Inflammation and Insulin Resistance

ZACHARY T. BLOOMGARDEN, MD

This is the first of two articles describing a symposium on insulin action, insulin resistance, inflammation, and atherosclerosis in Niagara Falls, New York, 20-21 September 2002. It will cover topics related to atherosclerosis pathobiology and the cell biology of insulin resistance.

Atherosclerosis pathobiology

Renu Virmani (Washington, DC) discussed aspects of the pathology of atherosclerosis. C-reactive protein (CRP) staining of plaques is a prominent histologic finding, and CRP levels are elevated in patients who die suddenly (1). Thincapped fibroatheromas (“vulnerable plaques”) are markers of unstable plaque seen in persons with unstable coronary artery disease (CAD). Those dying with acute rupture have the greatest number of these plaques, those with healed myocardial infarction have an intermediate number, and persons only showing erosion have still fewer fibroatheromas, which Virmani suggested may indicate different diseases grouped together by the term “atherosclerosis.” Healed ruptures are frequent in patients with coronary disease and with hyperlipidemia; 89% of ruptured plaques show evidence of prior rupture and healing, suggesting the chronicity of the process. Coronary calcification, Virami noted, is evidence of healed rupture rather than of total occlusion or fibrous plaque alone. Coronary calcification is an excellent marker of plaque burden, but is a relatively inaccurate predictor for residual lumen size. It correlates well with plaque instability, and weakens the plaque by increasing fibrous cap stress, potentiating the weakening effect of the lipid core. Among women, but not men, glycohemoglobin levels predict coronary calcification.

Jorge Plutzzy (Boston, MA) discussed the cell biology of atherosclerosis. The concept that greater angiographic stenosis is associated with greater clinical risk has been found to be incorrect on meta-analysis, with 68% of myocardial infarctions occurring in lesions with <50% stenosis (2). Furthermore, lipid lowering does not appear to act by reducing stenosis per se (3), leading to the concept that plaque rupture underlies acute coronary events. The role of inflammation in plaque rupture is important. It involves endothelial cells and vascular smooth muscle cells (VSMCs), as well as monocytes/macrophages and T-cells as mediators of inflammation, interacting with adhesion molecules on the surface of the endothelium in what is fundamentally an attempt to heal the lipid-filled lesion. Proliferation of smooth muscle cells and migration of these cells to set up the fibrous plaque may be thought of as healing processes, while degradation of the fibrous plaque with matrix metalloproteinases (MMPs), a large family of highly regulated proteases functioning at neutral pH, appears to underlie plaque rupture with clinical events.

The endothelium should, Plutzky suggested, be thought of as an “active organ” at the interface between circulating factors and the arterial wall. The endothelium secretes a variety of substances that influence the underlying arterial wall and have distant effects, and it is critical for atherogenesis (4). An increase in endothelial adhesion and permeability, in response primarily to inflammatory triggers, leads to leukocyte entry into the vessel wall via families of adhesion molecules. Selectins are involved in the early “rolling” of leukocytes, while intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 lead to subsequent adherence of leukocytes to the vessel wall. Chemoattractant cytokines, a large family of small proteins that signal through specific receptors, are produced by endothelial cells as well as other vessel wall cells. These are induced by other cytokines such as interferon (IFN)-γ, suggesting an amplifying effect.

The late atherosclerotic lesion is characterized by further entry of leukocytes, foam cell formation, T-cell activation, and adherence and activation of platelets (5). For a given total cholesterol–to–HDL cholesterol ratio, CRP levels further predict risk of subsequent myocardial infarction (6). Plaques prone to rupture are characterized by thinning of the fibrous cap, representing a decrease in arterial wall extracellular matrix via two mechanisms. Production decreases, with, for example, collagen production by VSMCs blocked by administration of IFN-γ, despite the effect of mitogens such as interleukin (IL)-1β and platelet-derived growth factor (PDGF). In addition, matrix is degraded by MMP. In vitro, IL-1 induces MMP-9 expression and activation of MMP-2 in VSMCs. Thus, there is a balance of forces between synthesis and breakdown of the matrix materials critically influenced by inflammatory cells. Tissue factor levels are high within the atherosclerotic plaque, in part because of the action of T-cell CD40 on tissue monocytes, so with exposure of plaque contents to the circulation, thrombus is produced.

Understanding of these processes suggests potential areas for therapy. Modulation of nuclear receptor ligands such as those for peroxisome proliferator–activated receptor (PPAR)-γ and -α, as well as estrogen, thyroid hormone, and aldosterone, may become important.
PPAR-γ is a mediator of adipogenesis and lipid and glucose metabolism highly expressed in fat, and it regulates lipoprotein lipase, leptin, and GLUT4. PPAR-α is a regulator of fatty acid (FA) metabolism expressed primarily in the liver, affecting FA binding protein and FA ω-oxidation. PPAR receptors are expressed throughout the vasculature, including atherosclerotic lesions. PPAR-γ agonists, such as prostaglandin F2α, inhibit MMP9 protein expression and activity in VSMCs (7). PPAR-γ agonists also inhibit human VSMC migration. The PPAR-γ agonist troglitazone decreases the degree of intimal hyperplasia and may prevent stent restenosis in diabetes (8).

The omega-3 fatty acid docosahexaenoic acid reduces cytokine-induced expression of pro-atherosclerotic factors. Chemokines are also PPAR-γ regulated, showing inhibition by a variety of PPAR-γ agonists, without effect by PPAR-α agonists. Lymphocytes overexpressing chemokines show decrease in chemotactic activity with PPAR-γ agonist administration. Both PPAR-α and -γ are expressed in T-cells, appearing to decrease their activity. Tumor necrosis factor (TNF-α)–induced VCAM-1 expression is inhibited by PPAR-α, with TNF-α–induced VCAM-1 promoter activity inhibited by fenofibrate, an effect not seen in animals deficient in PPAR-α. The mechanism of FA regulation of VCAM-1 may be mediated by a process such as that by which oxidized eicosapentaenoic acid (EPA) inhibits leukocyte adhesion, with oxidized EPA apparently acting as a PPAR-α agonist, a phenomenon again not seen in mice not expressing PPAR-α. This may explain some of the benefit of omega-3 fatty acids. Tissue factor is also a PPAR-α–regulated target gene.

Ronald Law (Los Angeles, CA) discussed animal models of atherosclerosis and the role of PPAR-γ ligands in the vessel wall, further discussing effects of the thiazolidinediones (TZDs). Law questioned whether insulin sensitizers exert anti-inflammatory effects that might decrease atherosclerosis. Carotid intima-media thickness is increased in insulin-resistant Caucasian and Hispanic individuals (9). Insulin resistance is defined as inadequate insulin response in tissues involved in glucose homeostasis, with a sequence of intracellular steps from the insulin receptor (IR) activating the insulin-sensitive glucose transporter GLUT4, particularly present in skeletal muscle and adipose tissue. TZDs improve the insulin resistance via activation of PPAR-γ, which is present in both type I atherosclerotic lesions containing primarily VSMCs and type II lesions containing infiltrating macrophages; both cell types express PPAR-γ. Atherosclerosis in diabetic and nondiabetic mice on high-fat or high-ω-3 fatty acid diet decreases by 30–80% with TZDs or the non-TZD PPAR-γ agonist GW7845, despite there being minimal effects on circulating lipids, thus suggesting direct vascular effects (10,11).

Early growth response gene 1 (Egr-1) is expressed at a fivefold higher level in the fibrous cap lesions of patients undergoing coronary endarterectomy than in adjacent media (12). High-fat diet–induced atherosclerosis in LDL receptor null mice results in progressive increase in Egr-1 expression, and in VSMCs angiotensin II is a potent Egr-1 inducer, as it upregulates Egr-1 as well as Egr-1 target genes, including TNF-α and ICAM-1, the effects of which are prevented by PPAR-γ ligands. Activator protein-1 (AP-1), another nuclear target negatively regulated by PPAR-γ ligands, mediates many mitogenic and inflammatory cellular effects. Thus, the PPAR-γ ligands may play a role in the treatment of persons with the IRS.

Derek M. Yellon (London, U.K.) discussed anti-apoptotic effects of insulin, with potential benefit in acute myocardial infarction. Cardioprotection in acute myocardial infarction involves the attempt to limit cell death. Initial approaches involved ß-blockers, calcium channel blockers, and nitrates, but none have been shown to directly limit cell death. Reperfusion via a variety of routes has, in contrast, clearly been successful, but it comes at the price of reperfusion-induced cellular injury. It may be possible to provide additional protection against this phenomenon. Yellon hypothesized that there may be a component of reperfusion cell death involving apoptosis, rather than necrosis, and that by upregulating cell survival pathways at the time reperfusion it may be possible to prevent this.

The caspases are cell proteins that contribute to apoptosis. A variety of caspase inhibitors inhibit cell death and reperfusion injury in an isolated perfused rat heart model in which ligatures are placed on coronary arteries and then removed. The heart has intrinsic “prosurvival” pathways, the phosphatidylinositol 3-kinase (PI3K) and extracellular signal-regulated kinase (ERK) signaling pathway, which are activated by the bradykinin B2 receptor, IR, the receptor for cardiotoxin-1, an IL-6–related cytokine acting on PI3K, and the urocortin and transforming growth factor (TGF)–β1 receptors acting on ERK. As insulin affects these pathways, it may act in part as a prosurvival factor in the heart. In isolated rat neonatal cells subjected to hypoxia and then reoxygenation, insulin decreases apoptosis, an effect blocked by inhibitors of PI3K (13). In the isolated perfused rat heart, insulin administered at the time of reperfusion decreases residual infarct size 40–50% via PI3K-induced phosphorylation of Akt, with the process blocked by inhibitors of tyrosine or PI3K (14). Interestingly, statins also activate PI3K in this model, a potential mechanism of cardioprotection.

Cell biology of insulin resistance

Boris Dzauzni (Denver, CO) described the enhancement of mitogenic effects of insulin by metabolic insulin resistance as an imbalance among insulin signaling pathways, pointing out that the question of whether hyperinsulinemia is a culprit or whether insulin resistance itself is the cause of the complications of diabetes and the metabolic syndrome cannot be answered by epidemiologic studies. Rather, he suggested, one must ask whether all aspects of insulin action and signaling are equally resistant to insulin, and whether insulin resistance and increased insulin action may coexist in the same tissue and even in the same cell in patients with this group of conditions.

Insulin responses in the vascular walls of insulin-resistant animals include marked decrease in phosphorylation of the IR, of IR substrate-1 (IRS-1), and of PI3K, but “absolutely normal” activity of mitogen-activated protein kinase (MAPK). Schematically, insulin action splits into two pathways after activation of the IR tyrosine kinase. One pathway leads predominantly to metabolic action via IRS-1/2 phosphorylation, increasing PI3K with subsequent activation of metabolic effects and nitric oxide stimulation, as well as to some extent stimulating mitogenic effects. The second pathway involves activation of the docking protein Shc (Src homologous and collagen-like protein), which in turn activates Ras,
leading to MAPK activation. She and MAPK prenyltransferases, which are activated by insulin, attach the isoprenoids farnesyl diphosphate and geranylgeranyl diphosphate to Ras and Rho, respectively. The isoprenoids are derived from the same biochemical pathway that produces cholesterol, and their production is blocked by the HMG-CoA reductase inhibitor, suggesting a mechanism of the “pleiotropic” effects of statins. Prenylation anchors these proteins to the plasma membrane, thereby activating the mitogenic pathways.

Endothelial cells express VCAM-1, ICAM-1, and the E and P selectins. Nitric oxide (NO) protects against vascular endothelial growth factor (VEGF) stimulation of adhesion molecule production. Thus, insulin counteracts the VEGF effect and the insulin-resistant state may prevent this. Using the PI3K inhibitor wortmannin, endothelial nitric oxide synthase (eNOS) stimulation by insulin is prevented, although the insulin effect on MAPK and Erk is unchanged, and prenyltransferase stimulation is enhanced. The VEGF effect then increases with increase in the “rolling interaction” rate of circulating monocytes with endothelial cells and doubling of in vitro monocyte arrest at endothelial cells.

Insulin maintains differentiation of VSMCs, but does not promote migration or proliferation, while PDGF enhances dedifferentiation, proliferation, and migration. Insulin increases smooth muscle actin and H-caldesmon, markers of VSMC differentiation. Administration of insulin with wortmannin halves the expression of VSMC α actin, and preincubating VSMCs with insulin enhances the PDGF response. The ability of insulin to potentiate the action of growth factors such as angiotensin II, advanced glycation end products, and increased glucose on nuclear factor (NF)-κB transactivation is similarly pronounced, and can be demonstrated in vitro at physiologic insulin concentrations. Thus, in the presence of metabolic insulin resistance, as defined by reduced PI3K activity, insulin does not stimulate NO production to counteract VEGF, and rather than maintaining differentiation, it potentiates the effects of growth factors, possibly enhancing the development of obesity, atherosclerosis, and other pathologic processes.

Morris White (Boston, MA) discussed potential sites of insulin resistance. The insulin/IGF-1 signaling cascade involves tyrosine phosphorylation of receptors, with phosphorylation of multiple enzymes, including PI3K, providing phospholipids which engage molecules promoting glucose transport, protein synthesis, and other processes. The MAPK cascade regulates a variety of transcription factors leading to gene transcription, with extensive “cross-talk” between these pathways.

The IR substrate family is a group of nonenzymatic scaffold proteins that provide anchoring points for PI3K and other intracellular enzymes, with related molecules present throughout evolution. IRS-1 and -2 are ubiquitous in human tissues, IRS-4 is present in the brain, and IRS-3 appears to not be expressed in humans. IRS-1 is involved in overall cell growth and insulin action, while IRS-2 plays roles in insulin action in β-cell, liver, brain, and retina, and in reproduction and food intake, suggesting a number of higher functions, with evidence of association of insulin resistance with neurodegeneration as in Alzheimer’s diseases and the memory loss of aging. Brains of IRS-2 null mice are reduced in size, while the brain size is relatively normal with decreased skull size in IRS-1 null mice. The IRS-2 signaling cascade is essential for hypoxia-induced retinal neovascularization.

IRS-2 null mice develop diabetes, which is not seen in mice lacking IRS-1, -3, or -4. IRS-2 null mice fail to compensate for peripheral insulin resistance, while insulin resistance is seen in IRS-1 null mice with hyperinsulinemia leading to metabolic compensation. Indeed, islet size is increased in IRS-1 null mice but decreased in those deficient in IRS-2, suggesting that IRS-2 mediates growth and survival of β-cells. Decreased insulin action contributing to insulin resistance may be caused by degradation or serine phosphorylation of IRS-1 or -2, as can be seen with a variety of mutations. Serine phosphorylation blocks tyrosine phosphorylation of the IR, particularly at serine 307, a process increased by certain fatty acids, by TNF-α, and by insulin via c-Jun kinase (JNK), a potential negative feedback cycle.

In a transgenic model increasing IRS-2 expression in β-cell precursors, β-cell growth can be demonstrated in IRS-2 null mice, leading to β-cell hypertrophy despite insulin resistance and to prevention of diabetes, although many other consequences of insulin resistance can be demonstrated in these animals. Mice overexpressing IRS-2 are relatively protected against islet apoptosis following low-dose streptozotocin administration. IRS-2 signaling in the β-cell appears to regulate the expression of a transcription factor, Pdx-1, by a cascade beginning with PI3K activation, with increase in β-cell GLUT2 and in proinsulin transcription. Pdx1 levels are low in IRS-2 null mice while high in IRS-1 null mice, and Pdx1 transgenic mice show restoration of β-cell mass in islets from IRS-2 null mice. White suggested that pharmacologic or genetic treatment increasing IRS-2 expression or activity in type 2 diabetes might ameliorate the associated insulin deficiency.

Gokhan S. Hotamisligil (Boston, MA) discussed adipose tissue cytokines, describing the insulin resistance syndrome as “a joint venture between metabolic and inflammatory abnormalities.” The view that adipocytes produce metabolically active molecules has very important implications related to the “distant contacts” of these immune system–like products, which are common to macrophages and adipocytes and include adiponectin, TNF-α, leptin, plasminogen activator inhibitor-1, TGF-β, angiogenin, and resistin, as well as the many bioactive lipids derived from these cells. Hotamisligil noted that the fat body found in the drosary of Drosophila melanogaster has functions of adipose tissue, liver, and hematopoietic and immune systems, with adipogenesis controlled in a factor similar to that of the mammalian adipocyte, which suggests evolutionarily ancient relationships between adipose tissue and the immune system.

Fatty acid binding proteins (FABPs) include at least 10 different small tissue-specific proteins. Two related FABPs that bind to fatty acids in white adipocytes are aP2 and mal1. With deletion of one and two copies of the aP2 gene, levels of mal1 increase progressively, suggesting a regulated need for these proteins. Mice lacking both aP2 and mal1 genes fail to develop insulin resistance with high-fat diets. Both aP2 and mal1 are abundantly expressed in macrophages as well as in adipocytes, but not in the precursor monocytes or preadipocytes. Mice lacking aP2 are less able to produce cholesterol esters and, thus, to form foam cells (15). In apoE-deficient mice, which are highly atherosclerosis prone, superimposing aP2 knockout de-
creases atherosclerosis by 60–80%, with failure to express ap2 in macrophages (rather than in adipocytes) appearing to be the major factor in this. ap2 null cells show a threefold increase in prostaglandin J2 (PGJ2), the endogenous ligand for PPAR-γ, which, perhaps, explains in part the anti-atherosclerotic effect. Indeed, with or without administration of a TZD, PPAR-γ activity is greater in ap2 null cells. PGJ2 also inhibits IkB in addition to interfering with NF-κB nuclear binding. Free FAs (FFAs) upregulate JNK, which affects nuclear NF-κB activity. FABP null mice show decreased JNK activity. Thus, the inflammatory and cholesterol efflux pathways are regulated by these small proteins that control lipid traffic.

Returning to the relationship between inflammation and insulin action, Hotamisligil suggested that obesity appears to be a state of chronic inflammation with increased production of cytokines and other acute-phase reactants that play a crucial role in regulation of systemic insulin action. TNF-α-deficient mice show increased insulin action. (16). Thus, while autophosphorylation of the IR leads to phosphorylation of docking molecules such as the IRS family, with subsequent phosphorylation of other kinases, TNF-α–induced serine phosphorylation of IRS-1 appears to disturb the propagation of the IR signal. JNK appears to mediate this serine phosphorylation by causing a reciprocal decrease in tyrosine phosphorylation. There is increased JNK activity in liver, fat, and muscle in obesity (17). As further evidence for this hypothesis, JNK1 null mice fail to develop IRS-1 serine phosphorylation, and they show decreased body weight and resistance to development of obesity and to development of hepatic steatosis in several obesity models; there is no change in mice that do not express the JNK2 isoform.

Michael Karin (San Diego, CA) discussed the molecular roles of NF-κB in inflammation. NF-κB is a ubiquitous, inducible, nuclear transcriptional activator composed of two DNA-binding subunits. NF-κB binds to enhancer elements in many different cell types and is activated by pathogenic stimuli. Cytoplasmic NF-κB is activated by cytokines, such as TNF-α and IL-1, and by various byproducts of viral and bacterial infection; thereafter, it migrates to the nucleus. NF-κB stimulates inflammation via NO and prostaglandin synthesis as well as production of TNF-α and IL-1, leading to amplification of the inflammatory response. NF-κB stimulates the innate immune response via secretion of defensins, chemokines, cytokines, and adhesion molecules. Finally, NF-κB activates a number of anti-apoptotic genes, including caspase inhibitors, an effect potentially relevant to the growth of atherosclerotic plaques, which can be regarded as “very small tumors that grow in blood vessels.” Inhibitor of κB (IκB) denotes a family of inhibitory proteins that bind to the one of the subunits of NF-κB in the rel family of transcription factors. NF-κB is generally present in an inactive cytoplasmic form, bound to inhibitory IκB proteins; activation causes dissociation and translocation of NF-κB to the nucleus. After lipids are taken up by a cell, cytosolic binding proteins may direct them to oxidation or to nuclear entry; and similar considerations apply to lipids mediating inflammation, with JNK and IκB the central controlling portions of these pathways.

The inhibitor of κB kinase (IκB kinase) proteins block NF-κB activation. IκB kinase degrades IκB with subsequent activation of NF-κB in conditions of inflammation and insulin resistance. Iκα and -β act in association with a regulatory protein, IKK-γ, which is involved in assembly of the active tetrameric complex. IKK-γ and -β are required for the stimulatory effects of TNF-α, IL-1, infectious agents, and insulin resistance on NF-κB. For example, TNF-α acts via a receptor, thereby activating IκK as well as the JNK cascade. NF-κα also activates caspase leading to apoptosis (which is opposed by IκK activation).

Mice lacking IκkB in specific cell types are able to survive the neonatal period and can be studied to assess the role of NF-κB in inflammation. A model of gut ischemia/reperfusion with marked increase in TNF-α, inflammatory pulmonary infiltrates appear, an effect not seen in TNF-α null mice. In the gut, there is intense mucosal IκK activation, which can be blocked by deletion of IκKB from the enterocytes; this prevents the increase in circulating TNF-α, which is responsible for the pulmonary effects. However, in the absence of IκKB, major damage to the gut mucosa is seen, suggesting that NF-κB prevents the apoptotic effect of ischemia otherwise seen. NF-κB activation inhibits apoptosis through suppression of both caspases and JNK activation. Thus, the inflammatory response may have both positive and negative effects, so that prevention of the IκK/NF-κB response can lead to both benefits and adverse actions.

Steven Shoelson (Boston, MA) discussed the interaction between anti-inflammatory drugs and insulin resistance, addressing the interrelationship between inflammation and the IκB–NF-κB axis. Fat cell cytokines, FFAs, and serine/threonine, rather than tyrosine phosphorylation via ERK, JNK, and perhaps other kinases, are potential mediators. Shoelson referred the audience to the review by Pickup and Crook (18) and to evidence from the Insulin Resistance Atherosclerosis Study (19) of the relationship between diabetes and inflammation, and he presented further information pertaining to potential therapeutic effects of modulation of IκB and IKK in states of insulin resistance.

Glucocorticoids promote insulin resistance and hyperglycemia, but nonsteroidal anti-inflammatory drugs (NSAIDs) have been known since the 1870s to have a glucose-lowering effect: early studies reported definite glucose-lowering action of aspirin in doses exceeding 5 g daily. In animal models, aspirin decreases fasting glucose, triglyceride, FFA, and insulin levels without evidence of weight loss, indicative of a direct insulin-sensitizing effect (20). Aspirin and salicylate both show muscle and liver insulin-sensitizing actions in this model and also in vitro with adipocytes made insulin-resistant by incubation by TNF-α. The molecular target of aspirin is platelet cyclooxygenase (Cox)-1 in low doses, both Cox1 and Cox2 in many tissues at a 650-mg aspirin dose, and IκKB in high (several grams daily) doses (21, 22). IκKB, -α, -β, and -γ are located within the cell on a scaffolding complex—termed NF-κB essential modi

In patients with type 2 diabetes treated with high-dose aspirin for 2 weeks, glucose and FFA levels decrease, with suppression of hepatic glucose production and an increase in peripheral glu-
cose utilization to an extent similar to that seen with metformin (23). The natural product parthenolide, an anti-inflammatory substance present in the herb feverfew, inhibits IKK (24) and may lower blood glucose. In contrast, activation of IKK in liver and fat induces whole-body insulin resistance, and activation of IKK in fat increases fat pad weight with an increase in total lipid and a decrease in insulin sensitivity. Whether this is a direct effect or reflects altered adipose tissue cytokine production is not yet known.

Shoelson concluded that IKKB inhibition reversed insulin resistance in obese animal models and in patients with type 2 diabetes, and that chronic activation may be involved in the insulin-resistant state. He noted that salisate, which produces less gastric irritation than salicylate, appears to show glucose-lowering effects at doses of 3–4.5 g daily, just at and above the upper portion of the usual therapeutic range. This finding suggests that it may be possible to develop salicylate-like products with clinical safety and the potential to inhibit IKKB.

References
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