Diabetologia (2004) 47:1650-1656
DOI 10.1007/s00125-004-1517-1

Diabetologia

For Debate

Animal models have little to teach us about Type 1 diabetes: 1. In support of this proposal

B. O. Roep[1] · **M. Atkinson**[2]

[1] Department of Immunohaematology and Blood Transfusion, Leiden University Medical Center, E3-Q, Leiden, The Netherlands
[2] Department of Pathology, University of Florida College of Medicine, Gainesville, Florida, USA

Sir Winston Churchill summed up our position in this debate when he said that "Men stumble over the truth from time to time, but most pick themselves up and hurry off as if nothing happened". To our thinking, we (the Type 1 diabetes research community) have stumbled both in terms of the way in which data derived from animal models of Type 1 diabetes have been handled, and in the manner in which the field continues to move forward without proper acknowledgement of what has been learned. Animal models such as the non-obese diabetic (NOD) mouse and the biobreeding (BB) rat develop immune-mediated disease with features resembling Type 1 diabetes in humans [1]. Although these animal models of autoimmune diabetes have proved to be valuable tools to study certain aspects of the disease process [2], they have also led to misconceptions and erroneous extrapolations, as well as false expectations with regard to the efficacy of immunotherapy. Hence, on a number of counts, we would argue that animal models have limited value when it comes to teaching us about Type 1 diabetes in humans.

Received: 30 June 2004 / Accepted: 10 August 2004
Published online: 13 October 2004

B. O. Roep (✉)
Department of Immunohaematology and Blood Transfusion,
Leiden University Medical Center, E3-Q, PO Box 9600,
2300 RC Leiden, The Netherlands
E-mail: boroep@lumc.nl
Tel.: +31-71-5263869, Fax: +31-71-5216751

M. Atkinson (✉)
Department of Pathology,
University of Florida College of Medicine,
Gainesville, Florida 32610, USA
E-mail: atkinson@ufl.edu
Tel.: +1-352-3920048, Fax: +1-352-3928464

The immune system

There are profound differences between the immune systems of mice and men. These have recently been summarised comprehensively by Mestas and Hughes, and include discrepancies in both innate and adaptive immunity [3]. Relevant examples of more than 80 known incompatibilities would include: balance of leucocyte subsets, defensins, toll receptors, inducible NO synthase, the NK inhibitory receptor families Ly49 and KIR, FcR, Ig subsets, the B cell (BLNK, Btk, and lambda5) and T cell (ZAP70 and common gamma-chain) signalling pathway components, Thy-1, gamma delta T cells, cytokines and cytokine receptors, Th1/Th2 differentiation, costimulatory molecule expression and function, Ag-presenting function of endothelial cells, and chemokine and chemokine receptor expression. Given the breadth of these functional differences, these discrepancies surely limit the usefulness of mouse models in studying Type 1 diabetes. Therefore, such differences should be taken into account when animals are used as preclinical models of human disease. Nonetheless, they are generally ignored.

Immunopathogenesis of diabetes

Similarities and discrepancies in autoimmune diabetes in mice and men have previously been summarised [4]. Genetic predisposition certainly belongs amongst the most striking similarities, and the resemblance between the human and murine MHC susceptibility molecules DQ8 and I-A^{g7} is truly remarkable [5]. In this regard, it is conceivable that the NOD mouse model might help to unravel the functional basis of the genetic predisposition to diabetes, despite evident disparities in disease between mice and men. However, while

multiple loci and alleles contribute to disease predisposition in humans, and genotypes rather than haplotypes determine the risk, it must be noted that NOD mouse strains, such as the C57BL/6 and 129 inbred strains, fail to express I-E antigen, the mouse orthologue of human DR4.

Another important difference relates to the phenotype of insulitis. Although BB rats display a degree of islet inflammation that closely resembles that in humans at disease onset [6], the infiltrate in NOD mice is characterised by two phases: a non-destructive infiltrate around the outer border of the islets (also referred to as peri-insulitis, benign insulitis or respectful insulitis), which in the event of conversion to diabetes is followed by infiltration of the islet core. In contrast, human insulitis is often sparse, with few leucocytes detectable in the inflamed islets. The pancreatic histology in mouse models expressing transgenes in islets (cytokines, costimulatory molecules) or effector cells (T cell receptors) represents an even greater artefact, which resembles lymph nodes and spleen but has little in common with insulitis in human Type 1 diabetes.

Another controversial yet equally important issue is the absence in the mouse of autoantibodies against islet antigens other than insulin [7], whilst antibodies against GAD65 and IA-2, particularly in combination, serve as excellent predictors of the development of clinical Type 1 diabetes in humans (Fig. 1). Intriguingly, autoantibodies to insulin alone (i.e. the NOD phenotype) provide the least predictive marker of prediabetes in humans [8]. A recent report identified the presence of maternal islet autoantibodies as a diabetogenic factor in offspring of diabetic mice [9, 10]. In humans, the situation appears to be the reverse; children of mothers with Type 1 diabetes are 50% less likely to develop the condition than children of Type 1 diabetic fathers. It is of interest that transplacental transfer of autoantibodies was frequently found in children born to diabetic parents, but appeared to be correlated with protection from islet autoimmunity rather than with increased risk [11]. A similar discordance has been identified with regard to B lymphocytes, which may be considered a prerequisite to the development of diabetes in NOD mice [12]. A recent report identified a case of development of Type 1 diabetes in a patient with severe inherited B lymphocyte deficiency due to a mutation in the *btk* gene, which is essential for B lymphocyte development in humans [13]. Although this patient did not produce antibodies and consequently showed no evidence of humoral islet autoreactivity, T cell autoreactivity against the islet antigens GAD65 and IA-2 (but not insulin) was increased relative to non-diabetic control subjects, and was similar to that of other new-onset Type 1 diabetes patients. Needless to say, this does not rule out a contribution of islet autoantibodies to the pathogenesis of Type 1 diabetes in humans, but merely indicates that these are not a prerequisite for the development of the disease. The latter notion is corroborated by the lack of efficacy of intervention strategies directed at B lymphocytes and their products in human Type 1 diabetes. For the record, mutations in the *btk* gene in mice would have led to normal levels of pre-B and immature B lymphocytes [3]. Whether or not the NOD mouse or XLA patient with Type 1 diabetes represents the odd case is a matter of another debate (see below). Finally, the presence of multiple immune abnormalities, including natural killer cell defects, lymphopenia, cytokine deviations and other autoimmune lesions apparently unrelated to diabetes itself (e.g. thyroiditis, sialitis) but characteristic of diabetes in both rats and mice, sets these models apart from the vast majority of cases of Type 1 diabetes in humans.

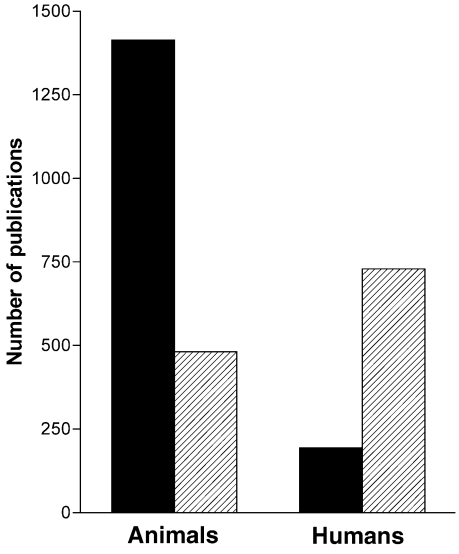

Fig. 1. Number of publications on studies on T cells (black bars) or autoantibodies (hatched bars) in animals and humans. Difference in subject of study between mice and men: $p=4.58\times10^{-160}$; $\chi^2=726.7$

Immunotherapy

We next raise the issue of prevention studies in animal models of Type 1 diabetes as an example of the process of collecting truth, viewing that information, and moving forward as if nothing had happened. We would indeed maintain that the Type 1 diabetes research community has developed a "selective blindness" as evidenced by its failure to recognise a number of shortcomings associated with animal models of the disease. First amongst these is the proper interpretation of information regarding the ease of disease prevention in the NOD mouse model. An often cited article noted that over 125 therapies were capable of preventing or delaying Type 1 diabetes in NOD mice [1]. For the purpose of this debate, that list has been updated, a task that revealed 195 published methods

that have reported this effect (Appendix). The phenomenon has not gone unremarked, with resulting comments such as "If you look cross-eyed at NOD mice, you prevent diabetes". The real problem, however, lies in the habitual practice of investigators who initiate therapeutic interventions in animals at 4 to 6 weeks of age. If we accept that a major goal of such studies is to identify therapies with application to human Type 1 diabetes, proper matching between the therapeutic agent and the time of administration should be attempted in terms of risk/benefit, safety and efficacy. Simply put, immunological "sledgehammers" will never be applied to those infants and adolescents at risk of Type 1 diabetes who are the candidates for therapeutic intervention. Fortunately, corrective action should be easy for the Type 1 diabetes research community to undertake. Future studies should utilise agents appropriate for the particular disease course, which means relatively benign agents early in the disease course and agents where safety profiles are more in question (e.g. immunosuppressive agents) at or immediately before disease onset in animal models. In addition to such matching in terms of ethics and safety, the financial costs of such interventions should also be included, and demonstrate a degree of feasibility.

Indeed, the issue of therapeutic safety (if applied to human Type 1 diabetes) also often represents an overlooked facet of studies in animal models. To provide support for this concern, the field of Type 1 diabetes prevention recently saw the publication of a surprising set of experiments indicating that administration of a beta cell self-antigen under a specific set of conditions induced a lethal form of shock [14]. In only a minority of published studies of animal models of Type 1 diabetes are investigations of safety, dosing and toxicity performed. Clearly, more attention should be given to the effects of an agent on the overall immune response, physiology, metabolism, and overall health of the animals.

Another issue that has led us to stumble has been the standard of cleanliness applied to housing animal colonies. Many environmental and behavioural components influence disease outcomes in NOD mice [15], and most infections (e.g. mouse hepatitis virus) have been associated with disease prevention or attenuation. Diets, handling and even cage height have also been claimed to modulate disease development in these animals. Hence, it is questionable whether the continued practice of failing to set standards for care and housing (i.e. that they are specific pathogen free) will allow for results that can be compared across all laboratories (at a minimum), or whether they might generate errant interpretations of facets related to pathogenesis and prevention. As such, groups involved in this field of investigation (e.g. the Immunology of Diabetes Society, the Animal Models for Diabetes Society) might well consider developing a set of standards and guidelines that would set matters straight. Such organisations could also introduce definitions into the field, which would allow comparisons of therapeutic efficacy between studies and would bring clarity to the field of endeavour. What would such a statement imply? It would establish a series of quantitative definitions of terms such as "delay", "prevention", or "marginal delay", each set to the degree of variance (e.g. 20% to 100% from the simultaneous control population). Additionally, standards for the observational period necessary for such lines of investigation (e.g. 52 weeks), definitions for the degree of hyperglycaemia necessary for diagnosis of Type 1 diabetes (e.g. >13.2 mmol/l on two occasions over a 24-hour period), and similarly, the use of glycosuria versus hyperglycaemia in disease diagnosis should be established.

It is common to hear the statement "Agent X (fill in the blank) prevents diabetes in NOD mice". Such declarations are usually derived from a statistical evaluation (e.g. life-table analysis, chi square at a set time etc.). However, a closer look at this issue would reveal that some agents provide absolute protection from disease (i.e. 0% Type 1 diabetes) while others provide what would more appropriately be described as a delay or partial protection (e.g. 30% rate in treatment group vs a 60% disease rate in control mice). So, in this example, two agents with very different degrees of "protection" might both be described as "preventing diabetes", without proper comparison. This notion is further complicated by the time periods involved in observing different disease therapies. Published reports normally have time periods ranging from 24 to 32 weeks, with a surprisingly low percentage of studies extending their observations beyond 32 weeks of age. Such short observation periods not only diminish the potential to strengthen statistical associations, but in addition, may eliminate the potential to uncover late therapeutic failures. Hence, it is highly recommended that this time period for observations be extended and uniformly adopted by the Type 1 diabetes research community.

A final problem with animal models and Type 1 diabetes prevention is one that has been exemplified in this rebuttal, namely exclusive reference to studies of NOD mice and a failure to consider other animal models of the disease (e.g. BB rats, LEW.1AR1 rats). While there are certain benefits of overweighting one animal model for therapeutic studies, this approach may come at the cost of ignoring another animal model, the BB rat, in which the insulitis lesion (i.e. the key phenotype for Type 1 diabetes destruction) is perhaps the most comparable to that in the human disease. Furthermore, if we accept the possibility that human Type 1 diabetes is not a single form of disease, but might in reality represent a collection of phenotypically similar cases with different aetiopathogeneses, surely the more animal models we have, the better.

Table 1. Roadmap to improved use of animals in Type 1 diabetes research in humans

Truth (often ignored)	Appropriate action (and acknowledgement)
Early prevention of disease (e.g. 4 weeks of age) in NOD mice is easy	Focus attempts with early interventions on agents suitable (i.e. ethics, safety, cost) for such use in humans
Non-specific pathogens influence disease rate in NOD mice	Perform investigations in specific pathogen-free environments
Late interventions for disease prevention or reversal in NOD mice are difficult	Attempt more studies of agents at onset of disease, with the goal of disease reversal or retention of C-peptide function
Not all disease interventions are safe (e.g. shock)	Perform studies of dosing and toxicity
Many studies utilise the word "prevent" when "delay" may be more appropriate	Establish criteria that define "marginal delay", "significant delay" and "absolute prevention"
A vast majority of Type 1 diabetes studies in animal models utilise NOD mice, a practice that carries risks for application to Type 1 diabetes in humans	Attempt prevention-based interventions in other animal models (rats)

Scientific impact

Care should be taken in interpreting data from adoptive transfer studies in animals and comparing them with data on Type 1 diabetes in humans, since the mechanism of action shares similarities with graft versus host disease rather than with "spontaneous" autoimmune disease in terms of treatment of recipients, priming and activation status of the lymphocytes and dosage of pathogenic lymphocytes. In addition, studies of transgenic animals or gene knock-out mice represent case reports that could suffer from cell biological and immunological artefacts unrelated to and incompatible with Type 1 diabetes in humans, or even in rats and mice. Furthermore, models of streptozotocin- or cyclophosphamide-induced diabetes generate chemically induced diabetes that has no clinical counterpart.

Do we need a philosophical change in academic science? The impact of evaluating important in vivo contexts in different animal models should be increasing, as far as acceptance in journals of higher scientific impact is concerned. As such, findings in animal models should be presented in parallel with representative pathology in human disease. Along with this, funding mechanisms should be established to re-evaluate findings in different experimental settings. Funding of animal experiments that will add little to what is known from human experiments should be discouraged. Overall, no single animal model should be considered the gold standard of investigations if this leads investigators to discount findings in alternative models that might be better suited to address certain questions.

Clinical impact

We now reach a point of major concern: inbred strains of rats or mice represent single case reports (Fig. 2). Consequently, results obtained from such models should be interpreted with great caution. In the past, various clinical reports have been rejected, downplayed, obstructed or ignored, as they were discordant with findings in any given animal model. For instance, the first report on an HLA-DR4-restricted T cell epitope of GAD65, initially rejected by a clinical journal, was only reconsidered by this journal after a study was published on HLA-DR4 transgenic mice immunised with GAD65, reporting data on the very same epitope [16, 17].

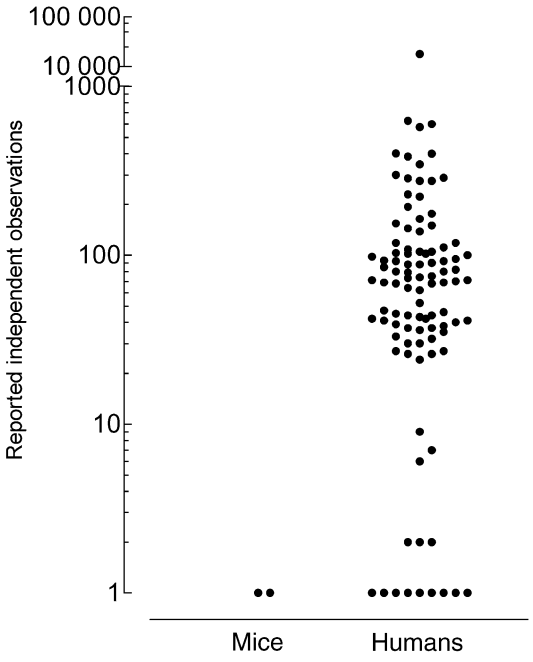

Fig. 2. Number of independent observations per report in mice and humans

In summary, our take-home messages are:

If animal data differ from clinical results, carefully consider why, reconsider, and possibly examine a different animal model for testing (if needed).

Appreciate the human anecdote "every patient bears a lesson to be learned" (as does the NOD mouse).

Animal models have, over time, proved to be more often inaccurate than accurate, especially with regard to therapeutic interventions.

Animal models should only be used to study specific aspects of the disease process, and not considered to represent the clinical disease.

Implementation of a proposed series of recommendations should be considered as we move forward with animal models (Table 1). The end result of this debate, even if not settled, will then be a positive move towards the recognition of the truth.

"You can't always get what you want,
But if you try sometimes
You might find
You get what you need."

(The Rolling Stones, 1969)

Appendix

Successful immunotherapies in NOD mice

AAV murine IL-10
AAV rat preproinsulin gene (vLP-1)
Adenovirus expressing mIL-4
Aerosol insulin
Allogenic thymic macrophages
Alpha galactosylceramide
Alpha-interferon (rIFN-alpha)
Alpha/beta T cell receptor thymocytes
Aminoguanidine
Androgens
Anaesthesia
Antioxidant MDL 29,311
Antisense GAD mRNA
Azathioprine
Anti-B7-1
Bacille Calmette-Guérin (BCG)
Baclofen
Bee venom
Biolistic-mediated IL-4
Blocking peptide of MHC class II
Bone marrow transplantation
Castration
Anti-CD3
Anti-CD4
CD4+CD25+ regulatory T cells
Anti-CD8
Anti-CD28 MAb
Cholera toxin B subunit-insulin protein
Class I-derived self-I-A beta(g7) (54-76) peptide
Cold exposure
Anti-complement receptor
Complete Freund's adjuvant
Anti-CTLA-4
Cyclic nucleotide phosphodiesterases (PDEs)
Cyclosporin
Cyclosporin A
DC deficient in NF-κB
DC from pancreatic lymph node
DC with IL-4
Deflazacort
Deoxyspergualin
Dexamethasone/progesterone/
growth hormone/oestradiol
Diazoxide
1,25 dihydroxy Vitamin D3, KH1060
1,25 dihydroxycholecalciferol
1,25 dihydroxyl Vitamin D3
Elevated temperature
Emotionality
Encephalomyocarditis virus (ECMV)
Essential fatty acid deficient diets
FK506
FTY720 (myriocin)
GAD 65 peptides in utero
Anti-GAD monoclonal antibody
Galactosylceramide
Glucose (neonatal)
Glutamic acid decarboxylase
(intraperitoneal, intrathymic, intravenous, oral)
Glutamic acid decarboxylase 65 Th2 cell clone
Glutamic acid decarboxylase peptides
(intraperitoneal, intrathymic, intravenous, oral)
Gonadectomy
Guanidinoethyldisulphide
Heat shock protein 65
Heat shock protein peptide (p277)
Haematopoietic stem cells encoding proinsulin
Housing alone
Human IGF-1
I-A beta g7(54-76) peptide
Anti-I-A monoclonal antibodies
Anti-ICAM-1
IgG2a antibodies
Immobilisation
Inomide
Anti-integrin alpha 4
Insulin (intraperitoneal, oral, subcutaneous, nasal)
Insulin B chain (plasmid)
Insulin B chain/B chain amino acids 9-23
(intraperitoneal, oral, subcutaneous, nasal)
Insulin-like growth factor I (IGF-I)
Anti-intercellular adhesion molecule-1 (ICAM-1)
Interferon-α (oral)
Interferon-γ
Anti-interferon-γ
Interferon-γ receptor/IgG1 fusion protein
Interleukin-1
Interleukin-4
Interleukin-4-Ig fusion protein
Interleukin-4-plasmid

Interleukin-10
Interleukin-10-plasmid DNA
Interleukin-10-viral
Interleukin-11-human
Interleukin-12
Intrathymic administration of mycobacterial heat shock protein 65
Intrathymic administration of mycobacterial heat shock peptide p277
Islet cells-intrathymic
L-Selectin (MEL-14)
Lactate dehydogenase virus (LDH)
Large multilamellar liposome
Lazaroid
Anti-leucocyte function associated antigen (LFA-1)
Anti-LFA-1
Linomide (quinoline-3-carboxamide)
Lipopolysaccharide-activated B cells
Lisofylline
Lymphocyte choriomeningitis virus (LCMV)
Anti-lymphocyte serum
Lymphocyte vaccination
Lymphocytic choriomeningitis virus
Anti-L-selectin
Lymphotoxin
LZ8
MadCAM
MC1288 (20-epi-1,25-dihydroxyvitamin D3)
MDL 29311
Metabolically inactive insulin analogue
Anti-MHC class I
Anti-MHC class II
MHC class II-derived cyclic peptide
Mixed allogeneic chimerism
Mixed bone marrow chimeras
Monosodium glutamate
Murine hepatitis virus (MHV)
Mycobacterium avium
Mycobacterium leprae
Natural antibodies
Natural polyreactive autoantibodies
Neuropeptide calcitonin gene-related peptide
Nicotinamide
Nicotine
Ninjin-to (Ren-Shen-Tang), a Kampo (Japanese traditional) formulation
NKT cells
NY4.2 cells
OK432
Overcrowding
Pancreatectomy
Pentoxifylline
Pertussigen
Poly [I:C]
Pregestimil diet
Prenatal stress
Preproinsulin DNA
Probucol
Prolactin
Rapamycin
Recombinant vaccinia virus expressing GAD
Reg protein
Reg protein
Rolipram
Saline (repeated injection)
Schistosoma mansoni
Semi-purified diet (e.g., AIN-76)
Short-term chronic stress
Silica
Sirolimus/tacrolimus
Sodium fusidate
Soluble interferon-γ receptor
Somatostatin
Non-specific pathogen-free conditions
Streptococcal enterotoxins
Streptozotocin
Sulfatide (3′sulfogalactosylceramide)
Superantigens
Superoxide dismutase-desferrioxamine
Anti-T cell receptor
TGF-β 1 somatic gene therapy
Th1 clone specific for hsp60 peptide
Anti-thy-1
Thymectomy (neonatal)
Tolbutamide
Tolerogenic dendritic cells induced by vitamin D receptor ligands
Top of the rack
Treatment combined with a 10% w/v sucrose-supplemented drinking water
TNF-α
TX527 (19-nor-14,20-bisepi-23-yne-1,25(OH)(2)D(3))
Vitamin E
Anti-VLA-4

References

1. Atkinson MA, Leiter EH (1999) The NOD mouse model of type 1 diabetes: as good as it gets? Nat Med 5:601–604
2. Leiter EH, von Herrath M (2004) Animal models have little to teach us about Type 1 diabetes: 2. In opposition to this proposal. Diabetologia 47:DOI 10.1007/s00125-004-1518-0
3. Mestas J, Hughes CCW (2004) Of mice and not men: differences between mouse and human immunology. J Immunol 172:2731–2738
4. Roep BO (2003) The role of T-cells in the pathogenesis of Type 1 diabetes: from cause to cure. Diabetologia 46:305–321
5. McDevitt H (2001) Closing in on type 1 diabetes. N Engl J Med 345:1060–1061
6. Homo-Delarche F, Drexhage HA (2004) Immune cells, pancreas development, regeneration and type 1 diabetes. Trends Immunol 25:222–227

7. Bonifacio E, Atkinson M, Eisenbarth G et al. (2001) International Workshop on Lessons From Animal Models for Human Type 1 Diabetes: identification of insulin but not glutamic acid decarboxylase or IA-2 as specific autoantigens of humoral autoimmunity in nonobese diabetic mice. Diabetes 50:2451–2458
8. Bingley PJ, Bonifacio E, Gale EA (1993) Can we really predict IDDM? Diabetes 42:213–220
9. Greeley SAW, Katsumata M, Yu LP et al. (2002) Elimination of maternally transmitted autoantibodies prevents diabetes in nonobese diabetic mice. Nat Med 8:399–402
10. Herrath M von, Bach JF (2002) Juvenile autoimmune diabetes: a pathogenic role for maternal antibodies? Nat Med 8:331–333
11. Koczwara K, Bonifacio E, Ziegler AG (2004) Transmission of maternal islet antibodies and risk of autoimmune diabetes in offspring of mothers with type 1 diabetes. Diabetes 53:1–4
12. Serreze DV, Chapman HD, Varnum DS et al. (1996) B lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new "speed congenic" stock of NOD.Ig mu null mice. J Exp Med 184:2049–2053
13. Martin S, Wolf-Eichbaum D, Duinkerken G et al. (2001) Development of type 1 diabetes despite severe hereditary B-lymphocyte deficiency. N Engl J Med 345:1036–1040
14. Pedotti R, Sanna M, Tsai M et al. (2003) Severe anaphylactic reactions to glutamic acid decarboxylase (GAD) self peptides in NOD mice that spontaneously develop autoimmune type 1 diabetes mellitus. BMC Immunol 4:2
15. Pozzilli P, Signore A, Williams AJK, Beales PE (1993) Nod mouse colonies around the world—recent facts and figures. Immunol Today 14:193–196
16. Endl J, Otto H, Jung G et al. (1997) Identification of naturally processed T cell epitopes from glutamic acid decarboxylase presented in the context of HLA-DR alleles by T lymphocytes of recent onset IDDM patients. J Clin Invest 99:2405–2415
17. Wicker LS, Chen SL, Nepom GT et al. (1996) Naturally processed T cell epitopes from human glutamic acid decarboxylase identified using mice transgenic for the type 1 diabetes-associated human MHC class II allele, DRB1*0401. J Clin Invest 98:2597–2603