Comparative Pathogenesis of Autoimmune Diabetes in Humans, NOD Mice, and Canines: Has a Valuable Animal Model of Type 1 Diabetes Been Overlooked?

Allison L. O'Kell,[1] Clive Wasserfall,[2] Brian Catchpole,[3] Lucy J. Davison,[4] Rebecka S. Hess,[5] Jake A. Kushner,[6] and Mark A. Atkinson[2]

Despite decades of research in humans and mouse models of disease, substantial gaps remain in our understanding of pathogenic mechanisms underlying the development of type 1 diabetes. Furthermore, translation of therapies from preclinical efforts capable of delaying or halting β-cell destruction has been limited. Hence, a pressing need exists to identify alternative animal models that reflect human disease. Canine insulin deficiency diabetes is, in some cases, considered to follow autoimmune pathogenesis, similar to NOD mice and humans, characterized by hyperglycemia requiring lifelong exogenous insulin therapy. Also similar to human type 1 diabetes, the canonical canine disorder appears to be increasing in prevalence. Whereas islet architecture in rodents is distinctly different from humans, canine pancreatic endocrine cell distribution is more similar. Differences in breed susceptibility alongside associations with MHC and other canine immune response genes parallel that of different ethnic groups within the human population, a potential benefit over NOD mice. The impact of environment on disease development also favors canine over rodent models. Herein, we consider the potential for canine diabetes to provide valuable insights for human type 1 diabetes in terms of pancreatic histopathology, impairment of β-cell function and mass, islet inflammation (i.e., insulitis), and autoantibodies specific for β-cell antigens.

The incidence of type 1 diabetes (T1D) is increasing worldwide (1), and despite immense research efforts, the inciting cause remains elusive. Animal models, in particular the NOD mouse, are often used to study T1D pathogenesis and have proven quite informative given certain similarities in disease-associated features with humans (2). However, the impact of physiological variances between mice and humans (e.g., immune system components, islet architecture, metabolism), taken together with limited success stories involving preclinical translation of therapies, has caused increasing concerns (3). Thus, a need exists for alternative animal models that may add additional insights to the human disease, with companion animals providing one potential avenue to fill this role. With this Perspective, we consider the current knowledge of naturally occurring canine diabetes and, following comparison with humans and the NOD mouse model of T1D, propose it may serve as an informative model of the human disease.

CLINICAL AND METABOLIC FEATURES

Diabetes mellitus is one of the most common endocrine diseases affecting pet dogs (4). Similar to human T1D (5), the incidence of canine diabetes also appears to be increasing: in the U.S., the prevalence of canine diabetes at veterinary teaching hospitals increased from 19 per 10,000 to 64 per 10,000 cases between 1970 and 1999 (6). Virtually all dogs require insulin therapy at diagnosis (4,7), regardless of the underlying cause. Canine diabetes can be classified into two major categories: insulin deficiency diabetes and insulin resistance diabetes (4). A canine equivalent to human type 2 diabetes does not seem to occur, and although

obesity is associated with insulin resistance, this does not progress to overt diabetes unless other predisposing factors are present (4). A variety of causes of insulin resistance diabetes have been suggested, which primarily involve hormonal antagonism of insulin activity, related to the diestrus period (progesterone associated), exogenous or endogenous excess of glucocorticoids, or the presence of acromegaly (4). Some studies report a female predominance (6,8,9), whereas others have not demonstrated a sex predilection (10,11). This discrepancy may be due to geographic location of the study population as dogs in Europe are more likely to remain sexually intact (and at risk for diestrus diabetes) than dogs in the U.S. There is no strong sex predilection in human T1D, but geographic variation exists, with Finland, Sardinia, and Sweden having the highest incidence of childhood-onset T1D (12). In the NOD mouse, however, a clear association with sex exists, with 60–90% of females and 10–65% of males developing the disorder (13,14).

Most diabetic dogs suffer from insulin deficiency diabetes, with the underlying cause of the pancreatic β-cell loss or destruction most likely a result of an inflammatory process in the exocrine or endocrine tissues and autoimmunity suspected in some cases (4). Pancreatitis may be diagnosed concurrently with diabetes in some cases (15). The role of autoimmunity is currently less clear than in human T1D and the NOD mouse, in which an immune-mediated pathogenesis is well established (14,16). The majority of dogs are middle-aged to older (>5–7 years) at diagnosis (6,8,10,11), although a relatively uncommon juvenile or congenital form of insulin deficiency diabetes has been reported in some breeds (4). This contrasts with the juvenile onset that is more common than adult-onset disease in people (16), though there is an emerging realization that T1D onset occurs more frequently in adults than once believed (17). It also parallels the late (postsexual maturation) onset of disease in the NOD mouse at 10–26 weeks of age (13,14).

Similar to humans, the reference range for normal blood glucose in dogs is 81–118 mg/dL (4.5–6.6 mmol/L) (Table 1). Clinical signs of symptomatic diabetes in all three species are similar (polyuria, polydipsia, polyphagia, and weight loss) (4,13,16). Canine diabetes is classically diagnosed when hyperglycemia (typically >250 mg/dL [13.9 mmol/L]) (18,19) and glucosuria are identified in the presence of clinical signs (Table 1). This is similar to NOD mice, in which a diagnosis is typically made when blood glucose is >250 mg/dL (13.9 mmol/L) on two consecutive readings (Table 1) (20); however, no single diagnostic criterion exists (21). Diabetes in people can be diagnosed using specific criteria for hemoglobin A1C or plasma glucose (16) (Table 1) and, thus, may be more likely to be recognized prior to the onset of symptoms. As glucosuria in dogs does not usually develop until blood glucose is between 180 and 220 mg/dL (10 and 12.2 mmol/L) (7), dogs that may have early or subclinical diabetes are often not identified. Ketoacidosis may be present at diagnosis or may develop

during therapy (4). This is comparable to human T1D in which diabetic ketoacidosis remains a somewhat common feature of clinical presentation (22). The NOD mouse develops ketosis without insulin therapy (13,23,24), but through evaluation for ketoacidosis in the literature, this complication appears rare (24).

Few reports exist describing β-cell function in dogs. In the majority of diabetic dogs, baseline fasting C-peptide was similar to or lower than healthy control canines, whereas the insulin and C-peptide response to glucagon stimulation was blunted, indicating insulin deficiency (25,26). These results parallel findings in human T1D in which there is insulin deficiency, assessed using C-peptide as a marker of β-cell function (27), and lifelong insulin therapy is required at diagnosis (22). Although the NOD mice can survive for several weeks without insulin therapy after diabetes onset (23), insulin therapy markedly prolongs survival (13). In addition to the NOD mouse, several other rodent models are used to study autoimmune diabetes, most notably the BioBreeding (BB) rat (14,23,28). Although an in-depth description of the BB rat is beyond the scope of this Perspective, important characteristics are summarized in Table 1.

PANCREATIC ISLET PATHOLOGY

Islet Architecture
The basic islet characteristics for each of the three species are summarized in Table 2. Canine islets have, on average, 78% β-cells, 11% α-cells, <11% δ-cells (29), and <4% pancreatic polypeptide (PP) cells (30). In comparison, human islets have fewer β-cells and more α-cells, averaging 50% β-cells, 40% α-cells, 10% δ-cells, and rare PP cells (30,31). NOD mice fall somewhere in between, with an average of 60–80% β-cells, 15–20% α-cells, <10% δ-cells, and <1% PP cells in their islets (30). Islet endocrine cell distribution is strikingly different between humans and mice. In mice, β-cells are located in the center of the islet and α- and δ-cells on the periphery (Fig. 1A), whereas in humans, endocrine cells are generally distributed throughout the islets without a distinct central and peripheral zone (Fig. 1B) (30,31). Canine islets do not have a distinct zonal distribution, with β-cells commonly located in the center of the islet but also in the periphery, α-cells located in either the center or periphery, and δ-cells having a random distribution (Fig. 1C) (29,32,33). In humans and dogs, the distribution of cell types varies with the region of the pancreas (33,34). Both species have a PP cell–dominant region that is located in the uncinate process of the head of the pancreas in humans (34) and in the right limb of the pancreas in dogs, which is analogous to the head of the human pancreas (33).

Pancreatic Histopathology in T1D
The histopathological characteristics reported in the pancreata of diabetic dogs have been variable. Historically, studies reported a slight to marked decrease in islet number and mild to marked reduction in β-cells with normal to proportionately decreased numbers of α- and

Table 1—Characteristics of T1D in humans, dogs, NOD mice, and BB rats

	Human	Dog*	NOD mouse	BB rat
Genetic susceptibility	+	+	+	+
Presence of autoantibodies	+	+/−	+	+
Insulitis	+	+/−	+	+
Disease heterogeneity	+	+	−	−
Inbred/outbred	Outbred	Outbred/inbred	Inbred	Inbred
Sex predilection	−	−	+ (female)	−
Diagnosis	a) Hemoglobin A1C ≥6.5% (48 mmol/mol) b) Fasting plasma glucose >126 mg/dL (7.0 mmol/L) c) 2-h plasma glucose ≥200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test d) Random plasma glucose ≥200 mg/dL (11.1 mmol/L) with classic symptoms Confirm diagnosis by a, b, or c with repeat testing	Presence of hyperglycemia (typically >250 mg/dL [13.9 mmol/L]), glucosuria, and clinical signs (normal fasting reference range: 81–118 mg/dL [4.5–6.6 mmol/L])**	Blood glucose >250 mg/dL (13.9 mmol/L) on two consecutive readings	Glucosuria (4+) in combination with blood glucose >250 mg/dL (13.9 mmol/L)
Ketoacidosis common	+	+	?	+
Insulin required at onset	+	+	+	+

*This table does not include congenital β-cell deficiency or insulin resistance diabetes (e.g., gestational/ progesterone or glucocorticoid induced). **At the time of this writing, there is no published standard range for canines. The range reported here is from the Endocrinology Section, Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI.

Table 2—Islet characteristics in humans, dogs, and NOD mice

	α-Cells	β-Cells	δ-Cells	PP cells
Human	40%; core + periphery	50%; core + periphery	10%; core + periphery	Core + periphery; rare except in uncinate process of head
Dog	11%; core + periphery	78%; core > periphery	<11%; core + periphery	<4%; core + periphery; predominantly right limb/head lobe
Mouse	15–20%; periphery	60–80%; core	<10%; periphery	<1% periphery

Adapted with permission from Steiner et al. (30). Information generated from refs. 29–31,34.

δ-cells compared with healthy control animals (35,36). Remaining β-cells were described as swollen, vacuolated, and degranulated (35). Inflammatory mononuclear islet infiltrates (described as insulitis) were observed in 6 of 13 dogs (36). These studies included female dogs with no information on breed or neutering status, and it is possible that some of these cases represented diestrus-related diabetes, which is considered to have a different pathogenesis than the adult-onset insulin deficiency diabetes that is the focus of this discussion. Ahlgren et al. (37) reported similar findings of varying numbers of β-cells in the islets of diabetic dogs, as well as enlarged and vacuolated islets but no insulitis. The majority of these dogs were female and neutering status was again unknown, so some of these dogs may also have had diestrus-related diabetes. A more recent study including predominantly males and neutered females reported small islets with few β-cells (Fig. 2A) (29). The composition of the islets in diabetic dogs (30% β-cells, 40% α-cells, and 30% δ-cells and PP cells) were markedly different than in controls (78% β-cells, 11% α-cells, <11% δ-cells (29), and <4% PP cells) (30). Additionally, diabetic islets were poorly defined compared with controls, and there was no evidence of lymphocyte infiltration except for in a single young puppy (29). Insulitis in a diabetic puppy has been reported previously but appears to be an uncommon finding in juvenile dogs (Fig. 2B) (38). Dogs with long-standing disease were essentially devoid of β-cells (29). Importantly, most pathological studies include few dogs at symptomatic onset; even then, it is plausible that a period of active insulitis early in disease (even prior to clinical diagnosis) may no longer be detectable.

The findings thus far in dogs contrast with pathological findings in human patients with T1D and the NOD mouse, in which insulitis is a defining feature. Insulitis is identified by the presence of a lymphocytic infiltrate affecting the periphery (peri-insulitis) or the interior of the islet (intra-insulitis) (39). A consensus definition in people requires ≥15 CD45$^+$ cells/islets in a minimum of 3 islets for diagnosis of insulitis combined with the presence of β-cell devoid islets (pseudo-atrophic islets) (Fig. 2C and D) (39). Usually <10% of all islets, predominantly insulin-positive islets (i.e., those with β-cells), are affected (39). The frequency of insulitis is reportedly higher in young patients with disease duration less than 1 month, with one review evaluating the collective literature suggesting 73% of those <14 years old having insulitis compared with 29% of those aged 15–40 years (40). Other studies, however, noted a higher prevalence of insulitis with shorter disease duration, but no correlation with age (41). With respect to the natural history of β-cell loss, although T1D patients have reduced β-cell area, residual β-cells exist for decades after the symptomatic onset of disease (42), which contrasts to the current findings in canine diabetes.

Insulitis is a prominent feature of diabetes in the NOD mouse and pathological characteristics have been far more widely and thoroughly studied than in humans or dogs because of the availability of pancreatic samples. Classically defined mononuclear infiltration begins at approximately 4 weeks of age, prior to the onset of diabetes (14,43); however, recent efforts have noted that cells of the innate immune system may enter as early as 2 weeks of age (44). Initially, the inflammatory infiltrate is predominantly located in the peri-islet region and begins focally (14,43). This focal mononuclear accumulation (predominantly lymphocytes) progresses to surround the islet and forms a "tertiary lymphoid organ" structure (43). As the mice age, intraislet lymphocyte invasion becomes prominent, along with a marked loss of β-cells by approximately 12 weeks of age (14). At the time of clinical onset of disease (i.e., 10–26 weeks of age), β-cell mass is severely decreased, pseudoatrophic islets are present, and virtually all islets are affected by insulitis (Fig. 2E and F) (43). Islet diameter decreases as duration of disease increases, along with the magnitude of lymphocytic infiltration; similar to humans, islets without β-cells often lack insulitis (45). Mice that do not develop diabetes also develop insulitis, but with high variability in severity (45).

Concurrent exocrine pancreatic changes have been found in conjunction with diabetes in all three species. There is somewhat conflicting evidence of a role for exocrine pancreatic disease as a predisposing factor for the β-cell loss seen in canine diabetes. Some histopathological studies have reported no or minimal/very focal areas of pancreatitis (29,37), whereas in others, 20–33% of dogs demonstrated acute or chronic pancreatitis with variable fibrosis (35,36). In the latter studies, it is unclear whether these lesions were severe or diffuse enough in all cases to cause diabetes (35,36). In dogs with histopathologically confirmed chronic pancreatitis, 5 of 14 (36%) had concurrent diabetes, of which 2 also had exocrine pancreatic insufficiency (15). β-Cell function was decreased in 5 of 6 (83%) nondiabetic dogs with chronic pancreatitis based
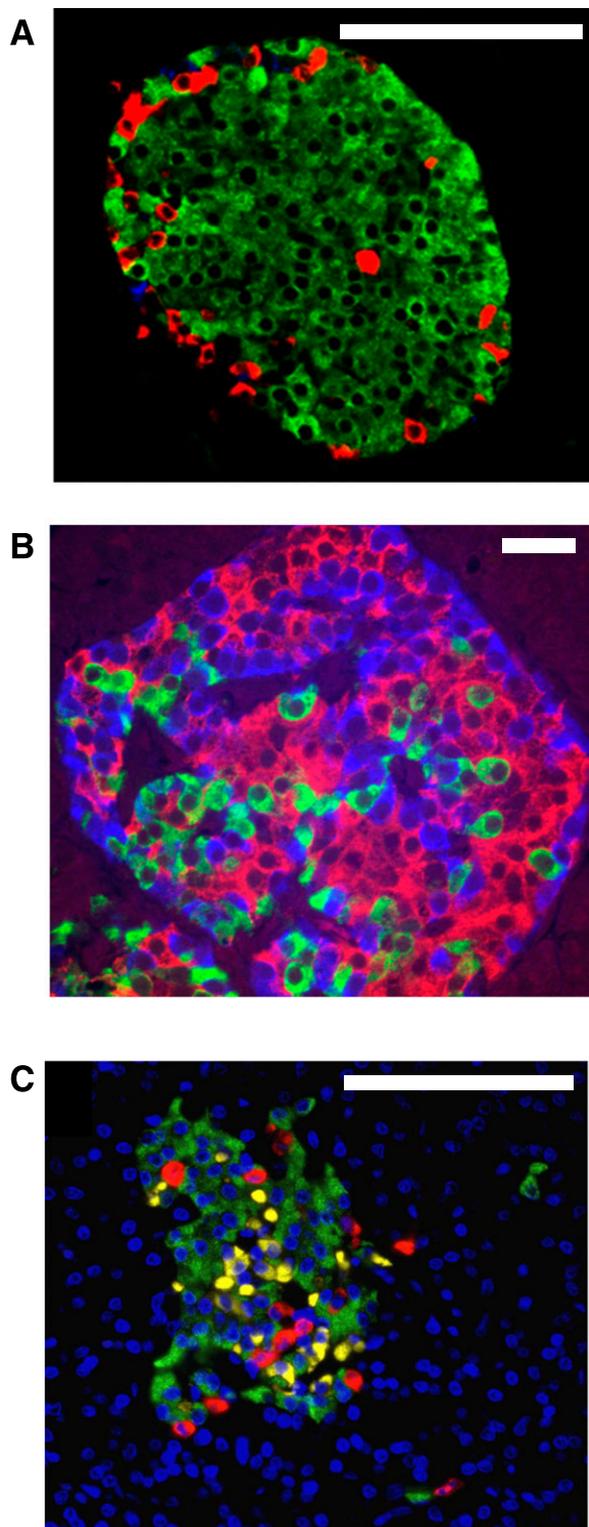
Figure 1—Normal islet architecture. Representative immunofluorescent images show islet composition for mice (*A*), humans (*B*), and canines (*C*) without diabetes. *A*: Insulin, green; glucagon, red; somatostatin, blue. Adapted with permission from Novikova et al. (45). *B*: Insulin, red; glucagon, blue; somatostatin, green. Previously unpublished image kindly provided by Dr. Martha Campbell-Thompson, of University of Florida. *C*: Insulin, green; glucagon, red; somatostatin, yellow. Adapted with permission from Shields et al. (29). Scale bars = 100 μm (*A* and *C*) and 20 μm (*B*).

on glucagon stimulation testing (46). Thus, the evidence supports pancreatitis as a cause or contributing factor in diabetes in some dogs, although the overall prevalence and significance of exocrine inflammation as a cause of β-cell destruction is unclear. In people, inflammatory infiltrates (e.g., lymphocytes and dendritic cells) can be found in the exocrine pancreas in patients with and without insulin-containing islets (47). The NOD mouse has the most well defined and consistent changes described. Prior to the onset of diabetes, exocrine pancreas is affected by mononuclear infiltration including infiltration of exocrine pancreatic venules, periductular infiltrates, and focal inflammation in connective tissue (focal pancreatitis) (48). In contrast to both dogs and people, however, at the time of disease onset, extraislet inflammation is absent (48).

In NOD mice, a prominent distinguishing feature from human and canine diabetes is the concurrent increase in lymphocytes in peripheral lymphoid organs, blood, other endocrine tissues, and glands (14,43), including lacrimal and salivary glands (i.e., a model of Sjögren syndrome) (49), a disorder not associated with T1D in humans or dogs.

GENETICS

The genetic basis for canine diabetes is not fully established and, similar to both humans and the NOD mouse, has proven to be somewhat complex. Compared with the NOD mouse, dogs have much higher genetic diversity and are divided into breeds with variable predisposition to diseases (50). There are remarkable breed differences in susceptibility to canine diabetes, suggesting an underlying genetic component (4) (Table 3). Dog leukocyte antigen (DLA) genes coding for MHC class II have been associated with both disease susceptibility and protection (51). Three different DLA haplotypes involving the DRB1-DQA1-DQB1 loci have been shown to confer increased risk among a variety of dog breeds, along with one protective haplotype (51). It is unclear at this time, however, whether these DLA haplotypes are markers of a susceptible or resistant breed rather than a susceptible or resistant individual (51). The possibility exists that genetic risk is fixed within a breed, and thus case-control studies within a breed may not be the best means to study genetic association. To date, associations between breed/genetics and autoimmune markers (autoantibodies, insulitis) have not been reported in diabetic dogs. In humans, genes coding for human leukocyte antigen (HLA, analogous to DLA) are the most established genetic risk factor for T1D (52). Similar to dogs, the DRB1-DQA1-DQB1 loci in the HLA class II region has the strongest association, with several different haplotypes conferring susceptibility or protection (reviewed in Noble and Valdes [52]). There is also evidence that HLA class I and III loci contribute to disease susceptibility, although strong linkage disequilibrium in the HLA region leads to challenges in analyzing these alleles individually (53,54). In the NOD mouse, MHC alleles ($H2^{g7}$) similarly confer the strongest susceptibility, and this region (termed *Idd1*) contains MHC class I and II genes (2,55). To the authors' knowledge,
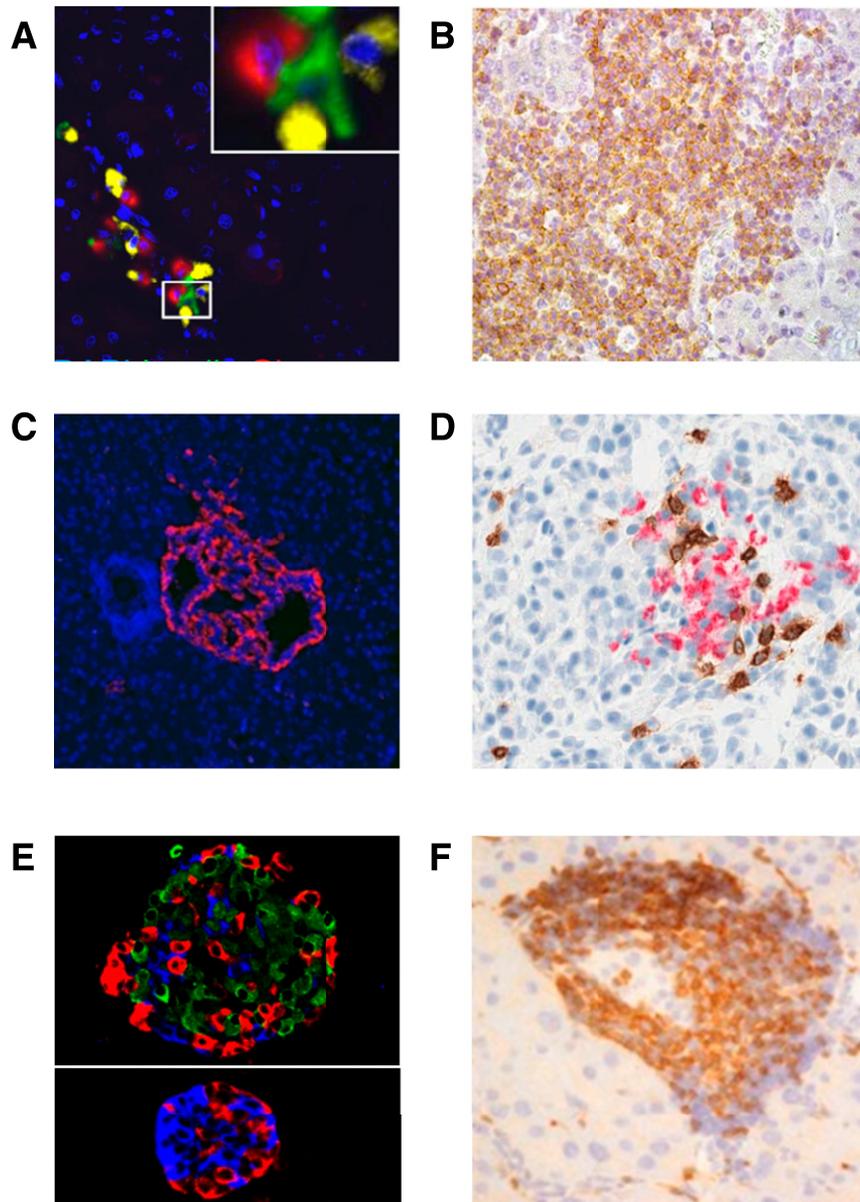
Figure 2—Islet architecture in diabetes. Representative immunofluorescent images (A, C, E) show islet composition, and immunohistochemical staining (B, D, F) shows insulitis for canines with insulin deficiency diabetes (A and B), humans with T1D (C and D), and NOD mice with diabetes (E and F). Panel B represents the rare finding of insulitis in a juvenile diabetic dog. A: Insulin, green; glucagon, red; somatostatin, yellow. Adapted with permission from Shields et al. (29). B: CD3, brown. Adapted with permission from Jouvion et al. (38). C: Insulin, green; glucagon, red. Unpublished image kindly provided by Dr. Peter In't Veld, of Brussels Free University (previously available at http://www.diapedia.org/type-1-diabetes-mellitus/2104434133/long-term-changes). D: CD3, brown; glucagon, red. Previously unpublished image acquired from the Network for Pancreatic Organ Donors with Diabetes (nPOD) online Aperio viewing platform, which is freely available with log-in credentials (nPOD 6396). E: Insulin, green; glucagon, red; somatostatin, blue. Progressive loss of insulin staining 1 week (top) and 3 weeks (bottom) after diabetes onset. Adapted with permission from Novikova et al. (45). F: CD3, brown. Adapted with permission from Koulmanda et al. (84).

there have been no published investigations into MHC class I or III in dogs.

More than 60 other loci have been associated with T1D risk in humans (56), highlighting the complexity of genetic determinants of the disease. Those with the highest odds ratios include *INS, CTLA4, IL2RA, PTPN22,* and *IFIH1* (57). These genes, aside from insulin (an apparent key autoantigen), are associated with the immune response and are also implicated in other autoimmune diseases (57). In the

NOD mouse, a number of other candidate genes have also been identified, including genes encoding IL-2, IL-21, CTLA-4, T-cell receptor, CD30, TNFR2, and β2-microglobulin (reviewed in refs. 2,55). Although only some of these genes have orthologs in human T1D, identification of these genes has improved understanding of the immunopathogenesis of the disease (2). Efforts to identify other candidate genes in dogs, similar to those contributing to risk in humans, have not found conclusive associations, although there may be

Table 3—Reported susceptible and protected dog breeds

Susceptible breeds	Protected breeds
Samoyed	Golden Retriever
Australian Terrier	Boxer
Tibetan Terrier	German Shepherd
Cairn Terrier	German Shorthaired Pointer
Miniature Schnauzer	Springer Spaniel*
Standard Schnauzer	Airedale Terrier
Miniature Poodle	American Pit Bull Terrier
Toy Poodle	Pekingese
Yorkshire Terrier	Collie
Pug	Shetland Sheepdog
Fox Terrier	Bulldog
Keeshond	Great Dane
Border Terrier	Cocker Spaniel*
Bichon Frise*	English Pointer
Border Collie	Norwegian Elkhound
Finnish Spitz	Old English Sheepdog
Siberian Husky	Brittany Spaniel
	Shih Tzu
	Boston Terrier
	Irish Setter
	Doberman Pinscher*
	Dalmatian
	Basset Hound
	Labrador Retriever*
	English Setter
	Beagle

As determined from refs. 4,6,85. Most studies were harmonious in reporting; those with variance are noted with *.

breed-specific genetic factors that are masked when assessing the diabetic dog population as a whole. An analysis of 18 genes associated with monogenic diabetes in humans did not find consistent associations in a large cohort of dogs (58). A large single nucleotide polymorphism (SNP)-based analysis of the human candidate genes *PTPN22, IL10, IL12B, IL6, IL4, RANTES, IFNG, INS, IL1A, TNFA,* and *IGF2* found 24 SNPs linked to susceptibility and 13 SNPs that were protective in dogs (59). However, the findings were variable comparing breeds, with some SNPs associated with increased susceptibility in one breed but protective in another (59). A similar cohort of dogs was further assessed for association of diabetes risk with SNPs related to genes encoding IFN-γ, IL-10, IL-12β, IL-4, and CTLA-4, and several susceptibility and protective associations were found in a variety of breeds (60,61). These findings are intriguing given the involvement of these genes in the immune response and similarities to observations in human T1D; however, the significance of any functional effects of these polymorphisms is unknown at this time. The investigators cautioned that these results require replication and should be interpreted with caution until further investigation into the associated genes can occur with focus on genetic factors in individual breeds (59–61).

IMMUNOPATHOGENESIS

The role of autoimmunity in the pathogenesis of diabetes in dogs has received far less study than in humans and mice, and although evidence for autoimmunity is present in multiple studies, the results to date are somewhat inconsistent. Evaluation of humoral immunity has primarily focused on circulating autoantibodies that have been documented in humans with T1D. In untreated diabetic dogs, circulating islet cell autoantibodies (ICA) were not detected using either frozen human or canine pancreas (37). Using purified islets from rat insulinoma as an antigen, approximately 12 of 23 (52%) untreated diabetic dogs demonstrated serum anti–β-cell antibodies detected via immunofluorescence (62). This may be analogous to humans who are positive for ICA yet negative for the other autoantibodies (63). Insulin autoantibodies have been detected in 3 of 109 (3%) (64) and 5 of 40 (12.5%) (65) untreated diabetic dogs. Proinsulin autoantibodies were found in 8 of 15 (53%) untreated and 6 of 15 (37.5%) insulin-treated diabetic dogs, but also in 3 of 15 (20%) of control dogs (66). Currently, it is unclear whether the latter is an incidental finding or a possible predictor of future diabetes development. In newly diagnosed, untreated diabetic dogs, autoantibodies against canine GAD65 or IA-2 were found in 4 of 30 (13%) and 3 of 30 (10%), respectively (67), in a population selected for those suspected as having insulin deficiency diabetes. However, in another study, only 1 of 122 (0.8%) treated diabetic dogs had GAD antibodies (37), although this population of dogs likely included a proportion that were affected with insulin resistance diabetes, and blood samples were obtained at variable (sometimes substantial) periods of time after diagnosis. Using human GAD, IA-2, and ZnT8 antibody assays in a recent report, none of 15 diabetic dogs had evidence of autoantibodies, but 3 of 15 control dogs were GAD antibody positive (68). These findings contrast with humans, in which autoantibodies to at least one of the major autoantigens (insulin, GAD65, IA-2, ZnT8) are present in >90% of patients at diagnosis (22,69,70). A number of additional autoantigens have been reported but with much lower sensitivity (69). These autoantibodies are often present prior to the symptomatic onset of disease and can be used to identify at-risk patients (70–72). In the NOD mouse, only insulin autoantibodies have been documented reliably, despite findings of insulin, GAD, and IA-2 as target autoantigens (73). Insulin is likely an early autoantigen in the NOD mouse as in humans (2), but whether the same holds true for dogs is currently less well defined. Autoantibodies themselves are not thought to be directly involved in disease pathogenesis and are more likely a biomarker of β-cell autoimmunity in humans (69). Similarly, NOD transgenic mice with B lymphocytes that cannot secrete antibodies still develop diabetes (74). B lymphocytes do heighten the immune response toward β-cells in NOD

mice (55), and research suggests that B lymphocytes may play a role in human T1D, likely through antigen presentation (75). To date, no studies have specifically investigated B lymphocytes in canine diabetes.

Other indirect evidence of autoimmunity in dogs was documented using mouse islets exposed to serum from diabetic dogs in vitro; the serum contained complement-fixing ICA that caused decreased stimulated insulin release and lysis of the islet cells (76). None of the minor autoantibodies in humans have been tested in dogs, and screening of an array of autoantigens may aid in picking out canine-unique or -specific autoantibodies. Given that autoantibodies have been detected in some diabetic dogs indicating a component of autoimmunity in a proportion of patients, this area is worth pursuing with additional studies and novel methodologies that may lead to more canine-specific indicators of autoimmunity in this disease.

Cellular immunity has had limited study in diabetic dogs. Using mouse islets in vitro, canine mononuclear cells caused increased basal insulin release and decreased stimulated insulin release, suggesting possible β-cell damage and functional impairment (76). Peripheral blood T-cell proinflammatory responses to insulin were found in two of four diabetic dogs, quantified by IFN-γ production (68). Although two of four control dogs also showed a response, preactivation of T cells was suggested as a cause of potential false-positive results (68). The lack of investigation in this area of canine diabetes is an important knowledge gap when comparing diabetes pathogenesis among species. In humans with T1D, β-cell destruction is largely considered to be mediated by cellular immunity, although direct evidence of this in humans is somewhat limited due to difficulties in studying these mechanisms in living subjects (77). Indirect evidence for the cellular immune response includes the dominance of T lymphocytes, particularly CD8^{+} (cytotoxic) T cells, within insulitis, and observations that therapies modulating T-cell frequency and function (e.g., regulatory T cells and T effector memory cells) possess the ability to preserve C-peptide production in those with recent-onset disease (78,79). In dogs, it is unclear, based on limited documentation of insulitis (36), whether T cells infiltrate the canine islets, and immunomodulatory therapy has not been reported. Additional evidence of cellular immune dysfunction in human T1D includes β-cell hyperexpression of MHC class I, which may increase their susceptibility to killing by cytotoxic T cells (78), and identification of peripheral T cells having reactivities with known β-cell molecules (80). β-Cell destruction is also predominantly mediated by autoreactive T cells in the NOD mouse (14), and the mechanisms have been far better elucidated. Similar to humans, upregulation of MHC class I on NOD β-cells increases their susceptibility to T-cell–mediated killing (2). Additionally, the MHC class II haplotype found in NOD mice is involved in the lack of immunological tolerance that is a feature of this disease (2). Multiple immunomodulatory methods are known to prevent or reverse diabetes in this species (3).

The innate immune system also plays an important role in diabetes in the NOD mouse, with macrophages identified as early infiltrators in islets that secrete cytokines that are toxic to β-cells and recruit dendritic cells (2). These dendritic cells have impaired maturation and tolerogenic function (55). Natural killer (NK) cells are present in NOD mouse insulitis lesions (2) and are reduced in number and function within peripheral blood (2,55), but the role of NK cells in potentiating or protecting against disease is unclear (81). In humans, NK cells are present in insulitis in addition to T and B lymphocytes, and a small subset of patients has NK cell–dominated insulitis (81). The role of the innate immune system in diabetes has received little attention in dogs. Diabetic dogs have higher serum CXCL8 and MCP-1 (82) and leukocytes that produce more proinflammatory cytokines in response to stimulation with lipopolysaccharide, lipotechoic acid, and peptidoglycan than control dogs (83). It is unknown whether these changes represent a cause or consequence of the disease, but they are suggestive of an inflammatory state.

CONCLUSIONS AND FUTURE DIRECTIONS

The limited success in identifying therapeutic strategies for disease prevention and reversal in T1D highlight the need for alternative animal models for comparative and translational research. Dogs with naturally occurring diabetes have the potential to fill this role, based on similarities to humans in metabolic characteristics, genetics, therapeutic needs, and suspected autoimmunity as components of their disease. In particular, studying β-cell biology in defined dog breeds that are highly susceptible or resistant to developing diabetes might provide important insights into pathways and mechanisms that might be exploited therapeutically. It is plausible that each species discussed herein resides on a continuum with respect to the severity and prevalence of autoimmunity, with NOD mice at one end of the spectrum (strongest autoimmune component), dogs at the opposite end (weakest autoimmune component), and humans somewhere in between.

Despite similarities to human disease, canine diabetes remains a relatively unexplored area of research, particularly with respect to the contributions of autoimmunity and genetics and more basic investigations into disease phenotype and metabolic characteristics. Complicating these studies also is the apparent heterogeneity of the diabetic dog population with multiple possible etiologies of β-cell destruction, including exocrine pancreatic disease. Additionally, concurrent diseases causing insulin resistance (e.g., hyperadrenocorticism) may cause or contribute to the disease in some cases, although a canine equivalent to human type 2 diabetes does not seem to occur. We suspect that autoimmunity is a component of disease in a proportion (or possibly, certain breeds) of diabetic dogs. Given that extensive islet destruction is already present at symptomatic onset of disease, earlier disease detection may be necessary in order to identify serological markers of autoimmunity, insulitis, or β-cell loss. Future studies in this area should be

performed using community diabetic dogs, rather than breeding diabetic dogs for research colonies, due to both ethical considerations as well as the potential benefits of the dogs living in a similar environment to their human counterparts. An exploration of canine diabetes as an alternative disease model represents a logical next step in the quest for meaningful progress in the diabetes field.

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