent the full heritability estimate for the risk involved, so therefore it should be determined how newly emerging data from post-GWAS research could be incorporated into existing risk algorithms.

Conclusion and future perspectives

Various studies have analyzed the influence of genetic components, especially that of TLRs, on the development of cardiovascular pathology. The different findings lead us to believe that genomics has a relevant role in the genesis of these processes. The TLRs participate in the innate immune response system, but they also modulate the responses that arise in situations of tissue stress. There are discrepancies in some findings, which might be explained by differing study methods. The disparate findings also illustrate how complex and difficult it is to define the role of the TLRs as modulators of inflammation and tissue damage. In the future, it is possible that developments in genomics will enable us to better understand disease evolution and predisposition, as well as facilitate the design of new molecules that will improve the prognosis of some of these pathologies.

Disclosure

The authors declare no conflicts of interest.

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