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Case Study

Spontaneous Exocrine Pancreas Hypoplasia in Specific Pathogen-free C3HeB/FeJ and 101/H Mouse Pups Causes Steatorrhea and Runting

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Under specific pathogen-free conditions, 1.3% to 1.8% of litters born in our inbred 101/H and C3HeB/FeJ mouse colonies had pups with steatorrhea and runting. Clinically affected male and female pups were first identified when they were from 14 to 25 d old. Unaffected littermates were healthy and were weaned successfully. Postmortem findings in 8 clinically affected mice included a small, poorly differentiated exocrine pancreas comprising cytokeratin-negative duct-like structures but lacking recognizable acinar cells with their normal carboxypeptidase B-positive zymogen granules. Endocrine pancreas islets were unremarkable and contained insulin-positive β cells and glucagon-positive α cells. There was mild inflammation of the hindgut but no evidence of intestinal pathogens or marked inflammation or necrosis of pancreas, either alone or as part of a multisystemic inflammatory condition. Sera from pups in 4 affected litters did not contain antibodies to reovirus 3, mouse coronavirus, rotavirus, or mouse adenovirus 2. Furthermore, 4 sets of parental mice and sentinel mice from the facility were negative for 13 viruses, bacteria, and parasites. C3HeB/FeJ and 101/H inbred strains may be genetically predisposed because the steatorrhea and runting was absent in 13 other mouse strains and subspecies bred in the specific pathogen-free facility. This condition resembles exocrine pancreas hypoplasia, but the inheritance is complex. A wider implication is that runting coupled with steatorrhea are phenotypic criteria to suspect pancreatic disease that could be used in the context of a mouse *N*-ethyl-*N*-nitrosourea-mutagenesis program to identify potential mutants with defects in pancreas development.

Abbreviations: FELASA, Federation of European Laboratory Animal Science Associations; SPF, specific pathogen free; TMEV, Theiler murine encephalomyelitis virus

Over a 29-mo-period, SPF colonies of C3HeB/FeJ and 101/H inbred mice in our facility had occasional litters containing runted pups with steatorrhea. From an infection control perspective, this was of concern because steatorrhea can be a clinical feature of infectious disease of the pancreas and gastrointestinal tract, with differential diagnoses including reovirus 3, mouse coronavirus (mouse hepatitis virus), rotavirus, and *Salmonella* infections.[19] However, throughout this period, the sentinel screening program showed stocks remained free of agents recommended by the Federation of European Laboratory Animal Science Associations (FELASA) for monitoring.[18] Because this clinical phenotype was not seen in 13 other strains and subspecies of mice in the same SPF unit, runting with steatorrhea in C3HeB/FeJ and 101/H mice might be strain-specific and attributable to inherited exocrine pancreatic hypoplasia similar to that described in calves and German shepherd dogs.[13] The genetics of pancreas development in the mouse is an area of intense study, in which transforming growth factor β, Hedgehog, and Notch signaling pathways have been identified as being of particular importance.[12,14] This case study describes a pancreatic disorder in inbred C3HeB/FeJ and 101/H strains that resembles exocrine pancreas hypoplasia, but the inheritance appears complex. As far as we are aware, spontaneous exocrine pancreas hypoplasia has not been reported previously in mice. Runting coupled with steatorrhea are criteria to suspect pancreatic disease. SPF mice generated in an *N*-ethyl-*N*-nitrosourea-mutagenesis program that had these clinical signs may represent potential mouse mutants with defects in pancreas development.

Case Studies

Mice. Inbred C3HeB/FeJ and 101/H strains were rederived by embryo transfer into SPF CD1 female recipients (Charles River Laboratories UK, Margate, UK) that were free of the following FELASA-listed agents: ectromelia, lymphocytic choriomeningitis virus, minute virus of mice, mouse adenovirus types 1 (FL) and 2 (K87), mouse cytomegalovirus, mouse hepatitis virus, mouse parvovirus, mouse rotavirus, pneumonia virus of mice, reovirus 3, Sendai virus, Theiler murine encephalomyelitis virus (TMEV), *Citrobacter rodentium*, *Clostridium piliforme*, *Corynebacterium kutscheri*, *Helicobacter* spp, *Mycoplasma* spp, *Pasteurella* spp, *Salmonella* spp, *Streptobacillus moniliformis*, streptococci β-hemolytic (not group D), *Streptococcus pneumoniae*, endoparasites, and ectoparasites. The CD1 embryo recipients were positive for *Entamoeba muris*, but we consider these organisms to be nonpatho-

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genic commensals. C3HeB/FeJ embryos were obtained from the Institute of Experimental Genetics (National Research Center for Environment and Health, Neuherberg, Germany); 101/H embryos were cryopreserved in 1992 from an inbred line maintained at Medical Research Council Harwell (Didcot, Oxfordshire, UK) for more than 50 y.

The humane care and use of C3HeB/FeJ and 101/H inbred strains as breeding stock in the Mary Lyon Centre is under the Home Office Certificate of Designation 30/4303. The rederivation of mice by embryo transfer is conducted under the Home Office Project License 30/1704, both of which are in accordance with the relevant UK legislation, the Animals (Scientific Procedures) Act of 1986.

Husbandry. To establish the SPF colonies, the mice were rederived into isolators in a separate quarantine facility. Two separate groups of CD1 embryo-recipient dams were screened as a precaution before moving their offspring into the barrier facility. The first 4 dams were screened when their pups were weaned, and the second 4 were screened 3 wk later. After 2 clear rounds of health screening, offspring were transferred into the main Mary Lyon Centre. This mouse-only facility has been open since June 2004. Staff in the production ward wet-shower into the facility and change their oversuits when entering the ward. Mice are housed on grade 6 sawdust bedding (Datesand Ltd, Manchester, UK) in individually ventilated racks (Techniplast UK Ltd, Kettering, UK) on a 12:12-h light:dark cycle (light phase, 0700 to 1900) at 19 to 23 °C and 45% to 65% relative humidity. All cages, wire racks, bedding, and water bottles and caps are sterilized by autoclaving before use in the wards. Sterilized water bottles are filled with chlorinated (9 to 13 parts per million) reverse osmosis-treated water. Water bottles are changed once a week; quality control tests show that after this period, chlorine concentration remains greater than 5 parts per million. Mice were fed irradiated chow (Rat and Mouse No. 3 Breeding Diet, Special Diets Services, Witham, Essex, UK). After a check for external damage, the diet packs are brought into the barrier after external sterilization treatment with vaporized hydrogen peroxide. Food and water was provided ad libitum.

The C3HeB/FeJ and 101/H colonies have been in continuous production since June 2004. The C3HeB/FeJ and 101/H colonies were each maintained by same litter sibling matings, using 1 male and 1 female (duo matings) or 1 male and 2 female (trio matings) mice. To increase production periodically in the 101/H colony to meet demands, some matings were between sibs from different litters from the same mating. Each mating was set up and allowed to produce a maximum of 7 litters. Litters usually were weaned at 21 d, but if mice were small, they were kept with the mother for as long as 25 d. For each mating, breeding records are kept regarding the sequence of litters produced; number and gender of pups born; and number of pups found dead (removed at daily cage checks), those missing at weaning, and those weaned. Husbandry manipulations are performed in a class II biologic safety cabinet. To reduce the possibility of cross-infection between colonies, cage changing stations were surface sterilized with 70% ethanol between colonies, and the animal technicians changed their gloves. At the end of a session, the cage station was sterilized with 2% Trigene spray (Medichem International Ltd, Sevenoaks UK). The C3HeB/FeJ and 101/H colonies are on different racks and have their own sentinel cages.

Microbiologic screening. Sentinel screening in the inbred strains in the production ward exceed those prescribed in the FELASA guidelines.[18] Screening occurs at 6- or 8-wk intervals rather than the 12-wk intervals recommended in the guidelines and includes the complete panel of agents rather than the recommended 3 quarterly partial screens followed by a full annual screen. Depending on their availability, either CBA mice or CD1 mice were used as sentinels. Cages of 4 male or 4 female mice were set up at weaning and challenged with a 12.5-ml scoop of dirty bedding collected from each of 8 cages every week, thus screening the 56-cage rack in a 7-wk rotation. In this system 1 mouse is sent from each cage each month, so that they tested when they are 8 to 24 wk old and have been exposed to dirty bedding challenge for 5 to 21 wk. There are 40 sentinel cages in the production ward.

Sera from 4 of the litters with affected pups were tested for specific antibodies to pathogens, and 4 sets of parental mice were screened for the full list of FELASA agents. Live mice were submitted to an outside laboratory (Harlan UK Ltd Technical Services Department, Loughborough, UK) for microbiologic testing, according to FELASA recommendations.[18] Each mouse was examined for obvious clinical and behavioral abnormalities and then was euthanized by use of an increasing concentration of CO_2; blood was withdrawn by cardiac puncture. After euthanasia, the mice were examined at necropsy for gross lesions of tissues and organs. Serum was separated and diluted 1:5 in sterile saline. The following agents were tested by enzyme-linked immunosorbent assay: ectromelia, lymphocytic choriomeningitis virus, minute virus of mice, mouse adenovirus types 1 and 2, mouse cytomegalovirus, mouse hepatitis virus, mouse parvovirus, mouse rotavirus, pneumonia virus of mice, reovirus 3, Sendai virus, Theiler murine encephalomyelitis virus, *Clostridium piliforme*, and *Mycoplasma* spp. Tests were carried out using commercially available reagents (Churchill Applied Biotechnology, Huntingdon, UK; EVL, Woerden, The Netherlands). Bacteriologic examination followed FELASA recommendations.[18] Bacterial samples from the nasopharynx were cultured on Columbia blood agar, MacConkey agar, colistin–nalidixic acid agar; samples from the large intestine were cultured on Columbia blood agar, MacConkey agar, colistin–nalidixic acid agar, and selenite broth. All cultures were incubated aerobically at 37 °C, and identifications of bacterial isolates were made by use of API identification strips (BioMerieux, Basingstoke, UK). Fecal pellets were tested by polymerase chain reaction assay for the presence of *Helicobacter* spp.[9,20,21] Mice were tested for ectoparasites by microscopic examination of the pelage involving removal of pelage into a culture dish, cooling to room temperature, and microscopic examination of the culture dish contents. Mice were tested for endoparasites by microscopic examination of wet preparations of contents of the small and large intestines for protozoa, helminths, and their eggs or cysts. Suspensions from the large intestine underwent microscopic examination against a dark background for testing for nematode worms.

Pathology. Clinically affected mice were culled humanely in light of welfare concerns. In 3 litters, 1 or 2 nonaffected siblings also were culled by overdose of intraperitoneal barbiturate (Pentoject, Animalcare, York, UK), and blood for serum was collected by cardiac puncture. Pups were weighed and then necropsied. The intestine and mesentery with pancreas and mesenteric lymph node were fixed en bloc in Bouin solution for 24 h. Liver, kidneys, adrenals, thymus, spleen, heart, lungs, trachea, skin, esophagus, stomach, and brain and, in some cases, ovary and uterus, knee, sternum, nasal cavity, middle ears, skeletal muscle, tongue, and eye were fixed in 10% neutral buffered formalin for 48 h. Fixed bones were decalcified in Formical (Decal Corporation, Congers,

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Table 1. Litters with clinical cases of runting and steatorrhea

Inbred mouse strain	Mating number	Mating type[a]	Litter	Litter size	Preweaning losses[b]	Gender, age, and weight[c]
C3HeB/FeJ	6	Duo	2nd	9	1	F, 17 d, 5.89 g
C3HeB/FeJ	24	Duo	4th	8	5	F. 17 d, 7.48 g NR, 17 d, 10.69 g, control[d] NR, 17 d, 12.69 g, control
C3HeB/FeJ	21[e]	Duo	1st	7	4	F, 16 d, NR
C3HeB/FeJ	48[e]	Trio	2nd	5	1	F, 25 d, 4.58 g
C3HeB/FeJ	53[e]	Duo	7th	6	1	F, 20 d, 6.17 g F, 20 d, 7.87 g, control
101/H	61	Trio	1st	5	3	M, 15 d, 6.39 g M, 15 d, 9.23 g, control
101/H	18[f]	Trio	1st	3	2	F, 19 d, NR
101/H	60[f]	Trio	1st	5	1	M, 14 d, 4.33 g

F, female; M, male; NR, not recorded.
[a]Matings were set up with 1 male and 1 female (duo) or 1 male and 2 female (trio) mice.
[b]Preweaning losses include pups found dead or missing at weaning and those culled due to a steatorrheic and runted phenotype and excludes nonaffected littermates taken as controls.
[c]Affected and nonaffected mice examined at necropsy.
[d]Nonaffected control littermate.
[e]C3HeB/FeJ mating 21 was G1, and matings 48 and 53 were G4 in the same family lineage.
[f]101/H matings 18 and 60 were G1 and G3 in the same family lineage.

NY) for 48 h. Tissues were embedded in wax and sectioned at 4 µm. Sections were stained with hematoxylin and eosin. Frozen sections of liver fixed in 10% neutral buffered formalin were stained for lipid with Oil Red O and for glycogen with periodic acid Schiff. Sections of pancreas fixed in Bouin solution were transferred to electrostatically charged slides (Superfrost Plus, Menzel Glaser GmbH, Braunschweig, Germany) and, without antigen retrieval, immunostained for insulin (guinea pig anti-pig insulin IgG, Dako UK Ltd, Ely, UK) or glucagon (rabbit anti-human glucagon IgG, AbD Serotec, Oxford, UK) according to the manufacturer's instructions. Other sections were immunostained for carboxypeptidase B (1:600; rabbit anti-pig carboxypeptidase B IgG, Abcam plc, Cambridge, UK) or cytokeratin (1:25; wide spectrum screening, rabbit anti-cow cytokeratin IgG, Dako). Biotinylated secondary antibodies (swine anti-rabbit or rabbit anti-guinea pig antibodies and avidin–horseradish peroxidase conjugate, Dako) were used as the detection system.

Insulin and glucose determination. For 1 affected C3HeB/FeJ litter, plasma glucose was measured by use of an automatic analyzer (model GM9, Analox, London, UK) and plasma insulin was measured by use of an enzyme-linked immunosorbent assay kit (Ultra-sensitive Mouse ELISA kit, Mercodia, Uppsala, Sweden) according to the manufacturer's instructions.

Analysis of preweaning losses. Preweaning losses and their temporal patterns were compared in affected litters (for example, litters with 1 steatorrheic and runted mouse) with other litters that had at least 1 preweaning loss due to other causes (but with no recognized steatorrheic and runted mice) using Fisher exact tests.

Results

Clinical features and prevalence. Over a 29-mo period, 5 C3HeB/FeJ litters and 3 101/H litters had runted pups that weighed approximately 22% to 36% less than age-matched siblings. This frequency represents 1.83% of C3HeB/FeJ (n = 273) and 1.27% of 101/H (n = 236) litters born during this period. Both male and female pups were affected and were first recognized when they were from 14 to 25 d old as being smaller than siblings and having greasy hindquarters sometimes stained with pale yellow feces (Table 1). With 1 exception, the affected pups were active and otherwise well—they did not appear to be fading pups. On the basis of histology from the first cases, serology on selected pups (discussed later), and the clinical well-being of littermates, we had reasonable confidence that we were not dealing with infection with mouse hepatitis virus, rotavirus, or reovirus 3. The affected matings were isolated (the rack was changed last and the cage-change station sterilized before other cages in the rack were cleaned), and the mating was left to produce 1 to 6 more litters. None produced a second affected litter, and the number of pups born in subsequent litters and the preweaning losses in these litters were unremarkable (data not shown). Some of the nonaffected siblings littermates were used in the breeding program to maintain the 101/H colony.

In individual matings, the affected litter was the first in 4 cases, the second in 2 cases, and the fourth or seventh (Table 1). In all, 65 of 236 (27.5%) of nonaffected 101/H litters and 88 of 273 (32.3%) of C3HeB/FeJ nonaffected litters lost 1 or more pups. The starting size of affected litters was comparable to litters with other types of preweaning losses, but the low numbers of cases (3 versus 65 for 101/H and 5 versus 88 for C3HeB/FeJ) preclude rigorous statistical evaluation using a nonparametric rank test (Table 2). For both 101/H and C3HeB/FeJ litters, the proportion of pups surviving to weaning versus the number of losses was the same in affected and nonaffected litters ($P > 0.50$). However the pattern of losses differed significantly between affected and nonaffected litters ($P < 0.001$). Losses in litters with a steatorrheic and runted mouse tended to be late, whereas those in nonaffected litters were both early and late.

With 1 exception, there were 2 to 5 mo between cases. In November 2005, there was a single case in each of the C3HeB/FeJ and 101/H colonies. Given the precaution of sterilizing cage-changing stations and gloves when dealing with different colonies, cross-infection with a potential pathogen seems unlikely.

Strain occurrence. None of the 13 other mouse strains and subspecies (C57BL/6J-J, BALB/cByJ, BALB/cAnNCrl, C3H/HeH, C3H/pde6b, 129/S2SvPas, 129/SvEv, 129/SvNIMR, CBA/CaH,

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Table 2. Preweaning losses in litters with 1 steatorrheic and runted mouse pup compared with nonaffected litters with at least 1 preweaning loss due to other causes

	101/H		C3HeB/FeJ	
	nonaffected	affected	nonaffected	affected
Number of litters	65	3	88	5
Number of pups	337 (5.18, 1–9)	13 (4.33, 3–5)	642 (7.30, 1–15)	35 (7.0, 5–9)
Losses				
0–5 d[a]	68 (1.05, 0–5)	0	131 (1.49, 0–15)	4 (0.8, 0–4)
5–10 d	43 (0.66, 0–7)	0	20 (0.23, 0–5)	0
10–15 d	7 (0.11, 0–2)	1 (0.33, 0–1)[b]	44 (0.50, 0–8)	0
15 d to weaning	29 (0.45, 0–4)	5 (1.67, 0–3)[b]	45 (0.51,0–5)	8 (1.6, 1–4)[b]

Data are given as total number (mean, range).
[a]Losses include pups found dead or missing at 0 to 5 d of age, 5 to 10 d, 10 to 15 d, and 15 d to weaning.
[b]Includes pups with runting and steatorrhea.

DBA2/B6, FVB/NCr1Br, CD1, *Mus musculus custaneus*) bred in this production ward showed a similar clinical phenotype. Affected litters were born in 3 of 9 separate C3HeB/FeJ family lineages. In 2 lineages, affected litters were in the first generation (G1), and these lines were terminated. In the third family lineage, G1 and G4 litters were affected; the condition skipped intervening generations. In the 101/H colony, 2 of 11 family lineages were affected. One family lineage had an affected G3 litter, the second had G1 and G3 affected litters (Table 1).

Microbiologic screening. Serum from the first affected pup was tested for antibodies against reovirus 3, mouse hepatitis virus, mouse adenovirus type 2, mouse rotavirus, and TMEV. TMEV was tested for because it was endemic in the old low-health status mouse colonies at MRC Harwell. The test results were negative. Subsequently another 7 pups from 3 affected litters were tested and were negative for reovirus 3, mouse hepatitis virus, mouse adenovirus type 2, mouse rotavirus, and TMEV. Four sets of parents (the male and 1 or 2 females from each mating) that produced 1 affected litter were screened after they were taken out of the breeding program. These 11 mice and the sentinels from the production ward (275 mice over 29 mo) and the rest of the facility (n = 532) remain free of FELASA-listed agents, with the exception of *Entamoeba muris.* Rather than an indication of a breakdown in barrier control, this organism is considered to be a nonpathogenic commensal that is present in current stocks because it was in the CD1 females originally used in the embryo rederivation program.

Gross and histopathology. One affected pup lacked a grossly visible spleen; otherwise there were no gross pathology findings. Affected individuals had a relatively small pancreas that required step-sectioning to locate. The exocrine pancreas lacked recognizable acinar cells with normal carboxypeptidase B-positive zymogen granules but had cytokeratin-negative duct-like structures (Figure 1 A through F). The endocrine pancreas was unremarkable, comprising round and irregular islets with normal proportions of insulin-positive β cells and glucagon-positive α cells (Figure 1 G, H). In places the islets were relatively crowded, reinforcing the impression that there was disproportionate under-representation of exocrine pancreas. The interstitial pancreatic tissue was either adipose tissue (Figure 1 F) or loose connective tissue with fibrocytes (Figure 1 B). In places there were light infiltrates of F/480-positive macrophages and CD45- and CD3-positive lymphocytes (data not shown), but these cells were interpreted as being from mesenteric lymphatics and lymphoid tissue rather than as indicative of inflammation. There was no evidence of necrosis.

Unlike their nonaffected siblings, affected pups had undigested milk in the small and large intestines (Figure 2 A, B). The colon epithelium was vacuolated in 5 of 8 cases, and 3 of 8 cases had mild multifocal epithelial erosion with neutrophil leukocyte exudation or increased numbers of inflammatory cells in lamina propria. In 1 of the 8 cases, there was focal ulceration in the small intestine and neutrophil exudation over a Peyer patch. No intestinal flagellates, pinworms, house hepatitis virus-infected enterocyte syncitial cells, or adenovirus inclusion bodies were seen in the intestine sections. Diffuse hepatocyte glycogen microvacuolation was present in 5 of the 8 livers, and in 1 of these there was marked Oil Red O-positive lipid vacuolation (data not shown). There was no evidence of inflammation of brain, liver, lymph nodes, spleen, heart, thymus, or lungs or noteworthy histologic abnormalities in other tissues examined.

Plasma insulin and glucose determination. In a single pairwise comparison between affected and nonaffected 20-d-old C3HeB/FeJ female siblings, the unaffected female had 7.79 mmol/l glucose and 0.20 µg/l insulin, and the affected sibling had 4.26 mmol/l glucose and 0.19 µg/l insulin.

Discussion

C3HeB/FeJ and 101/H colonies had litters with pups that had steatorrhea and were runted. The clinical signs of pancreatic insufficiency and maldigestion in affected pups were consistent with the pathologic findings of a small, poorly differentiated exocrine pancreas with multiple cytokeratin-negative duct-like structures but lacking fully differentiated exocrine gland cells. Unlike the exocrine pancreas, the pancreatic islets appeared well differentiated, with insulin positive β-cells and glucagon positive α-cells. We were able to compare blood insulin and glucose levels in 1 affected pup and its nonaffected sibling. The results showed that their insulin levels were comparable, and their glucose concentrations were within normal limits. These preliminary data indicate that functional islet cell mass is maintained in the face of the loss of exocrine pancreas mass.

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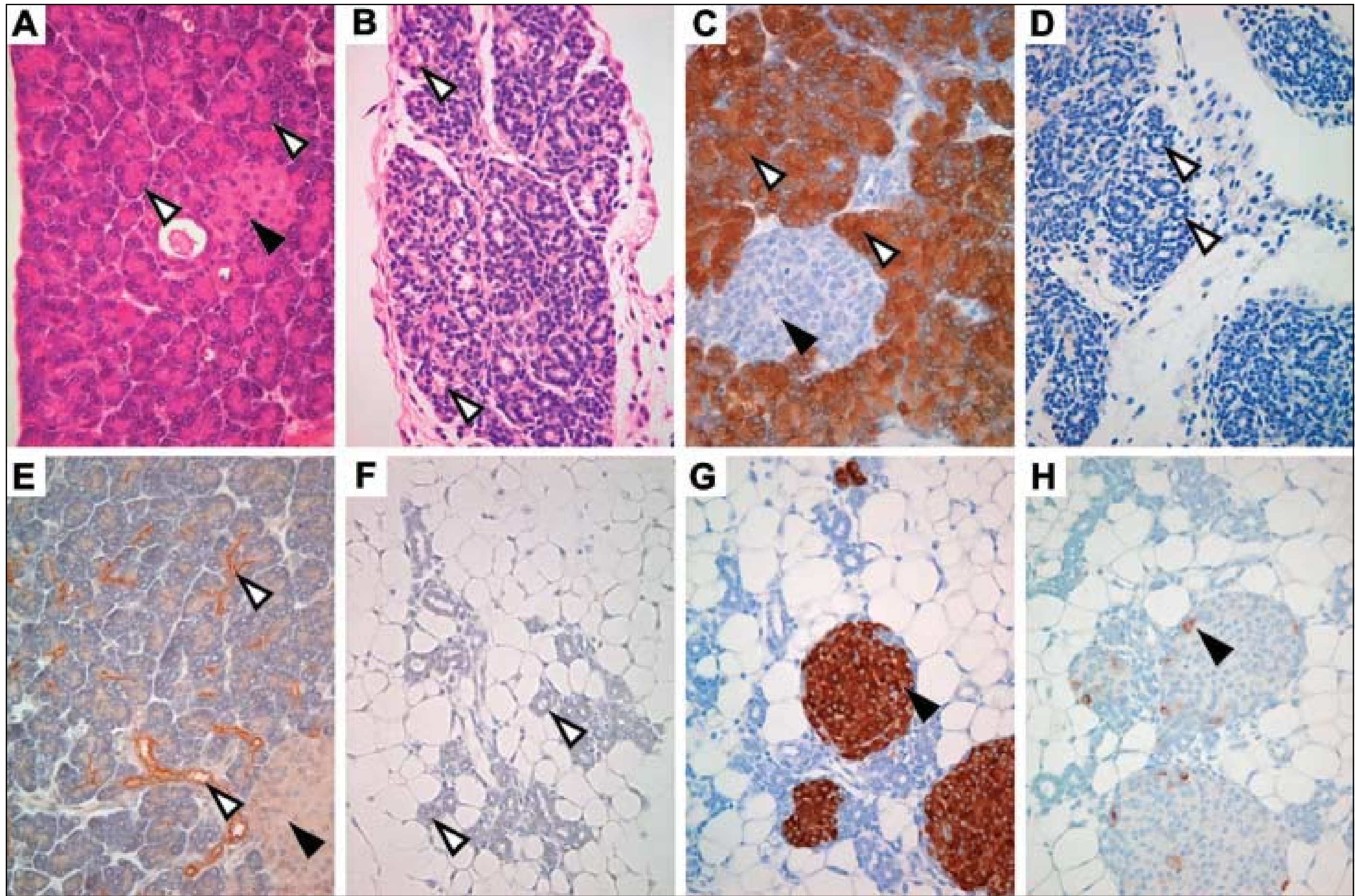

Figure 1. Pancreas from nonaffected normal (A, C, E) and affected (B, D, F–H) mice. For all panels, the original magnification was ×400, open arrowheads indicate exocrine pancreas features, and solid arrowheads indicate endocrine islet features. (A) Normal pancreas in a nonaffected 17-d-old C3HeB/FeJ mouse; hematoxylin and eosin stain. (B) Pancreas in an affected 19-d-old 101/H mouse is comprised of tubular duct-like structures and lacks normal acinar cells; hematoxylin and eosin stain. (C) Normal pancreas in a 16-d-old 101/H mouse, in which acinar cells have carboxypeptidase-positive zymogen granules, whereas the affected sibling (D) has duct-like structures that are negative for carboxypeptidase. (E) Normal pancreatic ducts in a 17-d-old C3HeB/FeJ mouse are cytokeratin-positive but are negative in an affected sibling (F). Endocrine islets in an affected 17-d-old C3HeB/FeJ mouse contain normal insulin-positive β cells (G) and glucagon-positive α cells (H). (See text for details about immunohistochemistry used in C-H).

The pathology findings have to be put in the context of individual serologic results as well as the results of the disease surveillance program. If we examine these in turn, the serology of pups in 4 affected litters showed no evidence of antibodies to reovirus 3, mouse hepatitis virus, mouse adenovirus type 2, mouse rotavirus, and TMEV, but the lack of seroconversion in itself does not exclude the possibility of viral infection. The difficulty with data from preweaned pups is that their immune system is immature. Furthermore, for particular agents, seroconversion may take place during a recovery phase.[19]

The sentinel screening program is based on transfer of soiled bedding to sentinel cages of immunocompetent mice and allows an adequate time for the sentinels to develop an infection or seroconvert. This method is widely accepted as a means of enhancing the detection of some agents in individually ventilated caging systems. In particular, the bedding transfer method is useful for detection of agents transmitted via the fecal–oral route such as mouse hepatitis virus, mouse parvovirus, intestinal flagellates and pinworms,[5,7,16] TMEV,[6] *Helicobacter* spp.,[23] *Clostridium piliforme*,[17] and vaccinia.[11] It is less useful for organisms transmitted by direct contact or aerosol such as *Pasteurella pneumotropica*, Sendai, lactate dehydrogenase-elevating virus, and cilia-associated respiratory bacillus.[19] The frequency and scope of our screening program exceeds those recommended in the FELASA guidelines, and the historical results since opening the Mary Lyon Centre suggests the barrier has not been broken. The strongest evidence for the absence of FELASA-listed agents are the negative results for full-panel serology, bacteriology, and parasitology tests in immunocompetent breeding stock: the 4 sets of parents that each had 1 litter with a steatorrheic and runted pup.

Although these data argue against an infectious etiology caused by agents on the FELASA list, there is a possibility we are dealing with an undescribed infectious agent. The properties of this putative agent might include vertical transmission that evades elimination by embryo rederivation, no detectable horizontal transmission to siblings or parents, and a narrow tissue tropism for exocrine pancreas. We have seen transmission of mouse hepatitis virus in 2 of 95 isolator cycles in the embryo rederivation program at MRC Harwell. These occurred in a separate quarantine unit and were identified in the isolator-screening program and eradicated.[10] Vertical transmission of a retroviral infection[19] might fit this epidemiologic pattern of steatorrhea and runting disease

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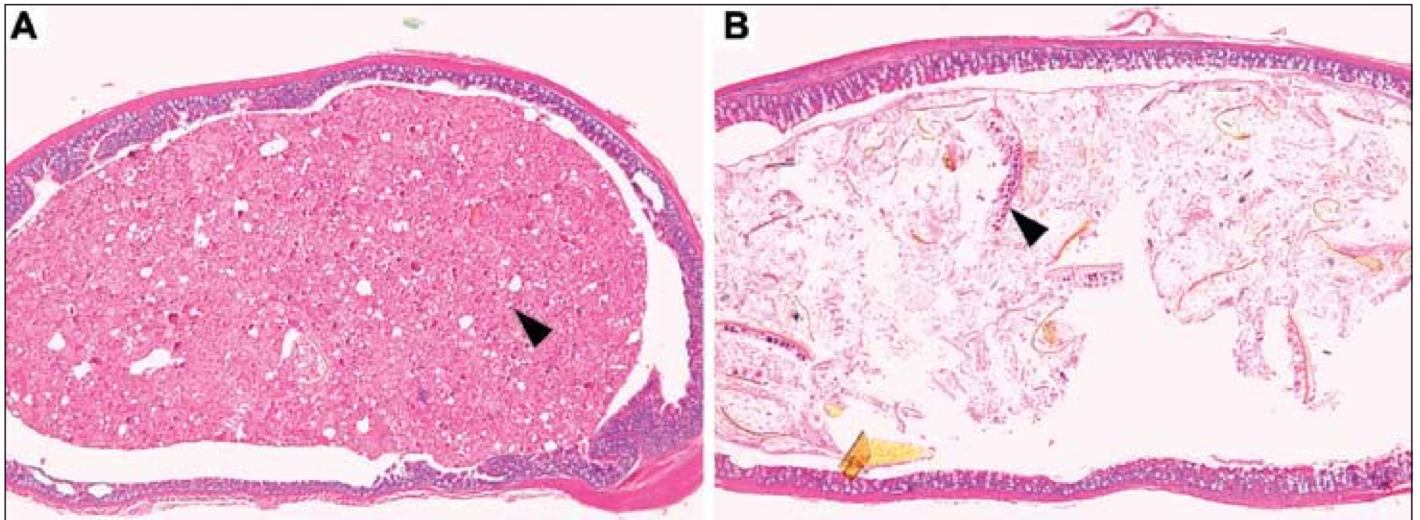

Figure 2. The intestines from (A) an affected mouse and (B) a nonaffected littermate control. The colon contents in the affected mouse are chiefly milk (arrowhead), whereas digested food mixed with plant ingesta (arrowhead) is present in the nonaffected mouse. Hematoxylin and eosin stain; magnification, ×40.

but would be difficult to distinguish from a purely murine genetic mechanism (discussed later).

With regard to the narrow tissue tropism of a putative infectious agent, it is noteworthy that the viral or bacterial infections on the FELASA list known to cause pancreatic lesions might be expected to cause lesions in a range of visceral organs, and such widespread disease did not occur. The only extrapancreatic lesion in affected pups was low-grade inflammation of the hindgut. This condition may be due to disruption of the normal bacterial ecosystem because of undigested milk in the large intestine.

Reovirus 3 is an example of an agent reported to cause pancreatic pathology as part of a multisystemic inflammatory process. In natural infections, reovirus 3 infection is associated with pancreatic duct inflammation and pancreatitis leading to steatorrhea in association with encephalitis, myocarditis, hepatitis, sialodacryoadenitis, and generalized lymphoid depletion.[19] Interestingly these early descriptions of reovirus 3 disease were in mice of undefined microbiologic status, and intercurrent virus infections may have played a role in this spectrum of lesions. Lesions developed in brain, intestine, liver, spleen, heart, thymus and lungs but not in pancreas in SPF mice oronasally inoculated with reovirus 3 when they were 2 d old.[1] In a similar way, some of the other infectious agents that could cause steatorrhea or pancreatic pathology or both, such as mouse hepatitis virus, mouse rotavirus, and *Salmonella* might also be expected to cause recognizable extrapancreatic pathology.

Leaving aside the possibility of an infectious etiology, pancreatic atrophy and hypoplasia are differential diagnoses. Exocrine pancreatic atrophy has been reported in mice on 2-y aging studies or after experimental pancreatic duct ligation.[4,8] A longitudinal study would be needed to confirm the normal development of the embryonic pancreas from embryonic day 9 to 10 onwards,[8] but the low prevalence makes this investigation infeasible.

Given the early onset of this condition and the well-established genetic mechanisms of pancreas development (see later discussion), inherited hypoplasia is more likely than atrophy. However, because the condition affects less than 2% of litters, often the first, and then skips later litters and generations in family lineages, inheritance is clearly complex. The pattern suggests putative inheritance of a single gene of low penetrance or a polygenic or multifactorial trait. The penetrance might actually be higher than currently appears to be the case, if the condition could be shown to affect more than 1 pup per litter. However investigating all case of preweaning morbidity is difficult because the mother can and does cannibalize pups. Furthermore, rigorous analysis of breeding records is hampered by having to make the assumption that nonaffected litters do not contain missed cases. Given this caveat, the available evidence from litter size and survivorship to weaning suggests that preweaning losses in litters with a steatorrheic and runted pup tended to be late in the postnatal period but that there was no excess of early losses that may have represented undiagnosed cases.

Embryologically, the pancreas arises from separate dorsal and ventral endodermal foregut primordia as buds that then expand and branch. Endocrine cells initially are intermingled with other pancreatic cells but then migrate out and form islets.[12] Genes in the transforming growth factor β, Hedgehog, and Notch signaling pathways are important in the early differentiation into exocrine, duct, and endocrine cells,[12,14] and the basic helix-loop-helix transcription factor PTF1-p48 is the earliest marker for the exocrine cell lineage. The PTF1-p48 null mutation leads to complete absence of the exocrine pancreas, but the cellular development of endocrine pancreas is normal; however the endocrine cells are directed by default to the spleen rather than forming mesenteric islets.[15] Mutations in genes in the transforming growth factor β, Hedgehog, and Notch signaling pathways may underlie a number of human diseases including annular pancreas, ectopic pancreas, pancreatic hypoplasia, congenital absence of islets, pancreas divisum, hyposplenism, and susceptibility to type 2 diabetes mellitus,[12,14] and mutations in the *SBDS* gene in human Shwachman-Diamond syndrome lead to exocrine pancreas insufficiency accompanied by neutropenia, thrombocytopenia, anemia, and metaphyseal chondrodysplasia.[3]

In comparison to these human phenotypes, affected C3HeB/FeJ and 101/H pups appear to have hypoplasia of the exocrine pancreas but normal mesenteric islets. In addition, ectopic pancreas, skeletal, and bone marrow abnormalities were not evident, but 1 of the affected 101/H pups lacked a grossly visible spleen,

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a feature it shares with mice carrying targeted mutations in type II activin receptors A and B; such mice have severe hypoplasia of the dorsal pancreas and spleen.[14] In the current study of C3HeB/FeJ and 101/H mice, we were unable to judge from the histology whether the dorsal or ventral pancreatic lobe or both were affected because the tissue was embedded en bloc.

This study was primarily a pathologic investigation, and tissue from early cases was not frozen for DNA. In light of these findings and the possible genetic component to the syndrome, we have started to collect tails at necropsy of suspected cases for DNA for genetic analysis.

In summary, the pathology, apparent strain predisposition, age of onset, and SPF microbiologic status of the affected mice is suggestive of pancreas hypoplasia that may have a complex genetic component. There is no reason to expect that both strains would necessarily carry the same genetic mutation(s). There is anecdotal evidence for greasy, runted mice in the old, conventional colonies at MRC Harwell,[22] suggesting that this pancreatic disease is not new a phenomenon that has arisen since the closing of that facility and its replacement by our new SPF facility. At the time when the affected pups were identified in our pre-existing facilities, there was a high prevalence of gastrointestinal pathogens such as mouse hepatitis virus, flagellates, and pinworms, and not all cases could be investigated in detail. The steatorrheic and runted phenotype we describe here is overt in our new SPF facility, and we can begin to exclude the effects of known infectious diseases. It remains to be seen whether this condition is confined to these inbred strains or will be found in other inbred strains. C3HeB/FeJ and 101/H inbred strains are only distantly related,[2] so there is no indication of inheritance of a single trait from a recent common ancestral strain.

Although the etiology of this condition is not completely clear, the recognition of postnatal pancreatic disease by its clinical signs of runting coupled with steatorrhea has a wider implication. An *N*-ethyl-*N*-nitrosourea-mutagenesis program run in an SPF facility could use this uncommon combination of clinical signs as a screen for mutants with defects in pancreas development. Because mice with pancreatic hypoplasia are unlikely to be reproductively viable, recognition of this phenotype might be useful only in a recessive screen of generation 3.

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