Supplementary Materials and Methods

Evaluation of Pancreatic Exocrine Function

Pancreatic exocrine function was evaluated by the secretin test, as before.[1] In brief, duodenal intubation was performed to collect pancreatic juice. After an acclimation period, 80 U/body of either porcine (SecreFlo, Repligen, Waltham, MA) or human secretin (ChiRhoStim, ChiRhoClin, Inc, Burtonsville, MD) was administered intravenously and pancreatic juice was collected into an ice-chilled tube every 10 minutes for 60 minutes. Total secreted volume (V:mL/h) and total amylase output (U/h) were measured. The HCO_3^- concentration in each sample was measured and the highest value was set as the maximum HCO_3^- concentration (MBC:mEq/L).

Secretin tests were performed at the time of diagnosis, 3 months after initiation of treatment (short-term) and 1 year (long-term) after initiation of therapy.

Immunohistochemistry

Biopsy samples or surgically resected tissues were fixed in 10% formalin and embedded in paraffin. Sections were deparaffinized, permeabilized, and used for immunohistochemistry.[2] AQP1 localization was examined using an anti-rat AQP1 IgG at 1:1000 dilution (AQP11-A; Alpha Diagnostics, San Antonio, TX). CFTR localization was examined using 3 separate monoclonal anti-human CFTR antibodies against different epitopes in CFTR (M3A7, Millipore Corp, Billerica, MA; #13-1 and #24-1, R&D Systems (Minneapolis, MN), all at 1:40 dilution). IgG4-positive plasma cells were immunostained with an anti-IgG4 antibody (The Binding Site, Birmingham, UK) at 1:200 dilution. CD133 localization was examined using an anti–prominine-1 antibody at 1:10 dilution (MB9-3G8; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Dilutions for all antibodies followed the manufacturer's recommendations. Immunoreactions were intensified using either Envision plus reagent (DAKO, Glostrup, Denmark) or Histofine Simple Stain MAX-PO (Nichirei Biosciences, Inc, Tokyo, Japan). Immunolabeling was visualized using 3,3′-diaminobenzidine tetrahydrochloride as substrate for horseradish peroxidase. Sections finally were counterstained with Mayer's hematoxylin.

Histologic Scoring

Tissue sections were examined and semiquantitated based on the intensity of 3,3′-diaminobenzidine tetrahydrochloride staining of CFTR at the cytoplasm in pancreatic duct cells by an independent observer (S.A.) who was blinded to the clinical information. The entire sections were examined for each sample and scored on a scale of 0–3 (0, no cytoplasmic staining of CFTR; 3, severe cytoplasmic staining of CFTR).

The extent of IgG4-positive plasma cell infiltration also was evaluated by an independent observer (S.A.) who was blinded to the clinical information. The extent was scored on a scale of 0–3 according to the number of immunohistochemically identified IgG4-positive plasma cells per high-power field in each specimen. Sections with less than 5 positive plasma cells/high-power field were scored as 0, sections with 5–10 cells were scored as 1, sections with 11–30 cells were scored as 2, and tissues with more than 30 positive cells/high-power field were scored as 3.

Genetic Studies

Genomic DNA was extracted from peripheral blood leukocytes by a standard isolation procedure using QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). All 27 CFTR exons of 5 AIP patients who agreed to the genetic analysis, including flanking intronic regions, were amplified using human CFTR gene-specific primers as shown in Supplementary Table 1. Amplicons were sequenced at Shimadzu Biotech (Kyoto, Japan) with the same primers used for fragment amplifications except for exon 9. Genetic analyses of a CFTR gene were performed at Nagoya University Hospital and the procedure was approved by the ethics committee of the hospital. Written informed consent was obtained from each patient.

Results

Cytoplasmic Mislocalization of CFTR Was Confirmed With Different Anti-Human CFTR Antibodies

To validate observations in Figure 4 and to ensure that the findings were not dependent on the antibodies used to immunolocalize CFTR, we have used 2 additional distinct anti-CFTR antibodies that recognize different CFTR epitopes. Identical immunolabeling of CFTR in the apical plasma membrane of pancreatic ducts in normal subjects and a large cytoplasmic mislocalization in tissue from AIP patients were confirmed by antibodies 13-1 (anti-R domain of CFTR) and 24-1 (anti-C terminus of CFTR), as reported by M3A7 (data not shown).

CFTR Genotypes in AIP

Mutations in CFTR have been reported in a subgroup of patients with idiopathic chronic pancreatitis in the Caucasian population. Some of the CF-causing mutations in CFTR are known to affect trafficking of the protein to the plasma membrane. However, the incidence of CF in Japanese people is extremely low (<1 per 350,000 live births).[3] In addition, none of the 20 common CFTR mutations reported in the white population and the 9 mutations causing CF in Japanese people[4] was found in normal subjects and chronic pancreatitis patients. Thus, it is unlikely that germline mutations in CFTR caused the mistrafficking of the protein in pancreatic duct cells in the Japanese patients with AIP. To further exclude the possibility that mutations in CFTR are responsible for the findings in our patient popula-

Supplementary Table 1. Primers Used in this Study to Amplify CFTR Gene Fragments

CFTR exon no	Forward primer	Reverse primer	Size, bp	Temperature
1-1	5′-GTT TTG CTG ACT GAG ACT AGG-3′	5′-CAA CGC TGG AGG ACA GAA GAA-3′	590	60°C
1-2	5′-AAA CGT AAC AGG AAC CCG ACT A-3′	5′-CTC AAC CCT TTT TCT CTG ACC T-3′	559	60°C
1-3	5′-GGA GAA AGC CGC TAG AGC AAA-3′	5′-GTG GCT CTC TAT TCA ATC AGC-3′	504	62°C
2	5′-TGT AAG AGA TGA AGC CTG GTA-3′	5′-GCT CCT ATT TTT AAA TAT AAG-3′	384	50°C
3	5′-CCA TGA GAT TTT GTC TCT ATA-3′	5′-GAG TTG GAT TCA TCC TTT ATA-3′	365	55°C
4	5′-AAG AGT TTC ACA TAT GGT ATG-3′	5′-TGC CAT TTA TTT AAT AGG CAT-3′	496	60°C
5	5′-GAA GAT AGT AAG CTA GAT GAA-3′	5′-AAT TGA CCT TTC TTA GTT TCC-3′	393	60°C
6a	5′-AGT GTG CTC AGA ACC ACG AAG-3′	5′-GTT CTA TGC ATA GAG CAG TCC-3′	428	60°C
6b	5′-TGG AAT GAG TCT GTA CAG CG-3′	5′-TGC ATG AAT ATT GAC AGA ACT-3′	475	60°C
7	5′-TAT AGG CAG AAA GAC TCT AGA-3′	5′-TCC TAG TAT TAG CTG GCA ACT-3′	504	60°C
8	5′-TGA ATC CTA GTG CTT GGC AAA-3′	5′-TCC TTC CAG TTC TAC CAG TTA-3′	400	60°C
9	5′-CCA TGT GCT TTT CAA ACT AAT TGT-3′	5′-TAA AGT TAT TGA ATG CTC GCC ATG-3′	578	60°C
9 (sequencing)	5′-CCA TGT GCT TTT CAA ACT AAT TGT-3′	5′-CAA CCG CCA ACA ACT GTC CT-3′	274	60°C
10	5′-TTG TGC ATA GCA GAG TAC CTG AAA-3′	5′-ATT GAT CCA TTC ACA GTA GCT-3′	506	60°C
11	5′-ACT GTG GTT AAA GCA ATA GTG-3′	5′-TAA ATG TGA TTC TTA ACC CAC-3′	359	55°C
12	5′-CTT CTG CAC CAC TTT TGA GAA-3′	5′-GCT ACA TTC TGC CAT ACC AA-3′	368	60°C
13-1	5′-GGT ACC AAT TTA ATT ACT ACA G-3′	5′-ATT TGT AAG GGA GTC TTT TGC-3′	562	60°C
13-2	5′-TGG GAT GTG ATT CTT TCG ACC-3′	5′-GGG AAG AGA TAT GTC CAT TGC-3′	767	62°C
14a	5′-ACA CTT AGA TTC AAG TAA TAC T-3′	5′-CAG AAG CTA AGA ACT ATA TGA-3′	471	55°C
14b	5′-GAC CCA GGA ACA CAA AGC AAA-3′	5′-TTC CAC TAC CAT AAT GCT TGG-3′	297	60°C
15	5′-GGT TAA GGG TGC ATG CTC TTC-3′	5′-AAG CCA GCA CTG CCA TTA-3′	472	60°C
16	5′-TCT GAA TGC GTC TAC TGT GA-3′	5′-GCA ATA GAC AGG ACT TCA AC-3′	298	60°C
17a	5′-AGA AAT AAA TCA CTG ACA CAC-3′	5′-CCA TGT GTA CTT TGT AAT ATA G-3′	410	55°C
17b	5′-TTT AAC CAA TGA CAT TTG TGA-3′	5′-ATA CCG ATT TCA AGG AAA TTA-3′	552	53°C
18	5′-TAG GAG AAG TGT GAA TAA AG-3′	5′-GAT ACA CAG TGA CCC TCA ATT-3′	303	60°C
19	5′-ATT GAA AAG CCC GAC AAA TAA-3′	5′-AGT TCA GAC TCT GCA AAT TAA-3′	574	55°C
20	5′-CAG GAT TGA AAG TGT GCA ACA-3′	5′-CTA TGA GAA AAC TGC ACT GGA-3′	470	60°C
21	5′-AAA ATG TTC ACA AGG GAC TCC-3′	5′-GTT GTG CAC ACA CAT ACA TGC-3′	434	62°C
22	5′-CAC GTA ATA GAC ACT CAT TGA-3′	5′-ATT TCC ACT GGG CAA TTA TTT-3′	571	57°C
23	5′-GCT GAT TGT GCG TAA CGC TAT-3′	5′-AGT AAA GCT GGA TGG CTG TAT-3′	401	60°C
24	5′-TTT CTG TCC CTG CTC TGG TC-3′	5′-ACT ATT GCC AGG AAG CCA TTT-3′	471	60°C

tion, we have sequenced the entire coding regions and flanking intronic regions of the CFTR gene of 5 patients with AIP. CFTR genotypes of these patients are summarized in Supplementary Table 2. None of the major CF-causing mutations in CFTR were found in these patients. The ratio of each haplotype, including polythymidine tract or TG repeats in intron 8 and the ratio of methionine or valine residue at position 470 in exon 10 were similar to the data in the previous analysis of CFTR genotypes in normal Japanese people.[4] A Q1352H mutation in one allele with a wild type in the second allele was found in one patient with AIP (case 3), and no other mutations were identified in coding regions of CFTR in all other patients.

Supplementary Table 2. CFTR Genotypes in Japanese Patients With Autoimmune Pancreatitis

		CFTR genotype			
Case no	Age/sex	n (TG)	Poly T	470 M/V	Mutation
3	61/M	11/12	7/7	M/V	Q1352H/WT
7	72/M	11/12	7/7	V/V	None
9	68/M	12/12	7/7	M/V	None
12	61/M	12/12	6/7	M/V	None
13	66/M	11/11	7/7	V/V	None

M, methionine; V, valine.

Discussion

Some of the mutations in CFTR are known to affect trafficking of the protein to the plasma membrane.[5] Sequencing entire open reading frames and flanking intronic regions identified a single mutated allele (Q1352H) out of 10 alleles in 5 AIP patients examined, excluding the possibility of mutations in CFTR as the cause of partial mistargeting of proteins in these patients. The allele frequency of TG repeats and polythymidine tract in intron 8, which affect the transcription efficiency of CFTR, were similar to our previous observation in normal Japanese subjects.[4] The Q1352H mutation in combination with a V470 haplotype was reported to show a decreased chloride channel activity in vitro that may result in pathogenicity.[6] However, our patient with the Q1352H mutation showed complete recovery of fluid and HCO_3^- transport by secretin test after several years of corticosteroids treatment (Shigeru B.H.K., unpublished observation). Therefore, at least in this patient, it is unlikely that Q1352H heterozygosity affects localization of CFTR, and thus aberrant HCO_3^- secretion, because steroids are not expected to correct mislocalization caused by mutations in CFTR.

CD133 is a membrane protein expressed in small pancreatic ducts in a fetal and adult pancreas and is considered a good marker for pancreatic stem/progenitor cells that can differentiate into endocrine and exocrine cells.[7,8] CD133 protein expression appears to be confined to the apical membrane of small pancreatic ducts in AIP and corticosteroid treatment did not alter the expression pattern and localization of the protein. Similarly, although expression of AQP1 was up-regulated markedly in AIP, localization of AQP1 remained unaltered. Therefore, it is unlikely that mislocalization of CFTR is caused by a general mislocalization of apical membrane protein. Differences in the expression levels in each protein or discrete functions in the plasma membrane may explain these discrepancies, although the function of CD133 remains unclear.

The finding that corticosteroid treatment regenerates the parenchyma and increases digestive enzyme secretion in AIP was surprising. The presence of CD133 immunolabeling in the apical membrane of small pancreatic ducts surrounded by regenerated acinar cells and the absence of CD133 expression in untreated pancreas or residual fibrotic area in tissues from patients treated with corticosteroids may be a clue to better understand the cellular mechanisms of pancreatic organ development and regeneration. Detailed roles of CD133 in pancreatic organ regeneration in chronic pancreatitis remain to be determined.

References

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