

# Natural Course of Preclinical Type 1 Diabetes

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## Key Words

Type 1 diabetes ·  $\beta$ -cell destruction · Autoantibodies · HLA genes

## Abstract

The clinical presentation of type 1 diabetes is preceded by an asymptomatic latent period characterized by the presence of diabetes-associated autoantibodies in the peripheral circulation, reflecting  $\beta$ -cell damage. This pre-diabetic period may last for months and years. Several studies observing genetically susceptible subjects from birth have shown that insulin autoantibodies (IAA) are the first or among the first autoantibodies to appear in young children, implying that insulin may be the primary autoantigen in most cases of childhood type 1 diabetes. About 12–16% of siblings of children with type 1 diabetes have been observed to test positive for at least one diabetes-associated autoantibody, whereas the risk of diabetes among siblings has been estimated to be 6–8%. In parallel, close to 4% of Finnish schoolchildren tested positive for at least one diabetes-associated autoantibody; the lifetime risk of type 1 diabetes in the Finnish population has been estimated to be close to 1%. These observations suggest that only 25–50% of those with signs of  $\beta$ -cell autoimmunity eventually progress to clinical type 1 diabetes. Accordingly there is a considerable proportion of children in whom  $\beta$ -cell autoimmunity remains subclinical or is aborted. Positivity for only one diabetes-associated autoantibody may actually repre-

sent innocent  $\beta$ -cell autoimmunity, while positivity for two or more autoantibodies seems to mark a point of no return. The autoimmune response is very dynamic in the early phase of prediabetes, with spreading from one antigen to another and from one epitope to another within a given antigen. In addition both isotype spreading and switching can be observed in early prediabetes. This indicates that the early prediabetic process may be a suitable target for immunomodulation aimed at delaying or preventing progression to clinical diabetes.

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## Introduction

Type 1 diabetes is an immune-mediated disease leading to chronic insulin deficiency due to extensive  $\beta$ -cell destruction in subjects with increased genetic susceptibility to diabetes. The clinical manifestation of type 1 diabetes represents end-stage insulinitis, since it has been estimated that only 10–20% of the insulin-producing  $\beta$  cells are still functioning at the time of diagnosis [1]. A so far unknown environmental factor is assumed to trigger  $\beta$ -cell destruction, whereas one or several other exogenous factors most probably affect the progression rate to clinical diabetes. The clinical disease presentation is preceded by an asymptomatic period of variable duration. Aggressive  $\beta$ -cell destruction may lead to disease manifestation within a few months in young children, whereas in other individuals the process will continue for years, even for

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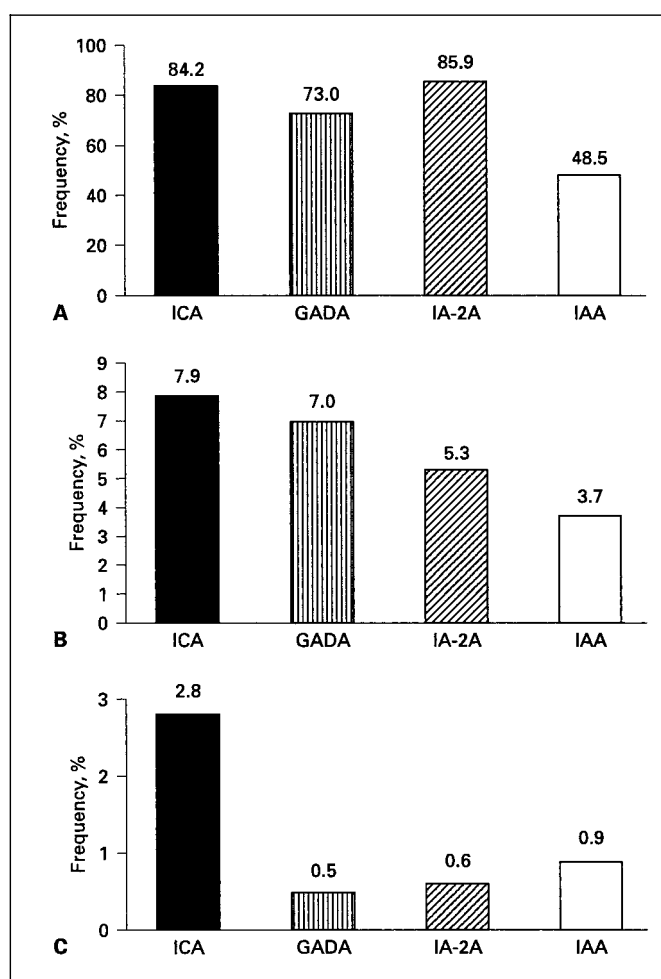
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more than 16 years. Human preclinical type 1 diabetes can be defined as a state where the individual has signs of  $\beta$ -cell autoimmunity with or without signs of reduced insulin secretory capacity.

### Diabetes-Associated Autoantibodies

Islet antibodies (ICA) were described for the first time in 1974 by Bottazzo et al. [2]. These antibodies are detected with conventional immunofluorescence and have been shown to bind to cytoplasmic components of all islet cells, not only to those of the  $\beta$  cell. The titres of ICA can be quantified by a dilution series of a strongly positive standard sample and are expressed in Juvenile Diabetes Foundation (JDF) units. Palmer and co-workers reported in 1983 that insulin autoantibodies (IAA) can be detected in patients with newly diagnosed type 1 diabetes before the start of exogenous insulin therapy [3]. Recently a microassay has been established for the measurement of IAA, with a substantial decrease in the serum volume needed for the analysis [4]. The IAA levels are expressed in relative units (RU), in relation either to a strongly positive standard serum or to a standard curve based on a dilution series of a strongly positive serum pool.

Antibodies to a 64 kD islet cell protein were detected in patients with newly diagnosed type 1 diabetes at the beginning of the 1980s [5], and this molecule was later identified as the enzyme glutamic acid decarboxylase (GAD), which catalyses formation of the inhibitory neurotransmitter  $\gamma$ -aminobutyric acid (GABA) from glutamine [6]. There are two isoforms of GAD, with molecular weights of 65 and 67 kD, respectively. Antibodies to the smaller, 65 kD isoform are associated with type 1 diabetes. GAD antibodies (GADA) are usually measured with a liquid phase radioligand assay and the levels are expressed in RU in a way similar to that described for IAA. Antibodies to 37 and 40 kD islet proteins were initially observed in studies aimed at characterizing the 64 kD antigen [7]. Some years later both Rabin et al. [8] and Lan et al. [9] identified an islet protein as a member of the protein tyrosine phosphatase (PTP) family. This islet antigen 2 (IA-2) protein, also referred to as ICA 512, was shown to be a host molecule of the 37 and 40 kD fragments, the 40 kD fragment representing the intracellular domain of the IA-2 molecule and the 37 kD fragment the corresponding domain of IA-2 $\beta$ . A majority of the autoantibodies to these two molecules detectable in patients with newly diagnosed diabetes bind to the IA-2 protein [10]. IA-2 antibodies (IA-2A) are mostly analyzed by a liquid



**Fig. 1.** Prevalence of ICA (■), GADA (▨), IA-2A (▩) and IAA (□) in 758 children with newly diagnosed type 1 diabetes (A), in 755 siblings of children with newly diagnosed type 1 diabetes (B) and in 3,652 schoolchildren representing the general population in Finland (C).

phase radioligand assay with a format similar to that of the GADA assay. The IA-2A levels are expressed in RU.

ICA represent a heterogeneous group of antibodies to a series of islet antigens including GAD65 and IA-2. ICA also comprise, however, antibodies to one or several so far unidentified antigens [11, 12], and so all the four antibodies mentioned above are here considered as separate antibody specificities, although some immunologists recommend that ICA should not be regarded as a separate antibody type. ICA, GADA and IA-2A have been shown to be present in a clear majority of patients with newly diagnosed type 1 diabetes, while IAA have been detected in about half of such patients [13]. Figure 1 presents the

frequency of the various autoantibodies in Finnish children with newly diagnosed type 1 diabetes (panel A), in 755 siblings of such children (panel B) and in 3,652 initially unaffected Finnish schoolchildren (panel C).

### Staging of Preclinical Type 1 Diabetes

We have shown that it is feasible to stage preclinical type 1 diabetes based on the number of autoantibodies detectable [14]. The staging procedure may also take into account the first-phase insulin response (FPIR) to intravenous glucose. In a staging process based on diabetes-associated autoantibodies, positivity for a single autoantibody represented early prediabetes, positivity for two antibodies advanced prediabetes and positivity for three to four antibodies late prediabetes. When FPIR was also considered, the term early prediabetes was applied to those with positivity for a single autoantibody and a normal FPIR ( $\geq 45$  mU/l, i.e. the third percentile in non-diabetic individuals), advanced prediabetes to those with two or more autoantibodies but still a normal FPIR, and late prediabetes to autoantibody-positive children with a reduced FPIR.

In the Finnish DiMe Study, when using the first classification (based on diabetes-associated antibodies), 6.5% of the siblings of children with newly diagnosed type 1 diabetes had early prediabetes, 1.7% advanced prediabetes and 4.6% late prediabetes [14]. According to the latter classification (also considering FPIR) 2.1% had early prediabetes, 3.2% advanced prediabetes and 1.8% late prediabetes. The risk of progression to clinical disease was clearly related to the stage of prediabetes in the sibling at the time of diagnosis of diabetes in the index case, being about 66% in those with late prediabetes according to the first classification and approximately 92% in those with late prediabetes according to the second classification. Preliminary data based on the observation of the siblings in the DiMe Study indicate that progression of the diabetic process is quite common in those who initially had advanced or late prediabetes, whereas regression is extremely rare among such siblings. On the other hand, regression to no prediabetes is seen in more than half of those with early prediabetes initially. This suggests that positivity for a single autoantibody specificity may represent harmless  $\beta$ -cell autoimmunity that is aborted in many children, whereas positivity for two or more autoantibodies seems to reflect progressive  $\beta$ -cell autoimmunity, i.e. a point of no return.

### Lessons from Observational Studies Starting from Birth

There are several ongoing prospective studies starting from birth in individuals at increased risk for type 1 diabetes. The German BABYDIAB Study has observed offspring of parents affected by type 1 diabetes, with sampling at the ages of 9 months, 2 years and 5 years [15]. The data showed that 11% of the offspring seroconverted to autoantibody positivity by the age of 2 years, and 3.5% tested positive for multiple ( $\geq 2$ ) antibodies at the age of 2 years. IAA were the first or among the first autoantibodies to appear in 96% (22/23) of the children who turned positive for multiple autoantibodies. Half of the subjects with multiple antibodies by the age of 2 years progressed to clinical diabetes by the age of 5 years.

The American DAISY study has two arms, one observing offspring in families with at least one affected family member and the other infants identified in the general population from HLA-conferred susceptibility to type 1 diabetes. Serum samples have principally been obtained at the age of 9 months, 15 months, 24 months and subsequently annually. Close to 6% of the offspring in diabetic families tested persistently positive for at least one autoantibody by a mean age of 5 years, whereas 1.6% had transient autoantibodies [16]. Among the genetically susceptible children derived from the general population, 0.9% had persistent autoantibodies, and about twice that proportion (1.6%) transient autoantibodies by a mean age of 2.2 years. Among the offspring from affected families with persistent autoantibody positivity 75% (21/28) tested positive for IAA, whereas this proportion was 85% (6/7) among the children derived from the general population.

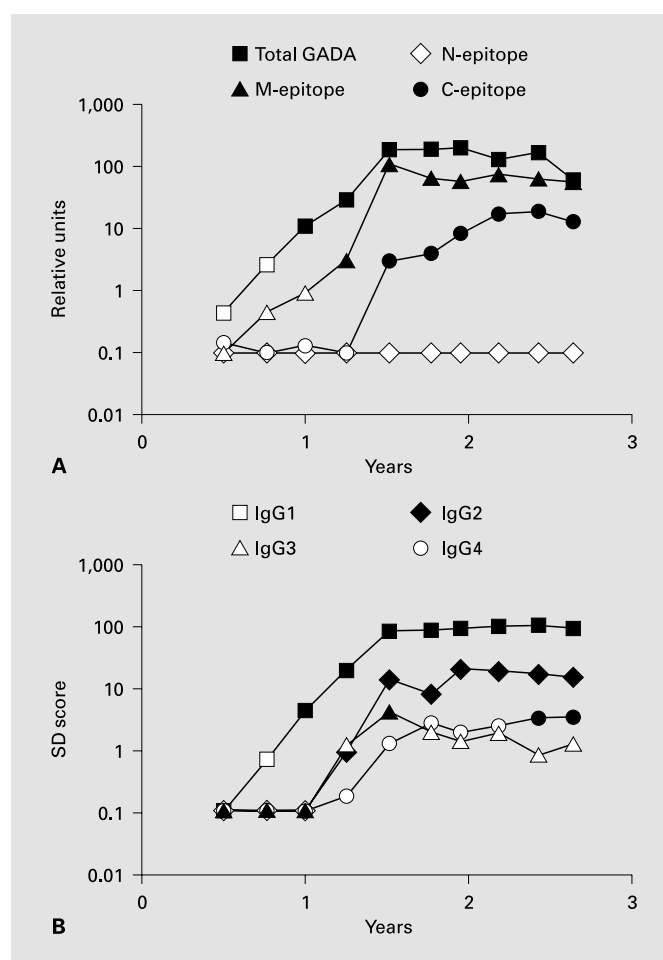
In the Australian BABYDIAB Study 357 children with at least one affected family member were followed from birth with sampling every 6 months up to a mean age of 3 years [17]. About 7% tested positive for at least two autoantibodies by the age of 3 years, 5% for a single antibody more than once and 14% for a single antibody only once. These proportions also include those with transplacentally transferred antibodies detectable in the sample taken at the age of 6 months. IAA were the first to appear in 64% of the children with two or more antibodies.

In the Finnish Diabetes Prediction and Prevention (DIPP) Study we screen all newborn infants born in three university hospitals for HLA DQB1 alleles associated with susceptibility or protection from type 1 diabetes, provided that the parents give their informed consent [18]. The families with a baby carrying increased HLA

DQB1-conferred disease predisposition (HLA DQB1\*02/\*0302 or 0302/x, where x ≠ \*02, \*0602 or \*0603) are invited to an observational study with regular study centre visits at the ages of 3 and 6 months and subsequently with at intervals of 3–12 months. When the first 2,448 subjects were observed up to the age of 2 years, ICA were detectable in 2.2% at the age of 2 years, with a higher proportion in those with the high-risk genotype (3.3%) compared to those with the moderate-risk genotype (1.6%) [19]. IAA were the first or among the first autoantibodies to emerge in 88% (21/25) of the young children positive for at least two antibody specificities by the age of 2 years. Transient antibody positivity was seen at a frequency ranging from 0.2% for IA-2A up to 1.9% for IAA by the age of 2 years. Less than 1% of the children had transiently positive ICA (0.9%) or GADA (0.5%). Fluctuating antibody positivity was most frequently observed for IAA (0.7%), whereas such a phenomenon was rare for the remaining three autoantibody specificities (only 0.1%).

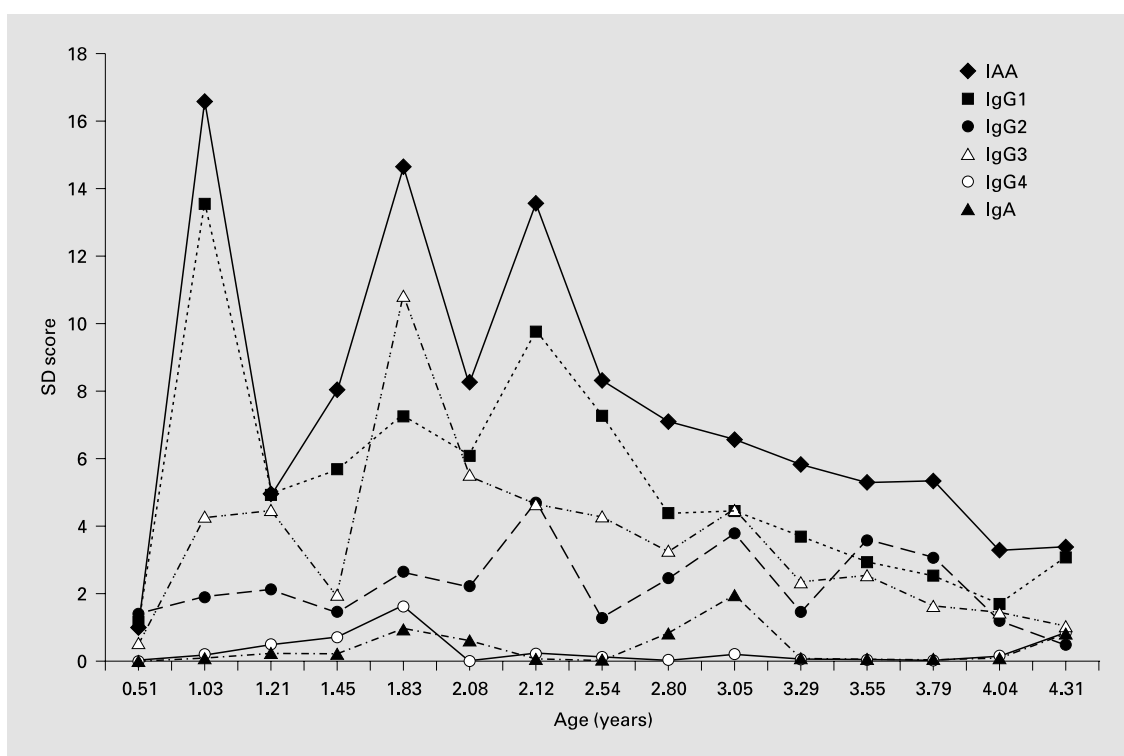
Fifteen index cases in the DIPP Study had progressed to clinical type 1 diabetes by the end of April 2000. Nine of these children had a very aggressive process progressing to clinical type 1 diabetes within a few months (range 4–8 months) after the appearance of the first disease-associated autoantibodies. Six subjects had a less aggressive pre-diabetic process lasting for >1 year (range 1.1–3.7 years). Those with a rapid process in most cases had increasing autoantibody titres up to the clinical diagnosis, whereas those with a slower process often experienced a pattern of decreasing autoantibody titres before disease manifestation. Most studies have shown that young age at the appearance of the first autoantibodies, high antibody titres (with the exception of GADA) [20], a high-risk HLA genotype and a reduced FPIR are all factors associated with an accelerated progression to overt type 1 diabetes [21–23].

In the DIPP Study we have had the opportunity to observe the maturation of autoantigen-specific humoral immune responses by observing young children from the time point of the first positive seroconversion. The upper panel of figure 2 presents the epitope spreading in a girl with the high-risk HLA-DQB1 genotype who converted to seropositivity for GADA at the age of 12 months. The lower panel shows the emergence of isotype-specific GAD antibodies. The maturation of the isotype-specific response to insulin in a young boy with the moderate-risk HLA-DQB1 genotype is illustrated in figure 3, revealing that the first IAA peak was mainly composed by IgG1, while the second peak was mostly due to IgG3. Both these cases illustrate that the humoral immune response is very



**Fig. 2.** Maturation of the humoral immune response to GAD65 in a girl carrying the high-risk HLA-DQB1\*02/\*0302 genotype. Epitope-specific antibody levels are shown in panel **A**, the open symbols marking antibody-negative samples and the closed symbols antibody-positive samples. The girl seroconverted to GADA positivity at the age of 1 year, antibodies to the middle region of GAD65 appeared 3 months later and C-terminal specific antibodies at the age of 18 months, while she remained negative for N-terminal specific antibodies throughout follow-up to the age of 33 months. IgG subclass antibodies are shown in panel **B**. The girl tested positive for IgG1 subclass antibodies in the first sample positive for total GADA at the age of 12 months. IgG2 subclass antibodies emerged 3 months later, IgG3 subclass antibodies at the age of 18 months and IgG4 subclass antibodies for the first time at the age of 30 months.

dynamic over the first 1–2 years. Thereafter the responses seem to flatten out and stabilize. This observation suggests that immunomodulatory therapies aimed at preventing or delaying the clinical manifestation of type 1 diabetes might be most effective in the early phase of the islet-directed immune response.



**Fig. 3.** The isotype-specific response to insulin in a boy with the moderate-risk HLA-DQB1\*0302/x genotype. The boy turned positive for IAA at the age of 1 year. The initial response was principally composed of IgG1 subclass IAA and some IgG3. A second peak of total IAA was seen at the age of 1.8 years, and this comprised mainly IgG3 subclass antibodies. A third peak was observed at the age of 2.1 years with a dominating IgG1 component. The child had in addition

low fluctuating levels of IgG2 subclass IAA, while he tested negative for IgG4 subclass and IgA IAA on all occasions. After the age of 2.8 years, i.e. 1.8 years after the appearance of the initial IAA response, the IAA levels decreased and flattened out – a phenomenon seen in a considerable proportion of young autoantibody-positive children 1.5–3 years after the emergence of the first autoantibodies.

## Conclusions

Studies on the natural course of preclinical diabetes are expected to provide new information on the pathogenesis of type 1 diabetes and facilitate the identification of high-risk individuals for intervention trials aimed at preventing clinical diabetes. Available data suggest that the natural history of preclinical disease varies considerably from one individual to another. Individuals with signs of  $\beta$ -cell autoimmunity can be classified into various categories of preclinical disease with conspicuous differences in the risk of progression to clinical diabetes. The frequency of preclinical diabetes is higher than the expected prevalence of overt diabetes, suggesting that some prediabetic subjects will never progress to overt type 1 diabetes. When the first clinically applicable treatment modality confirmed to be effective in delaying or preventing type 1 diabetes becomes available, diagnosis and grading of preclin-

ical diabetes will become integrated into clinical practice. The humoral immune response to various  $\beta$ -cell autoantigens is most dynamic over the first few years of the process, implying that immunomodulation may be most effective in early preclinical diabetes.

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