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A Trial With Heterologous Antilymphocyte Globulin in Man

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In the last 2 years, there have been several reports of the administration of heterologous antilymphocyte globulin (ALG) to humans. The objectives have varied. In our own institution[1–4] and more recently in several others[5–9] ALG prepared from immune horse serum has been added to the basic immunosuppressive regimen of azathio-prine and prednisone after the transplantation of vital whole organs including the kidney, liver, and heart. In addition, heterologous antilymphocyte derivitives have been used alone for the purpose of determining what changes in immunologic reactivity were thereby induced in volunteers,[10] or in an effort to treat autoimmune diseases[8] and lymphatic leukemia.[11]

There is no point in reviewing here the large body of incontrovertible evidence that ALG can slow or prevent the rejection of a variety of homografts in several species of lower animals as well as in subhuman primates. Suffice it to say that ALG also has an easily demonstrable immunosuppressive effect when used as the only treatment in man inasmuch as skin graft survival is prolonged[10] and the expression of pre-existing hypersensitivity states is blunted or eliminated.[2,5,8,12]

Equally unchallenged is the fact that there is a significant morbidity with the clinical administration of ALG as most fully described by Kashiwagi[13] and mentioned by others as well.[5,6,10] The intramuscular injections are almost always painful, often cause fever, may eventually evoke classical foreign protein reactions including anaphylaxis, and can precipitate thrombocytopenic crises. However, lethal complications must be rare. We have treated more than 100 recipients of renal or liver homografts with ALG without a drug-related death.

Conceding that the immunosuppressive effect of immune globulin is not doubted in any species including man in which it has been tested, other avenues of inquiry remain open including whether the benefit of ALG is outweighed by its side effects, if there is really a need to add globulin therapy to that with the standard drugs or if this practice will lead to increased survival, how the globulin might be refined and made less toxic, and what improved schedules of administration could be evolved for clinical use. It is upon these issues that this communication will touch.

THE QUESTION OF NEED

Transplantation from Related Donors

A trial of ALG therapy was begun at the University of Colorado in June 1966 because of dissatisfaction with the results obtained using azathioprine and prednisone together in the preceding 4 years. During that time, the mortality during the first 12 postoperative months after intrafamilial renal homotransplantation had remained almost fixed at about 30% despite the acquisition of extensive experience, adjustments in the way in which azathioprine and prednisone were administered, the use of ancillary measures such as local homograft irradiation, and even the application of histocompatibility matching.

The events leading to death or loss of the homograft in the significant minority of patients were relatively predictable. In an individual case, it soon became evident that continuing function of the transplanted kidney was dependent upon toxic doses of prednisone. If these were lowered, it usually became necessary to remove the organ and return the patient to chronic dialysis. If the doses were not reduced, the homograft could be saved but often at the cost of a lethal infection. In such unfavorable cases, the pattern leading to eventual failure was almost always identifiable within the first few postoperative months although some of these unfavored patients lived on for long periods as semi-invalids.

The way in which a one to 4 month course of ALG, added as an adjuvant to the basic azathioprine-steroid regimen, appeared to have influenced the outlook of subsequently treated recipients of consanguineous homo-grafts has been reported on several occasions[1–3] and will only be briefly summarized now. In comparison to our previous experience, the quantities of both azathioprine and especially prednisone were reduced, the overall quality of homograft function was better maintained, and the mortality was decreased. Eighty-five consecutive intrafamilial renal transplantations have been performed with several variations of ALG therapy, often with poor donor-recipient histocompatibility as determined by Terasaki.

All but 8 of the recipients are still alive, including 19 of the first 20 who received their kidneys from 21 to 27 months ago; 44 of the survivors have been followed for a year or more. Four of the deaths were due to non-renal medical complications: massive pulmonary embolization (18½ months), reticulum cell sarcoma (6 months), granulomatous colitis (3½ months), and acute yellow atrophy (25 days) which was first diagnosed on the morning after operation. The other 4 failures resulted from technical misadventures which either led to an immediate or delayed fatality (after 2 to 127 days). It should be noted that 2 of the 8 deaths were in a small subgroup of 13 patients to be separately discussed later who were treated with a specially prepared pure equine gamma G globulin that had apparently lost most of its immunosuppressive potency in the course of refinement.

In the total group, there were 3 examples in 2 patients of hyperacute rejection (Shwartzman reaction) of the initial transplants[14]; the homografts were removed promptly and replaced with functioning kidneys a few weeks later. There were no other retransplantations either early or late. None of the patients has been returned to a chronic dialysis program, and only one of the survivors is currently threatened with loss of his homograft. The exceptional patient was one who was treated with the highly refined globulin.

More will not be said about these statistics. Few groups could realize more completely than our own that long term patient and kidney survival can often be obtained with or without ALG treatment. It is only necessary to recall that 31 (67.4%) of our first 46 recipients of related homografts treated from 1962 to early 1964 (15) lived for at least one year and that 28 (61%) are still alive 4½ to almost 6 years after operation; only one of these patients has required late retransplantation.

In our hands, these results could not be significantly improved upon until the advent of ALG therapy. If in contrast, as has been said (but not yet well documented), other groups using standard azathioprine-steroid therapy have satisfactorily lowered their kidney loss rate and mortality without imposing stringent histocompatibility criteria for the selection of candidates, there is little need in those institutions to consider the use of ALG or any other modification of treatment. The decision about the adequacy of the immunosuppression is a local one.

Transplantation from Cadaveric Donors

We have not had sufficient experience to suggest what effect, if any, ALG will have on the long term results after cadaveric renal transplantation since we have accumulated only 12 such

cases in the last 2 years; 8 of the recipients are still alive, one in an anephric state. One of the patients destroyed 2 consecutively placed kidneys with Shwartzman reactions and died in 54 days. Two others, who had received organs from a common donor, died within a one day interval of massive pulmonary emboli 3½ months after operation. The 2 latter homografts had functioned well. At autopsy, there were no histologic signs of active rejection and little or no evidence of healed past rejection.[3]

The other 9 patients received their kidneys 4 to 22 months ago, the first 4 more than a year ago from donors with whom there were extremely poor histocompatibility (Terasaki) matches. Two of the 4 oldest homografts failed after one year and had to be removed; both of the recipients were returned to the chronic dialysis program where one committed suicide 6 months later by water ingestion. The 2 other recipients with the longest followup still have life sustaining but subnormal renal function after 14 and 16 months. Their creatinine clearances are 20 and 40 ml/minute.

The donors for the more recently treated 5 surviving patients were selected by Terasaki with prospective histocompatibility typing; a mismatch in a major HLA antigen group was present in only one case. These recipients all still have good renal function but the followup is only 4 to 10 months (average 6 months).

In spite of the small number of observations, some tentative conclusions might be justified. It was possible in all the 11 foregoing transplantations in which initial urine excretion was obtained to easily control rejection during the period of ALG therapy; in fact overt rejection was diagnosed during this interval in only 4 instances. However, with the discontinuance of ALG in all the recipients of histoincompatible kidneys, slow but progressive deterioration of the homografts was soon detectable. The situation was in marked contrast to that after intrafamilial transplantation where emergence from the 4 month level of convalescence with good renal function proved to be a highly reliable sign of a favorable long term prognosis. The fate of histocompatible non-related kidneys under these conditions of therapy can only be speculated upon until longer followups are available.

PURIFICATION AND POTENCY LOSS

In Kashiwagi's toxicity report,[13] it was stressed that the precipitin response of the treated patients was principally directed against the alpha and beta globulins in the ammonium sulfate precipitated ALG and that, in turn, there was a correlation between the level to which the precipitin titers rose and the likelihood of an anaphylactic reaction; high titers were common after several months of injections. In contrast, antibodies against the horse gamma G globulin were not usually detectable. Consequently, it was hoped that the incidence and severity of the side reactions might be reduced by the administration of pure gamma G globulin (hereafter called ALGG) which was prepared in bulk quantities with a DEAE batch technique.[16] The refined product was given Patients 58 through 70 in the intrafamilial ALG series. The ALGG batches had leukoagglutinin titers of 1:2,000–8,000 and protein concentrations of 1.9–3.5 gm %. The intramuscular doses were 4 to 8 ml depending upon the antiwhite cell titers.

Measurable precipitating antibodies against the ALGG developed in only one of the 13 patients. Although minor toxic manifestations were seen with about the same frequency as with the previously used ALG, there were no anaphylactic reactions. Unfortunately, it soon became apparent that the immunosuppressive effect of the product had been largely lost. All of the patients had satisfactory post-transplantation diureses. However, 7 of the 13 then developed severe rejection crises from ½ to 14 days later. Prednisone doses were increased in 2 cases to as much as 400 mg per day. Five of the recipients had secondary elevations of the BUN to more than 150 mg%. In 4 cases, resumption of hemodialysis was necessary for 1, 2½, 4, and 5 weeks before adequate urine excretion resumed. Two of the 13 patients eventually died,

although not from renal failure. Nevertheless, the high dose steroid therapy required for many postoperative weeks was a contributing factor to the unfavorable outcome.

The difficulties in this small group of patients can be appreciated by a comparison with the other 72 consanguineous recipients who received ammonium sulfate precipitated ALG, 58 before and 14 after the ALGG trial. Only 3 of the patients treated with ALG after technically satisfactory operations had rejections severe enough to cause secondary azotemia greater than 150 mg%, and there was only one instance where interim postoperative support (2 dialyses) with the artificial kidney became necessary because of uncontrolled rejection.

A plausible explanation of the apparent loss of ALG potency in these cases and in the recently reported dog experiments of Clunie et al.[17] may be related to the wider distribution of antiwhite cell antibodies in horse ALS as compared to that raised in the rabbit. The leukoagglutinins in the latter species are mostly in the easily separable gamma G globulins.[18,19] Attention was drawn by both Iwasaki[20] in our laboratories and Fateh-Moghadam[21] to the fact that the leukoagglutinins of horse ALS were also found in the T-equine globulin fraction, which consists mostly of “fast” gamma G and beta globulins; the potential practical importance of this finding was overlooked by most of the workers in the field.

More recently, Kashiwagi and Townsend in our laboratory[22] have re-examined the relative contribution of the different immunoglobulins to the total antilymphocyte activity of equine ALS. The horse studied had received an intensive immunization with human splenic lymphocytes for 2 months. More than half of the antileukocyte antibody was in the T-equine globulin. This fraction was eliminated by the technique used in preparing the ALGG used for the clinical trial. Moreover, Kashiwagi and Townsend showed in companion canine experiments, involving differential separation, that the discarded T-equine globulin had a lymphopenic effect at least as great as that caused by the highly refined “slow” gamma G globulin. Experiments with canine renal transplantation are underway to determine the relative immunosuppressive efficacy of the 2 fractions.

ALTERNATIVE REGIMENS

Two unsatisfactory aspects of the present methods of ALG use were mentioned earlier. First, there has been an increased incidence of classical foreign protein reactions with successive injections, particularly after several months. Second, evidence was cited that late rejection of cadaveric renal homo-grafts often developed once the serum therapy had been stopped in cases with poor histocompatibility matches; the same thing has been seen in recipients of livers.[23] Efforts have been made in recent months to deal with these problems.

One approach has been to give double doses of ALG in the early post-transplantation period with the objective of promoting prompt tolerance, such as that which can be produced with ALS in rodents.[24,25] The last 14 adult recipients of renal homo-grafts in the intrafamilial series described earlier were given daily doses of 8 ml of ammonium sulfate precipitated horse ALG which had a leukoagglutinin titer of 1:8000, a cytotoxicity titer of 1:2000 to 1:4000 and a protein concentration of 4.3 to 5.2 gm%. Highly significant lymphopenia, a finding not previously observed with half these doses, was invariably seen. Thus far, only 2 of the recipients have developed an unequivocal rejection. In these cases the process was easily controlled by increasing the steroid doses.

The use of these large quantities of ALG has usually led eventually to thrombocytopenia, severe enough in a few instances to require platelet transfusions. Otherwise, the treatment of this complication required only the temporary discontinuance of globulin therapy, which was then resumed each time the platelets returned toward normal. In these patients the initial lymphopenia occurred without platelet depression, but within 2 or 3 weeks there was an almost

invariable temporal correlation between the induced falls in the lymphocyte and thrombocyte counts.

The batches of ALG used for the foregoing patients had been absorbed with human thrombocytes[16] and had very low to undetectable titers of antiplatelet antibodies as measured by a standard thromboagglutination method. It is probable that the delayed thrombocytopenic effect was due to antibodies raised against antigenic determinants which are shared by human platelets and lymphocytes.

That this might be the case was suggested by Pichlmayr,[8] who found a reduction in leukoagglutinin titers of his horse ALG after absorption with platelets from the species against which immunization was conducted. Kashiwagi and Townsend[22] were unable to confirm these findings. However, they provided support for the concept of cross reactivity by showing that thromboagglutinin and leukoagglutinin titers in raw horse antidog ALS were both reduced by absorption with washed canine lymphocytes. Whatever the explanation for the thrombocytopenia, its appearance has imposed the most important practical limitation we have encountered on the doses of ALG that can be given to humans.

An alternative and possibly safer approach than that of high dose “blitzkrieg” therapy would be to extend the chronicity of heterologous globulin treatment. Probably this could often be achieved without any special measures since ALG was stopped in the majority of our patients only because an arbitrary 4 month period had passed and not because of the appearance of toxicity. Moreover, even with the development of immunity to horse globulin, desensitization by standard techniques may be feasible as has been shown in 2 of our patients.

Another solution could be the secondary use of ALG from another species. A transition from horse to rabbit ALG has already been made in 2 of our liver recipients. Whereas administration of the equine globulin caused fever and intense local reactions, the rabbit protein proved to be non-toxic. Reserve supplies of ALS are also being raised in our laboratory in the goat and cow.

SUMMARY

Heterologous ALG has been used in more than 100 human recipients of renal or hepatic homografts. There has been considerable consequent morbidity but no fatal toxic reactions. The results with both kinds of transplantation have been better than could previously be achieved in our institutions.

Two significant problems of ALG therapy have been emphasized in this report. First, rejection has been frequently observed after transplantation from non-related donors following the discontinuance of ALG treatment, indicating the need to test other ways of using this agent than that now being employed. Suggestions have been made about how therapy might be made more effective including switching from one heterologous donor species to another, or the administration of an intensified early course of ALG in an effort to Promote tolerance. Second a serious loss of Potency has evidently resulted from attempts to highly purify the gamma G globulin from horse ALS, necessitating a return at least temporarily to the crude ALG obtained by ammonium sulfate precipitation.

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