

Celiac Disease-Associated Autoimmune Endocrinopathies

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Celiac disease (CD) is an autoimmune disorder induced by gluten intake in genetically susceptible individuals. It is characterized by the presence of serum antibodies to endomysium, reticulin, gliadin, and tissue transglutaminase. The incidence of CD in various autoimmune disorders is increased 10- to 30-fold in comparison to the general population, although in many cases CD is clinically asymptomatic or silent. The identification of such cases with CD is important since it may help in the control of type I diabetes or endocrine functions in general, as well as in the prevention of long-term complications of CD, such as lymphoma. It is believed that CD may predispose an individual to other autoimmune disorders such as type I diabetes, autoimmune thyroid, and other endocrine diseases and that gluten may be a possible trigger. The onset of type I diabetes at an early age in patients with CD, compared to non-CD, and the prevention or delay in onset of diabetes by gluten-free diet in genetically predisposed individuals substantiates this antigen trigger hypothesis. Early identification of CD patients in highly susceptible population may result in the treatment of subclinical CD and improved control of associated disorders.

Autoimmunity as a concept evolved from the beginning of this century, when Ehrlich and Morgenroth (26) introduced the phenomenon of “horror autotoxicus,” i.e., fear of self-poisoning. Subsequently, many diseases were recognized with etiology arising from the abnormal reaction of the immune system to self antigens. These observations laid the foundation for establishing postulates that are characteristic of an autoimmune disease (4, 7). Since then, several hypotheses have been put forward regarding the mechanisms of autoimmunity (22, 76, 84, 110, 118).

In general, autoimmune disorders can be classified as either organ specific or non-organ specific. In organ-specific autoimmune diseases, the autoantibodies are specifically directed against antigens localized in a particular organ and are often detected in circulation. Examples of organ-specific autoimmunity include Hashimoto’s thyroiditis, type I diabetes, and myasthenia gravis.

In contrast, the non-organ-specific autoimmune disorders are characterized by the presence of autoantibodies directed against ubiquitous antigens (not specific to a particular organ). This results in the involvement of several organs and is often characterized by the presence of specific circulating immune complexes. Non-organ-specific autoimmunity includes diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and scleroderma.

Epidemiologic studies have shown that genetic factors are involved in host susceptibility to autoimmune disease. For example, the concordance rate of a particular autoimmune disease is much higher in monozygotic twins in comparison to fraternal twins (17). Moreover, this incidence is much higher in organ-specific autoimmune disorders in comparison to non-organ-specific disorders. Thus, in Grave’s thyrotoxicosis, Hashimoto’s disease, and type I diabetes, the concordance rate of the clinical condition is as high as 50% in monozygotic twins,

whereas in non-organ-specific autoimmune disorders such as SLE and RA 10% of identical twins are affected (64). Among the genetic factors associated with autoimmune diseases, the best characterized are major histocompatibility complex (MHC) class I and class II genes. The present review will focus on factor(s) involved in the pathogenesis of celiac disease (CD) and its coexistence with autoimmune endocrine disorders.

CD

CD is an enteropathy affecting mainly the proximal small intestine and is due to intolerance to gliadin, a cereal protein present primarily in wheat. The classical symptoms of CD are diarrhea, weight loss, and malnutrition. The severity of gastrointestinal symptoms and clinical signs generally are a reflection of the degree of intestinal malabsorption. Thus, patients with limited mucosal involvement may be devoid of gastrointestinal symptoms but not necessarily spared from extraintestinal complications such as anemia or osteopenia caused by malabsorption of iron and/or folate and calcium, respectively. In addition to the variable clinical presentations, several studies have reported a variable clinical course, where the CD symptoms would first appear in infancy with spontaneous remission during adolescence and recurrence at a later stage. Accurate diagnosis of patients with symptomatic or asymptomatic CD is therefore important because strict adherence to a gluten-free diet may prevent neoplastic and systemic complications associated with the disease (28, 31, 62, 74, 75, 94, 115). It has also been suggested that a gluten-free diet instituted on patients with CD early in life would prevent development of type I diabetes in genetically predisposed patients (56, 58, 93).

CD shares many features with autoimmune disorders in general, such as a polygenic mode of inheritance, a strong association with HLA-DQ2 and HLA-DQ8 antigens, the production of a local inflammatory response (lymphocyte infiltration and cytokine production), the presence of autoantibodies in the circulation, female preponderance, and an association with other autoimmune diseases (1, 2, 11, 14, 19, 30, 33, 88, 91,

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92, 97, 104). There is a strong association of CD with HLA class II molecules with >90% of CD patients having the HLA-DQ $\alpha 1^*0501$, $\beta 1^*0201$ heterodimer (19, 88, 91, 92, 117).

AUTOIMMUNE ENDOCRINE DISORDERS AND CD

Insulin-dependent diabetes mellitus. Insulin-dependent (type I) diabetes is an autoimmune disorder characterized by destruction of insulin-producing pancreatic β cells. Type I diabetes primarily affects children and accounts for 10 to 15% of the patients with diabetes. In the United States, a total of approximately 120,000 individuals have type I diabetes (108). Type I diabetes elicits both humoral and cellular immunity characterized by the presence of β -cell-specific autoantibodies and cytotoxic T cells. These autoimmune responses in type I diabetes are not restricted to pancreatic antigens and may also be directed against other autoantigens residing in the thyroid and adrenal glands (26). While there are some indications that type I diabetes may be triggered and possibly accelerated by environmental factors, a 30% concordance rate between monozygotic twins indicates a strong underlying genetic influence.

Genetic susceptibility to type I diabetes has been attributed to more than one gene. The major influence on susceptibility for type I diabetes is imparted by genes mapped to the histocompatibility leukocyte antigen (HLA) locus. The HLA locus maps to chromosome 6 in humans—specifically to 6p21.3, where it is localized to a region of DNA approximately 3 million base pairs (15). This region contains genes for the two major classes of HLA molecules (class I and class II); for the complement components C2, C4, factor B, and 21-hydroxylase; and for tumor necrosis factors alpha and beta. HLA A, B, and C genes control the expression of surface antigens representing class I gene. These molecules are found on all nucleated cells and platelets. The class II region encodes genes that cover approximately 1 million base pairs of the MHC (15). This region is divided into three major subregions: DR, DQ, and DP, where each encodes for at least one pair of α and β chains. Both class I and class II molecules are polymorphic. This polymorphism is further enhanced by the haplotype combinations produced by the mixing of α - and β -chain alleles within the same haplotype; a third level of HLA diversity is obtained by allelic recombination, i.e., mixing the HLA allele products within an individual. For example, the DQ α chain of each haplotype can pair with the DQ β chain from the same haplotype (“*cis*” pairing) or the opposite encoded haplotype (“*trans*” pairing). The resulting hypervariability is fundamental for the development of immune protection of a living organism against infectious agents. Each of the variabilities introduced in these molecules is designed to select, bind, and present peptides to the immune system for maximal effectiveness of the consequent reaction.

Since the antigen presentation for T helper cells and antibody production is mediated by the HLA class II molecules, it is not surprising to find that the susceptibility traits of various autoimmune diseases were mapped to different alleles of these genes. In the case of type I diabetes, an inheritable disorder thought to be of autoimmune etiology, possibly triggered by viral infection, approximately 60% of the genetic predisposition is related to the HLA gene, while the other 40% is not

HLA associated (18, 45). These results have been confirmed in various ethnic communities studied for their genetic linkage with type I diabetes. The emerging consensus is that the type I diabetes susceptibility trait is associated with the DR3 molecule with the DRB1*0301 β chain and the DQ2 molecule with the DQA1*0501 α chain and the DQB1*0201 β chain (9, 32). Thus, although it was initially believed that DR3 was the major molecule influencing the susceptibility to type I diabetes, it was found that it is actually the DQ2 molecule that influences the selection and binding to autoantigenic peptides (77). The apparent DR3 and type I diabetes association was merely due to linkage disequilibrium with the DQ2 α and β genes. In this regard, the aspartic acid residue at position 57 of the DQ β chain is extremely important since its presence confers resistance (DQ β 3.1 allele), while its absence (DQ β 3.2 allele) promotes susceptibility to type I diabetes. Besides the class II genes, it is now clear that there are other minor genetic components outside the HLA complex (the putative IDDM6 or diabetes 1 susceptibility gene) that do influence susceptibility to type I diabetes (86). Approximately two-thirds of type I diabetes patients have a specific predisposing HLA allele, although some of these alleles may not have full penetrance (i.e., do not elicit disease). A person with the predisposing allele DQ8 or DQ2 with a family history of type I diabetes has a 25% risk of getting this disease (99). Most interestingly, the same DQ2 molecule is associated with susceptibility to CD, suggesting the possibility that an individual may be susceptible to both CD and type I diabetes due to the inheritance of a single HLA haplotype. The HLA-DQ molecules differ from other MHC class II molecules in several aspects; mainly, in HLA-DQ molecules both polypeptide chains are polymorphic (99). The DQ α chain contains a hypervariable loop between residues 48 and 56, which is one of the most hypervariable structures known. An interesting feature about this loop is that in some α alleles it ends in such a manner that it creates a deletion mutation of an arginine at either position 55 or position 56. This, in turn, influences the adjacent first peptide binding pocket (P1) in the peptide-binding groove of the DQ2 molecule. Thus, extra hypervariability is generated, depending on which of the two arginines is deleted.

There have been several studies indicating an association between type I diabetes and CD (12, 13, 16, 21, 29, 34, 35, 43, 44, 47, 48, 51, 53, 59, 61, 62, 66, 68, 71, 78, 80, 81, 83, 100, 106, 112, 114, 116). (Table 1). Clinically, the coexistence of CD and type I diabetes is difficult to ascertain since many type I diabetes patients may have asymptomatic or occult CD. The prevalence of CD in type I diabetes patients is 10 to 30 times that in normal population. It is in fact desirable that CD, in such a highly susceptible population, is recognized, since a gluten-free diet may result in (i) better control of diabetes and (ii) decreased frequency of insulin reactions. The disappearance of diabetic instability after the introduction of a gluten-free diet in such patients emphasizes the importance of the early recognition and identification of CD.

There have been many studies performed to determine the incidence of CD coexisting with diabetes (12, 13, 16, 21, 29, 34, 35, 43, 44, 47, 48, 51, 53, 59, 61, 62, 66, 68, 71, 78, 80, 81, 83, 100, 106, 112, 113, 114, 116). An incidence of 2 to 8.5% has been reported, depending upon the diagnostic criteria used for CD. If the clinical and/or biopsy criteria are employed, this

TABLE 1. Incidence of CD in type I diabetes

Study group (yr)	Insulin-dependent diabetes		
	No. tested	No. positive	% Positive
Savilahti et al. (1986)	152	9	3.5
Koletzko et al. (1988)	1,032	20	2.0
Gadd et al. (1992)	180	4	2.2
Rossi et al. (1993)	211	10	4.0
Sategna-Guidetti et al. (1994)	383	12	3.2
Rensch et al. (1996)	47	3	6.4
Calero et al. (1996)	141	4	2.85
Talal et al. (1997)	188	12	6.4
Vitoria et al. (1998)	93	6	6.45
Fraser-Reynolds (1998)	236	12	5.1
Aktay et al. (2000)	212	18	8.5
Total	2,894	110	3.8

incidence of coexistence is on the low side, i.e., 2%. However, if serological criteria are used, incidences as high as 8 to 9% have been reported. This is obviously due to the fact that many cases of CD are asymptomatic and the gut morphological changes may be minimal and so cannot be recognized histologically. These findings have been documented by the studies of Chorzelski et al. (13, 14), in which the sensitivity of morphological gut changes was enhanced either by the administration of an increase in dietary gluten content or by the taking of multiple biopsy samples. Serological tests, such as use of the endomysial antibody, are virtually 100% sensitive and specific for diagnosing both symptomatic and asymptomatic, but untreated CD, especially when performed in an established laboratory experienced in performing serological tests for CD (23, 25, 82).

The association between CD and type I diabetes could be explained by the sharing of a common genetic factor or by one of the diseases being a pathogenic consequence of the other. This question was addressed by Pocecco and Ventura (79), who evaluated 4,500 type I diabetes cases from 19 different centers in Italy. These authors identified "silent" CD cases in type I diabetes individuals by using celiac-specific serology, followed by a jejunal biopsy confirmation. They observed that their patients primarily fell into two categories: group I consisted of patients diagnosed with type I diabetes who were later diagnosed as "silent celiacs" and group II consisted of patients with prediagnosed CD that was later found to be complicated with type I diabetes. Group I included 88% of all dual-diagnosis cases with minimal gastrointestinal problems and who were 11 to 17 years of age at the diagnosis of CD. Group II included 12% dual-diagnostic cases; the mean age of the subjects at diagnosis was younger than that for group I (6 to 10 years), and these cases experienced maximum gastrointestinal symptoms. While increased severity of type I diabetes symptoms leads to its earlier diagnosis of insulin-dependent diabetes mellitus, the lack of severity of symptoms and repercussions of silent CD delays its diagnosis. These observations therefore suggest a lack of specific precedence for one of the diseases over the other. Since this is not the case, there is equal likelihood for CD to precede type I diabetes and vice versa.

From the above observations, it is apparent that disease-related pathogenicity is not responsible for the association between CD and type I diabetes. Recently, considerable evi-

dence has accumulated that genetic factors may elucidate not only the coexistence of CD and type I diabetes but also its association with other autoimmune disorders. The key role of DQ2 molecules was evaluated by Vartdal et al. (110), who studied the amino acid residues determinant of binding affinity at different binding pockets. Using natural peptides eluted from purified DQ2 molecules and synthetic variant peptides in binding assays, Vartdal et al. (110) found that the DQ2 molecules have residues at relative positions of P1, P4, P6, P7, and P9 that interact critically with amino acid residues of the peptide. These anchor positions did determine the affinity of the peptide interacting with a given class II molecule, and formation of high affinity peptide-class II complexes were essential for the formation of long-term stable complex. This is important because these long-term stable complexes are necessary for initiating an effective T helper cell response.

Point mutation studies and amino acid replacement studies showed that P1 preferred the binding of positively charged residues such as lysine, P4 preferred smaller aliphatic side chain amino acids such as alanine and isoleucine, and P6 position favored a negatively charged residue, whereas the C-terminal P9 anchor favored the juxtaposition of a bulky phenylalanine residue. An aspartate-57 residue located in the groove of the fourth peptide-binding pocket (P4) could form a salt bridge with arginine-79 residue in the α chain, thereby decreasing the size of the pocket and changing the positive charge in the pocket. The DQ2 and DQ8 molecules responsible for the increased susceptibility to type I diabetes and other autoimmune diseases are devoid of an aspartate-57 residue. Residue 57 DQB seems to be critical for peptide binding, T-cell recognition, and stability of the MHC class heterodimer on the cell surface (24). Peptides that have the favorable sequences of amino acids, fulfilling the stringent conditions of the DQ2 peptide binding pockets, will bind avidly and generate a Th2 cell response, which in turn leads to maturity and proliferation of the antibody response.

In CD, the autoimmune responses have been studied in gliadin-specific T cells isolated from the celiac lesions of the intestinal mucosa (60). A majority of these T-cell lines were DQ2 restricted. Using such T-cell lines, CD-specific α - and γ -gliadin epitopes have been delineated (40). Most importantly, the criteria for gliadin-peptide binding to the DQ2 molecule have been shown to be fulfilled after deamidation (modification) of these peptides by tissue transglutaminase (67). In type I diabetes, the pathogenic insulin, GAD, and ICA512 probably provided active T-cell help by peptides derived from each of these antigens. Linkage analysis of type I diabetes-specific antibodies and HLA-DR and -DQ phenotypes showed that anti-ICA512 antibodies are associated strongly with DR3 alleles, while the anti-GAD and anti-insulin antibodies are associated with DQ2 alleles. This suggests that the DQ2 molecule may actually promote the T-cell help required to generate anti-GAD and anti-insulin antibodies.

Similarities in peptide sequences may lead to a cross-reaction of epitopes at the T-cell level, but it is unclear whether such short sequence similarities exists between gliadin or tTG and GAD or insulin. If cross-reactivity does exist, the coexistence of type I diabetes and CD can be explained by "molecular mimicry." External stimuli that can provide such molecular mimicry are represented by various viral proteins such as hu-

TABLE 2. Association of CD and autoimmune thyroid diseases

Patient group	% Association		
	Collin et al. (1994)	Velluzzi et al. (1998)	This study
Autoimmune thyroid disease	4.8	29.7	2
Grave's disease	4		3.5
Hashimoto's thyroiditis	6		0
Nonautoimmune thyroid disease	4		0
Blood donors	0.6	9.6	0

man cytomegalovirus protein IE2 and the Epstein-Barr virus gp110 protein that may initiate autoimmune disease (107). Thus, gliadin may have certain residues directly cross-reacting with sequences on insulin or GAD. Since both of these responses would be mediated through DQ2 molecules, the coinstitution and maintenance of an anti-gliadin Th cell response would provide T-cell help to CD-specific antibodies (anti-tTG, anti-EMA, anti-reticulon) and also aid in the production of type I diabetes-specific antibodies.

Thyroid autoimmunity. Thyroid autoimmunity is common and is due to an apparent immune reaction directed against self antigens of the thyroid. Three thyroid diseases are considered to have autoimmune etiology: Hashimoto's thyroiditis, idiopathic myxedema, and Grave's disease. The antigens against which the autoimmune reactions are directed to produce thyroid autoimmune disease include thyroglobulin (Tg), thyroid peroxidase (TPO), and the TSH receptor (5, 37, 38, 49, 65, 70, 73, 89, 96, 113). These autoantibodies cause direct thyroid dysfunction, as in Grave's disease caused by antibodies to the TSH receptor, or a destructive process, as in Hashimoto's thyroiditis and idiopathic myxedema.

CD and autoimmune thyroid disorders share a common genetic predisposition, namely, the DQ2 allele. This common predisposing genetic background would explain the higher incidence of thyroid autoimmune disorders in CD than in the general population. Because of the varied clinical presentations of CD, serological methods have been found to be very useful for detecting its existence in patients with thyroid autoimmunity. This association has been the subject of several investigations (5, 37, 38, 49, 65, 70, 73, 89, 96, 113) (Table 2). These studies include determining the occurrence of CD in thyroid patients, whereas other investigations focused on the incidence of thyroid autoimmunity in patients with CD. For example, Collin et al. (16) studied 83 patients with autoimmune thyroid disease by determining the presence of serum endomysial antibodies and found five to be positive for CD. Four of these five endomysial antibody-positive patients had, in fact, small bowel villous atrophy. Of 275 aged-matched controls included in the study, two cases were positive for endomysial antibodies: one with a solitary nonfunctioning thyroid nodule and one who was a healthy blood donor. Both cases also had villous atrophy consistent with CD. Thus, the frequency of CD in patients with autoimmune thyroid disease is 4.3% compared to nonautoimmune thyroid controls of 0.4%. The prevailing rate of CD of one in 300 among blood donors is similar to that determined in various epidemiological studies. Among 136 cases of autoimmune thyroid disorders studied in our laboratory, we found three cases to be positive for endomysial

antibodies. None of the 71 controls with nonautoimmune thyroid disorders (thyroid cancer and nodular goiter) had a positive serological antibody test for CD. The converse study, i.e., the incidence of autoimmune thyroid disease in CD, was investigated by Velluzzi et al. (111). They performed thyroid antibody tests and thyroid echography in 47 patients with CD and 91 healthy controls and found that CD patients had a three- to fourfold increase in the incidence of thyroid autoimmunity. They further determined that CD patients who were positive for DQ*1 0502/DQ*1 0102 had an increased prevalence of thyroid autoimmunity. Since a high incidence of DQB1*0502 has also been associated with CD, it is possible that genetic commonality may underlie the association of thyroid autoimmunity to CD. Amino acids 632 to 645 in the TPO peptide bind with high affinity to DQ2 molecules bearing correct residues as per the binding motif of the DQ2 molecules. As mentioned already, gliadin binds to the same pocket suggesting that this molecular mimicry could explain the coexistence of CD with thyroid disorders.

Parathyroid. Idiopathic hypoparathyroidism is considered to be an autoimmune disorder characterized by the presence of autoantibodies against parathyroid antigens and its association with other autoimmune disorders (6, 8, 10, 39, 72, 98, 105). Idiopathic hypoparathyroidism of onset in childhood is commonly associated with hypofunction of other endocrine organs. This is due to the presence of autoantibodies against antigen(s) of the organs affected, thus defining the syndrome of autoimmune polyglandular type I syndrome that involves the adrenal glands, pancreas, gonads, and pituitary and thyroid glands (111). Several studies have reported an association of idiopathic hypoparathyroidism with CD (6, 8, 10, 39, 72, 98, 105). CD has been reported to be associated with both hypo- and hyper (both primary and secondary)-parathyroidism. CD has been associated with significant bone loss and hypocalcemia. Most CD patients with bone loss and hypocalcemia have secondary hypoparathyroidism. A gluten-free diet in patients with CD with secondary hyperparathyroidism has been reported to result in normal bone mineral density. Therefore, the presence of hypocalcemia or normocalcemic hyperparathyroidism should prompt an examination for CD. Treatment with a gluten-free diet should result in clinical improvement and restoration of normal calcium levels. It is thus recommended that patients with idiopathic hypo- and hyperparathyroidism be routinely investigated for autoimmune polyendocrinopathy and that calcium homeostasis be monitored closely after the institution of a gluten-free diet for CD.

Addison's disease. Addison's disease is an autoimmune disorder characterized by the presence of autoantibodies to antigens in the adrenal cortex (57, 111). Adrenal cortex antibodies are directed against the steroidogenic enzyme 21-hydroxylase (36, 41, 95). Almost all normal but adrenal antibody-positive subjects will go on to develop Addison's disease with time, and the progression of the disease correlates positively with the antibody titers. A statistically significant association between Addison's disease and CD has been described (102). Since Addison's disease is rather uncommon, the association of Addison's disease with CD may not be coincidental. In a recent study, Heneghan et al. (35) found two cases of Addison's disease who also had selective IgA deficiency. The reported incidence of Addison's disease in European epidemiological

studies is three to six cases per 100,000 population and, hence, finding two Addison's disease patients among 700 CD patients (relative increase of 100-fold) provides strong support for a true association. Thus, it is suggested that patients with Addison's disease may be prone to subclinical CD and should be screened by serological methods.

Elegant studies have been performed to assess a genetic involvement in the progression of Addison's disease. These studies looked at the association of Addison's disease, the presence of 21-hydroxylase autoantibodies, and DQ2 or DQ8 class II molecules (27). It was shown that DQ8⁺ patients with 21-hydroxylase autoantibodies had clinical Addison's disease. It is noteworthy that DQ8 molecules are very similar in their sequence and their structure to the DQ2 molecules. The DQ2 and DQ8 molecules, which are positively associated with many autoimmune disease, have different surface electrostatic charges from the negatively associated DQ6 molecules. It is known that structural features, such as the hydrophilicity of the first pocket, the β 49–55 dimerization patch, the CD4 binding region β 134–138, or the β 167–169 RGD loop, influence peptide binding as well as T-cell stimulation function in the DQ molecules. The peptide binding motif of DQ2 and DQ8, although similar, are not identical. Therefore, analogue composition and structural confines on sequences derived from gliadin, tissue transglutaminase, or 21-hydroxylase may lead to a molecular cross-reactivity between these antigens at the T-cell level. However, the gliadin peptides that bind avidly to DQ2 can theoretically bind DQ8 with slightly lower affinity, determining disease susceptibility. We speculate that the gliadin peptide-MHC complexes can effectively overcome the T-cell activation threshold and induce the 21-hydroxylase-specific T cells to elicit a specific autoimmune response (Addison's disease).

Polyglandular autoimmunity. Autoimmune polyglandular syndrome is a rare endocrine disorder comprising a combination of at least two of the following autoimmune endocrine disorders: Addison's disease, autoimmune thyroid diseases, hypoparathyroidism, type I diabetes, or primary gonadal failure (20, 42, 46, 90). In addition, nonendocrine diseases that have been described in autoimmune polyglandular syndrome include mucocutaneous candidiasis, vitiligo, alopecia, pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, and intestinal malabsorption. The autoimmune polyglandular syndrome can be classified into three different groups. Malabsorption is commonly associated with one of them (type I autoimmune polyglandular syndrome). The association of malabsorption with types II and III has not been clearly established. It is thought that the malabsorption associated with type I autoimmune polyglandular syndrome is due to CD. Two case studies (57, 111) have been reported of autoimmune polyglandular syndrome and CD. Based upon the HLA haplotype analysis (DQ8 predominance), the association may represent genetic predisposition rather than casual association.

Infertility. CD is associated with infertility and miscarriage (52, 54, 85), and a gluten-free diet in such cases is associated with a substantial reduction of abortions, in the frequency of low-birth-weight infants and with an increased duration of breast feeding. In a study conducted by Lewis et al. (52), the incidence of subclinical CD was determined in patients with infertility and recurrent miscarriage. A total of 150 women

examined for infertility, 50 women with two or more subsequent abortions, and 150 females as a control group were examined for CD by serological tests. Four (4%) of the women in the unexplained infertility group but none in the control group were found to have CD. This observed frequency of 4% is at least 10 times higher than the incidence of CD in the general population. From these and other studies, it is obvious that CD is a risk factor for infertility resulting in (i) a deficiency or lack of micronutrients, vitamins, etc.; (ii) an increased frequency of endocrinological and autoimmune diseases; and (iii) a shortened reproductive period, with delayed menopause and early menopause. Thus, women with unexplained infertility should be screened for CD. Indeed, an increased frequency of successful deliveries has been reported after a gluten-free diet was started in such cases. In conclusion, CD should be suspected in women with spontaneous abortions, low-birth-weight newborns, and/or short or no lactation, even if malabsorption is clinically absent.

CONCLUSIONS

As elaborated in earlier sections, CD may coexist with several extraintestinal diseases. This association can be theorized based on a universal explanation of the clinical association based on cross-reactivities or molecular mimicry of antigens on a common class II DQ2 molecule. Because of the more extensive experience with the association between CD and type I diabetes, we believe we can reconcile the observations into two separate, compatible models.

In the first one, which we favor, the enzyme tissue transglutaminase has a central role. Tissue transglutaminase is a ubiquitous enzyme controlling apoptosis and the target for anti-tissue transglutaminase antibodies resulting from CD. These antibodies can bind to tissue transglutaminase and interfere with its physiological functions (3). Such functional derangement has been confirmed by a reduction in cross-linking activity that is associated with the presence of autoantibodies to tissue transglutaminase. This antibody-mediated interference of tTG activity results in abnormal accumulation of double-stranded DNA and lactate dehydrogenase in blood, which may lead to a necrotizing effect due to deregulated apoptosis. We propose that, due to deregulated apoptosis, certain tissue-specific endogenous proteins are released locally. In support of this interpretation are the reports indicating the presence of autoantibodies against substrate proteins with which tissue transglutaminase would normally react (101, 109). In light of this observation, it is tempting to speculate that tissue transglutaminase may normally modify these "tissue-specific" proteins and that, when tissue transglutaminase is inactivated by autoantibodies, targets for the class II molecules are generated that bind and present to the immune system as autoepitopes. This would trigger another specific autoimmune disease besides CD.

How do type I diabetes and other organ-specific autoimmune disorders associate with CD? We may elaborate this concept further, with type I diabetes as the primary example. It is well established that, in type 1 diabetes, the initial β -cell attack by CD8⁺ cytotoxic T cells causes destruction of these cells. Such an injury to the β cells can also be initiated by anti-tissue transglutaminase antibodies. This will invariably ex-

pose the endogenous GAD and insulin molecules to the exogenous antigen presentation pathway. Similarly, in CD patients, the anti-tissue transglutaminase antibodies may lead to interruption of the tissue transglutaminase functions as described above. In either case (type 1 diabetes or CD), there is an opportunity for tissue transglutaminase to interact with islet-specific antigens such as GAD and insulin. The tissue transglutaminase may now modify the diabetogenic antigens (GAD or insulin). During the deamidation (modification) process, tissue transglutaminase may make "neoepitopes" of GAD- and insulin-derived peptides. Such modifications may then lead to the appearance of peptides with sequences that satisfy the DQ2 peptide-binding motif. Hence, the subsequent presentation of these peptides (derived from GAD and insulin) to the DQ2 molecules forces the immune system to prime T cells that can now recognize "self" endogenous proteins such as GAD and insulin, eliciting type I diabetes-specific B- and T-cell response. It should be emphasized that proteins such as GAD or insulin may not possess any immunodominant T-cell autoepitope, yet the systematic and stepwise deamidation process at glutamine residues (by tissue transglutaminase) may promote the modified peptides to bind to DQ2 molecules. This model explains the propensity of DQ2-bearing patients to have a dual diagnosis of CD and type I diabetes. It is also in agreement with observations that CD or type I diabetes may arise in patients with a dual diagnosis in a mutually exclusive manner.

The novel physiological functions that tTG performs and their relation to the disease process, coupled with the coexistence of CD with several other autoimmune disorders, underscores the importance of CD as a model autoimmune disorder. We strongly believe that CD may yet play a critical role in the understanding of autoimmune responses.

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