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Models for Insulin Dependent Diabetes Mellitus in Humans

Abstract

Insulin-dependent diabetes mellitus is generally considered to result from a T-cell mediated autoimmune destruction of the insulin producing ß-cells in the pancreatic islets of Langerhans. Considerable progress has been made with regard to the unraveling of the pathogenesis of the disease, but neither the initiation of the disease process, nor the progression to overt insulin-dependency is understood. A major advantages in case of type 1 diabetes is the presence of spontaneous animal models of the disease that allow investigation of particular aspects of the disease. While in most autoimmune diseases, the process must be initiated, either by breakdown of tolerance through chemicals or induction of autoimmunity with specific autoantigens, diabetes develops spontaneously in non-obese diabetic (NOD) mice and biobreeding (BB) rats. Despite this major advantage, both models have considerable limitations. The present article deals with this notion, with focus on the best studied model in NOD mice. It is concluded that, although much can be learned from animal models, extrapolation to human disease must be done with great care. To put it extreme, an inbred mouse or rat strain can be considered as a case-report of the disease in humans.

Genetics

Type 1 diabetes in humans is characterized by a relatively strong but heterogeneous genetic predisposition, with a polygeneic trait (1-3). In particular genetic loci within the major histocompatibility complex on the short arm of chromosome 6 are strongly associated with either susceptibility to, or protection against development of diabetes. Interestingly, the major contributor, designated IDDM-1, consists of several HLA loci within the MHC region (4, 5). In Table 1, the frequencies of certain HLA class II alleles are indicated in a large cohort of juvenile onset type 1 diabetes in the Netherlands. From these data, it is evident that, although certain combinations of haplotypes of HLA-DR and –DQ are clearly far more frequently present amongst IDDM patients than in the general population, less than half of these patients have the predisposing haplotypes, while 11% of the general population contains these high-risk haplotypes. This implies that the vast majority of subjects with high risk

Table 1. Comparison of three most frequent HLA phenotypes in IDDM patients with general population.

HLA	IDDM patients (n=309)	General population (dutch) (N=504)
DR3/3 – DQ2/2	8.1%	3.0%
DR3/4 – DQ2/8	30.7%	3.4%
DR4/4 – DQ8/8	5.8%	0.2%
High risk total	*44.6%*	*6.6%*
Low risk (DR2 – DQ6)	*0.9%*	*25.4%*

haplotypes will never develop IDDM, since the incidence rates in the population at large are in the range of 0.1-0.4%. In families with IDDM, first degree relatives have an increased risk (ca. 5%), while identical twins show concordance rates of 25-55%. Thus, HLA genes are important, but neither required nor sufficient for the development of IDDM. Moreover, diabetes specific genes or alleles have not been identified. This suggests that particular combinations of predisposing genetic loci, rather than individual genes, will determine the actual risk of disease development. The results of the genetic studies also imply that environmental factors strongly influence the genetic trait.

While animal models are useful to study the genetic predisposition to diabetes, environmental issues associated with disease development in humans cannot easily be addressed in animals.

Genetic predisposition is also associated with disease progression in NOD mice (6-8). Again, a polygeneic trait is evident. Multiple genetic loci contribute to development of diabetes, but as in humans, MHC is most strongly associated with the disease (Table 2). The similarities with diabetes in humans is even more striking with regard to protection. Aspartic acid on position 57 of the DQß chain (e.g. DQB1*0602) in men (9), or mice (10) is associated with strong protection against development of diabetes. However, while a wide range of HLA class II alleles are associated with susceptibility to diabetes in humans, the mouse equivalent I-A^{g7} is the only MHC haplotype in mice to be associated with this disease. Nonetheless, several non-MHC regions contributing to disease predisposition appear to be homologous between mice and men.

Immunology

Type 1 diabetes in humans is characterized by rather general immunological phenotypes. A defective peripheral immunoregulation has been described in several studies (11-15). Even at the level of CD4/CD8 ratios, differences between patients and controls are reported. Few studies on prediabetic subjects have been published, but the data suggest that abnormalities on the levels of lymphocyte subsets exist prior to clinical presentation of diabetes. The relative increases in T-cells expressing both CD45RA and CD45RO, which represent in vivo activated T-cells as illustrated by the expression of HLA class II and CD25, may be caused by a maturation defect in

Table 2. Comparison of insulin-dependent diabetes in men and mice.

	Humans	NOD mice
Genetic predisposition, polygeneic trait	Yes	Yes
IDDM1/*idd-1*	Multiple haplotypes	One haplotype
Environmental effects	Probable	Yes
Endogenous retrovirus in ß-cells	?	Yes
Incidence	0.3%	80-90%
Age at diabetes onset	0 – >70 years	3 months
Gender bias	No	Yes
Defective peripheral regulation	Yes	Yes
T-cell driven insulitis	Mild	Severe
Peri-insulitis	(no)	Yes
Leukocyte infiltrates in other tissues	sometimes	Always
Disease transmission via BMT	Yes	Yes
Humoral reactivity to ß-cells	yes	(yes)
Autoantigens	GAD65, insulin, IA-2, 38kD, ...	GAD65, insulin, 38kD, peripherin, ...
Delayed onset with immunosuppression	Yes	Yes
Successful intervention therapies	?	OKT3, anti-MHC, intra-oral, -venous or-nasal autoantigens and peptides

diabetic patients (16). Alternatively, a constant priming, e.g. with islet autoantigens or viral products, may also result in the relative increase of activated lymphocytes (15). It is generally believed that the cytokine production pattern of pathogenic T-cells is Th1-like (i.e. interferon-g, IL-2, TNF), while non-destructive T-cell autoreactivity appears to be anti-inflammatory in nature (Th2; IL-4, IL-5 and IL-10) (17). This dichotomy is primarily based on animal studies (18, 19), but most studies on cytokine production of T-cells reactive with islet cell autoantigen support this concept (20, 21).

All evidence so far supports a T-cell driven pathogenesis of type 1 diabetes in humans. In addition to the abnormalities described above, studies using immunosuppressive agents (22, 23), as well as case reports on adoptive transfer with non-T-cell-depleted bone marrow from a diabetic patient to an immuno-incompetent relative of this patient (24) are in line with this hypothesis. In fact, recurrent insulitis and ß-cell destruction that was noted in patients receiving a pancreas segment of their non-diabetic twin was observed in the absence of islet autoantibodies (25). The latter are commonly used the predict onset of IDDM (26). Moreover, islet cell autoantibodies have been used the define the potential targets of pathogenic T-cells in IDDM. Thus far, GAD65, insulin, IA-2 and ICA69 have been defined on this basis (27-32), while other candidates were defined by T-cells, rather than autoantibodies (imogen-38, insulin-secretory granule protein of 38kDa) (33, 34). Thus far, none of these candidates were defined primarily on basis of animal models of diabetes. In fact, presence of various islet autoantibodies in NOD mice is still disputed.

The NOD mouse model has contributed significantly to the design of studies to define the pathogenesis of diabetes in humans. The notion that T-cells play a central role in ß-cell destruction were corroborated with the finding of insulitis in men (35) and mice (36), although the so-called non-destructive insulitis or peri-insulitis (36-40) has not been reported in humans so far (Table 2). Furthermore, the degree and extent of insulitis in humans is much less than that in NOD mice (41, 42). Interestingly, all NOD mice will develop a degree of insulitis, regardless of lack of progression to overt diabetes.

Other studies to support the role of T-cells in NOD mice include adoptive transfer (43-45), lack of diabetes in athymic mice and prevention of disease with monoclonal antibodies against T-cells (46). While several candidate autoantigens are recognized by T-cells of mice and men, such as GAD65 (28, 47), insulin (30, 48, 49) and insulin-secretory granule protein 38kDa (34, 50, 51), others have only been reported in either mice (peripherin (52), hsp60 (53)) or men (IA-2 (54)).

Recently, HLA-DR and –DQ transgenic mice have been used to study HLA restricted T-cell reactivity to human islet autoantigens *in vivo*. Interestingly, the immunodominant HLA-restricted epitopes that were defined after immunization with human autoantigens were identical to those identified by human T-cell clones isolated from type 1 diabetes patients (55-60). In fact, the HLA binding affinity of T-cell epitopes defined upon immunization of HLA transgenic mice and patient derived T-cells was relatively high, while epitopes of T-cells generated from non-diabetic subjects was relatively low (59). These finding implicate that in this context, mouse studies may be useful to study *in vivo* reactivity of HLA restricted T-cells specific to human autoantigen and the definition of human T-cell epitopes.

Immunotherapy

The experience with immunotherapy is relatively limited in human type 1 diabetes. Several trials using immunosuppressive agents have been applied with varying degrees of clinical benefit, but it is evident that general immunosuppression potentially delays the clinical onset of diabetes, or increases the rate of remission. The list of immunointervention trials in diabetes is long (61-63). At this stage, most emphasis is put on nicotinamide (64), insulin prophylaxis (37, 65) and oral or nasal tolerance induction with insulin (66-68). Clearly, studies in mice are a logical first step to assess efficacy of treatment, although failure in mice does not preclude potential success in humans. It has been convincingly shown that T-cells to either GAD65 (47, 69), insulin (48), hsp60 (53) or 38kDa secretory granule protein (50) can cause ß-cell destruction and diabetes in NOD mice. In fact, T-cells reactive to other yet undefined islet autoantigens have also been shown to be pathogenic in NOD mice (40, 70). The known candidate autoantigens have effectively been used to prevent or delay autoimmune destruction of ß-cells in NOD mice (37, 68, 71-75). However, it remains to demonstrated that these antigens indeed are suitable and effective in humans, without aggravating the autoimmune response to either ß-cells (76), or other tissue, since none of the candidate autoantigens are islet specific.

Caveats

As illustrated above, the similarities between men and NOD mice are astonishing in many respects, but evident discrepancies in the pathogenic processes between these two species must been appreciated. First, the homogeneous nature of inbred mouse strains such as NOD mice is an advantage with regard to synchronization of diabetes onset, high incidence rates and limited inter-individual biological variability, while at the same time it does not pay tribute to the large heterogeneity of type 1 diabetes in humans in terms of genetic background, clinical presentation and phenotype.

Second, particular aspects of the immune process in IDDM can be studied in great detail with help of transgenic (40, 77-85) and knock-out (86) mouse models. Unfortunately, such models have obvious disadvantages once extrapolated to the situation in humans for the obvious reasons, and can introduce cell biological artifacts that obscure 'natural' tolerance and autoimmunity. On the same line, the mechanisms involved in islet graft survival or destruction may be quite different from the 'natural' pathogenesis of type 1 diabetes, both in mice and men (87-89). For instance, intrathymic implantation of islets of Langerhans have only been successful in achieving tolerance in mice and rats (90), while such efforts failed in non-rodent mammals. Finally, the interpretations of studies on adoptive transfer in NOD mice must be done with great care. Clearly, the kinetics of the ß-cell destruction process are quite different, but more importantly, the treatment of the recipient mice with either irradiation or chemicals interferes with regulatory processes that occur *in vivo*. It may be argued that in fact, the mechanism of adoptive transfer is more similar to graft-versus-host reactions than spontaneous autoimmune reactivity in terms of effector cell numbers, treatment of recipients and kinetics. Nonetheless, diabetologists should be grateful to have models as close to the disease in humans as the spontaneous model in NOD mice. Surely, the mice have been very informative and helpful in shaping the experimental approaches to unravel human autoimmune ß-cell destruction.

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