Lectin Binding to Acinar Tissue for Complete Magnetophoretic Purification of Porcine Pancreatic Islets Depends on the Composition and pH of the Incubation Medium

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HIGH-grade purification of large amounts of crude collagenase-digested pancreatic islet preparations remains one of our major goals in future clinical islet transplantation. Behind our strategy lies the knowledge that exocrine tissue debris may damage the islet graft[1] and be highly immunogenic.[2] It is based on the methodology of magnetic cell separation.[3] This methodology has been adapted step by step to make it applicable to islet purification, including the important aspect of the possible use of xenogeneic islet donors.[4-6] In the past 3 years, our efforts to evaluate this method's potential for large-scale islet purification to satisfy future clinical requirements have been limited by the well-known problems involved in isolating large amounts of well-preserved and viable islets from the porcine pancreas.[7] However, our modification of the basic semiautomated isolation technique[7,8] made it possible to obtain large enough amounts of crude porcine islets for experimental analyses. This study is a report of our efforts to optimize further methodologic parameters that appear to be important for high-grade magnetic purification of porcine pancreatic islets.

This includes an analysis of the binding of our magnetic inducible microspheres (MIMS), which are coupled to the exocrine tissue-specific lectin Wistaria floribunda agglutinin (WFA). Investigated were various parameters of the incubation medium, that is, its composition, pH, and temperature. It is suggested that these parameters must be separately evaluated for each lectin and each xenogeneic islet donor species.

MATERIALS AND METHODS

Pancreatic Islet Isolation and Prepurification

Porcine pancreatic islets were isolated with the help of the semiautomated isolation technique described by Ricordi et al,[8] which was further modified in our laboratory.[7] Prepurification of crude islets was achieved using a discontinuous Euro-Ficoll density gradient.[9] The prepurified islets showed a purity of 40% to 80%. They were then submitted to magnetic cell separation. To differentiate islets from exocrine cells, crude islets were stained with dithizone.[10] The viability of the islets was determined by costaining them with fluorescein diacetate and propidium iodide (FDA/PI).

Preparation of MIMS and Lectin Coupling

MIMS were composed of heat-stabilized albumin and iron oxide (Fe_3O_4; Ferrofluid, Ferrofluidics Corp, Nashua, NH).[3] MIMS with a diameter of 1 ± 0.1 μm were prepared with a high pressure homogenizer (APV Gaulin GmbH, Luebeck, Germany). One milligram of tosylchloride-activated MIMS was coupled to 15 μg WFA lectin (Medac, Hamburg, Germany). These complexes were stored at 4°C in phosphate-buffered saline (PBS) containing 0.02% sodium azide, 1% bovine serum albumin, and 0.05% Tween. Stored like this, they retained their binding activity for more than 1 year.

Preincubation With D-Glucose

Before MIMS-WFA complexes were used for islet purification, they were incubated (24 hours, 4°C, slow rotation) in PBS that contained 2% D-glucose (pH 7.4). Thereafter, MIMS-WFA complexes were washed several times with sterile PBS and resuspended in Hank's balanced salt solution (HBSS) to be analyzed for altered binding specificity.

Magnetic Separation Procedure

Approximately 5000 to 7000 prepurified islets were incubated in various media with 0.3 to 5.0 mg MIMS-WFA complexes depending on the residual amount of exocrine tissue within the prepurified crude preparation: approximately 0.3 to 1.0 mg MIMS-WFA for 20% to 30% and 2.0 to 5.0 mg MIMS-WFA for 40% to 60% exocrine tissue. Incubation was carried out in 50-mL Falcon tubes for 30 minutes by slow rotation. The binding of MIMS-WFA complexes was evaluated microscopically. The strength of binding was expressed as the percent of complexes bound to exocrine (specific) or endocrine tissue (nonspecific). The islet suspension was then transferred into 50-mL culture flasks. Separation was carried out by positioning the flask vertically into a magnetic field (intensity $\approx 2 \times 10^3$ Oerstedt, inhomogeneity 10^3 Oerstedt/cm) for 10 to 15 minutes (stationary). Subsequently, the purified islets were collected from the bottom of the flask, while exocrine tissue debris, reacting with MIMS-WFA complexes, remained attached to the wall of the flask in the magnetic field. The procedure was repeated three times. Finally, the purified islets were resuspended in culture medium. The purification status and viability of each batch of separated islets were examined microscopically.

Incubation Media

The influence of three different incubation media on the binding specificity of MIMS-WFA complexes was tested: (a) complete RPMI-1640 (Gibco, Heidelberg, Germany) supplemented with 5% fetal calf serum (FCS; Conco, Wiesbaden, Germany), (b) complete CMRL-1066 (Gibco) supplemented with 5% FCS, and (c) serum-free medium (SF-3365; Serva, Heidelberg).

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Table 1. Analysis of Various Parameters for Optimized Magnetic Purification of Isolated Crude Porcine Pancreatic Islets: Preincubation of MIMS-WFA Complexes with D-Glucose (n = 5)

Preincubation in HBSS	MIMS-WFA Binding to Exocrine Tissue (%)	MIMS-WFA Binding to Endocrine Tissue (%)
Without glucose	30–70	0
With glucose	95–99	0

Note. Specificity and strength of binding were evaluated microscopically.

RESULTS

It is well known that optimum binding of lectins to their target structures (mainly carbohydrates) depends on a variety of parameters, for example, metal ions, ionic strength, temperature, and pH.[11] We therefore decided to investigate the influence of D-glucose on the specificity and strength of binding of MIMS-WFA complexes to crude porcine pancreatic islet preparations. MIMS-WFA complexes were preincubated in FCS-free HBSS medium with or without D-glucose. The result of the preincubation experiment was determined microscopically by the quality of subsequent binding of the complexes to a crude islet preparation. Table 1 documents the results, which may be summarized as follows: In contrast to a preincubation medium containing no D-glucose, addition of 2% D-glucose to the preincubation medium had a significant effect. It strongly increased both the specificity and the strength of binding of the MIMS-WFA complexes to the exocrine tissue in the crude islet preparation. Addition of 1% D-glucose was not sufficient to improve the binding, whereas the effect of 10% D-glucose resembled that of 2%.

In the second investigation we analyzed the effect of the composition of three different incubation media on the specificity and strength of binding of MIMS-WFA complexes (preincubated with D-glucose) to the porcine acinar cells as well as their purification potential during magnetic separation. The results were evaluated microscopically and are documented in Table 2. Incubation of MIMS-WFA complexes with crude islet preparations in two different media supplemented with 5% FCS, namely, RPMI and CRML, resulted not only in weak binding to porcine exocrine cells (30% to 70%) but also in nonspecific binding, that is, 1% to 5% binding to endocrine islet cells. In contrast to the above findings, a serum-free medium had a positive influence on the binding specificity (no binding to endocrine cells) and the binding strength (95% to 99%) of the MIMS-WFA complexes to their target cells. As to be expected from these data, purification in RPMI or CMRL media was poor, whereas purification in serum-free medium was optimal, that is, islets showed a purity of more than 99%.

Table 2. Analysis of Various Parameters for Optimized Magnetic Purification of Isolated Crude Porcine Pancreatic Islets: Comparison of Different Incubation Media (n = 7)

Incubation Medium (pH 7.4)	MIMS-WFA Binding to Exocrine Tissue (%)	MIMS-WFA Binding to Endocrine Tissue (%)
RPMI 1640 + 5% FCS	30–70	1–5
CMRL 1066 + 5% FCS	30–70	1–5
Serum-free medium	95–99	0

Note. Specificity and strength of binding were evaluated microscopically.

Table 3. Analysis of Various Parameters for Optimized Magnetic Purification of Isolated Crude Porcine Pancreatic Islets: Comparison of Different pH Values in Serum-Free Medium (n = 10)

Islet Preparation at pH	MIMS-WFA Binding to Exocrine Tissue (%)	MIMS-WFA Binding to Endocrine Tissue (%)	Islet Viability (%)*
7.0	50–65	0	50
7.2	60–70	0	80
7.3	70–90	0	92
7.4	95–99	0	98
7.5	95–99	0	93
7.6	99	0	80
7.8	99	1–5	50

Note. Specificity and strength of binding were evaluated microscopically.
*Islet viability was determined by costaining with FDA/PI.

The third investigation evaluated the influence of the pH of the medium on the binding parameters and on islet viability. For this purpose, incubation of MIMS-WFA complexes with crude porcine islet preparations was performed in serum-free medium at seven different pH values. Table 3 documents the results of this study, which may be summarized as follows: (a) The strength of specific binding of MIMS-WFA complexes to porcine exocrine cells gradually increases from 50% to 65% at pH 7.0 to 99% at pH 7.6. (b) However, binding specificity decreases with increasing pH values above pH 7.6, as can be seen from MIMS-WFA complexes that not only bind to exocrine cells, but also to endocrine islet cells (1% to 5% bound cells at pH 7.8). (c) Islet viability is highest at pH 7.4 (98%). Decreasing pH values (from 7.4 to 7.0) and increasing pH values (from 7.4 to 7.8) diminish islet viability to 50%. Thus, incubation at pH 7.4 not only shows the best binding quality, but also the best islet viability.

The fourth investigation, which is not documented in the tables, regarded the influence of the incubation temperature of a serum-free medium at pH 7.4 on complex binding. Variation of the incubation temperature from 4 to 24°C did not result in altered binding quality or altered islet viability, that is, it had no influence on the high quality binding of MIMS-WFA complexes to exocrine tissue debris.

DISCUSSION

The finding that preincubation of the MIMS-WFA complexes with D-glucose prior to their use in islet purification increases the specificity and strength of binding of the WFA lectin to exocrine cells is not well understood. It may be related to an allosteric alteration of the conformation of the WFA-specific binding site for N-acetyl-galactosamine[11] due to saturation of the lectin's other binding

site(s) by D-glucose. Changes in a lectin's biologic behaviour have been observed with Sophora japonica lectin as a result of treatment with a specific sugar.[12] Furthermore, the improved binding could be caused by eliminating nonspecific lectin binding to D-glucose-related carbohydrate structures on the exocrine and endocrine cell surface.

We observed that FCS in the incubation medium decreased the binding specificity of MIMS-WFA complexes to exocrine cells. Thus far it is unclear which components in the FCS, which is commonly used to supplement incubation media, are responsible for reducing the binding specificity. This happens with complexes that have been preincubated with D-glucose. Thus, it is conceivable that competitive binding of FCS components impairs the positive effect obtained with D-glucose. It may also be reasoned that FCS components may deposit on endocrine cells and thus cause nonspecific lectin binding.

The finding that the binding of the MIMS-WFA complexes to exocrine cells is pH dependent with a reaction optimum at pH 7.4 to 7.6 is not surprising because similar observations have been previously reported in the context of lectin research.[12] However, the optimum pH range is diminished because cell viability has to be taken into account as another important parameter. We propose that the optimum pH should be evaluated for each lectin and each donor species separately.

In spite of a recent report in which nonpurified human pancreatic islets functioned well in a diabetic patient under immunosuppression with a variety of drugs,[13] high-grade islet purification prior to (xeno)transplantation remains one of our main strategies. This is based on previous in vitro findings on human islets that high-grade purification diminishes islet immunogenicity.[2] These observations were confirmed in a congeneic rat model by grafting viable crude or pure islets under the kidney capsule.[14] The present study follows this rationale inasmuch as it investigated additional parameters and provided valuable additional information to improve the magnetic islet purification process. Recently we showed that the exocrine pancreatic tissue induces a strong xenogeneic cell-mediated immune response by human responder T cells in the mixed lymphocyte islet culture.[15,16] Thus, separation of potentially immunogenic exocrine cells from porcine islets may also be essential in the situation of xenogeneic transplantation. In this context high-grade magnetic purification may be the method of choice in addition to the low-grade prepurification method by density gradient centrifugation.

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