Acta Diabetol (1997) 34: 185–193

REVIEW

E. Bonifacio · P. J. Bingley

Islet autoantibodies and their use in predicting insulin-dependent diabetes

Received: 24 April 1997 / Accepted: 14 May 1997

Key word Autoantibodies

Introduction

Predicting type 1 diabetes mellitus refers to estimation of the probability that an individual will develop the disease in his or her lifetime or within a defined period. Since it is merely probability, certainty cannot be attained. Therefore, while it is possible to provide reasonably precise measures of this probability, we must remember that some individuals with a similar prior risk as determined by the predictive model used will develop the disease and others will not. The objective of studies developing predictive markers, parameters and models is to be increasingly discriminatory in the ability to identify which subjects will develop type 1 diabetes and when they will develop the disease [1]. These studies and in particular the measures of disease probability which they provide are necessary for the design of meaningful, cost-effective clinical trials which test therapies aimed at disease prevention or delay, and also for providing adequate and realistic information to persons to whom the prediction is applied.

We know that the probability of developing type 1 diabetes is affected by a genetic predisposition such that the risk in discordant monozygotic twins is several-fold higher than that in offspring or siblings of type 1 diabetic patients, which in turn is 10–20 times higher than in a person with no immediate family history of disease [1]. Currently, genetic markers can only provide a relatively low estimate of disease probability. Moreover, since the concordance rate in monozygotic twins is only of the order of 50% [2, 3] it will not be possible to assign disease probabilities greater than this figure using genetic markers alone. The most effective markers for predicting type 1 diabetes have been autoantibodies against the endocrine cells within pancreatic islets [4]. These can be detected in the majority of type 1 diabetic patients at and prior to disease onset. The evaluation of these markers in prospectively followed first-degree relatives of type 1 diabetic patients has provided a reasonably accurate and precise estimation of the disease probability, enabling a good discrimination of 'progressors' to disease [5–8]. Several factors such as age [9, 10] and genetics [11] independently influence the disease probability in first-degree relatives. In persons with no immediate family history of type 1 diabetes, the source of the majority of new cases, we generally believe that we will be able to apply parameters and models derived from families in order to predict disease, but estimates of disease probability in this group [12–20] remain anecdotal, and we cannot yet provide scientifically validated probabilities. The aim here is to discuss the autoantibody markers available for predicting type 1 diabetes, and how they might be used in models and strategies to provide disease probabilities.

Invited lecture presented during the 6th International Milano Meeting on Diabetes held in Milan on 21–23 March 1996

E. Bonifacio (✉)
Department of Medicine I, Istituto Scientifico San Raffaele, Via Olgettina 60, I-20132 Milan, Italy

P. J. Bingley
Diabetic Medicine, Department of Medicine, University of Bristol, Bristol, UK

Autoantibody markers of type 1 diabetes

A marker of disease can be defined as something which is more prevalent in – or prior to – disease than in non-disease. Few markers are totally specific for disease. Most markers are found in both health and disease but with varying degrees of skewness towards disease. Many autoantibodies have been proposed as markers of type 1 diabetes [21], and these will be discussed.

Islet cell antibodies

The traditional autoantibody markers of type 1 diabetes, islet cell antibodies (ICA), can be detected in around

70%–90% of type 1 diabetic patients at and prior to disease onset, and between 0% and 5% of healthy control subjects [7, 14, 16, 18, 22, 23]. They were first identified in patients with autoimmunity to multiple endocrine organs [24] and can also be found in other endocrine autoimmune disorders, though with prevalences much lower than that found in type 1 diabetes. ICA are detected by indirect immunofluorescence on frozen, unfixed human pancreas sections [25]. They generally bind to molecules contained in all islet endocrine cells [26], but there is considerable heterogeneity in the staining patterns of ICA, and this has suggested that the antibodies have multiple and variable target molecules [26–28]. They are predominantly of the immunoglobulin G1 (IgG1) subclass [29], and if of sufficiently high titre, ICA of other IgG subclasses can be detected [30]. Like most IgG antibodies, they can fix complement [31].

The role of ICA as predictive markers has been more extensively studied than other type 1 diabetes-associated autoantibodies. Early prospective studies in first-degree relatives of type 1 diabetic patients showed that ICA could be detected several years prior to the onset of the clinical disease [32]. Later, the probability of developing disease for relatives was shown to be directly related to the titre of ICA; relatives having the highest antibody titres almost always developed the disease, and those with low titres had a much lower risk [9, 10, 33]. Nevertheless, not all those with very high ICA titres develop type 1 diabetes, and estimates from pooled retrospective data in the ICARUS study indicate that the probability that a first-degree relative with high titre ICA (>80 JDF units) will develop type 1 diabetes within 5 years after testing is 53% [10]. While searching for reasons to explain the differences in progression in relatives with similar antibody levels, it was found that not all ICA are identical. Some ICA were shown to also stain islets of both rat and mouse pancreas, while some rat but not mouse islets (restricted ICA) [27]. An independent study found that some ICA had a predominantly beta-cell selective staining pattern on human islets and could be inhibited by a preparation of brain homogenate, while others stained all islet cells and could not be inhibited by brain homogenate [26]. A third study also identified two distinct ICA staining patterns: granular and homogeneous [28]. These studies noted that one of the patterns (restricted, beta-selective or granular) was associated with a greatly reduced likelihood of progression to type 1 diabetes. The antigen specificity of this ICA was found to be the islet and brain autoantigen glutamic acid decarboxylase (GAD) (vide infra). ICA specificity provides, however, only a partial explanation of the heterogeneity in disease progression, since having excluded those with restricted or GAD-specific ICA only, the remainder with high titre ICA do not all develop type 1 diabetes [5].

A paradox of the relatively low risk associated with GAD-specific ICA is that GAD is also one of the specificities of ICA found in type 1 diabetes [34, 35]. Therefore, whilst on their own GAD-specific ICAs may indicate only a marginal risk for type 1 diabetes, together with other ICAs they are a marker of the disease. What are the other ICAs? One has been shown to be another islet and brain autoantigen, the protein tyrosine phosphatase-like molecule, insulinoma associated cDNA 2 (IA-2) and its homologue IA-2β (vide infra) [36, 37]. It has been suggested that GAD and IA-2/IA-2β are the major targets of ICAs associated with type 1 diabetes [37]. Inhibition of ICA with GAD and IA-2, however, shows reduction of ICA staining but completely inhibits the ICA found at onset of type 1 diabetes in less than 20% of cases (our unpublished observations). This demonstrates that there is at least one other major ICA target which requires identification.

Glutamic acid decarboxylase antibodies

These were originally detected as immunoprecipitating 64-kDa proteins from islet homogenates [38]. One of the proteins within the 64-kDa band immunoprecipitated by sera from type 1 diabetic patients is the 65,000 M_r isoform of GAD [39]. Antibodies to GAD65 are detected in 70%–80% of patients at and prior to onset of disease and in <3% of control subjects [7, 18, 19, 23, 40–42]. Antibodies are also detected in the majority of patients with the rare neurological disorder stiff-man syndrome (SMS) [43], in occasional patients with disorders involving a GABAergic dysfunction [44] and in a minority of patients with endocrine autoimmunity other than type 1 diabetes [45–47]. The identification of GAD65 as an autoantigen of the 64 K antibodies has allowed high throughput sensitive assays for antibody measurement to be developed [41, 42]. The most effective of these are the radiobinding assays which use either ^{125}I-labelled recombinant GAD65 or ^{35}S-methionine labelled, in vitro translated recombinant GAD65 [48]. There is some cross-reactivity of antibodies with the 67,000 M_r isoform of GAD, but few if any patients have antibodies to GAD67 in the absence of GAD65 reactivity [40, 49, 50]. The major isotype detected is IgG, and GAD antibodies of all IgG subclasses have been reported [51]. In type 1 diabetic patients these antibodies recognise several, predominantly conformational epitopes, while in SMS patients where titres are 10–1000-fold higher, other epitopes are also recognised [52–54]. No studies have adequately reported the probability of type 1 diabetes associated with the detection of GAD antibodies without prior selection with ICA. With the possible exception of those cases where ICA is solely due to the presence of GAD antibodies (restricted or GAD-specific ICA), relatives with ICA who also have GAD antibodies have a higher probability of developing type 1 diabetes than those with ICA alone [5–8, 43]. Moreover, some patients have GAD antibodies in the absence of ICA at or prior to onset of disease [5–8]. GAD antibodies are, therefore, important markers for type 1 diabetes prediction.

IA-2 and IA-2β antibodies

Not all that is immunoprecipitated in the 64-kDa band by type 1 diabetic sera is GAD. It was found that mild prote-

olysis of the immunoprecipitates yielded fragments of 50-kDa, 40-kDa and 37-kDa [55]. The 50-kDa fragment derived from GAD65, while the others did not [56]. Antibodies to the 40-kDa and/or 37-kDa tryptic fragments are found in 50%–75% of type 1 diabetic patients at and prior to disease onset and in <2% of control subjects [23, 55, 57]. Their detection in individuals with ICA is associated with a markedly increased probability of developing type 1 diabetes [5–8, 45, 57]. The 40-kDa and 37-kDa tryptic fragments derive from the related tyrosine phosphatase-like proteins IA-2 (ICA512) and IA-2β (phogrin, IAR) [58–62]. These are transmembrane proteins expressed in the secretory granule membranes of islet cells and other so-called neuroendocrine cells [63, 64]. Identification of the antigens has enabled assays similar to those for GAD antibodies to be developed [65]. These have confirmed the prevalence to be >50% in type 1 diabetic patients, being highest in those with a disease onset before the age of 15 years [4, 36]. Antibodies to IA-2 have also been detected in a small minority of patients with SMS in the absence of type 1 diabetes [66] but so far remain relatively specific for type 1 diabetes. Antibodies react primarily to IA-2, and most, but not all, of the reactivity to IA-2β is due to binding to epitopes which are shared between IA-2 and IA-2β protein tyrosine phosphatase domains [61, 67]. Studies in sequential samples suggest that the initial epitopes recognised are specific to IA-2, with subsequent spreading to those shared with IA-2β ([67], our unpublished observations). Antibodies recognise several epitopes, but these appear to be exclusively within the cytoplasmic portion of the molecules [68]. As for GAD antibodies, studies of IA-2 antibodies as predictive markers are mainly confined to the probability of disease when detected in combination with other markers such as ICA, GAD antibodies and insulin autoantibodies (IAA) [5–8]. In these studies the probability of disease is higher in subjects also having IA-2 antibodies than those with ICA alone.

Insulin autoantibodies

Insulin is the only beta-cell-specific autoantigen so far identified. Antibodies to insulin are detected in insulin-naive (untreated) patients [69], the prevalence being almost 100% in very young individuals and almost absent in patients with adult onset of type 1 diabetes [4, 70]. Measurement is only reliable with liquid phase radiobinding assays [71] and appears to be most sensitive in assays using very large serum volumes and prolonged incubation [72]. Not all binding detected by these assays is due to IgG [73], and measurement with assays using protein A [74] to detect immunocomplexes may be more specific than those using polyethylene glycol (PEG) precipitation. Antibodies to insulin also recognise proinsulin [75], and additional proinsulin-specific antibodies have been detected [76, 77]. Cross-reactivity with insulin-like growth factor has not been reported. Relatives with IAA have an increased probability of developing type 1 diabetes, and this probability is greatest when IAA are detected in combination with ICA [78].

Islet autoantibodies: the remainder

As outlined above, the ICA reactivity seen in sera from type 1 diabetic patients cannot be totally accounted for by GAD, IA-2 and insulin, and there is clearly at least one other major islet autoantibody marker. A large list of putative targets of islet autoantibodies have been reported, but not all of these have been confirmed. Early biochemical characterisation of ICA targets suggested molecules with glycolipid properties [79, 80], but the major ICA specificities thus far identified are proteins, and the specificity of the biochemical characterisation of the early studies suggesting glycolipid targets has been questioned [81]. Glycolipid molecules which have been thought to be islet autoantigens include sulphatides [82] and the sialoganglioside GM2-1 [83]. Antibodies to GM2-1, which are detected using a solid-phase assay, have been found to be associated with an increased probability of type 1 diabetes when present in relatives with ICA [84]. Their relationship to ICA remains unclear. No specific inhibition studies have been reported, and our own unpublished observations suggest that gangliosides purified from human islets do not inhibit ICA staining.

Apart from antibodies to GAD, IA-2/IA-2β and insulin, few of the putative islet autoantibodies have been detected by liquid-phase immunoprecipitation assays. One exception is 38 K antibodies which immunoprecipitate a 38-kDa membrane glycoprotein from islets [85]. These are found in only a few (less than 20%) patients at and prior to disease onset, but do appear to be associated with an increased probability of disease. Antibodies to a 155-kDa rat insulinoma protein can also be detected in a liquid-phase assay [86]. These antibodies inhibit the binding of a monoclonal antibody (1A2) to antigen and are found in up to 90% of patients and 4% of control subjects, but also in the

Table 1 Putative targets for autoantibodies in insulin-dependent diabetes (+ reactivity reported by at least one study, **+** antibodies detected also in liquid phase assays, ? no reports on T cell reactivity)

Antigen	Antibodies	T cells	References
Insulin	**+**	+	[69–72]
Proinsulin	**+**	+	[75, 76]
GAD-65	**+**	+	[39–42]
GAD-67	**+**	+	[49, 50]
IA-2 (ICA512)	**+**	+	[36, 58–60]
IA-2 beta/Phogrin	**+**	?	[61, 62]
38kDa antigen (GLIMA)	**+**	?	[85]
52kDa antigen	+	?	[100]
155kDa antigen	**+**	?	[86]
Ganglioside GM2-1	+	?	[83, 84]
Ganglioside GT3	+	?	[101]
Sulphatide	+	?	[82]
ICA69	+	+	[87–90]
Carboxypeptidase H	+	?	[102]
Glucose transporter (GLUT-2)	+	?	[103, 104]
Peripherin	+	?	[105]
Jun B	+	+	[106]
Topoisomerase II	+	?	[107]
ICA12	+	?	[108]

majority of relatives of type 1 diabetic patients. The identity of the target of the 1A2 monoclonal antibody is unknown. The remainder of those reported have been detected in solid phase assays such as Western blot, where binding is to partially denatured antigen at very high concentrations. One of these, ICA69 antibodies [87], has spawned considerable interest due to a region of similarity between the ICA69 protein and the cow milk protein bovine albumin. Antibodies to ICA69, however, cannot be detected by liquid-phase immunoprecipitation assays [88], are not specific for type 1 diabetes [89], and in a workshop were found not be discriminatory between sera from type 1 diabetic patients and control subjects [90].

Islet antibody measurements

Assays used to detect and quantify autoantibodies provide the user with signals which need to be interpreted. It is important to remember that these signals reflect not only the number of antibody molecules, but also other factors, including antibody avidity, the number of epitopes recognised, non-antibody binding molecules, etc. Moreover, it is virtually impossible to quantify how many antibody molecules a signal represents and therefore impossible to determine when there is or is not antibody in the sample tested. This can be illustrated by comparing two assays which measure the same antibody. If we measure GAD antibodies using an enzyme-linked immunosorbent assay (ELISA), we would detect antibodies in only around 30% of type 1 diabetic patients, while if we used a radiobinding assay (RBA), we would detect antibodies in around 75% of patients [48]. We cannot conclude therefore that the absence of signal in the ELISA equates to no antibodies since we assume that the presence of signal in the RBA does indicate antibodies. Similarly, we cannot conclude that the absence of signal in RBA means no antibodies. We can take this further by saying that the absence of signal in a RBA assay today does not mean an absence of signal in a RBA assay tomorrow since the minimum number of antibody molecules the assay will detect varies from day to day. Rather than be discouraged by this, we need to grasp that our role is not to determine the presence or absence of antibody, but to ascribe to the assay readout diagnostic or prognostic meaning (probability of disease or health).

Much effort is expended on determining thresholds of positivity. This is useful for simplifying risk calculations, but tends to dichotomise the signals of an assay into present or absent, something we know is not true. There are several ways in which thresholds which distinguish the signals obtained in the majority of healthy individuals from those in the majority of patients can be calculated. Parametric methods based on mean and standard deviations in healthy control subjects are common, but the distribution of signals from autoantibody assays are rarely Gaussian, and therefore, unless signals are first transformed so that they fit a normal distribution, these methods are probably inadequate. An alternative method is to plot the distribution of the signals before or after log-transformation on a normal plot [91]. In that case it is also possible to pool signals from both patients and control subjects. Non-parametric methods are also common and are usually based on the interpolation of results from a distribution histogram to determine, for example, the upper 99th centile of control subjects. Other methods such as receiver-operating characteristics (ROC) plots [92] are very useful for comparing performances between different assays, but are not particularly helpful in determining thresholds. Each method for determining a threshold may not give identical endpoints. When we examined the measurements of IA-2 antibodies in 2801 school children, the mean plus 3SD corresponded to 20.4 units, the mean plus 3SD of log-transformed measurements 1.7 units, and the 99th centile 1.3 units. The proportion of new onset type 1 diabetic cases identified above these levels are 61%, 70% and 72%, respectively. One of the problems in determining thresholds from signals in control subjects for islet antibody measurements is that we cannot be sure that some of the subjects will not develop type 1 diabetes in the future. The use of the normal plot, in which signals falling outside the Gaussian distrubution can be distinguished, may partially overcome this problem.

In general, we would discourage the use of thresholds, as they dichotomise results into positive and negative. The information that can be obtained from the assay readouts will be most helpful if we treat the signals as a continuous or semi-continuous variable. We know that the probability of disease differs depending upon the magnitude of the signal. For example, in the ICA assay, a measurement of 5 JDF units is associated with a lower probability of developing type 1 diabetes than is a signal of 20 JDF units, which is in turn lower than that of >80 JDF units [9, 10, 33]. If the assay is a screening assay designed to exclude those with very a low probability of developing type 1 diabetes from further testing, then we would be more likely to choose a low threshold so as to avoid excluding too many subjects who will develop the disease. If, on the other hand, we want to select those with sufficient risk to enter into a clinical trial, a higher threshold is more appropriate so that not too many are treated unnecessarily. The use of several thresholds discriminating different probabilities of developing the disease will provide this flexibility. We would again stress that it is far better to view islet autoantibody measurements in terms of their probability of disease rather than the presence or absence of antibody. Finally, we give a warning to use caution when interpreting autoantibody measurements for purposes other than disease prediction or diagnosis, e.g. determining chronology of antibody appearance. It is tempting to conclude that because one autoantibody is usually detected earlier than another, the autoimmune response also occurs earlier. However, each islet autoantibody marker is measured by unique assays, and these assays will vary considerably in their ability to detect antibody. For example, just as low levels of GAD antibodies are not detected by some ELISAs, the non-GAD, non-IA-2 ICA antibodies which are currently detected in the indirect immunofluorescence assay will very likely be

easier to detect in radiobinding assays with specifically labelled antigen once it becomes available; the IAA assay which uses large serum volumes and several days of incubation may detect fewer antibody molecules than the radiobinding assays measuring GAD and IA-2 antibodies. It is not inconceivable that IAA may be detected earlier than ICA because of the assay used rather than autoimmunity occurring earlier.

Screening strategies based on autoantibody measurement

Several strategies can be applied. We cannot determine which is best, however, until we know when autoantibodies are first detectable and until sufficient numbers of people have been followed to disease onset. This can only be achieved with a long prospective follow-up of individuals from birth with sequential antibody measurement. These studies are in progress [93–95], and meanwhile we can make educated guesses based upon cross-sectional antibody distributions before and at onset of disease.

We have already discussed that there are currently four principle antibody markers available: ICA, IAA, GAD antibodies and IA-2/IA-2β antibodies. Studies show that ICA are detected in up to 90% of patients with only minor variations with respect to age or sex [23]. IAA are detected in the majority of patients with very young onset of disease, and are less prevalent in older onset patients [70]. GAD antibodies are detected in around 75% of patients, and are more prevalent in older onset patients, while IA-2 antibodies are detected in around 65% of patients and are more prevalent in younger onset patients (Fig. 1). GAD and IA-2 antibodies are therefore complementary, and one or the other is detected in over 90% of patients [8, 23, 97].

The first step in developing a screening strategy is to identify the initial screening test. This test will be applied to a very large number of samples and therefore should be cheap, relatively easy to perform and able to identify the majority of those who will develop type 1 diabetes. From the cross-sectional observations it is tempting to conclude that most patients have IAA early in life and that these gradually disappear over time. We cannot, however, be certain of this, and the most effective screening test is likely to be a combined GAD/IA-2 antibody test. The advantage of this is that both antibodies can be screened for in the same test [20, 36, 97, 98], and that measurement can be performed on whole capillary blood samples equally well as on serum [98, 99]. Currently, IAA cannot be incorporated into the same test, and even if its addition will lead to the detection of more cases early in life, it remains unclear how cost effective its addition in the initial screening will be.

The second step is to identify tests which can be applied to those selected by the screening test. After creating antigen-specific autoantibody assays, it has become clear that the probability of developing type 1 diabetes is not only related to the amount of antibody detected, but probably more so to the number of autoantibody markers detected [5–8]. Studies in type 1 diabetic families, schoolchildren and patients at and prior to disease onset show that in most patients or individuals who develop type 1 diabetes, two or more of the antibody markers are detected, and only a few have just one marker [4–8, 17, 85]. In contrast, most relatives or schoolchildren selected on the basis of elevated levels of either ICA or GAD/IA-2 antibodies have only a single marker. Therefore, those relatives, neonates, infants or schoolchildren in whom none or only one of the markers is detected have a low probability of developing type 1 diabetes. The presence of at least two markers is associated with a relatively high probability [50% or more) of disease [4–8], and the highest risk is found in those with three or four antibody markers. From these studies, it is clear that measurement of IAA and ICA in those selected on the basis of elevated levels of GAD and/or IA-2 antibodies in the screening test will provide a useful discrimination of type 1 diabetes probability. IAA is essential if

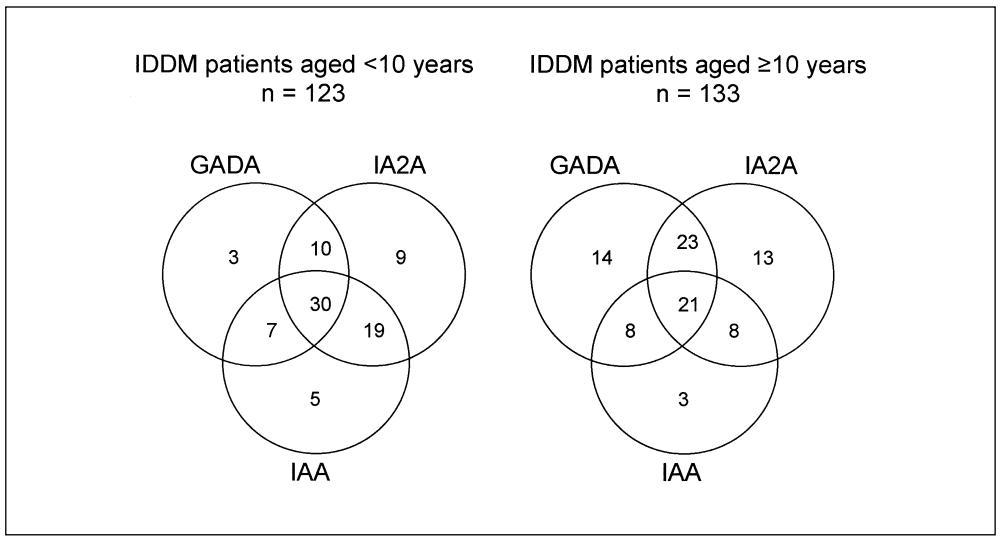

Fig. 1 The prevalence (%) of glutamicacid decarboxylase (GAD) antibodies, IA-2 antibodies and insulin autoantibodies (IAA) above the 99th centile of control subjects and antibody combinations at onset of disease in 256 type 1 diabetic patients from the Oxford region

type 1 diabetes prediction is performed in young individuals. The additional benefit of measuring ICA early in life is not clear. Vice versa, the benefit of measuring IAA in individuals over 15 years old is questionable because of their low prevalence at this age, and here ICA are still essential. Whether ICA should be considered an additional marker has been questioned [6] since in some cases the ICA is due entirely to the presence of GAD antibodies [26, 34, 35]. We would argue that this is uncommon and that in the majority of cases ICA recognising additional antigens is also present as demonstrated by competition studies (Bonifacio, unpublished observations). In addition, relatives with only ICA and GAD antibodies in the Barts-Oxford family study do have a probability of developing type 1 diabetes which is greater than those with just elevated GAD antibodies or ICA alone (Bingley, unpublished observations), and a substantial number (5 of 24) of relatives who develop type 1 diabetes had only ICA plus GAD antibodies or ICA plus IA-2 antibodies in samples prior to disease onset (Bingley, unpublished observations).

Another consideration is when and how often should autoantibody screening be performed. Data from prospective studies from birth onwards indicate that autoantibodies can be detected in the first years of life [93, 94], and therefore screening could start as early as 1 year of age. How often thereafter and for how long remains uncertain. We must also remember that the levels of antibody markers, and therefore also the number of markers detected in a single individual, can change over time. Other considerations such as whether antibody screening should be restricted to those in whom an a priori selection using genetic markers [95] has already been done are also unresolved. Clearly, this may have the benefit of reducing antibody screening costs, but how efficient this will be depends upon how effective (sensitive and specific) the genetic screen is.

In conclusion, whilst several islet autoantibodies have been reported, not all of them are associated with type 1 diabetes, and fewer still will be useful in its prediction. Currently, ICA, IAA, GAD antibodies and IA-2/IA-2β antibodies are the only established antibody markers for this purpose. Their combined use has improved our ability to predict type 1 diabetes and will allow the probability of developing the disease to be quantified based on the level and number of antibody markers detected. Useful screening strategies for both type 1 diabetic families and the general population can be proposed which first take advantage of a single test such as the combined GAD/IA-2 antibody test which has a high sensitivity to exclude those with a very low probability of developing type 1 diabetes from further testing, and second apply ICA and/or IAA to the remainder in order to discriminate those with the highest probability of disease. Prediction and prediction strategies should improve when the remainder of the ICA specifities are identified and when studies of autoantibody and genetic markers in sequential samples starting with birth are completed.

References

1. Bingley PJ, Bonifacio E, Gale EAM, Can we really predict IDDM? Diabetes 42:213–220, 1993
2. Barnett AH, Leslie RDG, Pyke DA, Diabetes in identical twins: a study of 200 pairs. Diabetologia 20:87, 1981
3. Kyvik KO, Green A, Beck-Nielsen H, Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. Br Med J 311:913–917, 1995
4. Roll U, Ziegler AG, Combined antibody screening for improved prediction of IDDM – modern strategies. Exp Clin Endocrinol Diabetes 105:1–14, 1997
5. Bingley PJ, Christie MR, Bonifacio E, Bonfanti R, Shattock M, Fonte MT, Bottazzo GF, Gale EAM, Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. Diabetes 43:1304–1310, 1994
6. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, Chase HP, Eisenbarth GS, Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. Diabetes 45:926–933, 1996
7. Gorus FK, Goubert P, Semaluka C, Vandewalle CL, De Schepper J, Scheen A, Christie MR, Pipeleers DG, and the Belgian Registry, IA-2 autoantibodies complement GAD65-autoantibodies in new onset IDDM patients and help predict impending diabetes in their siblings. Diabetologia 40:99–99, 1997
8. Christie MR, Roll U, Payton MA, Hatfield ECI, Ziegler AG, Validity of screening for individuals at risk for type 1 diabetes by combined analysis of autoantibodies to recombinant proteins. Diabetes Care 1997 (in press)
9. Riley WJ, Maclaren NK, Krischer J, Spillar RP, Silverstein JH, Schatz DA, Schwartz S, Malone J, Shah S, Vadheim C, Rotter JI, A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes. N Engl J Med 323:1167–1172, 1990
10. Bingley PJ, for the ICARUS group, Interaction of age, islet cell antibodies, insulin autoantibodies, and first-phase insulin response in predicting risk of progression to IDDM in ICA^+ relatives. Diabetes 45:1720–1728, 1996
11. Lipton RB, Kocova M, LaPorte RE, Dorman JS, Orchard TJ, Riley WJ, Drash AL, Becker DJ, Trucco M, Autoimmunity and genetics contribute to the risk of insulin-dependent diabetes mellitus in families: islet cell antibodies and HLA DQ heterodimers. Am J Epidemiol 136:503, 1992
12. Bruining GJ, Molenaar JL, Grobbee DE, Hofman A, Scheffer GJ, Bruining HA, Bruyn AM de, Valkenburg HA, Ten-year follow-up study of islet cell antibodies and childhood diabetes mellitus. Lancet 1:1100–1103, 1989
13. Bingley PJ, Bonifacio E, Shattock M, Gillmor HA, Sawtell PA, Dunger DB, Scott RDM, Bottazzo GF, Gale EAM, Can islet cell antibodies predict IDDM in the general population? Diabetes Care 16:45–50, 1993
14. Karjalainen J, Islet cell antibodies as predictive markers for IDDM in children with high background incidence of disease. Diabetes 39:1144–1150, 1990
15. Levy-Marchal C, Tichet J, Fajardy I, Gu XF, Dubois F, Czernichow P, Islet cell antibodies in normal French schoolchildren. Diabetologia 35:577–582, 1992
16. Schatz D, Krischer J, Horne G, Riley W, Spillar R, Silverstein J, Winter W, Muir A, Derovanesian D, Shah S, Malone J, Maclaren N, Islet cell antibodies predict insulin dependent diabetes in United States school age children as powerfully as in unaffected relatives. J Clin Invest 93:2403–2407, 1994
17. Genovese S, Bingley PJ, Bonifacio E, Christie MR, Shattock M, Bonfanti R, Foxon R, Gale EAM, Bottazzo GF, Combined analysis of IDDM-related autoantibodies in healthy schoolchildren. Lancet 344:756, 1994
18. Hagopian WA, Sanjeevi CB, Kockum I, Landin-Olsson M, Karlsen AE, Sundkvist G, Dahlquist G, Palmer J, Lernmark A, Glutamate decarboxylase-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. J Clin Invest 95:1505–1511, 1995

19. Verge CF, Howard NJ, Rowley MJ, Mackay IR, Zimmet PZ, Egan M, Hulinska H, Hulinsky I, Silvestrini RA, Kamathm S, Sharp A, Arundel T, Silink M, Anti-glutamate decarboxylase and other antibodies at the onset of childhood IDDM: a population-based study. Diabetologia 37:1113–1120, 1994
20. Wiest-Ladenburger U, Hartmann R, Hartmann U, Berling K, Bohm BO, Richter W, Combined analysis and single-step detection of GAD65 and IA2 autoantibodies in IDDM can replace the histochemical islet cell antibody test. Diabetes 46:565–571, 1997
21. Bosi E, Bonifacio E, Autoantibodies in insulin-dependent diabetes mellitus. J Endocrinol Invest 17:521–531, 1994
22. Boehm BO, Manfras B, Seissler J, Schoeffling K, Glueck M, Holzberger G, Seidl S, Kuehnl P, Trucco M, Scherbaum WA, Epidemiology and immunogenetic background of islet cell antibody-positive nondiabetic schoolchildren. Diabetes 40:1435–1439, 1991
23. Bonifacio E, Genovese S, Braghi S, Bazzigaluppi E, Lampasona V, Bingley PJ, Rogge L, Pastore MR, Bognetti E, Bottazzo GF, Gale AEM, Bosi E, Islet autoantibody markers in insulin dependent diabetes: risk assessment strategies yielding high sensitivity. Diabetologia 38:816–822, 1995
24. Bottazzo GF, Florin-Christensen A, Doniach D, Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiency. Lancet 2:1279–1283, 1974
25. Gleichmann H, Bottazzo GF, Progress towards standardization of cytoplasmic islet cell antibody assays. Diabetes 36:578–584, 1987
26. Genovese S, Bonifacio E, McNally J, Wagner R, Dean BM, Bosi E, Gale EAM, Bottazzo GF, Distinct cytoplasmic islet cell antibodies with different risks for type I (insulin dependent) diabetes. Diabetologia 35:385–389, 1992
27. Gianani R, Pugliese A, Bonner-Weir S, Shiffrin AJ, Soeldner JS, Erlich H, Awdeh Z, Alper CA, Jackson RA, Eisenbarth GS, Prognostically significant heterogeneity of cytoplasmic islet cell antibodies in relatives of patients with type I diabetes. Diabetes 41:347–353, 1992
28. Timsit J, Caillat-Zucman S, Blondel H, Chedin P, Bach JF, Boitard C, Islet cell antibody heterogeneity among Type 1 (insulin-dependent) diabetic patients. Diabetologia 35:792–795, 1992
29. Dean BM, Bottazzo GF, Cudworth AG, IgG subclass distribution in organ specific autoantibodies. The relationship to complement fixing ability. Clin Exp Immunol 52:61–66, 1983
30. Dozio N, Belloni C, Girardi AM, Genovese S, Sodoyez JC, Bottazzo GF, Pozza G, Bosi E, Heterologous IgG subclass distribution of islet cell antibodies. J Autoimmun 7:45–53, 1994
31. Bottazzo GF, Dean BM, Gorsuch AN, Cudworth AG, Doniach D, Complement-fixing islet-cell antibodies in Type 1 diabetes: possible monitors of active beta-cell damage. Lancet 1:668–672, 1980
32. Gorsuch AN, Spencer KM, Lister J, McNally JM, Dean BM, Bottazzo GF, Cudzorth AG, Evidence for a long pre-diabetic period in type 1 (insulin-dependent) diabetes mellitus. Lancet 2:1363–1365, 1981
33. Bonifacio E, Bingley PJ, Dean BM, Shattock M, Dunger D, Gale EAM, Bottazzo GF, Quantification of islet cell antibodies and prediction of insulin-dependent diabetes. Lancet 335:147–149, 1990
34. Atkinson MA, Kaufman DL, Newman D, Tobin AJ, Maclaren NK, Islet cell cytoplasmic autoantibody reactivity to glutamate decarboxylase in insulin-dependent diabetes. J Clin Invest 91: 350–356, 1993
35. Marshall MO, Moyer PE, Petersen JS, Hejnaes KR, Genovese S, Dyrberg T, Bottazzo GF, Contribution of glutamate decarboxylase antibodies to the reactivity of cytoplasmic islet cell antibodies. J Autoimmun 7:497–501, 1994
36. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E, Identification of protein tyrosine phosphatase-like IA-2 (Islet Cell Antigen 512) as the insulin-dependent diabetes-related 37/40 K autoantigen and a target of islet-cell antibodies. J Immunol 155: 5419–5426, 1995
37. Myers MA, Rabin DU, Rowley MJ, Pancreatic islet cell cytoplasmic antibody in diabetes is represented by antibodies to islet cell antigen 512 and glutamic acid decarboxylase. Diabetes 44:1290–1295, 1995
38. Baekkeskov S, Nielson JH, Marner B, Bilde T, Ludvigsson J, Lernmark A, Autoantibodies in newly diagnosed diabetic children immunoprecipitate human islet cell protein. Nature 298: 167–169, 1982
39. Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P, Identification of the 64 K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 347:151–156, 1990
40. Grubin CE, Daniels T, Toivola B, Landin-Olsson M, Hagopian WA, Li L, Karlsen AE, Boel E, Michelsen B, Lernmark A, A novel radiobinding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. Diabetologia 37:344–350, 1994
41. Petersen JS, Hejnaes KR, Moody A, Karlsen AE, Marshall MO, Hoier-Madsen M, Boel E, Michelsen BK, Dyrberg T, Detection of GAD65 antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. Diabetes 43:459–467, 1994
42. Aanstoot HJ, Sigurdsson E, Jaffe M, Shi Y, Christgau S, Grobbee D, Bruining GJ, Molenaar JL, Hofman A, Baekkeskov S, Value of antibodies to GAD65 combined with islet cell cytoplasmic antibodies for predicting IDDM in a childhood population. Diabetologia 37:917–924, 1994
43. Solimena M, Folli F, Aparisi R, Pozza G, De Camilli P, Autoantibodies to GABA-ergic neurons and pancreatic beta cells in stiff-man syndrome. N Engl J Med 322:1555–1560, 1990
44. Nemni R, Braghi S, Natali-Sora MG, Lampasona V, Bonifacio E, Comi G, Canal N, Autoantibodies to glutamic acid decarboxylase in palatal myoclonus and epilepsy. Ann Neurol 36:665–667, 1994
45. Christie MR, Genovese S, Cassidy D, Bosi E, Brown TJ, Lai M, Bonifacio E, Bottazzo GF, Antibodies to islet 37k antigen, but not to glutamate decarboxylase, discriminate rapid progression to IDDM in endocrine autoimmunity. Diabetes 43:1254–1259, 1994
46. Betterle C, Presotto F, Magrin L, Pedini B, Moro L, Caretto A, Zanchetta R, The natural history of pre-type 1 (insulin-dependent) diabetes mellitus in patients with autoimmune endocrine diseases. Diabetologia 37:95–103, 1994
47. Tuomi T, Bjorses P, Falorni A, Partanen J, Perheentupa J, Lernmark A, Miettinen A, Antibodies to glutamic acid decarboxylase and insulin-dependent diabetes in patients with polyendocrine syndrome type I. J Clin Endocrinol Metab 81:1488–1494, 1996
48. Schmidli R, Colman PG, Bonifacio E, and participating laboratories, Disease sensitivity and specificity of fifty-two assays for glutamic acid decarboxylase antibodies: the second international GADAb workshop. Diabetes 44:636–640, 1995
49. Hagopian WA, Michelsen B, Karlsen AE, Larsen F, Moody A, Grubin CE, Rowe R, Petersen J, McEvoy R, Lernmark A, Autoantibodies in IDDM primarily recognize the 65,000-M_r rather than the 67,000-Mr isoform of glutamic acid decarboxylase. Diabetes 42:631–636, 1993
50. Velloso LA, Kampe O, Hallberg A, Christmanson L, Betsoltz C, Karlsson FA, Demonstration of GAD-65 as the main immunogenic isoform of glutamate decarboxylase in type 1 diabetes and determination of autoantibodies using a radioligand produced by eucaryotic expression. J Clin Invest 91:2084–2090, 1993
51. Petersen J, Knip M, Kumala P, Dyrberg T, and the Childhood Diabetes in Finland Study Group, GAD65 antibody isotypes in first degree relatives suggest a TH2 immune response in non-progressors. Diabetologia 39 [Suppl 1]:A92, 1996 (abstract)
52. Richter W, Shi Y, Bakkeskov S, Autoreactive epitopes defined by diabetes-associated human monoclonal antibodies are localized in the middle and C-terminal domains of the smaller form of glutamate decarboxylase. Proc Natl Acad Sci USA 90:2832–2836, 1993

53. Butler MH, Solimena M, Dirkx R, Hayday A, De Camilli P, Identification of a dominant epitope of glutamic acid decarboxylase (GAD-65) recognized by autoantibodies in Stiff-man syndrome. J Exp Med 178:2097–2106, 1993
54. Daw K, Ujihara N, Atkinson M, Powers AC, Glutamic acid decarboxylase autoantibodies in stiff-man syndrome and insulin-dependent diabetes mellitus exhibit similarities and differences in epitope recognition. J Immunol 156:818–825, 1996
55. Christie MR, Vohra G, Champagne P, Daneman D, Delovitch TL, Distinct antibody specificities to a 64-kD islet cell antigen in type I diabetes as revealed by trypsin treatment. J Exp Med 172:789–795, 1990
56. Christie MR, Hollands JA, Brown TJ, Michelsen BK, Delovitch TL, Detection of pancreatic islet 64,000 Mr autoantigens in insulin-dependent diabetes distinct from glutamate decarboxylase. J Clin Invest 92:240–248, 1993
57. Ongagna JC, Lévy-Marchal C, Anti-37 kDa antibodies are associated with the development of IDDM in individuals with islet cell antibodies. Diabetologia 38:370–372, 1995
58. Rabin DU, Pleasic SM, Shapiro JA, Yoo-Warren H, Oles J, Hicks JM, Goldstein DE, Rae PMM, Islet cell antigen 512 is a diabetes-specific autoantigen related to protein tyrosine phosphatases. J Immunol 152:3183–3188, 1994
59. Payton MA, Hawkes CJ, Christie MR, Relationship of the 37,000- and 40,000-M(r) tryptic fragments of islet antigens in insulin-dependent diabetes to the protein tyrosine phosphatase-like molecule IA-2 (ICA512). J Clin Invest 96:1506–1511, 1995
60. Passini N, Larigan DG, Genovese S, Appella E, Sinigaglia F, Rogge L, The 37/40-kilodalton autoantigen in insulin dependent diabetes mellitus is the putative tyrosine phosphatase IA-2. Proc Natl Acad Sci USA 92:9412–9416, 1995
61. Lu J, Li Q, Xie H, Chen ZJ, Borovitskaya AE, Maclaren NK, Notkins AL, Lan MS, Identification of a second transmembrane protein tyrosine phosphatase IA-2beta, as an autoantigen in insulin-dependent diabetes mellitus: precursor of the 37-kDa tryptic fragment. Proc Natl Acad Sci USA 93:2307–2311, 1996
62. Hawkes CJ, Wasmeier C, Christie MR, Hutton JC, Identification of the 37-kDa antigen in IDDM as a tyrosine phosphatase-like protein (Phogrin) related to IA-2. Diabetes 45:1187–1192, 1996
63. Solimena M, Dirkx R, Hermel J-M, Pleasic-Williams S, Shapiro JA, Caron L, Rabin DU, ICA512, an autoantigen of type I diabetes, is an intrinsic membrane protein of neurosecretory granules. EMBO J 15:2102–2114, 1996
64. Wasmeier C, Hutton JC, Molecular cloning of phogrin, a protein tyrosine phosphatase homologue localised to insulin secretory granule membranes. J Biol Chem 271:18161–18170, 1996
65. Gianani, R, Rabin DU, Verge CF, Yu L, Babu S, Pietropaolo M, Eisenbarth GS, Recombinant ICA512 radioassay. Diabetes 44:1340–1344, 1995
66. Martino G, Grimaldi LM, Bazzigaluppi E, Passini N, Sinigaglia F, Rogge L, The insulin-dependent diabetes mellitus-associated ICA 105 autantigen in stiff-man syndrome patients. J Neuroimmunol 69:129–134, 1996
67. Lampasona V, Bonifacio E, Bingley P, Gale E, Development of IA-2 and IA-2b autoantibody epitope specificities in sequential samples from relatives. Autoimmunity 24 [Suppl 1]:8, 1996 (abstract)
68. Lampasona V, Bearzatto M, Genovese S, Bosi E, Ferrari M, Bonifacio E, Autoantibodies in insulin-dependent diabetes recognize distinct cytoplasmic domains of the protein tyrosine phosphatase-like IA-2 autoantigen. J Immunol 157:2707–2711, 1996
69. Palmer JP, Asplin CM, Clemons P, Lyen K, Tatpati O, Raghu PK, Paquette TL, Insulin antibodies in insulin-dependent diabetics before insulin treatment. Science 222:1337–1339, 1983
70. Vardi P, Ziegler AG, Mathews JH, Dib S, Keller RJ, Ricker AT, Wolfsdorf JI, Herkowitz RD, Rabizadeh A, Eisenbarth GS, Soeldner JS, Concentration of insulin autoantibodies at onset of type 1 diabetes: inverse linear correlation with age. Diabetes Care 11:736–739, 1988
71. Greenbaum CJ, Palmer JP, Kuglin B, Kolb H, and participating laboratories, Insulin autoantibodies measured by radioimmunoassay methodology are more related to insulin dependent diabetes mellitus than those measured by enzyme-linked immunosorbent assay: results of the Fourth International Workshop on the standardization of insulin autoantibody measurement. J Clin Endocrinol Metab 74:1040–1044, 1992
72. Vardi P, Dib SA, Tuttleman M, Connelly JE, Grindsberg M, Rabizadeh A, Riley WJ, Maclaren NK, Eisenbarth GS, Soeldner JS, Competitive insulin autoantibody assay: prospective evaluation of subjects at risk for development of type I diabetes mellitus. Diabetes 36:1286–1291, 1987
73. Bilbao JR, Calvo B, Urrutia I, Linares A, Castano L, Anti-insulin activity in normal newborn cord-blood serum: absence of IgG-mediated insulin binding. Diabetes 46:713–716, 1997
74. Williams AJK, Bingley PJ, Bonifacio E, Palmer JP, Gale EAM, A novel micro-assay for insulin autoantibodies. J Autoimmunity 1997 (in press)
75. Keilacker H, Rjasanowski I, Besch W, Kohnert KD, Autoantibodies to insulin and to proinsulin in type 1 diabetic patients and in at-risk probands differentiate only little between both antigens. Horm Metab Res 27:90–94, 1995
76. Kuglin B, Gries FA, Kolb H, Evidence of IgG autoantibodies against human proinsulin in patients with IDDM before insulin treatment. Diabetes 37:130–132, 1988
77. Bohmer K, Keilacker H, Kuglin B, Hubinger A, Bertrams J, Gries FA, Kolb H, Proinsulin autoantibodies are more closely associated with type 1 (insulin-dependent) diabetes mellitus than insulin autoantibodies. Diabetologia 34:830–834, 1991
78. Ziegler AG, Ziegler R, Vardi P, Jackson RA, Soeldner JS, Eisenbarth GS, Life-table analysis of progression to diabetes of anti-insulin autoantibody-positive relatives of individuals with Type 1 diabetes. Diabetes 38:1320–1325, 1989
79. Nayak RS, Omar MAK, Rabizadeh A, Srikanta S, Eisenbarth GS, 'Cytoplasmic' islet cell antibodies: evidence that the target antigen is a sialoglycoconjugate. Diabetes 34:617–619, 1984
80. Colman PG, Nayak RC, Campbell IL, Eisenbarth GS, Binding of 'cytoplasmic' islet cell antibodies is blocked by human pancreatic glycolipid extracts. Diabetes 37:645–649, 1988
81. Richter W, Eiermann TH, Endl J, Seissler J, Wolfahrt S, Brandt M, Jungfer H, Scherbaum WA, Human monoclonal islet specific autoantibodies share features of islet cell and 64 kDa antibodies. Diabetologia 36:785–790, 1993
82. Buschard K, Fredman P, Sulphatide as an antigen in diabetes mellitus. Diab Nutr Metab 9:221–228, 1996
83. Dotta F, Dionisi S, Misasi R, Tiberti C, Anastasi E, Carabba B, Farilla L, Di Mario U, Ganglioside antigens in autoimmune diabetes. Diabetes Nutr Metab 9:215–220, 1996
84. Dotta F, Gianani R, Previti M, Lenti L, Dionisi S, D'Erme M, Eisenbarth GS, Di Mario U, Autoimmunity to the GM2-1 islet ganglioside before and at the onset of type I diabetes. Diabetes 45:1193–1196, 1996.
85. Aanstoot H-J, Kang S-M, Kim J, Lindsay L, Roll U, Knip M, Atkinson M, Mose-Larsen P, Fey S, Ludvigsson J, Landin M, Bruining J, Maclaren N, Åkerblom HK, Baekkeskov S, Identification and characterization of Glima 38, a Glycosylated Islet Cell Membrane Antigen, which together with GAD65 and IA2 marks the early phases of autoimmune response in type 1 diabetes. J Clin Invest 97:2772–2783, 1996
86. McEvoy RC, Thomas NM, Ness J, Humoral immune markers: additional islet cell antigens – important clues or red herrings? In: Palmer J (ed) Diabetes prediction, prevention and genetic counseling in IDDM. Wiley, pp 97–107, 1996
87. Pietropaolo M, Castano L, Babu S, Buelow R, Kuo YLS, Martin S, Martin A, Powers AC, Prochazka M, Naggert J, Leiter EH, Eisenbarth GS, Islet cell autoantigen 69 kD (ICA69). Molecular cloning and characterization of a novel diabetes-associated autoantigen. J Clin Invest 92:359–371, 1993

88. Lampasona V, Ferrari M, Bosi E, Pastore RM, Bingley PJ, Bonifacio E, Sera from patients with IDDM and healthy individuals have antibodies to ICA69 on western blots but do not immunoprecipitate liquid phase antigen. J Autoimmun 7:665–674, 1994
89. Martin S, Kardorf J, Schulte B, Lampeter EF, Gries FA, Melchers I, Wagner R, Bertrams J, Roep BO, Pfutzner A, Pietropaolo M, Kolb H, Autoantibodies to the islet antigen ICA69 occur in IDDM and in rheumatoid arthritis. Diabetologia 38:351–355, 1995
90. Martin S, Lampasona V, Dosch M, Pietropaolo M, Islet cell autoantigen 69 antibodies in IDDM. Diabetologia 39:747, 1996
91. Dean BM, McNally J, Bonifacio E, Bingley PJ, Gale EAM, Bottazzo GF, Autoantibodies to human pro-insulin in first degree relatives of Type 1 (insulin-dependent) diabetic probands. Diabetes Nutr Metab 4:177–182, 1992
92. Zweig M, Campbell G, Receiver-operating characteristics (ROC) plots: a fundamental tool in clinical medicine. Clin Chem 39:561–567, 1995
93. Ziegler AG, Hillebrand B, Rabl W, Mayrhofer M, Hummel M, Mollenhauer U, Vordemann J, Lenz A, Standl E, On the appearance of islet associated autoimmunity in offspring of diabetic mothers: a prospective study from birth. Diabetologia 36:402–408, 1993
94. Roll U, Christie MR, Fuchtensbusch M, Payton MA, Hawkes CJ, Ziegler AG, Perinatal autoimmunity in offspring of diabetic parents: the German Multicenter BABY-DIAB Study: detection of humoral immune responses to islet autoantigens in early childhood. Diabetes 45:967–973, 1996
95. Rewers M, Bugawan TL, Norris JM, Blair A, Beaty B, Hoffman M, McDuffie RS, Hamman RF, Klingensmith GJ, Eisenbarth GS, Erlich HA, Newborn screening for HLA markers associated with IDDM: diabetes autoimmunity study in the young (DAISY). Diabetologia 39:807–812, 1996
96. Seissler J, Morgenthaler NG, Achenbach P, Lamperter EF, Glawe D, Payton M, Christie M, Scherbaum WA, and the DENIS Study Group, Combined screening for autoantibodies to IA-2 and antibodies to glutamic acid decarboxylase in first degree relatives of patients with IDDM. Diabetologia 39:1351–1356, 1996
97. Kawasaki E, Yu L, Gianani R, Verge CF, Babu S, Bonifacio E, Eisenbarth GS, Evaluation of islet cell antigen (ICA) 512/IA-2 autoantibody radioassays using overlapping ICA512/IA-2 constructs. J Clin Endocrinol Metab 82:375–380, 1997
98. Dittler J, Hummel M, Mollenhauer U, Ziegler AG, Development of a combined GAD/IA2-antibody test system in venous and capillary blood samples of type 1 and pre-type 1 diabetic patients. Diabetologia 39 [Suppl 1]:A38, 1996
99. Bazzigaluppi E, Bonfanti R, Sawtell P, Bingley P, Bosi E, Bonifacio E, Islet antibody measurement in whole blood samples. Autoimmunity 24 [Suppl 1]:38, 1996 (abstract)
100. Karounos DG, Thomas JW, Recognition of common islet antigen by autoantibodies from NOD mice and humans with IDDM. Diabetes 39:1085–1090, 1990
101. Gillard B, Thomas JW, Nell L, Marcus D, Antibodies against ganglioside GT3 in the sera of patients with Type 1 diabetes mellitus. J Immunol 42:3826–3832, 1989
102. Castano L, Russo E, Zhou L, Lipes MA, Eisenbarth GS, Identification and cloning of granule autoantigen (carboxypeptidase-H) associated with type 1 diabetes. J Endocrinol Metab 73:1197–1201, 1991
103. Johnson J, Crider BP, McCorkle K, Alford M, Unger R, Inhibition of glucose transport into rat islet cells by immunoglobulins from patients with new-onset insulin-dependent diabetes mellitus. N Engl J Med 322:635–659, 1990
104. Inman LR, McAllister CT, Chen L, Hughes S, Newgard CB, Kettman JR, Unger RH, Johnson JH, Autoantibodies to the GLUT-2 glucose transporter of beta cells in insulin-dependent diabetes mellitus of recent onset. Proc Natl Acad Sci 90: 1281–1284, 1993
105. Boitard C, Villa MC, Becourt C, Gia HP, Huc C, Sempe P, Portier MM, Bach JF, Peripherin: An islet antigen that is cross-reactive with nonobese diabetic mouse class II gene products. Proc Natl Acad Sci 89:172–176, 1992
106. Honeyman MC, Cram DS, Harrison LC, Transcription factor jun-B is target of autoreactive T-cells in IDDM. Diabetes 42:626–630, 1993
107. Chang Y-H, Hwang J, Shang H-F, Tsai S-T, Characterisation of human DNA topoisomerase II as an autoantigen recognised by patients with IDDM. Diabetes 45:404–414, 1996
108. Rabin DU, Pleasic S, Palmer-Crocker R, Shapiro J, Cloning and expression of IDDM-specific human autoantigens. Diabetes 41:183, 1992