Inflammation and Activated Innate Immunity in the Pathogenesis of Type 2 Diabetes

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There is increasing evidence that an ongoing cytokine-induced acute-phase response (sometimes called low-grade inflammation, but part of a widespread activation of the innate immune system) is closely involved in the pathogenesis of type 2 diabetes and associated complications such as dyslipidemia and atherosclerosis. Elevated circulating inflammatory markers such as C-reactive protein and interleukin-6 predict the development of type 2 diabetes, and several drugs with anti-inflammatory properties lower both acute-phase reactants and glycemia (aspirin and thiazolidinediones) and possibly decrease the risk of developing type 2 diabetes (statins). Among the risk factors for type 2 diabetes, which are also known to be associated with activated innate immunity, are age, inactivity, certain dietary components, smoking, psychological stress, and low birth weight. Activated immunity may be the common antecedent of both type 2 diabetes and atherosclerosis, which probably develop in parallel. Other features of type 2 diabetes, such as fatigue, sleep disturbance, and depression, are likely to be at least partly due to hypercytokinemia and activated innate immunity. Further research is needed to confirm and clarify the role of innate immunity in type 2 diabetes, particularly the extent to which inflammation in type 2 diabetes is a primary abnormality or partly secondary to hyperglycemia, obesity, atherosclerosis, or other common features of the disease.

Diabetes Care 27:813–823, 2004

There has been a recent explosion of interest in the notion that chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of type 2 diabetes. For example, since this hypothesis was first proposed in 1997 and 1998 (1,2), at least 12 studies have shown that circulating markers of inflammation, acute-phase reactants, or interleukin (IL)-6 (the major cytokine mediator of the acute-phase response) are strong predictors of the development of type 2 diabetes (3–14).

Although it is well established that insulin resistance and impaired insulin secretion are central to the pathogenesis of type 2 diabetes, it has been unclear how these abnormalities arise and how they are related to the many different clinical and biochemical features common in type 2 diabetes, including central obesity, hypertension, accelerated atherosclerosis, dyslipidemia, depression, disordered hemostasis, and altered metal ion metabolism, sleep, and reproductive hormone levels. Activation of innate immunity provides a new model for the pathogenesis of type 2 diabetes and the metabolic syndrome, which may explain some or all of these features, and points to research directions that might result in new therapeutic approaches for managing and predicting type 2 diabetes and its complications.

The purpose of this review is to discuss critically the evidence that now supports a role for inflammation and innate immunity in type 2 diabetes and to highlight the implications of this theory and the indications for future research.

METHODS — The Embase and Medline electronic databases were searched using the following key words: acute-phase response/reaction, innate/natural immunity/immune system, inflammation, stress, cytokines, C-reactive protein (CRP), sialic acid, type 2 diabetes mellitus, and noninsulin-dependent diabetes mellitus. Articles cited in key references, personal communications, and a personal database of relevant articles were also considered.

INNATE IMMUNITY, INFLAMMATION, AND THE ACUTE-PHASE AND STRESS RESPONSE: WHAT THEY ARE AND HOW THEY ARE RELATED

Innate immunity

The innate or natural immune system is the body's rapid first-line defense against environmental threats such as microbial infection and physical or chemical injury (15). A series of reactions are induced that prevent ongoing tissue damage, isolate and destroy infective agents, and activate repair processes to restore homeostasis (Fig. 1). Study of innate immunity has been somewhat neglected until recently, overshadowed by the complexities of the acquired or adaptive immune system (i.e., B- and T-cells) and suffering from the erroneous belief that this evolutionary ancient system is unsophisticated and now obsolescent for vertebrates (16).

A major component of innate immunity is a series of sentinel cells (classically macrophages, antigen-presenting B-cells, and dendritic cells, but probably also intestinal epithelial cells, endothelium,

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Received for publication 7 April 2003 and accepted in revised form 17 November 2003.

Abbreviations: AGE, advanced glycation end product; CRP, C-reactive protein; HPA, hypothalamic-pituitary-adrenal; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IL, interleukin; IRS, insulin receptor substrate; LC-NE, locus coeruleus-norepinephrine; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; PAI-1, plasminogen activator inhibitor 1; PPAR, peroxisome proliferator–activated receptor; PRR, pattern recognition receptor; TLR, toll-like receptor; TNF, tumor necrosis factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

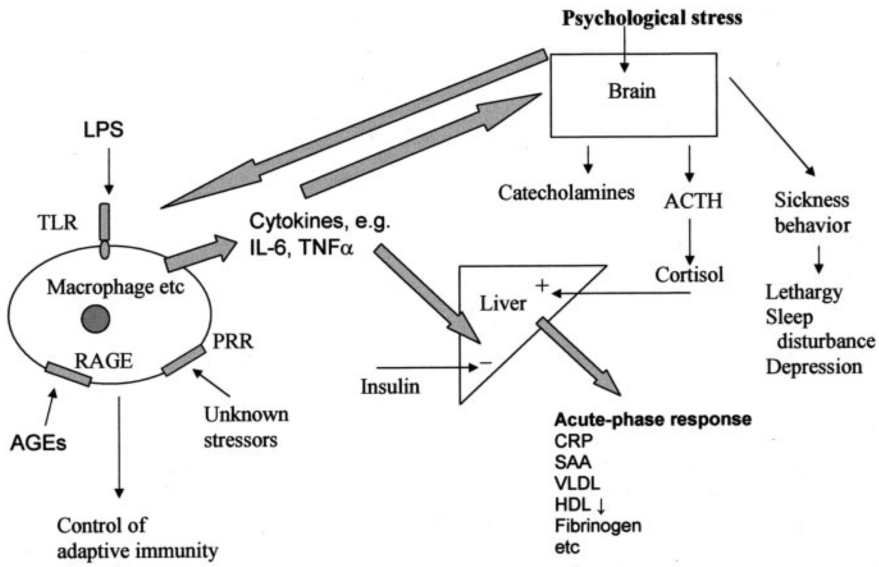

Figure 1—*The components of the innate immune system. Sentinel cells such as the macrophage detect potential environmental threats from infection, chemicals, and foods by PRRs that activate signaling pathways and release proinflammatory cytokines (IL-6 and TNF-α). Known PRRs include TLR-4, which senses bacterial LPS and the receptor for AGEs. Cytokines stimulate acute-phase protein production from the liver and also act on the brain to release adrenocorticotrophic hormone (and thereby cortisol from the adrenal gland) and activate the sympathetic nervous system with the release of catecholamines. Psychological stress can cause an acute-phase response via innervation of cytokine-producing cells and via activation of the sympathetic nervous system and adrenergic receptors on macrophages. Central cytokine-induced "sickness behavior" includes lethargy, sleep changes, and depression. The innate immune system also controls the adaptive (acquired) immune system via costimulatory molecule expression that is necessary for antigen presentation. SAA, serum amyloid A.*

Kupffer cells in the liver, adipocytes, and others) that act as "trouble detectors." A number of germ line–encoded (i.e., nonclonal) pattern recognition receptors (PRRs) on and in these cells recognize conserved molecular structures (pathogen-associated molecular patterns) that are characteristic of a class of harmful agents. The most studied PRRs are probably the family of at least 10 toll-like receptors (TLRs) (named after the toll receptor, first identified in the fruit fly, drosphophila) that are present at the cell surface as transmembrane receptors (17). TLR-4, for example, recognizes lipopolysaccharide (LPS) from Gram-negative bacteria, in conjunction with associated accessory molecules (CD14, MD-2). Other cell surface PRRs are macrophage scavenger receptors (18), the mannose receptor (15), and the receptor for advanced glycation end products (AGEs) (19). There are also intracellular PRRs, e.g., for double-stranded RNA (present in viruses). Binding to PRR activates nuclear factor-κB (NF-κB) signaling pathways that induce immune response genes, especially those for inflammatory cytokines, which are the main mediators of inflammation and the acute-phase response. Secreted and circulating PRRs such as CRP and mannan-binding lectin function as opsonins, binding to microbial cell components and flagging them for recognition by the complement system and phagocytes.

An important second function of innate immunity, which has only recently been appreciated, is to control the adaptive immune response (15,20). T-cells require two signals to be activated: the complex of presented antigen and the major histocompatibility complex class II molecule on the surface of an antigen-presenting cell and costimulatory molecules (CD80 and CD86), which are invoked by the innate immune system and the binding of pathogen-associated molecular patterns to PRRs. Thus, the innate immune system ensures that the adaptive immune system responds only to harmful antigens and that the biological context of a threat is recognized.

Inflammation

Inflammation is the local protective response to tissue injury (21). The word inflammation means "setting on fire" (16th century), and the process has been known since Egyptian times (c. 2500 B.C.). The cardinal signs of redness, swelling, heat, and pain were described by Celsus (first-century A.D.), and loss of function was added by Galen (130–200 A.D.). Microscopically, these features are due to vasodilation, accumulation of leukocytes, increased capillary permeability and interstitial fluid, and stimulation of nerve endings by mediators such as substance P.

The acute-phase response

In addition to local effects in inflammation, there is a systemic reaction known as the acute-phase response, best characterized by pronounced changes in the concentration of certain circulating proteins and other substances, called acute-phase reactants (22–24). Acute-phase proteins usually increase in concentration, with examples being CRP, complement, serum amyloid A, α1-acid glycoprotein, haptoglobin, and fibrinogen, but some such as albumin are negative acute-phase reactants that decrease in concentration. The acute-phase proteins are mostly synthesized in the liver, and production is stimulated by cytokines of the innate immune response—mainly IL-6 and tumor necrosis factor (TNF)-α (Fig. 1). In general, the acute-phase proteins limit injury or aid healing.

There are many other acute-phase responses induced by inflammatory cytokines, including leukocytosis, fever, and behavioral changes such as somnolence and lethargy. Despite the apparent oxymoron, an ongoing "acute"-phase response is seen in many chronic diseases, such as arthritis and cancer (and, as discussed below, type 2 diabetes and atherosclerosis).

The stress response

Innate immunity and the acute-phase response are integrated with the neuroendocrine system, particularly via the hypothalamic-pituitary-adrenal (HPA) axis and the locus coeruleus-norepinephrine (LC-NE) system of the sympathetic nervous system (25–27). Cytokines released by macrophages at the site of inflammation act on the brain to release corticotrophin-releasing factor from the hypothalamus, adrenocorticotrophic hormone from the pituitary gland, and cortisol from the adrenal cortex, which acts as an anti-inflammatory negative feedback by suppressing cytokine release and stimulating liver synthesis of acute-phase proteins. Psychological stress causes an acute-phase response by activating the HPA axis and the LC-NE system and by inducing IL-6, TNF-α, and other cytokine secretion from macrophages (via several mechanisms, including catecholamines acting on the macrophage β-adrenergic receptor, and corticotrophin-releasing factor and substance P release from local nerve endings acting on macrophages [27]). Thus, the brain can both produce and modulate inflammation.

THE ORIGINS OF THE ACTIVATED INNATE IMMUNITY PARADIGM

— A decade ago, we showed that, in comparison with nondiabetic subjects, circulating concentrations of commonly recognized acute-phase reactants were increased in type 2 but not type 1 diabetic patients who were matched for age, sex, glycemic control, and the absence of tissue complications (28). These acute-phase reactants included CRP, serum amyloid A, α1-acid glycoprotein, and sialic acid (the latter is an integrated measure of the acute-phase response because many of the acute-phase proteins are glycoproteins with sialic acid as the terminal sugar of the oligosaccharide chain). Serum levels of acute-phase reactants (including cortisol) and the cytokine mediator of the acute-phase response, IL-6, showed a graded increase with increasing features of the metabolic syndrome in type 2 diabetic and nondiabetic subjects, i.e., obesity, coronary heart disease, hypertension, hypertriglyceridemia, and low levels of HDL cholesterol (1).

We also noted that others had found that after experimental induction of the acute-phase response in animals (29) and in illnesses in humans likely to be associated with an acute-phase response such as malignancy (30) and infection (31), there are elevated serum concentrations of total cholesterol and VLDL triglyceride and lowered HDL cholesterol—typical features ("dyslipidemia") of type 2 diabetes and the metabolic syndrome. Also, many circulating analytes, which are known to have altered concentrations in type 2 diabetes, are established acute-phase reactants, e.g., fibrinogen, von Willebrand factor, plasminogen activator inhibitor 1 (PAI-1), ferritin, complement, lipoprotein(a), cortisol, testosterone (lowered), and zinc (lowered) (2).

Because there are many plausible mechanisms by which cytokines can lead to insulin resistance, impaired insulin secretion, dyslipidemia, and accelerated atherosclerosis, this led us to hypothesize that in type 2 diabetes, there is an ongoing cytokine-mediated acute-phase response (part of a wide-ranging activation of innate immunity), and this is closely involved in the pathogenesis of the disease (1,2).

How have recent studies provided evidence to support this theory?

1) Markers of inflammation are associated with type 2 diabetes and features of the metabolic syndrome in cross-sectional studies

Several cross-sectional studies in nondiabetic subjects or the general population (32–40), or in individuals with impaired glucose tolerance (IGT)/impaired fasting glucose (IFG) (41–44), have confirmed that acute-phase reactants such as CRP (and sometimes the cytokines IL-6 and TNF-α) are positively correlated with measures of insulin resistance/plasma insulin concentration, BMI/waist circumference, and circulating triglyceride and negatively correlated with HDL cholesterol concentration. In general, increasing components of the metabolic syndrome in individuals are associated with higher levels of inflammatory markers. In subjects with IGT or IFG, IL-6 but not TNF-α appears to be elevated compared with individuals with normal glucose tolerance (41), and in one study, inflammatory markers were related to insulin resistance but not to insulin secretion (42).

Additional cross-sectional studies in newly diagnosed (43) or established type 2 diabetic patients (45–48) have confirmed that acute-phase markers such as CRP and IL-6 are elevated in these subjects compared with nondiabetic control subjects. In the study by Leinonen et al. (47), all markers of inflammation, including CRP, serum amyloid A, secretory phospholipase A_2, and IL-6, and endothelial dysfunction (soluble cell adhesion molecules) correlated with the homeostasis model–measured insulin resistance. In studies with a small number of subjects (48), the elevated mean or median CRP and IL-6 levels in type 2 diabetes may not reach statistical significance—the concentrations of both analytes, although higher than in nondiabetic subjects, are low in comparison to other acute-phase conditions such as cancer and acute infections and require ultrasensitive assays to demonstrate accurately the circulating concentrations in diabetes.

In apparent contrast to IGT/IFG (where TNF-α levels are reportedly normal [41]), circulating TNF-α is usually elevated in established type 2 diabetes (49–51).

2) Markers of inflammation predict type 2 diabetes

Schmidt and colleagues (3,4), using data from the Atherosclerosis Risk in Communities study, were the first to show that a variety of inflammatory markers, including white blood cell count, low serum albumin, α1-acid glycoprotein, fibrinogen, and sialic acid, predict the development of type 2 diabetes in a middle-aged population. This has been confirmed over mean follow-up times from 2 to 20 years for women in the U.S. Women's Health Study (CRP and IL-6) (5), for elderly subjects in the U.S. Cardiovascular Health Study (CRP) (6), in Pima Indians (white blood count) (7), for multiethnic subjects in the U.S. Insulin Resistance and Atherosclerosis Study (CRP, fibrinogen, and PAI-1) (8), in Scottish men in the West of Scotland Coronary Prevention Study (CRP) (9), in the U.S. National Health and Nutrition Examination Survey (white blood count) (10), for Japanese men (white blood count) (11), for participants in the Hoorn Study in the Netherlands (CRP) (12), for participants in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Postdam Study in Germany (IL-6, with additional risk of IL-6 and IL-1β combined) (13), and in middle-aged men in the MONICA Augsburg Study in Germany (CRP) (14). Interestingly, CRP was a significant predictor of diabetes in women but not in men in the Mexico City Diabetes Study (52), indicating that the differential role of inflammation in men and women needs further elucidation.

In addition, low circulating levels of the recently identified anti-inflammatory adipose tissue–derived cytokine, adiponectin, predict type 2 diabetes in Pima Indians (53). Although slightly weakened by adjusting for obesity, the association of altered levels of acute-phase reactants and later diabetes in these studies is generally independent of age, sex, blood glucose concentration, family history of diabetes, physical activity, smoking, and baseline atherosclerosis. In the Pima Indian study (7), elevated white blood cell count was associated with a decline in insulin sensitivity but not insulin secretion, echoing the cross-sectional relationship in IGT between inflammatory markers and insulin resistance but not insulin secretion (42).

3) Inflammation is involved in the pathogenesis of atherosclerosis, a common feature of type 2 diabetes

Inflammation is now known to be involved in the pathogenesis of all stages of atherosclerosis (54,55). Numerous studies (e.g., 56–59) in the general population have shown that low-grade elevation of circulating markers of inflammation (CRP, sialic acid, and proinflammatory cytokines) is associated with the future development of myocardial infarction, stroke, and peripheral vascular disease and with cardiovascular mortality. The inflammatory marker, serum sialic acid, is cross-sectionally related to coronary heart disease in type 2 diabetes (60) and also predicts future cardiovascular mortality in type 2 diabetes, independently of baseline atherosclerosis (61). Taken together with the evidence that inflammation also predicts type 2 diabetes independently of atherosclerosis (above), these studies suggest that activation of the innate immune system is likely to be at least one of the long-postulated (62) common antecedents of both atherosclerosis and type 2 diabetes (61) (Fig. 2).

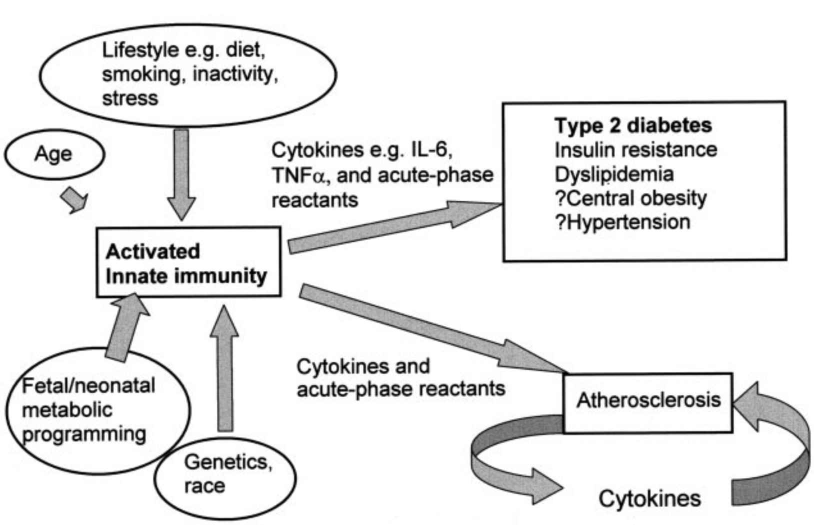

Figure 2—*Several factors such as altered nutrition, inactivity, age, fetal metabolic programming, and genetic propensity are known activators of the innate immune system. Cytokine production leads to insulin resistance (possibly impaired insulin secretion), type 2 diabetes, and other components of the metabolic syndrome, such as dyslipidemia. Activated innate immunity is a possible common antecedent of both type 2 diabetes and atherosclerosis.*

The acute-phase responses associated with type 2 diabetes thus offer plausible mechanisms that would explain why atherosclerosis is accelerated in type 2 diabetes, including mediation by acute-phase proteins themselves. For example, in addition to pro-coagulant acute-phase proteins such as fibrinogen and PAI-1, serum amyloid A displaces apolipoprotein A1 from HDL_3, redirecting HDL cholesterol from the liver to tissues, and increases binding to macrophages (23,29). CRP causes expression of endothelial adhesion molecules (63) and chemoattractants (64) and mediates LDL uptake by macrophages (65). Bound CRP activates complement, colocalizes with it in human hearts during acute myocardial infraction (66), and increases infarct size after experimental coronary artery ligation (67). Cytokines such as IL-6 and TNF-α have many pro-atherosclerotic actions, including promoting leukocyte recruitment to the endothelium by inducing adhesion molecule and chemoattractant synthesis and increasing capillary permeability (54). Such cytokines may be produced by the endothelium, smooth muscle cells, and macrophages at the site of atherosclerosis and contribute to a systemic acute-phase response, and/or cytokinemia and augmented acute-phase reactants inherent to type 2 diabetes may promote arterial disease.

4) Anti-inflammatory agents decrease the acute-phase response, may reduce the risk of developing type 2 diabetes, and improve control in established diabetes

Aspirin. High doses of salicylates such as aspirin have been known since the 19th century to lower glycosuria in diabetic patients (68), but only recently has the mechanism been shown as inhibition of NF-κB and its upstream activator, IκB kinase β, rather than via the classic cyclooxygenase targets of nonsteroidal anti-inflammatory drugs (69). Insulin resistance in genetically obese *fa/fa* rats and *ob/ob* mice is reversed by salicylates via an IκB kinase β–dependent mechanism (69). Two weeks' treatment of type 2 diabetic patients with high-dose aspirin causes a 25% reduction in fasting plasma glucose, a 50% reduction in triglyceride, and a 15% reduction in CRP concentration, independently of changes in plasma insulin concentration (70).

Statins. Assignment to pravastatin therapy in the West of Scotland Coronary Prevention Study resulted in a 30% reduction in the risk of developing type 2 diabetes (71), perhaps related to the drug's anti-inflammatory properties. Although the beneficial effects of statins (HMG-CoA reductase inhibitors) on cardiovascular disease have been generally attributed to cholesterol lowering, there is considerable in vitro and in vivo evidence that statins have a cholesterol-independent anti-inflammatory effect (72–74), for example, lowering CRP in post–myocardial infarction patients (independently of cholesterol levels) (75) and in subjects with type 2 diabetes (76). Statins can act through both HMG-CoA reductase–dependent mechanisms (inhibiting release of cytokines by upregulating peroxisome proliferator–activated receptor [PPAR]-α and -γ and inhibiting the NF-κB pathway) and HMG-CoA reductase–independent means (inhibiting the adhesion cascade by binding to the integrin lymphocyte function–associated antigen-1 and thus inhibiting leukocyte adhesion to intercellular adhesion molecule-1) (72).

However, the West of Scotland Coronary Prevention Study results should be interpreted with caution for several reasons: the study was not designed to examine the effects of this statin on diabetes development, it studied only men, and the multivariate hazard ratio for the prediction of diabetes by baseline pravastatin therapy was of only borderline significance (0.7 [0.50–0.99, 95% CI], P = 0.042). Also, any effect of pravastatin may include noninflammatory mechanisms such as reduction in the use of hyperglycemia-inducing cardiovascular drugs as the result of improved cardiovascular status or a secondary reduction in triglyceride and thus insulin resistance.

Glitazones. The recently introduced oral hypoglycemic agents thiazolidinediones ("glitazones") are PPAR-γ agonists that have been regarded as insulin-sensitizing through mechanisms such as altered transcription of insulin-sensitive genes controlling lipogenesis, adipocyte differentiation and fatty acid uptake, and GLUT4 expression. But glitazones are also anti-inflammatory (77), inhibiting cytokine production and macrophage activation (78–80) and reducing (to a varying extent depending on the study and the marker) circulating inflammatory markers such as CRP and white blood cell count in type 2 diabetic subjects (81–85). A failure to find a reduction of IL-6 accompanying the CRP reduction with glitazone treatment in some of these studies (81,84) is interesting and might indicate that statins alter the production of other cytokines involved in CRP synthesis (IL-1β and TNF-α) or inhibit the action of IL-6 at the liver or act through some other mechanism.

5) Gestational diabetes, a risk factor for type 2 diabetes, is associated with an inflammatory response

There is considerable evidence that nondiabetic pregnancy is a state of activated innate immunity, with increased acute-phase proteins and proinflammatory cytokines (86). First-trimester CRP levels are significantly higher in women who subsequently develop gestational diabetes later in their pregnancy than in women who remain euglycemic (87). Moreover, sialic acid, another inflammatory marker, is higher in women with previous gestational diabetes than in women without (44).

POSSIBLE MECHANISMS OF ACTIVATED INNATE IMMUNITY IN TYPE 2 DIABETES: CYTOKINES, FETAL PROGRAMMING, GENETICS, NUTRITION, INACTIVITY, STRESS, AND AGE

What are the factors that might cause activated innate immunity in type 2 diabetic patients or in patients destined to develop the disease?

Insulin resistance

We previously indicated how activated innate immunity may give rise to the features of type 2 diabetes, including cytokine-induced insulin resistance and impaired insulin secretion, increased capillary permeability and microalbuminuria, dyslipidemia, hypercortisolemia, hypertension, central obesity, and a hypercoagulant state (1,2). Mechanisms by which cytokines such as TNF-α can cause insulin resistance have been further clarified recently and include activation of the prototype stress-induced kinase, c-Jun NH_2-terminal kinase, which serine phosphorylates many signaling proteins including insulin receptor substrate (IRS)-1 and IRS-2, thereby inhibiting insulin signaling and stimulation of expression of SOCS [suppressor of cytokine signaling] proteins, which bind IRS-1 and -2 and mediate their degradation (88). Inflammatory cytokines such as TNF-α, IL-1β, and IL-6 also downregulate PPAR-γ expression (89).

It should be pointed out, however, that the exact effect of inflammatory cytokines on glucose metabolism in humans is still unclear. For example, Steensberg et al. (90) recently showed that acute (3-h) femoral arterial infusion of IL-6 in healthy men did not result in changes in glucose production or disposal or leg uptake. The presumably chronic elevated IL-6 levels in type 2 diabetes may or may not have different effects.

Interestingly, insulin is itself an inhibitor of acute-phase protein synthesis (91,92), and in animal models of diabetes, the acute-phase response is increased by insulin deficiency (93). This indicates that there could be a positive feedback in type 2 diabetes whereby cytokine-induced insulin resistance further augments the acute-phase response. The relatively normal levels of acute-phase reactants in type 1 diabetes (28) suggest that insulin replacement and the much lesser degree of hepatic insulin resistance in this type of diabetes is sufficient to restrain acute-phase protein production.

Fetal and neonatal programming

In the short-term, innate immunity has survival value and restores homeostasis after an environmental stress, but in type 2 diabetes and IGT, it may be that prolonged lifestyle or environmental stimulants cause maladaptation to the normal physiological responses to stress, causing disease instead of repair; a genetic or inborn propensity to a hyper-responsive innate immune system might exist in certain individuals (Fig. 2). This notion is supported by recent evidence that low birth weight or disproportionate size at birth is associated with elevated levels of acute-phase reactants such as cortisol and fibrinogen in adult life (94,95).

Genetics and race

Specific polymorphisms in the TNF-α gene promoter (96,97), TNF-α receptor gene (98), and IL-6 gene (99) are variously associated with insulin sensitivity or resistance. Nondiabetic subjects with a family history of type 2 diabetes have higher circulating CRP levels than age- and BMI-matched control subjects without a family history (100). The influence of race on the acute-phase response is not well studied, but serum sialic acid concentrations are higher in Asian type 2 diabetic subjects living in London but originating from the Indian subcontinent (who have a high frequency of type 2 diabetes) than in Caucasian type 2 diabetic subjects matched for age, sex, diabetes duration, and glycemic control (101).

Nutrition

Many dietary factors may contribute to activation of innate immunity in the genetically or metabolically programmed individual, including the effect of fat (102) and the n3:n6 fatty acid ratio (103) on cytokine production. Meal intake increases adipose tissue IL-6 production by some fivefold when measured by subcutaneous microperfusion (104), offering a mechanism by which repeated dietary excess might favor hypercytokinemia. Plasma CRP is reduced by dietary vitamin E supplementation, known to inhibit secretion of proinflammatory cytokines, probably independent of its antioxidant nature (105).

Although AGEs are best known as endogenous products of glycation of body proteins in diabetes, they are also present in food—the result of heat-generated reactions between sugars and proteins or lipids. Vlassara et al. (106) recently showed that administration of a high-AGE diet to diabetic subjects (type 1 and 2) caused plasma CRP and mononuclear cell TNF-α to increase, whereas a low-AGE diet caused CRP and TNF-α to decrease.

Age

The production of cytokines from monocytes and macrophages (107) and circulating acute-phase proteins (108) IL-6 and TNF-α (109) increase with age, as of course does the propensity to develop type 2 diabetes. Indeed, it has been argued that a major characteristic of aging is a global reduction in the capacity to cope with a variety of stressors and a concomitant increase in proinflammatory status (110).

Smoking and inactivity

Similarly, the risk factors for type 2 diabetes of smoking and lack of physical exercise are both associated with an increase in circulating acute-phase reactants (111–113).

Stress and multiple "hits"

There is a long history of largely inconclusive speculation about the relationship of psychological stress and the onset of type 2 diabetes (114,115). Thomas Willis (17th century) and Henry Maudsley (19th century) both believed that diabetes often follows nervous trauma or anxiety, and William Osler in his famous *Textbook of Medicine* actually comments that, of the two types of diabetes, it is the less severe obese (what we now call) type 2 diabetes that is associated with "mental strain" (116). To give some modern perspective to this notion, in the Hoorn Study of a city population in the Netherlands aged 50–74 years and without a history of diabetes, the number of stressful life events in the previous 5 years was positively related to the prevalence of newly detected diabetes (117).

There are many ways in which psychological stress might increase the likelihood of developing type 2 diabetes, for example, relating to central activation of the HPA axis and the LC-NE system with counterregulatory hormone release, and cytokine-induced insulin resistance (27). But two less obvious observations are of particular note. First, stress decreases splanchnic blood flow, increases intestinal permeability, and results in increased absorption of LPS from the gut (the greatest source of LPS). Elevated portal bloodstream LPS levels stimulate Kupffer cell receptors and cytokine release (27). Presumably, the absorption of other intestinal activators of innate immunity might also be augmented by stress, including AGEs present in food (see above).

Second, repeated stress with the repeated induction of corticosteroids can result in hippocampal damage, causing a failure in the downregulation of corticosteroid production by the feedback mechanism and thus persisting elevated circulating cortisol levels (118,119). This encourages the idea that resetting the control point of innate immunity at a higher level of activation might be caused by multiple stimuli over time—either a range of different stressors or repeated episodes of the same type.

PROBLEMS AND UNCERTAINTIES

A role also for adaptive immunity?

Lindsay et al. (120) reported that elevated serum total γ-globulin levels, a nonspecific measure of the adaptive immune system, predict the development of type 2 diabetes in Pima Indians. Cseh et al. (121) have also questioned whether both innate and adaptive immunity have a role in metabolic regulation and type 2 diabetes. It is unclear why γ-globulin is increased in type 2 diabetes, and further study in this area is needed. The observation may represent the interplay between innate and acquired immunity (see above); for example, a single injection of LPS in the mouse mobilizes up to 10% of the protein-encoded genome and some 60 (at least) genes involved in both innate and adaptive host defense (122).

The inflammatory response: primary or secondary? The role of hyperglycemia.

Does a cytokine-induced inflammatory response cause type 2 diabetes or is it just secondary to one or more biochemical and pathophysiological disturbances of the disease? A major uncertainty is whether hyperglycemia is a main determinant of the inflammation in type 2 diabetes—there is evidence for and against. Several cross-sectional studies of type 2 diabetes show that CRP and IL-6 are significantly correlated with blood glucose concentration or glycated hemoglobin percentage (45,47), although we found no relationship between serum sialic acid concentration and glycemia (60). Because the acute-phase response and cytokinemia are so closely related to insulin resistance, the relationship with hyperglycemia is not unexpected. Lowering of blood glucose levels in type 2 diabetic patients is accompanied by reduced levels of inflammation markers (46,123). In blood samples from nondiabetic subjects, high glucose levels stimulated IL-6 production from monocytes in vitro (124). AGEs are known to have a similar cytokine-stimulating effect on macrophages (125). And particularly cogent is the recent finding that acute hyperglycemia in nondiabetic and IGT subjects elevates plasma IL-6 and TNF-α concentrations, higher and longer in individuals with IGT and when the glucose was given as pulses (126). The effect was abolished by infusion of the antioxidant glutathione, suggesting that hyperglycemia-induced cytokine production is mediated by reactive oxygen species.

On the other hand, acute-phase markers are not elevated in type 1 diabetic subjects who have the same degree and duration of hyperglycemia as type 2 diabetic patients (28). In the large number of prospective studies mentioned above (3–14), the prediction of type 2 diabetes development in initially nondiabetic subjects by elevated inflammatory markers is generally independent of baseline glycemia. Thus, it seems that chronic hyperglycemia is not sufficient to induce inflammation, although it may contribute to it, and improving glycemic control may therefore reduce the inflammatory response.

The role of obesity and atherosclerosis

Obesity was strongly related to elevated circulating levels of inflammatory markers (mainly CRP) in several cross-sectional studies in the general population (32,34,37,39) and type 2 diabetes (47). Subcutaneous and intra-abdominal adipose tissue is a major source of TNF-α and IL-6 production (127–129). This raises the question of whether the acute-phase reaction of type 2 diabetes is mainly secondary to obesity.

The power of inflammatory markers to predict type 2 diabetes, although often markedly reduced, remains after adjustment for BMI (3,5–8,12,13), but the role of obesity in the activated innate immunity of diabetes needs more investigation.

In a recent study in which case and control subjects were matched by BMI and waist circumference, neither CRP nor IL-6 predicted the development of type 2 diabetes, although lowered levels of adiponectin did (130). These authors suggest, as an alternative hypothesis, that because inflammatory markers are associated with obesity, they only indirectly predict diabetes and act as surrogate markers of hypoadiponectinemia.

Atherosclerosis is another cosegregate of type 2 diabetes that is strongly associated with an acute-phase response in its own right (131). However, elevated inflammatory markers are also a feature of type 2 diabetic subjects without vascular complications (28) and, when studied, inflammatory markers were predictive of diabetes independently of baseline atherosclerosis (3). Present evidence supports the notion that atherosclerosis develops in parallel with type 2 diabetes (61), with both conditions sharing the common antecedent of activated innate immunity (Fig. 2), but like hyperglycemia and possibly some other manifestations of type 2 diabetes such obesity, macroangiopathy, once present, would presumably further enhance inflammation.

A particular puzzle is that type 1 diabetic patients without tissue complications do not have elevated acute-phase reactants (28) but remain at risk of accelerated atherosclerosis in the same way as type 2 diabetic patients. If the above model is correct, at least in part, one may speculate that specifically diabetes-related factors (possibly glucose) would need to additionally sensitize the arteries to cytokines and other atherogenic factors such as hypercholesterolemia, but little is known about this.

Glucose intolerance in other inflammatory diseases

One would predict that chronic inflammatory illnesses would be associated with either type 2 diabetes or the metabolic syndrome, unless the trigger is not constant hypercytokinemia but repeated bursts. There is too little information on glucose tolerance in chronic disease, but supportive research includes the association of rheumatoid arthritis with features of the metabolic syndrome, including an increased frequency of cardiovascular disease and type 2 diabetes not related to glucocorticoid use (132).

IMPLICATIONS AND FUTURE RESEARCH

— We need to know the temporal relationship of changes in circulating proinflammatory cytokines, acute-phase markers, insulin resistance, and glycemia during the development of IGT and type 2 diabetes. An interesting example might be a prospective study of children, many of whom are now developing type 2 diabetes in association with obesity. Also, there is still little information on inflammatory markers in ethnic groups at high risk of developing type 2 diabetes. The power of elevated acute-phase markers and IL-6 to predict type 2 diabetes development raises the question of whether these would be helpful in screening programs identifying individuals at risk of diabetes. And if type 2 diabetes is an inflammatory disease, can anti-inflammatory drugs, such as those targeted at the NF-κB signaling pathway, contribute to the management of the disease?

The realization that type 2 diabetes is a proinflammatory cytokine-associated disease leads us to question what other manifestations of type 2 diabetes are cytokine-induced and should join the usual features of the metabolic syndrome. For example, depression is common in type 2 diabetes (133), and many of the behavioral changes seen in depression are stimulated by IL-6 and TNF-α (134). Similarly, fatigue and alterations in sleep patterns, which are symptoms well known in the diabetic clinic, are linked with insulin resistance and elevated blood IL-6 and TNF-α (independently of obesity) (1,135,136). What else in type 2 diabetes is related to innate immunity?

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