

Review

Autoantigens IA-2 and GAD in Type I (insulin-dependent) diabetes

R.D.G. Leslie¹, M. A. Atkinson², A. L. Notkins³

¹ Department of Diabetes and Metabolism, St. Bartholomew's Hospital, London, UK

² Department of Pathology and Laboratory Medicine, College of Medicine, University of Florida, Gainesville, Florida, USA

³ Experimental Medicine Section, Oral Infection and Immunity Branch, National Institute of Dental Research, National Institute of Health, Bethesda, Maryland, USA

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Introduction

Type I (insulin-dependent) diabetes mellitus results from destruction of the beta cells of the islets of Langerhans, usually leading to absolute insulin deficiency. Although some patients who are insulin dependent have no immune changes, in the majority the destructive process is thought to be immune mediated [1]. The world-wide incidence of the disease varies, ranging from 1.7/100,000 person-years in Japan to 29.5/100,000 person-years in Finland [2]. In Western industrialised countries, Type I diabetes is the second most common chronic childhood illness after asthma. In the United States the life time prevalence of Type I diabetes approaches 0.4% [3]. Familial clustering of Type I diabetes emphasises the role of genetic factors in disease susceptibility. A major portion of this clustering is due to sharing of alleles at susceptibility loci in the major histocompatibility complex on chromosome 6, most notably HLA-DQB1 and HLA-DRB1, as well as at least 13 other loci on nine chromosomes [4]. None-genetic, probably environmental, factors are thought to operate in these genetically susceptible subjects over a limited period in early childhood to initiate the disease process [5]. The nature of these putative environmental

factors is still not known; candidates include viruses, toxins and dietary factors [6].

At the clinical onset of Type I diabetes, 60–80% of islets are deficient in beta cells and the islets can be infiltrated with mononuclear cells [7]. Mononuclear cell infiltration occurs principally around islet cells containing insulin, but is not a consistent feature. These mononuclear cells include macrophages and T lymphocytes and CD8 positive T cells appear to be most prevalent [8, 9]. In the years before the clinical onset of Type I diabetes, immune and metabolic changes can be detected in peripheral blood [1]. The immune changes involve both cellular and humoral responses which persist over a prolonged period up to diagnosis of the disease [10]. The nature, intensity, extent and persistence of these immune changes distinguishes people who develop Type I diabetes from those who do not [1, 5, 10]. Immune and metabolic changes can, therefore, be predictive of the disease. The long prediabetic period and the potential for prediction has led to attempts to prevent the clinical disease [11]. It is widely believed that Type I diabetes is due to an autoimmune process [1]. Whereas the evidence for autoimmunity in humans is circumstantial, animal models of the disease, such as the non-obese diabetic (NOD) mouse and the Bio-Breeding rat, support an autoimmune process leading to diabetes [12, 13].

Identification of disease-associated autoantigens, that act as targets of the immune response, has dual importance: firstly, antibodies to the molecules could act as predictive markers, and secondly, modulation of the immune response to an antigen might alter the disease course. Islet cell autoantibodies (ICA), recognising islet cytoplasmic antigens, were detected many years ago as a feature of newly-diagnosed Type I diabetic patients and comprise autoantibodies to a number of antigens including glutamic acid decarboxylase (GAD) and protein tyrosine phos-

Corresponding author: Professor D. Leslie, Department of Diabetes and Metabolism, St. Bartholomew's Hospital, London EC1A 7BE, UK

Abbreviations: IA-2, Protein tyrosine phosphatase-2; NOD, non-obese diabetic; PTP, protein tyrosine phosphatase; IA-2ic, intracellular IA-2; GAD, glutamic acid decarboxylase; ICA, islet cell antibodies; IAA, insulin autoantibodies; GABA, gamma-aminobutyric acid.

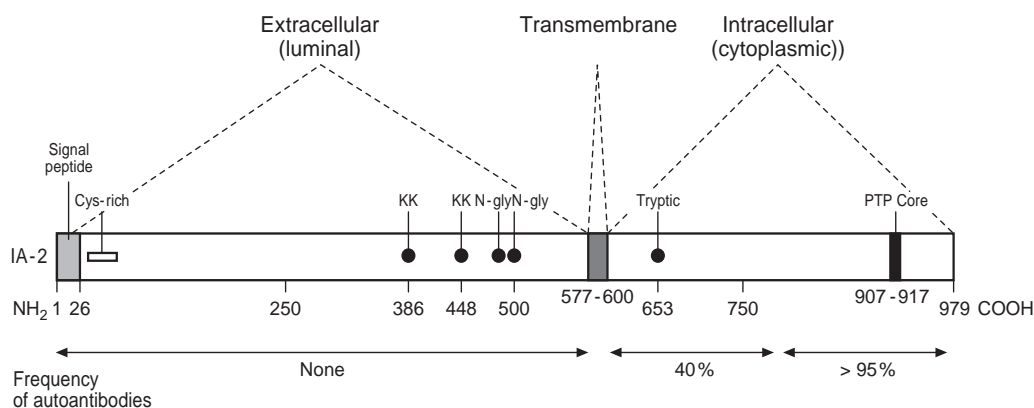


Fig. 1. Diagrammatic representation of IA-2 protein. Cysteine rich region (Cys); dibasic amino acid cleavage sites (KK); N-linked glycosylation sites (N-gly); tryptic cleavage site (tryptic); PTP core sequence. Frequency of autoantibodies in newly diagnosed Type I diabetic patients to different regions of the IA-2 molecule indicated by arrows

phatase-2 (IA-2), either of which can be detected in up to 90% of patients [14, 15]. This review focuses on these two antigens and discusses their potential role in the prediction and pathogenesis of Type I diabetes.

Properties of IA-2

Protein tyrosine phosphatase-2 is located on chromosome 7q36 and its cDNA encodes a 979 amino acid transmembrane protein (Fig. 1) [16, 17]. The extracellular and intracellular domains are, respectively, 576 and 378 amino acids in length. Based on sequence analysis, IA-2 is a member of the protein tyrosine phosphatase (PTP) family, but the expressed recombinant protein fails to show protein tyrosine phosphatase enzyme activity. Comparison of IA-2 with the highly conserved catalytic domain of other members of the PTP family indicated that IA-2 possesses several substitutions at sites critical for enzymatic activity [16]. Recent studies showed that by replacing aspartic acid with alanine at position 911 and alanine with aspartic acid at position 877, IA-2 becomes enzymatically active [18] (P. Li, A.L. Notkins, unpublished observations). Mouse and rat IA-2 have an aspartic acid in the catalytic domain and these molecules are also enzymatically inactive. The function of native IA-2 thus is still not resolved.

Northern analysis and immunostaining showed that IA-2 is expressed primarily in neuroendocrine cells [16, 19, 20]. It is found in the islets of Langerhans and in many parts of the central nervous system. In addition it is expressed in neuroendocrine tumours and can be useful as a marker to distinguish neuroendocrine from non-neuroendocrine tumour cells [20].

The extracellular domain of IA-2 appears to reside within secretory granules, whereas the intracellular domain protrudes into the cytoplasm [19]. Upon stimulation of cells, the secretory granules fuse with the plasma membrane and the extracellular domain of IA-2 becomes associated, at least transiently, with the plasma membrane. Although IA-2 cDNA encodes a protein of 979 amino acids with a molecular mass of 106 kDa, hyperimmune serum made against IA-2 fails to immunoprecipitate proteins of this size from radiolabelled insulinoma cells. Instead proteins with a molecular mass of 60–70 kDa are found [19, 21, 22]. It is thought that post translational processing results in protease cleavage of the IA-2 molecule at one or both of two dibasic cleavage sites located at amino acids positions 386–387 and 448–449, though the precise timing of the cleavage is not clear.

Autoantibodies to IA-2. Studies in the early 1990s showed that immunoprecipitation of insulinoma cell lysates with Type I diabetes sera yielded a 64 kDa protein which upon treatment with trypsin resulted in a 40 kDa fragment [21]. Although the molecular identity of this 40 kDa fragment remained obscure it was thought to be an important autoantigen because it was precipitated by a high percentage of Type I diabetic sera. Over the last couple of years the identity of this fragment has been determined and shown to be the tryptic cleavage product of IA-2 encompassing amino acids 653–979 [23–26]. Sera from patients with Type I diabetes also react with a protein designated ICA-512. Based on its reported sequence, this protein represents a 525 amino acid truncated version of IA-2 lacking 388 amino acids at the N-terminus and 65 amino acids at the C-terminus [27].

Autoantibodies to IA-2 have now been found in the sera of many patients with Type I diabetes. The assay that, thus far, has given the best results, in terms of the percentage of Type I diabetic patients positive for IA-2 antibodies, is the immunoprecipitation of radiolabelled recombinant IA-2 prepared in an *in vitro* transcription-translation system. The counts in the immunoprecipitate are determined directly or the immunoprecipitate is subjected to SDS-PAGE and the

Table 1. Percentage of autoantibodies to IA-2 and GAD as well as combinations of these autoantibodies in the general population, relatives of Type I diabetic patients (siblings and twins) and newly diagnosed Type I diabetic patients with estimates of disease risk for each group. The data is based on 5 studies [14, 29, 31, 94, 95]. The figures for autoantibody frequencies in a normal population include estimates based on signal levels above the 97.5th centile for that population [29]

	Type I diabetes Risk (%)	IA-2	GAD ₆₅	IA-2 and GAD	IA-2 or GAD
Normal	0.4	0–2.5	0–2.5	0–0.1	0–5
Siblings	5	1.5–5.3	6–9	0.3–1.4	7–8
Twins	35	36	26	19	36
Type I	100	55–75	52–82	37–57	87–94

characteristic full length 106 kDa IA-2 band made visible [17, 23]. These assays have proved to be highly specific, sensitive and reproducible in many laboratories. Other simpler assays, including ELISA, are now being tested.

The major antigenic regions of the IA-2 molecule with which autoantibodies react have been determined by deletion mutants. These experiments showed that contrary to initial expectation, none of the sera from patients with Type I diabetes reacted with the extracellular domain; all the sera reacted with the intracellular domain (Fig. 1) [28]. Approximately 95 % of the sera reacted with the carboxyl-terminus (amino acids 771–979) and 40 % with the amino-terminus (amino acid 604–776) of the intracellular domain of IA-2 (IA-2ic). Moreover, the epitopes with which the autoantibodies react are conformationally dependent. Reduction of the disulphide bonds within the intracellular domain of IA-2 results in almost total loss of reactivity with Type I diabetic autoantibodies, arguing that disulphide bond formation has a critical role in maintaining the antigenic structure of IA-2 [24]. These findings also suggest that assays which preserve antigenic conformation will be more effective than assays in which antigenic conformation is altered.

Many patients with Type I diabetes have been tested for the presence of serum autoantibodies to IA-2 [14, 15, 29–31]. It is estimated that about 65 % (range 55–75 %) of newly diagnosed Type I diabetic patients have autoantibodies to IA-2 (Table 1). In contrast, less than 2.5 % (range 0–2.5 %) of normal control subjects and patients with Type II (non-insulin-dependent) diabetes mellitus have autoantibodies to IA-2 [34–38]. The frequency of IA-2 antibodies in Type I diabetes varies with age and HLA genotype, being highest in the younger age groups and in patients with HLA DR4 and the HLA DQA1*030-DQBI*0302 genotype [14, 31–33]. There is also some evidence, requiring confirmation, that the percent positivity for IA-2 persists up to one year after diagnosis but decreases thereafter [14].

Cellular immune responses to IA-2. In contrast to the large number of studies on the autoantibody response to IA-2, there are very few studies on the cell mediated immune response to this antigen. Peripheral blood lymphocytes from patients with Type I diabetes have been shown to react with IA-2 [40, 41], but the magnitude of the response is low. There is also concern about the lack of consistency between laboratories when studying T-cell responses to specific antigens, this is the subject of current international workshops. Studies to determine the immunodominant epitopes of IA-2 using overlapping synthetic peptides are underway. In one study of T-cell responses in relatives of Type I diabetic patients, IA-2 peptide 805–820 was dominant, bound to HLA DR4 (*0401) and had 56 % identity with VP7, a major immunogenic protein of human rotavirus [42].

Comparison with IA-2 β . A second novel PTP, IA-2 β , has recently been isolated, localised to chromosome 7q36, and shown to be a major autoantigen in Type I diabetes [23, 43, 44]. It is similar in many respects to IA-2, especially in its intracellular domain which is 74 % identical to IA-2. The protein is enzymatically inactive and has the same aspartic acid substitution in the catalytic domain as IA-2. Autoantibodies from patients with Type I diabetes react only with the intracellular domain and almost exclusively with the carboxy-terminus. Overall, between 35 % and 50 % of Type I diabetic patients have autoantibodies to IA-2 β , which is considerably lower than the 55–75 % who have autoantibodies to IA-2 [23, 43, 44]. Moreover, since more than 95 % of Type I diabetic patients who have IA-2 β antibodies also have IA-2 antibodies, screening for autoantibodies to IA-2 β offers only marginal advantages for diagnosis. The possibility that autoantibodies to IA-2 β represent a late stage in the disease process is, however, being actively explored. Other molecules designated phogrin, IAR, ICAAR, PTPNE-6 have been isolated from several libraries and show nearly identical sequences to IA-2 β [45–48].

Potential pathogenic significance of anti-IA-2 immunity. Protein tyrosine phosphate-2 could be the target of the immune process which is believed to destroy the insulin secreting islet cells. Alternatively, antibodies to IA-2 could be a consequence of this destructive process, releasing the sequestered antigen IA-2 which induces an immune response. Protein tyrosine phosphate-2 has, however, only recently been identified as a major IDDM-associated antigen and studies of animal models and T cells are still at an early stage. In contrast, there have been extensive studies using another major Type I diabetes-associated antigen – the enzyme GAD.

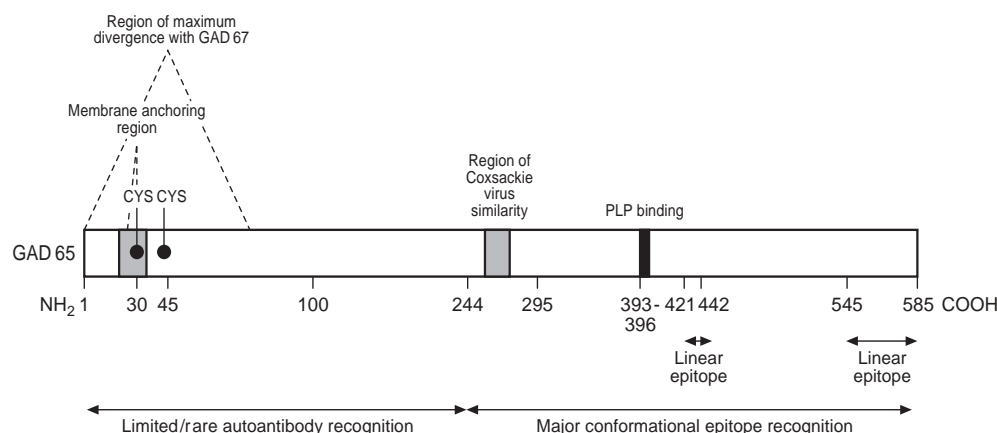


Fig. 2. Diagrammatic representation of GAD65 protein. Regions labelled include Cysteine rich region (Cys); pyridoxal phosphate binding site (PLP); membrane anchoring region; region with maximum divergence from GAD67; region of similarity with the Coxsackie virus; linear epitopes identified by GAD autoantibodies: major and minor regions recognized by GAD autoantibodies

Properties of GAD

Glutamic acid decarboxylase is found in nerves and islet cells as a doublet of proteins commonly referred to as GAD65 and GAD67 (i.e. molecular weight 65,000 and 67,000 Mr). Both isoforms of GAD contain a pyridoxal phosphate binding site, a cofactor required for enzymatic activity (Fig. 2) [49]. The human genes for these two isoforms lie on different chromosomes (i.e. chromosome 3 for GAD65 and chromosome 10 for GAD67) [50, 51]. The molecular sequences of GAD65 and GAD67 have been determined in a variety of mammalian species and show a remarkable degree of evolutionary conservation. The two human GAD isoforms are approximately 65% identical at the amino acid level, with much of the variance in primary structure located within their first 100 amino acids [50, 51].

Both GAD isoforms are synthesised within the cytoplasm as hydrophilic, soluble molecules. Whereas GAD67 remains soluble, much of GAD65 is post-translationally altered via a multi-step process involving modification of lipids within the N-terminal domain (e.g., palmitoylation), the result of which anchors the molecule within the membranes of synaptic vesicles in γ -aminobutyric acid (GABA) containing neurons or small synaptic-like microvesicles of islet beta cells [51]. Immunological staining of neural tissue with autoantibodies specific for GAD65 or GAD67 have shown a differential distribution of the two isoforms within the neural cytoplasm, with a preponderance of GAD65 in nerve terminals. Similar quantitative techniques have shown the marked predominance of GAD65 in human islet cells.

Whereas early reports of the tissue distribution and intracellular localisation of GAD were equivocal, it is now clear that the cellular distribution of GAD is very restricted. Apart from its presence in the central and peripheral nervous systems, GAD is observed only within pancreatic islet cells, epithelial cells of the fallopian tube, and spermatozoa of the testes. In terms of function, GAD is the rate-limiting enzyme in the pathway involving the conversion of glutamic acid to GABA, a major inhibitory neurotransmitter of both the central and peripheral nervous system [49]. The function of GAD within tissues other than neurons is not clear. The presence of both GAD and GABA within islet beta cells and the presence of GABA receptors on these cells suggests that GABA is involved in paracrine signalling in the islet.

Autoantibodies to GAD. The identification of GAD as a target autoantigen of Type I diabetes dates back to a report in 1982 of a 64,000 Mr antigen that was immunoprecipitated from human islets with sera from newly diagnosed Type I diabetic children [53]. Further biochemical characterisations of the 64 kD protein, taken together with studies of another autoimmune disease (i.e. Stiff-Man Syndrome) finally led to the identification of the protein as the smaller isoform of GAD, GAD65 [54].

The major antigenic region of GAD with which autoantibodies react has been determined using either expression of sub-regions (e.g. one-third fragments of GAD) or deletion mutants of GAD; these studies have identified the middle- and carboxyl-regions of GAD as the prime immunogenic sites for autoantibody recognition (Fig. 2) [55–58]. Additional studies using a panel of GAD65 specific monoclonal islet cell antibodies (i.e. MICA 1–7) derived from patients with Type I diabetes suggest that epitope recognition is conformational, consistent with epitope binding patterns with native serum [59]. The autoantibody epitopes using Type I diabetic sera have been mapped to the C-terminal region (amino acids 450–585) and the middle domain (amino acids 245–449) of GAD65.

The ability to isolate large quantities of native GAD from brains (e.g. pig, rat), along with the cloning and in vitro expression of the GAD65 gene, led to a series of studies identifying GAD autoantibodies using the enzymatic activity of immunoprecipitated GAD by measuring $^{14}\text{CO}_2$ liberated from ^{14}C -labelled glutamic acid, radioimmunoassay or ELISA. Results of the first and second GAD autoantibody workshops indicated that the specificity for recognition of GAD autoantibodies in diabetic sera using radioimmunoassay or ELISA was similar (approximately 90%), but radioimmunoassay was much more sensitive (76% compared with 36%) [54, 55]. Most current assays used to determine GAD autoantibodies are based on the ability of sera to immunoprecipitate radio-labelled recombinant GAD.

Numerous studies have determined the frequency of GAD autoantibodies in newly diagnosed Type I diabetic subjects from a wide range of populations. Autoantibodies to GAD67 are observed in 50–80% of newly diagnosed Type I diabetic patients but less than 2% of normal subjects [14, 55, 66]. (Table 1). Aside from variations due to the method of detection, additional factors contributing to this broad range of GAD65 autoantibody positivity in newly diagnosed patients include age and HLA-type. Conflicting results have been reported, but in general autoantibodies to GAD appear to increase with age and the frequency of these autoantibodies is highest in patients with HLA-DR3 type [14, 31, 33, 62–64]. Despite observations that GAD67 autoantibodies are present in 10–20% of newly diagnosed Type I diabetic patients, this molecule does not appear to represent an independent autoantigen as the autoantibodies recognise it by virtue of their cross-reactivity with GAD65 [65, 66]. The GAD65 autoantibodies, when compared with other autoantibodies in Type I diabetes (e.g. ICA), are unusual in that they tend to persist in sera for many years after diagnosis of the disease [67].

Autoantibodies to GAD have been identified in patients with Type II diabetes [34–39]. A substantial proportion (up to 20%) of such patients have GAD or other autoantibodies associated with Type I diabetes. This proportion of newly diagnosed Type II diabetic patients with ICA and GAD autoantibodies was recently shown to decrease with increasing age at diagnosis, e.g. from 34% aged 25–35 to 7% aged 55–65% for GAD. Overall autoantibody frequency in 1538 patients for ICA was 6%, for GAD 10% and for either autoantibody 12% [39]. In that study, patients with autoantibodies compared with those without autoantibodies were leaner, with a higher HbA_{1c} and a lower insulin response to intravenous glucose; of patients with both ICA and GAD autoantibodies, 94% required insulin treatment by 6 years against only 14% without either autoantibody [39]. Autoantibodies to GAD are a better predictor of progression

to insulin dependency than the combination of ICA and C-peptide values in Type II diabetic patients [68, 69]. Similarly, GAD autoantibodies may be valuable in predicting progression to Type I diabetes in patients with gestational diabetes of whom up to 5% can have these autoantibodies [70]. If Type II diabetic patients with autoantibodies do have a slowly developing form of Type I diabetes then the actual number of people with Type I diabetes will be increased substantially, since there are approximately ten times more cases of Type II than Type I diabetes.

Cellular immune responses to GAD. In contrast to the large number of studies on the autoantibody response to GAD, there are relatively few studies on the cell mediated immune response to this antigen. Peripheral blood lymphocytes from patients with Type I diabetes can react with GAD and GAD peptides though the magnitude of the response is low [40, 71–74]. Cellular immune responses to GAD have been reported to be inversely related to humoral immune responses so that enhanced cellular immunity was linked to progression to Type I diabetes whereas high autoantibody titres were detected in subjects who had not developed diabetes [62]. Reports identifying the cellular immune reactive epitopes of GAD in human Type I diabetes (via either direct stimulation of T cells, HLA-binding, or the generation of T-cell clones) have, in the main, been conflicting. Patients with Type I diabetes respond immunodominantly to region 473–555 of GAD65 or preferentially to region 247–279, in contrast to control subjects who respond mainly to the central region of the molecule [73, 74]. The epitopes of GAD identified in cellular immune studies of nonobese diabetic (NOD) mice, an animal model of Type I diabetes, and transgenic mice have, in some cases, been similar to those identified in humans [79–82]. For example, one immunodominant GAD epitope identified by T-cell clones from a diabetic patient (peptide 270–283) could be presented by HLA-DR B1*0401 (a Type I diabetes associated allele) and epitopes mapping around this region (peptides 274–286 and 271–285) were immunodominant in studies of HLA-DR B1*0401 transgenic mice [80–82].

Potential pathogenic significance of anti-GAD immunity. Immune responses to GAD could either be directly involved in the destructive process or merely ‘bystanders at the scene of an accident’, resulting from immunostimulation by sequestered antigens released from destroyed cells. Against this latter hypothesis, GAD autoantibodies were not detected in patients with islet cell damage associated with pancreatitis who had diabetes-associated HLA halotypes [83]. Studies in NOD mice, conversely, support a direct role for anti-GAD immunity predisposing to diabetes. These mice show early spontaneous T-cell re-

activity to GAD and modulation of GAD immunity attenuates the disease [76, 77]. A number of therapeutic approaches using GAD or GAD peptides given by intrathymic, intraperitoneal, oral or intranasal routes can prevent diabetes [76, 77, 84–89]. Although the exact method of disease protection using most of these therapeutic approaches is not clear, protection of mice from diabetes provided by oral GAD can be adaptively transferred by lymphocytes to naïve NOD mice, suggesting that disease prevention can occur through activation of regulatory T cells [79, 85]. Such antigen specific therapies to prevent diabetes are an attractive option, but we should be cautious as recent studies in animal models have shown that oral antigen therapy can induce or accelerate disease [87–89]. Since GAD autoimmunity is also associated with a neurological disease, Stiff-Man Syndrome, particular caution should be exercised in modulating the immune response to GAD lest the price of preventing Type I diabetes is to induce Stiff-Man Syndrome [56–58].

According to one hypothesis, Type I diabetes is due to a self antigen in islet beta cells, such as GAD, activating an autoimmune process. In support of this proposal, studies in NOD mice have demonstrated the ability of GAD peptide-reactive T-cell clones to adoptively transfer insulinitis and diabetes [78]. Interestingly, the CD4 + T-cell line specifically recognises the GAD65 peptide 524–543 which lies within the immunodominant region in humans [74]. A second hypothesis suggests that an immune response directed against a non-self antigen, containing a peptide sequence homologous with a self antigen, could activate an autoimmune process against that self antigen. This ‘molecular mimicry’ hypothesis receives support from studies of patients with Type I diabetes in whom the GAD65 peptide 247–279 is a major determinant with sequence similarity to the P2-C protein of the Coxsackie B virus [70]. Peripheral blood mononuclear cells from those Type I diabetic patients who respond to GAD peptides 247–279 also responded to a homologous Coxsackie viral peptide [73]. Thus, molecular mimicry could lead to an altered immune response against self antigens, even though the altered immune response was initially induced by non-self antigens.

Assuming that Type I diabetes is an autoimmune disease, antigen-specific therapy could be devised to modify the destructive process and prevent the disease in humans. A number of factors still, however, limit the potential for antigen-specific disease prevention: 1) the distribution and expression of autoantigens such as GAD and IA-2 is not tissue specific; 2) many patients are unresponsive to a given antigen to antigen-specific therapy could require several antigens, and 3) HLA associations in Type I diabetes are diverse, so HLA binding to epitopes will be variable and antigen-specific T cells may differ between patients [80, 81].

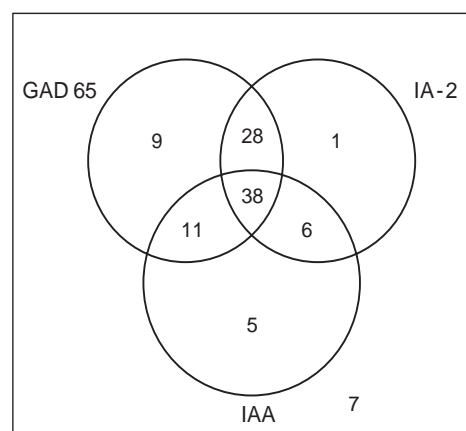


Fig. 3. The frequency of antigen specific autoantibodies measured by radioimmunoassay to IA-2 (intracellular fragment), GAD65 and insulin (IAA) in 105 prediabetic subjects who developed diabetes during follow-up identified from 3 578 relatives [15, 31, 94, 95]. Intersecting regions represent the number of prediabetic subjects positive for different combinations of autoantibodies. Seven did not have IA-2, GAD or insulin autoantibodies initially. Cut-off levels for positivity can vary between laboratories

Prediction of Type I diabetes

The induction of immune changes in early childhood and their continuous presence in the prediabetic period suggest that these changes might predict Type I diabetes. Changes in T lymphocytes including increased expression of the HLA-DR, a marker of activation, are a feature of the prediabetic period [1, 10]. Studies of T cell are, however, technically laborious. Moreover, none of the T-cell changes identified in the prediabetic period to date is antigen-specific or specific to Type I diabetes. As a result, attempts to predict Type I diabetes have focused on autoantibodies as disease markers. Numerous studies have demonstrated the value of ICA as a predictor of Type I diabetes [90, 91]. Determination of ICA though involves a technically difficult assay requiring human pancreatic tissue and the assay, therefore, is not readily applicable to large scale analysis [91]. A positive ICA reaction probably reflects the presence of autoantibodies to one or more islet cell antigens and reactivity to both IA-2 and GAD contribute to ICA [44, 92]. Autoantibodies to insulin are also found in the sera of some patients with Type I diabetes especially young children but do not contribute to the ICA immunofluorescence assay which uses unfixed tissue sections from which insulin has leached out (Fig. 3) [15, 31, 93–95]. Both the high prevalence of autoantibodies to IA-2 and GAD in prediabetic people and the availability of both molecules in recombinant form have encouraged the development of very sensitive assays which could replace the ICA immunofluorescence assay for population screening and disease prediction.

The aim of disease prediction is disease prevention. Type I diabetes could be prevented by avoiding those environmental factors which trigger the disease process (primary prevention); alternatively, therapy could modulate the destructive process before the onset of clinical diabetes (secondary prevention). Accurate disease prediction is vital for secondary prevention. Therapy should be given only to those people who are likely to become diabetic. If an autoantibody is used to predict the disease, then ideally: every non-diabetic subject with the autoantibody would eventually develop Type I diabetes (high disease positive predictive value); every subject without the autoantibody would remain non-diabetic (high disease specificity) and every subject who developed the disease would have that particular autoantibody (high disease sensitivity). Unfortunately, the higher the predictive specificity of a disease marker is, the lower is the predictive sensitivity and vice versa [91]. Further, the prognostic relevance of any marker varies in populations at differing levels of risk. If the disease risk is high then the predictive power can be high, but when the disease risk is low, as in the general population, then there is a corresponding reduction in predictive power [91]. These limitations in prediction can be overcome, at least theoretically, by using more than one marker, e.g. both an immune and a genetic marker. The potential of autoantibodies to IA-2 and GAD as predictors of Type I diabetes is discussed below. Although the predictive power, specificity and sensitivity of each of these autoantibodies alone usually falls short of 100 %, they could yet prove valuable in prediction when used in combination with other autoantibodies and with genetic screening. The predictive potential of these autoantibodies cannot be accurately estimated until large, long-term prospective studies, both in families and in the general population, have been completed.

Positive predictive values. The positive predictive values of autoantibodies can be estimated by calculating the number of subjects in a cohort with the autoantibody who develop Type I diabetes as a percentage of the overall number of autoantibody positive subjects. Predictions based on cross-sectional analyses must be verified in prospective studies. Of those reported prospective studies, none have been in a general population and all have had a limited period of follow-up (Table 1) [14, 15, 32, 33, 99–101]. Nevertheless, these family studies have yielded promising results. Thus, in a study over 12 years of 31 non-diabetic identical twins in whom 11 developed IDDM, the positive predictive value of IA-2 autoantibodies was 91 % and for GAD autoantibodies 88 % [14]. In 882 first degree relatives, 50 of whom developed diabetes, the positive predictive value at 5 years for autoantibodies to IA-2 (fragment 256–979) was 81 % and for autoantibodies to GAD 52 % [15]. These positive predictive

values might be increased further if only autoantibodies at high titre are considered. Positive predictive values of ICA are strongly related to the autoantibody titre, the higher the autoantibody titre the higher the predictive value [91, 94]. Autoantibodies to IA-2 and insulin also have increasing positive predictive values with increasing thresholds for positivity [94]. This relation between predictive power and autoantibody titre may, however, not hold for GAD [94].

Disease risk is most strongly related to the number of autoantibody markers present so that estimates of prediction are greatly improved by considering combinations of autoantibodies [14, 15, 29–31, 94–101]. The number of autoantibodies in combination to provide optimal prediction is yet to be established. All of the reported prospective studies of relatives of Type I diabetic patients found that combinations of two or more autoantibodies gave a higher positive predictive value than a single autoantibody alone (Table 2) [14, 15, 94, 95]. The positive predictive values in relatives with one, two or three autoantibodies was 2, 25 and 70 %, respectively in one study and 15, 44 and 100 %, respectively in another study [15, 94]. While current estimates of the positive predictive value of a combination of two autoantibodies in siblings and relatives falls short of 100 %, this figure will probably increase with longer follow-up as more autoantibody-positive subjects develop Type I diabetes (Table 2).

Specificity of prediction. Specificity of prediction with a disease marker reflects the chance that a person without that marker would remain unaffected. Specificity is important if a disease marker is to be used to identify people either for counselling or for therapy to prevent the disease from developing. Analysis of the autoantibody signals in normal subjects and Type I diabetic patients showed the expected reciprocal relation between sensitivity and specificity [29]. The higher the threshold for autoantibody positivity based on the normal population is the more specifically the autoantibody assay identifies patients with Type I diabetes, but at the cost of excluding patients with low autoantibody signals. Using two autoantibodies in combination increases the specificity of disease prediction but at the price of sensitivity; for example, in a prospective twin study the positive predictive value for a combination of IA-2 and GAD autoantibodies was 100 % but the sensitivity was only 55 %, i.e. 7 out of 12 twins [14] (Table 2).

Sensitivity of prediction. Sensitivity of prediction of a disease marker reflects the certainty that all people who develop the disease will have that marker. In four prospective studies following 3 578 first degree relatives, 105 developed diabetes of whom only 7 did not initially have an antigen-specific autoantibody giving a sensitivity of prediction, using an antigen-specific autoantibody, of 92 % (Fig. 3) [15, 31, 94, 95].

Table 2. The predictive values (%) of a combination of autoantibodies to GAD65 and IA-2 in non-diabetic subjects (identical twins, siblings or first degree relatives of Type I diabetic patients) followed prospectively [14, 15, 94]

Subjects	Number	Follow-up (years)	Prediction (PPV)	Specificity	Sensitivity
Twins	31	12	100	81	55
Siblings	755	7.7	41	95	81
Relatives	882	5	50	84	92

Estimates of: a) positive predictive value (PPV) based on calculating the number of relatives with autoantibodies in the initial sample who later developed Type I diabetes as a percentage of the overall number of relatives with the autoantibodies; b) specificity of prediction calculated by dividing the number of relatives without an autoantibody who did not develop

Type I diabetes by the total number of relatives who did not develop diabetes; c) sensitivity of prediction calculated by dividing the number of relatives with autoantibodies who developed diabetes by the overall number of relatives who developed diabetes

Since autoantibodies to IA-2 and GAD do not develop simultaneously and since many patients have only one antigen-specific autoantibody, using a panel of different autoantibodies should increase the sensitivity of prediction (Fig. 3; Table 2).

Why should autoantibody combinations be of any value as predictors? Presumably they reflect a spreading of the immune response to include more than one antigenic determinant with an associated increase in the risk of progression to Type I diabetes. Spreading of this immune response is possibly, in part, genetically determined [14]. Thus, the identification of genes associated with susceptibility to Type I diabetes could be valuable for disease prediction in conjunction with autoantibody screening. For example, non-diabetic relatives with ICA are unlikely to develop diabetes if they have the HLA allele DQB1*0602 [102]. The potential value of genetic screening is possibly reflected in the different positive predictive values for autoantibodies detected in identical twins (100%) as compared with siblings (41%) of patients with Type I diabetes (Table 2). Identification of those infants at birth who carry Type I diabetes-susceptibility genes would substantially reduce the numbers in a population who need to be screened for autoantibodies, and could increase the predictive value of a positive autoantibody test.

These observations hold out the prospect of population screening to identify people at high risk of developing diabetes using both genetic and autoantibody disease markers. A number of questions must, however be answered before screening becomes a reality.

Unresolved issues in prediction. The best age to screen the general population for Type I diabetes-associated autoantibodies is still not clear. Autoantibodies can appear very early in life and in many people can be detected by 5 years of age [5, 103]. Ideally screening should be done at birth and repeated at intervals thereafter. Alternatively a screening programme could test children aged 5 years, though a significant fraction of potential Type I diabetic patients

would be missed. Since the appearance of autoantibodies to different antigens appears to occur sequentially, disease risk based on autoantibody combinations will require repeated screening [103]. The screening strategies need to be flexible; for example, the predictive value of IA-2 autoantibodies, which are found more often in diabetic children than adults, probably varies with age [14, 32, 104].

Performance characteristics of autoantibody assays have an important influence on their predictive value. Of those autoantibodies currently used for prediction neither ICA nor insulin have provided consistent results, most likely due to technical differences in assay performance. Insulin autoantibodies, for example, gave a positive predictive value of 59% by 5 years follow-up in one family study but only 29% at 7.7 years in another study [15, 94]. Improving assay characteristics could improve their predictive value.

Some patients have ICA, but lack autoantibodies to IA-2, GAD or insulin. Thus, it is likely that other antigen-specific autoantibodies contribute to the ICA reaction and, once identified, could be valuable in disease prediction. Maximum predictive sensitivity and specificity in population screening may require testing of different sets of autoantibodies at different ages. Thus, in the context of predictive sensitivity, one study of recently diagnosed Type I diabetic patients detected multiple autoantibodies in 60% of patients aged less than 16 years but only 37% of older cases [96]. The close association between IA-2 and HLA DR4, the strongest single allele predisposing to Type I diabetes, suggests that IA-2 autoantibodies could be a more specific marker of the disease than GAD autoantibodies, which are associated with HLA DR3 as well as thyroid autoimmunity [104].

Concluding comments

Identification of an autoimmune response to specific autoantigens in the prediabetic period raises the prospect of prediction and prevention of Type I diabetes. The observations outlined here indicate just how far

we have progressed in identifying high risk subjects through antigen-specific autoantibodies. Screening the general population for Type I diabetes susceptibility is now feasible, though appropriate strategies have yet to be devised. It is likely that the predictive values for autoantibodies will be different in twin, family and population studies (Table 2) [29]. Potential limitations for prediction include the need for increased specificity with implies reduced sensitivity; in other words, we must exclude people at relatively low disease risk to find those in whom diabetes appears almost inevitable. Screening healthy populations to detect people at high risk is likely to become widespread if safe preventive measures become available.

Type I diabetes is probably due to immune-mediated destruction of insulin secreting cells containing antigens such as IA-2 and GAD. It is possible to induce immunological tolerance to antigens using a variety of strategies, including treatment with antigen peptides which bind to T-cell receptors [105]. Some of these approaches have been effective in animal models and have led to attempts to prevent the clinical onset of the disease (e.g. the Diabetes Prevention Trial DPT-1) [11, 13, 105]. The discovery of the Type I diabetes-associated antigens, IA-2 and GAD, certainly increases our chance of predicting the disease and might, in due course, enable us to prevent it.

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