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CURRENT CONCEPTS

Heterologous Antilymphocyte Globulin*

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THE immunosuppressive properties of heterologous antilymphocyte serum and its globulin derivative have been well known since 1961, when it was reported that a variety of immunologic responses could be weakened in guinea pigs by the administration of the antilymphocyte serum or its globulin derivative that had been raised in rabbits.[1] These included the marked suppression of delayed cutaneous hypersensitivity, a lesser effect on humoral-antibody formation and a relatively feeble mitigation of skin homograft rejection. Soon afterward, it was unequivocally demonstrated[2-5] that rabbit antilymphocyte serum had remarkable ability to prolong the viability of mouse or rat skin grafts even when they were transplanted across difficult histocompatibility barriers. Moreover, such an effect could often be obtained with less overt toxicity than with any previously known immunosuppressive agent.

As a consequence of these results, there was immediate interest in the possible clinical use of

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antilymphocyte globulin after human organ transplantation or for the treatment of various other immunologically directed pathologic states. It now seems clear that the globulin can be administered to man with both benefit and relative safety in spite of certain gaps in knowledge, which are alluded to below, but only under carefully controlled circumstances.

Preparation and Standardization

Some of the problems derive from the facts that there are neither standard routines for raising antilymphocyte serum nor fully accepted indirect methods for assaying the potency of the resulting product. Virtually all the aforementioned research on transplantation in rodents was done with antilymphocyte serum raised in rabbits. The lymphocytes obtained from the lymph nodes, thymuses or thoracic-duct lymph of the species to be immunized against were injected intravenously, subcutaneously or intradermally into the rabbits with or without Freund's adjuvant. The strength of individual batches of antiserum was usually assessed by test skin grafting. Little or no attention was paid to the levels of leukoagglutinins or lymphocytotoxins that the antilymphocyte serum contained, and when stated the titers of the antiwhite-cell antibodies were invariably low. For therapy, the serum was given intraperitoneally in volumes that, if extrapolated on a weight basis to adult human beings, would be 1 to 2 liters per individual dose.

Much of the work subsequently carried out in dogs was designed to surmount these practical difficulties. In our laboratories, the horse was selected as the heterologous serum source partly because of its large size and blood volume. Intensive subcutaneous immunization with spleen or lymph-node lymphocytes resulted in very high titers of antidog leukoagglutinins and lymphocytotoxins. When given subcutaneously in quantities that were only a fraction of those required for a comparable effect with low-titer antiserums, the resulting antilymphocyte serum or its globulin derivative prepared by ammonium sulfate precipitation of the horse serum, afforded protection to both canine kidney and liver homografts.

Nevertheless, the value of measuring antiwhite-cell antibodies to estimate the strength of antilymphocyte serum or its globulin derivative has been questioned by a number of authorities. In several laboratories, some high-titer antiserums have apparently had poor immunosuppressive qualities. The converse is not known to have been true in controlled animal experiments. At a symposium held at Davos, Switzerland, on March 25-28, 1968, Monaco, Woodruff and Lance, from the Harvard, Edinburgh and Mill Hill laboratories respectively, noted that their potent antiserums characteristically had high-grade lymphocytotoxicity. A potentially valuable method of directly relating antibody titers to the antirejection properties of antihuman-lymphocyte globulin is contained in a study[6] of the ability of such a globulin to prevent rejection of skin homografts in subhuman primates.

Antilymphocyte globulin for administration to human beings is not yet commercially available and has been locally produced only in centers using it clinically. In our own institution, cadaveric spleens are used as the source of lymphoid tissue, chiefly because of the ease of obtaining large enough quantities of antigen for equine immunization. At the same time, another horse is given comparable subcutaneous injections of dog-spleen lymphocytes. The antilymphocyte globulin is made from the blood of both horses in exactly the same way. The raw serum can invariably be shown with the appropriate serologic examination to require absorption with the red cells, platelets and plasma of the species against which immunization was conducted, to remove extraneous antibodies that can cause profound hemolysis, thrombocytopenia or other toxic manifestations. The globulin is then separated either by multiple ammonium sulfate precipitation or by a DEAE batch technic. The effectiveness of the antidog globulin is checked after canine renal homotransplantation before the companion human product is accepted. It is scarcely necessary to point out the deficiencies or possibly even the irrelevancy of the parallel immunization technic with two different donor species as a means of standardization.

It has been suggested that the quality of such antihuman globulin could be greatly improved by adjustments in the timing and route of immunization, by use of lymphocytes collected from the peripheral blood, lymph nodes, the thymus or the thoracic duct or by use of the cell membranes of the ruptured lymphocytes. The superiority of the antilymphocyte globulin with any of these deviations has yet to be established.

Guidelines of Therapy

Except for important experiments conducted with skin grafts in healthy volunteers[7] antilymphocyte globulin has not been used as the sole immunosuppressive agent after tissue and organ transplantation in human beings. There are good reasons for this.

The first is that antilymphocyte globulin does not always prevent rejection in inbred-mouse test systems involving skin homografts. Its effects are even less predictable in a given experiment after whole-organ transplantation between unrelated mongrel dogs. In the reported canine studies a minority of the recipients of vital organs lived for many months, about half had delay but not prevention of rejection, and a few rejected their kidneys or livers at the same time as would be expected in unmodified animals. The same kind of spectrum has been seen in the testing of other potent immunosuppressive agents such as azathioprine. Presumably, such variability is due to chance differences in the quality of

donor-recipient histocompatibility. Fortunately, antilymphocyte globulin has a synergistic action with adrenocortical steroids and several chemotherapeutic drugs including azathioprine.

A second consideration is the serious toxicity that can result from repeated foreign protein injections. Since antilymphocyte globulin is an immunosuppressant, it could be expected to reduce the host response to its own alien antigens. To some extent, this "self-antidotal" effect occurs, but it is not complete. Within a few weeks after institution of therapy in animals, immune elimination of the injected heterologous protein develops. Moreover, antibodies against the heterologous globulin can be demonstrated to develop by other kinds of immunologic tests. Serum-sickness nephritis or anaphylactic reactions have complicated the course of dogs being treated with either antilymphocyte serum or its globulin derivative, particularly after long-term therapy.

For both the foregoing reasons antilymphocyte globulin has been administered intramuscularly to our patients as an adjuvant immunosuppressive agent, and its use limited to the first four months after kidney or liver transplantation.[8] In the usual case the globulin was started three to five days before operation, continued daily for approximately two and a half weeks and then given every other day for two weeks, twice a week for two months and once a week for a final month. The individual doses were 1 to 5 ml, depending on the weight of the patient and the antiwhite-cell titer and protein content of the antilymphocyte globulin. Reduced doses of azathioprine and prednisone were also given, starting on the day of operation, and then continued after the course of antilymphocyte globulin had been terminated. The immunosuppression obtained from the combination of agents was expected not only to provide better control of rejection but also to decrease the danger from the injections of horse globulin.

There is no reason whatever to believe that the described schedule of therapy with this agent cannot be improved. For example, it has been suggested[7] that an appropriate way to give globulin therapy would be in "blitzkrieg" fashion, using very much larger doses but for a short period. The objective would be to deplete the long lived lymphocyte population thoroughly and acutely. This approach might result in a much more profound (and probably protracted) immunosuppressive effect, and it should reduce the chronic morbidity described below.

Effects of Therapy in Man

Adjuvant therapy with the four-month course of antilymphocyte globulin has been given to 86 patients who received renal or hepatic homografts at our institutions during the last 22 months; there has not been a drug-related mortality with more than 5000 injections. Before operation many of these recipients had positive skin tests to tuberculin, histoplasmin or other allergens. These became negative on retesting 48 to 72 hours after the institution of globulin therapy, indicating that the agent prevented expression of a previously established delayed hypersensitivity.

The evidence that antilymphocyte globulin was of value after clinical renal transplantation has been documented elsewhere.[9] In brief, mortality and homograft loss after intrafamilial homotransplantations were reduced to 5 per cent in the first year, the ultimate kidney function was superior to that in comparable past cases, and the quantities of azathioprine and prednisone necessary to achieve these results were reduced.

These advantages were not without corresponding penalties.[10] All of the patients had pain, which was often severe, at the site of the injections. Many had fever, urticaria, periorbital edema or skin rashes. In 15 per cent of the cases therapy had to be discontinued prematurely because of anaphylactic reactions. There was a rough correlation between the development of the anaphylactic reactions and the appearance in their serum of agglutinins against sheep red blood cells and antihorse-protein precipitins. The former probably measure the response to Forssman antigen in the equine protein. The latter antibodies were principally directed against the variable quantities of alpha and beta globulins that were in the antilymphocyte globulin produced by multiple ammonium sulfate precipitation. For the last several months, a pure gamma-G globulin prepared with a DEAE-cellulose batch technic has been used in patients. Rises in precipitin titers have not been seen, but elevations in hemagglutinin levels have been of the same magnitude as with the less refined antilymphocyte globulin originally used. Anaphylactic reactions have not yet occurred. However, the protection to the whole-organ homografts seem to have been considerably less than with the crude ALG originally used.

At the moment, it does not seem that the threat of either serum sickness or Masugi (direct nephrotoxic) nephritis will be a major deterrent to the clinical use of antilymphocyte globulin, at least in the context of the combination therapy that has already been tested. Eleven of 12 renal-homograft biopsies obtained from patients treated with this agent have not had horse protein detectable with immunofluorescence technics.

Future Prospects

Even now, it seems probable that heterologous antilymphocyte globulin will become a widely used immunosuppressive drug. With the clarification of standards for raising the antiserum and measuring its potency, many of the cumbersome control procedures and precautions mentioned earlier will no longer be necessary. Conceivably, the determination

of the optimum heterologous serum donor, the use of subcellular fragments as antigen and the application of technics for removal of only the desired immunoglobulin fractions could eliminate much of the risk and morbidity from the foreign-protein injections. As mentioned earlier, it would be surprising if better programs of administration were not devised. Under these circumstances, antilymphocyte globulin might eventually be used as the primary rather than as an adjuvant agent in clinical immunosuppressive regimens. At the present state of development, however, it must still be considered a drug that is undergoing experimental evaluation.

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