Anti-Serum to Cultured Human Lymphoblasts:

Preparation, Purification and Immunosuppressive Properties in Man

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HETEROLOGOUS anti-lymphocyte sera (ALS) have been used for the prolongation of skin,[6, 7, 9, 13] renal,[8, 11, 18] hepatic,[18] and cardiac allografts.[2] Such sera are potent immunosuppressants in animals, particularly when they are administered over prolonged periods of time[9, 11] or when used as priming agents in conjunction with other immunosuppressive modalities.[5, 7, 10, 17, 22, 23] Nevertheless, there are a number of theoretical hazards associated with the prolonged administration of any heterologous serum and a number of real limitations to its use in clinical practice. Serum administration has been followed by severe systemic reactions when given intravenously[11] and significant local irritation has been noted when given intra-muscularly or subcutaneously.[4, 12, 18] Thrombocytopenia,[19] anemia,[11, 18] wasting,[21] fever,[11, 18] anaphylactic reactions,[18] development of antibodies to the injected proteins,[18, 21] loss of potency with prolonged administration,[21] and deposition of proteinaceous material along the basement membranes of renal glomeruli have all been reported.[4] At the same time, only Monaco has provided evidence of immunosuppressive efficacy of anti-human lymphocyte sera in man.[12]

The present studies document the development of a high titer purified horse antiglobulin prepared against pure cultured human lymphoblasts. Such material can be given intravenously without the development of anemia or thrombocytopenia and can be given for prolonged periods of time because immunological tolerance to the administered globulin can be induced. The material is effective in prolonging human skin allografts and as an adjunctive agent with other immunosuppressive agents in the prolongation of renal allografts.

Materials and Methods

Preparation of Antilymphoblast Serum: Lymphocytes were obtained from the peripheral blood of two different normal volunteers and the cells maintained indefinitely in culture at the Roswell Park Memorial Institute. A standard media RPMI 1640 with 5% fetal calf serum added is used to promote the growth of the cells which proliferate in two hundred liter vats.

When antigen is required, 5 grams (wet weight) of cells are obtained by separating off a given volume of media. The cells are then collected either by sedimentation or slow, continuous flow centrifugation and are resuspended in 100 ml. of media in a sealed vial under sterile conditions. The vial of cells is shipped by air at ambient temperature to the University of Minnesota. The cells are washed twice with sterile saline, counted, diluted to 40 ml. with normal saline and injected at inter-

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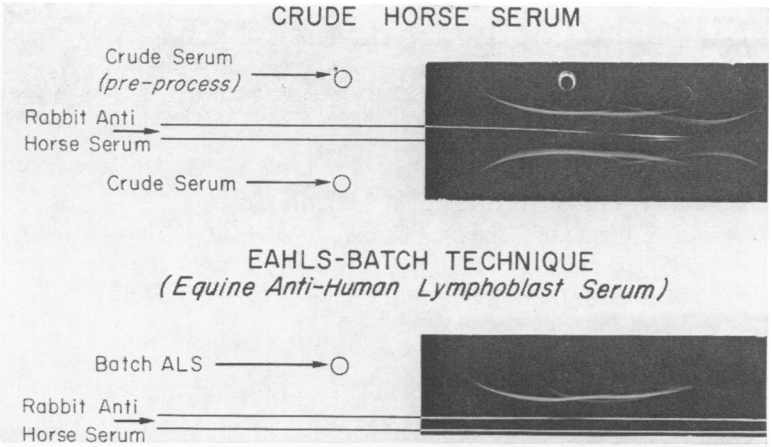

FIG. 1. Immunoelectrophoretic pattern of untreated horse serum and horse serum treated by batch chromatography technic (EAHLS). The batch preparation technic predominantly removes the albumin fraction from the horse serum.

vals of 10 days into multiple (4–8) intradermal and subcutaneous sites in the neck and hind quarters of a horse.* The number of cells in each injection ranges from 1–5 × 10^9. Each horse is bled of nine liters 7 to 10 days after the third and each succeeding booster injection, when a leukoagglutination titer of >1:1,000 has been reached. The horses are continually used for 6 months. The horse blood is allowed to clot and the serum removed.

In most of the experiments described below, crude purification of the serum was carried out by the batch chromatographic technic of Perper and Najarian.[14] The semipurified material is sterilized by millipore filtration and stored at −20° C. in 30 ml. sterile vials. The material is thawed just prior to use.

More recently new methods of purification have been utilized. At present the untreated serum is processed through a Canalco-CF-60 electrophoretic filter and bottled as before. Protein determinations on the final product are carried out by microbiuret technic. Each batch is cultured and assayed for pyrogens in rabbits. Only sterile and pyrogen free batches are used. Serum immune electrophoresis is performed against rabbit anti-horse serum to determine the horse serum components present in each batch of serum.

Administration of the Equine Anti-human Lymphoblast Serum (EAHLS) and Globulin (EAHLG): EAHLS or EAHLG is administered by slow intravenous drip in a dose of 4 mg. of globulin per Kg. body weight per day in approximately 250 ml. normal saline. Two groups of patients have received the EAHLS and EAHLG: Potential recipients of organ transplants and patients with multiple sclerosis who are receiving a course of immunosuppressive therapy as an experimental treatment for their disease. EAHLS or EAHLG therapy is begun in renal allograft recipients 2 days prior to scheduled renal transplantation. Recipients of cadaver organ transplants are administered one dose just prior to transplantation. Recipients of renal, hepatic and pancreatic transplants are given the serum daily for 10 days and every other day thereafter for five more doses. On occasion administration has been continued for more prolonged periods of time.

Full thickness skin allografts are exchanged between groups of five or six patients with multiple sclerosis of the same ABO blood type and are evaluated daily for total epithelial destruction. One group of six patients exchanged skin grafts without immunosuppressive therapy. One group of four patients exchanged skin grafts and received 4 mg./Kg. azathioprine daily after

* We would like to acknowledge the assistance of the University of Minnesota Veterinary School, particularly J. U. Usenick and John Bowran, in the maintenance of these horses.

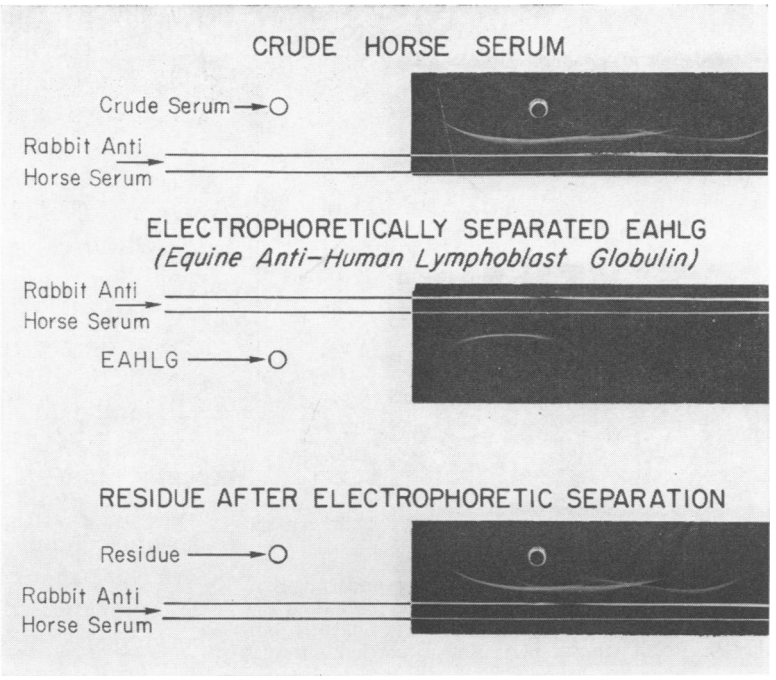

FIG. 2. Immunoelectrophoretic pattern of EAHLG compared with horse serum. The electrophoretically separated EAHLG consists of pure horse gamma globulin.

4–7 days of EAHLS treatment. A third group of seven patients exchanged skin grafts and were treated with EAHLS and EAHLG for 4–14 days without additional therapy. Histocompatibility typing of all donor-recipient combinations was performed by the laboratory of Terasaki.[20]

Leukocyte, differential and platelet counts were performed daily in duplicate on the peripheral blood of grafted patients. In addition, 500 lymphocytes on peripheral blood smears were counted and measured by micrometer. Lymphocytes measuring less than 7 micra were considered to be small lymphocytes; those measuring more than 7 micra were considered to be large lymphocytes.

Serum samples were collected at least weekly. Antibodies directed against horse serum proteins were determined by complement consumption, tanned sheep red blood cell agglutination and immune clearance. Immune clearance of pure horse gamma globulin was performed in 15 patients: 0.5 mg. ^{125}I labelled horse gamma globulin (Pentax) was injected intravenously, and the pattern of elimination was determined by measuring the residual radioactivity in daily serum samples.

Results

I. *Properties of the Cultured Human Lymphoblast Antigens:* Repeated histocompatibility typing of human lymphocytes in tissue culture over periods exceeding one year were performed by Terasaki.[20] There was no loss of histocompatibility antigens from the surface of the cultured human lymphoblast cells during this period. The two cell lines studied possess seven of the well defined histocompatibility antigens in humans, HLA 1, HLA 2, HLA 3, HLA 5, HLA 7, B 11, B 6.

When the cultured human lymphoblasts reach the University of Minnesota, they consist of 100% lymphoblasts free of other formed elements of the blood. Trypan blue exclusion studies have determined that 30–40% of the cells retain viability.

II. *Properties of Equine Anti-Human Lymphoblast Serum (EAHLS) and Globulin (EAHLG):* The composition of the semi-pure batch prepared antilymphoblast serum (EAHLS) is shown in Figure 1, where it

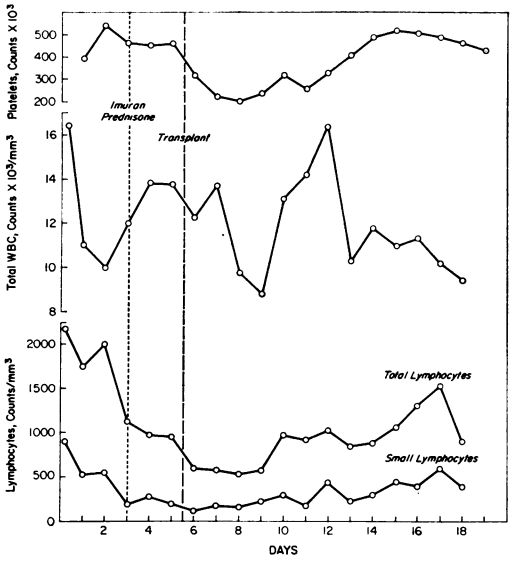

FIG. 3. Leukocyte, lymphocyte and platelet counts of patient T. L. who was treated with EAHLS, azathioprine and prednisone starting on day 3. Renal transplantation was carried out on day 5. Relative thrombocytopenia appeared only at the time of operation, but lymphopenia was persistent.

is compared by immunoelectrophoresis to normal horse serum. Semipurified serum contains almost all of the serum components except albumin. The leukoagglutination titer of this semi-pure serum was uniformly greater than 1:512. EAHLS was free of hemagglutinins, obviating the need for absorption with red blood cells or decomplementation.

Utilizing the Canalco-CF-60 serum electrophoretic separator, a highly pure horse globulin was obtained (Fig. 2). On occasion two components were present by immunoelectrophoresis, but further passage of this two-component material through the Canalco separator resulted in a single line electrophoretic gamma globulin. Leukoagglutination titer of this material (1.8 mg. gamma globulin/ml.) against random human peripheral blood leukocytes was 1:640. No hemagglutinins against human red blood cells were detectable. It is possible by repeated electrophoresis of horse serum to obtain a yield of 50 per cent of the horse globulin in immunoelectrophoretically pure form. The final concentrations of the horse globulin ranged from 2–12 mg./gamma globulin/ml. The serum was always sterile and was not pyrogenic to rabbits.

III. *Effect of Equine Antilymphoblast Sera and Globulin on Formed Elements of the Blood:* Figure 3 is an example of a typical response of a patient treated with semi-pure EAHLS, azathioprine and prednisone during renal transplantation. This combination of drugs induced a moderate fall in the small lymphocyte count over several days. The platelet counts fell abruptly at the time of operation but rose within a few days to pre-existing levels despite continued EAHLS administration. None of the recipients of renal allografts treated with EAHLS have developed thrombocytopenia.

Figure 4 reveals the mean peripheral blood counts of five patients with multiple sclerosis who received semi-pure EAHLS

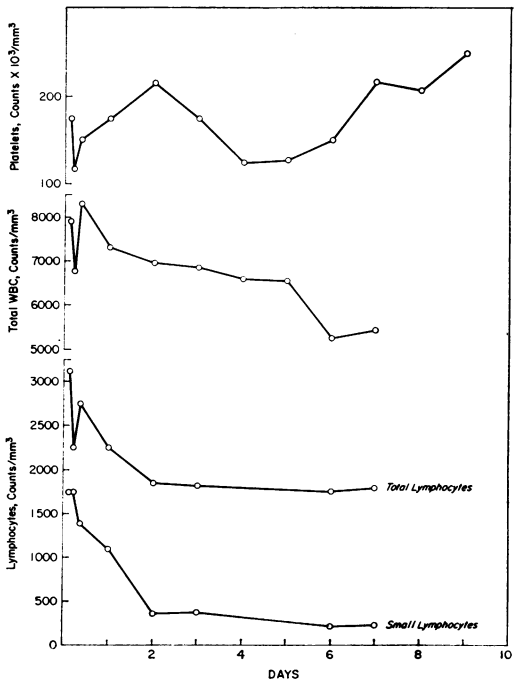

FIG. 4. Mean leukocyte, lymphocyte and platelet counts of five patients treated with EAHLS without other immunosuppression. Thrombocytopenia was absent but lymphopenia appeared.

TABLE 1. *Adjuvant Effect of ALG on Renal Allograft Survival*

Group	Follow-up Period (months)	Serum	No. Grafts	Graft Removal or Death	1 Rejection Episodes: None	One	More Than One	Mean Serum Creatinine (mg./100 m.)
Related donor	1–15	ALG	19	0	9	6	4	1.3
Related donor	15–21	No ALG	14	3	5	5	4	1.3
Unrelated donor	1–15	ALG	7	5	1	1	5	1.6
Unrelated donor	15–21	No ALG	5	4	0	1	4*	4.0

* Progress of 1st rejection to complete rejection recorded as >1 episode.

without other immunosuppressants. Skin grafts were exchanged on day 1. Within one hour of administration, the total lymphocyte count fell but returned promptly to normal. There was no thrombocytopenia. During the week, the small lymphocyte count fell to very low levels.

Figure 5 represents the mean lymphocyte counts of six patients with multiple sclerosis who received pure EAHLG. Skin grafts were exchanged on day 1. The lymphopenia was not marked in these patients in contrast to the effect of the semi-pure EAHLS. Thrombocytopenia was absent.

IV. *Survival of Renal Allografts:* Table 1 is a compilation of the kidney transplantations performed in the University of Minnesota Hospitals since July, 1967. Only patients followed for at least 30 days are included. The last 26 patients have received adjuvant treatment with semi-pure EAHLS in addition to azathioprine and prednisone. Of the 19 patients who have received kidney grafts from related donors (including two patients receiving allografts from an aunt and an uncle) there have been nine instances in which no clinical allograft rejection episode has been diagnosed. None of the 19 kidneys has been lost, and no patients have died during this period of time. Thus, more than half of those patients receiving allografts from siblings or parents have not experienced any allograft rejection episode at all. In comparison, of 14 patients with sibling or parental allografts prior to the period in which EAHLS was started, five did not experience any allograft rejection. Three of the patients not treated with EAHLS have totally rejected the transplant or have died after allograft transplantation. It is obvious that this latter group of patients have been followed for a considerably longer period of time, and no direct comparison is possible at present. There is no discernible difference be-

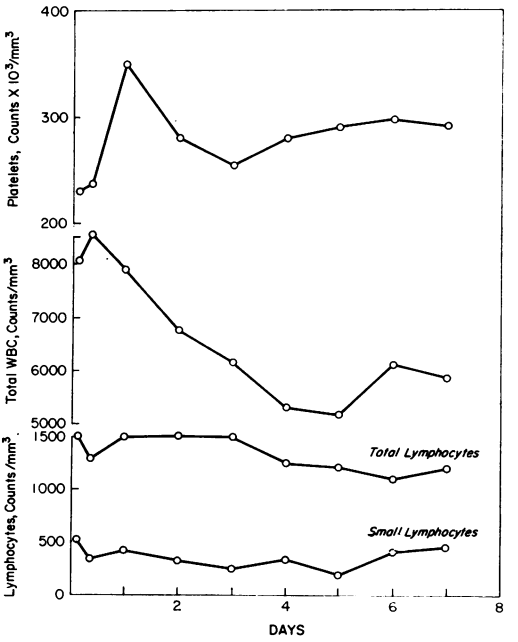

FIG. 5. Mean leukocyte, lymphocyte, and platelet counts of six patients treated with pure EAHLG. The lymphopenia seen in patients treated with the batch prepared EAHLS is absent after purification of the globulin.

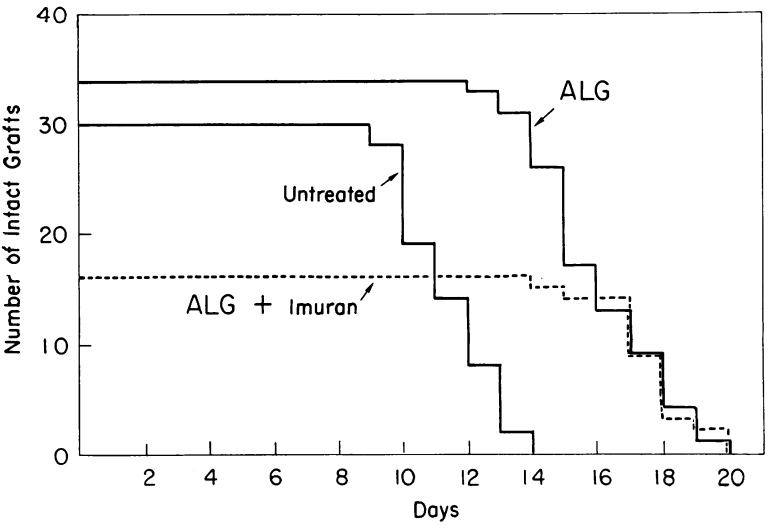

FIG. 6. Survival of full thickness skin allografts exchanged between patients with multiple sclerosis. There is marked prolongation of skin grafts on patients treated with EAHLG or EAHLS whether or not azathioprine was given.

tween the groups of recipients of unrelated kidney allografts whether or not EAHLS has been utilized.

Three patients received EAHLS as adjuvant therapy for cadaver liver transplantation and two patients for cadaver pancreatic transplantation. All of these patients either rejected their grafts or died of other causes.

V. *Effect of Antilymphoblast Serum* on *Skin Allograft Survival in Man:* Full thickness skin grafts were exchanged between groups of five or six patients with multiple sclerosis, who volunteered to receive antilymphoblast serum treatment as part of an experimental trial of immunosuppressive therapy for the disease. All patients within each group had the same ABO blood type, but at least one major histocompatibility antigenic difference (C or D match) was present between the skin graft donor and recipient. Three groups of patients were studied: One group (6 patients, 30 grafts) received no treatment; the second group (7 patients, 34 grafts) received EAHLS or EAHLG for 4–14 days and a third group (four patients, 16 grafts) received EAHLS for 4 days followed by 4 mg./Kg. azathioprine daily thereafter. The survival of the skin grafts are shown in Figure 6. Untreated patients with multiple sclerosis rejected the grafts in 9–14 days. Patients receiving EAHLS or EAHLG demonstrated considerable prolongation of graft survival.

VI. *Clinical Hypersensitivity Reactions to Antilymphoblast Serum:* Thirty-four renal allograft recipients (including recent transplants not included in Table 1) have received 704 doses of EAHLS and EAHLG over the past 2 years in addition to azathioprine and prednisone. In addition, three recipients of liver transplants and two recipients of pancreatic transplants received prolonged courses of treatment. The average period over which the material was given was 30 days and ranged from a single dose to 140 days.

Sixteen patients received semi-pure EAHLS and demonstrated no clinical reactions which could be attributed to the horse serum. No reactions have been detected thus far in four patients who have received the pure EAHLG. Two patients had previously been immunized to horse proteins and had immediate reactions to the first few drops of EAHLS. Of the 16 remaining patients, all of whom received the semi-pure EAHLS, mild clinical reactions to the serum were demonstrated between the 7th and 35th days of administration. The reactions consisted of fever,

TABLE 2. *Serological Studies on Recipients of Antilymphoblast Serum (EAHLS) and Antilymphoblast Globulin (EAHLG)*

Immunosuppression	Serum Treatment	No. of Patients	No. with Clinical Reaction	No. with Anti-horse Protein	No. without Clinical Reaction	No. with Antibodies to Horse Protein
Azathioprine Prednisone	EAHLS	18	11	11	7	2
None	EAHLS	7	6	6	1	0
None	EAHLG	4	1	1	3	0

urticaria, rash or the appearance of midline back pain during infusion. All were easily controlled by diphenhydramine hydrochloride (Benadryl). There were no anaphylactoid responses, no hypotension and no deaths. Eight of the reactions fell between days 28 and 35 following transplantation. The other reactions occurred on days 7, 9, 11, 19, 21, 22. The serum was always stopped as soon as the reaction was diagnosed or suspected.

Of seven patients with multiple sclerosis treated with semi-pure EAHLS without azathioprine or prednisone, significant reactions were seen in six. One patient developed erythema at the site of infiltration on the fourth day of administration, two developed urticaria, and three patients developed lower extremity purpuric rashes, arthralgia and angioneurotic edema. No life threatening complications ensued in any of the sensitized patients. The one patient who received 14 days of semi-pure EAHLS without clinical reaction was apparently tolerant to the serum and has since received a second course of EAHLS as treatment for multiple sclerosis without adverse reaction.

Four patients with multiple sclerosis received the purified EAHLG. Three of these have not developed any clinical reaction. One patient developed fever and lymphadenopathy on the 12th day of the administration of the pure globulin. Two of the 3 patients who did not develop any reaction had received a dose of disaggregated pure horse lgG one week prior to the 14 days of administration.

VII. *Serological Studies:* Summarized in Table 2 are the serological studies performed on the serum of those patients who received EAHLS as part of their immunosuppressive treatment following renal allotransplantation. In general, patients who manifested clinical reactions to the horse serum, demonstrated antibodies to one or more of the component horse proteins.

The tendency to form antibodies to the various components of horse serum was not equal (Table 3). In particular, there was a greater inclination to form antibodies to those horse proteins which are presumably contaminants of the antilymphoblast serum.

TABLE 3. *Development of Antibodies Against Specific Horse Proteins*

Immunosuppression	Serum Treatment	No. with Antibodies to Horse Protein	No. with Antibodies Directed Against			
			α	β	γ	Albumin
Azathioprine Prednisone	EAHLS	9	7	9	5	5
None	EAHLS	4	4	4	4	4

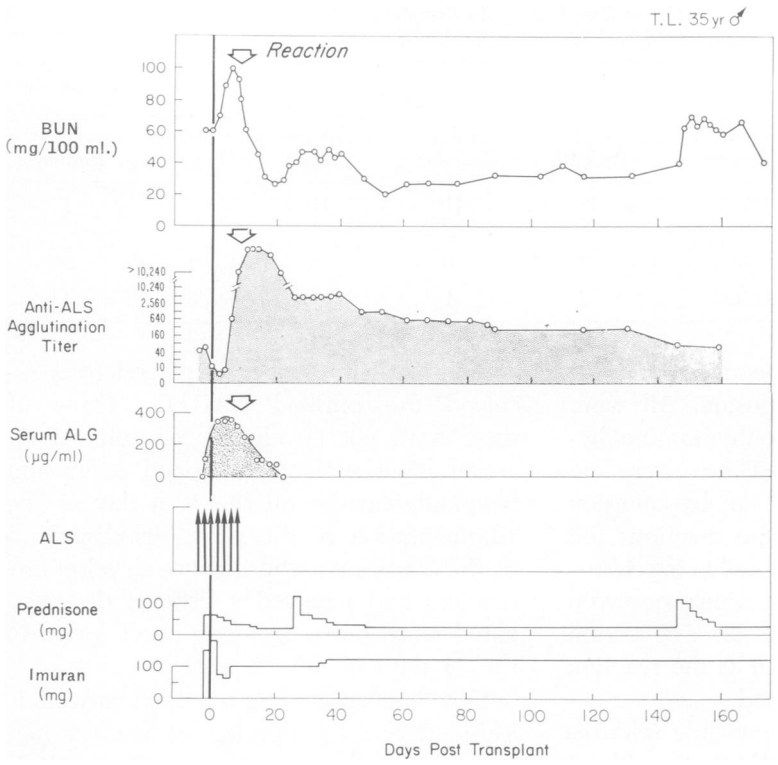

FIG. 7. Clinical and serological course of a recipient of a related renal allograft. Antibodies to horse globulin appeared soon after initiation of EAHLS therapy along with a clinical hypersensitivity reaction and rapid elimination of the horse protein from the circulation.

Although all patients who manifested clinical hypersensitivity responses to EAHLS developed antibodies to horse protein, the appearance of antibody did not always coincide with the clinical reaction. Several patterns of antibody development are shown in Figures 7–10. Frequently antibodies appeared between the 7th and 10th days following transplantation, but the reactions did not occur for several more weeks. Figure 9 demonstrates the course of one recipient of a cadaver pancreatic and renal transplant who did not develop either clinical reactivity or antibodies to horse proteins despite serum administration for 140 days.

Immune elimination curves of injected ^{125}I labelled pure horse gamma globulin were performed on several patients. The two basic patterns of elimination are shown in Figure 11. Those patients with circulating antibodies against horse globulin demonstrated rapid and complete elimination. Those patients without demonstrable antibodies eliminated horse globulin at a normal catabolic rate without evidence of immune elimination. Evidence for immunologic tolerance to horse gamma globulin in these patients was confirmed by the observation of circulating horse globulin which could be detected as long as 2 months after the last injection of EAHLG.

VIII. *Induction of Tolerance to Horse Gamma Globulin:* The previously discussed responses to EAHLS and EAHLG suggest that humans can be made tolerant to horse gamma globulin. Consequently, preliminary experiments have been conducted to determine if tolerance to horse gamma globulin can be induced intentionally for the purpose of prolonging the action of the injected EAHLG and minimizing hypersensitivity reactions.

A group of six patients with multiple sclerosis were injected with various potentially tolerogenic agents one week prior to the administration of anti-lymphocyte globulin. Two patients received saline, two

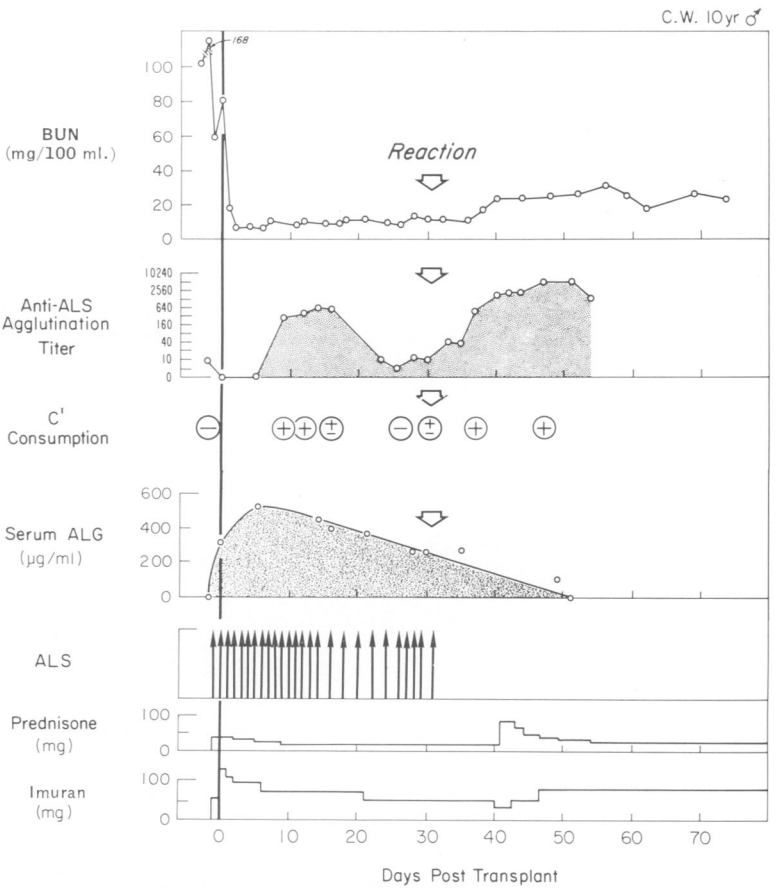

FIG. 8. Clinical and serological course of a recipient of a related renal allograft. Antibodies to horse globulin appeared soon after initiation of EAHLS therapy. The clinical reaction appeared after the anti-horse protein titer fell and was accompanied by a fall in serum complement. A secondary rise in serum anti-horse protein appeared after the clinical reaction but the disappearance of horse protein from the circulation was gradual.

semi-pure EAHLS and two disaggregated pure horse gamma globulin. The two recipients of disaggregated gamma globulin demonstrated no clinical reaction to a two week course of EAHLG. The immune clearance of a test dose of ^{125}I labelled gamma globulin demonstrated a non-reactive pattern of elimination. Similar patterns were observed by the recipients of the saline priming dose, but one of these developed a fever and cervical lymphadenopathy after 10 days of treatment. The two recipients of a priming injection of disaggregated semi-pure EAHLS developed urticaria and developed subsequent rapid elimination patterns.

Discussion

Antilymphocyte sera are excellent immunosuppressive agents in animals. Widespread clinical use will require that the following criteria be satisfied. 1. A high degree of immunosuppressive activity should be present. 2. An antigen should be found which is easily available and which does not elicit antibodies to red blood cells or platelets. 3. An antibody preparation should be purified to evoke minimal sensitization reactions over long periods of time. 4. The route of administration should minimize both toxicity and sensitization. 5. A mode of immunosuppressive assay should be available in order to control the efficacy of the product. The present studies have attempted to attack some of these problems.

Previous investigators have utilized spleen,[4] thymus,[7] lymph nodes,[9] and lymphocytes [15] as antigens for the preparation of antilymphocyte sera. The use of cul-

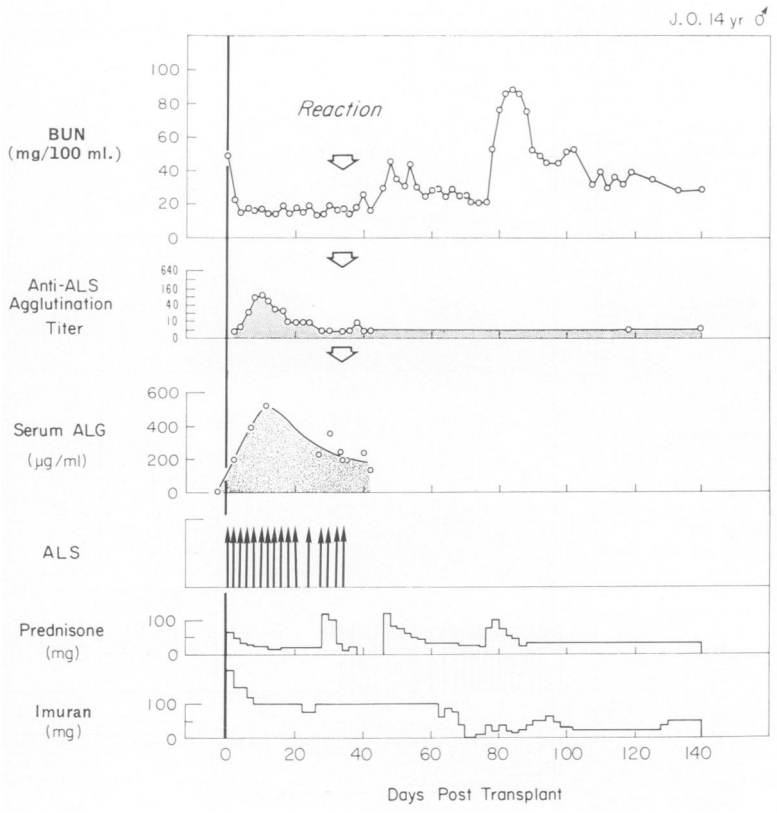

FIG. 9. Clinical and serological course of a recipient of a related renal allograft. Antibodies to horse globulin appeared soon after initiation of EAHLS therapy but the clinical reaction did not appear until the 33rd day. No secondary antibody response accompanied the reaction and the serum levels of horse globulin persisted.

tured lymphoblasts has the prime advantage of avoiding platelet, red blood cell, leukocyte and fibrocyte contamination, thus minimizing the problems of thrombocytopenia and anemia in the recipient. Antisera against such pure antigens can be administered intravenously without the systemic reactions which accompany intravenous administration of less specific antisera [11, 18] and avoid the local inflammatory reactions which accompany intramuscular injection.[12, 18] In addition, the use of cultured lymphoblasts has the advantages of sterility and availability of large quantities on demand. Apparently, viability of the cell is not necessary.[7]

Ideally the heterologous antibody administered should be free of all non-essential components in order to minimize sensitization of the recipient. The earlier semi-pure EAHLS discussed here contained a number of horse proteins other than the horse gamma globulin required. Use of the Canalco electrophoretic filter allows for complete separation of the gamma globulin from the other serum proteins in high yield, since any portion which possesses contaminants can be processed repeatedly until purity is established. Bacterial contamination is readily avoidable.

All preparations effective in experimental animals depend on the quality of the gamma globulin in the antiserum. Starzl has recently suggested that the T-equine globulin of horse serum may be necessary for full immunosuppressive activity.[19] Our studies on skin graft survival across strong histocompatibility barriers in man indicate that the highly purified gamma globulin fraction is as effective as the semi-pure preparation. However, less lymphopenia was produced by the purified

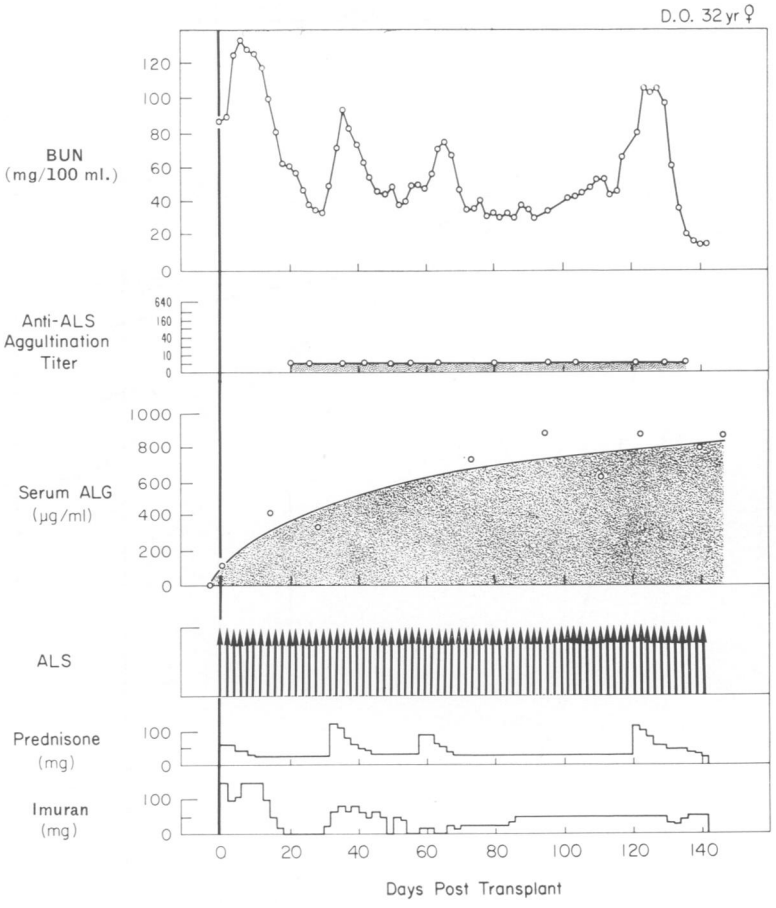

FIG. 10. Clinical and serological course of a recipient of a cadaver renal and pancreatic transplant. EAHLS was administered for 140 days without eliciting anti-horse protein antibodies. The serum horse globulin level continued to rise to the time of death.

EAHLG. Thus, globulins prepared against cultured human lymphoblasts were fully immunosuppressive without producing thrombocytopenia anemia or lymphopenia.

The intravenous route of administration was chosen to minimize the sensitization potential of the heterologous protein. In general the intravascular route tends to be more tolerogenic than direct injection of a foreign material into tissue. A by-product of this route is that local and febrile reactions were completely avoided. However, approximately half of the patients receiving organ transplants became clinically sensitized to horse proteins despite concomitant administration of immunosuppressive agents. Six of seven normal recipients of semi-pure EAHLS developed serum reactions. Nevertheless about ⅓ of the immunosuppressed recipients and ⅐ of the normal recipients did not react to the heterologous serums. The elimination pattern of the horse gamma globulin in these patients showed no evidence of sensitization. In addition, recipient antibodies to contaminating horse proteins were not always directed against the gamma globulin component. These findings suggested that it would be possible to deliberately induce immunologic tolerance to purified horse gamma globulin prior to the administration of EAHLG. A number of investigators have demonstrated that a small dose of disaggregated protein antigen administered intravenously will elicit tolerance to the antigen.[1] Howard has shown that tolerance to antilymphocyte globulins will further increase the

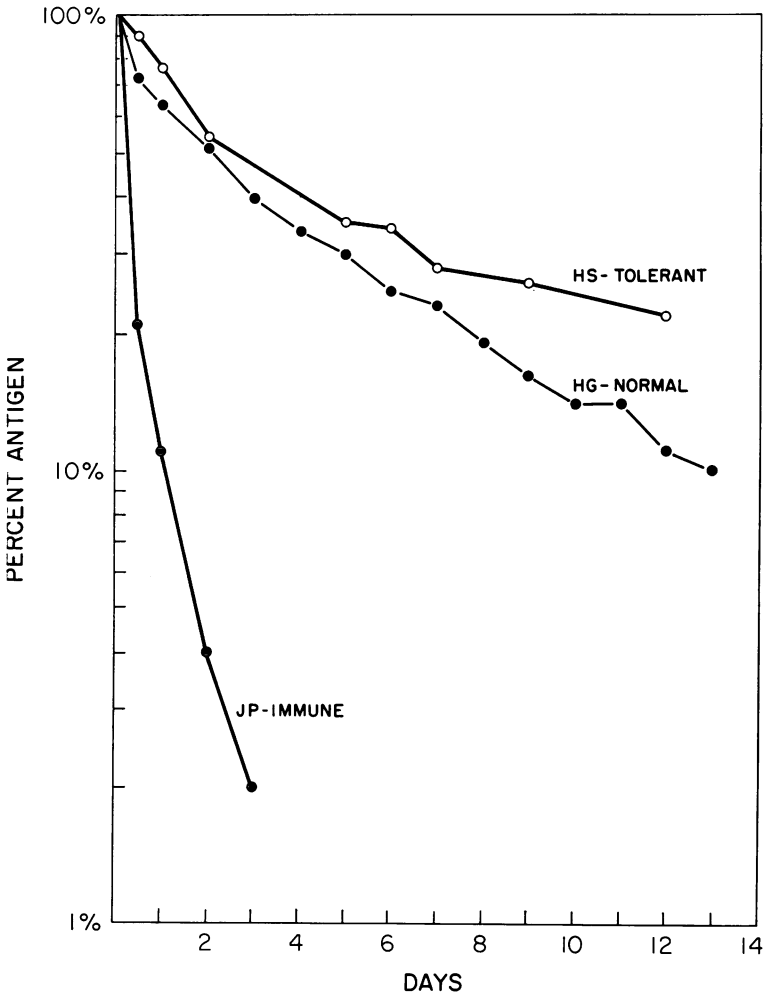

FIG. 11. Elimination of ^{125}I labelled horse gamma globulin in a normal patient and in two recipients of normally functioning renal allotransplants. Patient H. S. had no clinical reaction to EAHLS treatment, formed no detectable anti-horse protein antibodies and eliminated the labelled globulin slowly. Patient J. P. had a clinical reaction, had high titers of anti-horse protein antibodies, and rapidly eliminated the labelled horse globulin.

immunosuppressive properties of the globulin.[3] Thus, by inducing immunologic tolerance to EAHLG, one might expect to minimize the clinical reaction while increasing the effect of the EAHLG and prolonging the period of administration. Our results thus far suggest that induction of immunologic tolerance in this way can easily be done. The results of the clinical experiments with tolerance induction are in a preliminary stage and the possibility of increased effectiveness of immunosuppression in the tolerant patient cannot be determined at this time. However, the possibility of giving increasing doses of horse antiglobulin and continuing treatment for extended periods in the tolerant patient is now being investigated.

Summary

1. Cultured human lymphoblasts provide a readily available sterile antigenic source free of red blood cells, platelets, and leukocytes for the production of a horse antihuman lymphoblast serum (EAHLS).

2. Immunoelectrophoretically pure equine antihuman lymphoblast gamma globulin (EAHLG) can be easily prepared by continuous flow electrophoresis.

3. EAHLS and EAHLG will prolong skin grafts in human patients across HLA

histocompatibility barriers. EAHLS has been used as an adjunctive immunosuppressive agent in renal, hepatic and pancreatic transplantation. Of 19 related renal allografts performed in the past 15 months utilizing EAHLS, none has been rejected.

4. Intravenous administration of EAHLS avoids local reactions. Clinical sensitization to horse proteins is avoided in half of the recipients of renal allografts. The clinical sensitization reactions have been mild without serious morbidity or mortality.

5. It is possible to induce immunological tolerance to horse gamma globulin in order to avoid sensitization reactions and prolong the period of globulin treatment.

References

1. Golub, E. S. and Weigle, W. O.: Studies on the Induction of Immunologic Unresponsiveness. III. Antigen Form and Mouse Strain Variation. J. Immunol., **102**:389, 1969.
2. Halpern, B., Cachera, J. P., Lacombe, M., Hathaway, A., Crepin, Y., Hung, B. M., Lacassagne, J. P., Leandri, J., de Mendosa, M., Laurent, D., and Dubost, Ch.: The Efficacy of Sheep Anti-dog Antilymphocyte Serum (SADALS) in the Prolongation of Canine Heart Allografts. Transpl. Proc., 1:467, 1969.
3. Howard, R. J., Dougherty, S. F., Mergenhagen, S. E.: Prolongation of Skin Homografts by Rabbit Anti-mouse Lymphocyte Serum in Mice Rendered Tolerant to Rabbit γ-Globulin. J. Immunol., **101**:301, 1968.
4. Iwasaki, Y., Porter, K. A., Amend, J. R., Marchioro, T. L., Zühlke, V. and Starzl, T. E.: The Preparation and Testing of Horse Anti-dog and Anti-human Antilymphoid Plasma or Serum and its Protein Fractions. Surg. Gynec. Obstet., **124**:1, 1967.
5. Jeejeebhoy, H. F.: Effect of Rabbit Anti-rat-lymphocyte Plasma on Immune Response of Rats Thymectomized in Adult Life. Lancet, 2:106, 1965.
6. Levey, R H. and Medawar, P. B.: Some Experiments on the Action of Anti-lymphoid Antisera. Ann. N. Y. Acad. Sci., **129**:164, 1966.
7. Levey, R. H. and Medawar, P. B.: Nature and Mode of Action of Antilymphocyte Antiserum. Proc. Nat. Acad. Sci., **56**:1130, 1966.
8. Mitchell, R. M., Sheil, A. G. R., Slafsky, S. F. and Murray, J. E.: The Effect of Heterologous Immune Serum on Canine Renal Homografts. Transplantation, **4**:323, 1966.
9. Monaco, A. P., Wood, M. L., Gray, J. G. and Russell, P. S.: Studies on Heterologous Antilymphocyte Serum in Mice—II, Effect on the Immune Response. J. Immunol., **96**:929, 1966.
10. Monaco, A. P., Wood, M. L. and Russell, P. S.: Thymectomy: Effect on the Recovery from Immunologic Depression in Mice. Science, **149**:432, 1965.
11. Monaco, A. P., Abbot, W., Otherson, H. G., Simmons, R. L., Wood, M. L., Flax, M. and Russell, P. S.: Anti-lymphocyte Serum: Prolonged Survival of Canine Renal Allografts. Science, **153**:1264, 1966.
12. Monaco, A. P., Wood, M. L. and Russell, P. S.: Some Effects of Purified Heterologous Anti-human Lymphocyte Serum in Man. Transplantation, **5**:1106, 1967.
13. Nagaya, H. and Sieker, H. O.: Allograft Survival: Effect of Antiserum to Thymus Glands and Lymphocytes. Science, **150**: 1181, 1965.
14. Perper, R. J., Okimoto, J. T., Cochrum, K. C., Ramsey, M. and Najarian, J. S.: A Rapid Method for Purification of Large Quantities of Anti-lymphocytic Serum. Proc. Soc. Exp. Biol. Med., **125**:575, 1967.
15. Pichlmayr, R., Brendel, W. and Zenker, R.: Production and Effect of Heterologous Anti-canine Lymphocyte Serum. Surgery, **61**:774, 1967.
16. Russe, H. P. and Thompson, J. S.: Anti-thymus Serum-induced Wasting in Adult Mice. Fed. Proc., **26**:418, 1967.
17. Simmons, R. L., Ozerkis, A. J. and Hoehn, R. J.: Antiserum to Lymphocytes: Interactions with Chemical Immunosuppressants. Science, **160**:1127, 1968.
18. Starzl, T. E., Marchioro, T. L., Porter, K. A., Iwasaki, Y. and Cerilli, J. C.: The Use of Heterologous Antilymphoid Agents in Canine Renal and Liver Homotransplantation, and in Human Renal Homotransplantation. Surg. Gynec. Obstet., **124**:301, 1967.
19. Starzl, T. E., Brettschneider, L., Penn, F., Schmidt, R. W., Bell, P., Kashiwagi, N., Townsend, C. M. and Putnam, C. W.: A Trial with Heterologous Antilymphocyte Globulin in Man. Transpl. Proc., 1:448, 1969.
20. Terasaki, P. I., Vredevoe, D. L. and Mickey, M. R.: Serotyping for Homotransplantation. X: Survival of 196 Grafted Kidneys Subsequent of Typing. Transplantation, **5**:1057, 1967.
21. Waksman, B. H., Arbouys, S. and Arnason, B. G.: The Use of Specific "Lymphocyte Antisera" to Inhibit Hypersensitive Reactions of the Delayed Type. J. Exp. M., **114**: 997, 1961.
22. Weil, R., III and Simmons, R. L.: Combined Immunosuppression for Canine Renal Allograft Prolongation: Antilymphocyte Serum Plus Prednisone or Azathioprine. Ann. Surg., **167**:239, 1968.
23. Woodruff, M. F. A. and Anderson, N. F.: The Effect of Lymphocyte Depletion by Thoracic Duct Fistula and Administration of Antilymphocytic Serum on the Survival of Skin Homografts in Rats. Ann. N. Y. Acad. Sci., **120**:119, 1964.