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Immunohistochemical Analysis of Spontaneous Pancreatic Islet Amyloid Deposits in Nonhuman Primates

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Pancreatic islet amyloidosis has been previously reported in aged diabetic and nondiabetic humans, nonhuman primates, cats, raccoons (*Procyon lotor*), and degus (*Octodon degus*, a South American rodent).[4–6] In nonhuman primates, islet amyloidosis, often accompanied by diabetes mellitus, has been reported in Celebes crested macaques (*Macaca nigra*), rhesus monkeys (*Macaca mulatta*), cynomolgus monkeys (*Macaca fascicularis*), a Formosan rock macaque (*Macaca cyclopis*), and a drill baboon (*Mandrillus leucophaeus*).[1,2,7,8]

Human and feline islet amyloid deposits are derived from the polymerization of islet amyloid polypeptide (IAPP; amylin), whereas in the degu, they are derived from insulin.[4–6,9] IAPP is an islet beta-cell product secreted with insulin by humans and many other species.[5] The definitive biological functions of IAPP and the significance of IAPP in the pathogenesis of human type 2 (non-insulin dependent) diabetes mellitus and feline diabetes mellitus are unresolved.[5,6,9]

The purpose of this study was to examine the immunoreactivity of pancreatic islet amyloid in captive nonhuman primates. A review of necropsy records from several sources identified four cynomolgus monkeys, three Celebes crested macaques, and one orangutan (*Pongo pygmaeus*) with islet amyloid deposits. All of the nonhuman primates were adults ranging from 7 to 26 years of age. One of the Celebes crested macaques had diabetes mellitus, but the metabolic status of the other animals was not known.

Formalin-fixed, paraffin-embedded tissues were sectioned at 5 μm and stained with either hematoxylin and eosin (HE) or Congo red with or without $KMnO_4$ pretreatment. Congo red-stained sections were examined by both bright field and polarized light microscopy.

Using a modified avidin–biotin–peroxidase complex (ABC) method,[3] pancreatic tissues were evaluated for immunoreactivity with guinea pig anti-porcine insulin serum (Dako Corp., Carpinteria, CA) diluted 1 : 800 in phosphate-buffered saline (PBS), pH 7.4; 1 : 400 rabbit anti-human glucagon serum (Dako); 1 : 100 rabbit anti-human somatostatin serum (Dako); 1 : 400 rabbit anti-human pancreatic polypeptide serum (Dako); 1 : 800 rabbit anti-human calcitonin gene-related peptide (CGRP) serum (Peninsula Laboratories, Belmont, CA); and 1 : 200 rabbit anti-human amylin serum (Peninsula). Positive controls included internal controls in the tissue sections containing islet amyloid and application of the primary antisera to tissue sections from normal bonnet macaque (*Macaca radiata*) pancreas. Negative controls included replacing the primary antisera with the appropriate nonimmune sera and applying the primary antisera to tissue sections from a canine kidney containing glomerular amyloid.

Five-micrometer tissue sections were collected on positively charged glass slides (Fisher Scientific, Pittsburgh, PA), deparaffinized, rehydrated, and incubated in 1.5% normal goat serum in PBS for 20 minutes at room temperature in a humidified chamber. Sections were incubated with primary antibody for 1 hour under the same conditions, followed by a 30-minute incubation in 3% hydrogen peroxide in methanol to block endogenous peroxidase activity and 30-minute incubations of biotinylated goat anti-rabbit or anti-guinea pig IgG (Vector Laboratories, Burlingame, CA), and ABC (Vector). The chromagen was 0.016% diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO) with $NiCl_2$ (Digene Diagnostics, Silver Spring, MD) in 0.024% hydrogen peroxide and PBS. Sections were counterstained with nuclear fast red (Digene).

All the animals in this study had either multifocal or diffuse pancreatic islet amyloidosis. The extent and distribution of amyloid ranged from mild multifocal deposition surrounding islet capillaries with little or no loss of islet cells to marked diffuse deposition in enlarged islets with severely reduced

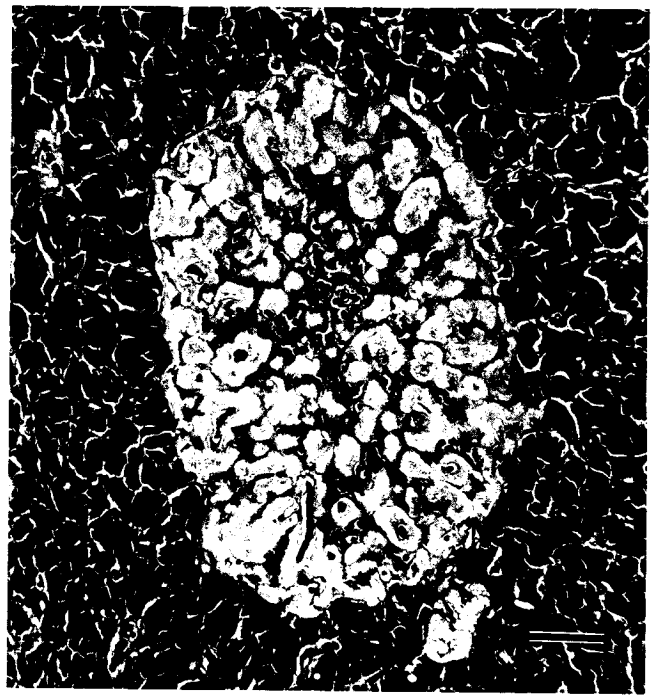

Fig. 1. Pancreas; Celebes crested macaque. Note perivascular amyloid deposits in a pancreatic islet. HE. Bar = 60 μm.

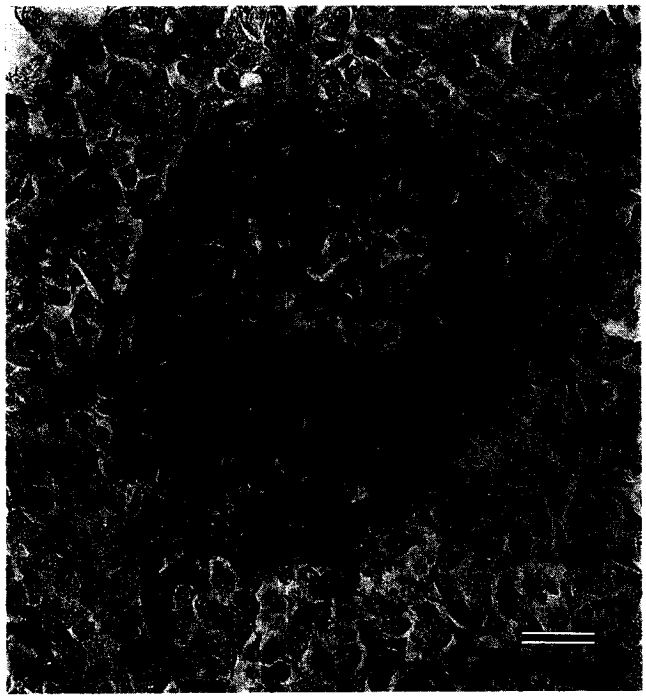

Fig. 2. Pancreas; orangutan. Amyloid deposits are immunoreactive with antisera to islet amyloid polypeptide. ABC method, nuclear fast red counterstain. Bar = 30 μm.

numbers of islet cells (Fig. 1). Two Celebes crested macaques with extensive islet amyloid deposits also had perivascular mineralization within the islets. The amyloid exhibited green birefringence when viewed with polarized light and was congophilic. Congophilia was retained after oxidation with $KMnO_4$. Amyloid was not noted in other organs.

Islet amyloid deposits in all the animals were moderately to strongly immunoreactive with antisera to IAPP (Fig. 2) and CGRP. Immunohistochemical stains accented the perivascular orientation of the amyloid deposits, which were frequently radially striated at the periphery of the deposits. No immunoreactivity of the islet amyloid was observed with antisera to insulin, glucagon, somatostatin, or pancreatic polypeptide.

The CGRP immunostaining of the amyloid deposits may have been a cross-reaction with polymerized IAPP. Immunoreactivity of islet amyloid deposits with antisera to IAPP and CGRP has been previously reported, and CGRP is not known to be amyloidogenic.[5,6] CGRP-1 and CGRP-2 are reported to be 43% and 46% homologous, respectively, with IAPP.[5] Perhaps antigenic epitopes in IAPP are exposed through structural realignment during the polymerization of IAPP to form islet amyloid fibrils. Resulting amyloid deposits could therefore be immunoreactive with both IAPP and CGRP antisera.

In this study, spontaneous pancreatic islet amyloid deposits in three species of nonhuman primates immunoreacted with antisera to IAPP and CGRP. The IAPP and CGRP immunoreactivity of islet amyloid deposits in these Celebes crested macaques agrees with results of a previous report.[6] This is the first report of islet amyloidosis in an orangutan and of IAPP and CGRP immunoreactivity of islet amyloid in cynomolgus monkeys and an orangutan.

Pancreatic islet amyloid deposits are a characteristic morphologic feature in more than 90% of humans with type 2 diabetes mellitus and in more than 80% of cats with a similar form of age-related diabetes mellitus.[5] The presence of islet amyloid and the amyloid's immunoreactivity in nonhuman primates imply that a non-insulin dependent-like form of diabetes mellitus may occur in macaques and orangutans.

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