Trapping of Peripheral Blood Lymphocytes in the Pancreas of Patients with Acute-Onset Insulin-dependent Diabetes Mellitus

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SUMMARY

Involvement of humoral and cellular autoimmunity in the pathogenesis of insulin-dependent diabetes mellitus (IDDM) is demonstrated by the presence of circulating autoantibodies and the early pancreatic lesion of insulitis. In an effort to detect the early pancreatic lesion in acute onset IDDM, we have labeled peripheral blood lymphocytes with indium oxine 111, reinjected these autologous cells intravenously into the patients, and followed their kinetics over 96 h using an emission computerized tomography (ECT) scanner. The reinjected cells are acutely distributed in the patients' lungs, liver, and spleen (2–12 h). At 24, 48, and 72 h, the labeled lymphocytes are no longer detectable in the lungs or the liver, but are clearly present in the spleen and in the pancreas. Lymphocytic pancreatic infiltration was observed in two of three acute-onset IDDM patients, but not in large number of patients undergoing similar scans for other diseases, suggesting ongoing mononuclear cell infiltration of the pancreas, a possible marker of the lesion of insulitis. Lymphocyte scanning may provide a powerful noninvasive tool for studying patients with IDDM and for selecting those who might benefit from immunosuppressive therapy. DIABETES *31*: 463–466, May 1982.

Most authors concur that the pancreatic lesion of insulitis, i.e., infiltration of mononuclear round cells, in and around the islets of Langerhans, is seen in the majority of patients (70–90%) coming to autopsy within a few months after the onset of insulin-dependent diabetes mellitus (IDDM).[1] Round cell infiltrates, however, are not found in long-standing IDDM, suggesting that the initial acute process has subsided.[1] Similar lymphocytic infiltrates are found in other endocrine tissues known to be affected by autoimmune diseases.[2–4]

Recent studies of IDDM lend support to the involvement of humoral and cellular autoimmunity in the disease process: the presence of circulating antibodies to islet cell antigens and islet cell surface antigens is well established.[5–9] While in vitro data on cellular immunity in IDDM remains inconclusive,[10,11] some authors have reported a significant increase of circulating K lymphocytes in newly diagnosed IDDM.[12]

To better define the involvement of the patient's own peripheral blood lymphocytes (PBLs) in the lesion of insulitis, we have isolated and radiolabeled newly diabetic patients PBL's with indium oxine 111, and were able to demonstrate active trapping of labeled PBL's in the pancreas, using an emission computerized tomography (CT) scanner.

CASE REPORTS

Case 1. A 14-year-old white male presented to Joslin Diabetes Center on April 6, 1981 with a 4-wk history of polyuria, polydypsia, weakness, pruritis, weight and strength loss. Glycosuria and hyperglycemia (>400 mg/dl) were documented 1 wk prior to presentation. He was on no therapy. The physical examination was normal.

The patient was admitted to the Diabetes Treatment Unit and was started on insulin. He rapidly regained weight and his glycohemoglobin fell from 17.1% at admission to 14.0% 1 wk later.

On April 13, 1981, the patient underwent emission computerized tomography scanning of the pancreas following injection of indium oxine-labeled PBL's as outlined below (Figures 1 and 2), according to a protocol approved by the Research and Human Studies Committees of the Joslin Diabetes Center and the New England Deaconess Hospital. The process of emission tomography adds no additional radiation beyond that used to label the patient's lymphocytes. (Figures 1 and 2)

At a later date, a liver-spleen scan was obtained with 2 mCi of Tc sulfur colloid with scintigraphy and tomographic images obtained in the same plane and projection. For anatomic correlation, an X-ray CT scan of the abdomen with oral

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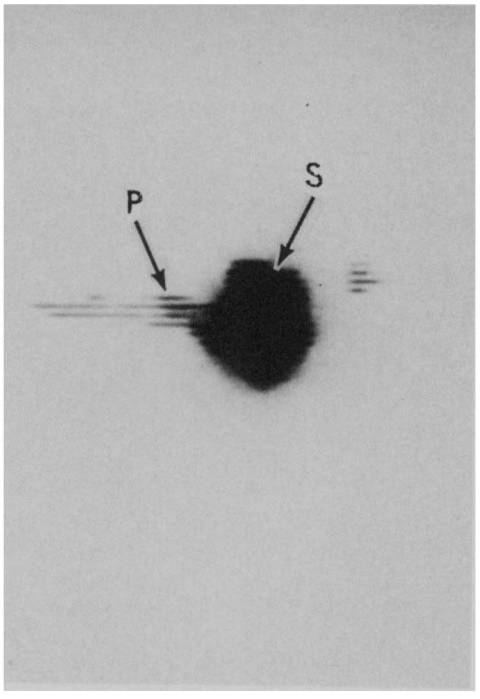

FIGURE 1. Emission computerized tomography scanning of ^{111}In-labeled autologous lymphocytes migrated into case #1 patient's pancreas (P) and spleen (S), at 24 h. The image was identical at 48 and 72 h. The uptake is greater in the tail of the pancreas.

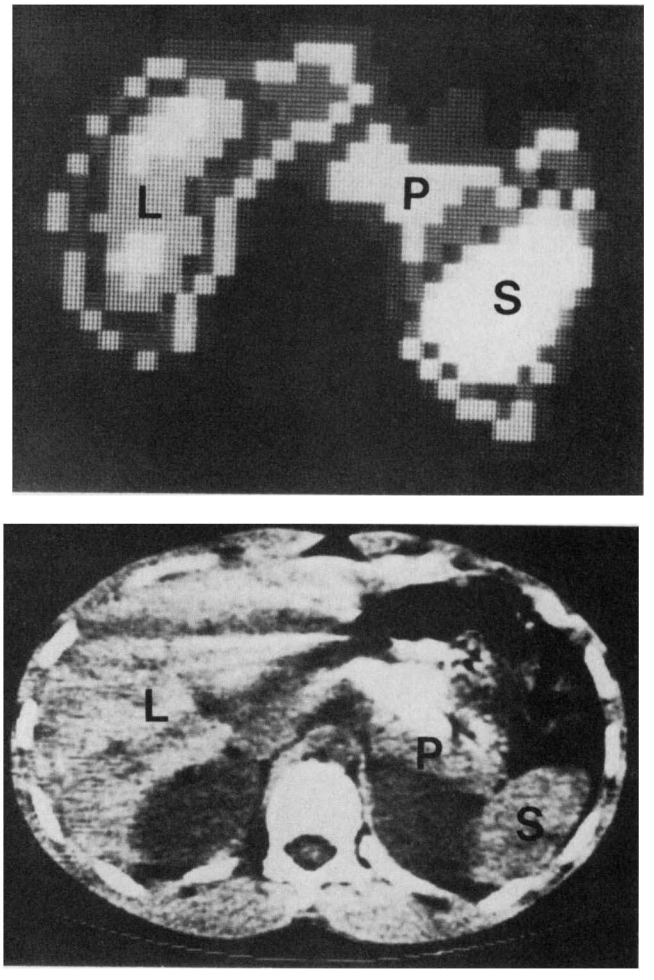

FIGURE 2. Anatomic correlation between abdominal X-ray CT scan and emission CT scan of case #1. Single photon ECT body images of the indium oxine scan and liver-spleen sulfur colloid scan combined by computer processing on A Digital PDP-11 computer. (S = spleen; P = pancreas; L = liver).

contrast was obtained on a General Electric 8800 Body CT scanner and the slice shown was selected in the region of indium oxine and sulfur colloid tomographic studies (Figure 2).

Case 2. This is a 10-yr-old white male with a 3-wk history of acute-onset IDDM accompanied by a weight loss, extreme weakness, and a blood glucose of 488 mg/dl on admission. His physical examination was otherwise unremarkable. He was started on insulin and 4 days after admission to the Diabetes Treatment Unit he underwent a pancreatic scan.

Case 3. This 37-yr-old white male presented to the Joslin Diabetes Center with a 7-day history of acute-onset IDDM with polyuria, polydypsia, weight loss, and ketonuria. His daughter had also developed acute-onset IDDM 40 mo earlier.

His physical examination was noncontributory and his admission laboratory values included glucose of 465 mg/dl and hemoglobin A1c of 13.3%.

He was treated with insulin with remarkable improvement. Six days after admission, he underwent emission CT scanning of his pancreas (Figure 3).

METHODS

Preparation of PBL's. Forty-five milliliters of heparinized venous blood are obtained from the patients. PBL's are separated using a ficoll-hypaque gradient. The cell yield is usually excellent: 65–75 × 10^6 lymphocytes. The lymphocytes are then incubated with 80–290 μCi of ^{111}In oxine (Medi-Physics, Emoryville, California) for 30 min, washed thoroughly, resuspended in 100 ml of normal saline solution, and then injected intravenously into the patient. Cell viability has consistently ≥90% by vital dye exclusion.

Scanning. Images were obtained at 6, 24, and 48 h after the reinjection of the labeled lymphocytes. Scintigraphy was obtained on a large field of view with a 37 tube Angle Camera, and tomographic images were obtained on the Harvard Multi Detector Body System. The single photon CT body scanner is an instrument consisting of a circular configuration of 10 sodium iodine detectors: 20 cm × 12.5 cm × 2 cm, each equipped with focus collimators. Each detector scans the circular field of view tangentially with its focal point moving toward the object. After a line is scanned, one detector is moved radially outward while its opposing detector is moved inward in the same direction. The next tangential line is then scanned. Each of the ten detector scans have a total field of view. Entire ring of detectors is then rotated 18° and the same pattern is repeated resulting in 20 projections at 18° intervals for the reconstruction of the transverse slice. The scan time is 5 min per slice. Detailed performance characteristics are published elsewhere.[13,14]

The single photon ECT body images of the indium oxine and sulfur colloid were computer-processed on a Digital PDP-11 computer with count normalization and superimposition of the images to represent a composite of the sulfur

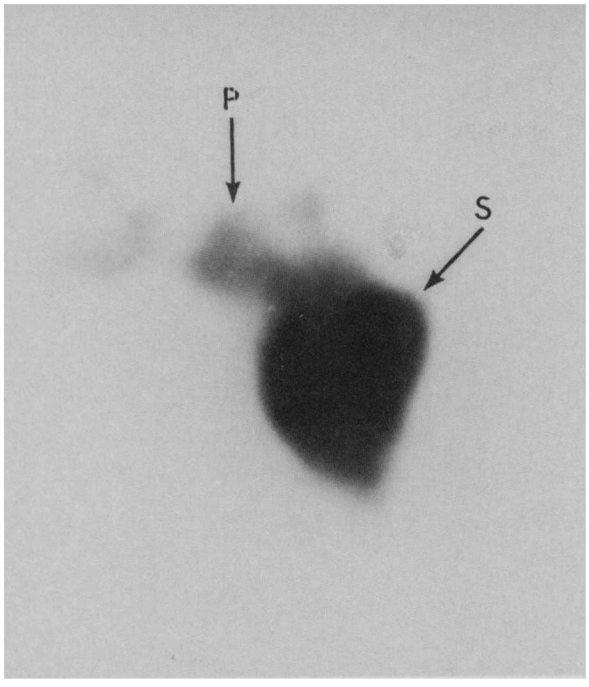

FIGURE 3. Emission computerized tomography scanning of ^{111}In-labeled autologous lymphocytes migrated into case #3 patient's pancreas (P) and spleen (S) at 24 h. The image was identical at 48 and 72 h. Most of the pancreatic uptake is observed in the tail of the pancreas.

colloid liver and the indium oxine pancreas and spleen uptake (Figure 2).

RESULTS

After the intravenous injection of radiolabeled cells, whole-body scans were performed immediately, at 6, 24, and 48 h. In all three patients, labeled lymphocytes were initially traced into the lungs, liver, and spleen. However, the pulmonary and hepatic uptake was transient, and most of the radioactivity (≥90% of the counts) was detected in the spleen.

In case nos. 1 and 3, indium uptake was detectable in the pancreas. Furthermore, the image matched the pancreas on computerized tomography images of the patient's abdomen (Figures 1, 2, and 3).

No pancreatic uptake was observed following injection of 99 Tc sulfur colloid, ruling out nonspecific uptake. Also, no pancreatic uptake was observed in a large number (N > 20) of patients undergoing diagnostic abdominal emission tomography scanning following injection of indium or gallium-labeled white blood cells. Finally, the image obtained with indium-labeled cells uptake, coincides exactly with the image of the patients pancreas obtained with computerized tomography (CT) scanning (Figure 2).

DISCUSSION

Indium 111 is an excellent cell marker for clinical use: it enters the cell by chelation with lipid soluble hydroxyquinolone (oxine); it leaks out slowly; it has a half-life that is long enough for most cell traffic studies (67 h); and the labeled cells remain viable. Finally, their emission is strong enough to allow the detection of labeled cells' accumulation with a gamma camera in deep tissues.[15–19]

In the absence of retroperitoneal pathology—no lymphadenopathy or organomegaly detected by abdominal CT scans—and without clinical or laboratory evidence of pancreatitis, it is likely that radiolabeled lymphocyte uptake by the pancreas reflects the lesion of insulitis associated with acute IDDM.

Endocrine tissues are particularly likely to be affected by organ-specific autoimmune disease. Early infiltration by mononuclear cells has been documented in the pituitary,[3] thyroid,[2] and pancreatic islets.[1] This infiltration of the islets may coincide with the presence of various autoantibodies: anti-cytoplasmic antibodies,[5–7] anti-islet cell surface antibodies,[8] and complement fixing antibodies.[9]

Our findings document for the first time the actual "homing" and trapping of the patients own lymphocytes in the pancreas.

Despite the reported increase of circulating killer lymphocytes in acute IDDM,[12] the role of cellular autoimmunity in the pathogenesis of IDDM remains to be understood. Data derived from animal models of insulitis suggest that T cells play a major role in this lesion which could be attentuated or prevented with specific immunosuppressive therapy.

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