Short-term treatment with mycophenolic acid and tacrolimus is tolerogenic for INS-1 cell clone transplantation and the deleterious effects of the drugs are limited: *in vivo* and *in vitro* studies

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Abstract

One of the major requirements for a successful and life-lasting organ transplant is the access to safe, least toxic and permanent tolerance-inducing drugs. In this study we wished to evaluate the effects of tolerogenic doses of the immunosuppressive drugs mycophenolic acid (MPA) and tacrolimus (Tac) on clonal β-cell lines, both *in vivo* and *in vitro*. Here we demonstrate that combined administration of low-dose MPA and Tac for 23 days induced permanent tolerance in an allogeneic β-cell line transplant in Wistar rat liver through the portal vein. This short-term treatment of tolerogenic doses of the two drugs was deleterious to the survival of the transplanted cells but a small percentage of the cells could resist the effect and become fully active when the drugs were removed. The surviving cells, retrieved from growth *in vivo*, did not exhibit increased resistance in comparison to the original cells when tested *in vitro* at two glucose concentrations, 10 and 20 mM. The presence of a small percentage of resistant cells at the two glucose concentrations was also detected in the *in vitro* study after a continuous 8-day treatment demonstrating that the *in vivo* resistance was not related to micro-environmental protection but possibly to a phenotypic cell state that is yet to be determined.

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Introduction

Since the successful Edmonton trial (Shapiro *et al*. 2000) allo-transplantation of human islets the definitive/long-term treatment of insulin-dependent diabetic patients by islet transplantation has become a matter of reality. The unprecedented success of this trial has been partly due to the prevention of the rejection of transplanted islets by effective and relatively safe immunosuppressive drug treatment. In the transplantations that led to insulin independence, between two and four cadaveric preparations of islets were used. The great hopes of thousands of insulin-dependent diabetic patients, however, are confronted with the serious obstacle of the scarcity of available human islets. The post-Edmonton trial challenge is to access a permanent and easily available source of functional β-cells on the one hand and to establish a safer and longer-acting immunosuppressive regimen, preferentially of the type that induces permanent tolerance, on the other.

Although the existing functional and stable ever-dividing β-cell lines could be considered for eventual transplantation the big challenge is to engineer a safe and tight proliferation-controlling protocol for such cells.

We have established highly glucose- and insulin-secretagogue-sensitive clones of a rat insulin-producing cell line, INS-1. The selected clones respond to glucose at physiological concentrations and have a relatively high insulin content. Before further elaborate design and manipulation of the growth control of these cells was carried out we were interested to see if they could survive the deleterious effects of immunosuppressive drugs and to find out what cell mass/insulin content is able to reduce high blood glucose levels following allogeneic transplantation in streptozotocin (STZ) diabetic rats.

As immunosuppressive drugs we chose mycophenolic acid (MPA) and tacrolimus (Tac; FK506; Prograf), known for inducing long-term tolerance in combination with other immunosuppressants. Mycophenolate mofetil (CellCept), a prodrug of MPA, induces activated T-lymphocyte apoptosis by depleting the dGTP pool required for DNA synthesis (Allison & Eugui 2000) and has been shown to induce long-term tolerance alone (Hao *et al* 1990, 1992, Wennberg *et al*. 2001) or in combination with other immunosuppressive drugs including Tac (Jones *et al*. 1999). Tac prevents interleukin-2 production by antigen-activated T-cells through inhibition of T-cell-specific and non-specific transcription factors required for the activation of interleukin-2 gene expression (Siekierka *et al*. 1994). Tac also has been demonstrated to induce long-term tolerance in combination with other immunosuppressants (Fealy *et al*. 1994, Vu *et al*. 1997, Qi *et al*. 1999, 2000) and is an efficient tolerogen in single injection

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if accompanied with the pre-sensitization by transfusion of donor splenocytes (Misao *et al.* 1997). Both of these immunosuppressants have been reported to inhibit insulin secretion and DNA synthesis in pancreatic islets and β-cell lines *in vitro* (Metz *et al.* 1992, Sandberg *et al.* 1993, Meredith *et al.* 1995, 1997, Redmon *et al.* 1996, Li *et al.* 1998, Sayo *et al.* 2000, Huo *et al.* 2002, Paty *et al.* 2002). Those studies, however, were mostly of short duration and studied each immunosuppressant alone. In this study we demonstrate the effects of tolerogenic doses of the two drugs on INS-1 clone survival both *in vivo* and *in vitro*. The *in vivo* doses of the two drugs in this study were taken from the previous studies in rodents and primates that induced long-term tolerance (Iwasaki *et al.* 1991, Sugioka *et al.* 1996, Mourad *et al.* 2001, van Gelder *et al.* 2001) and the *in vitro* doses matched the plasma concentrations of the same doses used *in vivo* according to pharmacokinetic studies (Arima *et al.* 2001, van Gelder *et al.* 2001).

Materials and Methods

MPA, STZ, Glybenclamide, glucagon-like peptide-1 (GLP-1), isobutylmethylxanthine (IBMX), forskolin and Percoll were from Sigma. Tac (Prograf; 5 mg capsules) was purchased from Fujisawa (Celle St Cloud, France), carboxymethylcellulose was from Merck VWR (Fontenay sous Bois, France) and Nateglinide was synthesized in our laboratory. Fetal calf serum (FCS), Hepes, pyruvate, RPMI 1640, glutamine, glucose-free medium and bicarbonate were purchased from Invitrogen.

Diabetic animals

Outbred Wistar male rats weighing 350–450 g (2–3 months old) were purchased from Janvier (Le Genêt-St-Isle, France). For induction of diabetes the animals were injected intraperitoneally with a STZ solution (freshly dissolved in citric acid and sodium citrate, at 100 mM and pH 7·5) at 55 mg/kg. After 48 h the glycemia was measured by glucometer (Glucotrend; Roche) in 10 μl blood samples collected from the tails of the animals. The rats were kept in groups of five per cage and had free access to water and food (A04; UAR, Augy, France).

Cell culture and preparation of cell aggregates for transplantation

INS-1 clones 327 and 368 (at passages 50–54) were cultured to full growth as explained by Asfari *et al.* (1992). 1 day before transplantation cells were trypsinized and cultured overnight in non-adhering 100 mm bacterial plates at 20×10^6 cells in 15 ml medium. This way the cells were clustered into aggregates of 5–50 cells per aggregate. The culture medium for preparation of aggregated cells was similar to complete medium (CM) except that FCS was replaced with heat-inactivated rat serum. Rat serum was prepared from blood taken from the abdominal artery of anesthetized outbred normal Wistar rats. Immediately before transplantation cells were centrifuged at 800 r.p.m. and 4 °C for 5 min and the cell pellets were resuspended gently in 0·8 ml CM with 10% heat-inactivated rat serum and kept on ice until the time of transplantation.

Transplantation

Diabetic rats with glycemia of 450–550 mg/dl were anesthetized with isoflurane (Florene, Abbott France, Rungis, France) inhalation for 3 min at 5%, which was then reduced to 2 and 1·5% in O_2/CO_2 (95:5) during surgery, which lasted 15 min. The animals received 30×10^6 cells in a total of 0·8 ml CM containing 10% rat serum (CM-R) instead of FCS by a 25-guage needle through the hepatic portal vein. After surgery the animals were injected with 1 ml 1% Xylocain/adrenaline (Astra-Zeneca) subcutaneously at the site of the sutures to reduce the eventual pain. Each transplanted rat also received a dose of 100 mg antibiotic i.m. (Clamoxyl; Smithkline Beecham).

All experiments conformed to the relevant guidelines of the French Ministry of Agriculture for scientific experimentation on animals, and our laboratory and personnel are authorized to conduct such investigation according to the Ministry's Executive Order no. 00764. The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health 1996).

In vivo *immunosuppressive drug treatment*

Both Tac powder (from Prograf, 5 mg/capsule) and MPA were dissolved at 5 and 4 mg/10 ml, respectively, in 0·5% carboxymethylcellulose in water (v/v). The animals received a mixture of MPA (at 5 mg/kg of rat body weight) and Tac (at 4 mg/kg of rat body weight) in volumes according to each animal's weight, by gavage on the same day before transplantation. The same dose and route of MPA and Tac administration continued for 23 days.

Control of blood glucose and insulin

Immediately before transplantation and at different time intervals after transplantation, the animals' tail-blood glucose was measured by Glucotrend (Roche). For insulin measurement 0·3 ml blood was taken from the tail at different time periods after transplantation and measured by ELISA (INSRAT 00-C1; Eurobio, Les Ulis, France).

Retrieval of the transplanted cells

At the time when glycemia was reduced to below normal levels, the animals were opened up and the developed

tumor was excised from the surrounding hepatic tissue and made into a single cell suspension for further study. The tumors were cut into small pieces in the culture medium and the suspended cells were washed and separated on a Percoll gradient of 35, 45, 55 and 65% in RPMI 1640 medium with 1% heat-inactivated fetal serum as explained by Asfari *et al.* (1992).

In vitro *proliferation and viability analysis of MPA-/Tac-treated cells*

Proliferation and survival were measured by two methods, as follows.

Colorimetric microtitration assay A colorimetric assay using a tetrazolium-dye-based microtitration assay (WST-1; Roche) was employed. Cell aggregates, prepared as indicated above, were cultured at a cell density of about 2×10^4 cells/500 µl CM in each well of 24-transwell plates (catalogue no. 7959601; Merck VWR) before addition of the test medium. The test medium containing 10 or 20 mM glucose and different concentrations of Tac and MPA were added on day 2 of culture, renewing the test medium daily for 4 days.

Trypan blue dye-exclusion assay The number of live cells was identified by trypan blue dye-exclusion assay and the percentage of the dividing cells was assessed by bromo-deoxyuridine incorporation assay (kit RPN20; Amersham Biosciences). Cell aggregates were cultured on to the 24-transwell plates (catalogue no. 7959601; Merck VWR) at 10^5 cells/well in a total volume of 800 µl CM containing Tac and MPA and the test medium renewed daily. At different time points the cells of two separate wells from each culture condition were incubated for 1 h in the presence of BrdU, then trypsinized and washed. One-third of the washed cells were used for the viability count by trypan blue-exclusion assay, and the rest were cytospinned for BrdU staining according to the manufacturer's protocol.

In vitro *immunosuppressive drug treatment*

Both Tac and MPA powder were dissolved in DMSO at 1000 times the highest final concentration of the tests. The dilution was made in the test medium and the control medium contained 0·1% DMSO.

Measurement of insulin secretion and content

The secretory response of the INS-1 cells and the selected clones to glucose and other insulin secretagogues was tested in static incubation. Cells were cultured at 10^5 cells/well on 24-well culture plates in 1 ml CM for 48 h with a medium change to 5 mM glucose on the evening before the experiment. The cells were then washed with glucose-free Krebs–Ringer bicarbonate Hepes buffer (KRBH; 135 mM NaCl, 3·6 mM KCl, 5 mM $NaHCO_3$, 0·5 mM NaH_2PO_4, 0·5 mM $MgCl_2$, 1·5 mM $CaCl_2$ and 10 mM Hepes, pH 7·4) with 0·1% BSA and preincubated in the same buffer for 30 min. The cells were then washed twice with the same buffer and incubated in 1 ml KRBH buffer containing glucose and other secretagogues at the indicated concentrations for 1 h. Insulin was measured by ELISA (INSRAT 00-C1) as indicated above.

For measurement of insulin content islets of four normal adult male Wistar rats, weighing about 350–450 g, were pooled into multiple groups of 30–40 islets and extracted in acid/ethanol (Gotoh *et al.* 1987). For the cell lines approximately 3×10^6 trypsinized cells were extracted in 1 ml acid/ethanol and the insulin was measured by the same method indicated above.

Results

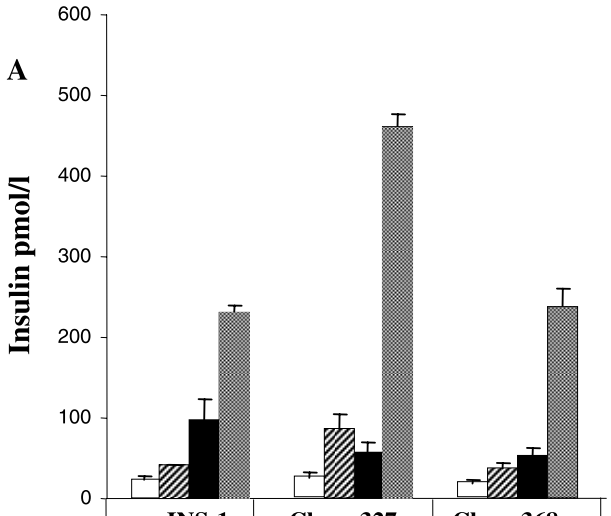
Insulin content and secretion of INS-1 clones

The INS-1 clones were selected for their superior response to glucose and other physiological and pharmacological insulin secretagogues. As indicated in the Fig. 1A these cells responded with better sensitivity to high glucose concentrations and their insulin content was comparable to that of INS-1 cells. By ELISA the insulin content of INS-1 cells after 48 h of incubation in CM at 5 mM glucose was $1{\cdot}574 \pm 0{\cdot}08$ µg/10^6 cells and that of clones 327 and 368 was $1{\cdot}318 \pm 0{\cdot}104$ and $0{\cdot}829 \pm 0{\cdot}056$ µg/10^6 cells respectively. With the same method the insulin content of islets from normal Wistar adult male rats of 350–450 g was found to be approximately 4·3 µg/10^6 cells ($13{\cdot}048 \pm 0{\cdot}174$ ng/islet from eight separate pools of 30–40 islets from four animals).

The clones were responsive to glucose and other known insulin secretory molecules such as forskolin/IBMX, GLP-1, Glybenclamide and Nateglinide (Figs 1A and B).

Transplantation

The outbred Wistar STZ diabetic rats with a glycemia of 350–570 mg/dl had approximately 50×10^6 cells/kg transplanted into the liver through portal vein. Control rats received the same volume of the medium, CM-R, alone. The oral MPA/Tac treatment started the same day and before transplantation. About 18 h after transplantation there was a reduction in blood glucose level that lasted for a short time (2–3 days). This was certainly due to the insulin release from the transplanted cells since no such reduction was detected in the control, sham-operated animals. Blood glucose rose rapidly afterwards to above pre-transplantation levels and remained high; approximately 593 ± 15 mg/dl during the whole period of



[Glucose	INS-1	Clone-327	Clone 368
□ 2.8	24.1	26.2	19.6
▨ 10	41.9	86.9	38.1
■ 2.8 + FI*	95.3	56.7	52
▩ 10 + FI*	230.8	460.1	236.1

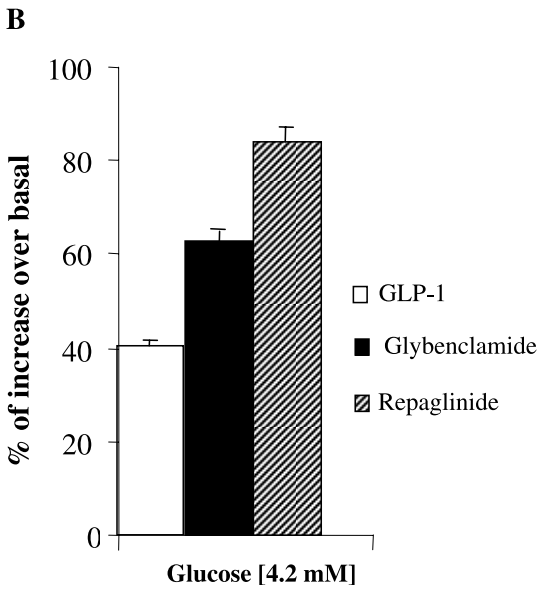


Figure 1 Glucose-induced insulin secretion of INS-1 and the two selected clones, 327 and 368. Cells were cultured for the insulin-secretion test as indicated in the Materials and Methods section. (A) Insulin secretion at 2·8 and 10 mM glucose alone and in the presence of a mixture of forskolin and IBMX (FI*) at 1 and 100 μM, final concentrations, respectively. (B) Insulin secretion of clone 368 in response to GLP-1 (10^{-6} M), Glybenclamide (10^{-6} M) and Repaglinide (10^{-6} M) at 4·2 mM glucose. Results are expressed as the percentage of induction over 4·2 mM glucose alone.

drug treatment, which lasted 23 days (Fig. 2A). After discontinuation of MPA/Tac administration a gradual decrease in blood glucose level and a rise in blood insulin were detected only in the transplanted animals. The decrease in blood glucose continued to very low levels and the animals died due to hypoglycemic shock. Out of the 17 transplanted animals, nine with clone 327 and eight with clone 368, 16 followed the same pattern, albeit at different intervals, after the termination of drug administration. One animal died within 48 h of transplantation for unidentified reasons. The period of the concomitant rise in blood insulin and reduction in blood glucose varied between the rats and it was not dependent on the level of glycemia on the day of the transplantation (Fig. 2B) nor on the type of the cells they received. No changes in blood glucose or insulin were observed in the seven diabetic sham-operated control animals that remained highly glycemic (601 mg/dl) with an average blood insulin of 38·1 ± 11·0 pM. The majority of control animals died around day 60 post-transplantation; the last animal survived until day 117. It should be noted here that with the same method of quantification the level of blood glucose of normal male Wistar rats of about 350–450 g was 100–140 mg/dl and blood insulin was 200–300 pM.

In vitro *sensitivity tests of the clones to MPA and Tac after transplantation*

At the time when the glycemia was reduced to normal levels, three animals were examined to evaluate the growth of the transplanted cells and their eventual recovery. All the examined transplanted rats developed a tumor, confined to the small lobe of the liver, of about 1·7 ± 0·7 ml in volume. A total number of (149·3 ± 48·9) × 10^6 cells was obtained from each dissected tumor and no detectable metastasis was observed in the transplanted animals.

The recovered cells grew under the *in vivo* conditions of high glucose concentration (601 mg/dl = 33 mM) and the presence of MPA and Tac, known to inhibit insulin secretion and cell proliferation in islets and β-cells. It was of interest, therefore, to see if the recovered cells that had grown under such unfavorable conditions had retained their sensitivity to MPA and Tac. So the recovered transplanted cells were tested for growth and survival under the high concentration of glucose and at the two relevant pharmacological doses of Tac and MPA; a minimum of 3·1 μM MPA and 3 nM Tac and a maximum of 46 μM MPA and 30 nM Tac. As indicated in Fig. 3, there were no differences in the sensitivity of the original clones

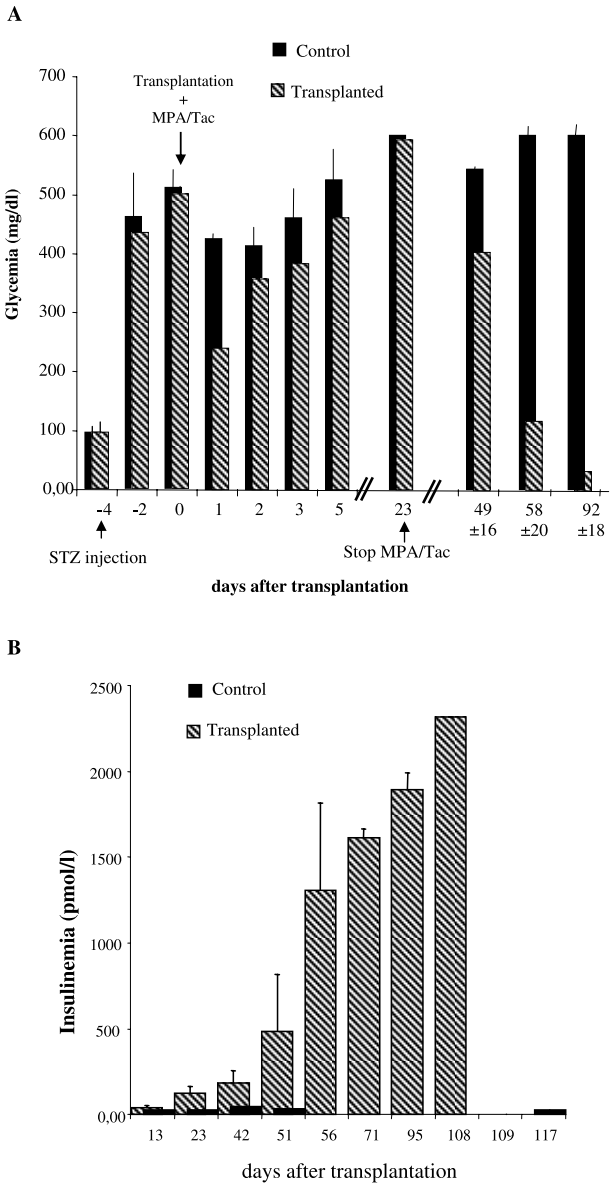


Figure 2 Glycemia and insulinemia of the transplanted and control rats. The STZ diabetic animals were transplanted with 50×10^6 aggregated INS-1 clone 327 cells per kg of rat body weight, through the portal vein as described in Materials and Methods section. Blood glucose and insulin from the tail were measured at different intervals post-transplantation. (A) Blood glucose of the transplanted and sham-operated control rats. (B) Blood insulin of the transplanted and sham-operated control rats.

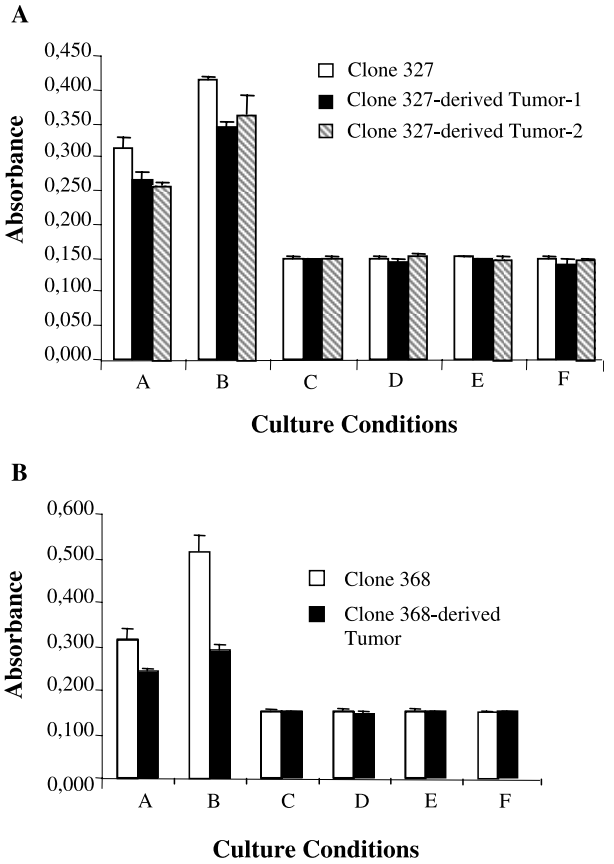


Figure 3 *In vitro* effects of Tac and MPA on INS-1 clones 327 (A) and 368 (B) and their *in vivo* derived tumors after 4 days treatment. Both the original clones and the *in vivo*-derived tumors were cultured as described in the Materials and Methods section and the cells were treated with two different concentrations of Tac and MPA at 10 and 20 mM of glucose for 4 days. Proliferation/survival was measured by colorimetric assay with WST-1 cell-proliferation reagent kit (Roche) according to the manufacturer's protocol. Culture conditions: A, 10 mM glucose; B, 20 mM glucose; C, 10 mM glucose with 3 nM Tac and 3·1 μM MPA; D, 20 mM glucose with 3 nM Tac and 3·1 μM MPA; E, 10 mM glucose with 30 nM Tac and 46 μM MPA; F, 20 mM glucose with 30 nM Tac and 46 μM MPA.

and their respective derived tumors to the proliferation-inhibitory effects of the two drugs. Furthermore, both clones and their respective derived tumors were equally affected at high and low concentrations of the two immunosuppressants and at the two concentrations, 10 and 20 mM, of glucose.

Identification of the reversibility of MPA and Tac effects after a prolonged treatment in vitro

Previous studies have reported a certain degree of reversibility of the effects of Tac and MPA on β-cells (Sandberg *et al.* 1993, Redmon *et al.* 1996). In those studies, however, the treatments were performed separately for each drug and the period of treatment was short; 72 h at most. We studied the survival of the clones under an 8-day *in vitro* treatment with both drugs at a mean concentrations of 15 and 21·85 nM for MPA and Tac respectively, used in our previous *in vitro* study, and in the presence of 10 and 20 mM glucose. Cells were cultured on to transwells and the culture medium was renewed daily. At day 8 the cells

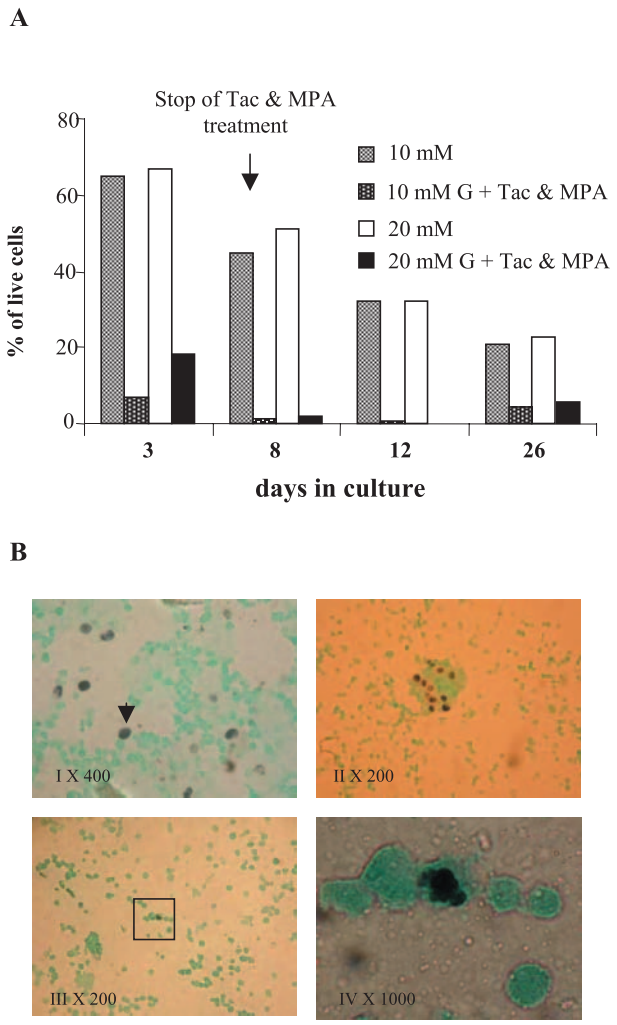


Figure 4 Viability and cell division of Tac- and MPA-treated cells *in vitro*. Cells were cultured on to 24-well transwells as described in the Materials and Methods section in CM with 10 and 20 mM glucose and in the presence of Tac (7 nM=5·6 ng/ml) and MPA (46 μM=15 μg/ml) for 8 days, with a change of the drug-containing medium every day. The medium was then changed to CM with 10 and 20 mM glucose on day 8 and the cells were kept for another 18 days with daily medium change. At different time intervals the cells of two transwells from the same culture conditions were tested (A) for viability by trypan blue dye-exclusion assay and (B) for visualization of the BrdU-incorporating (dividing) cells. (BI) Control and (BII) Tac- and MPA-treated cells at 10 mM glucose on culture day 22. The arrowhead in (BI) points to the immunostained BrdU-incorporating cell nuclei. (BIII) 20 mM glucose with Tac and MPA on culture day 26; (BIV) 1000× magnification of (BIII).

were transferred into drug-free culture medium, after which there was daily medium change up to 26 days. Under these conditions the number of live cells at both glucose concentrations was reduced dramatically during the treatment with the two drugs, as determined by trypan blue-exclusion test (Fig. 4A). At day 8, out of 2000 counted cells, only 1·3 and 2·1% live cells were detected at 10 and 20 mM glucose, respectively, and live cells were less than 0·5% on day 12. From day 22 the percentage of living cells showed a slight increase and at day 26 there was a net increase of the live cells at both glucose concentrations. In order to indicate that the live cells were indeed the result of newly dividing cells we identified, in parallel, the actively proliferating cells by BrdU-incorporation immunocytometric assay. At day 8 of treatment there were 18 ± 2·3% and 12 ± 4% BrdU-incorporating cells at 10 and 20 mM glucose, respectively, in the control cultures. No BrdU-incorporating cells, however, were detected in Tac- and MPA-treated cultures on day 8 at either glucose concentration. From day 22 – that is, 14 days after Tac and MPA removal – the scarce and isolated cell aggregates at both glucose concentrations were detected, with a majority of cells in the aggregates stained positively for BrdU (Fig. 4B). This was more prevalent at day 26, corresponding closely to the results of viability tests, indicating that a very low percentage of the cells could resist 8-day Tac and MPA treatment and regain cell proliferation when the drugs were removed.

Discussion

The aim of this study was to test the tolerogenic doses of MPA and Tac on the viability of glucose-responsive INS-1 clones *in vivo* on the one hand and the cell number that may be required to bring down a high blood glucose level on the other. The two cell clones that were used in this study were phenotypically different but were similar in their response to glucose and other insulin secretagogues. Clone 327 had higher insulin content and consequently secretion but there were no detectable differences in the blood glucose-reducing profile of the two transplanted clones or their *in vivo* or *in vitro* sensitivity to the immunosuppressive drugs. For the tolerogenic doses of MPA we used a lower dose than those reported in previous studies since it has been reported that the parallel administration of Tac increases the MPA exposure and, therefore, augments its effects (Mourad *et al.* 2001, van Gelder *et al.* 2001).

The short-term therapy with long-term tolerance to different organ transplants, including islets, in rodents and primates has been reported previously (Koulmanda *et al.* 1997, Levisetti *et al.* 1997, Thomas *et al.* 1999, Contreras *et al.* 2000, Buhler *et al.* 2002). Hao *et al.* (1990, 1992) reported on the induction of long-term tolerance with short-term therapy with classical immunosuppressive drugs in rats and mice. In those studies a very high dose of MPA, 80 mg/kg per day in mice and 40 mg/kg per day in rats, for a continuous 30-day administration, led to a more than 100-day islet allo- and xenograft acceptance in 70% of the animals with specific tolerance induction.

In our study we used a much lower dose of MPA, 5 mg/kg per day, in combination therapy with 4 mg/kg per day of Tac for a period of 23 days. This short-term treatment resulted in long-term tolerance in almost 100% of the transplanted rats. It should be mentioned, however, that induction of tolerance at such a low dose of MPA with Tac might be possible only in this allogenic β-cell transplantation where the genetic differences between the host and transplanted cells are quite limited. INS-1 cells are derived from an islet tumor of NEDH rat (Asfari *et al.* 1992), which is an inbred strain with the Wistar genetic background.

The lack of blood glucose reduction during drug administration and long time after arrest of drug application evidently was due to the deleterious effects of the two drugs on survival and function of transplanted cells, as was supported by the experiments performed *in vitro*. Nevertheless, the insulin levels of the transplanted animals during the 23 days of treatment were significantly higher than the controls. This could be due to either the very low levels of activity of the transplanted cells or to full activity of a few surviving cells that were resistant or were protected from the deleterious effects of the immunosuppressive drugs. To test this we compared the sensitivity of the recovered cells from the transplanted animals with those of the original cell lines, *in vitro*, and found that indeed they were equally sensitive to the drugs, even at high glucose concentrations. On the other hand when the cells were treated with the two drugs *in vitro* for 8 days very few cells were resistant or gained full proliferation activity when the drugs were removed. These results suggested that the resistance was neither a genetic modification as a result of selection pressure, nor due, or only due, to liver microenvironmental protection or high glucose concentration, but most probably due to some other inherent property of the cell aggregates. So, the very low levels of insulin in the transplanted animals were secreted from the few remaining resistant cells. This may suggest that MPA and Tac can affect only the cells that are on the periphery of the cell aggregates and can't diffuse through the cell mass reaching, at least in effective concentrations, to the cells in the center of the aggregates. So, depending on how long the drugs are present and the size of the aggregate, the number of surviving cells, for a defined drug dose, may vary. This can be determined by histological studies and identification of the state of proliferation/apoptosis of the cells in the aggregates. Whether the normal β-cells also exhibit such a property remains to be determined but if this hypothesis is true then the islets should be more resistant to the drugs because of their topographical structure.

A significant reduction of blood glucose in the transplanted rats was observed when blood insulin reached about five times the basal level in normal rats. This may reflect the development of insulin resistance in these STZ-induced diabetic rats due to STZ and prolonged hyperglycemia (Blondel & Portha 1989). It was also found that in these animals around 150×10^6 INS-1 clone cells, having a total insulin content of about 130 μg, are required to establish a considerable reduction in blood glucose, as was determined from the tissue mass retrieved from the transplanted animals at the stage of blood glucose reduction.

In conclusion the present study demonstrates that a short-term administration of MPA and Tac could induce long-term/permanent tolerance to INS-1 cell clones in an allogenic transplantation and that the resistance seems to be a phenotypic property of the cells, possibly related to the topographical position of the cells in the cell aggregates.

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