Is Latent Autoimmune Diabetes in Adults Distinct From Type 1 Diabetes or Just Type 1 Diabetes at an Older Age?

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Diabetes is classified clinically into two types: type 1 and type 2 diabetes. Type 1 diabetes is an autoimmune diabetes, whereas, in contrast, type 2 diabetes is nonautoimmune. However, there is a group of phenotypic adult type 2 diabetic patients (~10%) who have islet autoantibodies similar to type 1 diabetes. These patients are said to have latent autoimmune diabetes in adults (LADA) or type 1.5 diabetes. T-cells reacting with islet proteins have been demonstrated in type 1 and type 1.5 diabetic patients. In contrast, classic autoantibody-negative type 2 diabetic patients are also negative for T-cell responses to islet proteins. Therefore, we questioned whether type 1 and type 1.5 diabetes are similar or different autoimmune diseases. We have investigated the immunological and metabolic differences between type 1, type 1.5, and classic type 2 diabetic patients. We have identified autoantibody differences, differences in islet proteins recognized by T-cells, and differences in insulin resistance. We have also identified a small group of patients who have T-cells responsive to islet proteins but who are autoantibody negative. These patients appear to be similar to type 1.5 patients in having decreased stimulated C-peptide values. These immunological differences between type 1 and type 1.5 diabetes suggest at least partially distinct disease processes. *Diabetes* 54 (Suppl. 2):S62–S67, 2005

The finding nearly simultaneously by two groups in 1974 (1,2) that islet cell antibodies (ICAs) were common in the sera of patients with type 1 diabetes (type 1 diabetes) provided strong evidence that the β-cell lesion of type 1 diabetes was autoimmune in nature. Shortly thereafter, it was published that ~11% of patients with type 2 diabetes were also positive for ICAs and that this ICA^+ subset of type 2 diabetic patients tended to fail sulfonylurea therapy and needed insulin treatment earlier than ICA^- type 2 diabetic patients (3). Many other groups have also identified a subset of phenotypic type 2 diabetic patients who are positive for the antibodies commonly found in type 1 diabetes; this subset has been said to have type 1.5 diabetes, latent autoimmune diabetes in adults (LADA), slowly progressive IDDM, latent type 1 diabetes, youth-onset diabetes of maturity, latent-onset type 1 diabetes, and antibody-positive non-insulin-dependent diabetes (4–6) (Table 1). Although the different names have caused some confusion, the finding of this subset of phenotypic type 2 diabetic patients by many different investigators rather than just one or two groups confirms their existence as an important subset of phenotypic type 2 diabetic patients.

Probably the greatest area of confusion involves the distinction of LADA from type 1 diabetes occurring in individuals over the age of 30–35 years. Epidemiologic evidence suggests that type 1 diabetes peaks around puberty and again around age 40 years (7), and Nerup and colleagues (8) have suggested that the incidence of type 1 diabetes is approximately equivalent below and above age 20 years. We have discussed some of these nomenclature issues in an editorial (9), and most recently the Immunology of Diabetes Society proposed several criteria to try to standardize those patients referred to as having LADA. The Immunology of Diabetes Society proposed that patients be ≥30 years of age, positive for at least one of the four antibodies commonly found in type 1 diabetic patients (ICAs and autoantibodies to GAD65, IA-2, and insulin), and not treated with insulin within the first 6 months after diagnosis. The latter requirement, although admittedly subjective and likely to vary depending on the treating physician, is meant to distinguish LADA and type 1 diabetes occurring in patients >30 years of age (S. Fourlanos, F. Dotta, C. Greenbaum, J.P.P., O. Rolandsson, P.G. Colman, L.C. Harrison, unpublished data).

Another area of controversy involves the role of obesity and consequently the degree of insulin resistance in LADA. Kahn et al. (10) described the normal curvilinear relationship between insulin resistance and insulin secretion. Normal β-cells compensate for insulin resistance by secreting more insulin, and the product of insulin sensitivity and insulin secretion is normally a constant that Kahn (11) has termed the disposition index. The primary importance of this physiology is that insulin-resistant patients will present with hyperglycemia with a less severe degree of absolute insulin deficiency than insulin-sensitive patients. Because LADA patients span the spectrum from lean to obese, it is likely that differences in insulin sensitivity are an important variable in the physiology of LADA. We have become interested in this topic and will present some of our initial findings later in this article.

Whether the immunological damage to and destruction of the pancreatic β-cells involves the same mechanisms in all patients with autoimmune diabetes is unknown. That

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GADA, GAD antibody; ICA, islet cell antibody; LADA, latent autoimmune diabetes in adults; MICA, major histocompatibility complex class I chain–related gene A.

TABLE 1

Type 1.5 diabetes
Latent autoimmune diabetes of adults (LADA)
Antibody-positive type 2 diabetes
Latent-onset type 1 diabetes
Slowly progressive IDDM (SPIDDM)
Youth overt diabetes of maturity (YODM)
Progressive insulin-dependent diabetes mellitus (PIDDM)

question is addressed in the title of this article: "Is LADA different pathophysiologically from classic childhood type 1 diabetes or is LADA merely type 1 diabetes presenting at an older age?" This question is the focus of this review. This question also has important therapeutic implications. Because LADA is more common than classic childhood type 1 diabetes and because immunomodulatory therapies, such as anti-CD3, have been identified that are efficacious in modulating the type 1 diabetes disease process (12), it will be of great interest to see whether these treatments are similarly effective in LADA.

The genes predisposing to and protecting from classic type 1 diabetes have been extensively studied. Also, the islet autoantibodies in classic type 1 diabetes and as predictors of subsequent clinical diabetes in at-risk individuals have been very well characterized. Furthermore, because type 1 diabetes is largely a T-cell–mediated disease, several groups, including our own, have begun to describe the T-cell abnormalities characteristic of type 1 diabetes. Similarities or differences between classic type 1 diabetes and LADA in terms of genes, islet autoantibodies, and T-cells would provide important evidence to the question posed above: "Is LADA different pathophysiologically from type 1 diabetes or just type 1 diabetes presenting at an older age?" This article will review these data.

ANTIBODIES

The presence of islet autoantibodies in both LADA and classic type 1 diabetes provides strong evidence that the underlying disease processes are both autoimmune, but differences in antibodies in LADA and type 1 diabetes suggest potentially important immunological differences. All four islet autoantibodies—ICAs, GAD antibodies (GADAs), IA-2 antibodies, and insulin autoantibodies—are common in childhood type 1 diabetes, with many patients being positive for multiple autoantibodies. And, in nondiabetic relatives of patients with type 1 diabetes, risk of future type 1 diabetes is directly proportional to the number of autoantibodies positive (13–15). ICAs and GADAs are also common in LADA, but both IA-2 antibodies and insulin autoantibodies are much less common in LADA than in type 1 diabetes (5). As mentioned previously type 1 diabetic patients are very often positive for two or more autoantibodies, whereas single autoantibodies are common in LADA patients (Fig. 1).

In addition, the ICAs and GADAs found in LADA versus type 1 diabetes may differ. In one study comparing ICAs in LADA versus type 1 diabetes, it was found that over 90% of ICA^+ sera from type 1 diabetic patients was also positive for GADAs or IA-2 antibodies, whereas this was true in <20% of LADA patients. In this study, the investigators also found that GAD and IA-2 could block ICA staining in ~60% of type 1 diabetic patients' sera but in a much lower percentage of LADA patients'. This result suggests that antibodies to antigens other than GAD and IA-2 are more prevalent in LADA (16) and raises the intriguing possibility

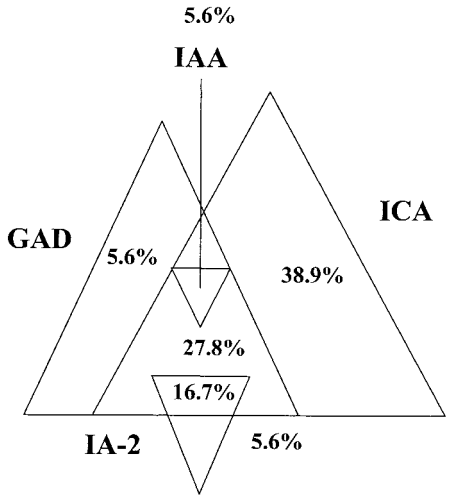

FIG. 1. Clustering of autoantibodies in autoantibody-positive patients. Numbers (%) refer to the percentage of the antibody-positive patients who were positive for the respective antibodies. Reproduced with permission from Juneja et al., *Metabolism* 50:10081–10013, 2001.

that some unidentified antigens are more commonly involved in LADA than type 1 diabetes.

In a relatively large study (n = 569, type 2 diabetes), GAD65/67 chimeric molecules were used to test for possible associations of GADA epitope specificity and clinical characteristics. Of the 11% of type 2 diabetic patients who were GADA positive, ~80% had antibodies directed to both middle and COOH-terminal epitopes. These patients had a lower BMI, lower basal C-peptide, and a higher frequency of insulin treatment than $GADA^-$ patients. The 20% of $GADA^+$ patients with antibodies directed solely at the mid-portion of GAD65 were indistinguishable from $GADA^-$ type 2 diabetic patients (17).

In collaboration with Chris Hampe and Åke Lernmark, we investigated whether there are differences in epitope specificity of GADAs in LADA versus type 1 diabetes. Using recombinant 35S-GAD65/67 fusion proteins, we found that sera from over 90% of type 1 diabetes bound to the middle or COOH-terminal portion of GAD65, whereas this was the case for only 65% of sera from LADA patients. In contrast, the NH_2-terminal portion of GAD65 was recognized by 20% of LADA patients compared with 5% of type 1 diabetic patients (18). More recently, we have found similar results using GAD65-specific recombinant Fabs. A total of 87 and 72% of type 1 diabetes sera were competed by two Fabs directed at the mid-portion of the GAD65 molecule compared with competition in 34 and 26% of LADA patients using the same Fabs (19) (Fig. 2).

Epitope differences between LADA and type 1 diabetes have also been confirmed in the Japanese equivalent of LADA, slowly progressive insulin-dependent diabetes. Using GAD65/67 chimeric molecules, Åke Lernmark in collaboration with Tetsuto Kobayashi and colleagues have identified unique NH_2-terminal linear epitopes in the GADAs of slowly progressive insulin-dependent diabetes that did not react with sera of adult-onset type 1 diabetes (20). Taken together, these observations demonstrate heterogeneity of GADA epitope specificity and suggest important differences between LADA and type 1 diabetes. GAD antibody IgG subclasses also appear to be different in type 1 diabetes versus LADA. The IgG4 subclass of GADA was more frequent in LADA than in type 1 diabetic patients.

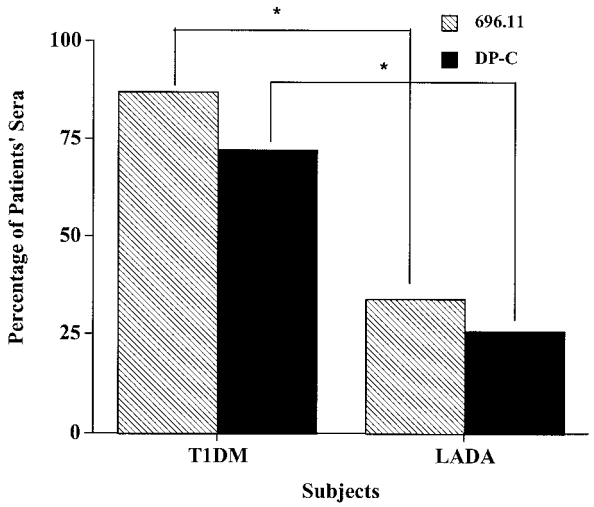

FIG. 2. Percent of type 1 diabetes (T1DM) and LADA patients' sera showing competition for GAD binding by Fabs directed against the mid-portion of GAD65, Fab DP-C, and Fab 696.11. *P = 0.001. Reproduced with permission from Padoa et al., *Diabetes* 52:2689–2695, 2003.

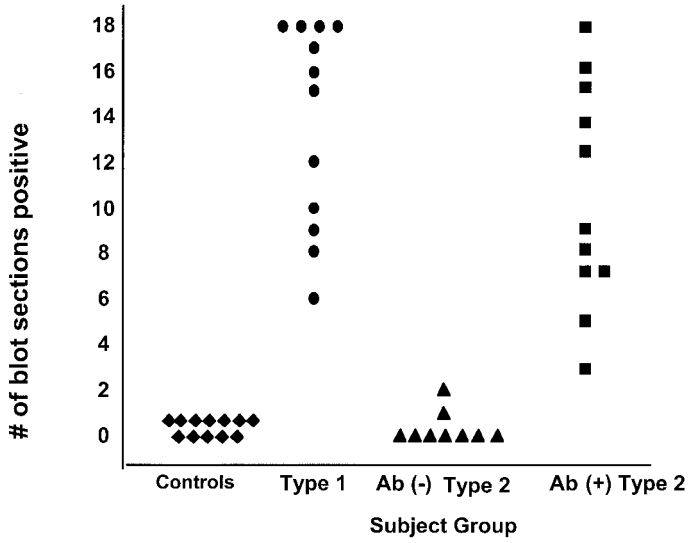

FIG. 4. Peripheral blood mononuclear cell responses of 12 normal control subjects, 12 type 1 diabetic patients, 9 autoantibody-negative (Ab−) type 2 diabetic patients, and 11 autoantibody positive (Ab+) type 2 diabetic patients. The number of molecular weight regions positive for each individual is shown. The different symbols represent individual subjects. A positive response is taken as SI >2.0. Reproduced with permission from Brooks-Worrell et al., *Diabetes* 48:983–988, 1999.

This would suggest a greater TH2 or regulated immune response in LADA (21) (Fig. 3).

T-CELLS

Because type 1 diabetes is a T-cell–mediated autoimmune disease, we have spent considerable time developing T-cell assays to measure reactivity to islet antigens in humans with type 1 diabetes. One assay called cellular immunoblotting uses proteins from human islets separated into 18 different molecular weight regions using SDS-PAGE. This assay demonstrated excellent sensitivity and specificity in a recent masked National Institutes of Health–Immune Tolerance Network Workshop (22). We have used this assay to describe the T-cell reactivity of recently diagnosed type 1 diabetic patients to multiple different molecular weight islet proteins (23), to describe the antigen spreading that occurs during the pre-diabetic (type 1 diabetes) period (24), and to compare T-cell responses of type 1 diabetes with LADA (25). T-cells from both type 1 diabetes and LADA commonly respond to four or more different molecular weight blot sections of islet proteins and in this regard are similarly distinguished from normal control subjects and antibody-negative type 2 diabetic patients who customarily respond to less than four blot sections (Fig. 4). But, there are differences in the

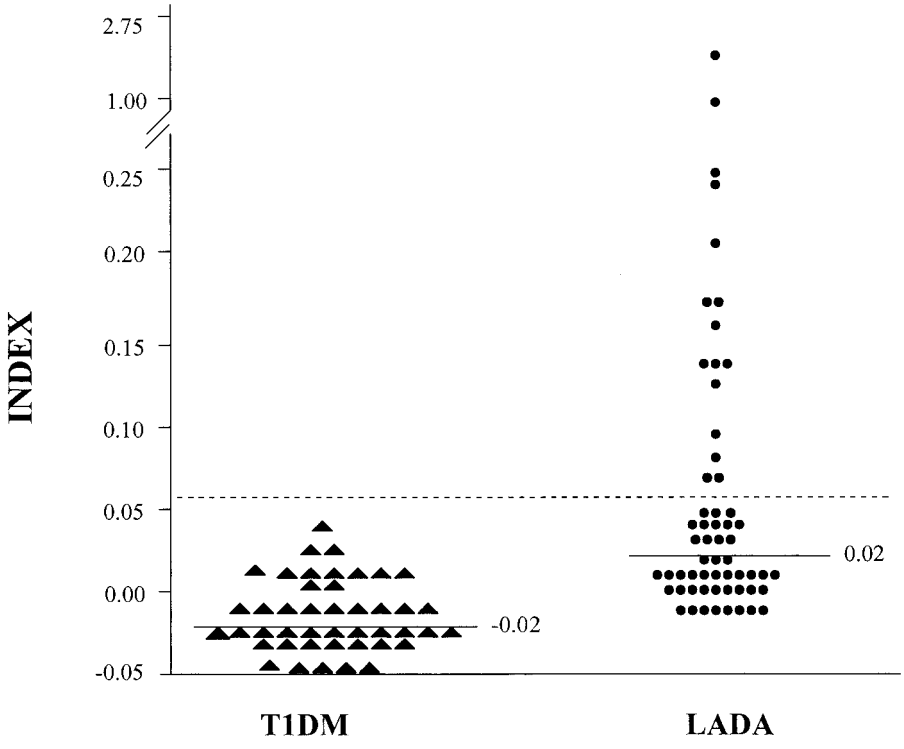

FIG. 3. GADA IgG_4 subclass distribution in patients with type 1 diabetes (T1DM; n = 45) and in patients with LADA (n = 60). Patients with an index below −0.05 are not shown in the figure. The cutoff level for negativity was fixed at 0.06 (represented by dotted line). The median index levels in each group are shown in the figure as −0.02 for type 1 diabetes and 0.02 for LADA. Reproduced with permission from Hillman M, *Diabetologia* 47:1984–1989, 2004.

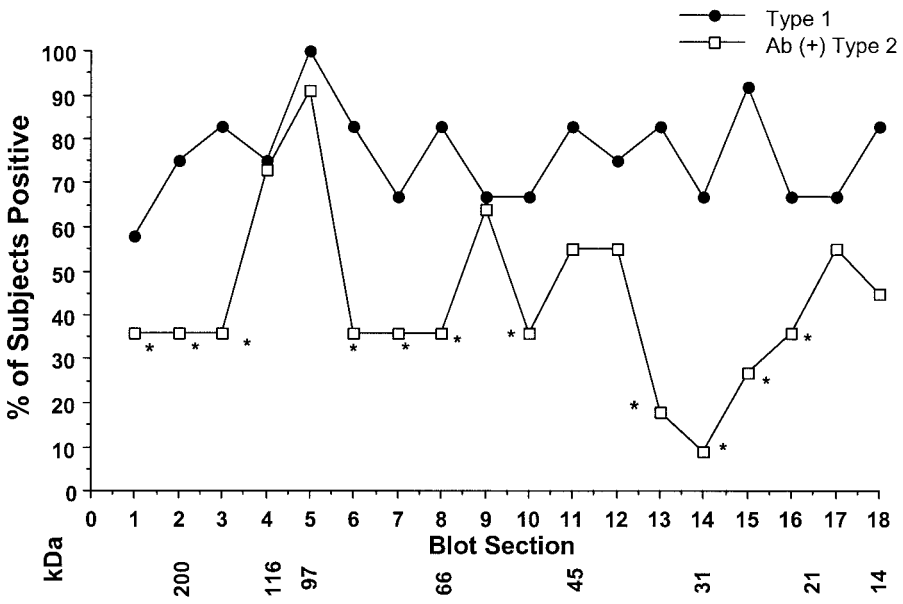

FIG. 5. Peripheral blood mononuclear cell responses of type 1 diabetic patients compared with antibody type 2 diabetic patients (LADA). The percentage of subjects responding to each molecular weight region is shown. A positive responses is taken as SI >2.0. Blot sections correspond to molecular mass regions >200 kDa (1) and <14 kDa (18). *P < 0.05, significant differences. Reproduced with permission from Brooks-Worrell et al., *Diabetes* 48:983–988, 1999.

specific blot sections stimulating T-cells from type 1 diabetes versus LADA. Some blot sections are stimulatory to a similar high percentage of type 1 diabetes and LADA patients, whereas other blot sections, especially in the lower–molecular weight regions, more commonly stimulate T-cells from type 1 diabetic than LADA patients (Fig. 5). These T-cell observations further support the role of autoimmunity in LADA but like the antibody data, suggest that there may be important antigenic differences between type 1 diabetes and LADA.

Because of our major interest in T-cells, we have been using the cellular immunoblotting assay on a relatively large number of phenotypic type 2 diabetic patients and have made a surprising discovery. We have identified some patients who are negative for GADAs, IA-2 antibodies, insulin autoantibodies, and ICAs but who have T-cell responses to islet antigens similar to type 1 diabetic patients. Similar to phenotypic type 2 diabetic patients who are antibody positive (LADA), these T-cell–positive but antibody-negative patients have a decreased stimulated C-peptide compared with antibody- and T-cell–negative phenotypic type 2 diabetic patients. We are currently investigating the prevalence of this interesting subset of autoimmune diabetes and will compare in these patients, versus antibody and T-cell–positive LADA and type 1 diabetes, other characteristics of the disease process, including cytokine responses to islet proteins and T-cell phenotypes.

INSULIN RESISTANCE

Insulin resistance is central to the pathophysiology of type 2 diabetes and is affected by many variables including age, BMI, ethnicity, physical activity, and medications. The contributions of insulin resistance to the pathophysiology of LADA is controversial and has been reported to be less than in type 2 diabetes and even comparable to type 1 diabetes (26,27). To address this issue, we compared insulin resistance in LADA, antibody-negative type 2 diabetes, and normal control subjects correcting for the effect of BMI. Insulin resistance was assessed by the homeostasis model assessment model. Glycemic control was comparable in the LADA and type 2 diabetic patients. As expected, BMI was positively correlated with insulin resistance overall. LADA and type 2 diabetic patients were significantly more insulin resistant than normal control subjects but did not differ from each other when corrected for BMI. Similar findings were obtained estimating insulin resistance using the QUICKI model. These observations suggest that both obesity and the diabetic state are important contributors to insulin resistance of type 2 diabetes and LADA. And, since many patients with LADA are obese, clinical outcome in LADA patients is determined by the interaction of insulin resistance (as in type 2 diabetes) and an autoimmune β-cell lesion (as in type 1 diabetes).

GENETICS

In type 1 diabetes, there is strong genetic control over both susceptibility to and protection from clinical disease. This is true for both animal models of type 1 diabetes, the NOD mouse and the BB rat, and for humans. The major histocompatibility complex region, HLA in humans, confers the greatest risk and protection, but other genes are also involved. Similarities and/or differences at the genetic level between type 1 diabetes and LADA would be of interest in trying to answer the question posed in this review: "Is LADA different pathophysiologically from type 1 diabetes?"

HLA. In type 1 diabetes, it is clearly established that HLA DR3, DR4 and DQβ1*0201 and 0302 confer risk of type 1 diabetes. In fact, it is estimated that ~50% of the risk of type 1 diabetes can be attributed to HLA. Furthermore, other HLA alleles including DR2 and DQβ1*0301 and 0602 confer protection against type 1 diabetes. Many groups have measured HLA in LADA patients, but unfortunately many of the reports do not agree, and consequently there is still considerable controversy in this area. LADA patients, like type 1 diabetic patients, have an increased frequency of HLA susceptibility alleles (4,5), but whether there are subtle differences between type 1 diabetes and LADA for specific alleles is controversial (4,5,28). Also, a greater association of LADA with type 1 diabetes HLA high-risk alleles in younger LADA patients and less in older LADA patients has been reported, but again this is not consistently observed (5). An early report suggested a relationship between HLA and insulin secretion in ICA^+ defined LADA patients. ICA^+ patients who were heterozy-

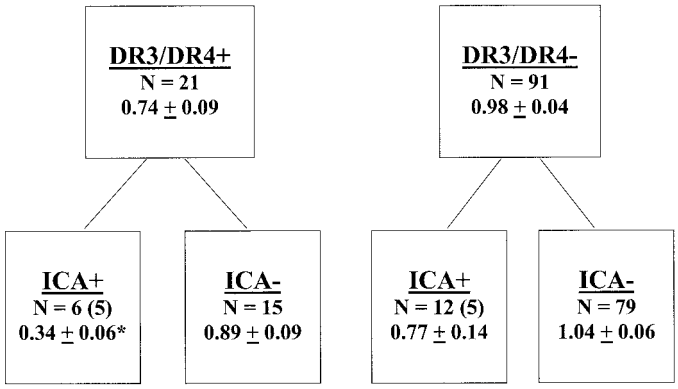

FIG. 6. C-peptide response to 1 mg i.v. glucagon in type 2 diabetic patients with (DR3/DR4+) or without (DR3/DR4−) heterozygous HLA-DR3/DR4 antigen and presence of (ICA+) or absence (ICA−) of islet cell antibodies. Numbers in parentheses are number of patients positive for complement-fixing islet cell antibodies. *$P < 0.001$, compared with patients positive for HLA DR3/DR4 but ICA negative. Reproduced with permission from Groop L et al., *Diabetes* 37:99–103, 1988.

gous for DR3 and DR4 had 60% lower stimulated C-peptide concentrations compared with ICA^- heterozygous DR3/DR4 patients. In ICA^+ patients who were $DR3/DR4^-$ stimulated, C-peptide was not decreased (29) (Fig. 6).

Non-HLA. Allelic variations at several non-HLA loci have also been associated with increased risk for and protection from classic type 1 diabetes, although these effects are weaker than for HLA. Comparisons between type 1 diabetes and LADA, like for HLA, have shown both similarities but potentially important differences. The cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene encodes a co-stimulatory molecule important for the repression of T-cell activation. The increased frequency of the CTLA-4 genotype A/G in both type 1 diabetes and LADA suggests a similar role in both types of diabetes (30). Allelic variation in the variable number of tandem repeats of the 5′ region of the insulin gene has also been associated with type 1 diabetes. In a recent study from Argentina, it was reported that the alleles were similar in type 1 diabetes and LADA and different from control subjects. But, the relative risk associated with the 1S/S genotype was significantly stronger for LADA than for type 1 diabetes (31). Major histocompatibility complex class I chain–related gene A (MICA) encodes polymorphic stress-inducible proteins recognized by γδ T-cells within the intestinal epithelium. The MICA microsatellite polymorphism has been associated with different autoimmune diseases including type 1 diabetes. MICA5 is associated with type 1 diabetes under the age of 25 years, whereas MICA5.1 is associated with both LADA and type 1 diabetes over 25 years of age (28,32). The gene for tumor necrosis factor-α is also in the major histocompatibility complex region and there is an allelic polymorphism within the promoter region. The TNF2 allele was significantly lower in LADA compared with type 1 diabetes or nondiabetic control subjects (33).

CONCLUSIONS

At the time of diagnosis, almost all patients with type 1 diabetes have autoantibodies that are reactive to islet antigens. In addition, ~10% of phenotypic type 2 diabetic patients also are positive for at least one of the islet autoantibodies. These antibody-positive phenotypic type 2 diabetic patients also commonly have T-cells reactive with islet antigens and share many genetic similarities with type 1 diabetes. These genetic and immunological similarities between LADA and type 1 diabetes strongly suggest that LADA, like type 1 diabetes, is an autoimmune disease. But there are also antibody, T-cell, and genetic differences between type 1 diabetes and LADA, which suggests the possibility of important differences in the underlying autoimmune disease processes of type 1 diabetes and LADA. Possibly there are differences in the degree of immune regulation or antigenic differences between type 1 diabetes and LADA. It is also possible that some of the observed differences are due to age-related effects on the immune system or because, in LADA, diabetes occurs earlier in the β-cell–destructive process because of the greater insulin resistance of LADA patients. This field has also been complicated by variable definitions of LADA and type 1 diabetes, and consequently comparing the results of one article with another is problematic. The recent definition of LADA suggested by the Immunology of Diabetes Society should help correct this problem. But the strongest and most important data to determine whether type 1 diabetes and LADA are different will likely come from intervention studies. As immunomodulatory therapies that slow or halt the type 1 diabetes disease process are discovered, testing these therapies in LADA will be essential. If therapies are efficacious in both type 1 diabetes and LADA, the genetic and immunological differences described in this article will be largely forgotten. But if some therapies are effective only in type 1 diabetes, or in LADA patients, this would constitute the strongest evidence for important disease process differences between type 1 diabetes and LADA, and consequently accurately diagnosing type 1 diabetes versus LADA would become clinically important.

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