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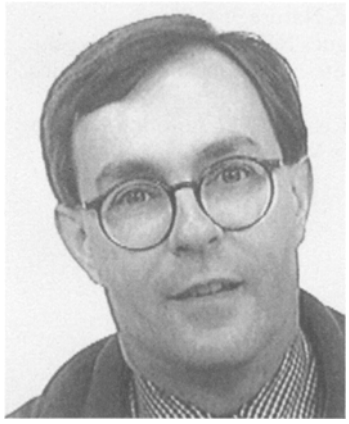

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IDDM: an islet or an immune disease?

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Summary Insulin-dependent diabetes develops as a consequence of the selective destruction of insulin-producing cells by an autoimmune reaction. However, the precise series of events which trigger anti-islet autoreactive T cells is still being investigated. Major issues will need to be raised before a comprehensive view of the anti-islet autoimmune reaction can be delineated. These include defining the primary site of activation of autoreactive lymphocytes and exploring hypotheses to explain the chronicity of the diabetes process. These issues all relate with the more general dilemma of the actual role of the islets of Langerhans in breaking self tolerance to beta-cell antigens. By studying non-obese diabetic mice deprived of beta cells following a single injection of a high dose of alloxan at 3 weeks of age, we recently obtained evidence that the activation of autoreactive T cells requires the presence of target islet cells in order to develop. [Diabetologia (1994) 37 [Suppl 2]: S90–S98]

Key words Diabetes mellitus, autoimmunity, animal models, autoantigens, autoantibodies, T cells.

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; GAD, glutamic acid decarboxylase; EAE, experimental allergic encephalomyelitis; NOD, non-obese diabetic; LCMV, lymphochoriomeningitis virus; BB, bio-breeding; TNF, tumour necrosis factor; CFA, complete Freund's adjuvant; Poly I:C, Polyinosinic polycitidilic acid; EMC, encephalomyocarditis; OS, obese strain; MHC major hisocompatibility complex

Insulin-dependent diabetes mellitus (IDDM) develops as a consequence of the selective destruction of insulin-producing cells by an autoimmune aggression. Autoantibodies associated with the disease process are detected against islet-cell cytoplasmic antigens [1], insulin [2], GAD [3], carboxypeptidase H [4], peripherin [5], a 37–40 kDa antigen [6], the gamma-interferon-inducible p69 antigen [7] and ubiquitous autoantigens [8]. Similarly, T cells which prolif-

Table 1. Major steps possibly involved in the development of autoimmune diseases

Genetic background
T-cell repertoire selection (role of MHC)
Capacity to present autoantigens (role of MHC)
Genetic defects
. decreased apoptosis (lpr, gld...)
. nul complement alleles
. target organ anomaly
Immune regulation
Biased B- or T-cell repertoire
. lymphoproliferative disorders
. expression of transgenic autoreactive B- or T-cell receptor
. transgenic expression of bcl-2
Cytokine overproduction[a]
. interferon, interleukin-2, tumour necrosis factor...
TH1/TH2 imbalance[a]
Idiotypic network imbalance[a]
Bypass of T-cell ignorance[a]
. molecular mimicry
. systemic presentation of "share" epitopes or antigens
Defective suppression[a]
Target organ
Aberrant expression of class II MHC molecules[a]
Quantitatively altered autoantigen expression functional alteration[a]
Delayed autoantigen expression during ontogeny[a]
Expression of an abnormal autoantigen[a]

[a] Possible target of environment

erate in the presence of human insulinoma cells, [9] murine [10] or porcine [11] islet cells, a 38 kDa secretion granule antigen [12–14], or GAD are detected in IDDM [15–17]. However, many islet autoantigens defined by B or T-cell recognition are not strictly beta-cell specific, and the detection of autoantibodies and autoreactive T cells does not infer their participation in tissue lesions. In experimental EAE, the activation of T cells specific for different basic myelin protein epitopes following immunization against the immuno-dominant 1–11 peptide indicates the difficulty of defining epitopes directly responsible for initiating an autoimmune process. In animal models, the transfer of diabetes by polyclonal T cells from diabetic animals or autoreactive T-cell clones in naive, irradiated, nude or SCID syngeneic recipients brings definitive evidence for autoimmunity to beta cells [19–24]. Interleukin-2 activated, K^d-restricted, $CD8^+$ T cells from diabetic NOD mice specifically lyse normal islet cells in vitro [25]. Class I-restricted cytototoxic T cells specific for normal islet cells have also been evidenced in mice expressing a gamma-interferon transgene on beta cells [26].

Immune effector mechanisms of beta-cell destruction are controversial. The presence of cytotoxic T cells and the immunodetection of perforin-expressing CD8 + T cells in diabetic NOD mice [25, 27], the extensive $CD8^+$ T-cell infiltration of syngeneic pancreas grafts at the time of diabetes recurrence [28] and the predominance of the $CD8^+$ T cell within insulitis at diabetes onset [29] in the human favour the role of $CD8^+$ cytotoxic T cells in beta-cell destruction. Similarly, $CD8^+$ T cells are required to achieve efficient transfer of diabetes in the NOD mouse [19, 20]. Diabetes occurs in $CD8^+/CD4^-$ mice expressing LCMV proteins on beta cells [30]. However, $CD4^+$ T-cell clones initiate islet-cell destruction in the apparent absence of $CD8^+$ T cells in (CBA×NOD)F1 recipients [23, 31]. The recurrence of diabetes against allogeneic islets deprived of class II-expressing cells in the BB rat and the NOD mouse [32, 33] points to non-MHC-restricted effector mechanisms. Autoantibodies are unlikely to be major effectors of beta-cell destruction [34]. The unique sensitivity of beta cells to cytokines in vitro [35] has been proposed to explain the non-MHC-restricted destruction of beta cells. However, in vivo exposure of histocompatible islets to cytokines during an allogeneic immune reaction does not lead to their destruction [36]. Diabetes is prevented in the NOD mouse by TNFα [37, 38]. Transgenic mice expressing TNF on beta cells develop insulitis but not diabetes [39, 40].

The precise series of events which trigger the anti-islet T-cell autoimmune reaction is still hypothetical. Effector mechanisms are under the control of a finely tuned immune balance between counteracting T-cell subsets [41, 42]. In most models, the immune activation traces back to an interaction between antigen presenting cells and CD4 + T cells [42]. The triggering role of environmental factors [43, 44] has not been directly proven in spontaneous forms of diabetes. The alternative hypothesis of a primary immune defect (i.e. defective T- or B-cell repertoire selection, abnormal expansion of a lymphocyte clone, T-cell regulatory defect) has also not received direct experimental support. Moreover, the association of different contributing factors is a reasonable hypothesis in the light of the polygenic susceptibility to diabetes [45].

Major issues will need to be raised before a comprehensive view of the anti-beta-cell immune reaction can be delineated. The primary activation site of auto-reactive lymphocytes remains undefined (Table 1). Whether the first activation of the immune system involves the direct recognition of beta-cell autoantigens or that of antigens sharing cross-reactive epitopes with islet cell autoantigens is an open issue. Whether the local heterogeneity of beta cells [46] or a step-wise activation of autoreactive T cells explain the chronicity [42] of the diabetes process is unsettled. Likewise, the long lag-time observed between the first detection of islet cell autoantibodies in subjects at risk for diabetes and first evidence for defective insulin secretion, leaves open the shape of the curve plotting the beta-cell mass against the duration of the autoimmune process following the postulated triggering event. These issues all relate to the more

general dilemma of the actual role of the islets of Langerhans in breaking self tolerance to islet antigens.

Environmental factors

Epidemiological evidence supports the role of environmental factors in the development of human IDDM. Concordance for IDDM in identical twins only reaches 30–40 %. An epidemiological relationship between viral infections and diabetes has been documented. Geographical differences in the incidence of diabetes and recent increase in the incidence of diabetes in Finland and in Sardinia cannot be explained by genetic differences. A modified incidence of diabetes is seen in migrants from geographical areas with a low prevalence to areas with a high prevalence of diabetes. Environmental factors modify the incidence and prevalence of diabetes in animal models. The incidence of diabetes is decreased in NOD mice by raised temperature, by diets containing no proteins [47], injection of streptococcal preparations, by infection with LCMV, lactate dehydrogenase, mouse hepatitis virus infections [48, 49]. In the BB rat, the prevalence of diabetes is decreased by non-protein diets [50], by low essential fatty acid diet [51], or by LCMV infection [52]. The occurrence of diabetes in the NOD mouse is also influenced by the hormonal status. Androgen treatment and oophorectomy of female NOD mice slows the progression to diabetes. The castration of males increases the incidence of diabetes [53, 54]. Non-specific interference with the immune system following a single injection of complete Freund's adjuvant (CFA) prevents the development of diabetes in the NOD mouse, while the injection of polyinosinic polycitidilic acid (poly I:C), an inducer of alpha-interferon, accelerates diabetes in the BB rat [55]. An outburst of diabetes has been observed in diabetes-resistant BB rats following Kilham's virus infection [56]. Exposure of BB rats to viral pathogens has been shown to influence the action of poly I:C [57]. The study of the T-cell repertoire in the NOD mouse has shown in vivo expansion of the Vβ8.3/CD8 subset which possibly relates to early exposure to an unidentified endogenous superantigen [58].

A major difficulty in studying the epidemiology of human diabetes is the long time period, possibly extending over several years, between the initiating event and the first detection of hyperglycaemia. Most studies previously referred to in animals indicate that environmental factors modulate the occurrence of diabetes on a susceptible genetic background but do not identify factors that may directly trigger the primary activation of autoreactive T cells, with the possible exception of the Kilham's virus. Historical models of organ-specific autoimmunity have relied on immunization against syngeneic tissues, antigens and more recently peptides, emulsified in CFA. Interestingly, there has not been convincing evidence that immunization against islet cells or insulin in CFA can induce beta-cell destruction although insulitis has been reported [59]. The only case has been the recent induction of diabetes in conventional mouse strains by immunization against heat shock protein 60 [60]. Little evidence has been provided in animals indicating that viral infections can induce autoimmune diabetes in conventional mouse strains carrying a genetic susceptibility background. Insulitis although not T-cell mediated transfer has been reported following reovirus infection [61]. Evidence that EMC virus infection leads to immune-mediated diabetes as conclusively demonstrated by transfer or anti-T-cell monoclonal antibody prevention experiments is controversial. The D-variant of the EMC virus induces diabetes in susceptible mice through a direct cytolytic effect on beta cells, including in athymic nude mice [62]. More convincingly, autoimmune models of IDDM have been developed in the mouse following repeated injections of low-dose streptozotocin, an agent with selective toxicity to beta cells [63]. In susceptible mouse strains, the injection of low-dose streptozotocin leads to insulitis and diabetes which can be transferred to syngeneic mice by T cells from diabetic animals [64]. Athymic mice are resistant to low-dose streptozotocin diabetes [65]. Diabetes in this model is prevented by the injection of anti-T-cell antibodies [66, 67], suggesting that beta-cell destruction is mediated by an autoimmune reaction. The break down of immune tolerance following streptozotocin-induced islet damage is thought to relate to changes in islet immunogenicity. The role of streptozotocin in inducing class II MHC antigens [68] as well as enhanced autoimmune, streptozotocin-induced diabetes by interferon gamma have been reported [69]. The prevention of streptozotocin-induced diabetes by intrathymic islet grafts points to the role of peripheral autoreactive T cells in the disease development and its possible prevention following negative selection in the thymus [70].

The development of transgenic mice has allowed the definition of possible links between environmental triggering events and breaking of immune tolerance to islet antigens. Transgenic mice expressing a LCMV glycoprotein or nucleoprotein on beta cells and the α the β chains of a CD8 + T-cell clone specific for the same LCMV glycoprotein as that expressed by beta cells have been established. The absence of insulitis and diabetes in these transgenic mice indicates that the presence of glycoprotein-specific T cells included within a biased T-cell repertoire is not sufficient to induce diabetes. However, peripheral T-cell ignorance was broken following infection of adult transgenic mice by the same LCMV strain as that encoding the glycoprotein transgene [43, 44].

Evidence that islet autoantigens, including GAD or the p69 antigen, share cross-reactive determinants with known pathogens (e.g. coxsackie virus B4) [71] or exogenous antigens (bovine serum albumin) raise the possibility that common environmental factors can be involved in breaking self tolerance. Finally, in most cases, the mechanisms by which environmental factors modulate or trigger the development of spontaneous forms of autoimmune diabetes remain elusive. A direct interaction with islet beta cells, as in the case of streptozotocin or coxsackie virus B4, may alter the antigenicity of islet autoantigens. An anti-beta-cell response can be elicited by cross-reactive antigens (molecular mimicry) as in the case of viruses or cow's milk protein. Alternatively, the interaction with lymphoid or antigen presenting cells as in the case of viral infections [48] can be proposed to explain disease acceleration or protection possibly conferred by environmental factors.

The case for primary immune defects

Whether mechanisms breaking self tolerance result from an intrinsic defect of the immune system or from a primary anomaly of target cells or organs remains an open issue in most human autoimmune diseases. Autoimmune reactions observed in lymphoproliferative disorders directly result from primary immune defects but do not make a general case in most forms of spontaneous autoimmune diseases. Nul complement alleles in human lupus and related disorders are more likely to be contributive elements to a genetic susceptibility background than directly responsible for primary triggering of autoreactive B- or T-cell clones. Enforcing a biased T- or B-cell repertoire following the expression of a transgenic T-cell receptor, IgM or IgG carrying anti-myelin basic protein, anti-erythrocyte, or anti-DNA specificity, respectively, has been shown to allow the development of autoimmunity [72–74], but still depend on the environment as in EAE in which transgenic mice only develop autoimmune lesions in a non-specific pathogen free environment [72]. Similarly, the expression of an expanded B-cell pool in mice carrying a bcl-2 transgene [75] drives a lupus syndrome, but a comparable situation in lupus-prone mice carrying the lpr or the gld mutations [76] indicates that such mutations bring acceleration factors on pre-existing susceptibility backgrounds. Thymic selection of peripheral autoreactive T cells is a perequisite for the development of autoimmune diseases as evidenced in collagen-induced arthritis in the mouse [77] but there is no experimental evidence that a thymic selection defect is a primary event responsible for pathologic autoimmunity. At the target cell level, a polymorphism of genes encoding autoantigens such as basic myelin protein in multiple sclerosis and the acetylcholine receptor in myasthenia gravis is controversial.

The hypothesis that autoimmune diseases primarily result from an immune regulatory defect has not received definitive experimental support. The case has been made for the local production of interferon gamma [26] or interleukin-2 [78] by beta cells in transgenic mice expressing a cytokine transgene. In the human, autoimmune reactions have been reported in patients treated with interferon alpha. Other immune defects resulting from an imbalance between regulatory T-cell subsets have been hypothesized to be predisposing factors. In the BB rat such imbalance is indicated by the depletion of T cells expressing the RT6 antigen, a marker on a subset of $CD4^+$ and $CD8^+$ T cells. Treatment of diabetes-resistant BB sublines with anti-RT6 monoclonal antibodies induces acute diabetes while diabetes-prone sublines are protected from diabetes by transfer of RT6 cells from diabetes resistant animals [79, 80]. In the NOD mouse, CD4 + suppressive T cells have been evidenced by cotransfer experiments in which irradiated adult recipients were protected from the diabetogenic effect of purified T cells derived from diabetic donors by coinjection of CD4 + T cells from non-diabetic mice [81]. The detection of protective T cells in this model depends on the thymus. Thymectomy at 3 weeks accelerates the development of diabetes in female mice. The detection of protective cells in diabetes prone animals (i.e. 8-week-old female NOD mice) suggests that they may appear as a transient protective barrier secondary to the presence or activation of autoreactive T cells responsible for islet-beta-cell damage. Evidence has been obtained that diabetes transfer can be achieved in the NOD mouse following the injection of spleen cells from diabetic mice into non-irradiated thymectomized adult recipients depleted of CD4 + T cells by the injection of anti-CD4 monoclonal antibodies. The development of diabetes in the NOD mouse is also prevented by the injection of autologous spleen cells exposed in vitro to cyclosporin and interleukin-2 [82]. In normal mice, thymectomy within 2 days following birth induces a lymphocytic infiltration of the thyroid, the gastric wall and the ovary, suggesting that regulatory T cells participate in maintaining self tolerance in the physiological state. Suppressor cells down regulating antithyroid autoimmunity have been evidenced in the rat following fetal thyroidectomy [83]. In the rat, the combination of thymectomy and sublethal irradiation or the transfer of normal spleen cells in athymic rats lead to the development of insulitis and diabetes [84, 85]. As in the BB rat, the development of autoimmunity in thymectomized and irradiated rats is prevented by the injection of normal syngeneic $RT6^+$ T cells.

An imbalance between TH1 and TH2 $CD4^+$ T cells is an attractive hypothesis which could explain most

of the observations on suppressor cells. TH1 cells secrete interleukin-2 and interferon gamma. TH2 cells show a predominant secretion of interleukin 4, 5, 6 and 10. A wasting disease sharing many features with graft vs host disease is induced in rat by the depletion of Ox-22 cells which targets TH1cells. The induction of diabetes in thymectomized, irradiated rats is dependent on $CD4^+$ T cells which carry the CD45 RC antigen, a putative marker of TH1 cells in the rat [84]. The thymic defect of interleukin 4 secretion [86] and the delay in diabetes onset following injection of interleukin 4 [41] have made a strong case for a TH1/TH2 imbalance in the NOD model. Similarly, the observation of a high interferon gamma/interleukin 4 ratio in invasive insulitis [87] and a corresponding imbalance between CD45 RA vs CD45 RO $CD4^+$ T cells in NOD lymph nodes and within the islet lymphocytic infiltrate after 1 month of age have been reported [88]. Other models involving a possible TH1/TH2 imbalance include mercuric chloride-induced autoimmunity in mice and rats [89].

The case for a target-cell anomaly

The indication that target cells and autoantigens are directly involved in the pathogenesis of autoimmune diseases was first obtained from the study of idiotypic markers carried by autoantibodies, the direct sequencing of autoantibody and the demonstration of target organ dysfunction in some autoimmune situations. The role of autoantigens in autoimmunity may be at different levels. The expression of autoantigens in abnormal forms, their delayed expression during ontogeny, or their quantitatively abnormal expression, possibly relating with target organ dysfunction, may directly trigger an autoimmune reaction. These may result from intrinsic as well as extrinsic defects possibly resulting from the action of virus or toxic agents on target cells. Alternatively, the physiological expression and presentation of autoantigens by MHC antigens may be a simple prerequisite to the activation of autoreactive lymphocytes, resulting in an antigen-driven immune response. The persistence of antigens has been shown to be required for maintaining immune tolerance in many experimental designs.

In autoimmune models characterized by polyclonal B-cell activation and a major role of autoantibodies, the preferential usage of V gene families or cross-reactive idiotypes by a significant proportion of autoantibodies with anti-DNA, anti-histone or rheumatoid factor activity and sequencing data showing a high rate of somatic mutations responsible for aminoacid changes on autoantibodies point to antigen-driven immune responses [90]. The clustering of autoantibodies specific of antigens localized in a subcellular particle in a given disease also points to antigen driven responses [91]. In lupus-prone MRL mice in situ hybridization shows a large variation in the extent of Vκ gene family usage by in vivo activated spleen B cells among individual mice, and a progressive restriction in the families used with disease development [92]. More direct evidence for the role of target cells in modulating the activation of autoreactive cells has been obtained in the obese strain (OS) chicken which shows increased iodine uptake and organification prior to any lymphocytic infiltration of the thyroid and decreased thyroid epithelial cell proliferation in vitro [93]. Diet supplementation with iodine increases the development of thyroiditis and treatments which inhibit iodine transport (KCLO4) or increase thyroid iodine release reduce thyroiditis development in the OS chicken [94]. The direct role of the presence of thyroid target cells in the activation of autoreactive B lymphocytes has been evidenced in thyroidectomized OS chicken which show decreased anti-thyroglobulin autoantibody levels [95].

In the case of autoimmune diabetes, the role of target islet cells has only received indirect experimental support. Both exogenous insulin treatment and glucose injections modulate the development of insulitis and diabetes in the BB rat and/or the NOD mouse [96–98]. A 50 % reduction of diabetes incidence has been obtained by prophylactic insulin treatment of BB rats. In the NOD mouse, treatment with protamine zinc pork insulin at the maximum tolerated dose from 4 to 26 weeks of age (0.25 IU/day up to weeks, 0.50 IU/day up to 10 weeks and 0.75 IU/day from then on) was shown to decrease the cumulated frequency of diabetes down to less than 10 %. Insulin treatment concommitantly resulted in significant reduction of islet inflammation and damage. The transfer of diabetes by spleen cells from diabetic NOD mice in irradiated male recipients is slightly delayed by prophylactic insulin treatment [99]. Similar evidence has been obtained in the BB rat [100]. In human IDDM, it has been suggested that intensive intravenous insulin therapy during 15 days from clinical onset of diabetes is beneficial by improving endogenous insulin secretion during the initial 12 months of disease [101]. Prevention of diabetes in normoglycaemic subjects at risk for developing diabetes, treated by insulin, has similarly been reported [102]. Several hypotheses could explain the beneficial effect of insulin on the development of diabetes. Insulin may modulate T cells through the activated T-lymphocyte insulin receptor. The insulin receptor is synthesized early on T cells, prior to the appearance of the interleukin-2 receptor, following antigen stimulation. Insulin has been shown to modulate cytotoxic T-cell functions and the regulatory role of T cells in providing help for B-cell insulin receptor synthesis, and to control intermediary metabolic functions and substrate oxidation in activated T cells. The reduction in

the synthesis of insulin receptors following hyperinsulinaemia during a glucose clamp is associated with a reduction in insulin-driven cytotoxic T-cell functions [103]. Other hypotheses postulate that insulin therapy directly modifies islet cells by decreasing the expression of beta-cell antigens or by "resting" beta cells. Evidence has been obtained in vivo in the rat for decreased antigen expression following the implantation of insulin-secreting tumours. Glucose modulates the expression of autoantigens such as GAD. Lastly, the injection of glucose in the neonatal period decreases the incidence of diabetes in the NOD mouse, possibly through the modification of maturing beta cells by increased exposure to glucose [104–106].

We directly tested the role of antigen expression in the primary activation of the anti-islet immune response in the NOD mouse by evaluating the activation of autoreactive T cells in beta-cell deprived animals. Beta-cell deprived NOD mice were obtained following a single injection of a toxic dose of alloxan and ultimately studied at 6 months of age. The capacity of spleen cells obtained from such mice to transfer diabetes in irradiated, 8-week-old, male recipients was lost, indicating the role of a low immune activation level against islet cells. The development of sialitis was not affected in these mice.

These data extend at the T-cell level those reported in the OS chicken in showing the role of target cells in eliciting organ-specific autoimmune reactions. They indicate that the expression of autoantigen(s) is required to allow the activation of autoreactive T cells. They bring strong evidence that autoimmunity is unlikely to result only from a primary immune defect but rather develop as a multifactorial process involving immune, environmental and target organ events. In transgenic mice expressing the Simian virus (SV) 40 T antigen on beta cells, evidence has been obtained that late expression of the T antigen is sufficient to elicit an immune reaction against beta cells [107]. Whether autoantigens involved in spontaneous diabetes in the NOD mouse relate with pathological autoantigen expression or simply require the physiological expression of autoantigens remains an open issue of possible importance in the perspective of designing new therapeutic strategies to block the autoimmune process.

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