Sweetness and light: perspectives for rodent models of type 1 diabetes

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Disease Models & Mechanisms • DMM

Type 1 diabetes (T1D) is a major disease affecting primarily young children with an incidence in Western societies of around 0.3% by 20 years of age. Although both genetic and environmental factors contribute to the disease aetiology, the precise nature of both the genetic and environmental contribution to human disease onset and progression remains poorly defined. Despite showing some differences from human T1D, rodent models for T1D (Leiter and von Herrath, 2004; von Herrath and Nepom, 2009) and, in particular the non-obese diabetic (NOD) mouse (Atkinson and Leiter, 1999; Kikutani and Makino, 1992), have provided important insights into the disease process, even if they have not yet allowed definitive identification of many of the genetic factors involved in the process. The recent isolation of germline-competent embryonic stem (ES) cells from the NOD mouse strain, and from the rat, will greatly facilitate the functional analysis of T1D in the mouse, and open up the possibility of improved exploitation of rat T1D models. This important technological breakthrough has the potential to remove bottlenecks from the identification of T1D genes, allowing the underlying metabolic pathways to be established and facilitating evaluation of the eventual role of the human homologues in the disease process. The current status and perspectives for an improved mechanistic understanding of the disease process will be addressed.

Characteristics of type 1 diabetes

Type 1 or juvenile diabetes onset is associated with the autoimmune destruction of the insulin-secreting B cells of the pancreatic islet of Langerhans. Clinical disease, which occurs early in life, often does not become apparent until over 70% of the insulin-secreting tissue has been destroyed, restraining treatment options (for a review of many aspects of T1D, see articles at www.uchsc.edu/misc/diabetes/books/type1/type1.html).

Disease onset and progression are under both complex genetic control and environmental influence. Although the major histocompatibility locus (HLA in humans, H-2 in the mouse) is

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known to be a major genetic determinant (Onengut-Gumusu and Concannon, 2005; Concannon et al., 2009), a large number of other genes (currently over 35 in the mouse) (Barrett et al., 2009; Maier and Wicker, 2005) appear to influence the course of the disease. Difficulties in identifying major gene loci other than HLA in human T1D, and the large number of identified loci in murine T1D, have led to suggestions that there may be some sort of threshold effect involved in the onset of T1D, to which both multiple genes and environmental factors would contribute. The nature of the environmental component(s) affecting disease aetiology is also poorly defined, although viruses such as rubella and coxsackie virus have been suggested to play a role (Zipris, 2009). Whilst insulin administration allows T1D patients to live normal lives, the failure of treatment to reproduce completely the stringent control of blood glucose levels in the different tissues of the body contributes to disease complications. Despite important progress, some 40% of diabetic patients will suffer from such complications, which can involve the kidneys, the cardiovascular system (Nathan et al., 2005) and the eye (retinopathies) (Rosenblatt and Benson, 2004).

Challenges to T1D research

Current research objectives aim at (1) restoring, partially or wholly, islet function by using islet cells developed ex vivo or in vivo from stem cells; (2) identifying robust biomarkers allowing the early detection of autoimmune onset prior to the establishment of clinical syndromes; and (3) the identification and characterisation of the genes and mechanisms involved in T1D and its specific targeting of the islet cells in order to develop strategies to neutralise the autoimmune response. It is important to realise that, even if islet cells can be derived successfully from the patient by stem cell technology and then transplanted as an isograft, or pancreatic function restored by the regeneration of the remaining islets (Nir et al., 2007), controlling the autoimmune response remains pertinent to long-term patient health status (Todd, 2009). Identifying ways of controlling the autoimmune targeting of the islets without interfering with the general immune status of the patient remains the gold standard for such research initiatives.

Animal models and their relevance to human T1D

The pathophysiological similarities between T1D in humans and the disease that develops in the murine non-obese diabetic (NOD) model, include both the crucial role of the MHC (major histocompatibility complex) in disease aetiology, defects in T-lymphocyte function, insulitis development, the presence of multiple types of autoantibody and the sensitivity of the disease to immunosuppression. Despite these similarities, there has been a continuing debate over the adequacy of the NOD mouse as a model for human T1D. This has revolved around the several dissimilarities in the disease processes, which include sex-dependent

susceptibility; the failure up until now of model studies to lead to successful human clinical trials (Roep, 2007); difficulties in assessing adequately the similarities in T1D in the two species, owing in large part to our lack of in-depth knowledge of the process of pathogenesis of the human disease (von Herrath and Nepom, 2009); and a general, if less-well defined, feeling that no single mouse model could adequately recapitulate the heterogeneity of the human disease process. Despite recent findings that the *CTLA4* gene (cytotoxic T-lymphocyte antigen 4) and IL-2 (interleukin-2) pathway are implicated in the genetic control of both human and mouse T1D (Rainbow et al., 2008), the large numbers of non-MHC loci, which individually have a small effect, that have been characterised as contributing to mouse T1D resistance/susceptibility has, in the past, been suggested to indicate the potential inappropriateness of the NOD model of T1D.

A recent direct genetic analysis of human T1D patients by large-scale whole-genome screening (GWAS) initiatives – made possible by progress in genotyping and biobanking – has, for the first time, allowed the identification of many of the common variants acting as T1D control elements. The 40 or so loci identified thus far (Barrett et al., 2009) appear to account for only a small proportion of the overall disease load observed in human populations (Goldstein, 2009; McCarthy and Hirschorn, 2008; Lettre and Roux, 2008). Although rather disappointing from the point of predictive medicine, these findings of multiple new loci affecting human T1D, each of small effect, strongly reinforce the resemblance at the genetic level of the human and mouse diseases. Strikingly, recent findings concerning the genetic control of diabetes susceptibility in the bio-breeding (BB) rat have revealed a similar genetic complexity in this species (Wallis et al., 2009; Leiter, 2009).

Although no-one should imagine that each and every gene defined in an animal model system for T1D will be directly transferable to the human disease, the pertinence of the NOD mouse and eventually rat models of diabetes in establishing the architecture of the complex genetic and epigenetic networks, and the interactions, that underlie T1D appear to have been markedly reinforced by these recent findings. In this context, it is also worth emphasising that the finding in human T1D, that a multiplicity of loci of small individual effect input to the end phenotype, clearly compromises hopes of direct functional genetic analysis in the human. And in a related area of great potential relevance to T1D, the difficulties, both scientific and ethical, in manipulating human stem cells have underlined the importance of advancing research in the area of stem cell/differentiation projects that are related to pancreatic cell differentiation, simultaneously, in the mouse and human.

The impact of models in T1D research
Research into animal models of T1D has centred on the use of the NOD mouse, and the BB and Komeda (KDP) diabetes-prone rat models (Mathews, 2005). Substantive insights into the complexity of the genetic controls in play, and the immunological mechanisms involved, have been achieved. However, both the definition of the underlying genetic networks, gene identification and characterisation (see Fig. 1), and extended functional analysis, have been hampered by the non-availability of one of the key elements in the mammalian geneticist's toolbox for experimentally altering
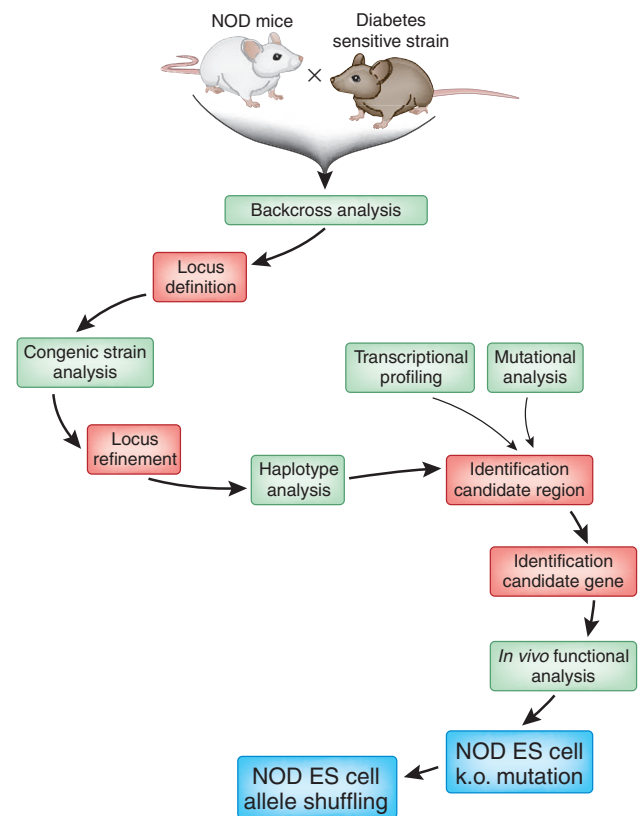
Fig. 1. Genetic and functional genomics analysis of T1D in the mouse: a flow diagram. The crucial defining steps are in red boxes, whereas the underlying technical approaches are in green boxes or, when relating to ES cells, in blue boxes.

gene function: robust germline-competent embryonic stem (ES) cells (Arai et al., 2004; Brook et al., 2003). In their absence, mouse geneticists have had to resort to the highly onerous, and not completely satisfactory, technique of integrating knockout mutations obtained on other mouse genetic backgrounds into the NOD strain by backcrossing, a strategy that tends to result in the transfer of relatively large genetic regions (often over 10 Mb), or consider the use of other techniques. Several years back, hopes were raised that RNA interference (RNAi) technologies, possibly in conjunction with lentiviral delivery, would provide a robust solution that would be widely applicable within different mouse strains. Despite one successful application of this technology to the in vivo study of T1D in the NOD mouse (Kissler et al., 2006), this technology has however proved more difficult, and certainly more onerous and less robust, in usage than expected (P.A. and U. Rogner, unpublished observations). Although by no means impossible, the fragility of NOD early mouse embryos under standard handling conditions provides an additional complication to such approaches. We conclude that RNAi approaches are unlikely to provide the long-awaited panacea for in vivo T1D functional studies.

In this context, the recent finding that germline-competent ES cells can be obtained from both NOD mice (Nichols et al., 2009; Ohta et al., 2009) and several rat strains (Buehr et al., 2008) is of major significance. Both sets of results depend on the finding that

established mouse ES cell lines could be efficiently grown and maintained in the undifferentiated state on so-called '2i' medium, in which the traditional culture medium that is used for ES cell culture and ES cell derivation, containing foetal calf serum, is replaced by inhibitors of the Erk and glycogen synthase pathways, with or without leukaemia inhibitory factor, or by pluripotin (Ying et al., 2008; Yang et al., 2009). Although strain- and species-specific differences between rodents may necessitate some tinkering with the derivation conditions that have been defined, the isolation of ES cell lines from a whole range of NOD congenic strains now appears possible (Nichols and Smith, 2009; Hanna et al., 2009). The isolation of germline-competent ES cells from both NOD and NOD congenic strains, and from diabetogenic rat strains, will underpin renewed efforts to undertake functional genomic analysis of animal diabetes models.

Genetics and ES cell technologies

It is clear that, in most common diseases such as T1D that are under multifactorial and multigenic control, the genetic input to the disease aetiology is likely to involve alterations to quantitative biological parameters rather than, as in many classical monogenic diseases, the complete presence or absence of a functional activity or gene/protein.

At the RNA level, such parameters may include both the steady-state levels and forms of crucial RNA transcripts through alterations in the levels of RNA transcription, or through post-transcriptional control mechanisms involving either RNA degradation or alterations in RNA splicing patterns. At the protein level, differences in protein substrate affinities, protein ligand binding, protein turnover rates, and differences in protein localisation levels within different cell compartments are all potentially capable of underpinning the type of quantitative variation that appears to be implicated in T1D aetiology.

The isolation of germline-competent NOD ES cells will allow approaches to be made at several levels. First, in implementing the straightforward gene knockout of potential *Idd* (insulin dependent diabetes) gene candidates. Second, and in my opinion more importantly, in allowing the implementation of allele-shuffling strategies in which deletion of the NOD gene is followed by its replacement with the identical gene from the diabetes-resistant strain under study. Unlike classical gene replacement, where the replaced genetic material would often be limited to the gene itself, it will probably be of interest to replace, through bacterial artificial chromosome (BAC) recombineering (Glaser et al., 2005), considerably larger genomic regions in order to include neighbouring control elements that may be implicated in resistance/sensitivity. The vectors and constructs that are available through the EUCOMM and KOMP gene-targeting programmes (www.eucomm.org; www.komp.org) may be of some use for targeting gene knockouts into the NOD strain, depending on the degree of sequence conservation between NOD and reference C57BL/6 strains. However, it is the availability of the genomic sequence for the NOD strain, and of ready-to-use end-sequenced NOD BAC clones, which is likely to prove of the greatest use to experimentalists (http://oct2007. archive.ensembl.org/info/data/docs/nod_mouse.html). Third, by providing the framework for testing by deletion studies the effects of human T1D candidate genes in a well-characterised experimental context.

Comparative transcriptional profiling of the resulting knockout/allele-shuffled mice should allow pathways contributing to the diabetes phenotype to be more easily defined, without the complications that are inherent in the use of congenic rather than co-isogenic mouse stocks. We expect such developments to lead to the identification of additional pathways or pathway members that contribute to T1D aetiology, and to the definition of potential therapeutic targets.

Icing the cake: opportunities for future models

Analyses such as those that we have performed on the *Idd6* locus on mouse chromosome 6 have suggested that, in many cases, genetically defined diabetes loci in the mouse are composed of several sub-loci contributing to the overall phenotype (Hung et al., 2006). Refined analysis of such haplotypes has been complicated by the difficulty in obtaining additional recombinants in the mouse, as the *Idd* candidate regions drop to megabase size, and difficulties in breeding very large numbers of mice. The ability to derive germline-competent ES cells from NOD congenic strains such as those that we have derived for the *Idd6* locus, either by using 2i medium or by generating germline-competent IPS cells from somatic cells of the congenic lines (Takahashi and Yamanaka, 2006; Hanna et al., 2009), then to delete one or several of the neighbouring presumptive *Idd* loci carried by these strains, recover the mice, and carry out detailed phenotypic characterisation on them, should allow such interactions to be productively studied for the first time. The implementation of conditional gene modification strategies aimed at specifically targeting modifications to the islet cells, or parts of the immune system, will require additional components such as Cre (Glaser et al., 2005) or Dre driver strains (Anastassiadis et al., 2009) to be established on the NOD or NOD congenic background – a not inconsiderable task at least in the short term. Despite this, there is no doubt that the extension of standard ES technologies to the main rodent models for T1D research is a major step forward that is likely to benefit research, not only the genetics, but also the physiology and immunology underlying this autoimmune disease.

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