Perspectives in Diabetes

The Heterogeneity of Diabetes

Unraveling a Dispute: Is Systemic Inflammation Related to Islet Autoimmunity?

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Diabetes is an emblematic example of a heterogeneous disease. Systemic inflammation has emerged as a prominent factor in the type 2 diabetes pathoetiology, but it remains ill-defined in type 1 diabetes. There is a wide spectrum of associations between inflammatory responses and diabetic syndromes. At one end of this spectrum, there is type 1 diabetes for which there is convincing evidence that chronic inflammation of pancreatic islets is a central aspect of disease pathogenesis. At the opposite end, is type 2 diabetes that is clearly associated with systemic inflammation, which could be either the cause or simply mark the underlying pathology. Accumulating evidence has substantiated that a subgroup of adult patients clinically diagnosed with type 2 diabetes exhibit autoantibody responses to islet autoantigens. The presence of these immunologic abnormalities is associated with a severe insulin secretory defect and the absence of signs of systemic inflammation as documented by plasma C-reactive protein and fibrinogen levels that are comparable with those of control populations. Islet autoantibody evaluation should be part of the diagnostic assessment for clinically diagnosed type 2 diabetes not only because it might predict the rate of progression to insulin requirement in adult populations but also to identify a pathogenically distinct disease phenotype characterized by the absence of systemic inflammation and its related disorders. A more appropriate characterization of this subgroup of clinically diagnosed type 2 diabetes, diabetes of autoimmune pathogenesis, will promote future research into the etiology, natural history, and treatment. *Diabetes* **56:1189–1197, 2007**

Diabetes is a heterogeneous dysregulation of carbohydrate metabolism, and there is no doubt that we are in the midst of an epidemic of this disease. The pathogenic equation for diabetes mellitus presents a complex interrelation of metabolic, genetic, and environmental factors as well as inflammatory mediators and, for most of which, is unclear if they reflect the disease processes or are simply measures of systemic or local responses to the disease. Since it is not entirely clear if systemic inflammation and the activation of the acute-phase responses are involved in the pathogenesis of type 1 diabetes as well as latent autoimmune diabetes of the adult (LADA), we will discuss its role in the pathoetiology of the latter two disorders and review background data on inflammation in type 1 and type 2 diabetes. We will pose the question as to whether or not markers of chronic inflammation are related to islet cell autoimmunity in older individuals clinically diagnosed with type 2 diabetes.

Type 1 diabetes, or insulin-dependent diabetes, is a chronic T-cell–mediated autoimmune disease characterized by selective destruction/dysfunction of pancreatic insulin-secreting cells. Type 1 and type 2 diabetes have distinct genetic bases. Although the association of specific *HLA* alleles and haplotypes with type 1 diabetes is very strong, this genetic locus is estimated to account for <50% of genetic contributions to disease susceptibility (1,2). Diabetogenic alleles are not fully penetrant, implying that not every individual who inherits the gene develops the disease.

Type 2 diabetes is another heterogeneous disease, and it is the most common form of diabetes, accounting for ~90% of cases and in many industrialized countries affecting 10–20% of individuals over the age of 45 years (3). In type 2 diabetes, both relative loss of β-cell mass and intrinsic β-cell secretory defects, including selective loss of sensitivity to glucose, may explain the failure of pancreatic β-cells to meet the increased secretory demand caused by insulin resistance. While there is a wealth of data indicating that systemic inflammation plays a role in the chain of events leading to type 2 diabetes, atherosclerosis, obesity, and fatty liver disease (4,5), it is not clear whether systemic inflammation and acute-phase proteins emerge as important elements of type 1 diabetes pathoetiology.

INFLAMMATION

Inflammation is a complex response that includes accumulation and activation of leukocytes and plasma proteins in and around the site of cell injury. The principal signs of inflammation consist of tumor, calor, rubor, and dolor (swelling, heat, redness, and pain) and are caused by vasodilatation, increased capillary permeability, local accumulation of leukocytes, and interstitial fluid and abnormal stimulation of nerve endings. Inflammation represents a protective response to control infections and promotes tissue repair, but it can also contribute to local tissue damage in a broad spectrum of inflammatory disorders.

The vast majority of investigations have demonstrated associations between systemic inflammation, also termed low-grade inflammation, and cardiovascular disease (CVD) (4–6). Other studies have also shown relationships between systemic inflammation and diabetes, insulin resistance, osteoarthritis, osteoporosis, Alzheimer's disease, muscle wasting, cancer, and rheumatoid arthritis and aging (7–10). The classic paradigm of inflammation reveals that traditional inflammatory processes, such as those involved in immunity, complement activation, and coagulation, are interrelated (11). Because of this interrelationship, it has been difficult to establish whether inflammatory mediators directly cause disease or whether they are merely markers of the underlying disease.

Inflammatory responses are associated with variations of a broad array of plasma proteins and proinflammatory cytokines. The acute-phase response is a systemic reaction in which a number of changes in plasma protein concentrations, termed acute-phase proteins, may increase or decrease in response to inflammation. Acute-phase proteins are defined as "positive acute-phase proteins" if their plasma concentration increases or "negative acute-phase proteins" if their plasma concentration decreases by at least 25% as a result of inflammation. The magnitude of the plasma increases varies from ~50% (i.e., ceruloplasmin) to as much as 1,000-fold in the case of C-reactive protein (CRP) and serum amyloid A, which is the plasma precursor of amyloid A (8). Both CRP and serum amyloid A are the most significant acute-phase proteins in humans. Other positive acute-phase proteins include fibrinogen, plasminogen, plasminogen-activator inhibitor 1 (PAI-1), α_1-acid glycoprotein, and proteins of the complement system. Negative acute-phase proteins consist of albumin, tranferrin, ferritin, and insulin-like growth factor 1.

Modifications in acute-phase protein plasma concentrations are largely dependent on their biosynthesis in the liver, and changes in their production are influenced by the effect on the hepatocytes by proinflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α). These cytokines are produced during the inflammatory process and are principal stimulators of acute-phase proteins. Acute-phase proteins and other markers of chronic inflammation can be readily detected in CVD, type 2 diabetes, and other chronic illnesses (12).

TYPE 1 DIABETES: A CHRONIC INFLAMMATORY DISEASE OF THE ISLETS

Type 1 diabetes is a T-cell–mediated autoimmune disease in which autoreactive cytotoxic T-cells recognize a number of antigenic determinants expressed in pancreatic β-cells. Similarly to other autoimmune disorders, in type 1 diabetes many components of the inflammatory responses, including CD4$^+$ and CD8$^+$ T-cells, macrophages,

dendritic cells, natural killer (NK) cells, cytokines, free oxygen, nitric oxide radicals, etc., contribute to β-cell destruction. The observation that in newly diagnosed diabetic patients, islet-infiltrating CD8$^+$ T-cells represent the prevalent cell type of insulitis (13,14) suggests that major histocompatibility complex (MHC) class I–restricted T-cells are likely to be as crucial to the development of autoimmune diabetes in human as they are in the NOD mouse. The autoantigens targeted by autoreactive CD8$^+$ T-cells in NOD mice appear to be insulin (15) and the islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRIP) (16).

An intriguing human investigation provided evidence that peptide 10–18 of the insulin B chain is associated with recurrence of autoimmunity and loss of β-cell function in islet grafted type 1 diabetic recipients (17). Other investigations indicated that islet destruction is caused by proinflammatory autoreactive T-cells, while the tolerant, nondiabetic state is characterized by autoreactive T-cells that secrete the immune suppressive cytokine, IL-10 (18).

The nervous system may also be implicated in initiating the chain of events leading to local inflammation and ultimately autoimmune diabetes. The TRPV1$^+$ pancreatic sensory neurons in NOD mice appear to function as a controller of islet T-cell infiltration and β-cell stress despite systemic presence of pathogenic T-cell populations (19).

It should be noted that a number of Japanese patients with clinically diagnosed idiopathic type 1 diabetes develop what is believed to be a nonautoimmune, abrupt-onset insulin-dependent diabetes characterized by the absence of insulitis and islet autoantibodies and the presence of high serum lipase concentrations (20). However, high serum pancreatic enzyme levels cannot rule out the presence of autoimmune type 1 diabetes, as it has been shown that 10% of newly diagnosed type 1 diabetic patients in Belgium and a Japanese patient diagnosed with fulminant-onset type 1 diabetes had elevated pancreatic exocrine enzyme concentrations along with clear signs of autoimmune abnormalities (i.e., GAD autoantibodies, etc.) (21,22).

Islet cell mass seems to play a role in the initiation of, but not progression to, auoimmune diabetes. As a matter of fact, in NOD mice using a pancreatectomy/islet transplantation model, Kimura et al. (23) have reported that the triggering of the diabetogenic autoimmune process needs to take place during a specific window of time (preinsulitis age 7 weeks) and that the presence of a large islet mass is not required for the progression to clinical diabetes. Although these are provocative findings, there is a need to have access to pancreatic tissue and pancreatic lymph nodes from subjects at risk of developing type 1 diabetes to uncover the role for islet cell mass in the development of islet autoimmunity during the natural history of the human disease (24).

Adaptive and innate immunity. Adaptive immunity is a type of immunity that develops as a response to infections and adapts to the infections. The components of adaptive immunity are T- and B-cells and their own cell receptors. These receptors are somatically generated during cell development to provide each cell with a structurally unique receptor. The adaptive immune system depends on the ability to assemble rearranged genes for both the TCR and the immunoglobulin gene. This ability results from two genes known as *RAG-1* and *RAG-2*, and their gene products encode a recombinase that is involved in somatic recombination. The adaptive immune system allows T- and B-cells to generate an enormously diverse response to

different pathogens. Both the naïve T- and B-cell receptor repertoire are generated by interaction with self-ligands, such as the MHC, which in turn can signal to T- and B-cells to mature and survive. T-cells that are selected and sustained on self-ligands are termed autoreactive T-cells.

A common characteristic of many autoimmune diseases, such as type 1 diabetes, is that both T-cell and autoantibody responses are directed against multiple self-antigens (25). As autoimmunity in type 1 diabetes progresses from initial activation to a chronic state, there is often an increase in the number of islet autoantigens targeted by T-cells and autoantibodies. This condition is termed "epitope spreading." This is a cascade process in that T-cells activate additional autoreactive B-cells, and B-cells present additional epitopes from different proteins, until there is autoreactivity to numerous autoantigens. In a similar fashion, multiple novel peptides within the same molecule can activate T-cells. A number of autoantibodies to islet antigens were molecularly characterized. These antigens include insulin, GAD65, ICA512/IA-2, and I-A2β (phogrin) (25). There is convincing evidence that multiple islet autoantibodies predict future development of insulin-requiring diabetes in individuals at risk of progressing to overt disease (26). Autoantibodies directed against islet targets serve as key markers to identify and enroll newly diagnosed type 1 diabetic patients and their family members in intervention trials. Recently, we provided evidence suggesting that a subset of cytoplasmic islet cell antibodies (ICAs) is related to a more rapid progression to insulin-requiring diabetes in GAD65- and IA-2 antibody–positive relatives as compared with relatives with GAD65 and IA-2 antibodies without ICA. This ICA reactivity more than likely is caused by a subset of ICA-recognizing unidentified islet autoantigen(s) (27). Although a pathogenic role of these islet autoantibodies has not been demonstrated, recent observations showed that injection of mice with IgG purified from type 1 diabetic patients induced bladder antibody–mediated dysfunction, which mimicked the effect of L-type voltage-gated calcium channel (VGCC) agonists (28). Further studies should establish clinical associations of these autoantibodies in large patient cohorts with well-characterized autonomic neuropathy.

Innate immunity is the phylogenetically oldest first line of defense against infections and physical or chemical injury. The innate immune system consists of circulating cells, epithelial barriers, and a number of proteins that all have the main scope to eliminate microbial elements. The main effector cells of innate immunity are neutrophils and mononuclear and natural killer (NK) cells. Each of these cell types plays a separate role against microbes. Innate immunity can also stimulate adaptive immune responses to effectively react against different types of microbes.

Some of the cell types that make up the innate immune system, such as macrophages (MΦ) and NK cells, secrete cytokines that activate phagocytes and stimulate the cellular reaction of innate immunity, termed "inflammation." The local protective responses to tissue microbial elements, which consist of recruitment of leukocytes and extravasation of several plasma proteins into a site of infection, represent the main components of inflammation.

Recent observations suggest that innate immunity might play a role in the development of islet autoimmunity (29). It has been postulated that toll-like receptors (TLRs), which are classically involved in a variety of microbial-derived molecules stimulating innate immune responses,

have also the potential to recognize self-antigens and trigger autoimmune diseases, such as type 1 diabetes, systemic lupus erythematosus, rheumatoid arthritis, and autoimmune heart failure (30).

The presence of an elevated number of dendritic cells (DCs) and MΦ within the islet cell infiltrate from both humans and nonobese diabetic (NOD) mice advocates a role of these cells in the diabetogenic process. Dysregulation of nuclear factor (NF)-κB enhances the antigen-presenting cell (APC) function of NOD DCs, which in turn would be expected to promote autoimmune responses (31). In humans, high-level monocyte prostaglandin synthase 2 expression might contribute to the defect in type 1 diabetes APC activation of T-cells through its negative effect on IL-2 signaling (32).

There is compelling evidence that autoimmune diabetes can be suppressed by draining pancreatic lymph node (PLN) DCs (33). In the context of autoimmune diseases, it has been proposed that DCs can induce either anergy or the development of regulatory T-cells (Treg cells) (34). Recent evidence suggests that in type 1 diabetes, there is a relative excess of islet β-cell–specific autoreactive T-cells and a deficiency of Treg cells (35). If Treg cells can routinely be expanded in vitro and in vivo, these cells could be harnessed to treat type 1 diabetes or facilitate tolerance of transplanted pancreatic islets (36).

NK cells are involved in killing target cells and interacting with APCs and T-cells. It has recently been described that there is a reduced activation of NK cells in longstanding type 1 diabetic patients (37). It is not clear if this anomaly is a consequence rather than a cause of disease as prolonged hyperglycemia could also explain this phenomenon. Candidate targets for NK cell recognition in the pancreatic β-cells are ligands for the NKG2D receptor expressed by NK cells and CD8$^+$ T-cells. It has been shown that NKG2D is involved in the development of autoimmune diabetes in NOD mice (38). Therapeutic strategies aimed at blocking NKG2D interactions with its ligands or NKG2D signaling might be promising therapeutic alternatives for type 1 diabetes by preventing the expansion and function of autoreactive CD8 T-cells.

The importance of NK T-cells (NKT cells) as regulators of autoimmunity has been suggested from studies conducted in NOD mice and SJL mice (lupus-prone mice). In both models, a reduced number and function of NKT cells has been demonstrated (39). In the NOD mouse, NKT cells harbor a defect in the potentially protective cell population (40). Unlike other T-cells, NKT cells recognize glycolipid antigens presented by the non-MHC CD1d molecule and express T-cell receptors (TCRs) that use an invariant Vα14-Jα1 chain and a limited number of TCRβ chains (Vβ2, Vβ7, and Vβ8). Autoimmune diabetes can be prevented by a number of strategies aimed at activating NKT cell function, such as upon treatment with α-galactosylceramyde (41). In humans, type 1 diabetes seems to be associated with a reduced frequency of Vα24JαQ$^+$ circulating T-cells and the defect of their capacity to secrete IL-4. Vα24JαQ$^+$ T-cells might be functionally related to the resistance to the progression of an autoimmune disorder such as type 1 diabetes (42).

Tumor necrosis factor-α and IL-1β. Tumor necrosis factor (TNF)-α is the primary mediator of the acute inflammatory response to gram-negative bacteria and other pathogens and can cause many of the systemic complications of severe infections. The major cellular source of TNF-α is

activated mononuclear phagocytes and antigen-stimulated T-cells; NK cells and mast cells can secrete TNF-α.

TNF-α may be involved in the development of insulin resistance by inhibiting the tyrosine kinase activity of the insulin receptor (43). As we discussed above, IL-1β induces nitric oxide (NO) production in pancreatic β-cells, and it may also promote an impairment of insulin secretion in β-cells (44).

In humans, it has been reported that TNF-α levels positively correlate with levels of insulin and BMI, suggesting that production or regulation of TNF-α may be involved in the pathogenesis of type 2 diabetes and insulin resistance observed in obesity (45). TNF-α may also impair insulin secretion in pancreatic islet cells (46) and may stimulate IL-6 production, which leads to β-cell destruction (47). TNF-α may also signal through two receptors, TNFαSR1 and TNFSR2, although the exact role of each has not yet been clarified, and it appears to interfere with the autophosphorylation of the insulin receptor and inhibits insulin signaling via pathways involving TNFαSR2 (43).

Several studies have suggested that pro-inflammatory cytokines, such as IL-1β, γ-interferon (IFN-γ), and free radicals, are mediators of pancreatic β-cell death in both human and rodent islet cells (48). There is compelling evidence that cytokines influence the expression of inducible NO synthase (iNOS), thereby leading to NO production. It has been reported that IL-1β + IFN-γ, via NO synthesis, markedly decreased SERCA2b protein expression, depleted Ca^{2+} stores, and activated the endoplasmic reticulum (ER) stress pathway, which is a potential contributing mechanism to β-cell death (49). Furthermore, cytokine-induced (IL-1β + IFN-γ) apoptosis of INS-1 cells appears to be dependent on NO production as demonstrated by the use of the NOD blocker N^G-methyl-L-arginine (50). In this study, Størling et al. (50) provided evidence that NO contributes to cytokine-induced apoptosis through potentiation of JNK activity and suppression of Akt. Although there is still discussion as to whether oxidative stress plays a key role in the pathogenesis of type 1 diabetes (51), a reduced antioxidant capacity has been demonstrated in type 1 diabetic patients as compared with healthy control subjects (52).

SYSTEMIC INFLAMMATION IN TYPE 1 DIABETES

An association between type 1 diabetes and the presence of islet autoantibodies and elevated levels of C-reactive protein (CRP) has been previously reported. This observation led to the conclusion that elevated CRP levels may provide an additional marker for risk progression to type 1 diabetes (53). These findings were not subsequently confirmed by others (54). In a much older population of diabetic patients from the Cardiovascular Health Study (CHS), we found that CRP as well as fibrinogen levels were not significantly higher in GAD65 autoantibody positive as compared with GAD65 negative counterparts (Figs. 1 and 2). The diagnosis of diabetes was based on both the American Diabetes Association (ADA) and 1985 World Health Organization (WHO) diabetes classification criteria. The CHS is a population-based longitudinal study of risk factors and subclinical disease related to the incidence and natural history of cardiovascular disease (CVD) in noninstitutionalized adults 65 years and older (55). The study population for the GAD65 antibody titer group analysis consisted of all CHS participants who were diagnosed with diabetes at
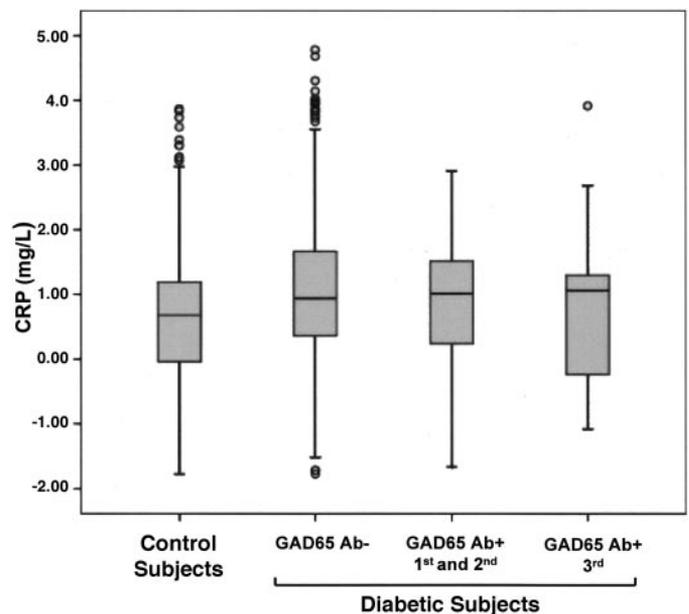

FIG. 1. CRP levels in relation to nondiabetic control subjects, GAD65 antibody–negative patients, and GAD65 antibody–positive diabetic patients. For the titer subgroup analyses, diabetic participants were divided into three subgroups that are represented as tertiles of GAD65 antibody–positive titers, from the lowest (first) to the highest (third) tertile. Only GAD65 antibody–positive samples were considered for determining tertiles. No statistically significant differences in CRP levels were found in these groups. (n = 399 control subjects; 1,284 GAD65 antibody–negative subjects; 33 GAD65 antibody–positive subjects, first and second tertiles; and 17 GAD65 antibody–positive subjects, third tertile). In both GAD65 antibody–negative and GAD65 antibody–positive adult diabetic patients, plasma CRP levels are comparable with those of a control population.

the baseline visit. CRP and fibrinogen levels (Figs. 1 and 2) did not differ significantly across GAD65 antibody titer groups.

Gathering evidence suggests that insulin resistance can herald the onset of both type 1 diabetes and type 2 diabetes (19,56–60) and that compensatory changes in β-cell mass and function in response to the insulin resistance state occur to maintain glucose homeostasis (59,61,62). Perhaps the most compelling demonstration of the effect of insulin resistance driving increased β-cell mass derives from experiments in mice heterozygous for null deletions of both the insulin receptor and insulin receptor substrate-1 (IRS-1) (63). These mice are extremely insulin resistant and ultimately progress to overt diabetes. In all the double-heterozygous mice, there was up to a 30-fold increase in β-cell mass compared with wild-type littermates. These initial studies have been strengthened by recent findings showing that mice haploinsufficient for β-cell glucokinase (Gck) were unable to increase their β-cell mass in response to insulin resistance (64). The latter study strongly advocates that insulin receptor substrate 2 (Irs2) has a substantial effect on β-cell mass and survival.

With regard to the potential relationship between islet cell autoimmunity and inflammatory markers, we believe that, for the most part, they follow two separate avenues and their pathogenic mechanisms leading to disease are diverse (Fig. 3). Insulin resistance can precede the clinical onset of both type 1 diabetes and type 2 diabetes (19,56–60), and in the initial stages of these two diseases, nondiabetic subjects adapt to insulin resistance by increasing β-cell mass and function in an effort to maintain euglyce-
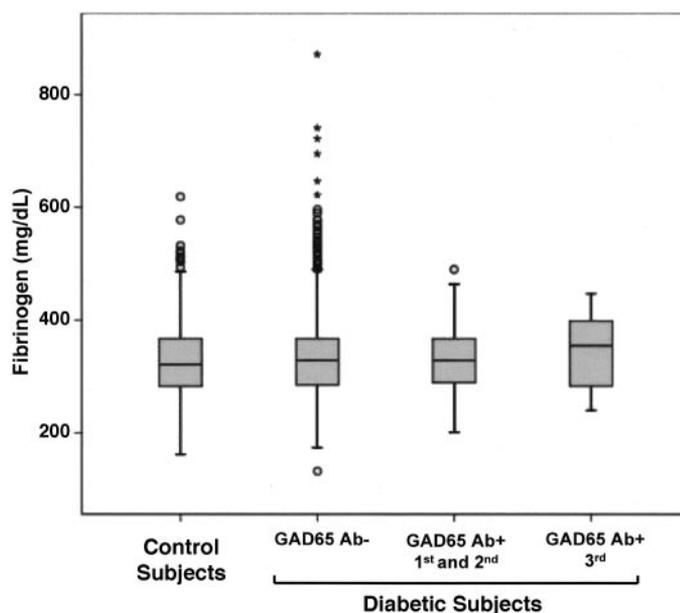
FIG. 2. Fibrinogen levels in relation to nondiabetic control subjects, GAD65 antibody–negative diabetic patients, and GAD65 antibody–positive diabetic patients. Diabetic participants were divided into three subgroups represented as tertiles of GAD65 antibody–positive titers from the lowest (first) to the highest (third) tertile. Only GAD65 antibody–positive samples were considered for determining tertiles. No statistically significant increase in fibrinogen levels was found in these groups ($n = 399$ control subjects; 1,281 GAD65 antibody–negative patients; 33 GAD65 antibody–positive patients, first and second tertiles; 17 GAD65 antibody–positive patients, third tertile). In both GAD65 antibody–negative and GAD65 antibody–positive adult diabetic patients, plasma fibrinogen levels are comparable with those of a control population.

mic values (59,61–64). In type 1 diabetes, there is convincing evidence that chronic inflammation of pancreatic islets leads the chain of events culminating in β-cell death. Conversely, the activation of the acute-phase response (i.e., elevated CRP levels) and systemic inflammation play a fundamental role for the pathoetiology of type 2 diabetes and metabolic syndrome (59,61–64).

SYSTEMIC INFLAMMATION IN LADA

The definition of LADA pertains to adults who have a slowly progressive form of autoimmune or type 1 diabetes that can be treated initially without insulin injections (65,66). This definition was intended to distinguish LADA from adult-onset type 1 diabetes, whereby insulin is necessary at disease onset, and from type 2 diabetes, whereby insulin therapy may or may not be required for a number of years after diagnosis (65,67,68). The term "LADA " was coined because without testing for islet-related autoantibodies, it would not be possible to diagnose autoimmune diabetes.

It has been estimated that in the U.K. Prospective Diabetes Study (UKPDS), ~10% of adults clinically diagnosed with type 2 diabetes exhibited evidence for islet autoimmunity documented by the presence of circulating GAD and/or ICAs (69). Many other studies have substantiated the identity of LADA. Nonetheless, the diagnostic criteria, natural history, genetics, pathophysiology, and inflammatory responses in LADA remain unresolved.

In clinically diagnosed type 2 diabetic patients, positivity for ICA and GAD65 autoantibodies correlates with some of the phenotypic features of type 1 diabetes, such as

younger age at diagnosis, lower BMI, and a relentless loss of β-cell mass (70). From the clinical standpoint, these patients should be classified as having LADA. In support of the role of islet cell autoimmunity in LADA, Shimada et al. (71) have reported their findings of a 65-year-old woman originally diagnosed as having type 2 diabetes with residual β-cell function. They found signs of insulitis, predominantly characterized by CD4$^+$ cells, but interestingly both NK cells and macrophages were also observed. GAD65 and IA-2 autoantibodies were detected in this patient; however, results on markers of activation of the acute-phase response (i.e., CRP) were not reported.

Thus far, there is no evidence that markers of systemic inflammation are linked with LADA, as the natural history of this condition is unknown. We recently found that among CHS participants aged ≥65 years with previously diagnosed diabetes at baseline ($n = 685$), the prevalence of GAD65 AA varied by current diabetes treatment (Fig. 4) (72). GAD65 autoantibodies were found in 2.3, 5.8, 7.8, and 8.3% of previously diagnosed diabetic participants ($n = 685$), respectively ($P = 0.02$, linear trend) (Fig. 4). We also found a remarkable stability of GAD65 antibodies in this population of diabetic persons aged ≥65 years.

A key observation in the UKPDS study has established that the presence of multiple islet autoantibodies to GAD65, the neuroendocrine molecule IA-2 and ICA, is associated with high risk of progression to insulin-requiring diabetes in newly diagnosed type 2 diabetic patients (69). A majority of patients with GAD antibodies at diagnosis developed insulin dependence within 6 years, which implies progressive autoimmune β-cell destruction. In individuals younger than 45 years of age, the rate of progression was remarkably similar to that of first-degree relatives of type 1 diabetic patients.

Despite a wealth of data on C-peptide assessment as primary outcome for intervention strategies designed to preserve β-cell function in type 1 diabetes, there are relatively a few studies addressing the issue of longitudinal changes of β-cell function in LADA. To the best of our knowledge, the majority of these analyses were performed in 15- to 35-year-old diabetic patients. Interestingly, in this age-group, a Swedish study on GAD65 autoantibodies and ICAs showed evidence of being independent predictors for the loss of measurable C-peptide (Å. Lernmark, personal communication). Although many cohorts of LADA patients have been identified, particularly in Europe, longitudinal changes of C-peptide measurements in these patients have yet to be thoroughly analyzed.

SYSTEMIC INFLAMMATION IN TYPE 2 DIABETES

The first indirect evidence that diabetes is associated with inflammation was reported in 1876 by Professor Ebstein, who observed that high-dose salicylates reduced glycosuria in diabetic patients (73). More than one century later, we learned that salicylate prevents the activation of NF-κB, which is a mediator of inflammation and apoptosis (74). Of note, a 2-week treatment of type 2 diabetic patients with high-dose aspirin seems to be sufficient to cause a 50% reduction in triglyceride and a 15% reduction in CRP levels, in addition to a 25% reduction of fasting plasma glucose levels, despite the absence of significant changes in plasma insulin levels (75). The anti-inflammatory effects of thiazolidindiones (TZDs), also known as insulin sensitizers that are peroxisome proliferator–activated receptor (PPAR)-γ agonists, appear to be secondary
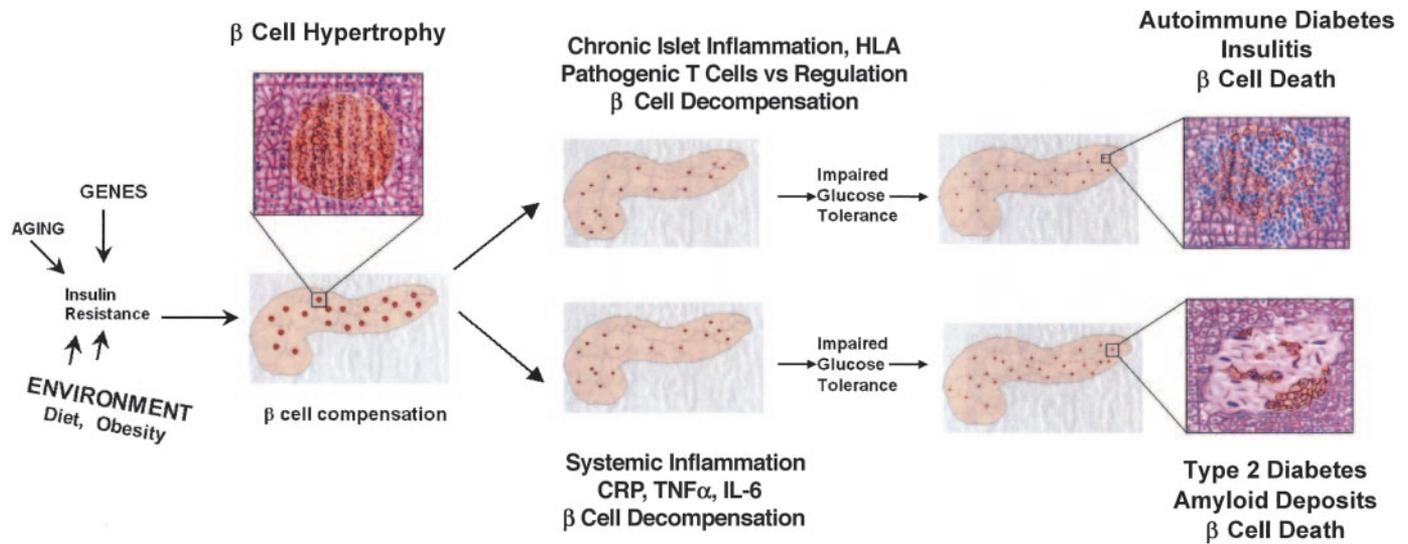
FIG. 3. Relationship between islet autoimmunity and systemic inflammation in adult patients with diabetes. Insulin resistance may precede the clinical onset of both type 1 and type 2 diabetes (19,56–60). In the initial stages of the natural history of these two diseases, nondiabetic subjects adapt to insulin resistance by increasing β-cell mass (β-cell hypertrophy) and function in an effort to maintain glycemic and metabolic homeostasis (59,61–64). Islet autoimmunity and systemic inflammation follow two separate avenues, and their pathogenic mechanisms leading to disease are diverse. In autoimmune diabetes, there is convincing evidence that chronic inflammation of pancreatic islets leads the chain of events culminating in β-cell death. Conversely, the activation of the acute-phase response and systemic inflammation play fundamental roles for the pathoetiology of type 2 diabetes and metabolic syndrome. Systemic inflammatory responses are associated with variations of a wide array of plasma proteins and proinflammatory cytokines (i.e., CRP, TNF-α, IL-6, etc.), whereas chronic islet inflammation is caused by an altered balance between pathogenic T-cells (that mediate disease) and regulatory T-cells (Tregs) that control autoimmunity.

to the inhibition of cytokine secretion and macrophage activation. It has been shown that TZD and perhaps also statins, which alter the secretion of a number of cytokines such as IL-1β and TNF-α involved in CRP synthesis, reduce inflammatory markers such as CRP and white blood cell count in type 2 diabetic patients.

At least three lines of evidence firmly indicate that systemic inflammation plays a role in the pathogenesis of insulin resistance and type 2 diabetes (8,9,12,76). Findings from numerous major epidemiologic studies indicate that the presence of different combinations of inflammatory markers, namely C-reactive protein, fibrinogen, IL-6, IL-1β, and plasminogen activator inhibitor (PAI)-1, are significant predictors of type 2 diabetes. In particular, these studies include CHS; the Atherosclerosis Risk in Communities Study; the Women's Health Study; the National Health and Examination Survey (NHANES); the U.S. Insulin Resistance and Atherosclerosis Study; the West of Scotland

Coronary Prevention Study; the Hoorn Study in the Netherlands; the Prospective Investigation into Cancer and Nutrition (EPIC)-Postdam Study and the MONICA Ausburg Study, both of which took place in Germany; and studies carried out in Pima Indians and in female Mexican populations.

The second line of evidence relates to the paradigm that chronic inflammation is involved in the pathogenesis of atherosclerosis, a common complication of type 2 diabetes and insulin resistance (4–7). A large number of investigations have convincingly shown that low-grade elevation of circulating markers of inflammation, such as CRP, sialic acid, and proinflammatory cytokines, is associated with cardiovascular mortality, coronary heart disease, peripheral vascular disease, and stroke.

The third line of evidence derives from the association of gestational diabetes, a risk factor for type 2 diabetes, and activated innate immunity with increased acute-phase proteins (77). Elevated CRP levels during the first trimester of pregnancy are more pronounced in women who develop gestational diabetes later in their pregnancy.

CONCLUSIONS

There is a wide spectrum of associations between inflammatory reactions and the various diabetic syndromes. At one end of the spectrum there is type 1 diabetes, for which there is convincing evidence that a chronic inflammation of the islets is an important feature of disease pathogenesis. At the opposite end is type 2 diabetes, which is clearly associated with systemic inflammation that could be either the cause or the consequence of some of the main features of the disease. Finally, somewhere between these two extremes, one finds LADA, which seems to share some features of both extremes, thereby raising the question of its pathogenesis.

Without testing for GAD65 autoantibodies, it would not be possible to identify a pathogenically distinct disease
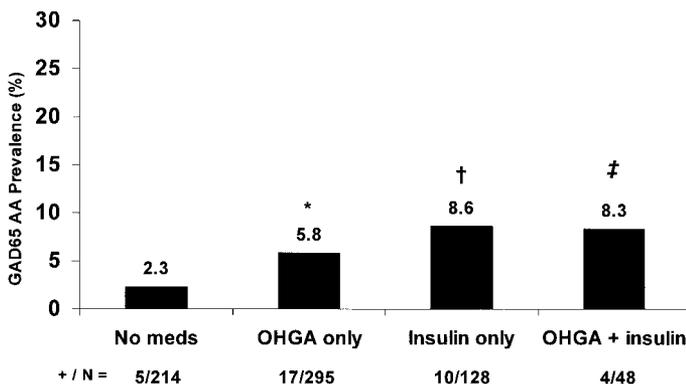

FIG. 4. Prevalence of GAD65 by diabetes treatment status among previously diagnosed diabetic participants at baseline, CHS ($n = 685$). OHGA, oral hypoglycemic agents. $P = 0.02$ for linear trend. *$P = 0.07$, †$P = 0.02$, ‡$P = 0.05$ for treatment group versus no diabetes medications group (No meds). Figure used with permission from *The Journal of Clinical Endocrinology & Metabolism* (72).

phenotype characterized by the absence of systemic inflammation and its related disorders, the presence of impaired insulin secretion, and the high tendency to be treated with insulin therapy (78). As a matter of fact, in these subjects, plasma CRP and fibrinogen levels are comparable to those of control populations (Figs. 1 and 2) (72,79). In this subset of older diabetic patients, the primary inflammatory injury seemingly resides in the pancreatic β-cells as for type 1 diabetes. We hypothesize that GAD65 antibody–positive diabetic persons are likely to benefit more from insulin therapy than obese, insulin-resistant, GAD antibody–negative, older diabetic individuals, especially early in their disease course.

The presence of GAD65 autantibodies in clinically diagnosed type 2 diabetic patients by conventional criteria (ADA or WHO) is not uncommon among older diabetic people, being 5–10% or higher, especially among those on insulin therapy. There are as many GAD65 antibody–positive older diabetic individuals as there are children affected by type 1 diabetes. This is not a trivial issue. Additional immunological markers of autoimmune diabetes might identify even a larger sample of clinically diagnosed type 2 diabetic patients. Given its relative simplicity, testing for GAD65 autoantibodies should be part of the diagnostic assessment for clinically diagnosed type 2 diabetes, as it might predict the rate of progression to insulin requirement in older populations. Those found to be GAD65 antibody positive are probably candidates for early insulin therapy or more aggressive oral therapies to lower their glycohemoglobin levels. Clinical trials and epidemiological observational studies should clearly separate GAD65 antibody–positive older diabetic patients from those who are GAD65 antibody negative.

Further research is clearly needed to focus on the etiology of GAD65 antibody–positive older diabetic patients clinically diagnosed as having type 2 diabetes. In particular, research should focus on how the etiology relates to genetics (i.e., *HLA*), other autoimmune disorders, other autoimmune markers, and the potential for environmental injury to the pancreatic islets. Clinical trials should evaluate what the best therapy is for GAD65 antibody–positive adult diabetic patients clinically diagnosed with type 2 diabetes by conventional clinical criteria alone.

ACKNOWLEDGMENTS

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