Transplantation of Cultured Fetal Pancreatic Islet Cells in the Treatment of Insulin-Dependent Diabetes Mellitus

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Fetal tissue grafting has been used in Russia for some 15 years for the treatment of insulin-dependent diabetes mellitus with complications. The use of cultured islet cells from the fetal human, as well as animal, pancreas has been successful even in the absence of immunosuppression. This review concerns only studies carried out in the CIS and the rest of the former USSR. It describes the treatment of complications of diabetes mellitus (retinopathy, nephropathy, polyneuropathy), the treatment of diabetes mellitus in children and pregnant women, and the preparation of patients with diabetes mellitus for surgery.

Key Words: *transplantation of human and animal fetal tissues; treatment of insulin-dependent diabetes mellitus and diabetic retinopathy, nephropathy, and polyneuropathy; treatment of diabetes mellitus in children and pregnant women; preparation of patients with diabetes mellitus for surgery*

The failure of traditional methods of treatment of insulin-dependent diabetes mellitus (IDDM) called for new, nontraditional methods of treating this severe disease. Since the main cause of IDDM, which usually develops during childhood or youth (before the age of 35), is the total or near-total death of β-cells of the pancreatic islets, the possibility of a pancreas transplant was explored. In the middle of the sixties, major advances occurred in transplantology and immunology, successful transplantation of the pancreas became a reality. To date, over 4000 clinical pancreas transplants have been performed all over the world [74]. Since transplantation of the pancreas is not a life-saving operation and the risk of adverse side effects and complications from the surgery per se, resulting from adjunct immunosuppressive treatment, is rather high, a transplant of the pancreas alone can hardly be justified in patients with IDDM. Such an operation is usually performed at the terminal stage of diabetic nephropathy, when a kidney transplant is required to save the patient's life. According to data of the leading centers of transplantation, successful transplantation of the pancreas performed simultaneously with kidney grafting provides for stable normalization of glycemia and for the abolishment of insulin dependence during the first year postoperation in almost 70% of patients with IDDM, the one-year survival attaining 90% [75]. On the other hand, no marked positive effect of transplantation of the pancreas has been noted with respect to the course of diabetic angiopathies [80], although, admittedly, these angiopathies had been severe at the time of transplantation. This operation, which is costly and not without risk for the patient with IDDM, is inadvisable at the earlier stages of diabetic complications. Furthermore, transplantation of the pancreas is severely limited owing to the lack of allogeneic donor organs.

Recently, the surgical techniques of pancreas grafting have virtually reached the limit of their

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potential, while the adjunct immunosuppressive treatment, despite continual improvements, is still apt to cause severe complications in a patient with IDDM. In this connection, interest in an alternative method of transplantation in the treatment of IDDM - transplantation of pancreatic islets (islet cells) - has increased. The advisability of such procedures is also supported by the fact that islets, which produce the insulin that is so crucial for the patient with IDDM, comprise only about 1% of the total weight of the pancreas in the adult donor, this naturally suggesting that just the endocrine (islet) tissue has to be transplanted rather than the entire pancreas. Such techniques of transplantation are not only more rational, but also safe, since separation of the islet tissue from the exocrine tissue helps eliminate the destructive effect of the digestive pancreatic enzymes, and the procedure of transplanting islet cells requires no serious surgical intervention.

Moskalewski (1965), who showed that intact islets can be isolated from the guinea pig pancreas following pretreatment of the pancreatic tissue with collagenase, was the first to separate the islet tissue from the exocrine tissue. On the basis of these findings, in 1967 Lacy and Kostianovsky developed a relatively simple method of isolating appreciable numbers of islets from the pancreas of rats and other small mammals. According to this method, the exocrine pancreatic tissue is primarily destroyed by the infusion of buffered salt solution into the system of efferent pancreatic ducts, followed by incubation of the material with collagenase. From the tissues thus treated islets are isolated with a small glass loop or pipette.

Although isolation of islets from the pancreas of rodents and some other animals very quickly became routine, isolation of considerable amounts of islets from the pancreas of adult humans (cadaveric donors) and large mammals was very complicated until recently and frequently impracticable. Largely because of this, from the middle 70s the human fetal pancreas (HFP) has been increasingly studied as a potential source of allogeneic islet cells. A large body of research has been carried out at the Research Institute of Transplantology and Artificial Organs (RITAO) (Moscow). Dead human fetuses at 16-24 weeks of gestation (spontaneous miscarriages and therapeutic abortions) served as the source of donor tissue. Classical isolation of islets from the human fetal pancreas was deemed impracticable, since at this stage anatomical separation of its endocrine and exocrine parts is incomplete [6]. Since the share of islet tissue can comprise up to 1/3 of the total weight of HFP [73], on the basis of the assumption that islet cells would exhibit a selective advantage vis-a-vis exocrine elements, different methods of fetal pancreatic tissue culturing were proposed. This contributed to the development of the method of islet cell culture [4,63], which involved the following main steps: treatment of the pancreatic tissue with collalytin (a collagenase-containing preparation), and careful dissection and incubation of tissue microfragments at 37°C for 5-10 days in medium 199 containing 10% bovine serum. As a result, islet cell cultures were obtained which were virtually devoid of exocrine elements, these having died and having been eliminated during culturing. Thus, it was established that islet cells exhibit a selective advantage during incubation of HFP. A high insulin-producing activity of these cultures was demonstrated by measuring the content of immunoreactive insulin in the culture medium for diverse regimens of islet cell incubation. The functional activity of the cultures *in vivo* was confirmed by their transplantation to animals with stable alloxan diabetes [49,72]. A manifest antidiabetic effect was observed in the case of introduction of cultured islet cells in the liver and spleen, as well as in the muscles [5,45,46,48,72]. Remission of experimental diabetes was noted in rat recipients before the completion of the experiment (20 weeks). A long-term survival of previously cultured islet cells in the organism of a xenogeneic recipient was verified by histological examination of the site of transplantation: groups of transplanted cultured islet cells which had preserved their structure were discovered [5,48]. Successful experimental studies provided grounds for clinical transplantation of cultured islet cells of HFP.

In October, 1979 the first in the USSR (and one of the first in the world) transplantation of cultured islet cells of HFP to a patient with diabetes mellitus was performed at RITAO [68]. Female patient E. L., aged 30, who had suffered IDDM since the age of 2, was the recipient. During the last few years, the course of diabetes mellitus in the patient had been labile: although the dose of injected insulin had virtually remained unchanged and the regimen of diet and exercise was stable, frequently (up to a few times a week), severe hypoglycemic states (sometimes including coma) would develop, often alternating with episodes of severe hyperglycemia, sometimes, with the development of ketoacidosis. Manifestations of late diabetic complications were appearing. The signs of distal diabetic neuropathy were most pronounced; over the last few months the patient had especially complained of intense leg pains and atrophy of the ankle muscles. Major symptoms of diabetic retinopathy, clinically manifested in small prereti-

nal bleedings, had developed moderately. The main manifestation of diabetic nephropathy was a moderate, yet stable, proteinuria. Over the last 2 years the patient had exhibited amenorrhea. The pretransplantation daily exogenous insulin requirements were 52 IU. Cultured islet cells obtained from 8 pancreatic glands of 18-24-week human fetuses (therapeutic minor cesarean section) were transplanted, under x-ray monitoring, to the splenic pulp. A marked therapeutic effect of transplantation was noted only 3 months postoperation: there were no complaints of pains in the lower extremities, and muscle atrophy disappeared. The course of IDDM stabilized: the disposition toward ketoacidosis and hypoglycemia no longer persisted. The improvement in the condition of the patient occurred against the background of compensated carbohydrate metabolism and a tendency toward decreased requirements for exogenous insulin, which made it possible to reduce the dose by 22 IU (from 52 to 30 IU) at the 6th month and by 10 IU more (to 20 IU a day) at the 8th month. Follow-up ophthalmological examination showed complete resorption of preretinal bleedings. Repeated urinary tests demonstrated stable aproteinuria. The menstrual cycle was restored. In spring 1981, i.e., 1.5 years after transplantation of cultured islet cells of HFP, its positive effect was largely preserved [66]. The promising results of the first transplantation have provided a basis for further transplantations of cultured fetal islet cells in clinical practice at RITAO.

The use of safe and simple techniques of intramuscular transplantation of cultured fetal islet cells was started in RITAO on April 16, 1981, and then in other medical centers of the Soviet Union [8,16,29].

By the end of 1993 some 240 transplants of cultured islet cells of HFP to patients with IDDM had been performed at RITAO, almost 220 transplants at the First Medical Institute (St. Petersburg), and 140 transplants at Lvov Medical Institute. Altogether, more than 800 allografts have been performed in Russia and in the republics of the former USSR. Analysis of these procedures has demonstrated a high efficacy of intramuscular transplantation of cultured islet cells of HFP, a positive effect being noted in almost 80% of recipients; in the main this effect involved the following posttransplantational changes [10,27,52,65,76,77]:

- stabilization of the course of labile forms of IDDM;
- reduced requirements for exogenous insulin against the background of stable compensation for disturbed carbohydrate metabolism;
- arrested development of late complications of IDDM.

As is well known, the labile course of IDDM not only aggravates disturbances in carbohydrate and other forms of metabolism, greatly impairing wellbeing, mental stability, and work capacity, but also triggers the rapid development of secondary diabetic complications.

Stabilization of the course of IDDM following transplantation of cultured islet cells of HFP, provides the optimal conditions for the choice of the appropriate dose of exogenous insulin [12,42,52,65], which contributes to stable compensation for disturbed metabolism. A reliable reduction of the blood level of glycosylated hemoglobin in the recipients testified to a marked improvement of carbohydrate metabolism; stabilization of the parameters of lipid metabolism, including normalization of indexes of lipid peroxidation, etc., was noted [10,28,35,52].

Reduced requirements for exogenous insulin (by 20-90%) in the recipients with IDDM, which occurred, as a rule, against the background of stable compensation for the diabetic state, were observed in the majority of patients 1-3 months posttransplant [64-70]. The reduced insulin requirements could have been due to stable compensation for carbohydrate metabolism per se; however, the appearance of peptide C (a marker of insulin secretion in β-cells) and its increased concentration in the blood of recipients with residual secretion of peptide C are indicative of active functioning of the β-cells which are present in successfully transplanted cultured islet cells [1,10,52,56]. The period of reduced insulin requirements lasts up to the 8th-12th month posttransplant, after which, in the majority of cases, the dose has to be restored to the pretransplantation dose. Changes of the serum level of peptide C in the recipients do not always correlate with changes in the requirements for exogenous insulin: a drop in the level of peptide C is often observed ahead of an increase in the requirements for exogenous insulin [51].

Many researchers who are concerned with transplantological methods of IDDM treatment, regard (probably by analogy with transplantation of the pancreas) the complete absence of insulin dependence in the recipient as the major (and, frequently, the only) criterion of successful transplantation of islet cells.

Shumakov *et al.* were the first [67,68] to discover the effect of transplanting cultured islet cells of HFP on secondary complications of IDDM. This criterion of efficacy must be regarded as being of crucial importance, since it is precisely diabetic angiopathies which underlie disability and early death of patients, while conversely, the re-

versal of angiopathies yields a much better prognosis for life and work capacity.

The most pronounced therapeutic effect of transplantation of cultured islet cells of HFP has been noted in patients with diabetic polyneuropathy [9,25,64-70,77]. In such patients manifestations of both distal (in 86% of cases) [64,65] and visceral (in 62% of cases) [53] neuropathy were markedly reduced and, in some cases, virtually disappeared; however, whereas in the case of distal neuropathy a positive effect was observed in the overwhelming majority of patients, in the case of visceral neuropathy an improvement was noted only in patients with a history of this complication of no longer than 3 years. The positive effect of transplantation of cultured islet cells of HFP on the course of diabetic angiopathy of the lower extremities has been convincingly documented [23,27,33,52].

The therapeutic effect of transplantation of cultured islet cells of HFP on the course of diabetic nephropathy depends on the severity of disturbances in renal function [23,38,39,52,64-66]. The best results have been obtained in patients in the prenephrotic stage of this complication: a marked reduction of proteinuria was attended by stable normalization of the arterial pressure (in 77% of cases) [51]. In patients with diabetic nephropathy in the nephrotic stage positive changes in clinical and laboratory indexes were less pronounced, being noted in 48% of cases. In patients with diabetic nephropathy in the nephrosclerotic stage a therapeutic effect of transplantation was virtually absent. Unsatisfactory results were also obtained in patients with chronic pyelonephritis [24].

Marked success of transplantation has been observed in patients with diabetic retinopathy [7,19,52,64,65]. According to data of Slovesnova [51,52], in addition to stabilization of the state of the retina, which was observed in 91% of cases, in one third of patients with common diabetic retinopathy gradual resorption of intraretinal bleedings and disappearance of retinal edema were noted posttransplant. Nine months after transplantation the fundus exhibited only the initial changes (diabetic angiopathy), attesting to regression of the pathological process. The improvement in the clinical picture of patients with proliferative diabetic retinopathy was unexpected, since, in the opinion of ophthalmologists, after the onset of fibrous proliferation, the further course of the disease is mainly determined by intraocular factors, and the possibility of stabilization, not to mention reversed development, of pathological processes seems unlikely at this stage. Nevertheless, in 42% of patients with proliferative diabetic retinopathy to whom cultured islet cells of HFP were transplanted intramuscularly, gradual resolution of preretinal hemorrhages, thinning of the fibrous tissue and vessel depletion in these hemorrhages, and smoothing out and rejoining of the zones of the earlier detached retina were noted [52,77].

An improvement in the fundus went along with a marked improvement in visual acuity in the 8th-12th month posttransplantation [17,52]. A stable picture of the fundus was maintained in patients over 3-4 years of the follow-up [52]. Evidently, the therapeutic effect of transplantation was due to a certain extent to the restoration of residual endogenous secretion of insulin in the recipients and/or its increase, since, according to findings of Vasyukova *et al.* (1981), a reduced residual secretion of endogenous insulin promotes the development and aggravation of diabetic retinopathy.

As was shown by Yugoslavian endocrinologists who performed allotransplantation of cultured fetal islet cells using the method developed at RITAO, the functional activity of transplanted β-cells may be corroborated by an increase of the basal level of peptide C after intravenous injection of glucagon (1 mg) to a patient with residual secretion of insulin and by the presence of peptide C in the blood of the recipient whose own islet apparatus does not secrete endogenous insulin (Djordjevic *et al.*, 1987). Similar results were obtained by Latvian researchers [57]. Undeniably, normalization of the state of the cell insulin receptors following transplantation of HFP promotes an improvement in the compensation for IDDM.

Improved parameters of the immune state were also noted in patients with IDDM after transplantation of cultured islet cells of HFP [7,14,59,61].

As is well known, the course of acute and chronic surgical diseases is more severe in the case of concomitant IDDM. At the hospital of Lvov Medical Institute, during abdominal operations for cholecystitis or pancreatitis, a suspension of precultured islet cells of HFP was slowly infused in the liver of patients with IDDM via the portal or superior mesenteric vein. Intraportal transplantation made it possible to reduce the mortality of surgical patients with concomitant IDDM from 19 to 0%, the number of postoperative complications from 61.9 to 33.3%, and the treatment period in the hospital from 32 to 16.8 days [9,10,34]. Similar results were obtained at the Riga Center of Transplantation, where transplantation of fetal islet cells was used to correct the metabolic state in patients with IDDM admitted for surgery [41]. In addition, transplantation of HFP markedly im-

proved the course of surgical septic infection in patients with IDDM [32,41].

Limitations in the availability of the allogeneic donor material, whether of the adult pancreas or from human fetuses, along with a considerable demand for clinical transplantations of cultured islet cells, have prompted research into alternative - xenogeneic - sources of islet cells: the pancreas of animals. Notably, studies of the immune characteristics of β-cells have provided theoretical grounds for the xenotransplantation of islet cells. An important result of these studies has been the conclusion that there are no species-specific differences between the antigenic characteristics of cells of humans and animals belonging to different taxonomic groups. The successful experiments with xenotransplantation of cultured islet cells to animals with induced diabetes mellitus have substantiated the possibility of effective xenogeneic transplantation in clinical practice [5,42,44,46-48,72,79]. It has been demonstrated that the use of precultured islet cells makes it possible to attain a long-term remission of the diabetic state even without immunosuppression.

The first clinical xenografts of cultured islet cells were performed in 1981-1982 at the Kiev Research Institute of Endocrinology and Metabolism [29] and then at RITAO [69] and some other medical centers. The pancreases of swine and bovine fetuses [40,69] and of neonatal pigs [29, 58] were used as the source for the cultures. By the end of 1993 in Russia and in the republics of the former USSR more than 2500 xenogeneic transplants had been performed.

Long-standing observations demonstrated that clinical xenotransplantation of cultured islet cells and clinical transplantation of HFP differ little with respect to their antidiabetic effect [3,29,36].

For instance, xenotransplantation of cultured islet cells proved to be no less effective in the case of a labile course of IDDM: against the background of stable compensation for carbohydrate metabolism the reduction in the requirements for exogenous insulin was the same as mentioned above, this being retrospectively indicated by a marked drop of the level of glycosylated hemoglobin [25,30]. Some scientists [30] suggest that normalization of indexes of lipid metabolism during the posttransplantational period should be regarded as the criterion of efficacy of xenotransplantation of cultured islet cells.

The clear-cut positive effect of xenotransplantation on the course of diabetic polyneuropathy was shown [25,29,30,37,64,65,69]. A marked therapeutic effect was also obtained in patients with diabetic retinopathy [18,19,30]. On the other hand, there are reasons to suppose that xenogeneic transplantation of islet cells has a lesser effect upon the severity of diabetic nephropathy.

The effect of intramuscular xenotransplantation of cultured islet cells obtained from the pancreas of neonatal pigs on the course of IDDM in children was studied in detail [2,3,11]. In all, the effect of more than 100 allo- and xenografts in 86 children and teenagers suffering from IDDM was assessed as positive in 81% of cases. Among the followed-up recipients 94% had diverse commonly associated complications: micro- and macroangiopathies of the lower extremities, hepatopathy, encephalopathy, retinopathy, nephropathy, disseminated lipodystrophies, lipoid necrobiosis, Mauriac and Nobecour's syndromes, etc. The effect of transplantation proved to be extremely marked with respect to complications of IDDM. For instance, a somewhat delayed physical and sexual development was still evident 6 months after xenotransplantation only in one half of the patients with Mauriac and Nobecour's syndromes, while 1-1.5 years later, the diagnosis of this complication could be canceled. Whereas before transplantation a hepatopathy was noted in one third of the children, one year later it was preserved in just 6±4%. The incidence of angiopathies of the lower extremities remained high at all times, but the severity of damage was reduced. For instance, whereas before transplant only 17±5% of the children exhibited the initial or functional stage of angiopathy, one year later the number of such patients, as well as of patients in whom angiopathies were no longer observed, reliably increased (47±7%). Not only the incidence, but also the severity of lipodystrophy and encephalopathy markedly decreased; a marked improvement was also observed with respect to the course of nephropathy.

As is well known, diabetes mellitus during pregnancy presents a high risk for the fetus. The diabetogenic effect of pregnancy determines a labile course of the disease, difficulties in correcting metabolic processes, and the development of secondary complications. At the Kharkov Medical Institute transplantation of cultured islet cells from the pancreas of neonatal pigs to pregnant women with IDDM is used as a means of preventing diabetic fetopathy. Over two years (1986-1987) Snopkova *et al.* [55] followed up 11 pregnant women with IDDM who had received xenografts of cultured islet cells of neonatal rabbit pups. The laboratory and clinical results testified to a positive effect on the course of diabetes mellitus during pregnancy. In the majority of women in the 3rd-4th months posttransplantation the daily re-

quirements for insulin were reduced by 10-20%, the increase in insulin requirements during weeks 20-25 which is typical of pregnancy was absent, and the course of the disease stabilized, facilitating proper dosage. In all cases the content of glycosylated hemoglobin was reliably reduced, and no vascular complications developed. A more favorable clinical outcome of pregnancy was noted (stillbirths and manifest fetopathy were absent, and a better adaptation was characteristic of the neonatal period). These facts offer hope that this technique will help solve problems afflicting pregnant women with IDDM [54,55].

Recently, the fetal or neonatal pancreas of rabbits has come to be used as a new source of cultured islet cells [50]. The new RITAO-developed techniques of preparing highly active cultured islet cells with a reduced immunogenicity have made it possible not only to markedly increase the number of transplantations, but also to raise their efficacy to almost 90%. A comparative analysis of the traditional intramuscular and the physiologically more appropriate intraportal techniques of xenotransplantation of cultured rabbit pancreatic islet cells was carried out. Infusion of preliminarily cultured islet cells in the liver proved to exert a more rapid and more marked antidiabetic effect [21,71]. Active functioning of intraportally injected xenogeneic islet cells was also confirmed [36,78].

For enhancement of the therapeutic effect of xenotransplantation of cultured islet cells on the course of diabetic retinopathy the method of introduction of islet cells in the retrobulbar tissue of the eye was developed and approved at RITAO in conjunction with the Department of Eye Diseases of the Moscow Stomatological Institute [15]. An analysis of nearly 40 long-term (up to 3 years) observations showed that following the xenografts resorption was ophthalmoscopically documented in 76% of cases, and a reduced intensity of proliferation and neovascularization in 47% of cases [15].

Encouraging results were obtained in cases of transplantation of cultured islet cells of the pancreas of neonatal rabbits to patients with newly diagnosed IDDM. In the majority of recipients compensation for the disease markedly improved, and the requirements for exogenous insulin dropped sharply. At the same time, normalization of the immune status was noted in patients, possibly attesting to attenuated autoimmune destruction of β-cells in their own pancreas [62].

Since a manifest immune response of the organism to both allogeneic and xenogeneic transplants of cultured fetal and neonatal islet cells [14,20,25,31,59-61] was absent in patients with IDDM, this confirmed the possibility of performing repeated transplantations. The manifest clinical effect of both allo- and xenogeneic transplantations lasted on average 12 months [2,25,30,41,52]. Repeated or alternated allogeneic and xenogeneic (including transplants from different animals) transplantations were performed. As a rule, the procedure is repeated after the effect of previous transplantations wanes [2,25,26,30]. For instance, as was reported by Ignatenko [24,25], 64 repeated transplantations of cultured fetal islet cells in different combinations were performed at RITAO in 31 patients. In 20 patients the procedure was done twice, in 3 patients three times, and in one patient four times. The results of repeated allo- and xenotransplantations proved to be similar to the clinical effect of the first transplantation. Analysis of 22 transplantations performed by Rozental' *et al.* [41-43] showed that the clinical improvement observed was virtually the same as with the previous transplantation in almost all patients. The therapeutic effect of a repeated transplantation was regarded by patients as an extension of the effect of the first successful transplantation. The assessment of 14 repeated transplantations reported by Benikova and Turchin [2] also attests to the efficacy of retransplantations.

Thus, allogeneic and xenogeneic transplantation of cultured islet cells obtained from fetal or neonatal pancreases can stabilize the labile course of IDDM and arrest its development, in a number of cases reversing the development of secondary diabetic complications. Repeated transplantations of fetal or neonatal pancreatic islet cells result in a marked prolongation of the favorable posttransplantational effect. The appropriate use of transplantation of cultured fetal islet cells in the treatment of IDDM can raise the quality of life and prolong the life span of patients afflicted with this severe disease.

REFERENCES

1. E. A. Benikova and E. V. Bol'shova, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 53-54.
2. E. A. Benikova and I. S. Turchin, *Probl. Endokrinol.*, № 4, 17-19 (1991).
3. E. A. Benikova, I. S. Turchin, L. S. Belyakova, *et al.*, *Ibid.*, № 2, 19-22 (1987).
4. V. N. Blyumkin, N. N. Skaletskii, N. I. Kaurichcva, *et al.*, *Byull. Eksp. Biol. Med.*, **97**, № 6, 764-766 (1984).
5. V. N. Blyumkin, N. N. Skaletskii, V. L. Popov, *et al.*, *Ibid.*, **95**, № 5, 89-91 (1983).
6. V. N. Blyumkin, B. I. Shal'nev, and R. A. Babikova, in: *Problems of Transplantology and Artificial Organs* [in Russian], Moscow (1977), pp. 67-70.

7. N. I. Boiko, *Allotransplantation of Cultured Islet Cells of the Pancreas to Surgical Patients with Diabetes Mellitus (Dissertation by Doctor of Medical Science)*, Moscow (1991).
8. N. I. Boiko and M. P. Pavlovskii, in: *Current Topics in Experimental and Clinical Endocrinology* [in Russian], Kiev (1987), pp. 41-42.
9. N. I. Boiko and M. P. Pavlovskii, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 54-56.
10. N. I. Boiko and M. P. Pavlovskii, *Probl. Endokrinol.*, № 1, 11-13 (1990).
11. E. V. Bol'shova, E. A. Benikova, and L. S. Belyakova, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 56-57.
12. N. P. Bychikhin, T. B. Romanova, V. P. Pashchenko, *et al., Ibid.*, pp. 58-59.
13. E. A. Vasyukova, E. S. Gulyaeva, A. A. Katsnel'son, *et al., Klin. Med.*, № 11, 42-46 (1981).
14. V. A. Vorob'eva, *Parameters of Cellular and Humoral Immunity in Patients with Diabetes Mellitus Following Transplantation of β-Cells of the Pancreas (Dissertation by Candidate of Medical Science)*, Moscow (1992).
15. N. A. Gavrilova, *Effect of Gravitational Plasmapheresis and Retrobulbar Transplantation of Cultured Islet Cells of the Pancreas on the Course of Diabetic Retinopathy (Dissertation by Candidate of Medical Science)*, Moscow (1993).
16. O. V. Galibin, L. V. Potashov, M. V. Chernikova, *et al.*, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 62-63.
17. S. V. Glukhoded, T. A. Slovesnova, S. N. Ignatenko, and N. N. Skaletskii, in: *Current Topics in Experimental and Clinical Endocrinology* [in Russian], Kiev (1987), pp. 86-87.
18. A. I. Danilova, in: *Organ Transplantation* [in Russian], Lvov (1990), pp. 50-52.
19. A. I. Danilova, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 65-67.
20. O. S. Dekhtyareva, E. A. Sakalo, P. M. Karabun, *et al., Ibid.*, pp. 67-68.
21. Yu. N. Evseev, *Comparative Assessment of Intramuscular and Intraportal Transplantation of Cultured Islet Cells of the Pancreas in Patients with Type I Diabetes Mellitus (Dissertation by Candidate of Medical Science)*, Moscow (1993).
22. A. S. Efimov, N. D. Tron'ko, I. V. Komissarenko, *et al., Ibid.*, pp. 71-72.
23. S. T. Zubkova, V. G. Naumenko, and S. M. Lisyanskaya, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 69-70.
24. S. N. Ignatenko, *Transplantological Methods of Treatment of Diabetes Mellitus (Dissertation by Doctor of Medical Science)*, Moscow (1989).
25. S. N. Ignatenko, T. A. Slovesnova, N. N. Skaletskii, *et al.*, in: *Problems of Transplantology and Artificial Organs* [in Russian], Moscow (1989), pp. 91-97.
26. S. N. Ignatenko, B. I. Shal'nev, and T. A. Slovesnova, in: *Current Topics in Experimental and Clinical Endocrinology* [in Russian], Kiev (1987), pp. 156-157.
27. G. A. Kazaryan, M. E. Basmadzhyan, R. A. Ovanesyan, *et al.*, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 74-75.
28. I. V. Komissarenko, A. G. Lysenko, G. V. Svetlova, *et al., Ibid.*, p. 77.
29. V. P. Komissarenko, I. S. Turchin, I. V. Komissarenko, *et al., Vrach. Delo*, № 4, 52-57 (1983).
30. A. A. Leinieks, *Efficacy of Transplanting Cultured Islet Cells of the Pancreas in Patients with Diabetes Mellitus (Dissertation by Candidate of Medical Science)*, Moscow (1989).
31. A. A. Leinieks, A. K. Shtifts, S. B. Vasipa, *et al.*, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 79-80.
32. T. N. Makarenko, N. I. Boiko, and M. P. Postranskii, in: *Organ Transplantation* [in Russian], Lvov (1990), pp. 88-89.
33. Yu. G. Odiyankov, E. G. Odiyankov, A. L. Urakov, *et al.*, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 83-84.
34. M. P. Pavlovskii, N. I. Boiko, and T. N. Makarenko, *Endokrinologiya*, **21**, 38-46 (1991).
35. M. P. Pavlovskii, N. I. Boiko, M. F. Tymochko, *et al., Probl. Endokrinol.*, № 3, 26-29 (1989).
36. A. V. Podshivalin, *Efficacy of Transplanting Cultured Islet Cells of the Pancreas in Patients with Diabetes Mellitus as Assessed with Radionuclides (Dissertation by Candidate of Medical Science)*, Moscow (1993).
37. V. A. Potykevich, I. A. Tsuranova, B. N. Man'kovskii, *et al.*, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 87-88.
38. O. Yu. Prozorova, I. A. Pronchenko, and T. A. Slovesnova, in: *Transplantation and Artificial Organs* [in Russian], Moscow (1984), pp. 21-24.
39. O. Yu. Prozorova, T. A. Slovesnova, and T. V. Potanina, *Ibid.* (1986), pp. 40-45.
40. R. L. Rozental', I. M. Il'inskii, Ya. B. Bitsans, and O. A. Fomina, in: *XXXI All-Union Congress of Surgeons*, Tashkent (1986), pp. 269-270.
41. R. L. Rozental', I. M. Il'inskii, V. A. Zakrevskii, *et al., Vest. Khir.*, № 5, 83-86 (1988).
42. R. L. Rozental', A. A. Leinieks, Ya. B. Bitsans, *et al.*, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 90-91.
43. R. L. Rozental', A. K. Shtifts, I. M. Il'inskii, *et al., Probl. Endokrinol.*, № 1, 10-12 (1988).
44. N. N. Skaletskii, *Xenotransplantation of Cultured Islet Cells of the Human Fetal Pancreas to Rats with Experimental Diabetes Mellitus (Dissertation by Candidate of Medical Science)*, Moscow (1987).
45. N. N. Skaletskii, in: *Problems of Transplantology and Artificial Organs* [in Russian], Moscow (1983), pp. 79-81.
46. N. N. Skaletskii, in: *Organ Transplantation* [in Russian], Kiev (1985), pp. 219-220.
47. N. N. Skaletskii, in: *Transplantation and Artificial Organs* [in Russian], Moscow (1984), pp. 79-81.
48. N. N. Skaletskii, in: *Current Topics in Experimental and Clinical Endocrinology* [in Russian], Kiev (1987), pp. 352-353.
49. N. N. Skaletskii, V. N. Blyumkin, B. I. Shal'nev, and M. I. Danilov, in: *Transplantation and Artificial Organs* [in Russian], Moscow (1986), pp. 67-68.
50. N. N. Skaletskii, L. A. Kirsanova, O. V. Zagrebina, *et al.*, in: *Organ Transplantation* [in Russian], Lvov (1990), pp. 238-239.
51. T. A. Slovesnova, *The Role of Allotransplantation of Cultured Islet Cells of the Human Fetal Pancreas in the Complex Treatment of Patients with Diabetes Mellitus (Dissertation by Candidate of Medical Science)*, Moscow (1989).
52. T. A. Slovesnova, S. V. Glukhoded, N. N. Skaletskii, *et al., Probl. Endokrinol.*, № 1, 25-28 (1989).
53. T. A. Slovesnova, S. N. Ignatenko, T. N. Goncharova, and O. S. Bulatova, in: *Organ Transplantation* [in Russian], Lvov (1990), pp. 127-128.
54. L. V. Snopkova, N. A. Volkova, and B. P. Sandomirskii, in: *Effect of Cooling on Biological Objects* [in Russian], Kharkov (1990), pp. 137-140.
55. L. V. Snopkova, V. I. Grishchenko, and B. P. Sandomirskii, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 91-93.

56. V. S. Suskova, E. N. Matsulenko, T. A. Slovesnova, *et al., Probl. Endokrinol.*, № 4, 16-20 (1988).
57. L. Ya. Farta, A. V. Galvin'sh, and P. S. Zavadskii, in: *Transplantological Methods of Treatment of Diabetes Mellitus* [in Russian], Riga (1988), pp. 95-97.
58. I. I. Fakhreev, E. A. Mitryukova, D. E. Kolodkin, et al., Ibid., pp. 97-98.
59. B. I. Shal'nev, V. S. Suskova, and V. I. Emets, in: *Organ Transplantation* [in Russian], Kiev (1985), pp. 143-144.
60. B. I. Shal'nev, V. S. Suskova, V. I. Emets, *et al.*, in: *Transplantation and Artificial Organs* [in Russian], Moscow (1986), pp. 55-59.
61. B. I. Shal'nev, V. S. Suskova, V. I. Emets, *et al.*, in: *Current Topics in Experimental and Clinical Endocrinology* [in Russian], Kiev (1987), pp. 419-420.
62. P. I. Shishko, *The Role of Alimentary Factors in the Modulation of the Immune Status and Residual Secretion of Insulin in Patients with Insulin-Dependent Diabetes Mellitus (Dissertation by Candidate of Medical Science)*, Moscow (1993).
63. V. I. Shumakov, V. N. Blyumkin, R. A. Babikova, *et al., Byull. Eksp. Biol. Med.*, **89**, № 8, 202-204 (1979).
64. V. I. Shumakov, V. N. Blyumkin, S. N. Ignatenko, *et al., Probl. Endokrinol.*, № 1, 18-22 (1985).
65. V. I. Shumakov, V. N. Blyumkin, S. N. Ignatenko, *et al., Ibid.*, № 5, 67-70 (1985).
66. V. I. Shumakov, V. N. Blyumkin, S. N. Ignatenko, *et al., Klin. Med.*, № 2, 46-51 (1983).
67. V. I. Shumakov, V. N. Blyumkin, S. N. Ignatenko, *et al.*, in: *Problems of Transplantology and Artificial Organs* [in Russian], Moscow (1982), pp. 5-7.
68. V. I. Shumakov, V. N. Blyumkin, B. I. Shal'nev, *et al., Probl. Endokrinol.*, № 1, 25-30 (1981).
69. V. I. Shumakov, S. N. Ignatenko, V. N. Blyumkin, *et al.*, in: *Transplantation and Artificial Organs* [in Russian], Moscow (1984), pp. 19-21.
70. V. I. Shumakov, S. N. Ignatenko, N. N. Skaletskii, and T. A. Slovesnova, in: *Problems of Transplantology and Artificial Organs* [in Russian], Moscow (1983), pp. 5-6.
71. V. I. Shumakov, N. N. Skaletskii, Yu. N. Evseev, *et al., Biosovmestimost'*, **1**, № 4, 187-195 (1993).
72. V. I. Shumakov, B. I. Shal'nev, V. N. Blyumkin, *et al., Byull. Eksp. Biol. Med.*, **89**, № 1, 48-50 (1980).
73. H. Ferner, *Gastroenterol. Stoffwechsel. Aktionen und Interaktionen*, Baden-Baden - Brussels (1974), pp. 75-79.
74. P. Morel, L. Buhler, Marini, *et al., Schweiz. Med. Wschr.*, **123**, № 13, 603-608 (1993).
75. R. P. Robertson and D. E. R. Sutherland, *Ann. Rev. Med.*, **43**, 395-415 (1993).
76. V. I. Shumakov, V. N. Blyumkin, S. N. Ignatenko, *et al., Transplant. Proc.*, **19**, 387 (1987).
77. V. I. Shumakov, S. N. Ignatenko, V. N. Blyumkin, *et al., Diabetes*, **38**, Suppl. 1, 314 (1989).
78. V. I. Shumakov, N. N. Skaletsky, Y. N. Evseev, *et al., Biomaterial-Living System Interactions*, **1**, № 4, 175-181 (1993).
79. N. N. Skaletsky, B. I. Shal'nev, V. N. Blyumkin, *et al., Diabetes*, **38**, Suppl. 1, 317 (1989).
80. J. Timsit, C. Legendre, L. Chatenoud, *et al., Biomed. Pharmacother.*, **46**, № 2/3, 71-78 (1992).