Diabetologia (1994) 37 [Suppl 2]: S73–S81

Diabetologia

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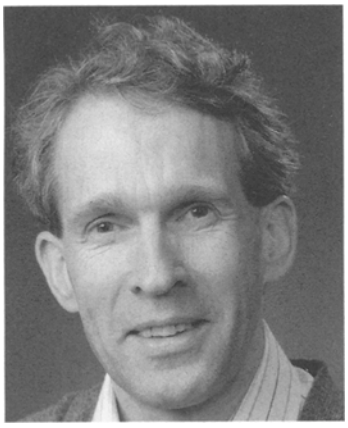
Minkowski Award, 1984, London



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Molecular biology of IDDM

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Summary The clinical onset of insulin-dependent diabetes is associated with several autoimmune phenomena including islet cell antibodies, glutamic acid decarboxylase (the GAD65 isoform) autoantibodies (GAD65Ab) as well as insulin autoantibodies. The molecular cloning of these autoantigens has permitted the development of precise and reproducible antibody immunoassays to identify marker-positive patients and control subjects. Among patients with new-onset diabetes about 70 % were GAD65Ab positive compared to 1.5 % among control subjects while 46 % of patients had IAA compared to 1 % among control subjects. The autoreactive sites or epitopes of GAD65 and insulin remain to be determined. The disease association with HLA on chromosome 6 may help to define the epitope specificity of the autoimmune reaction. Recent data suggest that 95 % of new-onset IDDM children (0–15 years of age) are positive for either DQ2, DQ8 or both compared to about 50 % of healthy control subjects. HLA-DQ6 is negatively associated with the disease. Both HLA-DQ2 and DQ8 therefore seem to be necessary, but not sufficent for diabetes. Molecular modelling suggests comparable physicochemical properties of DQ2 and DQ8 but are widely different from DQ6. In 1984, the conclusion was that molecular cloning of the genes for the autoantigens, antibodies, T-cell receptors, as well as HLA class I and II molecules associated with diabetes are essential for analysing the components which control the development of pancreatic beta-cell autoimmunity. In 1994, autoantigens and HLA molecules have been cloned and recombinant reagents developed to be used in experiments aimed at testing whether it will be possi-

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Abbreviations: RFLP, Restriction fragment length polymorphism; ICA, islet cell antibodies; IAA, insulin autoantibodies; kbp, kilobase pair; GAD, glutamic acid decarboxylase.

ble to predict IDDM. Our understanding of immune autoreactivity is, however, still inadequate and remains a major challenge to future Minkowski Award hopefuls. [Diabetologia (1994) 37 [Suppl 2]: S 73–S 81]

Key words Islet cell antibodies, glutamic acid decarboxylase (GAD) autoantibodies, GAD65, GAD67, insulin autoantibodies, HLA, major histocompatibility complex, chromosome 6.

Conclusions in 1984 brought new questions

In the 1984 Minkowski lecture presented at the Barbican Center, London, UK, interest in IDDM research was centered around the findings that 1) ICA are associated with a loss of beta-cell function; 2) islet cell surface antibodies may affect glucose-stimulated insulin release, 3) serum from patients with IDDM immunoprecipiate a 64 K protein, 4) the disease was associated with HLA-DQ rather than DR, 5) synthetic peptide HLA antibodies may be useful reagents with which to analyse the development of IDDM [1]. It was further speculated that molecular analyses of the autoantigen(s) and of class II antigen presentation would make it possible to test the hypothesis that: "presentation of an autoantigen to the immune system is associated either with the development of IDDM in susceptible individuals or with the progression of the disease" [1]. Then, as still is the case today, in 1984 it was unclear whether the beta-cell autoimmunity was primary or secondary to a beta-cell-killing disease process. What has been learnt in the past 10 years?

The clinical onset of IDDM is associated with a specific loss of the pancreatic beta cells. The other islet endocrine cell types remain intact. The clinical criteria for IDDM below the age of 15 years are succinct and permit reliable epidemiological investigations. Longitudinal investigations support the effects of age at onset on diabetes incidence. Epidemiological investigations, improving world wide [2] and particularly in Sweden where due to established Diabetes registers [3–6] indications are that the incidence rate of IDDM is increasing [6]. Similar reports are available from Finland and other countries [7]. The reason for this increase in incidence is not known but the consequences for a country with a small population such as Sweden are significant: the incidence among 0–14-year-olds has increased by 3 % per year between 1978 and 1987 [5]. What are the environmental factors and genetic determinants that have allowed this rise in incidence of diabetes?

The introduction of diabetes registers for adult patients have added other questions. In a Swedish registry for the 15–34-year-olds [8] it is reported that twice as many men as women develop IDDM over the age of 20 years. There is currently no explanation for this gender effect in this age group or if the difference will remain among older patients. This observation is puzzling since there are numerous reports among young adults and adults that several diseases of autoimmune character are more common among females than males. The development of precise and reproducible assays for autoantibodies against islet antigens in IDDM and for the HLA genes associated with this disease may help to clarify this and other phenomena. Our improved understanding of the HLA genes, the structure of the HLA class II molecules as well as of their function will be reviewed.

Progress towards a better understanding of the HLA association

The beginning of the 1980's were exciting since progress in molecular biology eventually allowed HLA cDNA to be cloned and sequenced (reviewed in [1]). Using the cDNA for the β chain of DQ, we were the first to demonstrate that HLA-DR4 could be divided into two subgroups – one was associated with IDDM (now known as DQ8), the other was not (now known as DQ7) [9]. In fact, recent analysis of population-based childhood studies suggest that DQ7 is negatively associated with IDDM [10]. Our cloning and sequencing of the β-chain gene of DQB1 [11], confirmed the association to diabetes of only one DQ-type which was often found on some, (those associated with diabetes) but not all, DR4-containing chromosomes [12, 13]. Further analyses were carried out by analysing the RFLP of the HLA genes (for a review see [14]) to make inferences to different DR and DQ alleles. Using the RFLP technique it was demonstrated that the greatest risk for childhood IDDM was conferred by HLA-DQ 2/8 heterozygocity [15]. In a study of patient and control families, BQB1*0302 indicated somewhat higher risk than DR4, while DR3 has a higher risk than DQB1*0201, however the 95 % confidence intervals overlapped [16]. More important, DQA1*0301 was present among 93 % of the patients compared to 50 % among control subjects and various possible transcomplementation combinations with DQB1 alleles showed closer association to IDDM than did any other alleles [10, 16]. The strong negative association of the DQB1*0602 allele also in the presence of DR4, DQB1*0302 or both suggests that DQ may primarily confer protection [17]. The mechanism by which these class II molecules confer susceptibility and protection remains an enigma.

Table 1. *Cis* and *trans* complementation of HLA DQA1 and DQB1 allele transcription products

DQB1 allele	DQA1 allele	*Cis* or *trans*	DQ specificity	Association to diabetes
0302	0301	*cis*	8	positive
0201	0501	*cis*	2	positive
0302	0501	*trans*	–	positive
0201	0301	*trans*	–	positive
0602	0102	*cis*	6	negative
0301	0301	*cis*	7	negative

It has been important that different HLA loci have been cloned and sequenced to analyse the structure of the genes for the α and β chains of all currently known class II molecules: DP, DQ and DR. A DR class II molecule is formed by a heterodimer between the non-polymorphic α chain and a polymorphic β chain derived from one of the several DRB loci (Table 1). About 700 kbp from the DRB1 locus is the location of the DQA1 gene. This gene has several different alleles; more than 12 have, so far, been detected in different populations [18]. Therefore, in contrast to DRA1, the DQA1 gene is highly polymorphic. This has consequences for the DQ α chain since the amino acid sequence of the functional part of the molecule varies markedly. Next to the DQA1 locus is the locus for the DQB1 gene which has also several different alleles. The DQB1 gene is coding for the β chain of the DQ class II molecule. Nomenclature committees [18] have named the different alleles of DRB, DQA1 and DQB1 alleles. For example, the DQ8 molecule is formed by the α chain coded for by the DQA1*0301 allele and the β chain coded for by the DQB1*0302 allele. The DQ7 molecule is formed by the gene products of DQA1*0301 and DQB1*0301 (Table 1). Interestingly, only 4 amino acids differ between DQB1*0301 and DQB1*0302 [13]. This is apparently sufficient for the former (DQ7) to confer protection and the latter (DQ8) susceptibility.

The presence of a single amino acid, Asp 57, in the DQB1*0301 β chain was proposed to control susceptibility or protection [13]. This hypothesis could not be confirmed since patients develop diabetes despite being Asp57 positive – the most prominent example being IDDM in the Japanese [19]. A combination between the amino acid on position 57 of the β chain and position 52 on the α chain was next proposed to confer susceptibility and protection [20], a hypothesis which could not be verified in other ethnic groups. It is therefore necessary to find explanations other than single amino acids with which to understand the association between HLA-DQ and IDDM. An important question to be answered is how four amino acids which differ between DQB1*0302 and DQB1*0301 are able to influence the risk of developing diabetes: increased for DQB1*0302 and decreased for DQB1*0301 [10].

In particular due to the efficiency of polymerase chain reaction [21], not only have the nucleotide sequences of numerous alleles been determined but the three-dimensional structures of both HLA class I [22] and class II [23] molecules have also been resolved. It has therefore been possible to determine the amino acid sequences which form the peptide binding groove of the class II molecules, the function of which is to present antigens to the T-cell receptor on T helper cells. Why should structural analyses be of interest in the understanding of IDDM? The answer is that they will make it possible to test the hypothesis that physicochemical properties of the HLA class II molecules control autoantigen peptide binding and presentation. Molecular modelling of HLA class II molecules is now feasible since the structure of HLA-DR has been resolved [23]. Comparing the molecular model of DQ7 with DQ8 did not reveal structural differences which would explain their opposite effects on diabetes susceptibility. On the other hand, comparing the electrostatic potential of the solvent accessible surface revealed major differences between the two DQ class II molecules (Sanjeevi and Lybrand unpublished data). The side chains of those amino acids located in the peptide binding groove therefore make considerable contributions to Class II molecule properties and enhance their function.

Class II molecules may be formed by at least two, perhaps three, different mechanisms (See [24] for review). One is that the A and B chain gene products come from the same chromosome, i.e. in *cis* configuration (Table 1). In this case the DQB1*0302 and DQB1*0301 translation products form the DQ8 molecule. The other possibility, [25, 26] is the phenomenon of transcomplementation in which the A chain is from one and the B chain from the other chromosome. For example, a diabetic child who is DQ2/8 heterozygous has the following DQ products formed in *cis* configuration from the two chromosome 6: DQ2 is from DQB1*0201 and DQA1*0501 and DQ8 is from DQB1*0302 and DQA1*0301. In such a child two further DQ class II molecules may be formed by transcomplementation i.e. DQB1*0201 with DQA1*0301 and DQB1*0302 with DQA1*0501. Molecular modelling suggest that these transcomplementation class II molecules have physicochemical properties indistinguishable from DQ8 and DQ2, respectively. The presence of transcomplementation may explain diabetes susceptibility in some children who may have a positively associated DQA1 allele together with a protective allele on the same chromosome in *cis* but a risk DQB1 allele in *trans* [24].

How many protective and susceptibility genotypes are there in the population? To answer this question a

Table 2. DQA1 and DQB1 allele combinations in Swedish children with IDDM and healthy control

DQ allele	Patients ($n = 425$)	Control subjects ($n = 367$)	Odds ratio	p-value
DQA1*0301	81 %	43 %	5.79	< 0.001
DQA1*0501, DQA1*0301 or both	95 %	69 %	9.01	< 0.001
DQB1*0302, DQB1*0501 or both	92 %	56 %	9.71	< 0.001
DQ8, DQ2 or both	91 %	48 %	10.4	< 0.001
DQA1*0301, DQB1*0302 or DQB1*0501	99 %	50 %	12.5	< 0.001

Data from previously published material [10]

total of 425 new-onset IDDM children and 367 healthy matched control children were typed by both RFLP [16] and polymerase chain reaction-based [10, 27, 28] typing. The data in Table 2 demonstrate that nearly all patients can be accounted for as DQA1*0301-positive. The second most common genotype was DQB1*0302 (DQ8) followed by DQB1*0201 (DQ2). These three alleles were present in 99 % of the patients. It was remarkable that only 3 of 425 (1 %) of the patients did not carry any one of these alleles. One interpretation of this result is that DQ8 or DQ2, which show similarities in physicochemical properties, present peptides of similar class II antigen-binding ability. For example, is it possible that DQ2 and DQ8 molecules are particularly prone to bind peptides from GAD65 or insulin? Perhaps this would explain why DQ2/8 heterozygosity is associated with the highest risk of developing diabetes. A total of 46 % of new-onset IDDM children were DQ2/8 heterozygous compared to 3 % of the background population. This means that about 1 in 12 of Swedish DQ 2/8-positive children would develop IDDM before 15 years of age. However, since the prevalence of diabetes is not more than about 0.2 % [3], the positive predictive value of HLA genotyping is low. It would be unethical to genotype children for HLA to identify individuals at risk to be treated with for example immunosuppressive agents as many subjects would be overtreated [29].

The negative association between IDDM and HLA is of equal importance to susceptibility in order to understand the role of HLA in the disease process. The negative association is most prominent for DQ6 which is formed by the α chain of DQB1*0602 and the α chain of DQA1*0102 (Table 1). DQ6 in fact also shows a negative association with the disease in patients who are DQ8 or DQ2 positive [16, 17]. Somehow the effect of DQ6 represents a dominant protection [16, 17, 20]. It is remarkable that about 30 % of the Swedish population is DQ6 positive and if the hypothesis of a dominant protection is accurate, one third of the population would not be at risk of developing IDDM. This means that if HLA typing was considered for neonatal screening of diabetes, it would be possible to exclude one third of the children from the risk of developing diabetes. In summary, the diagnostic sensitivity (positivity in disease) for IDDM of the HLA-DQ types DQA1*0301, DQB1*0302 and DQB1*0201, alone or in combination is nearly 99 %. The diagnostic specificity (negativity in health) is however more than 50 %. HLA typing for diabetes would therefore be unethical due to a very high frequency of false positive results.

Further analyses are necessary to explain the HLA association in IDDM. Two different hypotheses need to be tested, firstly that the trimolecular complex, i.e. the class II molecule with the peptide bound in the groove determine susceptibility to IDDM. This hypothesis will be tested first by comparing the physicochemical properties of DQ2 and DQ8 (susceptible class II molecules) with those of DQ6 (protective class II molecule) and to determine by peptide elution [30] the amino acid sequences of bound peptides. The second test is to determine if there is a difference between DQ2, DQ8 and DQ6 in the binding capacity of GAD65 and insulin peptides. Such analyses should help us to understand the functional role of these class II molecules in both normal and in disease-associated antigen presentation processes.

The second hypothesis is based on the historical background of the HLA association in IDDM. The first demonstration of an association was with HLA-B15 [31] to be followed by B8 [32]. These associations were later proven to be due to linkage disequilibrium with DR4 and DR3, respectively [33, 34]. It has not yet been proven that the HLA association in IDDM is HLA – DQ2, DQ8 or both because of linkage disequilibrium to DR3 and DR4 (see [14] for a review). In most studies the 95 % confidence intervals of the DR associations (relative risk values are calculated) overlap with those of the DQ association (see [16] for a review). The second hypothesis to be tested is that there is a specific diabetes associated gene located in the 700 000 bp region between the DQA1 and DRB1 loci. A 700 kbp region may harbour as many as 10 different genes. Polymorphic gene markers of DRB1 and DQA1 will be used to test this hypothesis. One approach is to investigate families with two or more diabetic children for polymorphic markers of this region and analyse the data by sib-pair analysis. The use of seven different methods to analyse risk in population-based patient and control studies showed that DQ could not be fully separated from DR [16] indicating the need for further analyses of the mechanisms by which HLA is associated with IDDM. Currently HLA-DQ2, DQ8 or both are markers for genes which seem necessary,

Table 3. Glutamic acid decarboxylase genes and their transcription and translation products as well as sites of expression

	GAD65	GAD67
Chromosome	10p11.2-p12	2q31
Gene	GAD2	GAD1
Transcript (mRNA)	5.6-kb	3.7-kb
Translation product	M_r 65,000	M_r 67,000
Property	amphiphilic	hydrophilic
Subcellular location	synaptic microvesicles	cytosolic
Human islet location	beta cells	not expressed
Human brain expression	+	+

but not sufficient, for the development of the disease. The diagnostic sensitivity is high while the diagnostic specificity is low. Combining HLA typing with antibody markers to improve prediction of IDDM is therefore a possibility.

The 64 K antigen is out and GAD65 is in

Using immunoprecipitation with IDDM sera and radiolabelled human or rat islets of Langerhans a M_r 64,000 autoantigen was detected [35, 36]. In 1984, it was concluded that antibodies against the M_r 64,000 antigen may be detected long before diagnosis of IDDM and that such antibodies may be a useful marker for prediction of IDDM. The approach to identify the autoantigen included expression libraries or determination of the primary structure by microsequencing. Neither one was used when the M_r 64,000 antigen was shown to represent islet GAD. The solution to the identification was instead made by the observation that patients with the rare disease Stiff Man Syndrome had antibodies which in frozen sections stained both GABA-ergic cells in the cerebellum and the islets of Langerhans in the pancreas [37]. The Stiff Man Syndrome sera stained an M_r 64,000 brain component on immunoblots [37]. Shortly thereafter it was demonstrated that the islet M_r 64,000 component immunoprecipitated by IDDM sera had GAD activity [38]. The Stiff Man Syndrome serum samples were able to detect the M_r 64,000 protein by immunoblotting while this was not the case however for IDDM sera. In addition, only those Stiff Man Syndrome patients who were DR3 or DR4 positive had IDDM. Several tests were developed using extracts of brain tissue to detect GAD antibodies [39, 40], however, the diagnostic sensitivity was low. GAD had previously been demonstrated in the islets of Langerhans [41] and GABA was the most abundant free amino acid in the islets of Langerhans [42]. Our use of a human islet expression cDNA library showed, however that GAD in human islets represented a novel isoform (GAD65) (Table 3). The previously known GAD (GAD67) had a molecular mass of 67,000 and the gene, localized to human chromosome 2 [43] was transcribed into a 3.7 kb mRNA [44–46]. The human islet GAD, the GAD65 isoform had a molecular mass of M_r 65,000 and the gene, localized to chromosome 10 was transcribed into a 5.6 kb mRNA [47]. The predicted amino acid sequence of GAD65 showed that 65 % of the amino acid residues were identical to those of GAD67 [47]. The presence of two different brain isoforms were confirmed both in the rat [48] and in man [49]. A full-length rat islet GAD67 cDNA was also reported to show a nearly complete sequence identity between human and rat islet GAD67 [50]. While human islets only express the GAD65 isoform, primarily in the beta cells [51, 52], both the GAD65 and the GAD67 isoforms are expressed in rat islets [50] (Table 3).

The molecular cloning of GAD65 and GAD67 allowed these proteins to be expressed in a variety of systems including bacteria [53], COS cells [43, 54], BHK cells [51, 55] and by in vitro translation [56]. The first study on GAD antibodies in IDDM claimed equal reactivity between GAD67 and GAD65 [53]. This study has been misleading, perhaps due to the use of a bacterial fusion protein [53] since subsequent investigations showed that IDDM sera are primarily reactive with the GAD65 and not the GAD67 isoform [54, 56, 57]. For example, in one study of children with new-onset diabetes only 10 % had GAD67 antibodies, and all of them GAD65 antibody positive [56]. These studies show that future investigations need to specify the antigen used in an assay for GAD antibodies.

The first GAD antibody workshop was carried out in 1993 to demonstrate a significant assay reproducibility and precision as well as an acceptable inter-laboratory variation in radioimmunoassays using labelled recombinant GAD65 (Coleman et al., unpublished data). Neither the ELISA nor the immunotrapping GAD enzymatic assays showed acceptable dilution curves and the inter-laboratory variation was significant.

Radioimmunoassays for GAD antibodies in studies of children and young adults with new-onset IDDM showed that the diagnostic sensitivity of GAD65 antibodies was 70–80 % [55–59]. In comparison, the diagnostic sensitivity of IAA was 43 % [60, 61]. Almost all patients with GAD65 antibodies were also ICA positive but the converse was not true [55, 56, 58]. In a population-based study, 84 % of children with new-onset IDDM were ICA positive [60]. In patients classified with NIDDM, GAD65 autoantibodies predict later insulin requirement [55, 58]. The positive predictive value of GAD65 antibodies for IDDM in one study of NIDDM patients [55] reached about 90 % (Hagopian, unpublished data). This positive predictive value was based on 5 years follow-up from the time the patients were first diagnosed with diabetes and classified with NIDDM. The treatment from the time of diagnosis was either oral hypoglycaemic agents, diet or exercise [55]. Furthermore,

about 50 % of Japanese diabetic patients classified with so-called "slow-onset diabetes" were also GAD65 antibody-positive compared to 70 % of new-onset Japanese IDDM patients [62]. It is suggested that GAD65 antibodies in patients with diabetes, irrespective of their classification mark loss of endogenous insulin.

The predictive values of markers for IDDM have most often been studied in first degree relatives [63–66], despite the fact that only about 10 % of new onset patients have the disease [3]. The life time recurrence risk for IDDM among siblings is only 6–8 % [67]. It is therefore of interest that the frequency of GAD65 antibodies determined by radioimmunoassay in a screen of more than 130 healthy, first degree relatives was 8 % (Falorni, unpublished data). Antibodies to GAD and tryptic fragments of the islet 64 K protein [68, 69] was reported to mark the development of IDDM in selected identical twins [70]. Further studies will therefore be of importance to define the positive predictive value of GAD65 antibodies for IDDM among first degree relatives.

In previous analyses we have demonstrated that 3 % of healthy Swedish schoolchildren have ICA [60, 71]. These ICA frequencies were later confirmed by others [72–74]. A diagnostic specificity of 97 % combined with 81 % specificity showed a predictive value for IDDM of 5 % when corrected for a prevalence of 0.15 % [71]. Another interpretation is that 1 of 20 ICA-positive individuals would develop IDDM. Similarly, GAD65 antibodies were found among 1.5 % healthy children [56] or found negative among control subjects [54, 58]. Hence the diagnostic specificity (negativity in health) for GAD65 antibodies might be higher in healthy school children (98.5 %) while compared to ICA the diagnostic sensitivity (70 %) would be lower. Only prospective analyses will tell whether GAD65 antibodies may be transient or to what extent these antibodies are primarily found in DQ2 or DQ8 positive individuals. Prospective studies are in progress since radioligand binding assays with recombinant GAD65 and GAD67 [56, 58, 62] permit the screening of large numbers of samples. Will one type of antibody marker do? Investigations in progress [75] in population-based patient and control studies should reveal whether the positive predictive value for IDDM may improve by the combination of markers including genotyping for HLA (see above) and beta-cell function tests [76]. Although a set of markers may improve the positive predictive value, this is often at the expense of sensitivity [60] which means that more patients are left undetected.

Future studies: possible ways of preventing diabetes

Since the review in 1984 [1], thousands of patients with new-onset diabetes have been subjected to a variety of immunotherapies. An open study [77] of cyclosporin was followed by controlled clinical trials [78, 79], however, the drug did not induce long-term insulin independency. The significant nephrotoxicity shown in 1984 [80] was confirmed in these controlled trials [81] and precluded further tests of cyclosporin in this patient group. The list of possible immune intervention approaches was long in 1984 [1] and has since grown even longer. In a recent review [82], 29 different immune intervention strategies tested in human IDDM are listed. None of these strategies were antigen specific. The molecular cloning of human islet GAD65 [47] will increase the length of the list. The multitude of expression systems makes it possible to produce human recombinant GAD65 in large quantities. Several strategies may therefore be developed to test the hypothesis that inhibition of self-reactivity against GAD65 will preserve residual beta-cell function or progression to clinical IDDM.

The human immune response is complex and there is currently no end to ideas and approaches for intervention or interruption of the disease. There are, however numerous questions which remain to be answered to allow interpretable results from antigen-specific immune intervention. Currently determined GAD antibodies are of the IgG type. When are GAD IgM antibodies present, if ever? This is interesting to investigate since IgM antibodies are usually found nearer the time of antigen presentation. When is GAD65 presented to the T lymphocytes? This is important since self-reactive T lymphocytes are thought to be part of the normal T-cell repertoire. Is the antigen presentation preceeded by the appearence of GAD65 in circulation? In tissue injuries, such as after a cardiac infarct, heart muscle proteins may be detected in circulation and transient myosin antibodies are formed. Our preliminary experiments on baboons indicate that transient GAD65 antibodies were detected after diabetes was induced by high-dose streptozotocin injections [83]. When would the GAD65-sensitized T cells be detected? Only preliminary studies, yet to be confirmed, have addressed the question of whether IDDM is associated with GAD-reactive T lymphocytes [84, 85]. Are GAD65-specific T lymphocytes predictive of IDDM? Are there environmental factors playing the game of molecular mimicry to induce pancreatic islet beta-cell autoreactivity? A coxsackie B virus [53] and a bovine serum albumin [86] found in a p69 protein [87] sequence are current candidates. The above is only a short list of unanswered questions, however, there are many more to be answered before the aberrant autoimmunity against the islet beta cells in IDDM has been dissected. Therefore, in 1994 the

conclusion is the same as in 1984 [1]: "recent developments in molecular biology have opened new avenues to study Type 1 diabetes in a way that could hardly be envisaged just a few years ago". In 1994, autoantigens and HLA molecules have been cloned and useful reagents developed to test whether IDDM can be predicted. However, our understanding of the possible role of the immune autoreactivity in the pathogenesis of diabetes is, still inadequate and remains a major challenge to future Minkowski Award hopefuls.

Acknowledgements. This review would not have been possible without my colleagues A. Falorni, C. Sanjeevi, I. Kockum, J. Robertson, C. Möller, M. Landin-Olsson, W. Hagopian, A. Karlsen, C. Grubin, L. Li, and L. Jobe for sharing their results and for the excitement of doing science together. Our research has been supported by the Swedish Medical Research Council (19X10408), the National Institutes of Health (grants DK 26190, DK 33873, DK 42654, DK 41801), the Novo Nordisk Foundation, the Hoechst Foundation, the Swedish Diabetes Association, the Karolinska Institute and the Petrus and Augusta Hedlunds Foundation.

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