Fcγ Receptors and Ligands and Cardiovascular Disease

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Abstract: Fcγ receptors (FcγRs) classically modulate intracellular signaling on binding of the Fc region of IgG in immune response cells. How FcγR and their ligands affect cardiovascular health and disease has been interrogated recently in both preclinical and clinical studies. The stimulation of activating FcγR in endothelial cells, vascular smooth muscle cells, and monocytes/macrophages causes a variety of cellular responses that may contribute to vascular disease pathogenesis. Stimulation of the lone inhibitory FγcR, FcγRIIB, also has adverse consequences in endothelial cells, antagonizing NO production and reparative mechanisms. In preclinical disease models, activating FcγRs promote atherosclerosis, whereas FcγRIIB is protective, and activating FcγRs also enhance thrombotic and nonthrombotic vascular occlusion. The FcγR ligand C-reactive protein (CRP) has undergone intense study. Although in rodents CRP does not affect atherosclerosis, it causes hypertension and insulin resistance and worsens myocardial infarction. Massive data have accumulated indicating an association between increases in circulating CRP and coronary heart disease in humans. However, Mendelian randomization studies reveal that CRP is not likely a disease mediator. CRP genetics and hypertension warrant further investigation. To date, studies of genetic variants of activating FcγRs are insufficient to implicate the receptors in coronary heart disease pathogenesis in humans. However, a link between FcγRIIB and human hypertension may be emerging. Further knowledge of the vascular biology of FcγR and their ligands will potentially enhance our understanding of cardiovascular disorders, particularly in patients whose greater predisposition for disease is not explained by traditional risk factors, such as individuals with autoimmune disorders. (Circ Res. 2015;116:368-384. DOI: 10.1161/CIRCRESAHA.116.302795.)

Key Words: atherosclerosis ■ C-reactive protein ■ hypertension ■ nitric oxide synthase type III

Fcγ receptors (FcγRs) are plasma membrane–associated receptors for IgG and the pentraxins C-reactive protein (CRP) and serum amyloid P component (SAP). As a result of recent interest in the role for inflammation in cardiovascular disorders, FcγRs and their ligands have been investigated in the context of cardiovascular disease. This review will highlight major observations made in both preclinical and clinical studies in that realm. After a brief description of FcγR subtypes and their functions, their IgG and pentraxin ligands will be discussed. Investigations performed in cell culture will then be highlighted, followed by queries of the impact of FcγR and ligands in animal models of cardiovascular disorders.

Attempts to understand the influence of IgG and pentraxins on cardiovascular disease risk and outcome in humans will then be summarized, including the voluminous studies of CRP as a risk factor and possible disease mediator. How genetic variants in FcγRs affect human cardiovascular health will be presented, including a recent interrogation of a variant in the lone inhibitory FcγR, FcγRIIB, that alters receptor function in endothelium. Finally, the current knowledge gaps in this area and the rationale to fill them will be presented. By recognizing what is known and not yet known about the influence of FcγR on cardiovascular health, it is hoped that this review will stimulate further work in this area. Such future efforts are warranted because FcγR biology may underlie a considerable component of vascular disease predisposition that is not currently explained by traditional risk factors.

FcγR Subtypes and Functions

FcγR Subtypes

The classical function of FcγRs is to invoke intracellular signaling on IgG binding in immune response cells and thereby modulate numerous inflammatory processes. FcγRs are categorized into activating receptors and inhibitory receptors (Table 1). In humans, the activating Fc receptors are FcγRI (CD64), FcγRIIA (CD32a), FcγRIIC (CD32c), FcγRIIIA (CD16a), and FcγRIIB (CD16b), and the sole inhibitory receptor is FcγRIIB (CD32b). In mice the activating FcγRs are FcγRI, FcγRIII, and FcγRIV, and in mice differential splicing of a single gene for inhibitory FcγRIIB yields FcγRIIB1, B2 and B3. FcγRs belong to the large immunoglobulin superfamily and they are type I transmembrane glycoproteins with the exception of the human subtype FcγRIIB, which is glycoprophosphatidylinositol (GPI) anchored. All FcγRs display a high degree of sequence identity in their extracellular portion (50%–96%) but differ significantly in their cytoplasmic domains.
Innate immune effector cells, such as monocytes, macrophages, dendritic cells, basophils, and mast cells, express activating and inhibitory FcγR. In human leukocytes, FcγRI and FcγRIIA are abundant in monocytes, macrophages, and granulocytes, FcγRIIIA are expressed primarily on macrophages but also in monocytes, and FcγRIIB are found in granulocytes.6,8 In mice, monocytes and macrophages express all activating and inhibitory FcγR, neutrophils mainly express the activating FcγRIII and FcγRIV and the inhibitory FcγRIIB, whereas the expression of FcγRI, FcγRIIa, and FcγRIII dominants on dendritic cells. There are 2 cell types that do not coexpress activating and inhibitory receptors; natural killer cells solely express the activating receptor FcγRIII, and B cells only express the inhibitory receptor FcγRIIB. In B cells, FcγRIIB functions as a regulator of activating signals transmitted by the B-cell receptor.3 In dendritic cells, the balance between activating and inhibitory FcγR activity influences their relative activation status.7 In addition to hematopoietic cells, FcγRs are expressed by follicular dendritic cells, endothelial cells, microglial cells, osteoclasts, and mesangial cells.3 Interestingly, ligand binding affects the abundance of cell surface FcγR. Immune complex binding to FcγRIIA leads to their endocytosis and ubiquitination and degradation, and engaged FcγRIIB also undergo internalization and degradation; in contrast, in human endothelial cells, CRP upregulates the expression of CD32 and CD64.9

### Activating FcγR Function

The activating FcγRs, with the exception of the human GPI-anchored FcγRIIB, activate signaling pathways through immunoreceptor tyrosine–based activation motifs (ITAMs) contained in their cytoplasmic regions. Human FcγRIIA and FcγRIIC comprises a single subunit with a cytoplasmic ITAM (Table 1) and they transduce activating signaling pathways autonomously. All other activating FcγRs (human FcγRI, FcγRIIA and mouse FcγRI, FcγRIII, FcγRIV) consist of a ligand-binding α-chain and a signal-transducing γ-chain that contains an ITAM in its cytoplasmic domain. In some instances the signal-transducing subunits for an FcγR can differ between cell types. Whereas human FcγRIIA is associated with an ITAM-containing γ-chain in monocytes and macrophages, it associates with the ITAM-containing CD2 ζ-chain in natural killer cells. Along with its signaling function, the γ-chain is important for the assembly and cell surface transport of the respective α chains. Because of their expression in innate immune effector cells, activating FcγRs have been proposed to link the specificity of antibodies generated by the adaptive immune system to the potent effector functions of the innate immune system.1,3

After crosslinking by immune complexes, the signaling pathways initiated by the different activating FcγRs are similar, beginning with tyrosine phosphorylation of the ITAM by kinases of the SRC (short for sarcoma) kinase family. This leads to the recruitment of SYK-family kinases to the ITAM, followed by the activation of various downstream targets, such as the linker of activation of T cells, multimolecular adaptor complexes, and phosphatidylinositol 3-kinase (PI3 kinase). By generating phosphatidylinositol (3,4,5)-triphosphate (PIP3), PI3 kinase creates membrane-docking sites for Bruton’s tyrosine kinase and phospholipase Cγ. Activation of phospholipase Cγ causes an increase in intracellular calcium and triggering of further downstream signaling events. In addition to calcium-dependent pathways, the RAS (abbreviation of rat sarcoma)-RAF (rapidly accelerated fibrosarcoma)-MAPK (mitogen-activated protein kinase) pathway is of central importance for cell activation after activating FcγR crosslinking. An additional major function of activating FcγR is to promote the endocytosis or phagocytosis of immune complexes, which include antibody-coated microorganisms and soluble proteins. In the case of granulocytes, monocytes, and macrophages, this will mainly result in the rapid degradation of the engulfed material in lysosomal compartments.1,3

### Inhibitory Fγc Receptor Function

The only known inhibitory FcγR, FcγRIIB, is the most broadly expressed FcγR, and it is present on virtually all leukocytes with the exception of NK cells and T cells. Because of the broad expression pattern, global genetic deletion of FcγRIIB can result in complex phenotypic changes affecting either innate or adaptive immune responses. FcγRIIB transmits inhibitory signals through an immunoreceptor tyrosine–based inhibitory motif (ITIM) contained in its cytoplasmic region.
FcγRIIB function is best understood in B cells, in which it serves as a checkpoint for humoral immunity, playing a critical role in preventing the generation of autoreactive antibodies. Partnering with B-cell receptor, the simultaneous triggering of the ITIM-containing FcγRIIB and the B-cell receptor results in the recruitment of phosphatases including SH2 domain–containing inositol polyphosphate 5’ phosphatase (SHIP) and SH2-domain–containing protein tyrosine phosphatase 1 that interfere with activating signaling pathways by hydrolyzing phosphoinositide intermediates. This prevents the recruitment of pleckstrin homology-domain–containing kinases, such as Bruton tyrosine kinase or phospholipase Cγ, to the cell membrane, thereby diminishing downstream events such as increases in intracellular calcium levels. Processes that disrupt FcγRIIB function in B cells result in a lower threshold for B-cell activation and stronger activating signals after B-cell receptor crosslinking.1–3

In addition to its participation in adaptive immunity by tempering antibody production by B cells, FcγRIIB is a regulator of innate immunity via its actions in mast cells, granulocytes, and macrophages. As these cells have the capacity to trigger strong proinflammatory responses, their activation needs to be tightly controlled. In the case of antibody-mediated responses, such as phagocytosis, antibody-dependent cell-mediated cytotoxicity, allergic reactions, and the release of proinflammatory mediators, this is the function of the inhibitory FcγRIIB. FcγRIIB contributes to varying levels of negative regulation depending on the specific IgG subclass that is bound to the receptor (see below).1–3

**FcγR Ligands**

**Immunoglobulins**

The complexity of the FcγR family is mirrored by the presence of 4 different IgG subclasses in humans (IgG1–IgG4) and in mice (IgG1, IgG2a/c, IgG2b, and IgG3), which bind with varying affinity and specificity to different FcγR via the Fc portion of the IgG. Overall, in humans, IgG1 and IgG3 are the most proinflammatory IgG subclasses, and in mice IgG2a and IgG2b are the most proinflammatory IgG molecules. FcγR vary in their affinity for IgG. FcγRI is the single high-affinity receptor in humans and in mice, particularly on binding of IgG1 and IgG3 in humans or IgG2a in mice. All other FcγR have a 100- to 1000-fold lower affinity and show a broader IgG subclass specificity. Relative binding affinity of mouse and human IgG subclasses to mouse and human FcγR is shown in Table 2.10 The low-affinity nature of IgG binding to most of the FcγR proteins serves as an important function in that it prevents the binding by monomeric antibody molecules that are always present at high levels in the circulation, thereby avoiding the potential nonspecific activation of proinflammatory responses. In contrast, the high-affinity FcγRI is constantly saturated with ligand. However, as has been described for the binding of IgE to the high-affinity FceRI, cell activation only ensues after the FcγRI receptors have been crosslinked by antigen. Considering the existence of both activating and inhibiting FcγR, and the varying affinities for IgG subclasses, the summary in vivo actions of IgG–FcγR tandems can be difficult to predict. To deal with this complexity, the ratio of affinities of a given IgG subclass for the activating versus the inhibitory receptors has been termed the A/I ratio and it has emerged as a helpful predictive value for the activity of a specific IgG subclass in vivo.1–3

**Pentraxins**

In addition to IgG, the acute phase reactant CRP is a ligand for FcγR. CRP is produced principally by the liver in response to a variety of pathological conditions including inflammation, infection, and trauma. CRP is a member of the pentraxin family of proteins, and it was originally described as a protein that binds to the C-polysaccharide of the cell wall of pneumococci. The protein consists of 5 identical 23 kDa subunits noncovalently associated in a flat pentameric disk. The primary stimulus for hepatocyte synthesis and secretion of CRP is the proinflammatory cytokine interleukin (IL)-6. Circulating CRP levels can rise 500-fold within 24 to 48 hours of the initiation of an inflammatory process. CRP was initially found to serve as an opsonin, binding to pathogenetic microorganisms and mediating the phagocytosis of sensitized erythrocytes. CRP also activates complement by binding to C1q, and studies in mice indicate that it is protective against infection and the development of autoimmunity. CRP levels are often used clinically as an indicator of infection or inflammation.11–14

In addition to the liver being a source of CRP, transcript for the pentraxin has been detected in human atherosclerotic lesions, with mRNA levels 10-fold greater in plaques than in normal arteries.15 CRP transcript has also been detected in areas of myointimal hyperplasia in a porcine arteriovenous graft model.16 Expression of CRP has been demonstrated in endothelial cells, particularly on treatment with macrophage conditioned medium17 or electro-negative low-density lipoprotein cholesterol.18 In vascular smooth muscle (VSM), angiotensin II, endothelin-1, and homocysteine promote CRP expression.19–21 Adipose tissue may be an additional important source of CRP because the mRNA is detectable in human adipose tissue, where it is expressed in both adipocytes and stromal cells.22,23 CRP mRNA is increased in the adipose tissue of obese individuals compared with controls and it is upregulated in vitro in adipose tissue explants by lipopolysaccharide or IL-6.24

As for the forms of CRP that affect the cardiovascular system, it is controversial whether the potential actions of the pentraxin of relevance to cardiovascular health are mediated by native, pentameric CRP, or monomeric (m)CRP. In some studies it has been reported that mCRP is detected in normal human blood vessels and in inflamed tissues,25–27 yet others indicate that mCRP is deposited in human atherosclerotic plaques but not in healthy vessels, and that pentameric CRP is not detectable in either healthy or diseased blood vessels.28 It has been reported that mCRP promotes a proinflammatory phenotype in cultured endothelial cells. However, in earlier investigations, mCRP actions on endothelial cells were modified by anti-CD16 (FcγRIII) blocking antibody and not by anti-FcγRII antibody,29 yet genetic studies in mice indicate that FcγRIIB is critically involved in the actions of endogenous CRP on endothelium.30 In addition, there is a recent report that mCRP actually mediates responses in human endothelial cells via plasma membrane insertion rather than by binding to surface FcγR.31 Furthermore, it has been demonstrated...
that native CRP impairs endothelial function in vivo, whereas mCRP does not.\textsuperscript{32} Activated platelets dissociate pentameric CRP to mCRP via lysophosphatidylcholine, which is only present on activated platelets,\textsuperscript{28} suggesting that it may remain challenging to definitively determine which form of CRP affects the cardiovascular system in vivo.

It was initially determined that CRP binds to the high-affinity receptor for IgG, Fc\(\gamma\)RI, in human monocytes and by reconstitution in transfected cells.\textsuperscript{33,34} Fc\(\gamma\)RI, in human monocytes and by \(\gamma\)-chain−/− mice displayed less CRP binding, whereas CRP binding was observed in leukocytes from \(\gamma\)-chain−/− mice, which express Fc\(\gamma\)RII.\textsuperscript{37} A role for Fc\(\gamma\)RII in CRP binding is less likely because natural killer cells from both humans and mice, which express Fc\(\gamma\)RIII as their sole Fc\(\gamma\)R, display negligible CRP binding.\textsuperscript{38} Thus, in both humans and mice, Fc\(\gamma\)RI and Fc\(\gamma\)RII most likely serve as the major receptors for CRP. The structurally similar pentraxin, SAP, which is the CRP equivalent acute phase reactant in mice, also binds to Fc\(\gamma\)R in leukocytes from double-deficient mice.\textsuperscript{37} A role for Fc\(\gamma\)RIII in CRP binding is conserved between CRP, SAP, and IgG.\textsuperscript{39}

**Fc\(\gamma\)R Ligand Actions in Vascular Cells**

**Endothelial Cells**

Based on observed associations between chronic modest elevations in CRP and cardiovascular disease in humans (see below), a variety of studies have been performed in cultured endothelial cells evaluating the effects of CRP. One of the first demonstrated that CRP decreases endothelial NO synthase (eNOS) mRNA and protein abundance and enzymatic activity in human aortic endothelial cells, and that there is an associated promotion of monocyte adhesion to the endothelial cells (Figure 1A).\textsuperscript{40} CRP upregulation of endothelium-derived plasminogen activator inhibitor-1 was then demonstrated,\textsuperscript{41} and this was followed by studies showing that CRP upregulates IL-8 and endothelial cell-monocyte adhesion by activating nuclear factor-kB, with these responses as well as CRP binding to endothelial cells being attenuated by anti-CD32 (Fc\(\gamma\)RII) and anti-CD64 (Fc\(\gamma\)RI) antibodies.\textsuperscript{3,42,43} Nuclear actions of CRP in endothelial cells were further delineated in studies of cells transfected with human eNOS 5′ flanking sequence fused to luciferase that indicated that CRP decreases eNOS gene transcription.\textsuperscript{44}

Acute effects of CRP on endothelial cell function were also demonstrated in studies that showed that the rapid activation of eNOS by diverse agonists is blocked by the pentraxin. SAP and aggregated IgG used to mimic immune complexes also inhibited eNOS activation. Fc\(\gamma\)RIIB mRNA expression was demonstrated in endothelial cells, and heterologous expression studies revealed that CRP antagonism of eNOS requires Fc\(\gamma\)RIIB. Demonstrating in vivo actions of CRP on the endothelium for the first time, it was found that acute CRP administration blunts acetylcholine-induced increases in carotid artery conductance in wild-type mice; in contrast, in Fc\(\gamma\)RIIB−/− mice, CRP actually enhanced the vasodilatory response to acetylcholine.\textsuperscript{45} Long-term actions of CRP affecting the endothelium have also been shown in vivo, with CRP transgenic mice (TG-CRP) displaying attenuated carotid artery re-endothelialization after perivascular electric injury. In parallel, CRP blunts cultured endothelial cell migration.\textsuperscript{46} CRP administration to rats for 72 hours resulted in decreased eNOS activity and blunted vasodilatory responses to acetylcholine in isolated, pressurized mesenteric arterioles.\textsuperscript{47} Endothelial actions of CRP have additionally been interrogated in mice harboring an inflammation-sensitive CRP transgene. Turpentine activation of the CRP transgene yielded serum levels of human CRP of 276 μg/mL, with CRP concentrations being <1.0 μg/mL in healthy humans and rarely exceeding 2 μg/mL in mice even in response to inflammatory stimuli.\textsuperscript{14,48} Aortic rings isolated from the mice with elevated CRP displayed impaired endothelium-dependent responses to acetylcholine, and NO release and eNOS-Ser1179 phosphorylation were decreased. In addition, CRP-overexpressing mice had increased perivascular fibrosis, greater endothelial vascular cell adhesion molecule (VCAM)-1 and monocyte chemoattractant protein (MCP)-1 staining, and enhanced macrophage infiltration.\textsuperscript{48}

The features of Fc\(\gamma\)RIIB and related signaling events required for CRP to antagonize eNOS were revealed in studies of eNOS activation by insulin,\textsuperscript{49} which stimulates the enzyme to promote vasodilation and capillary recruitment in the skeletal muscle microvasculature, thereby enhancing skeletal

### Table 2. Binding Affinity Between IgG Subclasses and Fc\(\gamma\) Receptors\textsuperscript{10}

<table>
<thead>
<tr>
<th>IgG</th>
<th>Human</th>
<th>Mouse</th>
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<tr>
<td></td>
<td>Activating</td>
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<tr>
<td></td>
<td>Fc(\gamma)RI</td>
<td>High</td>
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<tr>
<td></td>
<td>Fc(\gamma)RIIA</td>
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<td>Fc(\gamma)RIIC</td>
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<td>Fc(\gamma)RIIB</td>
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<td></td>
<td>Inhibitory</td>
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<td>Fc(\gamma)RIIB</td>
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<td>Fc(\gamma)RIIB</td>
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<tr>
<td></td>
<td>Fc(\gamma)RIIB</td>
<td>None</td>
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Fc\(\gamma\)R indicates Fc\(\gamma\) receptor.
muscle glucose disposal. In mice, the activating phosphorylation of eNOS-Ser1179 and also Akt phosphorylation in response to insulin were decreased by CRP treatment, and reconstitution experiments with wild-type and mutant FcγRIIB in NIH3T3 cells revealed that these processes require the ITIM of the receptor. It was further shown that endothelial cells express SHIP-1, that CRP induces SHIP-1 stimulatory phosphorylation in culture and in vivo, and that SHIP-1 knockdown by small interfering RNA prevents CRP antagonism of insulin-induced eNOS activation. How CRP causes the critical FcγRIIB ITIM phosphorylation in endothelial cells has been elucidated additionally. It was determined in cultured cells that FcγRI blocking antibodies prevent CRP antagonism of eNOS (Figure 1A), that CRP activates SRC via FcγRI, and that both ITIM phosphorylation and eNOS antagonism caused by CRP are SRC dependent. Parallel processes were found to mediate the inhibition of eNOS by aggregated IgG used to mimic immune complex. As such, FcγRI–FcγRIIB coupling by SRC kinase underlies both CRP and IgG attenuation of endothelial NO production. The partnership between FcγRI and FcγRIIB in the actions of CRP on endothelium was demonstrated in vivo in studies of carotid artery re-endothelialization in TG-CRP mice crossed with either mice lacking the γ-subunit of FcγRI (FcγRI−/−) or FcγRIIB−/− mice. Thus, both FcγRI and FcγRIIB have been implicated in the actions of CRP on endothelium in vivo.

Whereas various forms of FcγRIIB loss of function have been used to implicate the receptor in the actions of immune complex and pentraxin on endothelial cells, and FcγRIIB mRNA has been demonstrated in endothelium, evidence that FcγRIIB potentially affects cardiovascular health in humans would be strengthened if FcγRIIB protein expression could be demonstrated in human endothelial cells. This has been perplexing because of the lack of specificity of antibodies and the challenges involved in reliable detection of an antibody receptor using antibodies as probes. Fluorescence-activated cell sorting was therefore performed with human endothelial cells using an Alexa Fluor 488–conjugated monoclonal Ab (clone 8B5, MacroGenics) to human FcγRIIB generated by immunization of human FcγRIIA transgenic mice to prevent cross-reactivity with FcγRIIA in 8B5, Asn297 in the Fc domain has been mutated to Gln to prevent Fc-FcγR binding, thereby minimizing nonspecific binding. Using 8B5 Ab, FcγRIIB protein was readily detected in Raji-positive control cells (Figure 2A), and in human aortic, coronary artery, and umbilical vein endothelial cells (Figure 2B–2D). Further confirmation of Ab specificity was obtained by small interfering RNA knockdown of FcγRIIB (Figure 2E, green and red for scrambled and FcγRIIB-targeted small interfering RNA, respectively). Immunoblotting detected FcγRIIB as a 40-kDa protein in plasma membranes from Raji cells (positive control) and human aortic endothelial cells (Figure 2F). These findings indicate that FcγRIIB protein is expressed in human endothelial cells derived from a variety of vascular beds.

![Figure 1. Effects of Fcγ receptors (FcγR) and their ligands on vascular cells (A) and on preclinical models of cardiometabolic disorders (B). CRP indicates C-reactive protein; ET, endothelin; IL, interleukin; IVIG, intravenous immunoglobulin; LDL-IC, low-density lipoprotein–containing immune complex; LDLR, low-density lipoprotein receptor; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; ROS, reactive oxygen species; SAP, serum amyloid P component; TNF, tumor necrosis factor; and VSM, vascular smooth muscle (illustration credit: Ben Smith).](http://circres.ahajournals.org/)
Vascular Smooth Muscle Cells

As has been the case for studies of FcγR ligand actions on endothelial cells, CRP has been the principal ligand used in investigations in VSM cells. Using human coronary artery VSM cells, it was demonstrated that CRP induces caspase-mediated apoptosis (Figure 1A). Upregulation of the growth arrest–inducible and DNA damage–inducible gene 153 (GADD153) was also observed, the impact of CRP was primarily on GADD153 mRNA stability, and GADD153 silencing decreased the pro-apoptotic effect of CRP. Potentially supportive of a role for GADD153 in processes occurring in atherosclerotic plaques, GADD153 was specifically localized to apoptotic VSM cells in human coronary artery lesions.52 It has also been shown that CRP induces intracellular reactive oxygen species generation in VSM cells by NADPH oxidase 4 isoform, and AP-1/nuclear factor-kB activation, and MCP-1, IL-6, and endothelin (ET)-1 production.53,54 CRP additionally upregulates VSM cell matrix metalloproteinase-2 synthesis and activity55 and VSM cell tissue factor expression.56 Furthermore, CRP upregulates VSM cell angiogenesis II type 1 receptor (AT1-R) expression, and it promotes VSM cell migration and proliferation and enhances the effects of angiogenesis II on those processes.16,57

As for the potential FcγR operative in the actions of CRP on VSM cells, FcγRIIA expression has been demonstrated in cultured VSM cells isolated from human coronary arteries, and the receptor has been colocalized with α-actin–positive VSM cells in atheromatous regions in human coronary artery plaques.52 FcγRIIA blocking antibody attenuates the effects of CRP on VSM cell reactive oxygen species and cytokine production, and apoptosis (Figure 1A).52 FcγRIIA and FcγRIIIA transcripts have been detected in VSM cells, and the silencing of FcγRIIIA attenuates the upregulation of tissue factor expression by CRP.56 Thus, in studies limited to cell culture, CRP has direct adverse effects on VSM typical of responses observed with injury or inflammation, and the operative FcγRs are FcγRIIA and FcγRIIIA.

Leukocytes and Platelets

Evidence for potential actions of FcγR ligands and receptors in leukocytes has been primarily obtained in studies of low-density lipoprotein cholesterol–containing immune complexes (LDL-ICs). Human monocytes/macrophages exposed to LDL-IC display marked cholesterol ester accumulation such that they assume a foam cell phenotype (Figure 1A), and the LDL-ICs cause increased LDL receptor expression. The uptake of LDL-IC is mediated by FcγRI, and it results to and be activated by antibody-opsonized pathogens, thereby promoting their clearance.66,67,71,72 Because there is not a murine homologue for human FcγRIIA, the receptor is studied in vivo in transgenic mice expressing the human receptor.65,73 Platelets express 2 additional ITAM receptors. These are C-type lectin 2, which is the receptor for the snake venom toxin rhodocytin and for podoplanin, and FcγR γ-chain, which associates with the glycoprotein IV collagen receptor. Platelet ITAM receptors, their signaling mechanisms, and functions have recently been extensively reviewed,65 and therefore they will be mentioned in limited fashion in the remainder of this review.

FcγRs, Ligands, and CVD in Animal Models

Atherosclerosis

Atherosclerosis is considered to be a chronic inflammatory disease, and both innate and adaptive immunity play critical
roles in its initiation and progression.74 FcγRs are expressed in virtually all individual cell types participating in atherosclerosis (see above), and as such it is logical for FcγR to potentially influence atherosclerosis severity. This has been tested in vivo primarily in 4 studies in mice. The first entailed experiments in apoE−/− versus apoE−/−;FcγR γ-chain−/− mice, with the latter having genetic loss of function of activating FcγR. Without affecting plasma lipids, FcγR γ-chain deletion resulted in decreased atherosclerotic lesion size (Figure 1B), and lesion macrophage and T-cell content were cut approximately in half.75 In a separate study in mice fed a high-fat diet for 10 weeks, the global deletion of the FcγR γ-chain caused improved acetylcholine-induced relaxation of isolated aortic rings, and it lowered superoxide abundance in the vascular wall.76 These findings suggest that FcγR γ-chain–mediated processes may promote atherosclerosis by attenuating endothelial function or by increasing oxygen-derived free radical abundance. Alternatively, because the quantification of FcγR in the aorta in these studies revealed that the activating/inhibitory ratio changed from 2.5 to 0.4 with the deletion of the FcγR γ-chain,77 it is possible that the decrease in atherosclerosis observed in the absence of the FcγR γ-chain reflects an increase in the relative atheroprotective properties of the inhibitory FcγR FcγRIIB. In bone marrow reconstitution experiments, hematopoietic deficiency of FcγR γ-chain in apoE−/− mice resulted in decreased atherosclerotic lesion size, with fewer macrophages and T lymphocytes within lesions and also increased plaque stability. The expression of proinflammatory genes was attenuated and anti-inflammatory gene expression was increased. In vitro experiments using murine macrophages revealed decreases in foam cell formation, proinflammatory gene expression, and oxidative stress in cells lacking FcγR γ-chain, suggesting that the cell type in which the γ-chain influences atherosclerosis severity is the macrophage.77 Other work in a similar model suggested that with hypercholesterolemia, activating FcγRs promote atherosclerosis by increasing antigen-presenting cell IL-6 secretion, resulting in an enhanced Th17 response.78 The impact of loss of function of an activating FcγR was also evaluated in studies of LDLR−/− versus LDLR−/−;FcγRIIB−/− mice. At later stages of atherosclerosis development, FcγRIII silencing resulted in smaller lesions, and there was an associated increase in interferon-γ and IL-10 production by an expansion of CD4+ T cells.79 Potential modulation of atherosclerosis severity by FcγRIIB has also been evaluated, with either bone marrow reconstitution with FcγRIIB−/− versus FcγRIIB−/− marrow in LDLR−/− mice or global deletion of the receptor in apoE−/− mice. Independent of changes in plasma lipids, FcγRIIB omission by either strategy resulted in exaggerated atherosclerosis. In the study with global FcγRIIB silencing, there were both increased proinflammatory cytokines in the aorta and increased antibody titers to modified LDL.80,81 Thus, consistent with the inflammatory nature of atherosclerosis, and with impacts that parallel their activating versus inhibitory input into immune responses, FcγRs modulate atherosclerosis in mice.

Additional potential evidence of FcγR modulation of atherosclerosis severity comes from studies of the effect of immunoglobulin treatment in apoE−/− mice. The injection of polyclonal intravenous immunoglobulin preparations reduces fatty streak formation, and this is associated with reduced IgM antibodies to oxidized LDL and the inactivation of spleen and lymph node T cells.82 Whereas the administration of intact immunoglobulin causes a decrease in lesion size and fewer macrophages within lesions, treatment with Fab′/Fab′2 fragments of human immunoglobulin has no effect, thereby implicating involvement of the Fc region of IgG and FcγR.

The potential contribution of CRP to atherogenesis has also been investigated, using primarily gain-of-function strategies. Using generally similar strategies in male apoE−/− mice, the transgenic expression of human CRP has been found either to accelerate atherosclerosis progression83 or not to affect the development or severity of atherosclerosis, with the increased atherosclerosis in the former study being attributed to excessively high CRP levels.84,85 In apoE−/−;Leiden transgenic mice expressing a human CRP transgene, the CRP did not affect atherosclerosis.86 In atherosclerosis-prone ApoB100/100;LDLR−/− mice with human-like hypercholesterolemia, human CRP blunted atherosclerosis development.87 In apoE−/− mice–administered CRP via osmotic minipump for 4 weeks, atherosclerosis was unchanged.88 Compared with wild-type rabbits, transgenic rabbits expressing human CRP developed similar atherosclerotic lesions on a cholesterol-rich diet.89 As for loss-of-function studies, CRP-deficient mice displayed either equivalent or actually increased atherosclerotic lesions compared with controls,90 and whereas CRP antisense oligonucleotides effectively lowered plasma CRP levels in Watanabe heritable hyperlipidemic rabbits, they did not affect atherosclerosis in the rabbits.91 Recognizing that SAP is the acute phase reactant and CRP equivalent in mice, SAP concentrations have been measured during the development of atherosclerosis in apoE−/− mice, and they were found to be unchanged.84,85 Collectively, these observations in mice and rabbits do not support a role for CRP in atherosclerosis pathogenesis. Gain- or loss-of-function studies of potential participation of SAP in modulating atherosclerosis severity in mice have not yet been reported.

**Vascular Injury, Occlusion, and Thrombosis**

The potential participation of FcγR and their ligands in altering the response to vascular injury and degree of thrombosis has been interrogated in mice. Using human TG-CRP, it was demonstrated that the pentraxin causes exaggerated neointima formation after carotid artery ligation (Figure 1B).92 Follow-up studies were done crossing the human CRP transgenic mouse with FcγR null mice, and in them neointima development was decreased with either FcγRI or FcγR γ-chain deletion. In contrast, the deletion of FcγRIIB or FcγRIII resulted in neointima formation that was equal to or greater than that observed without FcγR manipulation; the findings with FcγRIII excision may have resulted from decreased competition for the γ-chain that is common between FcγRI and FcγRIII.93 Studies have also been performed in rats, with CRP administration causing increased neointima formation in a carotid artery angioplasty model.94 In addition to influencing neointima formation, it has
been demonstrated that CRP promotes thrombosis, with TG-CRP displaying exaggerated clot formation in both a transluminal wire injury model and a photochemical arterial injury model of thrombosis (Figure 1B).94 Furthermore, there is evidence that the impact of FcγR and their ligands on neointima development may relate to their modulation of thrombosis, because in a transfemoral artery wire injury model in which thrombosis was controlled with aspirin and heparin, TG-CRP displayed less neointima formation.95 The interpretation of findings on neointima development with FcγR manipulation may be complex because the FcγR γ-chain plays a key role in platelet activation by collagen, and platelet activation may be instrumental to neointima initiation and progression.96 Overall, the animal model data available to date indicate that FcγRs affect thrombotic and nonthrombotic vascular occlusion.

Hypertension
Because chronic elevations in CRP are associated with the development of hypertension in humans,97 to evaluate a possible causal relationship, blood pressure (BP) has been studied in transgenic mice expressing rabbit CRP under the regulation of the phosphoenolpyruvate carboxykinase promoter. Compared with controls, TG-CRP had hypertension that was predominantly systolic, and the severity of hypertension varied in parallel with changes in CRP levels modulated by dietary carbohydrate manipulation (Figure 1B). The regulated transgene made it possible to study CRP levels as low as 9 μg/mL, and such mice were hypertensive, indicating that modest elevations in CRP are sufficient to alter BP in the mouse. The TG-CRP displayed exaggerated BP elevation in response to angiotensin II but not in response to norepinephrine, and there was a reduction in vascular angiotensin II receptor subtype 2 expression. In contrast, the decline in BP with angiotensin II receptor subtype 1 antagonism and vascular angiotensin II receptor subtype 1 abundance were unaltered, indicating a selective effect of CRP on angiotensin II receptor subtype 2. Ex vivo experiments further showed that the CRP-induced decrease in vascular angiotensin II receptor subtype 2 is a direct effect on the vascular wall not requiring systemic responses, and that it is reversed by an NO donor, suggesting a role for NO deficiency in the in vivo process. In parallel, the chronic inhibition of NO synthase in wild-type mice attenuated vascular angiotensin II receptor subtype 2 expression without affecting angiotensin II receptor subtype 1. These findings provided direct evidence for CRP-induced hypertension in mice.98 In rats, human CRP expression via an adeno-associated virus similarly resulted in hypertension, and there was also evidence of decreased NO production indicated by a fall in serum NO and urine cGMP and impaired endothelium-dependent relaxation.99 In a complementary study, human CRP was expressed in the livers of spontaneously hypertensive rats using a transgene under the control of the apoE promoter. The rats with elevated CRP displayed both greater systolic and diastolic BP.100 These collective findings indicate that CRP causes hypertension in rodents.

Myocardial Infarction
Because the relative rise in plasma CRP provoked by a myocardial infarction in humans is strongly associated with postinfarct morbidity and mortality,101–103 a potential contribution of CRP to disease severity has been evaluated. In studies in rats, the administration of human CRP after coronary artery ligation caused a 40% increase in infarct size (Figure 1B).104 A small molecule inhibitor of CRP was then identified, 1,6-bis (phosphocholine)-hexane, which binds to CRP and occludes its ligand-binding B-face and thereby blocks its function. When administered to rats given human CRP after coronary artery ligation, the agent attenuated the increase in infarct size and the cardiac dysfunction caused by CRP.105 Using a rat CRP-specific antisense oligonucleotide, it was recently observed that lowering blood CRP resulted in reduced infarct size and improved cardiac function after myocardial infarction caused by ligation of the left anterior descending coronary artery in rats. In addition, in human TG-CRP mice, declines in circulating human CRP induced by a human CRP-specific antisense oligonucleotide were associated with decreased neointima formation after carotid artery ligation.106 The specific actions of CRP as well as possible isoforms of FcγR that influence myocardial infarction severity or outcome are unknown.

Insulin Resistance
Individuals with insulin resistance have markedly greater risk of developing cardiovascular disease, and there is mechanistic linkage between insulin resistance and endothelial dysfunction and vascular disease.50 Because chronic elevations in CRP are associated with increased risk of both cardiovascular disorders and type 2 diabetes mellitus in humans,107–111 the impact of CRP on glucose homeostasis has been queried in mice. Using the mouse transgenic for rabbit CRP or the administration of human recombinant CRP to wild-type mice, it was discovered that elevations in CRP cause insulin resistance (Figure 1B).112 Paralleling these findings, spontaneously hypertensive rats expressing CRP driven by an apoE promoter–regulated transgene display glucose intolerance and hyperinsulinemia.100 In the TG-CRP mice, animals lacking FcγRIIB were protected from CRP-induced insulin resistance, and immunohistochemistry revealed that FcγRIIB is expressed in skeletal muscle microvascular endothelium. Consistent with the receptor distribution, the primary mechanism in glucose homeostasis disrupted by CRP was skeletal muscle glucose delivery, and CRP attenuated insulin-induced skeletal muscle blood flow. In contrast, CRP did not impair skeletal muscle glucose delivery in FcγRIIB−/− mice or in eNOS knockin mice with phosphomimetic modification of eNOS Ser1176 (Ser1179 in human eNOS); eNOS Ser1176/9 is normally phosphorylated by insulin signaling to stimulate NO-mediated skeletal muscle blood flow and glucose delivery, and it is dephosphorylated by CRP/FcγRIIB.113 Thus, CRP causes insulin resistance in mice through FcγRIIB-mediated inhibition of skeletal muscle glucose delivery, and this may represent an additional mechanism whereby an FcγR and one of its ligands contribute to cardiovascular disease.

FcγR Ligands and CVD in Humans
IgG and Immune Complexes
Specific antigens and their respective IgG antibodies have been detected in the serum of patients with cardiovascular disease.
In addition, IgG deposits have been detected in atherosclerotic lesions both in humans and in mice.13,14 Two identified antigens in humans are oxidized LDL and heat shock protein,15,16 and along with their corresponding antibodies they have the potential to form immune complexes which may contribute to cardiovascular disease because of their proinflammatory properties involving either FcγR or complement activation. In an investigation in 52 patients undergoing carotid artery endarterectomy, there was a correlation between the levels of IgG against oxidized LDL at the time of surgery and arterial wall thickness 6 months later.17 In a study of >500 patients undergoing coronary angiography, IgG-oxidized LDL autoantibodies and IgG apoB100 immune complexes were positively associated with angiographically determined coronary artery disease in logistic regression analyses. However, in this population, the relationships were not apparent in multivariate analyses,13,14 and inconsistencies in correlations between IgGs and manifestations of cardiovascular disease have been observed between studies.118 A potential explanation for such inconsistencies may be the lack of reproducible antigens used to quantify the IgGs.119 Investigations in larger numbers of subjects, for example, in the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort, have failed to demonstrate a relationship between levels of IgG autoantibodies or immune complexes and coronary artery disease events.120 Interestingly, for decades it has been recognized that serum levels of antibodies are elevated in patients with both essential and pregnancy-associated hypertension.123-125 The antigens targeted by these autoantibodies include the angiotensin II type-1 receptor, L-type voltage-gated calcium channels, the α-1 adrenergic receptor, and the β-1 adrenergic receptor.125 Recognizing that these proteins regulate BP by governing sodium and water reabsorption by the kidney, vascular tone, and cardiac output, the potential contributions of the autoantibodies to disease pathogenesis have thus far logically been attributed to antibody recognition of their antigen targets, and not to FcγR activation. Systemic autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, and the antiphospholipid syndrome, are characterized by accelerated atherosclerosis and greater risk of coronary artery disease that is not explained by traditional risk factors. The increase in vascular disease risk associated with these autoimmune conditions hypothetically may be related to the presence of autoantibodies and autoantigens and the subsequent formation of immune complexes.126 However, evidence for a causal link between immune complex formation and cardiovascular disease risk or severity is currently lacking in patients with autoimmune disorders, as well as in individuals without known complications of autoimmunity.

**Pentraxins**

**Pentraxins and Coronary Heart Disease**

During the past few decades, massive data have accumulated indicating an association between modest elevations in circulating CRP and incident coronary heart disease.105 This is perhaps best exemplified by 2 meta-analyses that have been performed, the first entailing 22 studies including >7000 coronary heart disease cases and a mean follow-up period of 12 years. Comparing individuals in the top one third versus the bottom one third for baseline CRP level, the odds ratio (OR) for disease was 1.58 (95% confidence interval [CI], 1.48–1.68).127 The second analysis of 23 studies comparing subjects with CRP levels of >3.0 mg/L (>3.0 μg/mL) versus <1.0 mg/L (<1.0 μg/mL) yielded a similar OR of 1.58 (1.37–1.83).128

Another approach that has been used to evaluate potential linkage between CRP and cardiovascular disease incidence or severity is to study an intervention that alters CRP and then determine how outcome is modified. This has been done in studies of statins, which lower not only LDL cholesterol but also CRP.129 In a study of 502 individuals with angiographically documented coronary disease, the impact of moderate versus intensive statin therapy (40 mg pravastatin versus 80 mg atorvastatin daily, respectively) on atherosclerosis progression was evaluated using intravascular ultrasonography. Decreases in both LDL cholesterol and CRP predictably occurred with statin treatment, and after adjustment for changes in lipids, the declines in CRP were independently correlated with less atherosclerosis progression.130 In the Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial involving >18000 subjects without cardiovascular disease at baseline, LDL cholesterol levels <130 mg/dL, and CRP levels ≥2 mg/L, outcomes with placebo versus 20 mg rosuvastatin treatment daily were compared. The trial was stopped early at a median of 1.9 years of follow-up when a 44% decline in vascular events was observed with rosuvastatin. This was associated with a 50% fall in LDL cholesterol and a 37% decrease in CRP.131 The event rate in the placebo group and the clinical impact of CRP observed with treatment and the associated fall in CRP suggested that elevated levels of CRP increased vascular disease risk in these subjects, even when the LDL cholesterol level was within acceptable range based on guidelines at the time of the trial. However, recognizing that statins have numerous actions besides lowering CRP, the statin-based intervention trials shed only modest light on whether there is causal linkage between CRP and cardiovascular disease.

In addition to the effects of inflammatory insults and behavioral and environmental factors on CRP levels, 20% to 50% of the differences in CRP between individuals has been attributed to genetic variability.132 Several single nucleotide polymorphisms (SNPs) in the CRP gene influence its abundance, and loci in other genes involved in inflammatory pathways have also been identified to affect CRP.132,133 The identification of genetic modifiers of CRP allowed numerous studies to be performed involving Mendelian randomization, in which the genetic variants serve as likely unconfounded proxies for CRP levels to evaluate whether CRP has a causal role in coronary heart disease.134 The outcomes of the Mendelian randomization queries are exemplified by the collaborative study reported in 2011 involving close to 150000 control subjects and >46000 individuals with prevalent or incident coronary heart disease. Although 4 SNPs were demonstrated to influence CRP levels, and increasing CRP concentration was associated with increased risk of disease, no association was found between the SNPs and coronary heart disease.135 When combined with the overall neutral outcomes of animal studies of CRP and atherosclerosis, such findings indicate that CRP does not likely contribute to coronary heart disease pathogenesis in humans.


**Pentraxins and Hypertension**

In several reports CRP levels have been shown to predict the development of hypertension in previously normotensive individuals. This was the case in the Women’s Health Study, in which 5365 women of 20525 with normal BP at study onset developed hypertension during a median follow-up duration of 8 years.\(^{136}\) Even after adjustment for a variety of risk factors, baseline CRP was an independent predictor of the development of incident hypertension. Comparable observations were made in the Framingham Offspring Study, in which 232 subjects of 1456 initially normotensive individuals developed hypertension during a mean follow-up period of 3 years.\(^{137}\) Thus, there is epidemiological evidence linking CRP levels with incident hypertension.

As has been done in queries of possible linkage of CRP with coronary artery disease but in far fewer studies, the genetics governing CRP level and hypertension prevalence or development have been investigated. In a British Women’s Heart and Health Study investigation of a single CRP SNP, although the SNP was associated with a marked difference in CRP level, there was no relationship between the SNP and the prevalence of hypertension.\(^{138}\) In a study of \(\approx\)2000 Turkish subjects, CRP haplotypes associated with hypertension in both men and women,\(^{139}\) and 2 CRP SNPs were associated with hypertension in a study of 1400 control and 1331 Han Chinese with elevated BP.\(^{140}\) However, in a larger study of 2000 Han Chinese in which 8 CRP SNPs were investigated, although CRP levels were associated with increasing systolic as well as diastolic BP, none of the SNPs were associated with prevalent or incident hypertension.\(^{141}\) In a recent study a weighted genetic risk score was created to evaluate the combined effects of genetic variants associated with changes in CRP levels on BP in \(\approx\)750 Korean subjects. Individuals with the highest genetic risk score had CRP levels that were \(\approx\)2.5-fold higher than subjects with the lowest genetic risk score, and an elevated genetic risk score increased the likelihood of hypertension (OR 2.18).\(^{142}\) Because it has been demonstrated that elevations in CRP cause hypertension in rodents (see above), additional human studies of the genetics of CRP regulation and hypertension may be warranted, including the application of the weighted genetic risk score for CRP to other populations.

**FcγRs and CVD in Humans**

**Activating FcγRs**

Activating FcγRs, in particular FcγRI, FcγRIIA, and FcγRIIA, have been detected in human atherosclerotic lesions and in macrophages and other cell types in the medial and adventitial regions of the vascular wall.\(^{143}\) The observed functions of activating FcγRs in cell types of relevance to vascular health and the findings with receptor deletion in animal models of atherosclerosis (Figure 1B) provide sound rationale for studies determining whether genetic variants in FcγR influence the incidence or severity of vascular disease in humans. In a study of \(\approx\)900 subjects undergoing coronary angiography, the polymorphism FcγRIIIA-F158V was related to coronary artery disease, with individuals with FcγRIIIA-158 V/V having decreased risk of disease (OR, 0.53; 95% CI, 0.32–0.90). From a functional perspective, FcγRIIIA-158 V/V displays greater IgG1 and IgG3 binding than FcγRIIIA-F/F.\(^{144}\)

FcγRIIA is another activating FcγR for which genetic variation has been evaluated in several studies of cardiovascular disease. In a query of the polymorphism FcγRIIA-R131H involving >700 patients with a first acute coronary syndrome event compared with \(\approx\)500 individuals with stable angina pectoris, the FcγRIIA-R131R genotype was associated with acute coronary syndrome as the first manifestation of coronary disease (OR, 2.86; 95% CI, 2.06–3.99).\(^{145}\) In 553 individuals with either stable angina pectoris or unstable angina pectoris, those with FcγRIIA-131R/R were more likely to have unstable angina (OR, 4.02; 95% CI, 2.52–6.41).\(^{146}\) In an evaluation of 430 individuals with peripheral atherosclerosis and 411 controls in the Rotterdam Study, FcγRIIA-131H heterozygous and homozygous subjects were protected against advanced peripheral atherosclerosis. The age- and sex-adjusted ORs were 0.77 (95% CI, 0.54–1.12) and 0.65 (95% CI, 0.44–0.98), respectively.\(^{147}\) In 78 hypercholesterolemic subjects, homozygous carriers of the H allele compared with the R allele displayed better endothelial-dependent vasodilation as assessed by changes in forearm blood flow in response to intra-arterial acetylcholine infusion.\(^{148}\) In contrast to these 4 studies suggesting a cardiovascular health benefit of the H allele, in 1041 Finnish subjects, FcγRIIA-131H/H homozygotes had more premature atherosclerosis,\(^{149}\) and in several other investigations, the FcγRIIA-H131R polymorphism was found to have no impact on cardiovascular disease risk.\(^{144,150–153}\)

As for impact on receptor function, FcγRIIA-131R/R has increased signal transduction on CRP binding compared with FcγRIIA-131H/H.\(^{154}\) However, FcγRIIA-131H/H has higher binding efficiency for IgG2 and IgG3 than FcγRIIA-131R/R, resulting in decreased internalization of IgG2-opsonized particles by phagocytes in FcγRIIA-R/R individuals.\(^{155}\) In addition to these studies of FcγRIIA SNPs, differences in receptor expression have been investigated. An evaluation of FcγRIIA abundance on peripheral monocytes by flow cytometry found that the receptor expression is decreased in subjects with clinical atherosclerosis compared with controls.\(^{156}\) Recognizing that FcγRIIA participates with collagen in platelet activation, its abundance on platelets has also been assessed, and it was found to be increased in patients with acute myocardial infarction, unstable angina, or ischemic stroke.\(^{157}\) Not surprising considering the complex nature of FcγR biology, the cumulative available information about genetic variation in activating FcγR or differences in their expression has not yet added clarity to our understanding of how the receptors may influence cardiovascular health and disease in humans.

**Inhibitory FcγR FcγRIIB**

In an attempt to understand the potential participation of FcγRIIB in cardiovascular disease in humans, advantage has been taken of previous interrogations of genetic variation in the receptor in the context of lupus.\(^{158}\) One particular site of known single amino acid variation is amino acid 232, which is threonine (T) or isoleucine (I) depending on basepair 695 in exon 5 being C or T, respectively.\(^{159}\) The T allele is less common than the I allele, with the T allele frequency 0.13 in whites and 0.29 in blacks.\(^{160}\) Recognizing that this SNP
resides in the transmembrane domain and may thereby affect receptor function, the abilities of human FcγRIIB-T232 versus FcγRIIB-I232 to antagonize eNOS were compared in cultured endothelial cells. Doing so tests the impact of the variant in the cell type in which FcγRIIB actions potently influence vascular health.30,44,45 Cell context is critical because whereas prior studies of autoimmunity-related mechanisms showed less receptor function for FcγRIIB-T232 than FcγRIIB-I232 in monocytes, the opposite was found in B cells.160,161 Endogenous FcγRIIB was knocking down in bovine aortic endothelial cells by small interfering RNA, followed by sham transfection or introduction of either human FcγRIIB-T232 or FcγRIIB-I232 to equal abundance by transient transfection (Figure 3A). CRP (25 µg/mL) antagonism of eNOS activation by vascular endothelial growth factor (100 ng/mL) was then evaluated. Whereas sham-transfected cells deficient in FcγRIIB displayed no antagonism by CRP, CRP inhibited eNOS activation in cells expressing FcγRIIB-I232 (Figure 3B). In contrast, in cells expressing FcγRIIB-I232, there was no antagonism of eNOS by CRP. Thus, the identity of amino acid 232 in human FcγRIIB markedly affects the capacity of the receptor to alter endothelial cell function, providing a mechanism-based genetic entry point for the study of FcγRIIB in vascular disease pathogenesis in humans.

In light of the preclinical observations made on FcγRIIB, its ligands, eNOS, endothelial function and hypertension,30,44,45,98 FcγRIIB-I232/T232 and its potential influence on BP were then interrogated in the Dallas Heart Study (DHS). DHS is a population-based epidemiological study of Dallas County residents that has provided a cohort for successful genetic inquiry into processes underlying cardiovascular disorders and their risk factors.162-164 In the DHS, subjects between the ages of 30 to 65 years have undergone extensive cardiovascular phenotyping,164 including detailed studies of BP.162,169 A total of 3493 subjects in DHS were genotyped for FcγRIIB-I232 versus FcγRIIB-T232, and because of high homology between FcγRIIB and FcγRIIC, the strategy entailed polymerase chain reaction amplification of FcgRIIB genomic DNA followed by SNP genotyping by sequence-specific fluorescent hybridization probing.159 The prevalence of genotypes is shown in Table 3, and as previously observed,160 the T allele was less common than the I allele, particularly in nonblacks. The study population then consisted of 2925 subjects without active conditions affecting BP. In this subset then, the prevalence of IT or TT genotypes in whites and Hispanics (Table 3), statistical power was insufficient to assess the genotype–phenotype link in those groups. Importantly, the 3- to 4-mm Hg difference in systolic BP between genotypes observed for the SNP is similar in degree to the effect sizes for individual variants in known hypertension-susceptibility genes.172,173 Because several subjects in the DHS query were on antihypertensive treatment, the comparisons were repeated using the recommended strategy of adding a constant to the observed BP in treated subjects (15 mm Hg added to systolic BP).124 It was again observed that systolic BP is greater with IT or TT versus II genotype in the combined racial/ethnic groups and in blacks (Table 4, lower). Importantly, subject age and sex distribution did not differ by genotype. These findings in a single population indicate that the FcγRIIB-I232/T232 variant affects BP in the setting of elevated CRP in humans. Queries of the FcγRIIB-I232/T232 variant are now warranted in additional populations.

Conclusions and Current Unknowns

The recent interest in the contribution of inflammation to cardiovascular disorders has led to both preclinical and clinical studies of FcγR and their ligands in the context of cardiovascular disease. The activating FcγRs, particularly FcγRI, FcγRIIA, and FcγRIIIA, cause a variety of cellular responses in endothelial cells, VSM cells, and monocytes/macrophages that may contribute to vascular disease pathogenesis.
Protein >2.0 mg/L

Blood Pressure in Dallas Heart Study Subjects With C-Reactive Protein

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<th>II</th>
<th>IT</th>
<th>TT</th>
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<tr>
<td>Total population (n=3493)</td>
<td>2297</td>
<td>1058</td>
<td>138</td>
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<tr>
<td>Blacks (n=1792)</td>
<td>948</td>
<td>721</td>
<td>123</td>
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<tr>
<td>Whites (n=1032)</td>
<td>806</td>
<td>218</td>
<td>8</td>
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<tr>
<td>Hispanics (n=595)</td>
<td>492</td>
<td>101</td>
<td>2</td>
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<tr>
<td>Other (n=74)</td>
<td>51</td>
<td>18</td>
<td>5</td>
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<td></td>
<td>69%</td>
<td>24%</td>
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Corrected for antihypertension therapy

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<tr>
<td>IT or TT</td>
<td>130.6±20.2 (725)</td>
<td>134.0±21.0 (536)</td>
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<td>P&lt;0.05</td>
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Values in parentheses represent n values. FcγR indicates Fcγ receptor.

Table 4. Impact of FcγRIIB-I232/T232 Genotype on Systolic Blood Pressure in Dallas Heart Study Subjects With C-Reactive Protein >2.0 mg/L

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<th>Overall</th>
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<tr>
<td>IT or TT</td>
<td>134.7±22.9 (725)</td>
<td>138.2±23.7 (536)</td>
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<td>P&lt;0.05</td>
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Disclosures

None.

References


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