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Light Scattering Analysis of Rat and Mouse Islet Cells in the Fluorescence-activated Cell Sorter

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INTRODUCTION

The complexity of the endocrine pancreas being composed of at least four cell types, insulin-, glucagon-,somatostatin- and pancreatic polypeptide (PP)-producing cells, was early appreciated in numerous studies by light- and electron-microscopy (22). While the B- and A-cells were identified as the cells producing insulin and glucagon, respectively, there was a remainder of cells not fulfilling the specific staining criteria. In 1960 Hellerström and Hellman (5) classified cells stained by a modified silver impregnation technique as A_1 -cells to distinguish them from the A_2-cells which remained silver negative. More recent studies by light- and electron-microscopic immunocytochemistry suggest that the A_1- or D-cells contain somatostatin (3,9) and have revealed the presence of islet PP-cells (11). The B-cell predominates the endocrine cell population throughout the pancreas and it was noted that the D-cells were more often in a close association with B-cells than with the A-cells in a variety of species (7). In addition, the A-cells are primarily found in islets of the splenic part, while PP-cells predominate in the duodenal portion of the pancreas (1).

The possibility that the A- and D-cells would act as local regulators of insulin secretion (6,7), a concept further substantiated by the demonstration of specific cell contacts between endocrine islet cells (21), prompted us to prepare single cell suspensions from isolated pancreatic islets (8,12) with the view of isolating each individual cell type. It would then be possible to investigate cell-cell interactions in reconstitution experiments.

We are analyzing and sorting suspensions of islet cells in a Fluorescence -activated Cell Sorter (FACS III or IV, Becton-Dickinson, California (4) on the basis of their light scattering properties. In the present study the ability of mouse and rat islet cells to scatter light was compared.

METHODS

The Fluorescence-activated Cell Sorter utilizes single cell suspensions to be ejected in a stream which passes an incident laser beam before the stream is broken into droplets trapping individual cells. Islets of Langerhans were prepared by collagenase-digestion (10,18) of the pancreas from rats or mice using the protocols indicated as follows.

Sprague-Dawley rats (150-200 g) were injected with pilocarpine about one hour before sacrifice and the minced pancreata incubated with collagenase (13). Following Ficoll-gradient centrifugation the islets were washed by centrifugation and maintained in a modified Swim's S-77 medium containing 1 mmol/l ethylene -glycol-bis(oxy-ethylene nitrilo)tetra acetic acid (EGTA) and 10 g/l bovine serum albumin (14,19). The islets were individually collected under a stereomicroscope and dispersed into single cells by mechanical shaking (12,14).

Adult NMRI-mice were fasted overnight before sacrifice. The intact pancreata were subjected to collagenase-digestion by repeated incubations, each with vigorous shaking. The first incubation was for 20 min and the following 5-6 incubations were for 5 min each; only the undigested tissue was transferred. After each incubation period the supernatant containing free exocrine cells and islets was removed and cells and islets washed by sedimentation before the islets were separated from the remaining digest by centrifugation on a Percoll gradient (2). The islets were individually collected under a stereomicroscope and transferred to Swim's medium with EGTA and 10 g/l bovine serum albumin (BSA). Single cells were prepared by pipetting the islets repeatedly (5-6 times) through a Pasteur -pipette melted to a blunt-ended tip with a small (0.1-0.5 mm) orifice.

The dispersed cells from either rat or mouse islets were added to a gradient of albumin composed from bottom to top of 1 ml, 300 g/l and 10 ml, 40 g/l BSA in Swim's medium (19). After centrifugation for 10-20 min at 50xg the cells were harvested from the 40/300 g/l BSA interphase. The cells were diluted in Swim's medium with 40 g/l BSA and pelleted by centrifugation (50xg, 10 min). The cells (10^5-10^6 cells) were carefully resuspended in a modified Hank's buffer (19) containing 100 g/l BSA and placed on ice until they were diluted with one volume phosphate-buffered saline before analysis in the Fluorescence-activated Cell Sorter (FACS IV, Becton Dickinson). The FACS IV was aligned for both forward low angle scattering (1-15°) and fluorescence (>520 nm) prior to each analysis as described (19). Glutaraldehyde-fixed chicken red blood cells (CRBC) were used for this purpose allowing comparisons to be made from experiment to experiment (15,16).

RESULTS AND DISCUSSION

Forward-angle light-scattering analysis showed that live cells from either rat or mouse islets were contained within a broad peak (Fig. 1). The islet cells were distinctly separated from dead cells, cellular debris and other particles of low scatter intensity (19). The scatter intensity of the islet cells was greater than that of the CRBC used to align and standardize the instrument in each experiment. The histogram of the CRBC is composed of two peaks, the major peak is seen to the left of the broad islet cell peak (Fig. 1). The CRBC preparation used has proved stable for several years allowing the same batch to be used throughout our experiments.

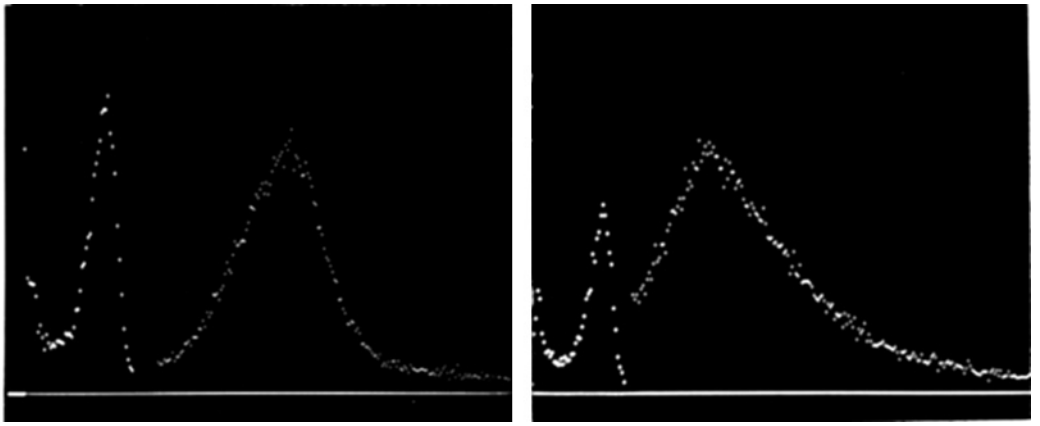

Fig. 1. Histogram of near forward angle light scattering of mouse (left) and rat (right) islet cell suspensions. The abscissa shows the relative intensity of the light scattering signal and the ordinate the number of cells. The major peak of glutaraldehyde-fixed chicken red blood cells (the distinct peak to the left in each histogram) is also shown. 4.1×10^4 mouse and 5.8×10^4 rat islet cells were analyzed.

Taking the major peak of the CRBC-preparation as 1, the scatter intensity of the islet cells in the peak channel was calculated (Table 1).

Table 1. Forward low angle light scatter of islet cells

Cell type	No. of experiments	Light scatter intensity
Chicken Red Blood Cells (CRBC)		1
Mouse islet cells	6	2.8 ± 0.04
Rat islet cells	4	2.2 ± 0.1

The difference between mouse- and rat islet cells is significant $p < 0.001$ (t-test). Mean ± SE for the number of experiments shown.

The analysis of islet cells by light scattering in the FACS flow cytometer showed that the histogram derived from the light scattering at a forward low angle was well-defined and reproducible. The peaks of rat and mouse islet cells had greater light-scattering intensities than the peaks produced by the CRBC or other cell types such as exocrine cells, erythrocytes, spleen cells or lymph node cells (19,20). Since several of the latter cells have a diameter similar to the islet cells, the forward low angle light scattering at 488 nm, the wave length generated by the argon laser, seems to be dependent upon a variety of parameters such as cell and nuclear size and symmetry, in addition to physical parameters such as refractive index and absorption by cellular constituents (15,17). The difference in light scattering between rat and mouse islet cells remains to be explained but may simply reflect species differences in biochemical composition and structure.

However, the relative contribution of the various endocrine cell types may also be important, in addition to differences in morphology and granule structure.

The complex mixture of single cells from either rat (19) or mouse (20) islet cells has been analyzed by sorting cells from various parts of the histogram shown in Fig. 1. The sorted cells were subjected to radioimmunological determination of islet hormones, electron microscopy as well as metabolic labelling of the sorted cells (19). In the latter experiments sorted cells are incubated with ^{35}S-methionine to allow labelling of hormonal precursors (19). There was a heterogenous distribution of the different endocrine cell types within the light scattering peak (19,20). Cells containing and synthesizing glucagon were found in the left slope, somatostatin in the far right slope, while insulin was confined to the center of the peak. The results indicate that A-cells have a lower ability to scatter light than B-cells, while the D-cell light scattering is more

intense, indicating that endocrine islet cells can be partly resolved into enriched cell populations by flow cytometry and sorting.

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