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Attributes of clinically used immunosuppressive drugs: possible future uses of antilymphoid sera[1]

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WE WILL TRY to cover three general areas of clinical interest by providing: *a*) a brief summary of accomplishments which have already been possible with the admittedly imperfect immunosuppressive regimens now in use; *b*) a critique of the drugs or drug combinations currently being employed; and *c*) a brief description of research which suggests that heterologous antilymphoid serum derivatives may be of clinical value in the future.

PAST EXPERIENCE

Interest in the practical application of homografting procedures is justified by the chronic survival which has already been attained by many patients after renal homotransplantation. In Denver, for example, a series of 64 consecutive uremic patients was treated from November 1962 to March 1964 with renal homografts obtained from volunteer living donors. A follow-up on these recipients is now available (to October 1966) of 31–47 months. Exactly half of the patients are still alive (Fig. 1). After a very steep early mortality, the life survival curve has been relatively stable. Similar long-term results in large series have been reported by Hume et al. (4) and Hamburger et al. (2).

In our experience, a most important determinant of the ultimate outcome has been the source of the homograft, as demonstrated by a breakdown of the foregoing series. Twenty-nine of those patients who received kidneys from blood relatives (excluding identical twins) are still alive (63%). Only two deaths have occurred from the 6th month onward, one of these as the consequence of myocardial infarction after 32 months in a patient with excellent kidney function. In contrast, only 3 of the 18 recipients of nonrelated kidneys survive to date. In the latter group, the early mortality was high, and unlike the situation with related cases, the chance of late death remained significant (Fig. 2).

The above series was compiled at a time when practical methods of donor-recipient matching were not available, except for the assurance of compatible blood types. The results, therefore, were obtained with relatively random donor selection from related and unrelated populations.

Since that time, clinically usable techniques have been developed for measuring antigens in the human lymphocytes, one system being that of Terasaki and co-workers of Los Angeles (16). Since most antigens in the white cells are shared in other tissues including the kidney, the demonstration of close compatibility in the leukocytes of a donor-recipient pair might be predicted to be a favorable prognostic sign providing the antigens being studied had any relation to histocompatibility. Terasaki's retrospective studies of the original Denver series provided evidence that this was the case. The truly outstanding clinical results in the chronic survivors were in those patients shown to have received a kidney from a well-matched donor (15). The kidney injury found in biopsies of these homografts after 2 years was inversely related to the quality of the antigen compatibility (12).

Because of these suggestive findings, donor selection for subsequent cases has been made by Terasaki on the basis of the best available antigen match (16). The selectivity which was possible for transplantation between relatives was, of course, limited by small numbers, for which reason the matching actually achieved was little better than could be obtained by random intrafamilial pairing. In contrast, the donor pool of 30–100 in the nonrelated cases permitted a considerable improvement of choice compared to that previously possible.

Twenty-six cases have been studied with these new conditions, half with related and half with nonrelated donors. The survival for the first 14 postoperative months was the same for both groups (Fig. 3). One of the recipients of a nonrelated homograft died of hepatitis after 17.5 months. All others from both series were still alive after 15 months to 2 years.

Although the above data suggest that typing permits discrimination of suitable donors, particularly for nonrelated cases, it is unlikely that such techniques will eliminate an inherent and unsatisfactorily high mortality.

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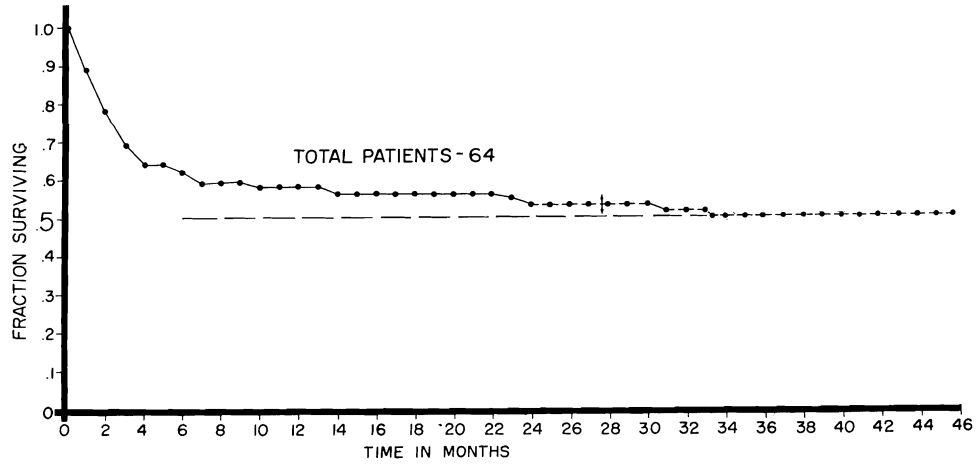

FIG. 1. Life survival curve of the first 64 patients treated with renal homotransplantation from November 1962 to March 1964. All donors were living volunteers. Recipients now have been followed for 2.5 to almost 4 years. Exactly one-half of the patients were still alive at the end of September 1966.

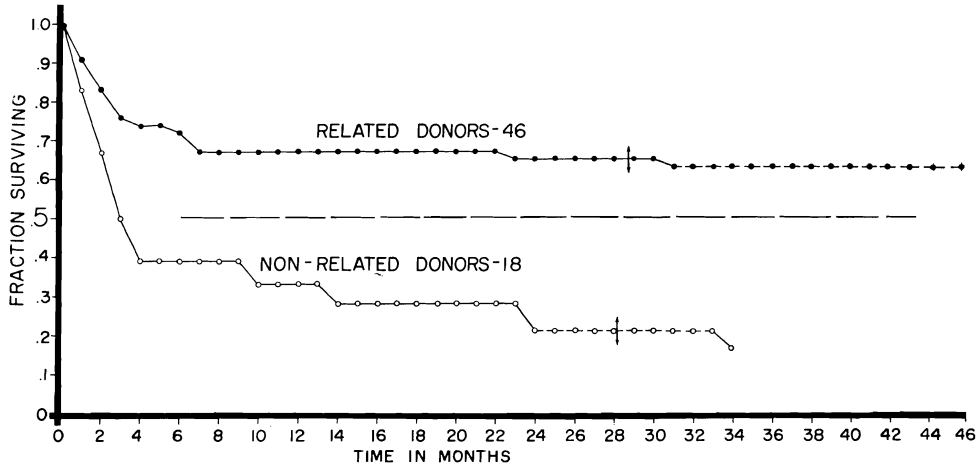

FIG. 2. Breakdown of the results shown in Fig. 1 according to the source of the homograft. Note that 63% of the patients who had consanguineous donors were still alive compared to only 3 of 18 who received nonrelated kidneys.

The reason is that virtually all of the deaths in the later series, and most of those in the original group as well, were due to the immunosuppressive agents used to prevent rejection, and not through failure to control this process.

CRITIQUE OF IMMUNOSUPPRESSIVE REGIMENS

The foregoing point can be easily demonstrated by a study such as that illustrated in Fig. 4. In this study, 18 dogs had removal of one of their kidneys and transfer of the other organ to the contralateral iliac fossa, in essence providing them with a perfectly matched transplant. They were then treated with azathioprine in the same way as if homotransplantation had been done. The life survival curve was remarkably similar to that previously demonstrated in the clinical series. Infectious disease complications were the chief causes of death. A contributory factor often appeared to be liver injury. It is known that azathioprine is markedly hepatotoxic agent (Fig. 5) in dogs (14), although the evidence is tenuous that it similarly affects the human liver (3).

Description of these experiments does not imply dissatisfaction with azathioprine which is the single best immunosuppressive agent available today. Patients who can be effectively treated with this drug alone are remarkably well. Unfortunately, therapy solely with azathioprine is usually insufficient to prevent homograft repudiation. It is the necessity for additional treatment which imposes great risk. The use of prednisone, the most effective drug to control and reverse rejection, has created the most serious problems.

The way in which such combination drug therapy contributes to survival or to death can best be appreciated from specific examples. In Fig. 6 the course of a patient is illustrated who was operated upon in April 1963. He had immediate diuresis of the homograft and excellent subsequent renal function which continued for 2 weeks. At that time rejection developed in spite of therapy with azathioprine. There was swelling of the transplant, deterioration of renal function, and fever. All of these adverse findings were reversed with the institution of 200 mg/day of prednisone.

In this patient it became possible to reduce and after 5 months to discontinue prednisone. During the 3 ensuing years the patient has received only azathioprine, that drug which at the beginning was insufficient to

prevent rejection. The serial events show, first, that rejection is a reversible process and, second, that some change in the host-graft relationship transpires ultimately allowing relaxation in many patients of the stringency of immunosuppression. In animal experiments it has been shown that the change is complete enough to allow survival for years after discontinuance of all therapy. The frequency with which this might be expected in man is not known.

The early need for maximum immunosuppression is the most important etiology of failure as shown in Fig. 7. As in the previous case, this patient received a fraternal homograft. There was good initial function followed by a very severe rejection and temporary anuria. The addition of high dose steroid therapy was followed by return of urine excretion. After it appeared that the worst of the rejection crisis had passed, the patient died from bone marrow depression and sepsis. Here, overdosage with

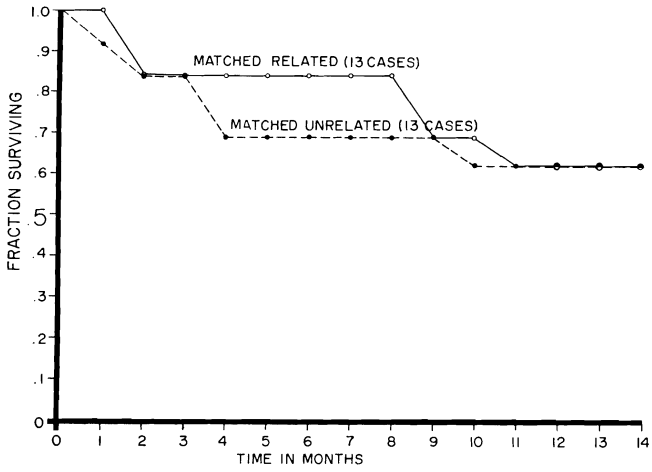

FIG. 3. Results of renal transplantation in a more recent series of patients whose donors were selected by Terasaki's antigen-matching method. Note that survival after 13 months was exactly the same in both the related and nonrelated cases. Compare with results shown in Fig. 2.

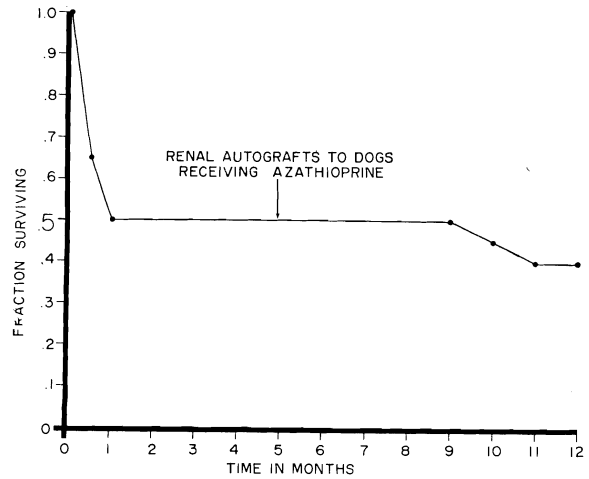

FIG. 4. Life survival curve of 18 dogs which received renal autografts and subsequent therapy with azathioprine.

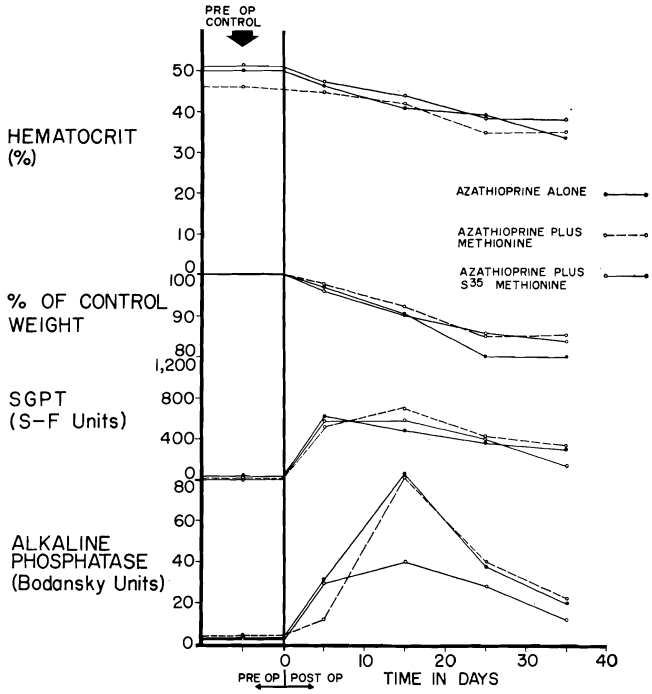

FIG. 5. Toxicity of azathioprine when used alone, with ^{35}S-methionine, and with L-methionine. Six dogs were in each of the three test groups depicted. Despite the abnormalities of liver function, jaundice did not develop. (By permission of C. V. Mosby Co., from Starzl et al. *Surgery* 58: 131, 1965.)

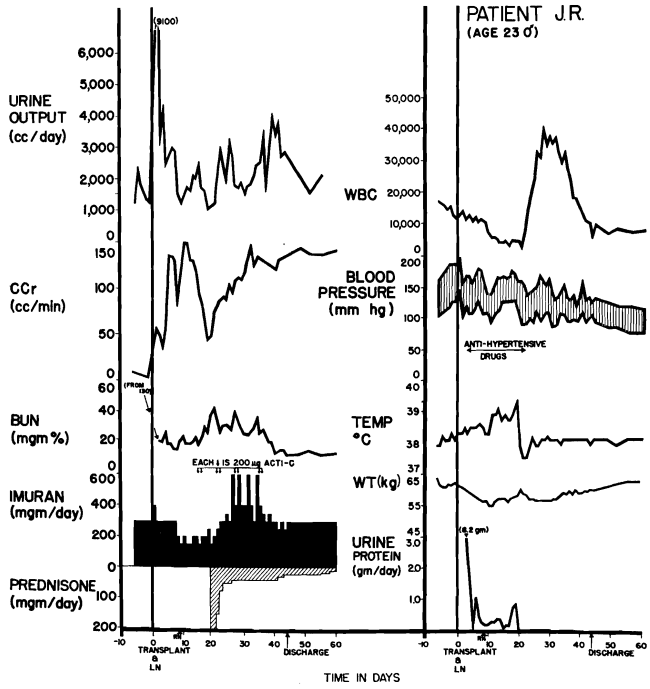

FIG. 6. Classical rejection crisis in patient treated with drugs alone. Deterioration of renal function began 19 days after transplantation. All stigmata of rejection are present except for acute hypertension and weight gain, which were successfully prevented by medical treatment. Acti-C = Actinomycin C; LN = Left nephrectomy at time of transplantation; RN = Right nephrectomy. Imuran is synonymous with azathioprine. (By permission of *Surg. Gynecol. Obstet.* 117: 385, 1963.)

azathioprine was the immediate cause of death. The intensive steroid therapy played a secondary role.

Such acute toxicity with azathioprine has been virtually eliminated since it is now thoroughly appreciated that this drug and its by-products are partially excreted in the urine. With the deterioration of renal function that accompanies a severe rejection crisis, it is often necessary to reduce the dose. No such simple formula to decrease the hazards of steroid therapy is available.

In many cases it is found that continued function of a homograft is dependent upon continuation of unacceptably large quantities of prednisone. The complications which follow are exceedingly troublesome at best, and lethal at worst. These include cosmetic deformity, bone demineralization often with spontaneous fractures, muscle wasting, arrest of growth in infants, fatty infiltration of the liver, pancreatitis, and gastrointestinal ulceration and hemorrhage to name just a few. Most serious, however, is the consequent susceptibility to microorganisms of all types.

If the resultant infections are due to common pathogenic bacteria they can be treated effectively with properly chosen antibiotics. Very often, however, these are caused by fungi, protozoa, or viruses for which specific therapy is not available. The tragic consequences are illustrated in Fig. 8. This patient, who received a homograft from his brother, had an early rejection crisis followed by excellent renal function for the next 9 months. After reduction of his prednisone to 10 mg/day, he had a delayed rejection which was controlled by increasing the prednisone dose to a level from which subsequent withdrawals were not possible without further deterioration of kidney function. He died 15 months later but not from renal failure. He had fatty infiltrations of the liver, a duodenal ulcer, and pancreatitis. There were cytomegalic inclusion viruses in the lungs and liver, and diffuse pneumonitis due to *Pneumocystis carinii* and *Aspergillus fumigatum*.

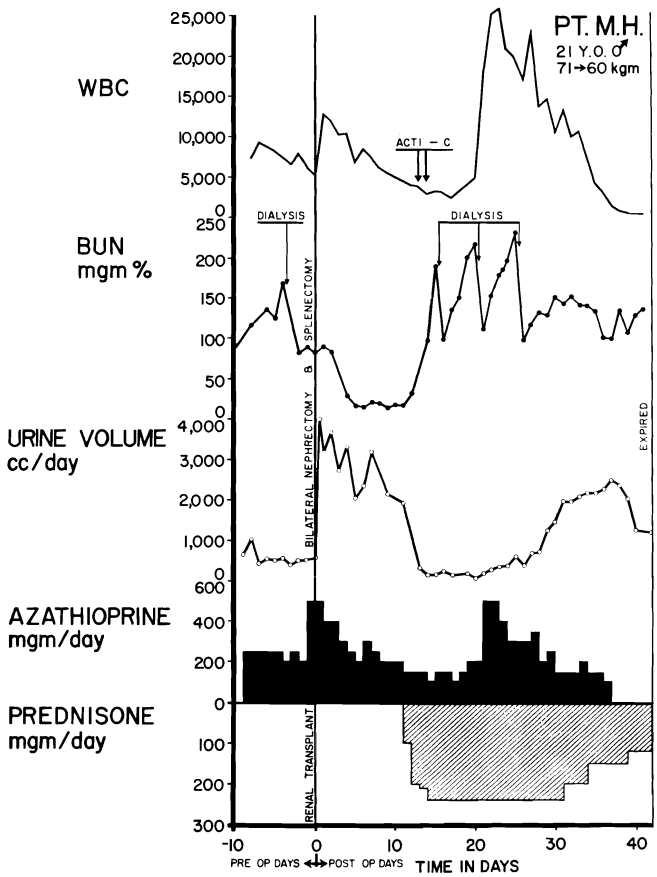

FIG. 7. Typical unsuccessfully treated case. The donor and recipient were brothers, both of A+ blood type. A violent rejection crisis followed good early function, and anuria developed which lasted 2 weeks. Although the rejection was reversed and a secondary diuresis began, the patient died from drug toxicity, leukopenia, and septicemia. Acti C = Each arrow is 200 μg intravenous actinomycin C. (By permission of C. V. Mosby Co., from Starzl et al. *Surgery* 56: 296, 1964.)

HETEROLOGOUS ANTILYMPHOID SERUM

We would now like to discuss some recent studies which could conceivably lead to improvement in patient care. These investigations have been with heterologous antilymphoid serum or its globulin derivatives. Waksman et al. (17) and Woodruff (18, 19) were the first to suggest the possible value of such substances for mitigation of homograft rejection. Their predictions have been amply confirmed by several other investigators (1, 6–11), also working with inbred mice, guinea pigs, or mice treated with serum raised in the rabbit. Skin grafts were used as the test system. Our efforts have been oriented to the testing of these biologic materials for protection of whole organ grafts, and to the development of a product which could practically and safely be given to man (5).

Serum was raised in horses which were subcutaneously inoculated with lymphocytes from the lymph nodes or spleens of dogs, or alternatively with cadaveric human lymph nodes, thymuses, and spleens. When large numbers of cells (10–196 billion) were injected weekly or even more often, leukoagglutinating titers in the equine serum rose to as high as 1:16,000 within 20–75 days.

The serum or plasma collected from these horses and injected unchanged into dogs' peritoneal cavities was toxic, causing death in 30% of the animals treated daily for 2 weeks. Nevertheless, the prolongation of renal homograft survival in those dogs that did not die from treatment was significant.

A critical step in reducing toxicity was absorption of the horse serum with canine red cell pack and serum. When the hemagglutinins and precipitins were thus removed, it was possible largely to eliminate the acute anemia which the original product had caused. Furthermore, injections by either the intraperitoneal or subcutaneous routes did not cause death. Further absorption with canine liver and kidney markedly reduced the leukoagglutinating titer without increasing the safety.

The location of the leukoagglutinating antibodies was studied by column chromatography, electrophoresis, and immunoelectrophoresis (5). The activity was in the

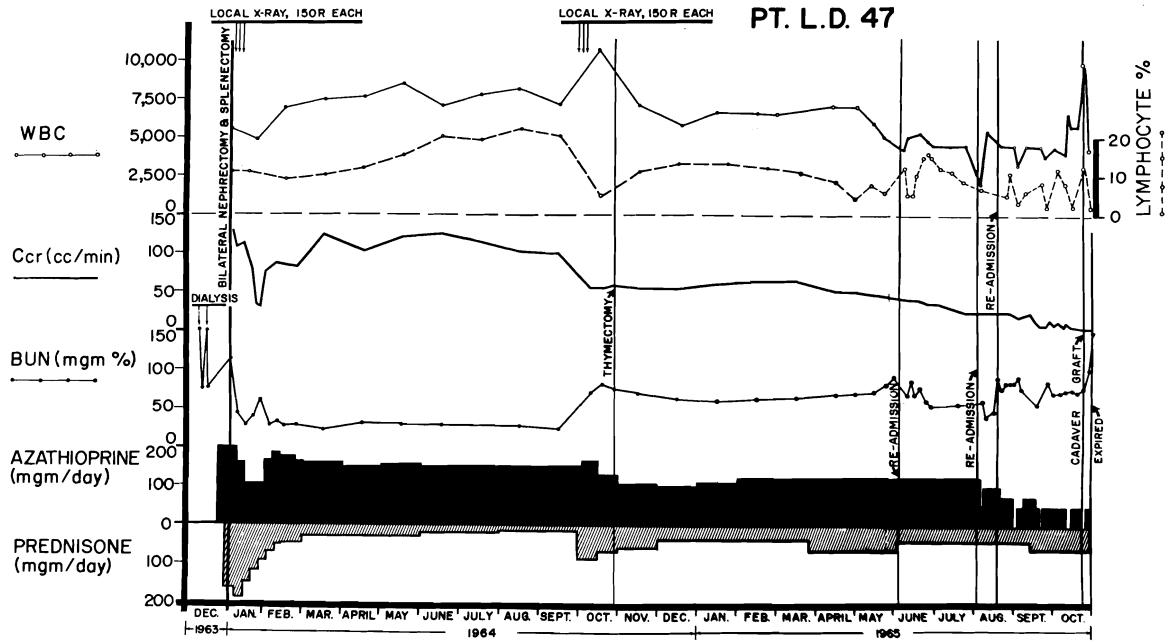

FIG. 8. Course of a 37-year-old man who received a kidney from his younger brother. Both were A+ blood type. Note the severe late rejection after 9 months and the subsequent slow deterioration of renal function. Late thymectomy did not induce either lymphopenia or make easier the subsequent management; the post-thymectomy changes in lymphocyte counts were related to adjustments in steroid dosage. (By permission of *Ann. N. Y. Acad. Sci.* 129: 598, 1966.)

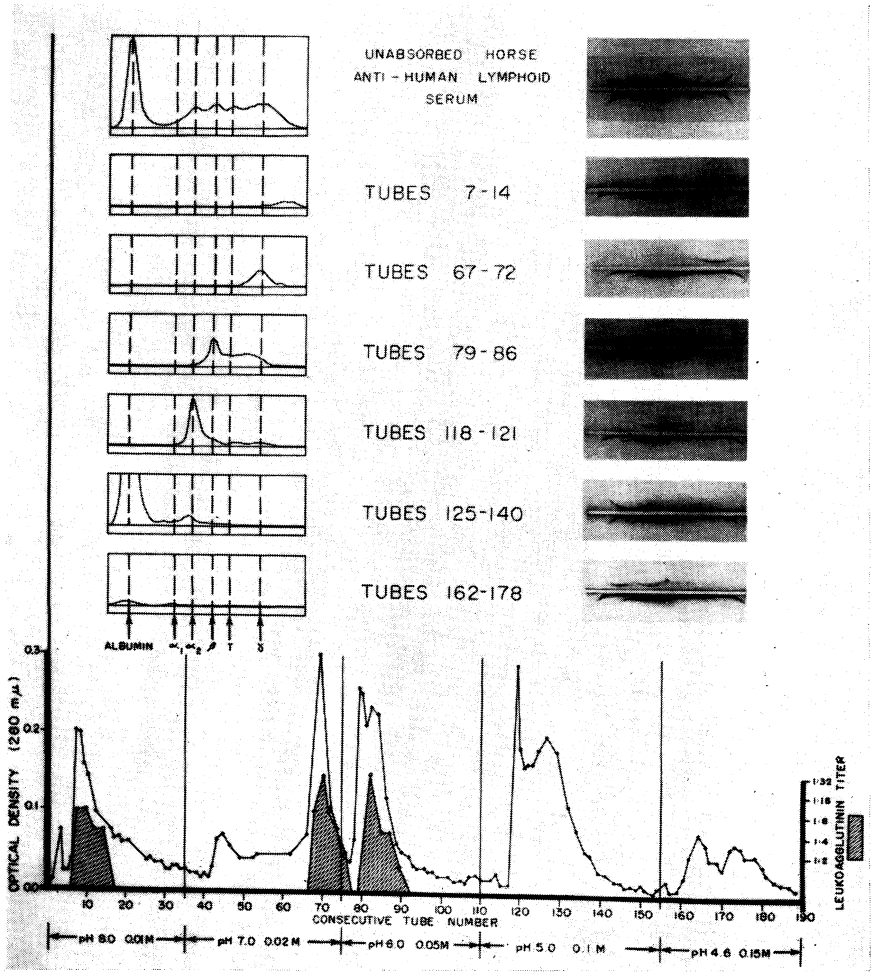

FIG. 9. Studies of the leukoagglutinin-containing fractions in antihuman-lymphoid serum employing column chromatography, electrophoresis, and immunoelectrophoresis. Various eluates from the DEAE-cellulose column were analyzed spectrophotometrically for protein content (expressed as optical density), and the presence or absence of leukoagglutinins determined for each collection tube. Electrophoresis and immunoelectrophoresis permitted relatively complete classification of the active immunoglobulins. (By permission of *Surg. Gynecol. Obstet.* 124: 1, 1967.)

gamma and T-equine globulin fractions, and to a lesser extent in the beta globulin (Fig. 9).

Ammonium sulfate precipitation at 0.4 saturation was used to remove these components in bulk for biologic testing. The electrophoretic and immunoelectrophoretic qualities of a batch of antihuman-lymphoid globulin obtained with two precipitations is shown in Fig. 10.

The plasma, serum, and globulin prepared from the blood of immunized horses all caused lymphopenia as compared to the absence of this effect in control dogs (Fig. 11). A further important difference was noted. The canine precipitin titer against horse protein either did not rise or increased sluggishly to low levels when the immune serum or globulin was administered daily, compared to rapid high rises with comparable doses of normal serum. These findings suggested that the risk from serum sickness might be less with antilymphoid materials than might otherwise be feared, due presumably to their immunosuppressive qualities.

It has, in fact, been found that dogs tolerate daily injections of the antilymphoid substances for months without overt toxicity if the serum has initially been absorbed with red cells and serum. Changes were not seen in either standard liver or renal function tests. However, renal biopsies revealed electron-micrographic densities in the glomeruli of a significant percent of kidneys from these animals, providing a clear warning that clinical use of such agents might have to be on a short-term basis (5).

The protective effect upon canine renal homografts of immune horse plasma, serum, and globulin has been extensively evaluated (13). The results are summarized in Fig. 12 with statistical computations that limit survival credit for any individual dog to 70 days. All three agents significantly prolonged survival providing therapy was given before as well as after transplantation. The effect was inconstant when treatment was confined to the post-transplant period and it was not demonstrable if injections were only given preoperatively. All kidneys were eventually rejected, the maximum survival being 144 days.

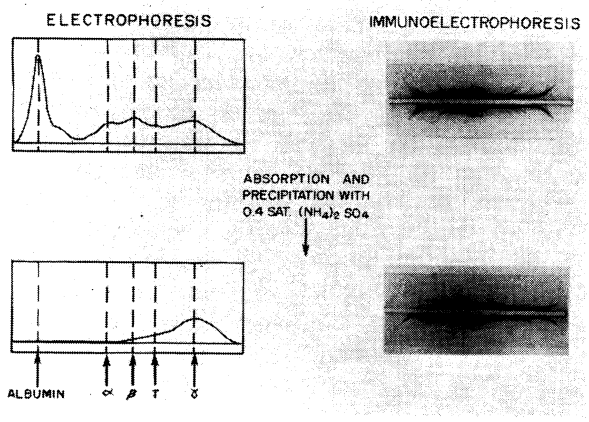

FIG. 10. Electrophoresis and immunoelectrophoresis of absorbed antihuman-lymphoid serum and the protein obtained from it by two precipitations with 0.4 saturated ammonium sulfate, two dialyses and lyophilization. The final product, which was used clinically, consists almost entirely of gamma G globulin. (By permission of *Surg. Gynecol. Obstet.* 124: 1, 1967.)

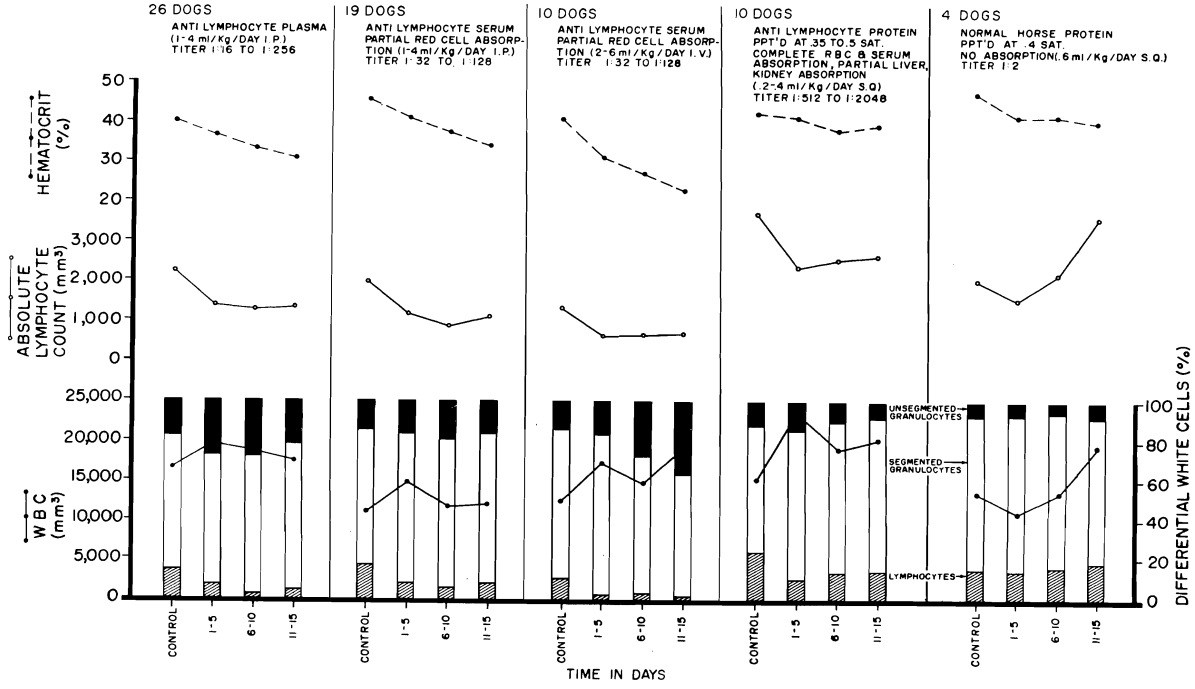

FIG. 11. Effect of horse plasma or serum and crude horse globulin upon the hematocrit, lymphocyte count, total white count, and white count differential during 15 days of daily administration. In all but the control experiments on the right, the agents were prepared from horses immunized against dog lymphoid tissue. Note that acute anemia was largely prevented only when complete absorption had been carried out with canine red cell pack. (By permission of *Surg. Gynecol. Obstet.* 124: 1, 1967.)

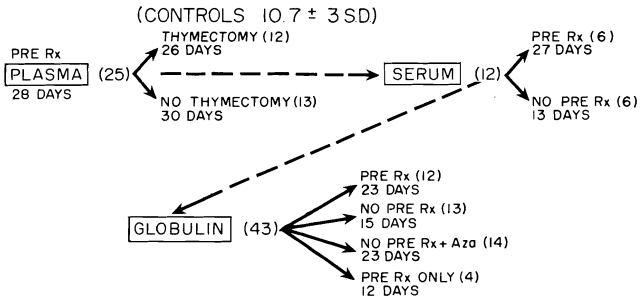

FIG. 12. Summary of survival (days) in 80 dogs with renal transplantation treated with antilymphoid plasma, serum, or globulin. Note that thymectomy was not useful, and that the best results were with treatment before and after operation. PreRx = treatment before and after. No PreRx = treated only after. PreRx only = therapy solely before operation. No PreRx + Aza = globulin and azathioprine both started at time of transplantation. Details and statistical analyses have been published (13).

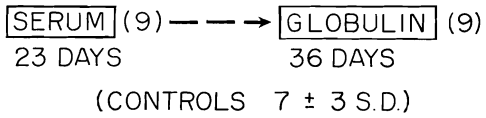

FIG. 13. Effect of antilymphoid serum or globulin upon survival after orthotopic liver transplantation. For both these mean values and those in Fig. 12, dogs living longer than 70 days were given a 70-day survival credit.

Prolongation of survival after orthotopic homotransplantation of the canine liver was also easily demonstrable (Fig. 13), apparently with a somewhat greater regularity than with renal homografts. The mean survival values depicted are computed with a limitation of 70 days credit for any individual animal, but three of these dogs are still alive after 4–6 months. The course of one illustrates two important points (Fig. 14). First, it was not necessary to have prolonged lymphopenia for success. Second, rejection did not necessarily follow cessation of therapy, or if it did, it was often indolent in its development. The animal whose course is depicted has not received any treatment for more than 5 months.

These studies provided some guidelines for the clinical application of antilymphoid agents. It seemed clear that an immunosuppressive effect was present, but that this was too incomplete to warrant sole or even primary dependence upon such therapy. In addition, it was thought that the threat of serum toxicity might preclude long-term treatment. Consequently, a clinical trial was given horse antihuman-lymphoid globulin as an adjuvant agent. It was started in intramuscular injections from 5 to 35 days before operation and continued thereafter as shown in Fig. 15. Therapy with azathioprine was given in the customary way. During the 1st postoperative month prednisone was administered only if required for

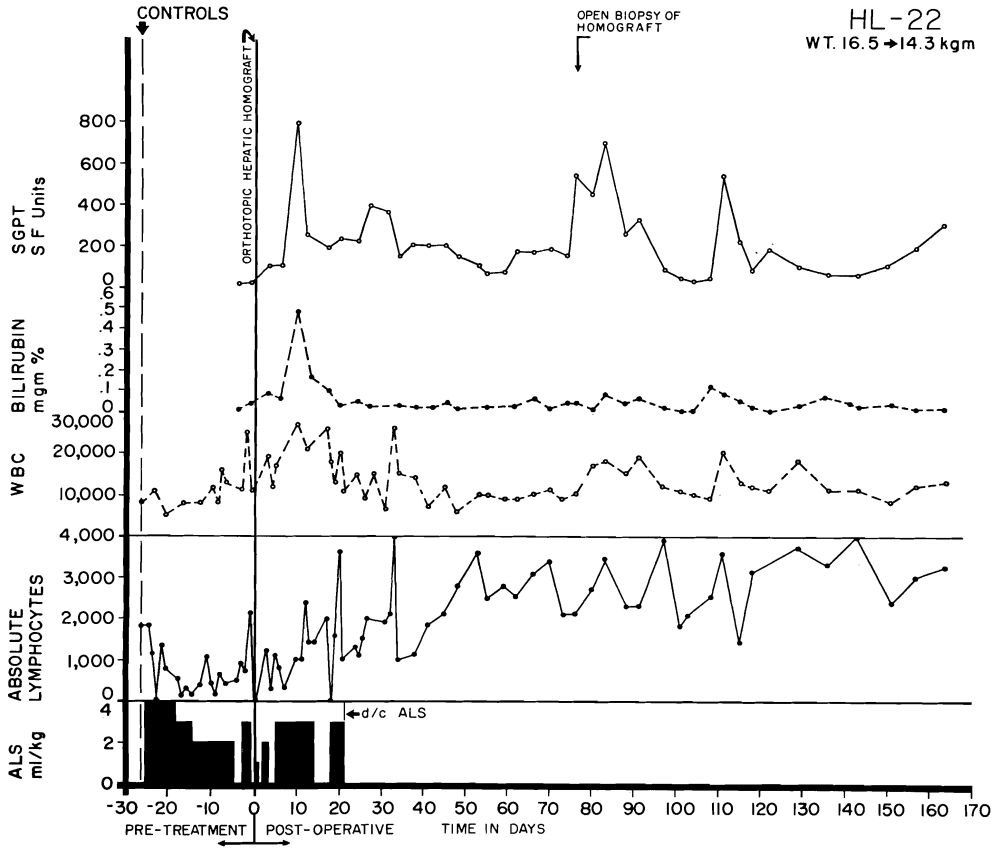

FIG. 14. A chronically surviving dog which was treated before and for 20 days after orthotopic liver transplantation with intraperitoneal antilymphoid serum (ALS). Note the pronounced lymphocytosis late in the postoperative period. The animal is in excellent health after almost 6 months. (By permission of *Surg. Gynecol. Obstet.* 124: 301, 1967.)

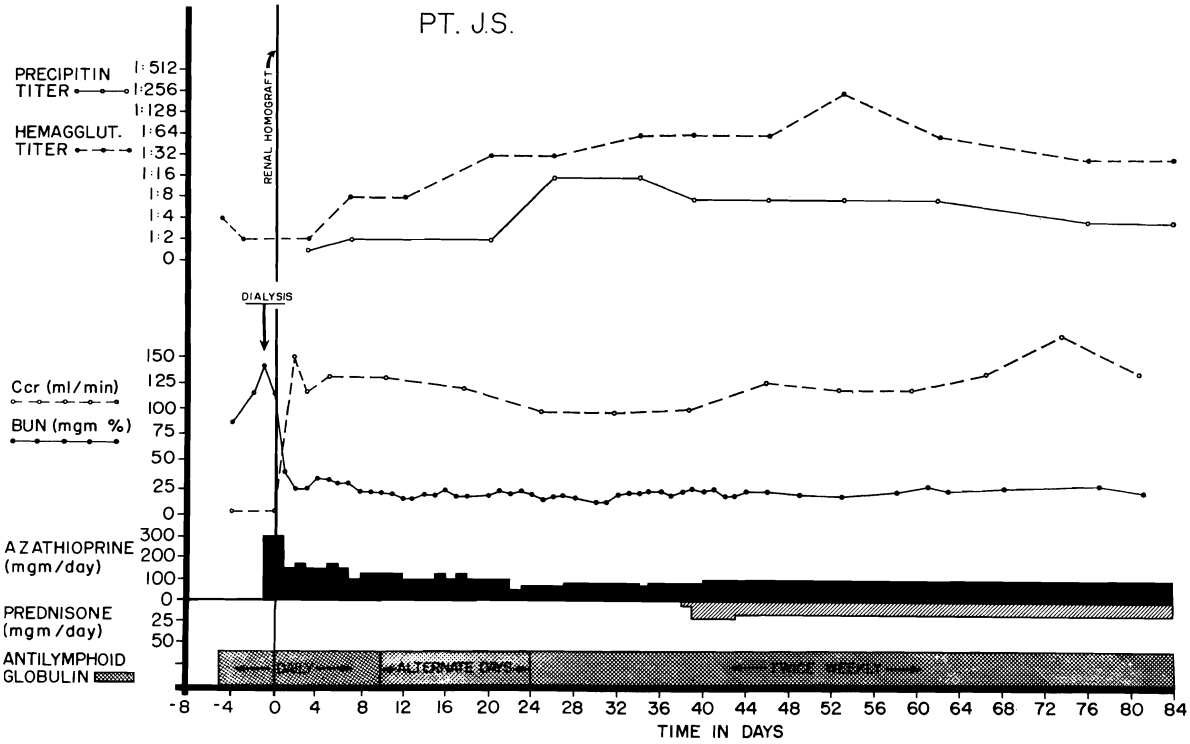

FIG. 15. Course of a patient treated before and after renal homotransplantation with antilymphoid globulin. No rejection occurred. Note the rises in precipitin and hemagglutinin titers, findings which prompted institution of prednisone therapy. These titers subsequently fell. This patient had a good antigen match with his sibling donor. (By permission of *Surg. Gynecol. Obstet.* 124:301, 1967.)

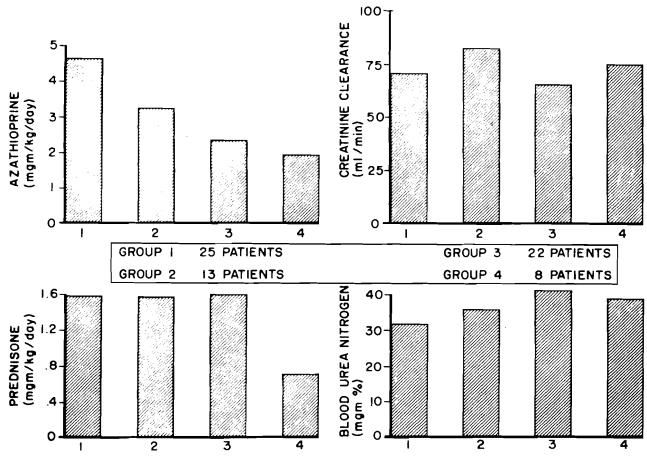

FIG. 16. Variations in immunosuppression and renal function during the first 63 postoperative days in four successive groups of patients who received kidneys from blood relatives. Since the blood urea nitrogen and creatinine clearance were not determined each day, these were compiled on a weekly basis. Those in *series 4* received adjuvant therapy with antilymphoid globulin. Note the drastic reduction in average prednisone dose which was achieved in these patients without significant loss of renal function. Progressive diminution of azathioprine dosage in the succeeding series is evident. (By permission of *Surg. Gynecol. Obstet.* 124: 301, 1967.)

the treatment of rejection. After this time, small steroid doses were started even if rejection had not occurred.

Eight patients with homotransplantation from related donors have been treated with this regimen. All eight are well from 2 to 3.5 months after operation. The convalescence in these cases was compared with that observed in three similar groups of patients treated in the past. The conditions of cross analysis and the other details of this study are fully described elsewhere (13), but some brief summary comments are in order.

The most striking change was that the amount of prednisone necessary during the first 9 weeks of convalescence was considerably less than half of that used in any previous series (Fig. 16). The doses of azathioprine were also smaller. This reduction in standard immunosuppression was not paid for with a loss of homograft function (Fig. 16). The pooled results of daily BUN and creatinine clearance in the three retrospective control groups were not significantly different than in the antilymphoid globulin series. Life-threatening toxicity has not yet been observed, although fever and injection-site tenderness were seen in almost every case. For the complete data as well as a discussion of the validity and interpretation of these observations, the reader is referred to a complete account of the results (13).

Thus far, there appears to have been some benefit during the critical early postoperative period when the mortality has been the greatest in all clinical series, here and elsewhere. Whether this gain will be canceled by a later morbidity is an open question. Meanwhile, it is important to stress the experimental nature of anti-

lymphoid globulin therapy in view of the unknown risk from serum sickness. The magnitude of this hazard and the related question of serum nephrotoxicity alluded to earlier can be completely assessed only with further observation and by study of renal homograft biopsies in those patients now under treatment. Wider clinical trial is not recommended until this information has been obtained.

SUMMARY

The status of immunosuppression in the field of organ homotransplantation has been discussed from three points of view: *a*) the results obtained in the past; *b*) a critique of the pharmacologic agents and treatment protocols now being used; and *c*) the possible future clinical role of heterologous antilymphoid sera and their derivatives.

REFERENCES

1. Gray, J. G., A. P. Monaco and P. S. Russell. *Surg. Forum* 15: 142, 1964.
2. Hamburger, J., J. Crosnier, J. Dormont, R.-J. Reveillard, J.-H. Hors and J. Alsina. *Presse Med.* 73: 2793, 1965.
3. Hill, R. B., K. A. Porter and C. G. Massion. *Arch. Pathol.* 81: 71, 1966.
4. Hume, D. M., H. M. Lee, G. M. Williams, H. J. O. White, J. Ferré, J. S. Wolf, G. R. Prout, Jr., M. Slapak, J. O'Brien, S. J. Kilpatrick, H. M. Kauffman, Jr. and R. J. Cleveland. *Ann. Surg.* 164: 352, 1966.
5. Iwasaki, Y., K. A. Porter, J. Amend, T. L. Marchioro, V. Zühlke and T. E. Starzl. *Surg. Gynecol. Obstet.* 124: 1, 1967.
6. Jeejeebhoy, H. F. *Immunology* 9: 417, 1965.
7. Levey, R. H., and P. B. Medawar. *Ann. N. Y. Acad. Sci.* 129: 164, 1966.
8. Levy, R. H., and P. B. Medawar. *Proc. Natl. Acad. Sci. U. S.* 56: 1130, 1966.
9. Monaco, A. P., M. L. Wood, J. G. Gray and P. S. Russell. *J. Immunol.* 96: 229, 1966.
10. Monaco, A. P., M. L. Wood and P. S. Russell. *Science* 149: 423, 1965.
11. Nagaya, H., and H. O. Sieker. *Science* 150: 1181, 1965.
12. Porter, K. A., J. M. Rendall, C. Stolinski, P. I. Terasaki, T. L. Marchioro and T. E. Starzl. *Ann. N. Y. Acad. Sci.* 129: 615, 1966.
13. Starzl, T. E., T. L. Marchioro, K. A. Porter, Y. Iwasaki and G. J. Cerilli. *Surg. Gynecol. Obstet.* 124: 301, 1967.
14. Starzl, T. E., T. L. Marchioro, K. A. Porter, P. D. Taylor, T. D. Faris, T. J. Hermann, C. J. Hlad and W. R. Waddell. *Surgery* 58: 131, 1965.
15. Starzl, T. E., T. L. Marchioro, P. I. Terasaki, K. A. Porter, T. D. Faris, T. J. Hermann, D. L. Vredevoe, M. P. Hutt, D. A. Ogden and W. R. Waddell. *Ann. Surg.* 162: 749, 1965.
16. Terasaki, P. I., K. A. Porter, T. L. Marchioro, M. R. Mickey, D. L. Vredevoe, T. D. Faris and T. E. Starzl. *Ann. N. Y. Acad. Sci.* 129: 500, 1966.
17. Waksman, B. H., S. Arbouys and B. G. Arnason. *J. Exptl. Med.* 114: 997, 1961.
18. Woodruff, M. F. A. *The Transplantation of Tissues and Organs.* Springfield, Ill.: Thomas, 1960, p. 100, 150.
19. Woodruff, M. F. A., and N. F. Anderson. *Ann. N. Y. Acad. Sci.* 120: 119, 1964.