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Some controversial problems kidney transplantation

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To-day, 30 years after the first successful kidney transplantation between identical twins, the results of renal allograft transplantation are not yet satisfactory. Several problems are still open for discussion and many opinions are controversial. In the present discussion, I should like to comment on problems connected with:

1. postoperative oliguria/anuria, its prevention and impact on allograft longterm function,
2. different posttransplant immunosuppressive regimens,
3. pretransplant assessment of immune status of the recipient,
4. immunological monitoring of the transplant patient.

The following presentation is based on my personal experience with 500 kidney transplantations performed in the Warsaw Transplantation Center since January 26, 1966. This material includes 484 first and 16 second grafs. 434 organs —including 28 live donor kidney— were taken locally, 66 outside Warsaw, in other Polish cities or in DDR, USA, CSSR, The Netherlands and Hungary. All but one local unrelated allograft were taken from non-beating heart cadavers, wheras organs delivered from abroad were harvested after donor brain death. Surprisingly enough no statistically significant differences were found neither between cumulative patient and graft survival up to at least 4 years after transplantation nor the frequency of postoperative oliguria/anuria between these two groups of recipients.

Since 1966 three periods can be distinguished in our Center, according to the immunosuppression used.

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In the first years we have followed the commonly used immunosuppresion regimen, in which azathioprine and high oral doses of prednisone were given. Acute rejections were treated with large doses of oral prednisone, actinomycin C infusions, ATG and local graft irradation. Some of the recipients were pretreated with thoracic duct lymph drainage. Such methods as HLA matching, cross-matching, renocystography, ultrasonography or graft biopsy were yet not at hand. The differential diagnosis between postoperative acute tubular necrosis (ATN) and acute graft rejection was extremely difficult. And —first of all— our experience in the field of transplantation was inadequate.

At that time overt signs of hypercorticoism with frequent infections were commonly observed. For these reasons, the mortality rate during the first posttransplantation year was rather high —about 25 %, almost the same (30 %) as that reported from other more experienced centers. The intensive oral corticothepary was especially dangerous in young patients, in whom such complications as Cushingoid syndrome, steroid diabetes and impaired growth were usually observed. We have lost our first patients 6 months after transplantation because of acute haemorrhagic pancreatitis which developed while the function of her graft was excellent, but when —according to the regime of that time— she was still on high doses of oral prednisone.

With the introduction in 1974 of the new immunosuppressive regime, based partly on intravenous application of methylprednisolone (MP), an improvement of patients survival was achieved.

With this method the number of complications significantly diminished and both the mortality rate and graft survival improved by approximately 10 %, but postoperative ATN remained a challenge.

To assess the impact of posttransplant oliguria/anuria on long term patient and cadaver allograft survival 285 consecutive recipients were allocated to 5 groups: A. non-oliguric, 69 patients; B. Oliguric-anuric of one week's duration, 45 patients; C. Oliguric-anuric of two week's duration, 82 patients; D. Oliguric-anuric of three week's duration, 45 patients; E. Oliguric-anuric of more than three week's duration, 22 patients, and F. with non-functioning graft, 28 patients. Immunosuppression consisted of azathioprine, prednisone and promethazine. Rejections were treated with i.v. pulses of methylprednisolone. No differences were found between A, B, C, D and E group patient survival up to the end of the 3rd year. However, postoperative recipient survival was significantly lower (75 %) in patients with nonfunctioning graft, than in members of remaining group (78-92 %). Nonoliguric patients had higher graft survival than patients of groups B, C, D and E, but these differences reach statistical significance only with regard to recipients with oliguria/anuria of more than 3 week's duration. Practically no differences were observed between cumulative graft survival of group B, C, and D.

In an attemps to improve the immediate function of non-beating heart cadaver grafts an appropiate study on 22 dogs were performed. It was shown that resected kidney submitted to warm ischemia of 90 minutes duration and autotransplanted to original host is unable to support its life. Both lidocaine pretreatment and dopamine infusion after revascularization improved the mortality rate of animals by 25 %. The best protection against ischemic kidney damage was obtained in animals pretreated with lidocaine and receiving constant low-dose infusion on revascularization of the autograft. Preliminary clinical studies in our center have shown that lidocaine pretreatment in the donor before harvesting combined with constant low-dose (2 mg/kg/min) dopamine infusion in the recipient decreased the frequency of oliguria/anuria after transplantation from 75 % to 30 %. Nevertheless, further studies are needed to clarify the exact mechanism of these protective actions. The prevention of posttransplant oliguria/anuria is especially important in patients treated with cyclosporine (CsA), whose nephrotoxic action can prolong the duration of ATN and increase the damage of already injured organ.

Posttransplant immunosuppression

Looking for further improvement of graft function several randomized prospective trials using different adjuvant immunosuppressants were performed. These studies were done between 1978 and 1985, at the time, in which our transplantation protocols, surgical techniques and diagnostic procedures were already well developed. All recipients were followed by myself and my close co-workers. The patients involved were randomized on alternative basis. The following parameters, considered as important for graft acceptance and survival were taken into account during comparison of all experimental groups: age, sex, the primary renal disease, preformed antibody levels, dialysis age, pre- and perioperative blood transfusions, degree of HLA A-B match, duration of graft ischemia and handling, and of postoperative oliguria/anuria. Unfortunately at that time HLA-DR matching was not available.

With first adjuvant studied —Niridazole— no beneficial effect on posttransplant course of patients treated was found. Therefore this trial was soon discontinued and Promethazine hydrochloride was chosen for further studies.

Promethazine hydrochloride, an antihistaminic drug has weak immunosuppressive action of its own. Nevertheless, given intraperitoneally it prevented early cardiac allograft rejection in the rat, and in combination with standard immunosuppressants, + it prolonged kidney allograft survival in rabbits and rats. The exact mode of immunosuppressive action of Promethazine is not known.

Promethazin may act analogically to another H_1 antagonist —clemastine fumarate—, which —as was shown by Nair et al— when added directly to the mixture of effector and target cells decreases the target binding capacity of effector lymphocytes, and inhibits also NK activities of T cells. In fact, we have found recently that promethazine inhibits proliferative responses in «one way» MLR, as well as NK activities, and to a much lesser degree ADCC reaction.

We have also shown, that «in vitro» low promethazine concentrations caused enhancement, while higher-inhibition of PWM and MLR-induced Ig synthesis. Moreever promethazine was found to inhibit both the generation of memory cells of secondary humoral response to alloantigens assessed in MLR, as well as secondary responses themselves. It also prevents graft capillary endothelium damage, blocking platelets aggregation. Its beneficial effects can also be party connected with inhibition of antibody production and delayed hypersensitivity-type reactions.

Taking into account the dominant role of all these activities during allograft rejection, we decided to study the effect of addition of promethazine to a standard immunosuppressive protocol, despite preliminary negative results obtained in a few human cases already reported.

In November 1979 a prospective randomized trial of two appropriate immunosuppressive regimens was started. 102 cadaver graft recipients were assigned on an alternative basis to either C (control) or Pm (promethazine) groups. The basic regimen consisted of prednisolone administered intravenously during the first 3 weeks postgrafting in the doses 1 g on the first, 500 mg on the second, 250 mg on the third day and 125 mg on the 4th-21st days. Thereafter oral prednisone was given in a single morning dose of 30 mg/day up to the end of 3rd month, with slow tapering to 10-15 mg/day at the end of the first year. Acute allograft rejections were treated with 1 g/day methylprednisolone pulses given i.v. 2-6 times. Azathioprine was given at a dose of 1-2,5 mg/kg of body weight/day according to the WBC and platelet count. The Pm group received 25 mg b.d. of promethazine given concomitantly with steroids and azathioprine.

The dignosis of acute rejection in patients without ATN was made on the basis of clinical criteria, such as fever, graft enlargement and pain, arterial hypertension, fall of urinary output with hyponatriuria, and first of all, rise of serum creatinine level without any other reasons such as infection, dehydration, electrolyte disturbances, heart failure, urinary tract obstruction, internal urinary leakage or vascular accidents. Other confirmatory signs of rejection, such as fall of C_{PAH} and presence of typical echographic pattern of allograft oedema were also looked for.

About 66 % of our patients developed early postoperative oliguria/anuria of 1 to 6 weeks duration. In this situation the diagnosis of acute rejection was especially difficult, because of such signs as serum creatinine level and urinary output —the main criteria of rejection in nonoliguric recipients— fluctuated independently of graft function relation with frequence of HD sesions.

In such cases C_{PAH} was frequently estimated and, if it increased with time, no antirejection therapy was given. Lack of improvement was considered as a result of acute rejection or of very severe ATN. For differential diagnosis suction or needle biopsy was performed. If for some reasons it was impossible to do it, the anuric patients were given 1 g of MP i.v. on the 6th, 7th and 8th days. If no improvement of graft function followed, the same MP therapy was repeated on the 13th, 14th and 15th days post operation. If recipient still remained oliguric/anuric, further treatment was given according to the results of repeated graft biopsies.

At the time of trial analysis (April 20, 1985) no statistically significant differences between cumulative patient survival in Pm and control groups were found. However, 65 % of grafts in the promethazine group were functioning compared with only 47 % grafts in the control group. Actuarial graft survival was significantly higher in the Pm group up to at least 4 years (72 vs 47 %, $p < 0.025$). The function of surviving grafts, as assessed by serum creatinine levels, was comparable.

About 20 % of the recipients in both groups did not have rejection during the observation period. The number of irreversible rejections and/or deaths was significantly ($p < 0.05$) higher in control (26/51) than in the Pm (15/51) group. The fact that one of the control patients died in a car accident with a well functioning graft did not alter these results.

The difference in graft survival was caused by higher graft loss in control group during the first 3 months after surgery (12/51 vs 3/51, respectively). Thereafter, the frequency of graft failure was comparable in both groups.

Late failures —more than 1 year after transplantation— were observed in 10 recipients of Pm and 6 of the control group. 5 Pm and 4 control patients died with functioning graft because of cardiovascular (2 Pm and 2 control) and hepatic (2 Pm and 1 control) complications or of infection (1 patient in each group). One control recipient lost his life in a car accident. There were 3 irreversible chronic rejections in Pm and 2 in control group. De novo glomerulonephritis and graft artery stenosis contributed to transplant loss in remaining 2 Pm patients.

Whereas irreversible graft rejections were more frequent in control than in Pm group, the number and timing of reversible rejection episodes were comparable in both group of patients. The Pm group was given some more prednisone during the first 18 months of therapy (22.7 ± 5.7 vs. 19.9 ± 5.1; $p < 0.025$), and more boluses of prednisolone were required to control their rejection episodes during the first two years after transplantation (7.9 ± 5.1 vs 5.5 ± 3.8; $p < 0.05$). Nevertheless, this was not the decisive factor responsible for better results obtained in Pm group, because all but one of the control patients, who rejected their graft, received steroids in higher amount than the average doses used in Pm group. In all but 3 of them, the number of prednisolone boluses given was also higher than in Pm group.

No serious side effects or complications related to the use of promethazine were encountered. The frequency of cardio-vascular, gastrointestinal, bone, hepatic and mental disorders, as well as bacterial, fungal and viral infections was comparable in both groups. The only significant difference was a higher rate of urinary tract infections: episodic during the first 3 months, and recurrent during the second year, in the control

group patients. These long term observations confirm our previous suggestion that promethazine significantly improves the kidney graft survival rate in man and that this action is not related to less intensive corticotherapy in the control group.

In 1980 Mc Geown et al showed that good results can be obtained in kidney allograft transplantation with azathioprine in combination with low doses (20 mg/day) of prednisone. The efficiency of this method was confirmed by Morris et al, Buckley et al and Salaman et al.

In 1980 we performed an uncontrolled trial with low dose steroids, but because of poor results, it was soon abandoned. The second randomized trial, this time with the addition of Promethazine was started in 1982. Thirty adult first cadaver allograft recipients were randomized to receive either high-dose steroids or low-dose regime. In both groups azathioprine and promethazine in our standard doses were given. The patients were well randomized by age, sex, original kidney disease, period on dialysis, pretransplant blood transfusions, pretransplant anti-HLA antibodies, HLA-A and B match and warm total ischemic and handling times.

Patients have been followed for up to 38-40 months. No patient has been excluded from results. Actuarial recipient survival was comparable in both groups.

The low steroid group received i.v. 50 mg of MP, on the first, 25 mg on the second day, and 25 mg/day of prednisone from the 3rd day to the end of the sixth month. These doses were then tapered to 10 mg/day at the end of the first year. Azathioprine and promethazine were given according to the basic regimen. Acute rejection was treated with oral prednisone: 250 mg, 175 mg, 125 mg, 100 mg and 75 mg, each dosis for three consecutive days.

Only one patient from the control group died in the 36 months after transplantation because of myocardial infarction. No mortality was observed with low-dose-steroid regimen. However, wheras cumulative graft survival in experimental group was almost the same as reported by Morris et al (47 % after 2, and 3 years), the control values were significantly higher, 86 and 79 %, respectively. No late irreversible rejection episodes were observed. The function of surviving grafts were comparable in both groups studied.

An explanation for the absence of beneficial influence of promethazine in low-steroid regimen is not at hand. However, it was recently reported that prolonged reduction of azathioprine predispose to allograft failure, especially in recipients treated with low-doses of steroids. This may be relevent to our results obtained with low-steroid group, because all our recipients, who rejected their graft did not tolerate azathioprine well, and could not be given more than 25-75 mg/day.

Because of the very poor results obtained and the lack of serious complications with high-dose steroid regimen, this study was abandoned and the triple-drug regimen (high-dose prednisone, azathioprine and promethazine) was accepted as standard immunosuppressive therapy for kidney allograft recipients.

In a further attempt to get still better results of graft survival by prevention of vascular damage of transplanted kidney, two antiplateled drugs dipyridamole and aspirin were added to our standard prednisone-azathioprine-promethazine regimen.

Aspirin and dipyridamole acts on different phases of platelet function. The former blocking irreversibly thromboxane A_2 (TXA2) synthesis inhibits platelet aggregation and release and prevents TXA2 and serotonine mediated vasoconstriction. In higher doses however, it inhibits prostacycline synthesis. It was shown that as little as 30 mg/day of this drug decreases TXA2 activity for 2 weeks, and that a daily dose of 80 mg reduces TXA2 synthesis, without inhibiting endothelial production of prostacycline. This was the rational for administration in our trial both low-dose (75 mg/day) of aspirin and the recently recommended higher dose (300 mg/day).

Dipyridamole diminishes platelet adherence to damaged endothelium and inhibits their aggregation induced by different stimuli.

For this reasons it was used —sometimes with beneficial results— in combination with aspirin for prevention of intravascular clotting. As it seems likely that the early application of antithrombotic drugs can be crucial for their beneficial effect on graft function, they were started in our trial before surgery.

Sixty-two patients completed the low-dose and 60 higher-dose aspirin trial. There were no statistically significant differences between aspirin groups and their control with regards to the already mentioned parameters considered important for the outcome of kidney transplantation. All recipients were kept on our standard steroid-azathioprine-promethazine therapy. In addition to dipyridamole in a dose 75 mg t. i. d. low-aspirin group received 75 mg/day and higher-aspirin patients 300 mg/day of acetyl-salicylic acid.

In the lower-dose aspirin trial after 21-36 months of observation cumulative 3 year patient and graft survivals were lower in antiplatelet than in control group (91 vs 97 % and 63 vs 72 %, respectively), but these differences did not reach statistical significance. The frequency and timing of reversible rejections were comparable

in both groups. In any time after the transplantation the graft function evaluated on the serum creatinine levels did not differ significantly between the two groups.

Likewise in the higher-aspirin group no beneficial effect of addition of antiplatelet drugs was observed. Cumulative one year patient and graft survivals were 97 and 83 % in the experimental and 97 and 80 % in the control group. No significant differences in accepted grafts function were noted.

Last year we started the randomized prospective trial in which promethazine regimen is compared with cyclosporin-low prednisone immunosuppression. But up to now we have not collected enough data to discuss the results of this study.

Since January 1979, 260 patients were treated in our Center with triple drug (promethazine, azathioprine and high-dose prednisone) immunosuppression. Cumulative graft survival after six years amounted to 58 %. Only 21 (8 %) recipients died. These results are comparable to that obtained with cyclosporine therapy in several randomized studies. It seems therefore, that this method can be recommended as immunosuppressive therapy for patients in whom high-dose prednisone treatment is acceptable, especially when postoperative oliguria/anuria dangerously increases nephrotoxicity of cyclosporine.

Immunologic monitoring

The success of renal allograft transplantation depends among others on 2 factors: The immunological responsiveness of the recipients and on early diagnosis and treatment of allograft rejection.

For these reasons several methods were elaborated to estimate the degree of responsiveness of the recipient in pre- and post-transplantation period and to detect the appearance of rejection in its very beginning.

Pretransplant immunologic monitoring

To assess the immunologic responsiveness of the recipient several methods were used with controversial results. Among them the most popular are tests based on

1. the skin response to sensitivization with common antigens
2. detection of specific LDA in prospective recipient serum, and
3. «in vitro» ADCC assays with and without methylprednisolone addition.

The skin response to sensitivization with common antigens is believed to be a valuable measure of the immune status of potential graft recipients. In our studies three kinds of antigens were used: PPD and SKID —to which more them 70 % of adults respond— and DNCB (dinitrochlorbenzen). In the latter test two doses of DNCB (2 μg and 100 μg dissolved in 0,1 ml of acetone) were applied to an area of forearm skin in a ring of 2 cm diameter. Acetone alone serves as control. Afterwards examinations where performed after 2, 7 and 14 days, and —if no response were present— testing doses of DNCB 100, 200 and 400 μg were applied in day 14-18 and read at 48 h. Significant induration involving at least one half of the surface area at any of the test site was considered as positive.

In all, 113 tests were performed in 52 patients. Neither the result of any particular test, nor of their combination could reliably predict the degree of immunological responsiveness of recipient, as jugded on the basis of cumulative graft survival after 1 and 2 years postoperatively.

As the presence of LDA is considered as a more sensitive parameter of immunisation than positive CDC test, the relationship between the pretransplant presence of LDA against donor specific HLA AB antigens and the frequency of graft rejection and recipient survival was studied. Again no relationship between these phenomena could be demonstrated.

Recently the ADCC assay as such or in the presence of methylprednisolone (MP) was recommended to distinguish the so called immunologic unresponders from responders. In this test the activity of the patient's killer cells is assessed in a system in which L_{1210} mouse lymphoma cells covered with specific rabbit antibodies were used as target cells. No relationship between pretransplant ADCC activity and cumulative patient and graft survival after 1 and 2 years was seen in our 35 patients studied in this respect.

Recently Priscilla Kincaid-Smith suggested, that basing on the results of ADCC test performed in the presence of MP, all patients can be divided into 2 groups: Steroid-sensitive and non-sensitive. The latter should not be transplanted, because they will reject their graft very soon. We could not confirm her results, as we have not found any relationship between sensitivity to MP as detected in «in vitro» ADCC-MP test and:

a. frequency of cadaver graft rejection episodes,
b. 3 years cumulative patient and graft survival,

c. cumulative steroid dosage given during the first year after surgery.

Neither could we find any constant correlation between pretransplant MP sensitivity «in vitro» and:

d. «in vivo» — as measured by ADCC activity after first 3 days of intensive immunosuppressive therapy and expressed as a percentage of pretransplant activity or
e. by the «in vivo» respones to 1-6 i.v. boluses of 1 g.

Therefore, according to our present experience, any single test besides donor specific CDC cross-match can reliably predict the recipient's response to the transplant already before grafting.

Taking into account the unreliability of all these immunological tests we have abandoned them altogether.

Posttransplant monitoring

Three «in vitro» immune tests were performed to determine wether their posttransplant value correlate with graft long-term survival. It was found that:

The diminished ADCC «in vivo» activity measured on the 4-5th day after grafting did not have predictive value, whereas fall of in vivo ADCC activity below 50 % of pretransplant value was associated with better 3 year graft survival, although this difference has no statistical significance, maybe because of low number of patients studied. On the other hand, neither the presence in the recipients serum of LDA against donor specific HLA AB ags nor donor specific CDC could predict early graft loss.

It seems, that up to now a good and reliable prognostic test is not at hand.

Graft rejection

Tests used for early diagnosis of graft rejection were performed from 0 to 7 days preceding the clinical diagnosis of this event, confirmed usually by graft biopsy and/or positive response to intensive antirejection therapy. The assays utilized were based on response of recipients lymphocytes to different stimuli, on their activity, on percentage of their particular subpopulations, on limphokine production and on influence of rejection on bone marrow function and antibody production. Nonspecific reactants were also studied. The % of false positive and false negative results were the main criterium of evaluation.

The following tests were found to have low diagnostic value because of high % of false negative (F—), and/or false positive (F+) results:

Increased $>$ 2.000 cpm spontaneous blastogenesis of peripheral blood lymphocityes (40 % F— and 50 % F+).

Increased $\geqslant$ 30.000 cpm reactivity to PHA stimulation (F— 40 % and F+ 0 %).

Low response to Con A stimulation (F — 0 and F+ 80 %).

Low ($<$ 0.5) rate of PHA/Con A stimulation (F— 20 % and F+ 12 %).

We have not established any relationship between changes of ADCC activity and graft rejection, neither in the early postoperative period, nor in the period of acute rejection.

Also a combination of LDA and ADCC tests were not useful in this respect.

Two tests monitoring T-lymphocytes were also found to have little value:

ARFC test gave 20 % of false positive and 47 % false negative results.

No clear relationship could be documented between changes of subpopulations of lymphocytes bearing IgG and IgM receptors and graft rejection.

Several tests measuring limphokines production were also unreliable, as correlates of graft rejection.

Assay of LIF production in the presence of donor specific gave 34 % false negative and 64 % false positive results. Considering that both LIF and LSF did not improve test value.

If instead of donor specific GBM antygen was used, there were 34 % false negative and 57 % false positive results.

During acute rejection, nonspecific suppressor cells were found, which inhibited MIF production in the third party assay. Cells isolated from the blood of patients undergoing acute graft rejection, when added to the assay system, abolished SKID induced LIF production by normal lymphocytes, taken from blood buffy coats from individuals known to have strong positive skin test to SKID. This assay gives no false negative or false positive results. Unfortunately it is a time consuming and quite complicated procedure.

CRP, another nonspecific assay recently recommended for kidney graft rejection diagnosis, was in our experience very unreliable, giving 75 % false negative and 50 % false positive results.

Studying the influence of rejection on hemopoiesis we have found that CSF production is diminished not only during acute rejection, but unfortunately also during viral infections. This phenomenon can sometimes be reversible by

i.v. boluses of MP. Less characteristic patterns of CSF secretion in urine were also found in these patients.

The most striking observation was a marked reduction of GM-CFU cells in the blood of patients undergoing acute rejection, as compared with patients with stable graft function and healthy blood donors ($p < 0.001$). Usually these phenomena occured 3-7 days prior to definite signs of rejection. The number L-CFU cells, reduced in graft recipients, did not show any relationship to graft function.

Most recently we have found that during acute graft rejection the production of Ig by peripheral blood lymphocytes is markedly increased. It can happen: spontaneously, after stimulation with PWM and with Salmonella antigen.

Till the present all too few patients have been studied with these last methods. But even if further studies confirm their predictability, their practical value will be restricted because they are rather time-consuming and very sophisticated.

In summary, the good and practically useful immunologic method of detection of graft rejection is yet to be found.

Conclusions

1. Postoperative oliguria/anuria of more than 3 weeks duration significantly decreases long term cumulative graft survival.
2. The presence of non-functioning graft increases recipients' early mortality rate.
3. Triple drug (Promethazin-Azathioprine-high dose-steroid) regimen can be recommended especially for recipients with postoperative oliguria/anuria in whom high dose prednisone treatment is acceptable.
4. Positive donor specific CDC crossmatch is the only immunologic reliable test for prediction of early graft rejection.
5. The reliable immunologic tests for prediction of acute and chronic allograft rejections are yet to be found.