Clinical Experience With Horse Antihuman ALG

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THERE will be little point in dwelling on our clinical experience with ALG, though this has been extensive. The reason is that we have not carried out a contemporaneous control study omitting ALG. We do not have, as a consequence, proof that our results were improved by the clinical introduction of ALG. Nevertheless, there is evidence supporting this point of view which we will briefly describe.

Related Transplantations

Our first renal homotransplantations were carried out in 1962. If related donors could be found, substantial benefits for the majority of recipients were obtained from the very beginning of our experience.

In Fig. 1 is shown graphically the fate of 131 consecutive patients who were given kidneys by siblings, parents, or more distantly related donors such as aunts, uncles, or cousins, between 1962 and Spring 1968. The follow-up in Series 1 is now 8–9½ yr, in Series 2 from 6–7½ yr, and in Series 3 from 4–6 yr.

The 71 patients of Series 1 and 2 were treated with azathioprine and prednisone. Tissue-typing techniques were not used in Series 1 since they were not yet available. In Series 2, an effort was made by Dr. Paul Terasaki of Los Angeles to select the best donor among those available in any given case. This effort of donor discrimination was not beneficial (Fig. 1) and, in fact, the eventual survival was somewhat inferior to that in Series 1.

The 60 patients of Series 3 were administered horse antilymphocyte globulin (ALG) in addition to azathioprine and prednisone. With minimum follow-ups of at least 4 yr, the survival of both patients and grafts appears to have been increased (Fig. 1). Even today, after 4–6 yr, 75% of these ALG-treated patients are still alive and all but three of the survivors (42 of 45) have acceptable function of their original homografts.

Generally, the ALG-treated patients had globulin therapy for about 4 mo post-transplantation, with intramuscular injections of 3–6 mg/kg. The ALG was started several days before operation and given daily for 2 wk, every other day for 2 wk, twice a week for 2 mo and once a week for a final month. Because they were the only available source of human lymphoid tissue in large quantities, human spleens were used in this era to raise the ALG, with careful attention being paid to separating the leukocytes from stroma, platelets, and red cells before their inoculation into horses. After absorption and purification, the eventual product usually had lymphocytotoxic and leukoagglutinin titers of 1:4000 or preferably greater. Anti-GBM activity was not demonstrable or very minimal in the Dixon rat model.

Nonrelated Homografts

As with the consanguineous cases, the introduction of ALG as part of a triple drug treatment program was associated with an improvement in results. This is demonstrated in Fig. 2, in which Series 1

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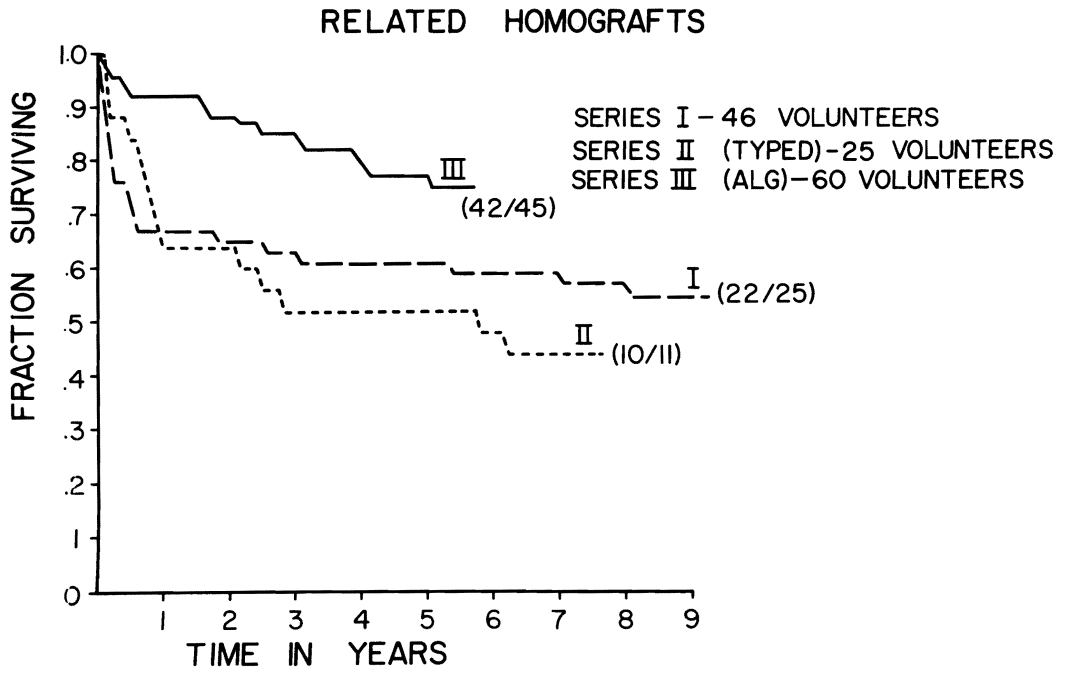

Fig. 1. Life survival curves of three groups of recipients of consanguineous kidneys. The patients in Series 1 were treated with the double drug program of azathioprine and prednisone from 1962 to March 1964 and, consequently, have potential follow-ups of 8 to 9½ yr. The patients of Series 2 were treated from October 1964 to April 1966 and, consequently, have potential follow-ups of 6–7½ yr. They received the same drug therapy as in Series 1 and, in addition, an attempt was made at donor selection by HL-A matching. The recipients of Series 3 were treated with the triple drug program of azathioprine, prednisone, and ALG between June 1966 and April 1968; they have potential follow-ups of 4–6 yr. In all three series, the denominator indicates the surviving patients whereas the numerator tells the number of originally transplanted kidneys that are still functioning in those survivors.

and 2 conform to the same intervals defined in the preceding section, and in which Series 3 is also comparable except that cases were included through April 1969. In the 1962–66 era, the patient survival rate at 1 yr was between 33% and 52%. In Series 3, this 1-yr figure rose to 82% (14 of 17 patients). After 1 yr, in all three series, including the final ALG group, the ultimate survival has drifted off far more rapidly than with the use of related homografts.

Is There an Indispensable Drug?

Subsequent to the completion of Series 3, and continuing until February 1971, more than 160 additional patients were given either related or cadaveric renal homografts under immunosuppressive therapy with azathioprine, prednisone, and ALG. In a general way, the results were confirmatory of those in Series 3, although the survival was not quite so high, primarily because the medical criteria for acceptance to the program were so drastically relaxed. Finally, for more than 1 yr, cyclophosphamide has been used in place of azathioprine in a triple drug program that includes steroids and ALG. Here, too, the results have been good, both in related and nonrelated cases

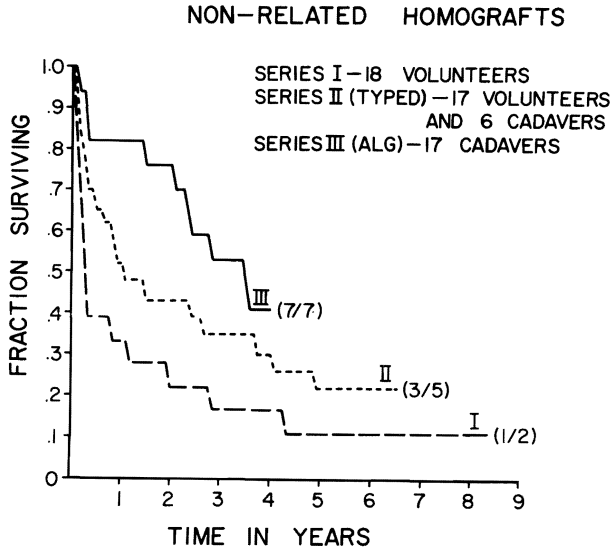

Fig. 2. The results after transplantation of non-related kidneys. The conditions of treatment and the meaning of the figures in the parentheses are identical to those in Fig. 1. Note that the results with nonrelated kidneys have been inferior to those with related grafts at all levels of our experience. The follow-ups are also the same as in Fig. 1 except in Series 3, in which the potential follow-ups are 3–6 yr.

as has been described in another paper of this symposium.

In reviewing our experience of the last decade, the point can be made that the one absolutely indispensable agent that has made renal transplantation practical is prednisone. More than occasional successes are possible with the omission of either one or two of the three other major agents we have mentioned, namely azathioprine, cyclophosphamide, or ALG. However, without adrenal corticosteroids, we believe that the management and reversal of rejection would be so seldom achievable that the clinical discipline of renal transplantation would vanish.

It is in this context that ALG must be evaluated. We have always considered ALG to be an adjuvant immunosuppressant, and one which is of the greatest value for short-term use during the often difficult early postoperative period when the issue of graft acceptance or failure is most commonly decided. When used in this way, rather than as a panacea, we have been convinced that ALG is an important part of our therapeutic armamentarium and one that has not only improved survival but also the quality of convalescence.

Problems in the Future of ALG

There is probably not an appropriately informed responsible scientist in the world who does not concede that ALG is a potent immunosuppressive agent in humans. But that is not the question that perplexes clinicians interested in renal transplantation. Rather, the issue is whether or not ALG fills some unique role that cannot be equally well met by the clever manipulation of other agents such as steroids, azathioprine, and cyclophosphamide. For this purpose more controlled trials such as those of Sheil will be welcomed.

One reason why the true clinical value of ALG must be settled is the tremendous investment of personnel and material resources that have been required to make ALG available for human use. At a recent conference in Bad Soden, Germany, examples were cited in which the cost of ALG accounted for half the financial investment to treat a renal recipient. In addition, the amount of talent that is required to ensure a supply is amazing. These efforts and expenses will be worthwhile only if tangible and substantial benefits are demonstrable. This is particularly true since in addition to the nuisance of

procuring it there are potential dangers with the administration of ALG. Anaphylaxis, which has led to several deaths, is the most terrifying side effect, but there are others, including injection site pain, thrombocytopenia, and injury to the homograft itself, to mention only a few.

No matter how useful heterologous ALG proves to be, there will remain very major problems of standardization. There are four exceptionally sensitive points that must be clarified: (1) the best animal in which to raise ALS; (2) the most effective immunization schedule to be used; (3) the correct antigen; and (4) the in vitro techniques for evaluating the effectiveness of the product. All of these matters were considered at the Bad Soden, Germany, ALG conference in April 1972, as well as at an earlier meeting at San Diego, California, held in December 1971.

The consensus from these discussions was that the choice of animal is probably not a crucial factor. The schedule of immunization is probably also not critical except that if the course is a short and standard one according to the Monaco–Medawar principle the ultimate product is apt to be relatively the same from animal to animal. In horses, this has been shown in our laboratories in five animals submitted to three-pulse immunization with large numbers of lymphoblasts (Fig. 3). The resulting high titer ALG prolonged survival of rhesus skin homografts to 24 days (controls <12).

The third question about the best antigen source is still open for discussion. The thymocyte has a number of advocates, but from the viewpoint of convenience and purity, a contender, as we have heard from Condie, is the cultured lymphoblast which, so far as we know, represents a pure B-cell population.

Concerning the fourth point, there has been a gradual acceptance of at least four in vitro tests. Even a year ago there were flat denials that the leukoagglutinin and lymphocytotoxicity tests had any correlation with immunosuppressive effect, although it was commonly conceded that correlations were good with the rosette inhibition test of J. F. Bach. Yet at the German conference, data were presented with rabbit ALG showing that cytotoxicity titers and potency in the monkey skin test system had an almost perfect correlation. In other studies with the horse it was demonstrated from more than one laboratory that four current titration methods yielded about the same answer (cytotoxicity, leukoagglutination, rosette inhibition, and microcomplement fixation) and that the heights of these titers were a relatively direct measure of the immunosuppressive quality as cross checked in the surrogate monkey model. It was pointed out that methodologic artifacts in measure-

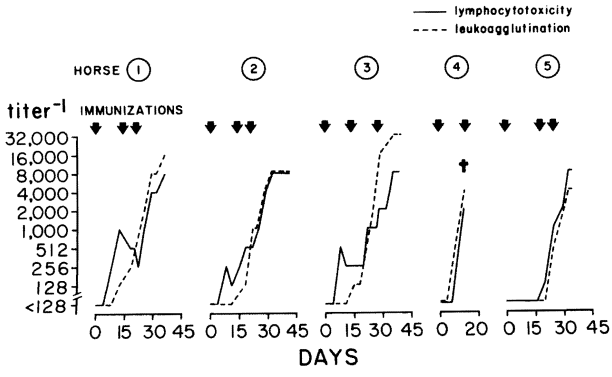

Fig. 3. Anti–white-cell titers in five horses undergoing three-pulse immunization with human cultured lymphoblasts.

ment of anti–white-cell titers may have accounted for discrepancies in the past.

It may be superfluous to engage in much debate about the dose and administration schedules of such a poorly standardized agent as ALG. Nevertheless, it is important to attempt this by those who are or are planning to give ALG now. In our center we have tried to use ALG with a minimum leukoagglutination and cytotoxicity titer of 1:8000, in volumes for adults of 4–5 ml per injection intramuscularly. Since the protein content is 5 g% the dose per injection is usually 4–5 mg/kg. It should be noted that this kind of dose in the Simmons dose-response curve, worked out in patients with multiple sclerosis, caused an easily detectable prolongation of human skin graft survival. If these doses are further reduced either by using poor titer material or by decreasing the volume of injectate one may easily enter into a homeopathic range. If alternating case studies of renal transplantation are carried out, it is to be hoped that ALG will not be discredited by making this mistake of underdosage. Because of the danger of sensitization to the foreign protein, ALG therapy will probably have to continue to be restricted to the first few postoperative weeks or months.