RAT SERUM IMPROVES RAT PSEUDOISLET FORMATION AND INSULIN GENE EXPRESSION

L. Lione, A. Puddu, A. Pedemonte, G.L. Viviani

DI.M.I., Dipartimento di Medicina Interna, Università di Genova

INTRODUCTION

Langerhans islets are constituted by four different cellular populations with a central core of β cells surrounded by α, δ and PP cells. Islets may be dissociated to single component cells that re-establish contacts to form organized and functional aggregates capable of responding appropriately to islet secretory stimuli (1-5). In fact, dissociated islet cells have the ability in vitro to form aggregates with the same cell-type organisation as native islets: pseudoislets (6-8). Engraftment of purified islets, single islet cells or their aggregates represents alternative strategies to whole pancreas transplantation. Islet allograft can enjoy prolonged survival if cultured in vitro before transplantation. Transplantation of pseudoislets, compared to islets, results in a longer survival time of the graft, due to a lower immunogenicity of aggregates (9-11). In fact, examination of pseudoislets through electronic microscopy demonstrated the absence of capillaries containing blood cells, passenger leukocytes, endothelial, ductal and endocrine cells, which are indicated as responsible of the main immunogenicity of islet tissue (12). A major obstacle for successful pseudoislet transplantation is to obtain a sufficient amount of aggregates (3). However, if the re-aggregation of islet cells could be markedly stimulated before implantation, the transplantation may have a better chance of success. We investigated the effects of Fetal Bovine Serum (FBS) and Rat Serum (RS) at 3 different concentrations on islet cell aggregation. Here we reported that rat serum improves rat pseudoislet formation.

MATERIALS AND METHODS

Animals - Rat pancreas were obtained from animals weighing approximately 200 g. Pancreas were inflated by injection of collagenase solution (Blend Collagenase Type F, Sigma Chemical Co.). The pancreas was then excised, incubated at 37°C and digested for 26 min. Purification of the islets was performed by centrifugation on histopaque gradient.

Cell cultures - Isolated islets were cultured in RPMI 1640 supplemented with 10% FBS to allow recovery from the isolation procedure. The day after, the isolation procedure islets were disrupted in single cells by a chemical, mechanical and enzymatic method. Single cells were cultured in static condition with RPMI 1640 5.6 mM glucose at three different serum concentrations (2%, 5%, 10%) in 24-well microplates at 37°C in a humid atmosphere with 5% CO_2. Fetal Bovine Serum and Rat Serum were used obtaining six different culture conditions. RS was obtained during the surgical process. Inactivation of complement was achieved by heating serum at 56°C for 60 min.

RT-PCR - After the culture period total RNA was extracted using the RNA fast method and 1 μg of total RNA was used to obtained cDNA with M-MLV Reverse Trascriptase using oligodT primer. cDNA was processed to amplified preproinsulin and actin with polymerase chain reaction (PCR) using specific primers. Sense primers of both preproinsulin and actin were labelled using $\gamma AT^{32}P$ and T4 Polinucleotide Kinase. 20 μl of PCR products were analyzed on 12% polyacrylamide electrophoresis gel and detected by autoradiography. Radioactivity of interested lines was quantified with a β-Counter and results were expressed as ratio between preproinsulin and actin expression.

Insulin secretion - Insulin release was measured from triplicate groups of 10 pseudoislets that were incubated for 3 h consecutively at 37°C (5% CO_2/95% O_2) in 24-well microplates containing 1 ml Krebbs Ringer Buffer (KRB). Pseudoislets were allowed to estabilish a basal insulin secretion for 1 h in KRB supplemented with 2.8 mM glucose. After 1 h supernatant was discarded and pseudoislets were incubated for the second hour in the same buffer. Pseudoislets were washed twice with PBS and incubated in

KRB supplemented with 16.7 Mm glucose. Pseudoislets were sonicated and extracted overnight with acid-ethanol at 4°C before estimation of hormone content. The insulin concentration of the samples was measured by radioimmunological technique with I^{125}-labelled insulin as tracer.

Thymidine incorporation - Islet cells replication of pseudoislets cultured in presence of different serum conditions was estimated by adding methyl-H^3thymidine at a concentration of 1 μCi/ml for 24 hours to the culture media.

Statistical evaluation - All results were expressed as mean + or - SEM and analyzed with the paired Student's t test. Differences with a p value of less than 0.05 were considered significant.

RESULTS

In our study we investigated the effects of different concentrations of rat serum and foetal bovine serum on aggregation of single rat islet cells. Purified rat islets were cultured overnight to recovery and then disrupted into single cells. Within 24 hours cells formed long chains and after 72 hours spheroids appeared of about 50 μm diameter. After 7 days culture islets cells completely re-aggregated in pseudoislets. Islet cells cultured with rat serum required a shorter time to form pseudoislets than those in foetal bovine serum (fig. 1). The increased recruitment of cells in rat serum cultures has been observed since first 24 hours.

Evaluation criteria of different sera effects took in account only pseudoislets with a diameter >100 μm and considered: a) mass of pseudo-islets; b) number of pseudoislets and c) media diameters of aggregates. Rat serum produced a significant increment in pseudoislet formation in all tested concentrations (fig. 2). Number of aggregates is significatively increased when islet cells were cultured in presence of 5% RS (fig. 3), while pseudoislet diameter is enhanced at 2% and 10% RS (fig. 4).

Islet cell replication during culture was evaluated through ^{3}H-thymidine incorporation, no significant difference has been found between groups of cells cultured in FBS and those cultured in RS (data not shown). Many authors reported that homologous serum improved insulin secretion (13),

but in our model no difference in insulin secretory response was observed between pseudoislets incubated with rat serum and those cultured with fetal bovine serum, however pseudoislets cultured with RS showed a greater insulin content compared with those cultured with FBS. We investigated whether serum could also effect insulin gene expression and our data showed that insulin gene expression increased in pseudoislets cultured with rat serum (fig. 5).

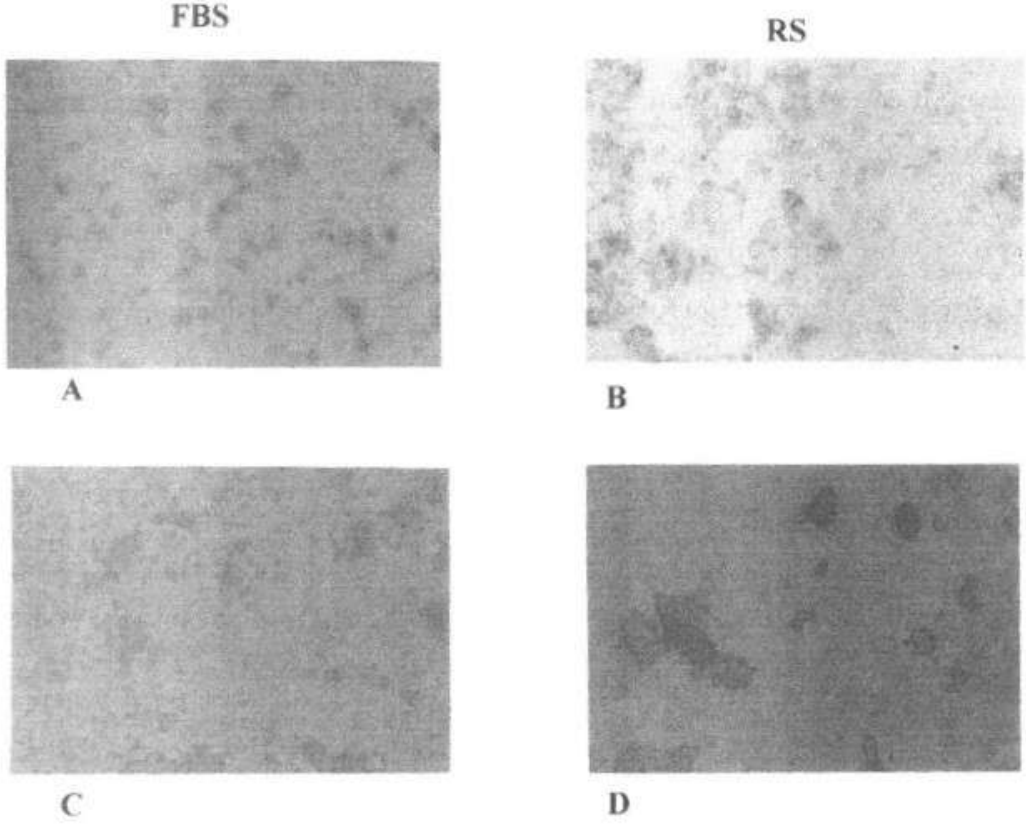
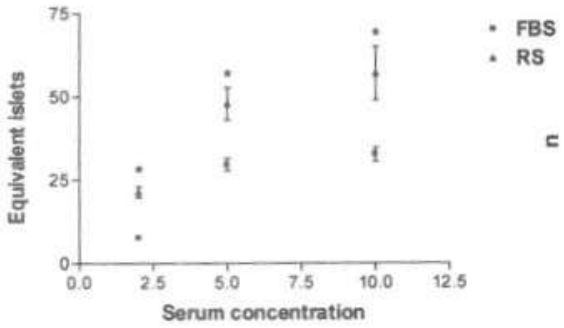


Fig. 1 - Islet cell re-aggregation: 1 day culture (A,B); 3 day culture (C,D).



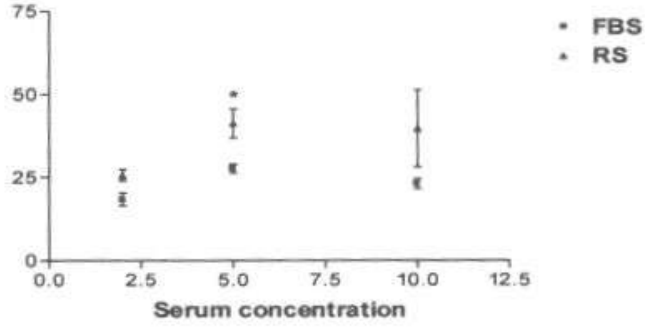


Fig. 2 - Pseudoislet formation; Fig. 3 - Number of aggregates.

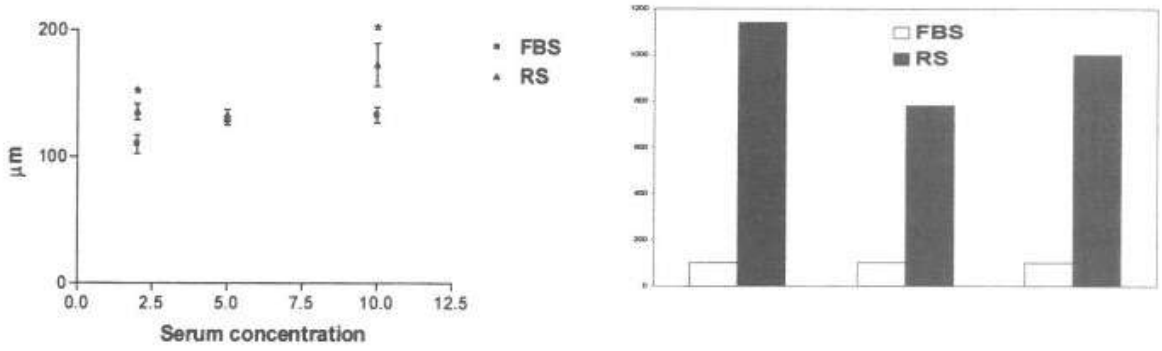


Fig. 4 - Pseudoislet diameters;
Fig. 5 - Increment in insulin mRNA expression.

DISCUSSION

This work showed that rat serum improved islet cell re-aggregation increasing cell to cell association and number of pseudoislets. This is very important in light of one of the limits of pseudoislets in transplantation in a poor re-aggregation mass.

The enhanced rat serum stimulated insulin gene expression is not correlated with increased insulin secretion, but with an increased insulin content. This could suggest a role of homologous serum in preserving long-term function.

Adaptation of rat pseudoislets to a new extracellular environment could be favoured by the presence of homologous serum. Thus rat serum could supply more efficient growth factors than FBS. Since the composition of serum is complex its difficult to identify which factors may be involved in these phenomena. For example, rat serum could contain factors more capable in stimulating chemoattraction between islet cells than those contained in foetal bovine serum. Moreover, rat serum could enhance survival of islet cells in culture.

As pseudoislets are a useful research model for islet function studies and a physiologically relevant substitute for transplantation studies we suggest the use of a medium supplemented with homologous serum.

Rat islet cells in culture are able to form tridimensional aggregates with an architecture and functional activity similar to native islets: pseudoislets.

Pseudoislets represent an alternative source for islet transplantation, because their transplant results in a long term allograft acceptance without immunosuppression of the host. Use of pseudoislets has been limited by their reduced yield and by poor reaggregation mass. Since culture conditions have been reported to affect reaggregation, the aim of this study was to evaluate the effects of different concentrations of two sera (Fetal Bovine Serum [FBS] and Rat Serum [RS]) on reaggregation and insulin gene expression in pseudoislets. Islets were isolated from male Lewis rat by means of histopaque gradient centrifugation. The day after islets were disrupted into single cells and cultured in RPMI 1640 5.6 mM glucose with 2%, 5% and 10% solutions of both FBS and RS. Cells spontaneously reaggregated to form pseudoislets. After seven days of culture, pseudoislets were counted and analysed for insulin secretion and insulin gene expression using RT-PCR. Rat serum increased the number of aggregates and their diameters. Insulin gene expression of pseudoislets cultured with RS showed a ten fold increase in comparison to those cultured with FBS. These data show that the culture medium supplemented with RS improves total reaggregate volume and increases insulin gene expression. With the perspective of pseudoislets' use in transplantation RS is better indicated than FBS for the production of rat pseudoislets.

---

1) JUN TZE W., TAI J., Transplantation, 1982, 34 (4), 228-231.
2) HALBAN P.A., POWERS S.L., GEORGE K.L., BONNER-WEIR S., Diabetes, 1987, 36, 783-790.
3) MATTA S.G., WOBKEN J.D., WILLIAMS F.G., BAUER G.E., Pancreas, 1994, 9 (4), 439-449.
4) BRITT L.D., STOJEBA P.C., SCHARP C.R., GREIDER M.H., SCHARP D.W., Diabetes, 1981, 30, 580-583.
5) KUO C.Y., HERROD H.G., BURGHEN G.A., Pancreas, 1992, 7 (3), 320-325.
6) HOPCROFT D.W., MASON D.R., SCOTT R.S., In Vitro Cell Dev. Biol., 1985, 21 (8), 421-427.
7) SHIZURU J., TRAGER D., MERRELL R.C., Diabetes, 1985, 34, 898-903.
8) OTONKOSKI T., J. Clin. Endocrinol. Metab., 1988, 67, 734-740.
9) BEGER C. et al., Langenbecks Arch. Chir. Suppl. Kongressbd, 1997, 114, 233-236.

10) WOLF-JOCHIM M., WOHRLE M., FEDERLIN K., BRETZEL R.G., Exp. Clin. Endocrinol. Diabetes, 1995, 103 (Suppl 2), 118-122.
11) PIPELEERS D.G., PIPELEERS-MARICHAL M., VANBRABANDT B., DUYS S., Diabetes, 1991, 40, 920-930.
12) GILL R.G., WOLF L., Cell Transplantation, 1995, 4 (4), 361-370.
13) KIN T. et al., Cell Transplantation, 1996, 5 (5 S1), S45-S47.

KEY WORDS: diabetes, pseudoislets, transplantation.

Lectured at the meeting held in Genova on December 21, 2000.
Received: December 27, 2000; accepted: January 10, 2001.

Address reprint requests/correspondence to Prof. G.L. Viviani, DIMI, Dip. di Medicina Interna, Università di Genova, Viale Benedetto XV 6, I-16132 Genova.