Supplementary Materials and Methods

Animal Model

Neonatal male Balb/c mice (<24 h old) were obtained from SLC in Japan. IgG fractions purified from patients or controls were injected subcutaneously into neonatal mice through a 27-gauge needle at a single dose of 10~20 mg human IgG per gram body weight in 200 μl PBS. We also purchased human IgG (I4506, Sigma-Aldrich, Germany) as control IgG. The IgG1 or IgG4 subclass fraction purified from human serum was divided into two equal fractions. One of each (IgG1 or IgG4) was injected as a single dose, and the others were mixed (IgG1+IgG4) and injected as a single dose. We defined IgG purified from patient serum as patient IgG and that purified from healthy controls or disease controls as control IgG, and purchased IgG as purchased IgG.

Histologic Evaluation

The mice were sacrificed at certain time-points after IgG injection and each organ (salivary gland, heart, lung, liver, bladder, bile duct, pancreas, kidney, intestine, bladder, prostate, and aorta) was immediately removed and placed in formalin for a minimum of 1 d before embedding in paraffin. All organs were cut into 5-μm thick coronal sections, and every 10th section of each entire organ was stained with hematoxylin and eosin (H&E). Edematous area was calculated by subtracting the areas of the acini, pancreatic duct, and vasculature from whole pancreas areas in all H&E-stained sections and is shown as the percent of the whole photo field. Image Pro Plus 5.0.1 software (Media Cybernetics) was used to quantify the areas of the acini, pancreatic duct, vasculature, and whole pancreas. A cut-off value of the edematous area was defined as the mean

value + 2 SD of mice given control IgG or PBS. The mean edematous area of controls (IgG and PBS) was 15.1 ± 3.0%, resulting in a cut-off value of 21.1%.

Necrosis was evaluated by the same method as in the acute pancreatitis mouse model.[1] Cells with swollen cytoplasm, loss of plasma membrane integrity, and leakage of organelles into the interstitium were considered necrotic. Necrosis was graded with H&E staining as: 0, absent; 0.5, focal occurrence of 1-4 necrotic cells/high-per field (HPF); 1, diffuse occurrence of 1-4 necrotic cells/HPF; 1.5, same as 1 + focal occurrence of 5-10 necrotic cells/HPF; 2, diffuse occurrence of 5-10 necrotic cells/HPF; 2.5, same as 2 + focal occurrence of 11-16 necrotic cells/HPF; 3,diffuse occurrence of 11-16 necrotic cells/HPF (foci of confluent necrosis); 3.5, same as 3 + focal occurrence of >16 necrotic cells/HPF; and 4, >16 necrotic cells/HPF.

Hemorrhage was graded with H&E staining with the modified method described by Schmidt et al. (23) as: 0, absent/whole pancreas; 0.5, one focus/whole pancreas; 1, two foci/whole pancreas; 1.5, three foci/whole pancreas; 2, four foci/whole pancreas; 2.5, five foci/ whole pancreas; 3, six foci/whole pancreas; 3.5, seven foci/whole pancreas; and 4, eight or more foci/whole pancreas.

The histologic evaluation and scoring were performed by two independent pathologists who were blinded to the study conditions.

Immunoelectron microscopy Study

The immunoelectron microscopy study was performed according to standard methods for mouse and human tissue sections. For mouse tissue sections, pancreatic tissue was placed in 4% paraformaldehyde with 2% glutaraldehyde for a minimum of 12 h, and cut into 50-μm thick coronal sections. For human tissue sections, paraffin-embedded pancreatic tissue was cut,

deparaffinized, and placed in 4% paraformaldehyde with 2% glutaraldehyde for 10 min. The primary antibodies used were human IgG 1:100 (ab109489, Abcam) and IgG1 (AF006, The Binding Site), following antigen retrieval with 10 mM citrate buffer pH 6.0; and IgG4 1:100 (418051, NICHIREI), following antigen retrieval with proteinase K. For detection, pre-embedding 1.4-nm gold particles (Nanoprobes, USA) were used according to the manufacturer's instructions. The sections were then post-fixed in 1.0% osmium tetraoxide in 100 mM phosphate buffer, pH 7.4, for 1 h at room temperature, then dehydrated in a graded series of ethanol solutions. After immersion in propylene oxide, samples were again immersed in a mixture (1:1) of propylene oxide and LUVEAC-812 (Nacalai Tesque, Japan) overnight, embedded in Epon812 resin according to the inverted beam capsule procedure, and polymerized at 60°C for 3 d. The tissue samples were cut into ultrathin sections (70 nm) on an ultramicrotome EM UC6 (Leica, Germany). Ultrathin sections were examined with an H7650 electron microscope (Hitachi, Japan).

References for Supplementary Materials and Methods

1 Schmidt J, Rattner DW, Lewandrowski K. A better model of acute pancreatitis for evaluating therapy. Ann Surg. 1992;215:44-56.