

Use of the Göttingen Minipig as a Model of Diabetes, with Special Focus on Type 1 Diabetes Research

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Abstract

Animal models of type 1 diabetes remain essential tools for investigation of the etiology and pathogenesis of the disease and, importantly, for the development of effective new treatments. Although a range of well-characterized and widely used models of type 1 diabetes in rodents are currently available, large animal models are a valuable complement to rodent models for both physiological and practical reasons. The pig is very useful in many aspects as a model for human physiology and pathophysiology because many organ systems of this species, as well as physiological and pathophysiological responses, resemble those of the human. The Göttingen minipig is particularly suitable for long-term studies because of its inherent small size and ease of handling, even at full maturity. Of particular relevance to the field of type 1 diabetes are the many similarities evident between humans and pigs with regard to pharmacokinetics of compounds after subcutaneous administration, structure and function of the gastrointestinal tract, morphology of the pancreas, and the overall metabolic status of the two species. Because spontaneous type 1-like diabetes is very rare in pigs, a model of the condition must be induced experimentally, either surgically or chemically. This process is discussed, and the use of the pig as a model in islet transplantation and diabetic complications is briefly summarized.

Key Words: animal model; comparative physiology; large animal model; pig; streptozotocin; type 1 diabetes

Introduction

Animal models are essential tools for investigation of the etiology and pathogenesis of human type 1 diabetes and for the development of new treatments for the disease. To ensure the quality of data derived from experimental procedures, the use of animals as models should ideally be limited to studying specific and well-defined characteristics that both resemble and are predictive of the disease in humans. Furthermore, the choice of animal model must in each case depend on the aspect of the human disease

in focus. Therefore, the utility of animal studies requires well-characterized models in which both similarities to and differences from the disease in humans are explored in detail. A range of well-characterized and widely used models based on rodents are currently available. However, with certain aspects of type 1 diabetes, large animal models are a valuable complement to rodent models in many ways, both for practical and physiological reasons. This article focuses on the use of the Göttingen minipig as a model in type 1 diabetes research. Aspects specifically related to type 2 diabetes and obesity are not discussed herein.

General Considerations

The pig is very useful in many ways as a model for human physiology and pathophysiology because many of the pig's organ systems, as well as physiological and pathophysiological responses, resemble those of the human (Brown and Terris 1996; Douglas 1972; Reeds and Odle 1996; Swindle and Smith 1998). Of special relevance in the field of type 1 diabetes are the many similarities between the two species in pharmacokinetics after subcutaneous administration of compounds, in gastrointestinal tract morphology and function, and in metabolic physiology. These characteristics are discussed in more detail below.

Due to the size of the pig, it is possible to obtain a larger volume of blood in these animals than in rodents, thereby enabling studies similar to those performed in humans. Furthermore, the pig can be trained to allow performance of experiments in conscious, unstressed animals, thus allowing studies to be carried out in relation to ingestion of meals. Although the lack of easily accessible superficial veins in the pig makes blood sampling a potentially stressful procedure, it is possible to circumvent this potential by implantation of either temporary (Carroll et al. 1999; Matte 1999; Smith and Ficken 1991) or permanent central venous catheters, which can be adequately maintained for long periods of time (Moritz et al. 1989; Wingfield et al. 1974). Implantation of catheters also poses a considerable risk for catheter-related infections (Barth et al. 1990); however, this risk can be reduced to a large extent by appropriate use of hygienic measures (Moritz et al. 1989; O'Grady et al. 2002).

Göttingen Minipig

The Göttingen minipig is especially suitable for long-term studies because of its inherent small size and ease of han-

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ding, even at full maturity, which is reached at 2 yr of age (compared with 3 yr for domestic pigs) (Barth et al. 1990; Bollen and Ellegaard 1996, 1997; Holtz 1986). The Göttingen minipig was developed in the early 1960s at the Institute of Animal Breeding and Genetics of the University of Göttingen, Germany. The animal is the result of a cross-breeding between the Vietnamese swine, the Hormel or Minnesota miniature swine, and the German improved Landrace, which gives the white color. This cross-breeding resulted in the Göttingen minipig's characteristic small size and stable genetics and phenotype (Bollen and Ellegaard 1997; Glodek 1986). Its small size is most likely due to reduced levels of growth hormone (GH¹) binding protein or other growth factors, whereas GH and insulin-like growth factor-1 levels are normal (Elsaesser et al. 2002; Lauterio et al. 1988). However, peak amplitudes of GH have been reported to be lower in Göttingen minipigs (Johansen et al. 2001) compared with larger strains (McCusker et al. 1985).

In the Danish colony of Göttingen minipigs, selection is based on keeping the colony out-bred (Bollen and Ellegaard 1996; Brandt and Möllers 1999). The colony is microbiologically standardized to reduce interference of pathogens in experimental results (Bollen and Ellegaard 1996), and reference values for several clinical chemical and hematological parameters have been established for the colony (Canavan et al. 1997; Ellegaard et al. 1995; Jacobsson 1989; Jørgensen et al. 1998; Larsen et al. 2001). Based on its well-described genetics and biology and the inherent small size, in combination with the many similarities in terms of physiology and pathophysiology between pigs and humans, the Göttingen minipig is of particular interest for long-term studies in relation to human type 1 diabetes.

Pharmacokinetics

The high degree of similarity of the skin and subcutaneous tissues in pigs and humans (Benech-Kieffer et al. 2000; Meyer 1996; Qvist et al. 2000; Swindle and Smith 1998) is of particular relevance in the field of type 1 diabetes in which patients are treated with subcutaneous injection of insulin. In addition, the serum albumin concentration in pigs is very similar to concentrations observed in humans (Ganong 1991a; Mersmann and Pond 2001). Together, these characteristics result in kinetics and dynamics after subcutaneous administration of compounds that closely resemble those in humans (Agero et al. 2002; Gutniak et al. 1994; Knudsen et al. 2001; Kurtzhals et al. 1996; Markussen et al. 1996; Nauck et al. 1996; Pieber et al. 2002).

¹Abbreviations used in this article: ALX, alloxan; FFA, free fatty acids; GH, growth hormone; HbA_{1c}, hemoglobinA_{1c}; HLA, human leukocyte antigen; IAPP, islet amyloid polypeptide; MHC, major histocompatibility complex; NAD, nicotinamide adenine dinucleotide; NIA, nicotinamide; PERV, porcine endogenous retroviruses; pp, pancreatic polypeptide; SLA, swine leukocyte antigen; STZ, streptozotocin.

With respect to biotransformation, the phase I metabolism involving the microsomal cytochrome P-450 enzyme system of the pig and the human appear similar in many aspects although some differences have been documented (Fujimori et al. 1986; Witkamp and Monshouwer 1998). In contrast to other species, a typical characteristic of the pig is the lack of sulfation of most (but not all) drugs during phase II metabolism in the liver, where glucuronidation and acetylation appear to be the main metabolic pathways in this species (Witkamp and Monshouwer 1998). Nevertheless, the pig is a useful model for pharmacokinetic investigations.

Structure and Function of the Gastrointestinal Tract

The digestive system of the pig displays many functional similarities to that of the human (Brown and Terris 1996). The pig is a true omnivore, which is especially interesting because despite anatomical differences, its nutritional requirements and physiology of digestion are very similar to those of humans (Bentouimou et al. 1997; Dixon et al. 1999; Huge et al. 1995; Miller and Ullrey 1987; Phillips et al. 1979; Swindle and Smith 1998). Both pigs and humans are highly dependent on dietary quality, because symbiotic micro-organisms in the gut play a relatively minor role in modifying ingested nutrients (Miller and Ullrey 1987). Intestinal transit times are slightly greater in pigs (Barth et al. 1990), whereas digestive effectiveness is comparable (Miller and Ullrey 1987). Furthermore, the possibility of feeding well-defined meals to pigs is a great advantage in the study of glucose tolerance compared with the more continuous feeding pattern seen in most rodents.

Pancreas

The porcine pancreas resembles the human pancreas in size, shape, and position. In both species, a substantial portion of the pancreas is retroperitoneal. The blood supply of the endocrine and exocrine tissues in the pig pancreas shares several similarities with those of humans (Murakami et al. 1997) whereas in the pig, a single pancreatic duct separate from the common bile duct enters the duodenum (Stump et al. 1988). Porcine and human insulin differ by only a single amino acid at the 30 position of the B-chain (Ganong 1991b).

Endocrine cells are mainly found in the islets of Langerhans although single cells or small clusters of alpha- and/or beta-cells have also been observed (Jay et al. 1999; Wiczorek et al. 1998). The islet structure in young pigs is more diffuse than in adult humans, and the reticular capsule separating endocrine and exocrine tissues is not as evident as in humans (van Deijnen et al. 1992; Ulrichs et al. 1995; Wiczorek et al. 1998). Islets become more compact with age (Jay et al. 1999; Krickhahn et al. 2002) so that islets from adult pigs are more similar to adult human islets (Brand-

horst et al. 1995). The number of islets and islet volume density vary widely between different breeds of pig (Ulrichs et al. 1995).

As in the human pancreas (Klöppel et al. 1985; Orci et al. 1979; Rahier et al. 1981; Stefan et al. 1983), the alpha-cells in the pig exist mainly in the dorsal pancreas, both in the core and periphery of islets (Jay et al. 1999; Wieczorek et al. 1998), whereas in both species, the pancreatic polypeptide (pp¹)-containing cells are almost exclusively in the ventral pancreas (Jay et al. 1999; Orci et al. 1979; Rahier et al. 1981; Stefan et al. 1983; Wieczorek et al. 1998). In humans, the pp-cell is reported to be the predominant endocrine cell type in the ventral portion of the pancreas (Orci et al. 1979; Rahier et al. 1981; Stefan et al. 1983) whereas in the pig, the beta-cell is predominant in both the ventral and dorsal pancreas (Jay et al. 1999). As in humans, islet amyloid polypeptide (IAPP¹) is expressed mostly in beta-cells but also in some alpha- and delta-cells (Lukinius et al. 1996). The sequence of IAPP in the amyloidogenic domain is dissimilar in pigs and humans, and pigs are *not* prone to formation of pancreatic amyloid whereas humans are (Betsholtz et al. 1989; Clark et al. 1988; Klöppel et al. 1985).

The beta-cell content of the endocrine tissue in the normal minipig is in the same range (i.e., 60-80%) as reported in humans (Clark et al. 1988, Gepts and Lecompte 1981; Kobayashi et al. 1997; Larsen et al. 2003). However, beta-cell mass, relative to body weight, is almost twice as high in the minipig compared with that in humans (~20 vs. 10 mg/kg) (Larsen et al. 2003; Maclean and Ogilvie 1955). The high beta-cell mass relative to body mass in the minipig could indicate a greater insulin secretory reserve compared with the human. The sensitivity of islets to damage by a range of reactive oxygen and nitrogen species is very similar in the pig and human pancreas, and both are more resistant than rat islets (Wacker et al. 1995).

Metabolism and Glucose Tolerance

The postabsorptive metabolism of the pig is, in many aspects, similar to the processes seen in humans (Miller and Ullrey 1987). Metabolic adaptations induced by fasting are quite similar in humans and the pig, and the kinetics of the increase of plasma free fatty acids (FFA¹), glycerol, and ketone bodies and decrease in glucose and insulin are similar. Pigs have lower fasting FFA and ketone body levels than humans, perhaps due to the longer intestine and approximately doubled transit time in pigs (Barth et al. 1990). During fasting, hepatic glucose production is almost twice as high in conscious pigs compared with humans (4 vs. 2 mg/kg/min) (DeFronzo et al. 1983; Müller et al. 1983), and it has been reported that hepatic gluconeogenesis plays a greater role in the maintenance of normoglycemia in the anesthetized pig than in humans, where extrahepatic gluconeogenesis can maintain normoglycemia during anesthesia (Lauritsen et al. 2002).

The minipig has lower fasting plasma levels, compared with humans, for both insulin (Eriksson et al. 1989; Faber et al. 1978; Fritsche et al. 2000; Larsen et al. 2001, 2002c; Matthews et al. 1983; Reaven et al. 1976), C-peptide (Faber et al. 1978; Larsen et al. 2001, 2002c), and glucose (Canavan et al. 1997; Ellegaard et al. 1995; Eriksson et al. 1989; Matthews et al. 1983; Fritsche et al. 2000; Larsen et al. 2001, 2002c; Reaven et al. 1976; Weyer et al. 1999), whereas domestic pigs have fasting glucose levels in the same range as in humans (Barb et al. 1992; Ramsay and White 2000; Wilson et al. 1986). Plasma levels of glucose and insulin increase with age both in minipigs (Larsen et al. 2001) and humans (Rosenthal et al. 1982), indicating reduced insulin sensitivity and/or beta-cell function (Matthews et al. 1985). In addition, glucose tolerance can be expected to deteriorate slightly with age in both species (Broughton and Taylor 1991; Rosenthal et al. 1982) because fasting and postprandial glucose levels are closely related in the minipig (Larsen et al. 2002c) and in humans (ADA 1998, Anon 1998; Bruce et al. 1988).

Pigs are reported to have greater glucose tolerance (i.e., less increase in plasma glucose) after an oral glucose load (Ferrannini et al. 1985; Hanawalt et al. 1947; Kruszynska et al. 1993; Larsen et al. 2002c; Reed and Kidder 1971; Weyer et al. 1999) and to dispose an intravenous glucose load more efficiently than humans (Ahren and Pacini 1998; Anderson 1973; Anderson and Elsley 1970; Cerasi and Luft 1967; Deacon et al. 1998; Hanawalt et al. 1947; Larsen et al. 2002b; Ritzel et al. 1995). Based on these characteristics of the pig, one might question the relevance of using accepted human criteria for abnormal glucose tolerance in the diagnosis of glucose intolerance and diabetes in minipigs when one interprets glucose tolerance data. It is imperative to take these differences into consideration.

When assessing long-term glucose control in diabetic pigs, it is important to consider that porcine erythrocytes contain only marginal (small) intracellular glucose concentrations in the normal animal. Accordingly porcine hemoglobin contains very few glycosylated components (hemoglobinA_{1C} [HbA_{1C}]¹) due to the limited permeability of erythrocytes to glucose (Higgins et al. 1982). For this reason, measurement of HbA_{1C} should not be considered as an appropriate indicator of glycemia in pigs as it is in humans (Gabbay et al. 1977; Koenig et al. 1976), and fructosamine should therefore be preferred as an intermediate-term (1- to 3-wk) marker of glucose control as it is also used in humans (Baker et al. 1983, 1985; Cockram et al. 1990; Lloyd et al. 1985).

With regard to insulin secretion, normal pigs have an extensive capacity for insulin secretion after stimulation with glucose and/or arginine in vivo (Kjems et al. 2001; Larsen et al. 2003). Furthermore, the pig may be regarded as a model of particular interest in the area of dynamics of insulin secretion because peripheral insulin concentrations show very rapid dynamics that facilitate insulin pulse detection (Kjems et al. 2001; Larsen et al. 2002a).

Diabetes in Pigs

Ethical Considerations

A single case of spontaneous diabetes with islet atrophy and degeneration has been reported in the domestic pig, and the etiology of this case was proposed to be infectious (Biester 1925). Due to the extreme rarity of type 1-like diabetes in the pig, models of diabetes must be induced experimentally, either surgically or chemically, as discussed below. However, before inducing a disease experimentally in any animal, the ethical aspects of the procedure should always be carefully considered, and the potential benefits of the project to society must outweigh the costs to the animals. Both the induction itself as well as the long-term diabetic state can give rise to adverse effects. Nevertheless, suffering and lasting harm can be avoided by defining humane endpoints and by keeping the pigs under competent veterinary supervision to ensure monitoring and correction of the health status of each individual animal. With such veterinary care, diabetic pigs can be kept for a long time with good regulation of the diabetic state and only minor adverse effects.

Pancreatectomy has been investigated as a method of inducing diabetes in pigs, and impairment of glucose tolerance has been shown to be related to the extent of pancreatectomy. Lohr and colleagues (1989) reported that 40% pancreatectomy resulted only in mild changes, whereas an 80% lesion resulted in significant hyperglycemia (Lohr et al. 1989). Total pancreatectomy has been reported to result in severe hyperglycemia (Mellert et al. 1991, 1998; Stump et al. 1988; Wilson et al. 1986). Based on the invasive surgery necessary to perform pancreatectomy, this method should be considered only when other methods are not feasible due to the welfare considerations associated with surgery. Another disadvantage is that surgical induction of diabetes includes removal of both exocrine and endocrine (including alpha and delta cells) tissue, which is not characteristic of the disease in humans (Wilson et al. 1986).

Chemical Induction of Diabetes

Streptozotocin (STZ¹) is a 1-methyl-1-nitrosurea linked to position C2 of D-glucose (Herr et al. 1967). The compound is transported into the beta-cells by the GLUT2 receptor (Elsner et al. 2000; Ledoux and Wilson 1984). STZ induces DNA strand breaks and subsequently activates repair mechanisms that result in a reduction in cellular Nicotinamide Adenine Dinucleotide (NAD) and ATP-levels below physiological levels, which leads to cell death (Yamamoto et al. 1981).

Several stable models of diabetes have been established in domestic pigs and minipigs by using pharmacological induction of beta-cell damage with STZ (Barb et al. 1992; Canavan et al. 1997; Gabel et al. 1985; Gerrity et al. 2001; Grussner et al. 1993; Marshall 1979; Marshall et al. 1980, 1975; Mesangeau et al. 2000; Pattou et al. 1994; Wilson

et al. 1986). These models have markedly increased fasting plasma glucose and decreased glucose-stimulated insulin secretion, which resemble the functional aspects of type 1 diabetes.

Relatively low doses of STZ (35-40 mg/kg) have no major effects on glucose tolerance in domestic pigs or minipigs (Gabel et al. 1985; Marshall et al. 1975), whereas a dose of 85 mg/kg has been found to induce diabetes in pigs that was reversible within 2 wk (Gabel et al. 1985). Doses of 100 to 150 mg/kg of STZ have been reported to induce insulin-dependent diabetes in both domestic and minipigs in several studies (Barb et al. 1992; Canavan et al. 1997; Gabel et al. 1985; Grussner et al. 1993; Larsen et al. 2002c; Wilson et al. 1986). One study has reported that 150 mg/kg of STZ failed to induce diabetes in 7- to 8-mo-old Göttingen minipigs, whereas 200 mg/kg resulted in insulin-dependent diabetes (Liu et al. 1998). This variation in response could be due to age differences (in rats, younger animals have been found to be less susceptible to STZ than older animals [Masiello et al. 1975]) or to gender differences (in rats, females have been found to be less susceptible to STZ than males [Ostenson et al. 1989]).

A dosing scheme using two low doses of STZ (60 mg/kg followed by 30 mg/kg 8 days later) has been used for induction of diabetes in Hanford minipigs, resulting in lack of residual insulin secretory capacity (Marshall 1979). The diabetic state induced in the study by Marshall (1979) was stable in four of seven animals, whereas metabolic control improved again over the subsequent 12 mo in three of seven animals. The same approach has recently been used in Yucatan minipigs, utilizing two doses of 55 and 50 mg/kg given 8 days apart. These animals were severely diabetic, and likely even in a catabolic state, because their weight decreased dramatically (Mesangeau et al. 2000). Similarly, in Yorkshire swine, 50 mg/kg of STZ given on 3 consecutive days induced stable, severe diabetes (Gerrity et al. 2001), and in a single Göttingen minipig, islet antibodies have been reported after two doses of 40 mg/kg of STZ with 11 days interval, thus indicating a role of immune processes in this type of model (Rolandsson et al. 2002). However, in most of the reported models of STZ-induced diabetes in pigs, no involvement of inflammatory processes has been described in the development of diabetes—a major difference compared with human type 1 diabetes.

Models of moderate hyperglycemia can also be induced in pigs, but because the dose-response relation between STZ and level of hyperglycemia in the pig is very steep (Larsen et al. 2002c), it is difficult to obtain consistent, moderate degrees of diabetes after STZ. Nicotinamide (NIA¹) can prevent the acute decrease in NAD and ATP levels in beta-cells by inhibiting the DNA repair mechanisms associated with STZ (Masiello et al. 1990; Yamamoto et al. 1981). In agreement with previous reports in rats (Masiello et al. 1998), a combination of NIA and STZ in pigs can result in a more moderate degree of fasting and/or postprandial hyperglycemia with retained residual insulin secretory capacity that is stable for at least 2 mo (Larsen et al. 2002c).

Alloxan (ALX¹) is another compound used specifically to induce beta-cell damage. The mechanism of action is similar to STZ (Yamamoto et al. 1981), and their diabetogenic actions are very similar (Junod et al. 1967; Rerup and Tarding 1969). ALX has also been used for induction of diabetes in Yucatan and Göttingen minipigs; doses around 100 to 200 mg/kg result in severe diabetes (Boullion et al. 2003; Dixon et al. 1999; Otis et al. 2003; Phillips et al. 1980). ALX in a dose of 80 mg/kg has been reported to induce diabetes with moderate hyperglycemia and partial loss of beta-cell mass in Göttingen minipigs (Kjems et al. 2001).

Models of diabetes based on chemical damage of beta-cells cover a wide spectrum of hyperglycemia (Barb et al. 1992; Boullion et al. 2003; Canavan et al. 1997; Gabel et al. 1985; Grussner et al. 1993; Kjems et al. 2001; Larsen et al. 2002c; Otis et al. 2003; Wilson et al. 1986), yet all are characterized by reduced insulin secretion and beta-cell mass, two of the major characteristics of human type 1 diabetes (Anon 1998; WHO 1999). However, another very important component in the development of type 1 diabetes—the formation of autoantibodies (Anon 1998; WHO 1999)—has been reported in one minipig to date (Rolandson et al. 2002). Additional studies to address this issue are warranted to clarify further the usefulness of porcine models of type 1 diabetes.

Because the human leukocyte antigen (HLA¹) complex of the major histocompatibility system (MHC¹), and especially the HLA class II molecules, is an important determinant of the risk of type 1 diabetes in humans (Pociot and McDermott 2002), it is relevant to consider whether the same is true in pigs. However, because spontaneous diabetes is very rare in pigs, it is not possible to evaluate whether variants of the swine equivalent of the HLA complex, the SLA complex, play a role as risk factors for spontaneous type 1 diabetes in the pig. To our knowledge, no studies have to date looked at the involvement in the SLA complex in induced models of diabetes in pigs. Nevertheless, it is indeed possible that increased knowledge about the SLA complex will provide valuable information for evaluating the immunological processes of diabetes in the pig.

Transplantation

Transplantation of islets of Langerhans is, at least potentially, a therapeutic approach to type 1 diabetes. Shapiro and colleagues (2000) have described the feasibility of this approach. Although a major obstacle for this concept is the limited availability of human pancreatic islets, xenotransplantation of islets might be a solution to the problem. The resemblance between the structure of human and porcine insulin and the similarities in metabolism and glucose levels in the two species make the pig a potentially relevant source of islets. Furthermore, the almost unlimited availability of islets from pigs is an obvious, major advantage over other possible species such as nonhuman primates.

Isolation of islets from porcine pancreases poses several technical challenges (Ricordi et al. 1986, 1990) that, to some extent, are due to the very thin peri-insular capsule that surrounds the islets. Because of the diffuse structure of porcine islets, islets are often disrupted during the isolation procedure (Brandhorst et al. 1995; van Deijnen et al. 1992; Ulrichs et al. 1995). Isolation of islets from older pigs (2–3 yr) improves the yield considerably compared with the prospective yield from younger animals (10–12 mo) (Socci et al. 1990; Ulrichs et al. 1995). It is possible to preserve insulin secretion from the islets up to several weeks during culture conditions (Niwa et al. 2001), although others have reported reduced responsiveness after only 1 wk in culture (Nielsen et al. 2002). These discrepancies can most likely be explained by differences in characteristics of donors, the purification procedure, and culture conditions because rate of apoptosis is affected by several factors (Stadlbauer et al. 2003).

Autotransplantation of islets in adult pigs has shown a linear correlation between fasting blood glucose and grafted islet mass. In addition, intraportal siting of islets leads to a longer period of normoglycemia compared with grafting of islets under the kidney capsule, probably due to less vascularization in the latter location (Mellert et al. 1998).

Successful allotransplantation of islets from adult pigs to diabetic recipient pigs has been reported (Mellert et al. 1998; Morsiani et al. 2001). Studies indicate that multiple subtherapeutic doses of islets delivered into the portal vein results in better long-term metabolic control compared with a single administration of the same amount of islets (Morsiani et al. 2001).

Neonatal and fetal islet grafts have been transplanted into human type 1 diabetic subjects, demonstrating the ability of the grafts to survive in the human body (Elliott et al. 2000; Groth et al. 1994, 2000). Recently, neonatal pancreatic cell clusters have been cultured and implanted in microcapsules in immunocompetent diabetic mice, resulting in reversion of hyperglycemia for more than 5 mo (Omer et al. 2003). Similarly, microencapsulated islets from 6- to 8-month-old pigs have been transplanted to pancreatectomized dogs, and successful resultant graft function without immunosuppression has been documented for weeks to months (Kin et al. 2002). A recent study has opened the possibility of subcutaneous transplantation of encapsulated porcine pancreatic endocrine cells in diabetic mice, resulting in normalization of hyperglycemia for up to 2 mo (Wang et al. 2003).

In nonhuman primates, the function of adult pig islets in both nondiabetic and diabetic immunosuppressed animals has been shown to be very short lasting, indicating almost immediate graft destruction in this species (Buhler et al. 2002; Cantarovich et al. 2002). In contrast, a regimen including splenectomy and irradiation prolonged graft survival to more than 2 wk, although it failed to prevent rejection thereafter (Buhler et al. 2002).

Pigs, like most nonhuman mammals, express α -1-3 galactosyltransferase, which is involved in formation of the carbohydrate galactose α (1-3)-galactose. Galactose α (1-3)-

galactose has been detected on fetal porcine islets (McKenzie et al. 1995; Rydberg et al. 1995), but not on adult animal islet cells (McKenzie et al. 1995). Because more than 2% of human immunoglobulin M and immunoglobulin G in the circulation represents α -gal antibody (McMorrow et al. 1997), the difference between the species is of great importance for rejection of porcine xenografts via humoral mechanisms. However, islets from adult pigs are also rejected due to the presence of other xenoantigenes recognized by human antibodies; and several strategies, including transgenic pigs, are being developed to block the humoral and cell-mediated destruction of xenografts (Rayat et al. 1999).

For xenotransplantation in general, an important factor for graft survival is the relation between the HLA and SLA systems. The HLA complex is localized to a specific region on chromosome 6 in humans, whereas in the pig, the SLA genes are localized on chromosome 7 and are interrupted by the centromere (Chardon et al. 1999; Geffrotin et al. 1984). The length of the SLA region is considerably shorter than what has been observed in humans. This difference is important for the comparative study between SLA and HLA regions (Chardon et al. 2001; Genet et al. 2001). As in other mammals, SLA has three major regions containing class I, II, and III genes, and the overall gene order between pigs and humans is conserved although in some regions inversions are observed in the SLA complex (Genet et al. 2001). The SLA complex has several polymorphic amino acid positions that are similar in localization to those in the human HLA complex, whereas other parts of the SLA complex are almost invariable (Chardon et al. 1999).

Between strains of pigs, there is considerable variability in the SLA sequences as in other species (Chardon et al. 1999, 2001), but whether they would match the sequences in the HLA system remains to be determined. MHC inbred miniature pigs with well-defined haplotypes for SLA have been established (Sachs et al. 1976), and parts of the SLA complex of several breeds of pigs have been analyzed (Chardon et al. 1999). However, only a few Göttingen minipigs have been included in published studies (Ando et al. 2003; Omi et al. 1999). For more details on the SLA complex, readers are referred to the review of Chardon and colleagues (1999) as well as to web sites where detailed information on the pig genome can be found (homepage of the Roslin Institute of Edinburgh: <http://www.projects.roslin.ac.uk/pigmap/reports.html> and the National Center for Biotechnology Information homepage (<http://www.ncbi.nlm.nih.gov>)).

One major concern with regard to transplantation of porcine islets to diabetic patients is the risk of infection with both known and new agents. In particular, the risk of infection of human cells by porcine endogenous retroviruses (PERV¹) has caused concern (Patience et al. 1997). Studies in humans who have received grafts from pigs have not shown any evidence for infection with PERV (Elliott et al. 2000; Groth et al. 2000; Heneine et al. 1998; Paradis et al. 1999). To reduce the risk of transferring infective agents

during xenotransplantation from pigs, systematic screening programs have been developed (Bjoersdorff et al. 1995; Kumar et al. 2002).

Diabetic Complications

To our knowledge, no reported studies have looked specifically at diabetic late complications in Göttingen minipigs, although studies in other strains of pigs have been published. Increased oxidative stress resulting in inflammatory responses in the coronary arteries has been described in diabetic pigs, and a combination of diabetes and hyperlipidemia reportedly accelerates atherosclerosis in pigs greatly (Gerrity et al. 2001; Natarajan et al. 2002; Zhang et al. 2003). Within 6 mo after induction of diabetes, intimal proliferations in arteries consisting of mixed parietal microthrombi, irregular endothelium surfaces, and detachment of endothelial cells have been demonstrated in minipigs (Marshall et al. 1980). It has also been reported that in combination with high-fat high-cholesterol feeding, diabetes accelerates atherosclerosis in domestic pigs (Gerrity et al. 2001). Hallmarks of diabetic late complications such as mesangial expansion and nodular changes have been shown in the kidneys of diabetic Hanford minipigs (Marshall et al. 1980). Additionally, cataracts and increasing thickness of capillary basement membranes in retinal capillaries and muscle tissue have been observed in Yucatan and Hanford minipigs with increasing duration of diabetes (Hainsworth et al. 2002; Marshall et al. 1980; Phillips et al. 1980).

Concluding Remarks

Well-characterized animal models of type 1 diabetes are a valuable tool to increase our understanding of the disease in humans. Due to the high degree of similarity to humans, both physiological and pathophysiological, the pig is of particular interest as a large animal model to complement the range of models available in rodents. Based on the well-described genetics and biology and the inherent small size, the Göttingen minipig is particularly suitable for long-term studies.

Because type 1-like diabetes does not develop spontaneously in pigs, it must be induced either surgically or chemically. Pancreatectomy has several disadvantages and should therefore not be the preferred method for induction of diabetes in pigs. Several groups have reported promising models of diabetes in pigs after different degrees of chemical damage to the beta-cells. Especially since the late 1990s, interest in developing models of moderate diabetes in pigs has increased. Chemically induced models are very useful for studying some aspects of type 1 diabetes in humans, such as beta-cell function and mass as well as the effect of pharmacological interventions, but because diabetes must be induced in the pig, many questions relating to development of the disease cannot readily be studied in the cur-

rently available models. Similarly, studies that explore the involvement of inflammatory processes and the genetic resemblance to humans with regard to development of type 1 diabetes would be most relevant to substantiate the use of the Göttingen minipig further as a model in type 1 diabetes research.

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