

# A Simple Methodological Principle for Large Scale Extraction and Purification of Collagenase-Digested Islets

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**L**ARGE SCALE preparation of purified islets of Langerhans represents an obstacle, especially for human islet transplantation. At present, collagenase digestion and Ficoll density gradient preparation achieve only a 10% to 40% yield of purified endocrine tissue.<sup>1</sup> Hand-picking, well established in rat and mouse models, may provide sufficiently purified preparations. But for the number of islets needed in human transplantation, this method is far too consuming in both man power and time and thus, unacceptable. On the other hand, recent findings<sup>2</sup> demonstrated that certain lectins bind selectively to the exocrine portion of pancreas tissue sections (Table 1). Therefore, it was reasoned that it should be possible to use such lectins for islet separation, and a new methodological principle, consisting of magnetic microspheres (MMS) coupled to lectins of adequate specificity, was investigated in preclinical model studies.

## MATERIAL AND METHODS

### *Rat Islets*

Rat islets were isolated from male 2- to 3-month-old LEW (RT<sup>1</sup>) rats by collagenase digestion according to the technique of Lacy and Kostianovsky.<sup>3</sup> After Ficoll density centrifugation of the digested material, the crude islet preparation was microscopically checked for sufficient digestion and the islets were counted.

### *Human Islets*

Human islets were isolated from cadaver pancreata according to the technique of Gray et al.<sup>1</sup> The preparation contained 10% to 40% endocrine tissue consisting of large and small single viable islets. The majority of the preparations consisted of exocrine debris, small blood vessels, and occasionally, small lymph nodes.

### *Preparation of MMS and Lectin Coupling*

The MMS were prepared by modification of the basic procedure described recently.<sup>4</sup> Briefly, 7.5 mg serum albumin and 18 mg Fe<sub>3</sub>O<sub>4</sub> are dissolved in 250 µL distilled

water, mixed with 10 mL cottonseed oil, and sonicated in an ice water bath six times for one minute each. The emulsion is immediately squirted into 120 mL oil at 120 °C. The heat-stabilized MMS are purified of oil by washings with ether. Thereafter, they are activated with 2.5% glutaraldehyde and, after several washings, incubated with a lectin (100 µg lectin/mg MMS) in phosphate buffered saline (PBS) for two hours. Finally, they are washed with PBS and resuspended in RPMI medium. The following lectins were used: maclura pomifera agglutinin (MPA), ulex europaeus agglutinin-I (UEA-I), and soybean agglutinin (SBA).

### *Magnetic Separation Procedure*

Approximately 1,500 nonpurified rat islets were incubated with 5 to 6 mg UEA-I-coupled MMS in 5 mL RPMI/2.5% fetal calf serum (FCS) for 25 minutes at 22 °C. Binding of these MMS to exocrine cells was checked microscopically. The suspension was transferred into a 50-mL culture flask containing 20 mL RPMI/5% FCS. This flask was positioned in an electromagnetic field of 800 Gauss for four minutes and 1,400 Gauss for two minutes. Thereafter, purified islets were collected from the bottom of the flask, while exocrine tissue bound to MMS-lectin complexes and unbound complexes remained attached to the flask wall in the magnetic field (Fig 1). The procedure was repeated several times. Each batch of separated islets was analyzed microscopically for purification status and yield.

### *Immunofluorescence Studies of Isolated Islets*

Based on the reactivity pattern of the various pancreas tissue portions with different lectins, outlined in Table 1A, the purity of rat and human islet preparations was evaluated. Fifty rat islets or 100 µL of human islet suspensions were incubated with 100 µL of fluorescinated

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**Table 1. Binding Pattern of Lectins to Pancreatic Tissue. Immunofluorescence Staining of Tissue Section Portions Following Incubation With Fluorescinated Lectin\***

Tissue	Rat			Human		
	MPA	UEA-I	SBA	MPA	UEA-I	SBA
Endocrine	++	0	0	0	++	++
Exocrine	++	++	++	++	++	++
Connective	+	0	++	++	+	++

\* ++, strong; + weak; 0, none.

(FITC)-labeled lectin (1:5 diluted with PBS, 30 minutes, 4 °C in the dark). After three washings, islets were placed on glass slides with 10  $\mu$ L veronal buffer (pH 7.2). A cover slide was sealed with Eukitt-adhesive. Immunofluorescence was determined under an inverted light Zeiss microscope.

### Transplantation of Rat Islets

Islets were transplanted syngeneically via the portal vein into the liver of male LEW rats, made diabetic by a single intravenous (IV) injection of 55 mg/kg streptozotocin. Nonfasting blood glucose levels of the recipients were measured daily for the first ten days and then once weekly.

## RESULTS

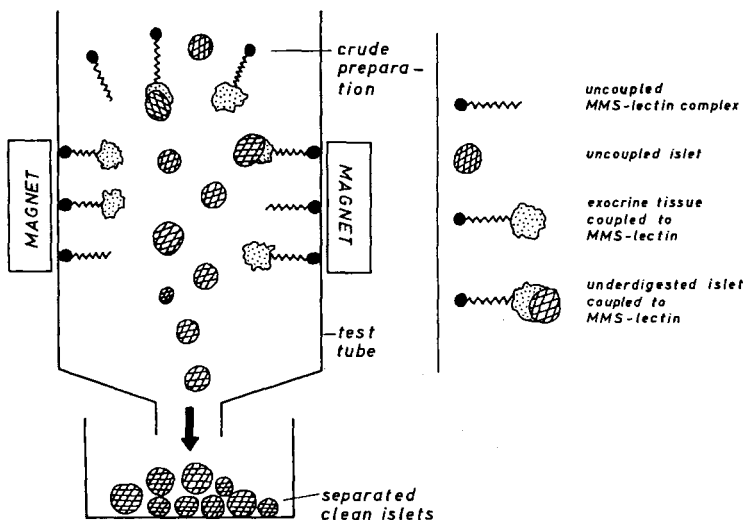
### Binding Pattern of Lectins

The data of Petersen et al<sup>2</sup> on lectin-binding patterns of pancreatic tissue sections were fully confirmed in our MMS-binding and immunofluorescence studies with disintegrated rat and human tissue suspensions, as shown in Table 1. For example, crude rat islet prepara-

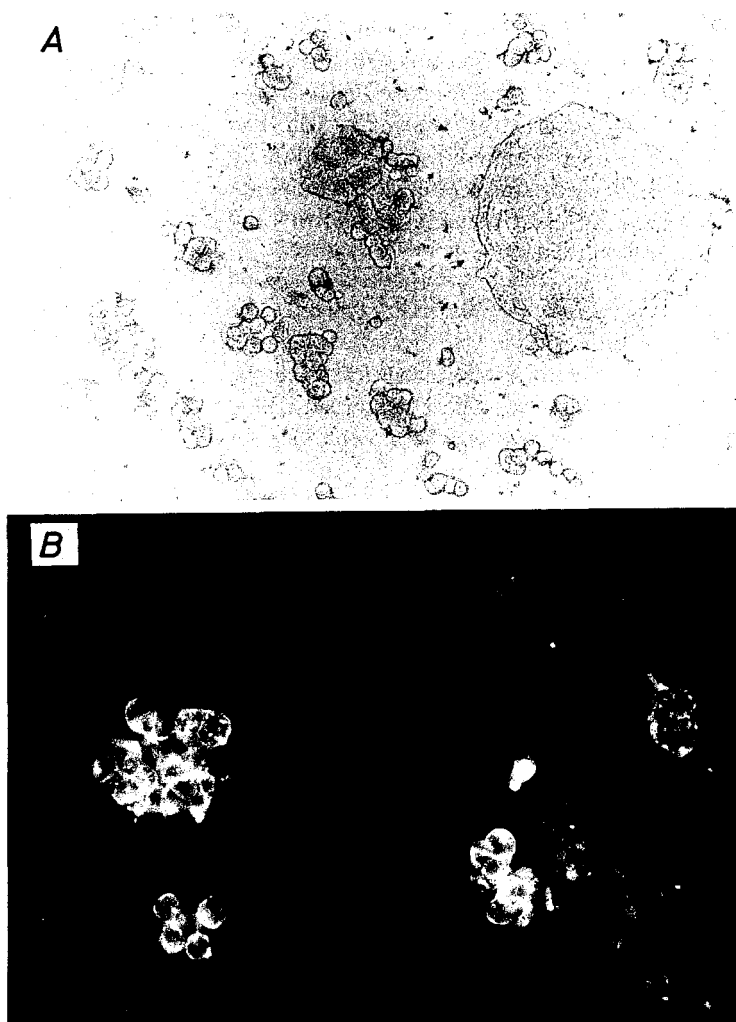
tions with UEA-I-coupled MMS demonstrated that many microspheres were clearly attached to the exocrine material, whereas only a few or none could be observed on the islet surface (Fig 2A). Figure 2B shows the corresponding immunofluorescence staining pattern with UEA-I. Similarly, the MPA-binding pattern of human islet suspensions was seen to be restricted to the nonendocrine tissue portions in both kinds of microscopical investigation (data not shown).

### Purity and Yield of MMS-separated Rat Islets

In general, the purity of separated islets was found to be in the order of >98% and the yield in the order of 75% (Table 2). However, both parameters were clearly codetermined by the grade of disintegration obtained by the preceding collagenase digestion of the pancreatic tissue and the power of the magnetic



**Fig 1. Composition of the various MMS complexes and principle of islet separation.**



**Fig 2.** Crude rat islet preparations. (A) Light microscopy; MMS-lectin complexes bind to exocrine, but not to endocrine tissue; (B) fluorescence microscopy; UEA-I reactivity is only detectable on exocrine, but not on endocrine tissue preparations.

**Table 2.** Separation Efficiency of Lectin-coupled MMS: Purity, Yield, and Viability Data of Separated and Isografted LEW Rat Islets

Graft Recipient (animal no.)	Nonpurified Islets (Crude Preparation)		Microsphere-separated Islets (Purified Preparation)			Islet Graft Survival (d)
	No. of Islets	Purity (%)	No. of Islets	Purity (%)	Yield (%)	
1	1,700	40	1,265	>98	74.4	>170
2	1,530	40	1,135	>98	74.2	>155
3	1,550	35	870	>98	56.1	>127
4	1,130	40	846	75	75.0	>81
5*	700	30	516	>98	73.8	>72
	900	35	684	>95	76.0	
6	2,056	35	1,314	>95	64.0	>40

\*Animal no. 5 was regrafted two days after the first transplantation.

field. For example, in experiment no. 3 in which the crude islet preparation contained larger numbers of underdigested islets (confirmed microscopically), the final yield was only 56.1%. In experiment no. 4 with regularly digested islets, the degree of purity was only 75%, since an electromagnetic field of only 800 Gauss was used.

#### *Viability and Functional Integrity of Separated Rat Islets*

Immediately after separation, the viability of rat islets was investigated in trypan blue dye exclusion tests. Approximately 98% of islets were found to be viable. The functional integrity of these islets was demonstrated by grafting and survival time in syngeneic diabetic recipients. Normoglycemia reoccurred within the first 24 hours after transplantation in all graft recipients except animal no. 5, which required regrafting. As seen from the data in Table 1B, a number of islets, in the order of 1,000, allowed long-term restitution of streptozotocin-diabetic animals. This number is known from our extensive earlier studies to be required when hand-picked islets are grafted. Only much smaller numbers were found to be insufficient, requiring regrafting (see experiment no. 5), which is also in accordance with our experience in grafting hand-picked islets.

#### *Preliminary Studies With Human Islet Suspensions*

The binding pattern of MPA-FITC to human islet preparations and, thus, their MMS separation potential, depended strictly on the amount of exocrine tissue still being attached to the endocrine material after collagenase digestion. Using freshly prepared islet suspensions, MPA staining was still found at the periphery of the endocrine tissue as revealed by immunofluorescence microscopy. However, this staining pattern changed significantly when islets were cultured for ten hours (37 °C, RPMI/10% FCS, 5% CO<sub>2</sub> in air): MPA reactivity could only be observed on

residual exocrine tissue, but only very rarely and only in minute amounts on endocrine tissue, which in general appeared completely dark (unstained) in UV light.

#### DISCUSSION

In spite of a recent suggestion that exocrine debris contaminating mouse islet allografts may be nonimmunogenic,<sup>5</sup> there is some evidence that this may not hold for human exocrine tissue: acinar cells and vascular endothelial cells from crude human islet suspensions express major histocompatibility complex (MHC) class II antigens,<sup>6</sup> which are known to greatly contribute to allograft immunogenicity. Thus, separation of endocrine material from exocrine debris continues to be a prerequisite for successful islet allografting, particularly in the human. The approach presented here with MMS-coupled lectins appears to fulfill this request, since it is effective, simple, and especially, fast. The degree of islet purity, studied in the preclinical rat model, nearly reaches hand-picking quality, which is known to be satisfying. The good visibility of MMS particles in the light microscope allows for an optical control of the binding specificity during the separation procedure. In all preparations, unspecific MMS binding to clean islets was hardly observed. However, the final yield of islets did not exceed 75%. It may be suggested that some 25% of the islets still contained exocrine cells undetectable in the light microscope, but detected by the magnetic field. The data of graft recipient no. 3 support this hypothesis. Therefore, it is expected that a reduction of the magnetic field power may improve the final yield, although at the expense of the purity of the preparation (eg, animal no. 4).

The long-term survival of MMS-separated rat islets in syngeneic recipients clearly indicates that their function is unaltered by the technical procedure. It may be pointed out that the same limiting number of MMS-separated islets, as required in former experi-

ments with hand-picked grafts (approximately 800 islets for a 250-g animal), was required for restitution of diabetic recipients to normal glucose levels.

There is good reason to expect that the satisfying rat data also hold for human islet grafts, since exactly the same technical separation procedure is applied. However, one problem still to be solved, consists in the fact that a number of human islets remain attached to exocrine tissue portions after enzymatic digestion. A second milder approach with collagenase should solve this problem in possible combination with the above described short-term cultivation proce-

dures. First attempts in this direction, including our present data, appear promising.<sup>7</sup> After having solved the digestion-dependent problem of purity, the MMS separation procedure will help to solve the problem of quantity in human islet transplantation, since hand-picking is impracticable in the clinical situation for obvious reasons. Considering the fact that both the technique of human islet isolation and the magnetic separation with MMS-coupled lectins are recent and promising, it is hoped that the field of human islet transplantation profits from the combination of such methodological approaches in the near future.

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