Antimicrobial-Sensing Proteins in Obesity and Type 2 Diabetes

The buffering efficiency hypothesis

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Obesity is well known to be associated with a cluster of metabolic diseases such as dyslipidemia, hypertension, insulin resistance, type 2 diabetes, and atherosclerosis (1). Alterations of the innate immune system are increasingly recognized to be intrinsically linked to metabolic pathways in humans (2). Central to metabolic diseases is insulin resistance associated with a low-grade inflammatory status (3). The mechanisms through which proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1α, interact with cellular insulin signal transduction cascades have been better understood in the last few years (4–6). In vivo, a direct correlation between increased circulating proinflammatory cytokines and insulin resistance has been well demonstrated (3,7). The origin of this increased inflammatory activity in obesity and type 2 diabetes is virtually unknown. Immune system homeostasis is challenged by continuous external insults, such as saturated fatty acid–rich diets (8), pathogen-associated molecular patterns like lipopolysaccharide (LPS) (9), advanced glycation end products (AGEs) (10), burden of infection (11), and oxidative stress (12). These continuous insults could result in a chronic low level of inflammation associated with insulin resistance.

Here, we review the potential significance of neutrophil dysfunction in subjects with type 2 diabetes and the consequence of altered antimicrobial-sensing protein profile in obesity-related metabolic disturbances.

NEUTROPHIL DYSFUNCTION IN METABOLIC DISEASE—Given that 60–70% of blood leukocytes are granulocytes and over 90% of granulocytes are neutrophils, polymorphonuclear cells (PMNs) are the largest fraction of white blood cells. PMNs possess a variety of functions, including chemotaxis, adhesion to the endothelium and foreign agents, phagocytosis, and microbicidal activity. PMNs are able to penetrate and migrate into infected tissues and destroy invading microorganisms after internalization by producing multiple toxic agents such as reactive oxygen species (ROS), proteases (elastase), and proteins interfering with bacterial development.

Chronic disease (such as type 2 diabetes), age-associated insulin resistance, nutrition, and lifestyle have a significant effect on PMN function. Of note, the risk of infectious diseases is two- to fourfold higher in patients with diabetes, or even impaired glucose tolerance without hyperglycemia, than in healthy subjects (13). The neutrophils of diabetic patients show enhanced production of ROS, increased apoptosis, and significantly lower neutrophil chemotactic responses. It is notable that the circulating levels of proinflammatory cytokines are elevated in diabetic patients, and it has been suggested that the impaired functions of neutrophils contribute to the increased susceptibility to infections observed in these patients. Hyperglycemia, or the presence of AGEs, leads to persistent activation of neutrophils, as evidenced by the increased activity of neutrophil alkaline phosphatase (14). Furthermore, both an increased basal release of TNF-α, IL-8, and IL-6 (14,15) and a low secretion of some granular proteins by neutrophils from patients with type 2 diabetes (16,17) have been reported. In addition, the impaired actin polymerization in neutrophils from type 2 diabetic patients was a main factor in the inability of neutrophils to downregulate integrin CD11b/CD18 and to exocytose primary granules (CD69), altering neutrophil exocytosis (16).

It has previously been shown that insulin has a strong regulating effect on the functional activities of immune cells (18,19). Generally speaking, the priming action of insulin on PMN activity may be seen as the body providing a global defense to support primary immune response against exposure to antigens, which is enhanced by food intake (20). Walrand et al. (21) showed that aging-induced reduction in insulin sensitivity plays a role in the age-related weakening of the immune system, particularly after food intake (20). Therefore, alterations in immune cell function may partly explain the higher prevalence of infective episodes in the type 2 diabetes and older population. Previous studies have shown that the clearly altered PMN functions of diabetic subjects could be restored by controlling hyperglycemia with insulin. Interestingly, although PMNs do not require insulin to uptake glucose, glucose use and glycogen metabolism inside PMNs are both insulin dependent. In addition, insulin receptor expression was correlated with PMN chemotaxis in both young and elderly subjects after insulin treatment (21). Antimicrobial protein production in PMNs is also altered in association with insulin resistance and in the elderly (21) (as reviewed below) and is decreased under hyperglycemic conditions.
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in humans after intravenous endotoxin administration (22).

Elgazar-Carmon et al. (23) reported that high-fat feeding causes a significant recruitment of neutrophils to intra-abdominal adipocyte tissue, peaking at 3–7 days and subsiding thereafter. For this reason, the authors speculated that this recruitment of neutrophils could constitute a key event in initiating the inflammatory cascade in response to high-fat feeding. These neutrophils might produce chemoattractant factors, allowing macrophage infiltration and a chronic inflammatory state in adipose tissue. The notion that “chronic inflammatory infiltrate” is preceded by a transient “acute inflammatory infiltrate” dominated by neutrophils is a well-established paradigm in systemic inflammatory processes.

SPECIFIC MARKERS OF TYPE 2 DIABETES-ASSOCIATED NEUTROPHIL DYSFUNCTION

Several antimicrobial proteins produced by neutrophils, such as lactoferrin, bactericidal/increasing permeability protein, and α-defensins, are decreased in association with insulin resistance and type 2 diabetes. The circulating concentration of these proteins is in parallel with the low antimicrobial capacity of neutrophils from type 2 diabetic subjects. Furthermore, one of these proteins (lactoferrin) displayed a significant association with insulin resistance and type 2 diabetes. The circulating concentration of these proteins is in parallel with the low antimicrobial capacity of neutrophils from type 2 diabetic subjects.

Lactoferrin

Lactoferrin is a pleiotropic glycoprotein of the innate immune system that is involved in LPS buffering. Lactoferrin is a monomeric 80-kDa glycoprotein, with a single polypeptide chain of ~690 amino acid residues and two sialic acid molecules, that is produced by neutrophils and several epithelial cell types. Neutrophils are the only source that contributes to significant amounts of circulating lactoferrin in the bloodstream (26). Lactoferrin is folded into homologous N- and COOH-terminal lobes, each comprising two domains that enclose a conserved iron binding site. This protein is positively charged in the NH2-terminal region (the first 60 amino acids) of the N-lobe at a physiological pH because it is rich in arginine (26). Lactoferrin is able to bind and buffer other pathogen-associated molecular patterns in addition to LPS, viral DNA and RNA, CpG sequences, and soluble components of the extracellular matrix. This ability is associated with lactoferrin anti-inflammatory activity, as demonstrated in several studies (26), in which lactoferrin downregulated proinflammatory cytokine production in cell lines acting via nuclear factor (NF)-κB (27) and to decreased secretion of TNF-α and IL-6 in mice.

In humans, fasting circulating lactoferrin concentration was inversely associated with BMI, waist-to-hip ratio, fasting triglycerides, and fasting glucose and directly associated with HDL cholesterol and insulin sensitivity (17,28). Lactoferrin secretion decreased significantly in whole blood under proinflammatory stimulus (IL-6 coinubcation) and increased significantly after insulin sensitization (rosiglitazone) (17). Furthermore, circulating lactoferrin concentration was associated with vascular function in obese subjects with altered glucose tolerance.

On the other hand, two nonsynonymous LTF gene polymorphisms, which produce two amino acid changes in the NH2-terminal region, were associated with dyslipidemia according to glucose tolerance status (28). Circulating lactoferrin concentrations, both at baseline and fat stimulated, were also inversely associated with postprandial lipemia, parameters of oxidative stress, and fat-induced inflammation in severely obese subjects after acute fat intake (24). In high-fat diet–induced obesity in C57BL/6 J mice, lactoferrin cotreatment led to weight loss, decreased body fat content, and adipocyte size (25).

In vitro, lactoferrin administration improved insulin action (increasing insulin-induced +73SerAKT phosphorylation) in the mouse 3T3-L1 cell line and in human HepG2 cell lines, even in those conditions where the response to insulin was downregulated (under proinflammatory conditions and dexamethasone administration). Furthermore, lactoferrin led to blunted adipogenesis in the context of increased phosphorylation of 172ThrAMPK and retinoblastoma activity in 3T3-L1 cells (29).

Bactericidal/increasing permeability protein

Bactericidal/increasing permeability protein (BPI) is located in the azurophilic granules of neutrophils and is an ~55-kDa cationic protein with selectivity toward Gram-negative bacteria, most likely because of its strong affinity for LPS (30). Besides being bactericidal, BPI also neutralizes the cytotoxic effects of LPS. Most of the antibacterial and LPS binding activity of holo-BPI is found in the 20- to 25-kDa NH2-terminal fragments of the protein (30). rBPI21, representing a recombinant 21-kDa protein and corresponding to amino acids 1–193 of the NH2-terminal human BPI (with the exception that a cysteine is replaced by an alanine at position 132), is bactericidal and binds to and neutralizes endotoxin (31).

Plasma BPI concentration was directly correlated with insulin sensitivity and HDL cholesterol concentrations and was inversely associated with metabolic parameters (waist-to-hip ratio, fasting triglycerides) and serum lipopolysaccharide binding protein (LBP) and LPS concentration (32). BPI genetic variations that lead to lower serum concentration of BPI were associated with insulin resistance and increased circulating inflammatory markers (32). In addition, circulating BPI level was recently reported as a useful maker for endothelial dysfunction (33).

Human α-defensins

Human α-defensins are arginine-rich peptides, containing 29–35 amino acids. Their three disulfide bridges connect cysteines 1–6, 2–4, and 3–5. Human α-defensins are synthesized as 93–100 amino acid prepropeptides with a 19 amino acid signal peptide and a 41 to 51 amino acid anionic pro-segment. α-Defensins are predominantly found in neutrophils (mainly DEFA1–3) and in small intestinal Paneth cells. Stimulus-dependent releases of presynthesized defensin-containing cytotoxic granules contribute to the local antimicrobial response (34). Significant positive associations among plasma α-defensin (DEFA1–3) concentrations, insulin sensitivity, and nonatherogenic lipid profile and vascular function in apparently healthy Caucasian men were reported (35).

From these findings, it is evident that metabolic dysfunction is associated with decreased production and/or secretion of lactoferrin, BPI, and α-defensins from neutrophils. To counteract the decreased production of these proteins from the first line of defense, it seems that the body increases the production of other antimicrobial proteins from the liver, fat, and lungs, as described below.
ANTIMICROBIAL-SENSING PROTEIN PROFILE IN METABOLIC DISEASE

Soluble CD14

The earliest cell-mediated events after endotoxin release appear to involve the transfer of LPS to the GPI-linked protein CD14. Different lines of evidence support a central role for CD14 in LPS-mediated responses. Specific monoclonal antibodies against CD14 inhibit the ability of LPS to stimulate monocytes (36). Transfection of CD14 into the 70Z/3 pre-B cell line enhances the responsiveness of these cells to LPS by more than 1,000-fold (37). CD14 also exists in a soluble form (sCD14) (38), and its levels are significantly raised in septic patients (39). The physiological role of sCD14 is not yet completely understood. sCD14 has been shown to inhibit the LPS-induced TNF-α production in whole blood and monocytes (40), and in a mouse model of endotoxin shock, sCD14 was shown to inhibit lethality as well (41). However, contrary to this inhibiting effect of sCD14 on LPS effects, sCD14 facilitated the activation of endothelial cells that do not express membrane CD14 (42). Troelstra et al. (43) reported that the effect of sCD14 on neutrophil response to LPS was a balance between activation and inhibition, depending on the concentration of circulating LBP in serum. However, sCD14 could play a key role as an intermediate in the neutralization of LPS under physiological conditions. sCD14 accelerates the transfer between LPS micelles and lipoproteins by acting as a carrier. sCD14 also enhances the release of monocyte-bound LPS, transferring LPS into plasma and lipoproteins and, thus, decreasing cellular responses to LPS, such as induction of TNF-α and IL-6 synthesis (44).

sCD14 was significantly and inversely associated with insulin resistance, waist-to-hip ratio, systolic and diastolic blood pressure, and inflammatory markers (soluble receptors of TNF-α, sTNFR1 and sTNFR2), after controlling for fasting triglycerides and smoking status (45). Interestingly, genetic variations that lead to lower serum concentration of sCD14 were associated with insulin resistance and increased inflammatory markers (45). sCD14 could also be a marker of hepatic insulin resistance and dysfunction. In fact, decreased serum sCD14 concentration was associated with the highest alanine aminotransferase activities in serum (46). These apparently protective associations of sCD14 with metabolic parameters (insulin sensitivity, blood pressure, hepatic injury) are supported by the anti-inflammatory activities of sCD14, neutralizing LPS effects in vitro models. In addition, a direct relationship between sCD14 and endothelial function in type 2 diabetic subjects was found to be opposite to the inverse association of these parameters in nondiabetic subjects (47).

LBP

LBP is an important LPS marker. LBP is a 65-kDa protein present in blood at high concentrations (~2–20 μg/mL) (48). LBP is an acute-phase reactant, predominantly derived from the liver, and plasma levels rise dramatically after inflammatory challenge, including bacterial sepsis (48). Although the molecular structure of LBP is not entirely known, LBP clearly binds LPS (and LPS substructures, such as lipid IVa) through the recognition of lipid A (48). The plasma protein LBP dramatically accelerates the binding of LPS monomers from aggregates to CD14 (49), thereby enhancing the sensitivity of cells to LPS. Furthermore, LBP acts as a lipid transfer protein, a function in keeping with its sequence homology to lipid transferases (phospholipid transfer protein and cholesterol ester transfer protein). LBP copurifies with HDL particles, and additional studies have shown that LBP can transfer LPS to lipoproteins, neutralizing LPS effects (50).

Serum LBP reflected the serum endotoxin (LPS) concentration and was negatively associated with insulin sensitivity, obesity, and cardiovascular disease (32,51). Interestingly, serum LBP concentrations were increased in patients with type 2 diabetes in a recent study (52).

Neutrophil gelatinase-associated lipocalin

A recently characterized factor produced by the adipose tissue is lipocalin 2 (also known as 24p3 and neutrophil gelatinase-associated lipocalin [NGAL], siderocalin). NGAL is a 25-kDa secretory glycoprotein that belongs to the lipocalin family. The members of the lipocalin family contain a common tertiary structure with an eight-stranded β-barrel surrounding a cup-shaped ligand binding interior, covered with hydrophobic amino acid residues. This structure confers lipocalin the ability to bind and transport a wide variety of small lipophilic known ligands for lipocalins including retinol, steroids, odorants, pheromones, and, in the case of NGAL, siderophores (53). NGAL is expressed in many tissues and cells in addition to adipose tissue, including kidney, liver, lung, thymus, small intestine, mammary tissue, and leukocytes (macrophages and neutrophils). Expression of NGAL in liver, macrophages, and adipocytes is markedly induced by a variety of proinflammatory stimuli through activation of NF-κB (33). NGAL was elevated in multiple murine models of obesity, and reduction of NGAL in cultured adipocytes improved insulin sensitivity. Data from db/db mice (53,54) indicated an elevated NGAL expression in the liver, whereas in high-fat–fed mice, liver NGAL expression tended to be lower. The authors concluded that the contribution of extra-adipose sources of NGAL to serum was unclear and may differ between obesity models. Studies in humans showed a positive relationship between circulating NGAL concentration and fasting insulin and homeostasis model assessment values. However, the origin of increased circulating NGAL in humans is poorly known. Because NGAL concentrations were positively correlated with several adiposity variables, including BMI, waist circumference, and percent body fat, some authors suggested that the increased fat mass might also account for the increased circulating concentrations of this protein in obese humans (55). Recently, it was reported that both metabolic endotoxemia (metabolic LPS concentration, which was not enough to produce acute endotoxemia) and saturated fat might contribute to circulating NGAL concentration in patients with insulin resistance (56). LPS-induced NGAL production in whole blood culture was significantly increased in subjects with type 2 diabetes (56). Law et al. (57) reported that NGAL increases insulin resistance, stimulating the expression and activity of 12-lipoxygenase (increasing the amounts of arachidonic acid) and TNF-α production in fat tissues.

Surfactant protein A and surfactant protein D

Some components of the lung surfactant have been shown to be important host defense components against respiratory pathogens and allergens. Pulmonary surfactant is a complex mixture of lipids (90%) and proteins (5–10%) that constitutes the mobile liquid phase covering the large surface area of the alveolar epithelium. It maintains minimal surface tension within the lungs to avoid lung collapse during respiration. Four surfactant proteins

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SPs (SP-A, SP-B, SP-C, and SP-D) are intimately associated with surfactant lipids in the lung (58). SP-A is the major surfactant-associated protein, constituting 3–4% of the total mass of isolated surfactant and 50% of the total SP. These SPs occur physiologically in small amounts in blood (59), and because they are secreted into the respiratory tract, their occurrence in serum can only be explained by leakage into the vascular compartment. Intravascular leakage increases in conditions characterized by pulmonary inflammation and/or pulmonary epithelial injury (59). By upregulating SP-A and SP-D synthesis, the innate immune system can immediately respond to intrusion of foreign agents by helping to prevent further invasion. Circulating SP-A concentration was significantly higher among patients with glucose intolerance and type 2 diabetes than in subjects with normal glucose tolerance, even after adjustment for BMI, age, and smoking status (ex/never) (59). On the contrary, serum SP-D concentration was significantly decreased in subjects with obesity and type 2 diabetes and was negatively associated with fasting and postload serum glucose, HbA1c, serum lipids, insulin sensitivity, and inflammatory parameters (60). These findings suggest that lung innate immunity, as inferred from the alteration in circulating SP-D and SP-A concentrations, is at the crossroads of inflammation, obesity, and insulin resistance.

**BUFFERING EFFICIENCY HYPOTHESIS**—Chronic low-grade inflammation and associated insulin resistance might be viewed in the context of an unbalanced innate immune system. The evidence reviewed here led us to propose the buffering efficiency hypothesis (Fig. 1). An altered production of antimicrobial-sensing proteins (low sCD14, BPI, Lactoferrin, DEFA1–3, and SP-D, and high LBP, NGAL, and SP-A) were associated with insulin resistance, obesity, vascular dysfunction, hepatic dysfunction, and dyslipidemia. A partial loss in the buffering efficiency of external insults (saturated fatty acids, LPS, AGEs, and ROS) could increase their negative effects on metabolism. Furthermore, insulin resistance might result in a vicious cycle, decreasing the concentration of those buffering proteins (Table 1).

Antimicrobial efficiency of neutrophils is decreased in insulin-resistant conditions, as evidenced by the decreased circulating levels of lactoferrin, BPI, and other antimicrobial proteins (α-defensins, SP-D). Neutrophil activity may be restored by controlling hyperglycemia using insulin (20,23). Stegenga et al. (22) reported that hyperglycemia led to impaired neutrophil degranulation after intravenous endotoxin administration in humans.

**Figure 1**—The effects of altered antimicrobial-sensing protein profile and neutrophil dysfunction in the relationship between chronic low-level inflammation and obesity-related metabolic disturbances. External insults are as follows: fatty acid–rich diets, pathogen-associated molecular patterns (endotoxin, LPS), AGEs, burden of infection, and ROS. Lf, Lactoferrin.
This impairment of neutrophil function was associated with a poor metabolic profile in subjects with type 2 diabetes, including decreased neutrophil deformability and increased production of ROS and proinflammatory cytokines.

Insulin resistance and chronic low-grade inflammation seem to be mutually potentiated, leading to a vicious cycle, strengthened by an unbalanced innate immune system. To cope with the continuous challenges from the environment, the body builds different barriers of defense (Fig. 1). Epithelial cells of the skin constitute the first barrier of defense. Some of the proteins described here in association with insulin action are also synthesized in epithelial cells (lactoferrin, SP-D, α-defensins). Beneath the skin, the body has built an important second line of defense. Almost 50% of adipose tissue is distributed in the subcutaneous fat depot, beneath the skin throughout the whole body. Interestingly, an increased amount of subcutaneous adipose tissue is associated with a decreased risk of developing type 2 diabetes (61).

Epithelial cells of mucosa also cover each centimeter of the digestive tract, the other surface of interaction with the environment. If pathogens are able to disrupt mucosa, the body again has built a strong second line of defense—visceral adipose tissue. However, this depot is metabolically very active, unstable, and in close contact with ~1 kg of bacteria in the gut. If this barrier is overwhelmed, bacteria and bacterial products from the gut reach into the liver, an important structured buffer.

Our body also interacts with the environment through the alveolar space and epithelial cells of the respiratory tract. SPs are also important members of the armamentarium defense.

Metabolic disease can be envisioned as a relative failure of all these body defenses (innate immune proteins of the skin, subcutaneous adipose tissue, and the gut and respiratory tract). This failure leads to chronic inflammatory disease, to insulin resistance in the long term, and finally to type 2 diabetes (Fig. 1).

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