Review

Molecular biology of Type 1 (insulin-dependent) diabetes mellitus*

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Diabetes mellitus has been known to man since ancient times. There are many documents available or quoted throughout several centuries and, closer to our times, accounts of experiments to explain the origin of diabetes mellitus. In the classical series of experiments in dogs involving pancreatectomies, von Mering and Minkowski [1] in 1889 concluded that complete removal of the pancreas caused diabetes. It was not a transient glycosuria but a true, lasting diabetes mellitus which, in every respect, mimicked the worst type of this disease found in man. In a second study, Minkowski [2] reported on a larger number of dogs in which pancreatectomy was performed, further emphasizing the striking disorders in glucose metabolism which are associated with removal of the pancreas. It was concluded that diabetes mellitus was associated with an hitherto unknown function of the pancreas. The possible relationship to the islets of Langerhans did not become evident until several years later. In fact, although pathologists in the early years of this century reported morphological alterations in the islets of Langerhans of patients with diabetes, it was not until 1965 that Gepts [3] by careful morphological investigations showed major abnormalities in a number of β cells and the presence of inflammatory cells in the islets of Langerhans in newly diagnosed Type 1 (insulin-dependent) diabetic patients. Recent studies with morphometric techniques [4–5], which allow a quantitative measure of both the total volume or mass of the endocrine pancreas of individual cell types visualized by sensitive and specific immunocytochemical techniques, have shown that Type 1 diabetes in man is associated with a specific loss of the pancreatic β cells (Fig. 1). The disappearance of β cells is linked to a diminished mass of the endocrine pancreas, but the total mass of A (glucagon), D (somatostatin) and pancreatic polypeptide cells seemed to remain within the normal range [4]. Type 1 diabetes seems, therefore, to represent nature's own β -cytectomy, a procedure yet impossible to carry out in specific experiments. Chemical agents, such as alloxan and streptozotocin, are β -cytotoxic but their effects are harmful to other cells also.

What are the molecular mechanisms which govern the extermination of β cells? Numerous investigations, particularly during the last 25 years have suggested that β cells are lost in association with a specific autoimmune reaction (Table 1). It is possible that a precise and concerted activity of the immune system might be directed against certain molecules which amongst the endocrine islet cells occur only on the β cell (Fig. 2). The particular features of this cell is its ability to synthesize, store and release insulin. The biosynthetic pathway of insulin is well described [21]. However, the critical components which are involved in the control of β -cell function, i. e. recognition of D-glucose as a major stimulus for insulin release, initiation of secretion and glucose control of insulin biosynthesis, are yet to be identified. One hypothesis to explain the specific loss of the pancreatic β cells in Type 1 diabetes is that an abnormal immune reaction, being controlled by certain HLA-D region genes, is directed explicitly against molecules expressed solely on the β cell. Recent advances in biology and medicine,

Fig. 1 A and B. The endocrine pancreatic mass in **A** healthy control subjects and **B** Type 1 (insulin-dependent) diabetic patients and the relative distribution of the endocrine B, A, D and pancreatic polypeptide (PP) cells (Data from 4)

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Table 1. Immune phenomena associated with Type 1 diabetes

Immune phenomena	Reference
Cellular immune abnormalities	
Insulitis	Gepts [3]
Cellular hypersensitivity to pancreatic antigens	Nerup et al. [6]
Some alterations in T- and B-lymphocytes	Mascart-Lemone et al. [7] Jackson et al. [8]
Signs of polyclonal activation	Horita et al. [9] Papadopoulos et al. [10]
Humoral immune abnormalities	
Autoantibodies reactive with islet cells	Bottazzo et al. [11] MacCuish et al. [12] Lernmark et al. [13] Doberson et al. [14] Bækkeskov et al. [15]
Other organ-specific autoantibodies	Nerup and Binder [16] MacCuish and Irvine [17]
Genes linked to HLA-region Class II antigens important in controlling the immune response	Platz et al. [18] Cudworth and Wolf [19] Owerbach et al. [20]

Fig. 2. Schematic diagram of a pancreatic β cell showing the major pathways and cellular localisation of gene activities, protein synthesis and formation of organelles involved in storage and secretion of insulin

most prominently the rapid development of molecular biology, now offer new and powerful means by which to study these aspects of Type 1 diabetes mellitus.

Effects of islet cell antibodies

The immune system is able to generate innumerable variants of immunoglobulin molecules that can bind to their corresponding antigen or to part of the antigen.

The variable region of immunoglobulin heavy and light chains is coded for by several genes, present in germ-line DNA, which, following antigen-specific stimulation, are re-arranged somatically to form the variable region gene. The expression of immunoglobulin genes and the resulting secreted form of antibody molecules are also highly diverse. There are differences in binding capacity to one and the same antigen and the ability to mediate innate immune functions, such as complement-dependent cytotoxicity, may vary. The tissue preparation which harbours the antigen and the method used to detect the antibody-antigen complex is therefore of importance in the detection and characterization of an antibody. During the last few years, a number of assay systems have been developed to permit the detection of islet cell antibodies associated with the onset of Type 1 diabetes (Table 2).

Islet cell cytoplasmic antibodies (ICA) are detected by an indirect immunofluorescence test on frozen sections of human pancreas [11, 12, 22, 23]. The ICA reaction usually covers all endocrine islet cells, but it is possible, with a specific monoclonal antibody to human proinsulin [27] to demonstrate that the reaction clearly involves β cells [28]. The antigen(s) detected by ICA remain to be identified. It has been much debated whether ICA are involved in the pathogenesis of Type 1 diabetes or whether they appear after lesions to the β cells. The reaction pattern of ICA towards intracellular molecules is often used as an argument that these antibodies represent a secondary response to an unknown primary lesion. It is conceivable that damage inflicted to β cells and resulting in a specific lysis, could release intracellular antigens that would be recognized by the immune system thus leading to the formation of ICA. In this respect, ICA would represent a marker for β -cell destruction, on-going or already passed. If ICA levels are regulated as immunoglobulins in general, one would assume that the ICA levels decrease with decreasing levels of antigen. It was, therefore, of considerable interest that early cross-sectional studies showed a decreased prevalence of ICA with increasing duration of Type 1 diabetes [12, 23]. However, it was not clear from these studies to what extent the decrease reflected varying levels of ICA. Thus, efforts were made to standardize the ICA assay, to control its reproducibility and to estimate end-point titres [29]. This approach was used to follow 82 newly diagnosed Type 1 diabetic patients, with an age of onset representative of the age-related incidence of Type 1 diabetes in Denmark [30]. The end-point titre of ICA was determined on nine occasions during a 30-month follow-up period, together with fasting C-peptide levels as a measure of residual β -cell function. The median fasting C-peptide level was below the lower limit of normal at diagnosis, but rose rapidly, yet temporarily, for 1–6 months following initiation of insulin therapy. However, after 1 year, fasting C-peptide levels decreased continuously, reaching a median level below the lower limit of normal control subjects 30 months after commencing insulin therapy. The decrease in fasting

Table 2. Islet cell antibodies in Type 1 diabetes

	References
Islet cell (cytoplasmic) antibodies	Bottazzo et al. [11] Lendrum et al. [22] Irvine et al. [23]
Islet cell surface antibodies	Lernmark et al. [13] Maclaren et al. [24] Lernmark et al. [25]
Cytotoxic (complement mediated) islet cell antibodies	Dobersen et al. [14] Soderstrum et al. [26]
Immunoprecipitating islet cell antibodies	Bækkeskov et al. [15]

Fig. 3. Inhibition of glucose-stimulated insulin release from column-perfused rat islet cells by human immunoglobulin prepared from the sera of Type 1 diabetic children positive for ICSA. Following a 60-min perfusion period in the presence of D-glucose (5 mmol/l) alone, the dispersed islet cells were exposed either to immunoglobulin from healthy control subjects (○—○) or from ICSA-positive Type 1 diabetic patients (●—●). Mean $\pm$ SEM values for six different individuals in each group are shown. * $p < 0.05$, ** $p < 0.025$. [Data from 36, reproduced with permission from the American Diabetes Association]

C-peptide levels reflected the corresponding increase in insulin requirement.

With regard to ICA, 57% of the 82 patients had antibodies at the time of diagnosis, while only 29% remained positive at 30 months. The median titres were 1:81 and 1:3, respectively, which suggest that the decrease in prevalence of ICA with increasing duration of Type 1 diabetes, is in fact due to higher levels of ICA at the time of diagnosis. Patients with a high ICA titre at onset tend to remain ICA-positive even after a prolonged period, and it was noted that the median C-peptide levels among patients with ICA persisting for 30 months were lower than corresponding values for ICA-negative patients, decreasing from 0.11 pmol/ml at 18 months to 0.09 pmol/ml at 24 months and 0.06 pmol/ml at 30 months compared with 0.18 ($p = 0.04$), 0.15 ($p = 0.05$) and 0.16 ($p < 0.003$) pmol/ml, respectively. The increased rate by which fasting C-peptide levels declined in the ICA-positive patients was mirrored by the significantly increased amounts of insulin injected ($p = 0.01$) to achieve the same metabolic control as for the ICA-negative patients. Careful quantitation of ICA, therefore, may be important for the prognosis of Type 1

diabetes as it relates to insulin requirement. Although a possible cause-relationship between the decline in endogenous insulin-production as reflected in fasting C-peptide levels, and diminished ICA titres cannot be defined in this study, it is possible that ICA levels somehow reflect previous β -cell destructive processes. Thus, determination of end-point titres for ICA may be a useful way to predict an increased rate of loss of endogenous β -cell function. In several recent studies (31–34), it has been documented that ICA may precede the diagnosis of Type 1 diabetes by several years. It remains to be determined whether healthy individuals with a high titre of ICA have an increased risk of developing Type 1 diabetes.

The existence of islet cell surface antibodies (ICSA) (Table 2) offers the intriguing possibility that β -cell function may be influenced by the binding of these antibodies to their antigen on the β -cell surface [13]. In initial studies attempts were made to test whether ICSA were able to affect β -cell function either directly [35, 36] or indirectly by their ability to mediate complement-dependent cytotoxicity [37]. Using column-perfused dispersed rat islet cells [36] and immunoglobulin prepared from sera of newly diagnosed Type 1 diabetic children and matched controls, it was found that the immunoglobulin fraction from ICSA-positive Type 1 diabetic patients inhibited glucose-stimulated insulin release (Fig. 3). These experiments suggested that ICSA in sera from Type 1 diabetic children may interfere with β -cell function in vitro, an observation which has been confirmed recently [38, 39].

Influence of ICSA on cell function in vitro does not necessarily mean that these cells are affected under conditions in vivo. Although ICSA may circulate at high levels, it does not follow that they can cross the capillary barrier to reach the target antigen on the β -cell surface. Immunoglobulin prepared from ICSA-positive plasma of Type 1 diabetic children was injected daily for 14 days into immunosuppressed mice [40]. Following the last injection, the pancreas was perfused to determine the dynamics of insulin release in response to glucose, and the pancreas was extracted for insulin determination as a measure of total pancreatic β -cell mass [41]. Although glucose-induced insulin release was diminished in these mice, the total pancreatic insulin content was unaffected, which suggests that the ICSA injected were inadequate to cause sufficient β -cell destruction to result in overt Type 1 diabetes. It is concluded that ICSA inhibit β -cell function and that long-term exposure to these antibodies may be deleterious and contribute to the development of Type 1 diabetes.

What is the β -cell autoantigen?

The possible influence of ICSA on β -cell function raises the question of which antigen(s) these antibodies are able to recognize. Since ICA are designated autoantibodies, i.e. antibodies that recognize self, the antigen

Fig. 4. Pathways of cellular interactions involved when the immune system mounts a response towards a foreign antigen. Important components which may serve as targets in future attempts to control an abnormal immune response are (1) the MHC class II antigens, (2) the antigen-specific T lymphocyte receptor, (3) the cytolytic T lymphocyte receptor, or (4) the membrane-bound antibody on the B lymphocyte

detected by an autoantibody is referred to as an autoantigen. The pathway of cellular interactions involved when the immune system mounts a response towards a foreign antigen is complex and not yet explained fully (Fig. 4). It is assumed that a similar pathway is also involved when an immune response occurs against an autoantigen. In brief, the antigen is presented to a T-helper lymphocyte (also called T-inducer cell), having a specific receptor for the antigen. The T-helper lymphocyte receptor is thought to be able to recognize the antigen only if it also recognizes simultaneously an MHC Class II antigen (HLA-DR antigen in man). Antigen presentation is therefore restricted, since the antigen-presenting cell must have the same Class II antigenic specificity as the T-helper lymphocyte. The activated T-helper lymphocyte can promote specifically proliferation and differentiation of cytotoxic T lymphocytes and/or B lymphocytes. The latter have receptors, i.e. membrane-bound antibody molecules on their cell surface. The former cells are able to recognize and kill the target cell if it is expressing the antigen on its cell surface. However, the killing is restricted since the cytolytic T lymphocyte will kill only if the MHC Class I antigen is shared between the T-lymphocyte and the target cell. Although the B-lymphocyte can recognize a circulating antigen, it will not proliferate and differentiate unless help, in the form of a B lymphocyte growth factor(s), is provided by the T-helper lymphocyte.

The formation of antibodies is controlled partially by the availability of antigen, but the levels of circulating antibodies may be controlled also by feed-back mechanism. This phenomenon involves the formation of antibodies which recognize the antigen-binding portion on the original antibody. This part of the antibody is the idiotype and antibodies which are formed specifically against the idiotype are referred to as anti-idiotyp-

ic antibodies. Such anti-antibodies are thought to play an important role in controlling the immune response to antigens.

In order to test the hypothesis that an abnormal immune response towards the pancreatic β cell is involved in the pathogenesis of Type 1 diabetes, it is essential to identify and characterize relevant autoantigens. In a first approach [15], pancreatic islets from human cadaver kidney donors were isolated and cultured in vitro in the presence of a radioactive amino-acid, ^{35}S -methionine. Proteins synthesized (Fig. 2) during culture will incorporate this amino-acid and become radioactive. The islets were lysed in a detergent which permits solubilization of plasma membrane-proteins, and this lysate was incubated with sera from Type 1 diabetic patients and control subjects. Immune complexes formed between autoantibody and autoantigen during incubation were first adsorbed to a Protein A-Sepharose immunosorbent, washed, and boiled in sodium dodecyl sulphate (SDS) to denature and solubilize bound antibody and antigen. The solubilized material was subjected to SDS-gel electrophoresis, thus separating the proteins according to their molecular mass [15, 42]. It was found that sera from Type 1 diabetic patients specifically detected an M_r 64000 molecular weight component [15]. Cross-reactivity between Type 1 diabetes sera and rat pancreatic islets was also demonstrated [36]. The M_r 64000 autoantigen represents a minor islet component and it is thought to be a pancreatic β -cell specific membrane component since the presence of M_r 64000 autoantibodies coincided with that of ICSA (also β -cell specific) in the sera of Type 1 diabetic patients [15]. Current studies are aimed at the isolation and characterization of this antigen, using recent techniques of electrophoretic elution of immunoprecipitated antigens [43] and at the determination of its primary structure by microsequencing [44].

The identification and isolation of any cell-specific autoantigen would be of considerable interest since it will allow not only investigations on the mechanisms by which an autoimmune response against the autoantigen develops and is controlled in susceptible individuals, but also the development of more sensitive and specific immunoassays for autoantibodies. This is of particular importance since ICA and other immune abnormalities may be present several years before the clinical onset of Type 1 diabetes [31–34]. In one individual followed for 2 years before the diagnosis of Type 1 diabetes [35], consecutive serum samples were analyzed for their ability to immunoprecipitate the M_r 64000 autoantigen (Table 3). This analysis indicated that autoantibodies against this component also may be present before the diagnosis of Type 1 diabetes and, in fact, may precede ICA. Similar observations have been made in the spontaneously diabetic BB rat (Table 3) which develops insulin-dependent diabetes with many similarities to that in man [45]. In some animals which later developed insulin-dependent diabetes, both ICSA and autoantibodies against an M_r 64000 component were present even at the time of weaning [46, 47].

Table 3. Presence of autoantibodies before the diagnosis of Type 1 diabetes

<i>Studies in man</i> [44]					
	Months before diagnosis				
	24	21	9	3	0
M _r 64000 antibodies	+	+	+	+	+
ICA	—	—	—	—	—
<i>Studies in the spontaneously diabetic BB rat</i> [45]					
	Days before diagnosis				
	35	25	20	10	0
M _r 64000 antibodies	+	+	+	+	+
ICSA	±	+	+	+	+

Fig. 5. The human MHC on the short arm of chromosome 6 with the centromere shown to the left. α and β indicate the number of genes currently thought to code for these polypeptide molecules in each of the three known loci in the HLA-D region **Fig. 6.** The HLA-DQ-chain cDNA probe used to determine restriction fragment length polymorphism in Type 1 diabetes. The cDNA probe was derived from mRNA formed by transcription of one of the HLA-DQ β -chain genes. This gene is composed of intervening sequences (introns) interrupting the coding sequences which contain the genetic information for the amino acid sequence of the signal peptide, the first domain (D1), the second domain (D2), the transmembrane region (TM), the cytoplasmic portion (CT) and the 3' non-coding region

It is concluded that antibodies against the M_r 64000 antigen may be detected long before Type 1 diabetes is diagnosed and that such antibodies may be a useful marker to predict Type 1 diabetes.

Genetic control of the autoimmune reaction against the pancreatic β cell

The autoimmune reaction against certain pancreatic β -cell antigens, often associated with the onset of Type 1 diabetes, appears not to occur in all individuals, but only in those who are HLA-DR3- and/or DR4-positive [18, 19]. The well-documented association between Type 1 diabetes and these HLA-DR specificities is im-

portant, since it may signify that genes coded for in the MHC somehow control abnormal immune reactions directed against the pancreatic β cell. However, it is notable that although 93–98% of Type 1 diabetic patients lie within this category, the frequency of these specificities in the control population is as high as 57–58% [18, 19]. In other words, nearly half the population in a northern European country, such as the UK or Denmark, do not have the propensity to develop Type 1 diabetes. However, there remains an explanation why Type 1 diabetes, the prevalence of which is about 0.3%, affects mainly the HLA-DR3- and/or DR4-positive population. One possibility is that these HLA-DR specificities are linked to a diabetogenic gene in the HLA-D region of the MHC on the short arm of chromosome 6 (Fig. 5). It is equally feasible that environmental factors may influence an abnormal process which eventually leads to sufficient disappearance of pancreatic β cells and clinical onset of insulin-dependent diabetes. The role of viruses in the aetiology of Type 1 diabetes is much debated [48, 49] as is the possibility that chemical agents present in the environment might be of importance [50]. The hypothesis that environmental factors are important to the pathogenesis is supported by observations that the concordance rate for Type 1 diabetes among monozygotic twins is only 50% or less [51, 52]. The seasonal variation in incidence is yet another argument for an influence of environmental factor(s) [53, 54].

Why is it then of interest that only individuals of certain HLA-DR types develop Type 1 diabetes? The HLA-DR molecules are Class II transplantation antigens which are coded for in the HLA-D region (Fig. 5) and represent two dissimilar subunits called α and β chains, being approximately M_r 34000 and M_r 28000, respectively. These heterodimeric proteins are expressed on the surface of immune competent cells and are important to the regulation of the immune response [55–57]. Recent molecular cloning of HLA-D region genes [58, 59] have revealed that each locus of the HLA-D region codes for one or several α or β chains (Fig. 5). A cloned probe (Fig. 6) for one of the HLA-DQ β -chain genes [61, 62] (previously named HLA-DC but renamed at a recent meeting [60]), was used in a DNA-DNA hybridization analysis by Southern blotting [63]. This technique, referred to as restriction fragment length polymorphism analysis, was applied to homozygous tissue typing cells [64], HLA-DR identical control and diabetic individuals [65] and to a linkage analysis in HLA-DR typed control and diabetic families [66]. These studies showed that the polymorphism detected by the HLA-DQ β chain, and confirmed by others [67, 68], was associated with HLA-DR and that HLA-DR identical control and Type 1 diabetic patients differ in the presence of certain restriction fragments [65]. It was concluded from these studies that the restriction fragment length polymorphism with an HLA-DQ β -chain probe is more closely associated to Type 1 diabetes than tissue type alone and that DNA probes may better de-

Fig. 7. A circuit of molecular biology indicating how an approach to obtain partial structural information at one level could lead to discovery of the entire structure by methods involving molecular cloning, chemical synthesis and protein chemistry

fine the risk of developing this disease. In particular, after digestion with the restriction enzyme BamHI, a fragment of about 3700 base pairs (3.7 kb) in size, was found rarely among Type 1 diabetic patients [65, 66]. Instead, these patients had a BamHI (12 kb) fragment which was lacking in BamHI (3.7 kb) fragment-positive individuals (B. Michelsen, personal communication). Cloning of the BamHI (3.7 kb) fragment from the HLA-DR4-containing chromosome of an HLA-DR4/DR7-positive healthy female individual without a family history of diabetes mellitus [69] demonstrated that Type 1 diabetic patients may lack a BamHI restriction site in their genomic DNA, not in a part of a gene coding for a HLA-DQ β -chain, but rather in one of two possible intervening (intron) sequences. This observation suggests that Type 1 diabetes is either more closely associated to this HLA-DQ β -chain gene or that this particular genomic sequence is in close linkage to a hypothetical diabetes susceptibility gene(s).

Since intervening sequences may be important to the function of eukaryotic genes [70, 71], studies were initiated to determine the cell-surface expression of HLA-D region β chains. Since these proteins are highly polymorphic, we have predicted the amino-acid sequence from cloned genes [72, 73], synthesized peptides representing intramolecular sequences and raised antibodies against the peptides. These antibodies are used in an indirect immunofluorescence assay to test whether they react with the native protein. A rabbit antibody against a sequence present in the second domain of a HLA-DQ β chain was found to recognize, not only denatured β chains in a plasma membrane preparation, but also monocytes known to express both HLA-DQ antigens [74]. It is concluded that the generation of antibodies against synthetic short peptides may permit the detection of cell surface expressed molecules which are important to the regulation of the immune function.

Future studies: possible ways of preventing diabetes

There have been numerous attempts to intervene with the course of diseases which are associated with abber-

rant immune functions. A wide variety of hormones and drugs are available to test to what extent they interfere with the appearance and the course of an autoimmune disease. In fact, in some instances, therapeutic effects of immune-modulating agents are used as a proof that the pathogenesis of a disease has an immune component. Immune-intervention, therefore, spans a broad area of medicine covering vaccination, therapy of inflammation as well as tumour and transplantation immunology. In fact, development in some of these areas has progressed very rapidly with increasing specificity of the reagents being developed. The documented precision of the immune response to recognize a foreign antigen or its ability to attack and kill a cell infected by a virus has, however, not yet been met by current approaches to influence the immune response. The major task is to be able to intervene with an immune response against a foreign antigen and only against that antigen. Dramatic examples of the opposite process, that of no precision, is total lymphoid radiation, use of cortisone or prednisone, plasmapheresis or cyclosporin A therapy. These drugs or procedures have one thing in common, namely, they inhibit or affect the entire immune system.

In Type 1 diabetes several attempts have been made to influence the course of the disease by immunotherapy [75]. Plasmapheresis, combined [76] or not [77] with steroids, prednisone [78, 79], interferon [80, 81] inosiplex [82], or methisoprinol [83] have been administered to newly diagnosed Type 1 diabetic patients without producing long-lasting insulin-independence or improvement in metabolic control. The possibility by a number of techniques, to modulate or alter the immune response to decrease the incidence of diabetes in the spontaneously diabetic BB rat [45] have further stimulated efforts to carry out early immune intervention in man. Since cyclosporin A was found to decrease the incidence of diabetes in the BB rat [84], this drug was tested in an open study in man [85]. In this study some patients were not only able to discontinue their insulin therapy but their course of endogenous insulin production showed some increase compared with other patient populations. It is recognized that cyclosporin A is an effective immunosuppressive agent but it also has several side effects. A recent report indicates nephrotoxicity at therapeutic dose-levels [86]. That Type 1 diabetes is associated with powerful immune activity against the pancreatic β cell is illustrated by the recent observations in pancreatic transplantation between identical twins who were discordant for Type 1 diabetes for more than 15 years [87]. A recurrence of Type 1 diabetes was observed in three diabetic twins receiving a pancreatic transplant without immune suppression. Not only did the function of the β cells cease but a biopsy before and after transplantation revealed the appearance of inflammatory cells in the islets, an insulinitis which was not seen before transplantation. If pancreatic transplantation is to become a possible means by which to increase the life span of Type 1 diabetic patients, it may well become necessary to administer immune suppressive therapy

for life. In future studies, would it be possible to prevent a specific immune response directed against one particular β -cell antigen? By carefully controlling the different arms of the immune response towards an antigen, would a β -cell destructive process be influenced?

Several major approaches to influence the immune response to an antigen may be envisaged (Fig. 4). Some of them have been attempted already in other autoimmune disorders, such as experimental allergic encephalitis [88, 89], acetylcholine receptor-induced myasthenia gravis [90, 91] or in thyroiditis [92]. Molecular cloning of the genes for antigen(s), antibodies and T lymphocyte receptors, as well as MHC Class I and II antigens, are important for the analysis of those components which are central to the development of an immune response to an antigen. The structure of these components, which may form the basis on which to obtain the necessary reagents, might be obtained by a number of methods and strategies (Fig. 7), all of which are available and are being utilized in a wide variety of systems [93]. Once any of these components have been identified, for example, by antibodies specifically raised against these proteins or parts of them, it should become possible to test whether any such reagent would influence the immune response (Fig. 4). A targeted approach, for example, would be: (1) to raise an antibody which is directed against a specific Class II antigen molecule controlling the immune presentation mechanism. Unless such a reagent would remove only those cells which are involved in antigen presentation of pancreatic β -cell specific antigen, a complete inhibition of other important immune functions would be induced as well. (2) Another antibody of interest would be that directed against the T-cell receptor for a pancreatic β -cell specific antigen. Such an antibody may be highly specific and remove the T-lymphocyte from the circulation, thereby making an immune response towards the antigen impossible. (3) A third reagent would be an antibody to the receptor on a cytolytic T lymphocyte which is able to recognize the pancreatic β cell. Such an antibody would be ideal to influence the cellular immune killing of pancreatic B cells. (4) A fourth approach would be a specific reagent against the B-lymphocyte receptor, i.e. the antibody molecule expressed on the cell surface. This reagent could be either the antigen itself, or the antigen coupled to a toxin [92], which by being internalized would be able to kill the B-lymphocyte. One could also envisage the preparation of antibodies against the antigen-binding part of the antibody molecule. Such anti-idiotypic antibodies would be able also to bind to the T-lymphocyte receptor and influence the viability of both the B- and T-lymphocytes. Once these reagents have become available it will be possible to test the hypothesis that presentation of an autoantigen to the immune system is associated either with the development of Type 1 diabetes in susceptible individuals or with progression of the disease after diagnosis. Recent developments in molecular biology, which have opened up new avenues, will now make it possible to study Type 1

diabetes in a way that could hardly be envisaged just a few years ago.

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