Review articles

β -Cell damage in diabetic insulinitis: are we approaching a solution?*

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Key words: Type 1 diabetes, insulinitis, autoimmunity, islet cell antibody, cell-mediated immunity.

Was it a pure coincidence or a genetically determined trait which led to the original description of islet-cell antibodies? Whatever the answer to this intriguing question, the fact is that the young researcher who observed the reaction under an ultraviolet microscope was born in Venice. He was immediately attracted by the green fluorescent shape of the islets of Langerhans, standing out brightly against the dark background of the surrounding exocrine pancreatic acini, much as the islands of his native city stand out against the dark blue waters of the Venetian Lagoon. In the initiation of this saga, the 'geographical' genetic influences of Alex Florin-Christensen, born in Buenos Aires of a Danish/Spanish ancestry, and of Deborah Doniach, born in Geneva of mixed Russian parentage, remain to be established. Nevertheless their contributions to it were very real.

Islet cell antibodies (ICA) were first detected in patients suffering from 'Schmidt's syndrome', an uncommon polyendocrine disease centred on adrenocortical insufficiency caused by autoimmune adrenalitis [1]. Interestingly enough, the sera of these patients were already known to contain multiple autoantibodies to endocrine glands, and they were also the first sera which later were found to react specifically with prolactin cells in the human pituitary gland [2]. These findings gave a clear indication that autoimmune processes can be implicated in lesions affecting the pituitary. Previous observations on 'lymphocytic hypophysitis' were based entirely upon post mortem material and retrospective clinical deductions tinged with speculations of possible autoimmunity [3].

Several laboratories had previously tried to identify specific ICA in Type 1 (insulin-dependent) diabetic patients. However, their efforts proved unsuccessful. Historically it is of interest to recall that the patients tested in these initial attempts had been diabetic for many years and that the majority of them had no other overt endocrine abnormalities [4]. In retrospect it is also worth noting that the introduction of Ploem's epi-illumination system for ultraviolet microscopy, together with the availability of fresh normal blood group O human pancreas from kidney donors, greatly improved the sensitivity of the immunofluorescence test for ICA. Based on the previous negative findings and on the low prevalence of ICA in long-standing diabetes, which could now be confirmed, both our group and that based in Edinburgh [5] concluded that ICA were found mainly in a rare subgroup of diabetic subjects.

By definition, medical science is a dynamic discipline which accepts new findings reluctantly unless they are fully substantiated. In the early 1970's the British Diabetic Association established a register of newly diagnosed diabetic children. This scheme was set up primarily to investigate the epidemiology of the disease throughout the United Kingdom and to study the possible role of viral infections in its causation [6]. It was thanks to the availability of a large number of sera collected from diabetic children within weeks of initial diagnosis that Richard Lendrum and his colleagues were, surprisingly, able to demonstrate ICA in this material. The reactions were similar to those found in polyendocrine cases [7]. The negative results obtained previously in patients with long-standing disease were soon explained by the subsequent demonstration that in a large proportion of diabetic children ICA tended to disappear from the circulation within months, or at least within the first few years, after onset of symptoms. In classical 'endocrine autoimmunity', specific humoral and cell-mediated immune phenomena tend to persist for many years, even when the affected gland is largely atrophic. We now know that in polyendocrine diabetes there is a slower destruction of the pancreatic islets with

* Based on the Minkowski Lecture delivered at the 18th Annual Meeting of the EASD, Budapest, September 1982

residual C-peptide secretion persisting for some years. By contrast, in so-called 'juvenile' Type 1 diabetic patients, only a minority show persistence of various immune reactions to islet cells over a period of years. This small minority also has clinical and serological features often described in polyendocrine autoimmunity [8]. These early results sustained the credibility of the concept that Type 1 diabetes was a heterogeneous group of disorders and that several causes must be implicated in the initiation of the β cell damage [9].

1974 and 1975 were exciting years in diabetes research and the work accomplished in several laboratories laid the foundations for the subsequent expansion of this chapter in human pathology [10, 11].

Humoral immunity in diabetes

ICA are organ-specific for endocrine pancreas, they cross-react with other species, and they are invariably of IgG class. Several antigens, including some that are present only in β cells, are now envisaged in what is basically a polyclonal autoimmune response with subclass restrictions [12]. The antibodies may be complement fixing (CF-ICA) and this is a separate variant [13]. The more common ICA are not able to fix complement (ICA-IgG). As the islet-cell antigens have not yet been isolated and are at present only partially characterized [14], cytoplasmic ICA are still normally detected by standard indirect immunofluorescence. Unfixed group O human pancreas is still the substrate of choice [15]. Fixed pancreas substrates give controversial readings, especially in complement-fixation immunofluorescence tests with anti-C3 conjugates used to detect CF-ICA [16].

In the screening test, ICA-IgG reacts with all four endocrine cells of the islet [17]. This 'shared' autoantigen is not represented on the cell surface and therefore cannot be in direct contact with sensitized lymphocytes in the living gland. Since circulating antibodies exert cytotoxic effects through complement-dependent mechanisms, extensive parallel tests for ICA-IgG and CF-ICA have been undertaken. In all diabetic groups tested, only half the ICA fixed complement [13].

Most interestingly, CF-ICA also constituted about half the total ICA-IgG reactivity in healthy, genetically predisposed, first-degree relatives of diabetic probands. Non-diabetic 'autoimmune endocrine' patients gave similar results. In both groups some individuals with persistent CF-ICA later became diabetic. This led to the conclusion that CF-ICA reactions obtained on pancreatic sections include the β -cell-specific autoantibodies that are the most relevant markers for ongoing insulinitis. We now know that some CF-ICA selectively stain β cells [18]. We also found some sera containing complement-fixing variants specific for glucagon or somatostatin cells. These single-cell antibodies were known to exist in some diabetic patients [19].

Islet cell surface antibodies (ICSA) are detected on viable cultured human fetal [20] or adult animal pancreas [21], and here too separate specificities exist for α and β cells as shown by Van de Winkel et al. [22]. These authors used a cell-sorter to separate ICSA-positive cells. In addition to the reactions specific for α , β , and δ cells, they identified a small number of sera containing surface reactive antibodies to 'pancreatic polypeptide' (PP) cells. Antibodies to these cells have not been detected so far on cryostat sections, probably because it is necessary to employ the dorsal portion of the head of the pancreas known to be rich in PP cells (the PP lobe described by Orci [23]). By analogy with the surface expression of other organ-specific 'microsomal' systems, such as the thyroid, gastric, or adrenal, in which the cytoplasmic autoantigens are also expressed on the cell surface [24], it is probable that some 'selective' CF-ICA represent the cytoplasmic portion of those ICSA that react with β cells and are therefore cytotoxic to cultured animal islets, or interfere with glucose-stimulated insulin secretion in these cultures [25]. However, islet-cell surface staining is obtained in about 30% of diabetic sera that give negative results on sections [26]. This strongly suggests the involvement of an additional antigen that is expressed entirely on the plasma membrane of β cells. These data support the existence of multiple antibodies specific to different islet antigens, and it is only when the antigens are isolated and characterized that we will be in a position to study their effects separately. The 'monoclonal revolution' has already produced hybrid clones that permit the analysis of human polyclonal antibody mixtures in 'receptor' diseases, such as myasthenia gravis and thyrotoxicosis.

Humoral immunity in predisposed individuals

Because Type 1 diabetes often starts as an abrupt illness, it was a surprise to discover, by regular testing of 'unaffected' relatives of diabetic children, that autoimmune phenomena exist for years in some siblings who are HLA-identical or haplo-identical with the proband (and thus have probably inherited the diabetogenic genes), and possess CF-ICA during the latency period [27]. These siblings proved to be the most vulnerable for future diabetes.

In the Barts/Windsor/Middlesex Family study, 13 of approximately 700 relatives had persistent CF-ICA and seven of these became diabetic over a follow-up period of 5.5 years. A recent analysis of the family data showed interesting new features [28]. Five unaffected siblings who had CF-ICA for 11–44 months became negative. A further four siblings had these antibodies intermittently for 55–61 months of observation, while one unaffected brother has shown this reactivity for over 56 months without developing evidence of glucose intolerance. Surprisingly, one sibling and one parent, who were constantly negative for conventional ICA-IgG as

well as CF-ICA, became positive after 24 and 39 months of follow up, and the antibodies have so far (late 1983) persisted in their sera for 20 and 11 months, respectively.

In the diabetic families, fluctuations of ICA-IgG reactions are often seen, and sometimes the antibodies appear simultaneously in more than one member including the diabetic proband. Overall, ICA reappeared in 27% of diabetic children who had given many negative results for up to 5 years and had been diabetic for up to 20 years. This was somewhat unexpected in view of previous studies on large groups of diabetic children showing disappearance of ICA between 6 months and 3 years after onset of the disease in the great majority of cases [29]. The unexpected disappearance of CF-ICA in unaffected members of these families might explain why no fresh cases of diabetes have appeared since November 1981. The fluctuations in ICA perhaps reflect silent intermittent infections with insulotropic viruses and we are looking at viral antibody patterns in these individuals. A recent retrospective study on a set of identical diabetic triplets revealed the first appearance of ICA-IgG in a non-diabetic member, 5 years before he became diabetic; the total period of discordance was 39 years [30]. Identical twins are known to remain discordant in at least 50% of cases [31]. Prospective tests for ICA over a period of 7 years in 46 discordant co-twins showed that only two had CF-ICA and one of these became diabetic 5 years later (C. Johnston, Personal communication).

Practical value of ICA tests

During the past 10 years ICA determinations in epidemiological studies have helped to clarify the role of autoimmunity in diabetic populations of different ethnic and genetic backgrounds [32, 33]. However, it is now clear that the test does not help the final diagnostic assessment of the patient. A positive or negative ICA result does not alter the natural history of the disease or the absolute need for insulin in these patients. Similarly, regular monitoring of ICA during the 'honeymoon' period has not clarified the obscure events underlying the remission phase (C. Coscelli, personal communication).

Various unsuccessful attempts have been made to see whether the presence of ICA can be of any value in explaining the severity of clinical diabetes, or its 'brittle' variant. Similarly, ICA has not helped to assess residual β -cell function as measured by C-peptide determination [34]. Microvascular diabetic complications do not correlate with the presence of ICA or other autoantibodies [35]. Raised levels of circulating immune complexes are the only abnormal immunological variable detected in these patients [36]. However, these markers are also found in Type 2 (non insulin-dependent) diabetic patients with similar complications. In pancreatic transplantation, ICSA appear to be better markers than

cytoplasmic ICA for monitoring islet rejection in progress [37]. It remains to be seen whether this surface reactivity is induced by transplantation antigens or results from reactivation of memory autoreactive lymphocytes.

As previously mentioned, ICA and especially CF-ICA testing greatly helps in the identification of high risk of diabetes in genetically susceptible individuals in Type 1 diabetic families. In addition, it is advisable to screen for ICA non-diabetic patients with other autoimmune endocrine disorders [38]. Polyendocrine patients who show mild glucose intolerance have a greater tendency to possess ICA [39]. Interestingly, when Type 2 diabetic patients with no evidence of other endocrine disorders are tested for the two ICA specificities, CF-ICA selects out a subgroup with lower C-peptide responses (approximately 10%) compared with similar cases lacking ICA (L. Croop, personal communication). The presence of ICA may indicate a future need for insulin therapy in these 'pseudo' Type 2 diabetic subjects. The issue concerning the use of immunosuppressive drugs in the early treatment of Type 1 diabetes is at present controversial. There is hope that in the near future more selective immunosuppressive agents will be developed and safely used. Controlled clinical trials could then select patients in whom various islet cell antibodies and cell-mediated immune parameters are still present at the time of entering the study and compare the efficacy of the treatment here with that in similar groups of patients who had already lost these markers. Such protocols can be justified if the disappearance of specific anti-islet cell immune phenomena, in particular CF-ICA, is interpreted as indicative of irreversible β -cell damage [40].

To screen for persistent ICA in long-standing diabetic patients is of diagnostic help. The presence of ICA is often associated with antibodies to other endocrine glands [41]. This may alert the physician to investigate and follow subclinical abnormalities in other glands [42]. For similar reasons, it is advisable to widen the autoantibody screen to include other organ specific antibodies in newly diagnosed diabetic children [43].

Re-examination of the insulinitis process

The lesions characteristic of autoimmune insulinitis, especially mononuclear cell infiltration, can be seen only in pancreatic tissues of newly diagnosed diabetic patients. Lymphocytes surrounding and infiltrating individual islets have been described mainly in young children, but occasional reports indicate similar appearances in elderly female patients with polyendocrine autoimmune disorders [44]. Previous histopathological studies on insulinitis were carried out on formalin-fixed pancreases, which do not permit evaluation of lymphocyte subsets by the monoclonal surface marker antibodies currently available. Furthermore, there are no previous histologi-

cal reports attempting to demonstrate immunoglobulin deposition in relation to islets of Langerhans.

A direct demonstration and a better definition of various immune phenomena occurring *in vivo* have recently been achieved by examining fresh-frozen blocks of pancreas obtained from a newly diagnosed diabetic child who accidentally died 24 h after diagnosis [45]. Active insulinitis was seen in this pancreas as anticipated. Immuno-histological studies confirmed the presence of pseudoatrophic islets, interspersed irregularly with islets showing milder lesions and a fairly well organized architecture. The majority of the islets were devoid of insulin cells, whereas cells secreting glucagon or somatostatin were well-preserved. The inflammatory process was scattered in foci throughout the gland and was more obvious in the tail and body of the pancreas. Multiple blocks had to be sectioned in these areas to find the cellular infiltrates of the insulinitis. Nevertheless, when such inflammatory foci were identified, the mononuclear cells were abundant and tended to surround or embrace individual islets or small groups of these. When various monoclonal reagents were applied and located by immunofluorescence with single or double fluorochrome techniques, the majority of mononuclear cells were found to be T-lymphocytes. Cytotoxic/suppressor lymphocytes appeared to constitute the main bulk of the infiltrate but the other known lymphocyte subpopulations, including natural killer and killer lymphocytes, were also represented. Ninety percent of autoreactive T-lymphocytes expressed HLA-DR antigens, indicating that they were 'activated' T-cells and suggesting a specific immune response directed against islet antigens.

When anti-IgG reagents were applied, several interesting phenomena could be observed. Pre-plasma cells synthesizing IgG, as shown by their bright intracytoplasmic fluorescence, were seen as if emerging from pancreatic vessels; they appeared to be streaming in the interstitium between exocrine lobules, ultimately to converge towards islets. In other fields these IgG positive immunocytes were seen stationed around individual islets. It is of interest that mature plasma cells are rarely seen by conventional histology in diabetic insulinitis.

Perhaps the most intriguing finding was the presence of IgG within some of the islets. In certain fields, endocrine cells, varying from a few isolated cells to large groups constituting up to two-thirds of the islet, appeared to be coated with a smooth layer of IgG on their outer membrane. This observation may be relevant for antibody-dependent complement-mediated cytotoxicity. The Ig coat could also serve as bridging molecules for killer cell activation [46]. Granular Ig deposits, resembling those seen in the glomerular basement membrane in 'immune complex nephritis' or on the thyroid follicular basement membrane in autoimmune thyroiditis [47], were not seen in this pancreas.

The most striking picture, however, was the pres-

ence of IgG inside the cytoplasm of islet cells. This finding suggests penetration of antibodies following injury to the cell membrane. Similar appearances have been observed affecting thyrocytes in autoimmune thyroiditis and in renal tubular cells in nephritis.

Class I major histocompatibility antigens or HLA-A, B, C molecules are known to be synthesised by and expressed on virtually all nucleated cells in the body. This was confirmed for pancreatic islet cells when monoclonal antibodies to the non-polymorphic portion of HLA-A, B, C molecules were applied to frozen sections of normal human pancreas. However, the same monoclonal reagents stained some of the diabetic islets much more strongly, indicating an overproduction of these membrane glycoproteins in the diseased pancreas. These preliminary results suggest a more precise role for cytotoxic T-lymphocytes in the recognition of islet surface antigens. Such cells are in fact known to operate exclusively by their recognition of Class I molecules when they eliminate tissue cells infected by environmental agents, i.e. viruses [48].

Could aberrant HLA-DR expression be relevant to autoimmune 'insulinitis'?

The Class II major histocompatibility antigens [Ia in mice), encoded in man by genes in the HLA-D region, play a key role in the presentation of antigens and regulation of the immune response. The expression of these cell-surface glycoproteins is normally restricted to B-lymphocytes, macrophages, dendritic and other antigen-presenting cells and capillary endothelium [49]. Modulation of HLA-DR expression has been observed only exceptionally outside the immune system; guinea-pig mammary gland/duct/epithelium becomes Ia positive during pregnancy and lactation [50]. Ia antigen expression can be induced during graft-versus-host disease and in some cancers [52].

HLA-DR molecules are not expressed on resting endocrine cells under physiological conditions although the Class I major histocompatibility products HLA-A, B, C can be demonstrated on the membrane and in the cytoplasm of some endocrine organs including the thyroid gland [53]. Recently, it has been demonstrated that normal thyroid cells in culture can be stimulated by mitogens and under these circumstances they actively synthesize and express HLA-DR molecules on their plasma membranes [54]. Perhaps more relevant to human autoimmune disease is the finding that thyrocytes isolated from Graves' disease glands did not require any mitogenic stimulus and spontaneously showed DR molecules when cultured in monolayers. This abnormal phenomenon was demonstrated by immunofluorescence with monoclonal antibodies to the non-polymorphic region of these surface markers. On cryostat sections from glands removed at partial thyroidectomy, the same phenomenon was more clearly seen and could be

analysed in relation to the other histological and immunological features of the goitres. The brightest DR staining was seen in Hashimoto's thyroiditis; in Graves' disease it appeared to be a very early index of focal lymphocytic thyroiditis [47]. Induction of HLA-DR expression on human adult islet cells has not been attempted as yet, but preliminary experiments on human fetal islets in culture showed that at that stage of embryonal development i.e. 13–20 weeks' gestation, islet cells are refractory to stimulation by phytohaemagglutinin. These experiments confirmed what had been observed using human fetal thyroid cells. When adult rat islets and thyroid glands were examined for DR expression by immunofluorescence using appropriate monoclonal antibodies for this species, they failed to show the phenomenon and it could not be stimulated by adding phytohaemagglutinin to the cultures. This difference between rodent and human endocrine organs is at present unexplained.

In the human 'diabetic' pancreas examined in detail by immunofluorescence, preliminary experiments have shown that some cells in diseased islets are stained with the monoclonal anti-DR antibodies used in the thyroid study. The shape and size of the DR-positive cells suggest that they may be endocrine in nature, rather than activated T-lymphocytes, macrophages or dendritic cells. We are now trying to identify these DR-positive cells more precisely by applying several combinations of monoclonal reagents and observing them with different fluorochromes. We have so far been able to show that the cells are neither glucagon nor somatostatin cells. It would indeed be most interesting if it could be demonstrated that β cells expressed DR inappropriately in diabetic insulinitis, as was shown for thyrocytes in Graves' disease and Hashimoto's thyroiditis.

Immune responses are initiated by HLA-DR positive cells presenting antigen to T-lymphocytes. Based on the thyroid experiments, and on the finding that DR molecules are found on thyrocytes in autoimmune thyroid diseases (and possibly also in the islet cells in diabetic insulinitis), a new hypothesis has been put forward for the mechanism of induction of autoimmunity in endocrine organs [55]. It is known that interferon is the best inducer of DR antigen expression. It was postulated that its production could be stimulated by viruses or other environmental agents, which might be present in the relevant endocrine gland of genetically predisposed individuals without causing signs of infection. The series of events which would follow is that of aberrant DR expression, presentation of surface autoantigens and subsequent induction of autoreactive T-cells. These T-cells would in turn activate effector B- and T-lymphocytes. Whether the initial induction of autoimmune T-cells led to autoimmune disease would depend on a variety of other factors such as abnormalities of the suppressor T-cell pathway, reported to coexist with autoimmunity and necessary to induce autoimmune diseases in mice. This mechanism of autoimmune disease induc-

tion explains vague associations with viral infections and long latency periods before disease becomes manifest, and gives an explanation for the well-documented HLA-DR association of autoimmune diseases in man, including Type 1 diabetes.

How do other endocrine glands contribute to diabetes?

Diabetologists have noticed for some years that prepubertal boys are tall for their age when they present with diabetes [56] and this has prompted a search for evidence of pituitary involvement. Previous studies showed anterior pituitary antibodies in about 7% of Type 1 adult diabetics who also suffered from 'polyendocrine' autoimmunity, and most of these antibodies appeared to react with prolactin cells [57]. In family studies, predisposed relatives having CF-ICA proved to have, in addition, antibodies reacting with several pituitary cell types [58]. The highest prevalence (4/7) was in those who became diabetic during the follow-up. In the prediabetic latency period, 36% of ICA-positive relatives had these antibodies; in newly diagnosed diabetes, 16% were positive by immunofluorescence on pituitary tissue; and in long-standing diabetes 2% only were positive. These results suggest either that similar viruses may infect pituicytes and β cells [59], or that the antibodies detected are markers of some as yet unknown stimulating immunoglobulins akin to those found in thyrotoxicosis [60], but acting on the pituitary receptors either directly or through increased secretion of as yet uncharacterized hypothalamic [61] or pituitary [62] hormones that affect insulin secretion. In this context it is of interest that some HLA-identical relatives in other prospective studies show increased insulin responses to arginine [63], suggesting that something stimulates the pancreas to 'regenerate' its β cells, which we know from the presence of CF-ICA are gradually being damaged. In polyendocrine patients who have similar long latency periods, there is usually a decreased insulin production [39].

Because some diabetic patients showed diminished responses of gastric inhibitory peptide (GIP) to protein test meals, immunofluorescence tests were carried out on human duodenum. A small proportion of Type 2 diabetic subjects proved to have antibodies to the GIP-secreting cells, and when sera from Type 1 diabetic patients were applied to gut sections, a proportion were positive on GIP and/or secretin cells [64]. The significance of these unexpected organ-specific reactions either indicates further heterogeneity in this complex syndrome or suggests that in some cases the enteroinsular axis also is involved in the final development of diabetes. Another interesting development is the report of human hybridomas raised with lymphocytes taken from young diabetics whose sera were positive for antibodies by immunofluorescence on pancreas and thyroid sections [65]. The monoclonal antibodies secreted by some

of these clones reacted with several endocrine glands, suggesting the existence of cross-reactive antigens in these organs. In human sera, each of the 'microsomal' autoantibodies is strictly organ-specific and it is possible, for instance, to adsorb out all the thyroid cytoplasmic antibody activity without affecting the titre of gastric parietal cell antibodies coexisting in the same serum. Also, the human organ-specific antibodies are mainly of IgG class whereas the multiple organ monoclonals were all IgM. Another important difference was that some clones cross-reacted with cytoskeletal elements and this is not seen in human organ-specific autoimmunity.

Are we ready to treat diabetes by immunosuppression?

Partial success was obtained with massive immunosuppressive therapy in a few newly diagnosed diabetic patients [66, 67] and more selectively with cyclosporin-A [68]. In the BB rat, it has proved possible to prevent the onset of diabetes by a variety of conventional immunosuppressive agents [69] and recently whole-blood transfusion from non-predisposed control rats to young BB animals has prevented clinical expression of the disease and diminished the frequency of insulinitis [70]. Now that we can identify predisposed individuals in human diabetic families, the question remains: is it justifiable to immunosuppress them in order to slow down the insulinitis damage and possibly prevent diabetes? We fully agree with Rossini [71] that the available immunosuppressive regimens do not select the right subsets of islet reactive immunocytes and are therefore too drastic. At the same time, it is probable that blockage of the autoimmune response to insulin-secreting cells would effectively prevent the disease, irrespective of whether the insulinitis is a primary autoimmune phenomenon or a secondary event initiated by environmental agents. The fact that 50% of CF-ICA-positive relatives in our study escaped clinical expression of the disease shows that we are not yet ready. It is impossible to envisage giving any of the available forms of non-selective immunosuppression to symptomless individuals. Furthermore, the unexpected recent finding that, in our family study, individuals carrying CF-ICA for many years have now lost these damaging antibodies, warrants even greater caution in embarking upon adventurous clinical trials.

There is an urgent need to dissect further the autoimmune immune networks in order to identify future diabetics with 100% certainty. 'Activated' T-lymphocytes are present in increased numbers in the blood of newly diagnosed Type 1 diabetics [72, 73]. A possible new strategy is to establish continuous lines from such cells in the presence of T-cell growth factor, antigen-presenting cells and 'feeders' [74, 75]. Some of the circulating DR-positive T-cells are likely to be specifically involved in recognizing relevant autoantigens in the islets. Such self-replicating T-cell lines already exist for exper-

imental thyroiditis in the rat [76]. These cell lines have been shown to provide 'help' in the synthesis of thyroglobulin antibodies by B-lymphocytes and it is hoped to achieve a parallel result with 'diabetic' lymphocytes, which might be expected to act as 'helper cells' for the synthesis of islet-cell surface-reactive antibodies.

A possible future treatment strategy may result from such experiments. If a T-cell line specific for a dominant islet-cell surface antigen can be developed, it might also be possible to produce monoclonal antibodies able to inhibit this 'subset' of T-cells in the patient's own circulation and thus prevent triggering or expansion of organ-specific effector/cytotoxic lymphocytes.

Future prospects

Ideas on the role of autoimmunity in the causation of diabetes are in a state of flux due mainly to prospective immunological studies and to the new cloning techniques.

A detailed analysis of HLA-DR molecules by DNA probing techniques [77] underlines the polymorphism of the DR region and reinforces the concept of genetic heterogeneity in diabetic patients. New monoclonal reagents to the other molecules of the HLA-D region will soon be more generally available [78] and when applied to population studies they are bound to disclose further differences between diabetics and non-diabetic subjects [79].

Another interesting new development in the study of Type 1 diabetes is the unexpected finding of insulin-receptor antibodies of the IgM class in untreated juvenile diabetics [80]. There are also reports of spontaneous antibodies to insulin in such cases [81] appearing before any insulin injections. Although these preliminary results await confirmation, the presence of these two types of autoimmune reactions may suggest that insulin acts as a powerful immunogen, especially if an inappropriate secretion of immature hormone molecules is envisaged during the slow process of autoimmune β -cell damage as suggested by J. Rotter (personal communication). HLA-DR4 positive individuals are more liable to produce insulin antibodies, both to animal and human preparations [82] and these patients may be the best candidates for spontaneous autoimmunization to their own insulin or proinsulin [83]. The operation of anti-idiotypic networks can then explain the apparent insulin-receptor antibodies as 'internal image' anti-idiotypes made in response to the original anti-insulin idiotype epitope [84, 85]. Finally, the possible participation of other endocrine glands in directly influencing the metabolic status of β cells has great potentiality for future studies. The present hypothesis implies an active rôle for the pituitary gland and possibly for hypothalamic factors and gut endocrine cells in the regeneration of islet cells. These factors could be decisive in explain-

ing the events occurring during the long latency period which precedes the abrupt onset of the disease [18].

There is always a danger of being 'trapped' in pre-conceived notions and established dogmas. This is one of the most important lessons we have learned from Oscar Minkowski, the great experimental pathologist, who is annually celebrated by our European Association. He intuitively elucidated the primary role of the pancreas in the regulation of glucose homeostasis, after centuries of unsuccessful attempts, but his scientific curiosity moved him to explore other unknown pathological entities. He was one of the first to identify the pituitary gland as the major organ involved in acromegaly [86].

It is by pursuing unconventional lines of research that, we hope, the cause of Type 1 diabetes will be disclosed in the not too distant future.

Acknowledgements. I would like to pay tribute to my friend, Andrew Cudworth, who died so soon after this lecture was delivered, but made such an effort to be present in Budapest to hear me. Professor Mario Austoni of Padua University guided my first steps into medical science and encouraged my entry into the field of immunopathology. Professor Deborah Doniach took me by the hand and opened the gate into this fascinating field for me. After 10 years I still greatly enjoy her warm friendship and respect her scientific wisdom. My particular thanks go to Luca Chiovato, Betty Dean, Hemmo Drexhage, Toshiaki Hanafusa, Emilio Khoury, Marco Londei, Rita Mirakian, Paolo Pozzilli, Ricardo Pujol-Borrell, Anick Pouplard, Werner Scherbaum, Carmen Vandelli and Eva Wolf with whom it was fascinating to explore new venues in the mysterious world of 'endocrine autoimmunology'. Andrew Gorsuch, Kate Spencer and Ann Tarn have played a major part in clarifying the events leading to clinical diabetes and I am indebted to them. The 'autoimmunity team' in the Department of Immunology, The Middlesex Hospital and the 'immunogenetic team' in the Department of Diabetes, St. Bartholomew's Hospital were the unseen operational forces for moving our knowledge forward. Professor Ivan Roitt, head of my department, encouraged and supported the expansion of the subject which he and Deborah Doniach had initiated and developed more than 25 years ago. The recent collaboration with Dr. Marc Feldmann has already opened new exciting horizons for future research. I would like to thank the MRC in the United Kingdom, the Joint Research Board of St Bartholomew's Hospital, the British Diabetic Association, the Wellcome Trust Foundation, the Juvenile Diabetes Foundation in the USA, and Novo Laboratories, for their continued and generous support throughout the years. I am grateful indeed to have entered a special field of research which has allowed me to make so many enduring friendships around the scientific world. The manuscript was edited by Mrs. M. Clark.

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