Effect of Culture on Islet Rejection

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SUMMARY

In order to diminish or prevent rejection of transplanted allogeneic islets of Langerhans, in vitro culture was used. After digestion of the rat pancreas and Ficoll separation, islets were handpicked to be free of vascular and ductal tissue. Phenol red in the culture medium imparted a pink color to the islets when observed with a diffuse green light against a black background. Islets cultured at room temperature (24°C) remained functionally and morphologically intact for 1–4 wk. Insulin secretion was 1–3 μU per islet per hour, increasing to 16 μU per islet per hour at 37°C, Culture alone resulted in a modest prolongation of function across a major histocompatibility barrier, Wistar Furth to Lewis (mean survival time, 11.6 ± 1.2 vs. 7.2 ± 0.5 days). However, one injection of antilymphocytic serum (ALS) into 10 recipients at the time of transplantation prolonged survival to greater than 100 days in nine rats. In the combination ACI to Lewis, also a major barrier, the same regimen prolonged function to greater than 100 days in five out of five recipients. Injection of donor peritoneal exudate cells resulted in prompt rejection of islets. These results suggest that culture and ALS either damage or alter passenger leukocytes in the donor tissue, thereby preventing rejection of the islets. DIABETES 29 (Suppl. 1):93–97, 1980.

The use of in vitro culture for the possible prolongation of allograft survival is based upon the concept that passenger leukocytes contaminating the graft are responsible for initiation of immune rejection by the recipient and that these passenger cells could be altered or diminished by in vitro culture of the graft before transplantation. Snell[1] first suggested that donor lymphocytes, carried over in grafts, might play a significant role in invoking immunity. It appears that passenger leukocytes do play a primary role in initiating a local immune reaction against the host within the graft; however, it is not clearly established that passenger leukocytes play a primary role in host-versus-graft reactivity.[2] Lafferty et al.[3] demonstrated that in vitro culture of the thyroid for 26 days in an atmosphere of 95% O_2 before transplantation produced marked prolongation of thyroid allograft survival. The period of time required for in vitro culture could be decreased to 7 days if the donors were pretreated with cyclophosphamide to diminish the lymphoid population[4] or by culturing the thyroid in hyperbaric oxygen.[5] Rejection of the thyroid allografts could be restored by injecting peritoneal exudate cells syngeneic to the donor strain.[6] Kedinger et al.[7] have reported that the survival time of diabetic rats could be prolonged significantly by culturing islets in vitro 4–5 days before transplanting islet allografts directly into the hepatic parenchyma of the liver.

The purpose of this report is to review our studies on prolongation of islet allograft survival across major histocompatibility barriers by procedures that were directed towards elimination or alteration of passenger leukocytes in the islet grafts before transplantation.

EFFECT OF DONOR PRETREATMENT REGIMENS ON ISLET ALLOGRAFT SURVIVAL

We attempted initially to use the in vitro techniques described by Lafferty et al.[3] on isolated adult islets and found that exposure to 95% O_2 resulted in disintegration of the islets after 4–5 days of in vitro culture. In addition, the raft technique of culture used by Lafferty et al.[3] could not be applied to the islets since the individual islets became flattened and disintegrated on the surface of the raft.

Because of these limitations for in vitro culture of the islets, we determined the effect of pretreating donor rats with total body irradiation and intravenous silica to diminish the lymphoid and macrophage population in the islets in conjunction with a brief period of in vitro culture of the islets with rabbit anti-rat lymphocyte serum (ALS) before transplantation.[8] The islets were isolated by the collagenase technique[9,10] and were separated by centrifugation on a Ficoll gradient.[11] In all of the studies reviewed in this report, the isolated islets were transplanted via the portal vein tech-

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nique,[12] and the recipient Lewis rats were made diabetic by the intravenous injection of streptozotocin (65 mg/kg). Rejection was considered to have occurred in individual recipients when the posttransplant, 24-h urine glucose level was equal to the mean minus one standard deviation of the pretransplant urine glucose level for that particular rat. This procedure made it possible to follow the pattern of rejection as well as to determine the time of rejection.

Unfortunately, none of these donor pretreatment regimens had any effect on prolongation of survival of Ficoll-isolated islets transplanted across either a minor (Fischer [AgB^1] to Lewis [AgB^1]) or a major histocompatibility barrier (WF [AgB^2] to Lewis [AgB^1]). After these discouraging results were obtained, we carried out a careful examination of the tissue present in the islet layer removed from the Ficoll gradient. In this layer we found some islets with attached ductular and vascular tissue, free fragments of vascular and ductular tissue, and, occasionally, small lymph nodes. Since these contaminating structures could be contributing to the immune rejection, the studies were repeated using only islets that were handpicked and free of associated vascular or ductal tissue.

Transplants of handpicked islets across a minor histocompatibility barrier (Fischer to Lewis) after pretreatment of the donors with irradiation and silica in conjunction with in vitro culture of the islets with ALS for 2–3 days before transplantation produced prolongation of the survival time of the transplants.[8] Two of the recipients remained aglycosuric for 90 and 127 days, with rejection still not occurring at 210 and 279 days after transplantation. Pretreatment of the donors alone with irradiation and silica and without in vitro exposure of the islets to ALS did not prolong islet allograft survival.

Since exposure of the islets to ALS appeared to be of importance, we determined the effect of the pretreatment regimen in conjunction with a single injection of ALS into the recipient at the time of transplanting the islets rather than exposing the islets to ALS in vitro.[8] Islet allograft survival across a weak histocompatibility barrier (Fischer to Lewis) was markedly prolonged by either the pretreatment regimen and a single injection of ALS or only a single injection of ALS without donor pretreatment.

These studies were repeated across a major histocompatibility barrier (ACI [AgB^4] to Lewis [AgB^1]). The mean survival time in the control group receiving only freshly isolated islets was 5.2 days. A single injection of ALS into the recipients at the time of transplanting freshly isolated islets produced a slight prolongation of allograft survival, with six of the rats rejecting between 7 and 20 days and one rejecting at 91 days. Pretreatment of the ACI donors with irradiation and silica and a single injection of ALS into the recipients produced a further prolongation of survival, with rejection occurring between 30 and 68 days in four rats; one rat still had a functional transplant at 189 days.[8]

These findings indicated that pretreatment of donor animals with irradiation and silica in conjunction with a single injection of ALS into the recipient would produce prolongation of islet allograft survival across a major histocompatibility barrier; however, the length of survival of the transplant was not comparable to the markedly prolonged survival of thyroid allografts obtained by Lafferty et al.[3]

EFFECT OF IN VITRO CULTURE (24°C) AND ALS (ACI TO LEWIS)

Opelz and Terasaki[13] reported that in vitro maintenance of lymphocytes at a low temperature (22°C) for more than 4 days resulted in a loss of the ability of the lymphocytes to stimulate allogeneic lymphocytes in mixed lymphocyte cultures. Since we were attempting either to diminish or alter the lymphoid elements in the islets before transplantation, we determined whether isolated islets would survive when they were incubated in vitro at room temperature (24°C).

We were surprised to find that the islets remained functionally and morphologically intact after in vitro incubation at 24°C for 1–4 wk.[14] The islets were incubated in tissue culture medium containing glucose (1.5 mg/ml), and the rate of insulin secretion ranged from 1 to 3 μU per islet per hour during the 28-day period of culture at 24°C. When the temperature was increased to 37°C at the end of each of the weekly intervals, the rate of insulin secretion increased to approximately 16 μU per islet per hour and remained stable during a subsequent 7-day period of incubation. This rate of secretion was comparable to control islets, which had been cultured at 37°C for corresponding periods of time.

Since the islets remained morphologically and functionally intact when incubated at 24°C, studies were accomplished to determine the effect of a 7-day period of incubation of ACI (AgB^4) islets at 24°C before transplantation in conjunction with a single injection of ALS into diabetic Lewis (AgB^1) recipients.[15] This pretreatment regimen produced an islet allograft survival of greater than 100 days in all five of the recipients. The mean total weight gain for this group was 100.2 g for the 100-day period. Three of the recipients returned to their individual pretransplant levels between 135 and 150 days after transplantation, whereas the other two recipients still had not rejected their islets at 220 days after transplantation.

EFFECT OF IN VITRO CULTURE (24°C) AND ALS (WF TO LEWIS)

The effect on islet allograft survival of in vitro culture (24°C) for 7 days before transplantation and a single injection of ALS into the recipient was determined across another major histocompatibility barrier (WF [AgB^2] to Lewis [AgB^1]). In 10 recipients receiving islets cultured for 7 days at 24°C and a single injection of ALS, nine had not rejected the allografts at 100 days and one rejected at 12 days.[16,17] Eight of the recipients were aglycosuric throughout the 100-day interval, and one, which remained aglycosuric for 80 days, demonstrated increased urine glucose to 1.75 g/24 h at 100 days. This study confirmed our initial findings and indicated that in vitro culture at 24°C, in conjunction with temporary immunosuppression of the recipient, would produce marked prolongation of islet allograft survival across major histocompatibility barriers.

The effect of only a single injection of ALS on allograft survival of transplants of Wistar Furth islets to Lewis rats was greater than that obtained in our previous investigation using ACI rats as donors. Two recipients receiving only ALS and freshly isolated islets had not rejected the transplant at 100 days.[16,17] The ALS used in these studies was prepared from Wistar Furth lymph node cells. The same lot of ALS was used for the transplantation of ACI and Wistar Furth (WF)

islets to Lewis recipients. Thus, it is possible that the ALS may have affected or destroyed passenger leukocytes in WF islets more effectively than passenger leukocytes from the ACI strain.

In vitro incubation of WF islets at 24°C for 7, 14, 21, and 28 days before transplantation without the injection of ALS into diabetic Lewis recipients did not produce a marked prolongation of islet allograft survival.[16,17] Islets incubated for 21 days (24°C) survived slightly longer than control recipients receiving freshly isolated islets (mean survival time, 11.6 ± 1.2 vs. 7.2 ± 0.5 days). These findings indicated that the single injection of ALS into the recipient was required, in conjunction with culture of the islets at 24°C, to obtain marked prolongation of islet allograft survival.

INDUCTION OF REJECTION BY PERITONEAL EXUDATE CELLS

The underlying hypothesis for the experiments on prolonging islet allograft survival by in vitro culture at 24°C and the single injection of ALS was that these procedures were altering or diminishing the passenger leukocyte content of the islet tissue. In order to test this hypothesis, we determined whether the intravenous injection of peritoneal exudate cells (PEC) obtained from the donor strain would induce rejection of established islet allografts.

The two recipients in our initial study[16,17] that had not rejected allografts of ACI islets at 220 days were injected intravenously with 6×10^7 PEC obtained from ACI rats on day 220. Six days after the injection of PEC, rejection was initiated in both animals with a rapid rise in the 24-h urine glucose levels and a precipitous loss of body weight. Similar findings were obtained when Wistar Furth (WF) PEC were injected intravenously into recipients with established WF islet allografts at 219–233 days after transplantation.[17]

These findings indicated that allografts of islets remained immunologically undetected by the recipient until PEC from the donor strain were administered.

REPEATED ISLET TRANSPLANTS AFTER INDUCTION OF REJECTION BY PEC

After induction of rejection of ACI islet allografts by donor PEC[16,17] at 220 days after transplantation, one of these animals was used to determine whether subsequent islet allografts from other strains could be established and whether these allografts could be induced to reject by PEC. A transplant of WF (AgB^2) islets into this animal after in vitro culture (24°C) and ALS in the recipient was successful, and the WF allograft was induced to reject only by Wistar Furth PEC, whereas PEC from ACI rats had no effect on the allograft.[17] A third transplant with islets from the Buffalo strain (AgB^6) was then successfully established after in vitro culture (24°C) and a single injection of ALS. Thus, islet allografts from three different strains of rats (ACI, Wistar Furth, and Buffalo) were successfully established even though rejection of ACI and then WF islets had been induced by PEC syngeneic to the donor strain.

These findings indicated that a remarkable specificity for the PEC was required to induce rejection of the islets and that subsequent islet transplants from different strains could be accomplished, even though rejection of islets from other strains had already been induced in the recipient.

"GREEN-LIGHT" TECHNIQUE FOR ISLET IDENTIFICATION IN VITRO

One of the problems that concerned us was whether small lymph nodes the size of islets would be mistakenly identified and be present even in the handpicked islets used for the transplants. Recently, we have developed a technique that permits the specific identification of islets in vitro.[18] Isolated islets in tissue culture medium containing phenol red have a slightly pinkish color when viewed with ordinary light. This pink color was enhanced by simply viewing the islets under a dissecting microscope with a diffuse green light against a black background. With this lighting arrangement, the islets appear distinctly pink, whereas small lymph nodes, ductal tissue, acinar tissue, and vascular tissue are a bright green. When preparations of handpicked islets were examined with this technique, we found occasional small lymph nodes that had been mistakenly identified as islets. This simple procedure is now used routinely to screen all of the islets before transplantation in order to be certain that small lymph nodes are not included in the transplanted material and that the islets are free of attached acinar, ductal, or vascular tissue.

MECHANISM RESPONSIBLE FOR PROLONGATION OF ISLET ALLOGRAFT SURVIVAL

It would appear that the period of in vitro culture of the islets at 24°C and the administration of ALS to the recipient diminished and/or altered the passenger leukocyte content of the islets below a threshhold level required to initiate rejection. Evidence in favor of this concept is as follows: (1) clean islets, free of associated vascular or ductal tissue, are required for prolongation of islet allograft survival; (2) ALS, prepared against WF lymph node cells, appeared to be more effective in prolonging islet allograft survival of WF islets than of ACI islets when only a single injection of ALS was administered without in vitro culture of the islets; (3) rejection of established allografts of WF islets and ACI islets could be induced by the intravenous injection of PEC lymphocytes and macrophages) that were syngeneic to the donor strain; and (4) the islet cells were apparently not altered since immunologic recognition and rejection of established allografts could be induced by PEC.

Other mechanisms which could play a role in the prolongation of islet allograft survival are the diabetic state of the recipient, the site of implantation of the islets, and the effect of ALS on the recipients. Chemically induced diabetes in mice has been reported to partially suppress the immune system[19–21] and to reduce the nucleated cell content of the thymus and spleen.[22] Obviously the diabetic state did not play a major role in prolonging islet allograft survival, since rejection occurred in control rats regardless of whether the transplants were across a minor or major histocompatibility barrier. In addition, it has also been reported that islet allografts in diabetic rats may be even more susceptible to rejection than other tissues, since islets rejected more rapidly than either skin or heart allografts using the same strain combination.[23,24] Thus, it appears unlikely that the diabetic state played a major role in prolonging islet allograft survival in our investigations; however, a minor effect on suppressing the immune system of the recipients cannot be excluded.

Barker et al.[25] reported a slight increase in the median survival time of islet allografts transplanted across a weak histocompatibility barrier after intraportal transplantation than after intraperitoneal transplantation. When a major histocompatibility barrier was present, the intrahepatic site did not provide prolongation of survival. Since the transplants in our investigations were across major histocompatibility barriers, it appears unlikely that the site of transplantation played a major role in prolonging islet allograft survival; however, a possible synergistic effect of this site on prolonging survival cannot be excluded.

The role of the single injection of ALS in prolonging survival appears to be the destruction of passenger leukocytes in the islet allografts as well as the destruction of passenger leukocytes that may have been altered or damaged after in vitro incubation at 24°C. It is also possible that ALS may have produced a temporary, critical, immunologic window in the recipient, which permitted the establishment of tolerance to the transplanted islet tissue.

FUTURE PROBLEMS

The finding that in vitro incubation of the islets at 24°C and a single injection of ALS into the recipients produces a marked prolongation of islet allograft survival across a major histocompatibility barrier raises several fascinating problems for future investigations. Studies are in progress to attempt to determine whether the T cell, B cell, or macrophage is responsible for the induction of immune rejection of established islet allografts. Once the identity of the stimulator cell or cells has been accomplished, then it will be possible to determine whether the islet cells lack certain loci in the major histocompatibility complex, such as the Ia region.

Techniques are now available for the dispersion and reaggregation of islet cells into small islets,[26,27] making it feasible to determine whether ALS is essential for prolongation of islet allograft survival since these preparations should contain only a few or no passenger leukocyte cells.

It will be of great interest and importance to determine whether the simple procedure used for prolongation of islet allograft survival can be used for prolonging xenograft survival of islets. Sollinger et al.[28] have reported prolongation of thyroid xenografts (rat to mouse) by utilizing the technique described by Lafferty et al.[3]

Studies on different sites of implantation should determine whether the intrahepatic location of the transplants plays a role in the procedures used for prolongation of islet allograft survival.

These studies, in addition to long-term metabolic studies with the optimum method developed for prolongation of islet allograft survival, will be required before islet allografts are attempted as a means of therapy for the diabetic patient.

SUMMARY

This report is a review of our investigations directed toward prolongation of islet allograft survival in the rat. In vitro culture of isolated rat islets at 24°C in conjunction with a single injection of ALS into the recipients produced a 90–100% survival at 100 days after transplantation of islets across major histocompatibility barriers. Rejection of established islet allografts was induced by the intravenous injection of peritoneal exudate cells syngeneic to the donor strain. The simplest explanation for the marked prolongation of islet allograft survival is that the procedure either diminished or altered passenger leukocytes in the islet allografts and that these cells play a primary role in the induction of recognition and rejection of the islet allografts by the recipient. We have discussed other factors that may also be involved in prolongation of the islet allograft survival as well as future problems to be resolved.

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