

*Review***Lessons from the NOD mouse for the pathogenesis and immunotherapy of human Type 1 (insulin-dependent) diabetes mellitus**E. F. Lampeter<sup>1, 3</sup>, A. Signore<sup>2, 4</sup>, E. A. M. Gale<sup>1</sup> and P. Pozzilli<sup>1, 4</sup><sup>1</sup> Department of Diabetes and Immunogenetics, St. Bartholomew's Hospital,<sup>2</sup> ICRF, HTIG, Faculty of Clinical Sciences, University College London, UK,<sup>3</sup> City Hospital Leipzig, Hospital of Internal Medicine, Leipzig, GDR, and<sup>4</sup> Cattedra Endocrinologia (I), Clinica Medica (II), University of Rome "La Sapienza", Rome, Italy

**Summary.** Suitable animal models of human Type 1 (insulin-dependent) diabetes mellitus have long been sought, in particular a model that would permit detailed histological and immunological investigation of changes in the islet preceding the metabolic disorder. This would allow hypotheses as to pathogenesis of the condition to be examined and interventions such as immunotherapy to be tested. The most widely studied models include the low-dose streptozotocin induced diabetic mouse and the BB rat, but both differ in important respects

from the human disease. In this review we describe one highly successful model, the non obese diabetic mouse. Selected aspects of pathogenesis and immunotherapy are presented and analogies with human Type 1 diabetes discussed.

**Key words:** Non obese diabetic (NOD) mouse, pathogenesis Type 1 (insulin-dependent) diabetes mellitus, immunotherapy Type 1 diabetes.

The NOD mouse was derived from a cataract-developing substrain of the outbred JcI-ICR mouse by selective breeding from 1974 to 1980 [1]. Diabetes develops spontaneously between the 12th and 30th week of age, with an onset characterized by polydipsia, glycosuria, rapid weight loss, hyperglycaemia and ketoacidosis (Table 1). The onset of hyperglycaemia is preceded by insulinitis, progressive B-cell destruction and decreasing circulating insulin levels leading to insulin dependency [2–4]. Without insulin treatment the animals die within 4 to 8 weeks (unpublished observations). Thus, clinical and pathological features in the NOD mouse closely resemble human Type 1 (insulin-dependent) diabetes mellitus. Since all conclusions drawn from animal models are, however, based on analogy with human disease, the analogy needs detailed validation. For this reason we describe similarities and differences relating to pathogenesis and immunotherapy in the NOD mouse and human Type 1 diabetes.

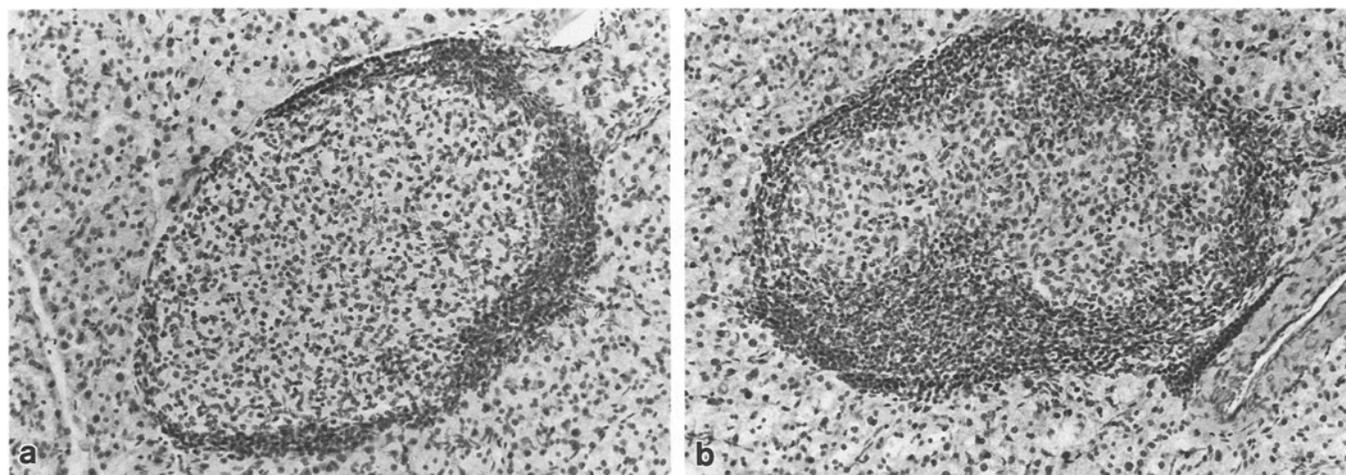
**Genetic background**

Continued in-breeding of the strain has resulted in high genetic uniformity as shown by morphology, allele distribution of enzymes and other proteins, and immunological studies including mixed lymphocyte reaction and skin grafting [5]. Based on this, the genetic back-

ground of insulinitis and overt diabetes has been investigated by backcross experiments with C57BL, NZB mice and a non obese non diabetic subline (NON) of the same origin as the NOD [6–8]. The results indicate three recessive diabetogenic genes, two of which are non MHC-linked. One controls the development of severe insulinitis and appears to be incompletely dominant, and the other is involved in the progression to diabetes, probably mediated by a lack of specific suppressor cells. The third, MHC linked, gene is not required for insulinitis but apparently influences the autoimmune response [7]. It has been suggested that the NOD mouse has a unique class II MHC, which may lead to the autoimmune insulinitis [9]. Furthermore, treat-

**Table 1.** Comparison of clinical features at onset of diabetes in the human and the NOD mouse

	Type 1 (insulin-dependent) diabetes mellitus	
	human	NOD mouse
Weight loss	Present	Present
Polydipsia	Present	Present
Polyuria	Present	Present
Hyperglycaemia	> 15 mmol/l	20–30 mmol/l
Ketoacidosis	Common	Less severe
Serum insulin	Very low	Very low
Outcome without insulin	Lethal	Lethal
Sex preponderance	Female $\geq$ male	Female $\gg$ male



**Fig. 1a and b.** Micrographs of islets of Langerhans in a 20-week-old female NOD-mouse showing periinsulinitis (a), followed by invasion of the islet by lymphocytes penetrating the capsule (b) (Magnification 145 $\times$ , haematoxylin and eosin staining)

ment with anti-I-A monoclonal antibody prevented diabetes in NOD mice [10].

Human Type 1 diabetes is associated with MHC products [11], most closely linked with the HLA DQ region [12]. In mice the equivalent to DQ-beta is the A- $\beta$  chain, and this has interesting similarities with human diabetes [13]. Back-cross experiments have shown that homozygosity at this gene is necessary for the development of diabetes. The NOD A- $\beta$  allele is unique in the species in having serine in position 57 instead of aspartic acid (Asp). Similarly in humans DQ- $\beta$  Asp 57 negative homozygosity is found in 90% of Caucasian Type 1 diabetic patients, whereas, Asp 57 positive homozygosity at DQ- $\beta$  gives almost complete protection from Type 1 diabetes [13].

Despite strong evidence for an association with a genetic factor or factors, the concordance rate for Type 1 diabetes is surprisingly low in identical twins (30–50%), suggesting that susceptibility is inherited rather than the expressed disease [14]. The NOD mouse resembles man in this respect, since the animals are genetically identical but not all develop diabetes. Abnormal immunological parameters including islet cell antibodies (ICA) and increased numbers of circulating activated T cells are, however, concordant in human twin studies [15, 16] and NOD mice also have a concordant immunological process, as shown by the fact that all females and more than 90% of males exhibit insulinitis [7, 17, 18]. The incidence of diabetes is, however, at least twice as high in female NOD mice than in males, and castration experiments suggest that this difference is related to female sex hormones [17]. Castration of mice up to the age of 7 weeks results in an increase of diabetes incidence in males and a decrease of incidence in females.

Administration of testosterone prevents diabetes in castrated animals, whereas oestradiol raises the incidence of diabetes in castrated animals of both sexes. The rate of development of diabetes is also influenced by diet [17]. Although hormonal and dietary influences have not been shown in human diabetes, human Type 1 diabetes and the NOD model suggest that genetic factors predispose to the autoimmune disorder, but have limited importance for clinical expression of the disease.

The genetic uniformity in this inbred strain has great advantages in the experimental situation as a guarantee of identity, but for the same reason has limited relevance to the human situation. Thus, even though 95% of Type 1 diabetic patients are HLA DR3 or DR4 positive [20], there is marked genetic heterogeneity. The mechanism of inheritance of the disease in the NOD mouse cannot, therefore, be identical to that in man, although it constitutes one of the possible alternatives.

### Histopathology

Insulinitis is the pathological hallmark recent onset Type 1 diabetes and is observed in the NOD mouse from at least the 4th week of age [21, 22]. The earliest change is periinsulinitis adjacent to the pancreatic ducts, followed by invasion of the islet capsule by small lymphocytes which penetrate the islet (Fig. 1). The final stage is characterized by small islets from which B-cells have disappeared, with resolution of insulinitis. The different stages of this process can, however, be found within the same pancreas at any age. Phenotyping of lymphocyte subsets involved in the insulinitis has produced conflicting results [23–26]. We have found that monocytes and B-lymphocytes are the predominant cell population [25]. Previous studies have reported L3T4 cells (mainly helper/inducer) and MHC class-II cells as the most represented subsets [23, 26]. Within the T-lymphocyte population L3T4 cells are more frequently found than Lyt-2 cells (mainly cytotoxic/suppressor) [24].

**Table 2.** Comparison of morphological features in human diabetes and the NOD mouse

	Type 1 (insulin-dependent) diabetes mellitus	
	Human	NOD mouse
Periinsulinitis/insulinitis	Present	Present
Insulinitis in subjects without diabetes	?	Present
Small islets lacking B-cells at the end stages	Present	Present
Lymphocytic infiltration in other organs	Rare	Present

The prevalence of insulinitis is high in humans who have died soon after the onset of Type 1 diabetes, and in one early study insulinitis was present in 16 of 23 who died within 6 months of onset [27, 28] and in 47 out of 60 patients with a diabetes duration of less than one year [29]. The smouldering nature of the process is equally apparent, with normal islets, insulinitis and “end-stage” islets depleted of B cells within the same histological field. While there is still some controversy concerning the prevalence of insulinitis in man, there is agreement on the histological pattern of lymphocytic infiltration (Table 2). As in the NOD mouse, insulinitis develops in man as periinsulinitis and progresses to infiltration of the islets and B-cell destruction [28]. There has been only one report concerning the phenotype of lymphocyte subsets, based on the pancreas of a child who died at the time of diagnosis [30]. The majority of infiltrating lymphocytes were T-cells, predominantly CD8 positive although other inflammatory cells were present. Thus, despite possible differences between the lymphocyte subsets infiltrating the islets, the NOD mouse is a good model from the histopathological point of view. Diabetes has a strong female preponderance in the NOD mouse (70% vs 20% at 30 weeks of age) but insulinitis is present to a similar degree in both sexes. Thus, about 80% of males and 30% of females show insulinitis without developing diabetes up to the 30th week of age [4]. In the human situation we remain ignorant as to the time of development of insulinitis prior to the disease, although it is assumed to coincide with the appearance of ICA and other autoimmune markers, and it is not known whether individuals with insulinitis inevitably progress to diabetes.

In NOD mice lymphocytic infiltration is not restricted to the islets but occurs also in salivary tissue [31] and occasionally in the thyroid and adrenal glands [32], suggesting a wider disturbance of immune tolerance in this animal. Type 1 diabetes is also associated with overt polyendocrine disease and there is an increased prevalence of autoantibodies to thyroid, adrenal or gastric parietal cells, although figures concerning this vary [33]. Infiltration of salivary glands has not, however, been described in human diabetes.

## Immunological observations

### Autoantibodies

ICA have been found in about 50% of NOD mice up to the 21st week of age but tend to disappear later [34, 35]. Islet cell surface antibodies (ICSA) appear at 3–6 weeks, reach peak incidence and titre at around 12–18 weeks, and decline thereafter [26, 34]. There is no evidence that these autoantibodies are directly involved in B-cell destruction, and both ICA and ICSA might be secondary to islet cell destruction and massive release of cellular antigen, a view which accords with the time course of insulinitis in this animal model. Insulin autoantibodies (IAA) have also been reported; they may antedate insulinitis [35] and are present in almost all animals later in life [34]. Finally, autoantibodies which immunoprecipitate a 64,000 mol.wt.islet antigen have recently been described [36]. As in humans, the pathogenetic relevance of these autoantibodies remains uncertain, and the prognostic significance of ICA, ICSA, insulin and 64 kilodalton autoantibodies has yet to be investigated in the NOD mouse.

### Cell mediated immunity

Several successful attempts have been made to transfer insulinitis and diabetes via lymphocytes derived from NOD mice, using a variety of protocols. Diabetes appears within a few weeks of lymphocyte transfusion, providing further support for the autoimmune hypothesis. Recipient animals were either newborn or very young normal NOD mice [37], totally irradiated NOD mice [38, 39], or athymic nude mice of NOD origin [40]. Despite these differences, similar results were obtained with regard to the age of lymphocyte donors, and 100% successful transfer of diabetes/insulinitis can only be achieved with lymphocytes from mice at least 16–19-weeks-old [37, 39]. Interestingly the transfer can be made with either diabetic or non-diabetic donor lymphocytes.

**Table 3.** Comparison of immunological features of Type 1 (insulin-dependent) diabetes in humans and the NOD mouse

	Type 1 diabetes mellitus	
	Human	NOD mouse
Insulin autoantibodies	Present	Present
Islet cell antibodies	Present	Present
Islet cell surface antibodies	Present	Present
Islet cell specific cellular immunity	Present	Present
Abnormal T-helper/T-suppressor	Present	Present
Major T-lymphocyte subset in the insulinitis	CD 8 <sup>+</sup>	L3T4 <sup>+</sup>
Aberrant expression of class II MHC on insulin positive cells	Present	?

phocytes. In older non-diabetic recipients (i.e. > 25 weeks) the transfer is much less effective and only 5 of 16 developed diabetes [39]. When separated lymphocyte subsets were used, both Lyt-2<sup>+</sup> (mainly cytotoxic/suppressor) and L3T4<sup>+</sup> (mainly helper/inducer) cells appeared to be necessary. In addition, both subsets should be derived from a donor of appropriate age (16 to 19 weeks) as shown in transfer experiments in which L3T4<sup>+</sup> cells from an appropriate donor were reconstituted with Lyt 2<sup>+</sup> cells from a 6-week-old donor (or vice versa) but failed to induce diabetes when transferred. It was further shown that newborn mice are susceptible to transfer until the 3rd week (females) and the 5th week (males).

The NOD mouse, therefore, appears susceptible to the transfer of diabetes until the time at which insulinitis develops spontaneously. At approximately 16 to 19 weeks the animals acquire the ability to transfer the disease with lymphocytes, but this capacity is often lost in non-diabetic mice from the 25th week onwards. These findings may reflect time dependent differences in the development of necessary lymphocyte subsets (i.e. T-helper/inducer first, antigen-specific effector second and T-suppressor cells, last). If this is the case B-cells might disappear too rapidly for the induction of T-suppressor cells in animals which develop diabetes, whereas animals with slower destruction of B cells may produce sufficient specific T-suppressor cell activity to protect themselves from further B-cell loss.

A variety of cellular cytotoxicity systems have been investigated in search of an active effector cell mechanism in the NOD mouse. Direct cellular cytotoxicity (CTL) was increased as compared to ICR mice using Balb/c islets as targets in a chromium release assay. Antibody dependent cellular cytotoxicity (ADCC) and natural killer (NK) cell activity have been tested in NOD and ICR mice. Both ADCC against chicken erythrocytes in the presence of anti-chicken erythrocyte antibodies and NK activity against Chang liver cells are decreased in the NOD mouse [41]. Another interesting observation is that athymic nude mice with NOD background [40] or NOD mice undergoing neonatal thymectomy [18] did not develop insulinitis and diabetes - suggesting a pivotal role for T-lymphocytes in the autoimmune process. Administration of monoclonal antibodies (mAb) specific to some lymphocyte surface markers can block function or destroy the corresponding cell subset. Thus, treatment with anti Thy 1.2 mAb (T cells) prevents diabetes but does not influence the progression of insulinitis [40]. Administration of L3T4 mAb abolishes insulinitis and diabetes [42, 43]. In addition, Lyt 2<sup>+</sup> cells (suppressor/cytotoxic) and macrophages are necessary for the development of insulinitis since treatment with anti-Lyt2 antibody and silica particles prevents B-cell destruction [44].

Thus, macrophages and Lyt2<sup>+</sup> cells are required for induction of the autoimmune process by appropriate antigen presentation and for generation of specific ef-

factor cells, respectively. On the other hand, cyclophosphamide (known to impair T-suppressor cells) promotes overt diabetes and increases its incidence in the NOD mouse [45]. These data suggest the presence of specific T-suppressor cells in the NOD mouse, although these are clearly not efficient enough to maintain tolerance in all cases.

Aberrant expression of HLA class II on B cells has been claimed to play an important role in the initiation of the autoimmune process leading to diabetes [46]. Class II expression was found in a child who died soon after clinical presentation [30] and confirmed by an immunohistological study of formalin fixed paraffin embedded tissue from post mortem cases with recent onset of diabetes [47]. In the NOD mouse conflicting results have been obtained. Hanafusa et al. [48] described aberrant expression of class II molecules prior to insulinitis, as identified by anti-IA mouse antibodies, not only in the NOD but also to some extent in BALB/C and B10.GD mice. These results could not be confirmed using P7/7 rat MAb [23] which recognizes class-II molecules of b, d and k haplotype [49], and all islet cells appear negative with this antibody [25].

### Immunotherapy

Cyclosporin A reduces insulinitis in the NOD mouse but is unable to abolish it [50], while ICSA titres were similar or even higher than in control animals. In another report low-dose cyclosporin treatment has been shown to protect against insulinitis [51]. These data indicate that cyclosporin A can partially suppress the cell mediated reaction but not the production of ICSA. Unfortunately the incidence of diabetes in cyclosporin treated animals was not investigated.

Nicotinamide, an inhibitor of poly-ADP-ribose synthetase, reduces the incidence of insulinitis and diabetes in the NOD mouse [52]. Cyclophosphamide increases the incidence of diabetes but this effect can be blocked by nicotinamide [19]. ADCC is naturally elevated in the NOD mouse but falls after nicotinamide treatment [53]. This suggests an important role for ADCC and also indicates that nicotinamide has immunomodulatory properties. This is supported by the observation that single injections of nicotinamide prior to allogeneic islet transplantation prolong graft survival, a treatment which was much more successful if nicotinamide was combined with desferrioxamine, an iron-chelating agent [54]. Nicotinamide seems to have some benefit in newly diagnosed Type 1 diabetic patients [55, 56] and increases C-peptide secretion in the first year after diagnosis [57].

### Conclusion

The NOD mouse model shares a number of important characteristics with human Type 1 diabetes. The disease develops spontaneously and is not accompanied by

general immunodeficiency as in the BB rat. Differences include simultaneous lymphocytic infiltration of salivary glands and other organs, and a strong female predominance. Even so, study of mechanisms involved in insulinitis, B-cell destruction, and the generation of other immunological disturbances allows hypotheses concerning human Type 1 diabetes to be developed and tested. The availability of high and low incidence lines may, in addition, offer clues to factors involved in the onset of diabetes.

Insulinitis is in progress well before overt hyperglycaemia in the NOD mouse, and this is important for two reasons: (1) it allows the autoimmune process to be defined before complete B-cell destruction and hyperglycaemia have occurred. This might prove very useful in the search for new markers during this crucial phase of the natural history. (2) the prolonged and well defined prodromal period provides an excellent opportunity to test different approaches to immunotherapy early in the prediabetic stage.

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