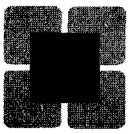

Prophylaxis and treatment of post-renal transplant rejection

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■ Antilymphocyte preparations are effective immunosuppressive agents for treatment of post-transplant rejection in renal transplantation. Polyclonal preparations have been used for more than 15 years, and more recently monoclonal antibodies have been employed. These agents prevent rejection when used prophylactically soon after renal transplantation and they effectively treat acute rejection episodes either as first-line therapy or for steroid-refractory rejection episodes. In the past, polyclonal antilymphocyte preparations were poorly reproducible, contained contaminating antibodies against normal blood cell constituents, and required administration of large doses through a central vein or an arteriovenous fistula. The monoclonal antibody preparation Orthoclone OKT3 has proven as effective as the polyclonal preparation ALG to prevent or treat acute rejection episodes in the early post-transplant period. Compared to polyclonal preparations, monoclonal preparations are preferable because of their uniformity, absence of contaminating antibodies, and ease of administration. The development of antibodies to mouse proteins in the recipient may limit the usefulness of monoclonal preparations when given for an extended period or in repeated courses.

□ INDEX TERMS: ANTILYMPHOCYTE PREPARATIONS; RENAL TRANSPLANTATION □ CLEVE CLIN J MED 1991; 58:125–130

SINCE THE early years of organ transplantation, antibody preparations to various lymphocyte populations have been used as adjunct immunosuppressive agents. Despite many clinical studies[1–7] that have assessed the efficacy of these agents, their effectiveness and exact immunosuppressive role in clinical transplant therapy remain controversial. Recently, a new agent, muromonab-CD3, better known as Orthoclone OKT3, has revived interest in antilymphocyte preparations and provided new information about their efficacy.

The renal transplant program at The Cleveland Clinic Foundation has employed antilymphocyte preparations for immunosuppressive therapy since 1977. Until the end of 1985, a polyclonal preparation obtained from the University of Minnesota (Minnesota ALG) was used. This antibody preparation was developed in horses inoculated with cultured human lymphoblasts.[1] Since 1986, the new monoclonal antibody Orthoclone OKT3 has been employed to treat certain rejection episodes. Its

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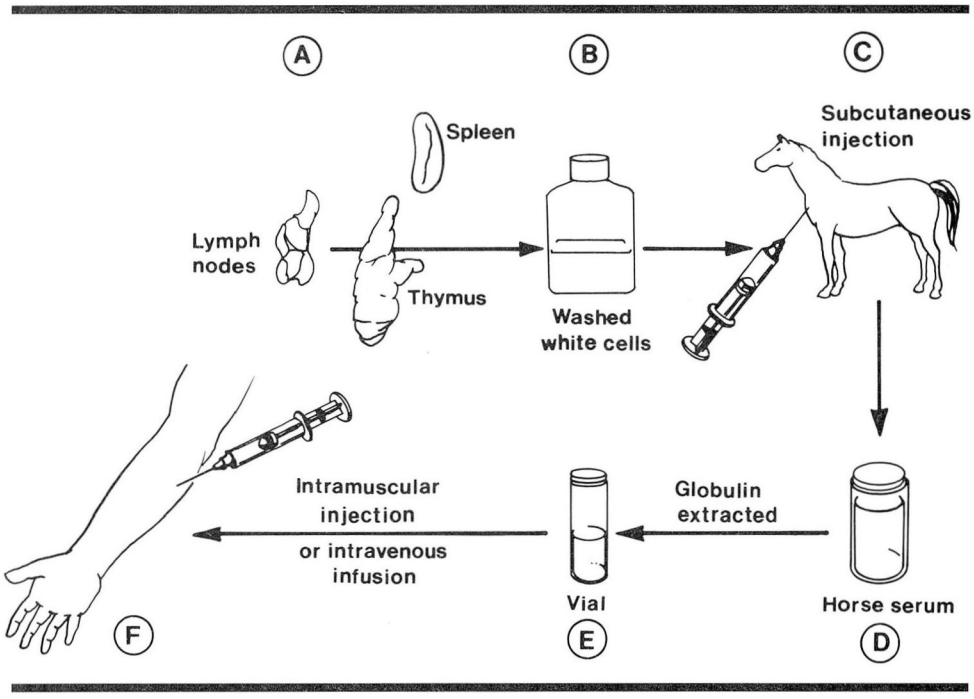

FIGURE 1. The making of a polyclonal antilymphocyte preparation. A. Lymphocytes, thymocytes, or lymphoblasts are isolated from the spleen, lymph nodes, thymus, or peripheral blood of humans. B. Cells are washed. C. Cells are inoculated into horses (sometimes rabbits or goats). D. Serum is collected from the immunized animal. E. A gamma globulin fraction is isolated from the serum. F. The gamma globulin fraction is administered to a patient.

preparation and pharmacologic characteristics have been well described.[8]

This article summarizes and compares clinical experience with the antilymphocyte preparations ALG and OKT3 in the early post-transplant period, both at the Cleveland Clinic and at other centers.

PRODUCTION TECHNIQUES FOR ANTIBODY PREPARATIONS

Polyclonal antilymphocyte preparations such as ALG are developed in animals (generally horses, rabbits, or goats) after a purified lymphocyte cell preparation has been injected into the animal (*Figure 1*). Various lymphocyte preparations have been isolated as the inoculating cell from the spleen, lymph node, thymus, or peripheral blood. For example, the Minnesota ALG preparation uses lymphoblasts isolated from the peripheral blood,[1] whereas an antilymphocytic preparation previously available from the Upjohn Company (ATGAM) used thymocytes.

After a routine series of immunizations, serum is collected from the immunized animal, and a gamma globulin fraction is then isolated from the serum. One of the limitations of polyclonal antibody preparations is that although the inoculating cells are purified, some contaminating peripheral blood material, such as red blood cells, polymorphonuclear leukocytes, or platelets, always remains. These contaminating cells may result in contaminating antibodies to antigens on the surface of cells in the final antibody preparation.

Monoclonal antibody preparation is an entirely different process from that employed for polyclonal antibodies (*Figure 2*). Mice are generally used to produce the antibodies, and various lymphocyte preparations are injected into the mice. Spleen cells are then hybridized with a myeloma cell line, resulting in perpetual cell culture material. Finally, the hybridized cells are isolated and cultured into separate wells in order to produce specific antibodies. For large-scale production, the cell material is cultured either in vitro or within special animals, generally mice.

This unique hybridization and selection process is responsible for the distinguishing characteristics of monoclonal antibodies compared with polyclonal preparations. OKT3, the only monoclonal antibody approved by the Food and Drug Administration (FDA), is a murine (mouse) antibody directed against the CD_3 (T_3) antigen on the surface of all T cells.

CLINICAL ADVANTAGES AND DISADVANTAGES

Because of their varying production techniques, polyclonal and monoclonal preparations have distinctly different pharmacologic characteristics (*Table 1*). Polyclonal preparations have contaminating antibodies to leukocytes, red blood cells, or platelets that were present in the inoculating cell preparation. As a

result, patients treated with polyclonal preparations often have significant cellular reactions that require reductions in dosages to prevent clinically significant complications.

Platelet antibodies and thrombocytopenia are the most common problems seen, but leukopenia and Coombs' positive hemolytic anemia may also occur. In a study[2] at the Cleveland Clinic in which polyclonal ALG was compared with a placebo control during the early post-transplant period, ALG-treated patients had a significantly ($P < .001$) increased risk of leukopenia and thrombocytopenia (*Table 2*). By contrast, monoclonal antibody preparations contain no contaminating white blood cell, platelet, or red blood cell antibodies because these preparations have been selected to identify a single cell line that produces a single, uniform antibody. Thus, complications such as leukopenia or thrombocytopenia do not occur.

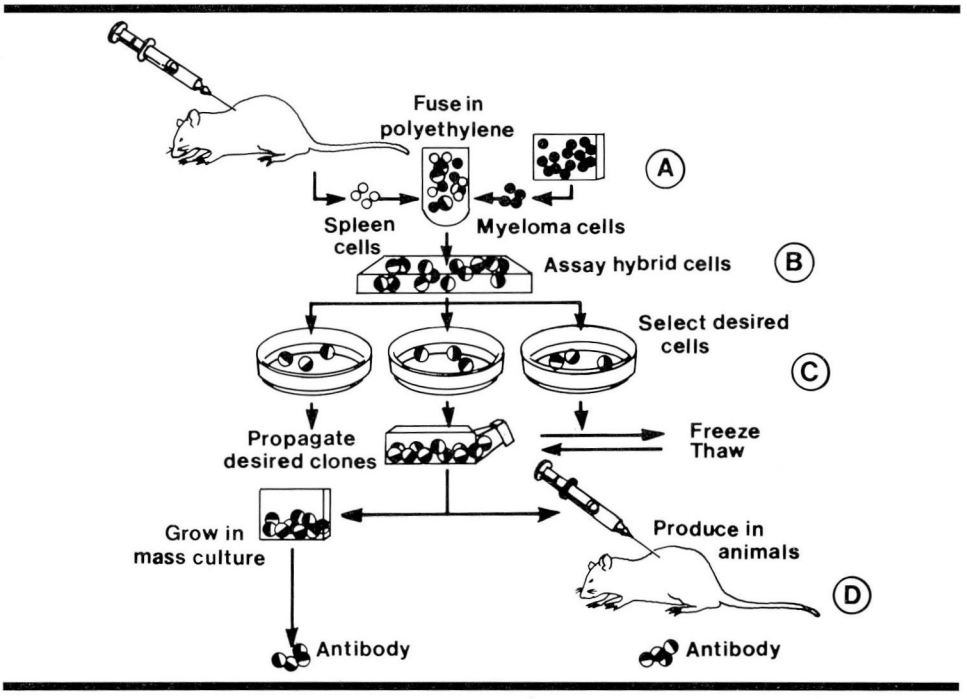

FIGURE 2. The making of a monoclonal antibody. A. Spleen cells from immunized animals are fused with myeloma cells to create a hybridoma. B. Individual cells are assayed for their specific activity. C. The cells that make the desired antibody are selected. D. These cells are then cloned in culture or within ascitic fluid in mice.

Because the monoclonal antibody is produced from a perpetual cell line and is a single antibody to a defined antigen component of the lymphocyte, it is reproducible, uniform, and extremely potent. These features of monoclonal antibodies, and of OKT3 in particular, give them important advantages over polyclonal preparations. Most polyclonal antibodies have had problems with batch-to-batch variability of potency and with side effects caused by variations in the amount of therapeutic and contaminating antibodies. For these reasons, it has been difficult to obtain FDA approval for polyclonal preparations, and they have not been widely available.

CLINICAL USES OF ANTILYMPHOCYTE PREPARATIONS

Prophylaxis of rejection

One of the initial therapeutic uses of polyclonal ALG was as prophylactic therapy for acute cellular rejection early post-renal transplant. Several studies[2–4] reported that administration of polyclonal antibodies not only delayed rejection episodes during the early post-transplant period, but it also prevented rejection in some patients and improved overall 1-year graft survival. These benefits were confirmed by a randomized, prospective, placebo-controlled, double-blind study performed at the Cleveland Clinic.[2] In this study, the onset of rejection was delayed (*Figure 3*), and very few rejection episodes were observed during the 2-week course of ALG administration. After ALG was discontinued, rejection episodes occurred, but, overall, the patients treated with prophylactic ALG had significantly ($P < .001$) fewer rejection episodes than the placebo group. Graft survival was also improved at 1 year in the active-treatment group; 78% of ALG grafts were functioning at 1 year compared with 48% of placebo grafts (*Figure 4*). Study patients were maintained on prednisone and azathioprine immunosuppressive therapy and were not treated with cyclosporine.

Orthoclone OKT3 has also been used as prophylaxis (without cyclosporine) in a therapeutic trial in

TABLE 1
MONOCLONAL *v* POLYCLONAL ANTIBODY PREPARATIONS

Monoclonal antibodies	Polyclonal antibodies
Homogeneous	Heterogeneous
Consistent, measurable reactivity	Batch-to-batch variability
Predictable reactions	Variable reactions
Smaller doses required	Larger doses required
Peripheral IV administration	Central vein or AV fistula administration

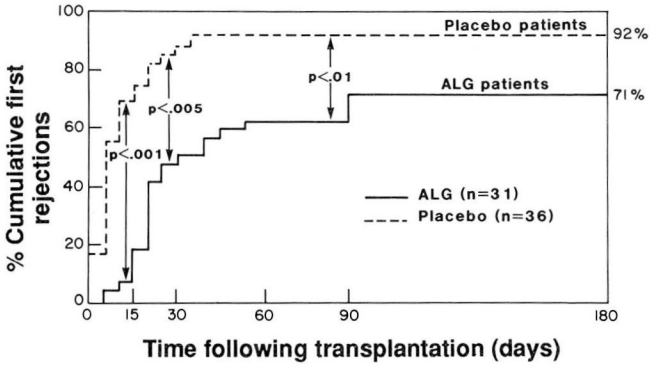

FIGURE 3. First rejection episodes in antilymphocyte (ALG) *v* placebo groups. From Novick et al.[2]

TABLE 2
ADVERSE EFFECTS DURING TREATMENT WITH ALG OR PLACEBO*

Effect	ALG (n = 31) n	(%)	Placebo (n = 36) n	(%)
Allergic reactions				
Fever	11	(35.5)	8	(22.2)
Chills	3	(9.7)	5	(13.9)
Diarrhea	2	(6.5)	0	
Rash	2	(6.5)	0	
Pruritus	1	(3.2)	0	
Leukopenia† (WBC <4000)	21	(67.7)	4	(11.1)
Thrombocytopenia* (Platelets <100,000)	21	(67.7)	2	(5.6)

*From Novick.[2]
†$p < .001$

France.[9,10] During the first month after transplantation, rejection episodes were significantly reduced with OKT3 therapy, from 1.1 episodes per patient to 0.32 episodes per patient (*Table 3*). Furthermore, in the absence of the development of anti-mouse antibodies that may eliminate the drug from the blood and thus limit its therapeutic effect, the prophylactic administration of OKT3 was also found to be effective in preventing rejection during the administration period. A large multicenter, prospective, controlled study is currently under way to assess the efficacy of OKT3 prophylaxis in the early post-transplant period.

The advantage of using prophylactic antilymphocyte preparations in the early post-transplant period is that renal function is allowed to recover from the ischemic injury of the transplant procedure. If, for example, cyclosporine administration is delayed until such recovery has occurred, the deleterious effects of cyclosporine nephrotoxicity may be minimized, while still preventing damage to the graft that might be caused by early rejection.

From these preliminary data it appears that monoclonal OKT3 and polyclonal ALG preparations are equally effective in preventing rejection episodes. The development of anti-mouse antibodies in the recipients of OKT3, however, has been a source of concern[11]; in patients who develop antibodies to the mouse protein, the therapeutic antibody may be eliminated from the circulation, thereby offsetting its efficacy. The risk of anti-mouse antibody production may be effectively eliminated by administering immunosuppressive agents (especially azathioprine or cyclosporine, alone or in combination) concomitantly with OKT3.[12]

Antibodies to horse proteins (or whatever animal protein is used) have also occurred after polyclonal ALG preparations have been administered, although the efficacy of subsequent courses of therapy has been maintained.[5] This difference between polyclonal and monoclonal preparations may be due to the much greater amount of polyclonal antibody administered. In addition, the large amount of nonspecific antibody present may serve either to desensitize the patient or to bind the anti-horse antibodies so that the therapeutic polyclonal antibodies maintain their efficacy. However, systematic studies to assess the clinical and therapeutic effects of such antibody production in recipients of polyclonal ALG preparations have not been performed.

Treatment of acute rejection episodes

Polyclonal antilymphocyte preparations have been shown to be effective as the primary treatment of acute cellular rejection[6,7] and as treatment of steroid-resistant rejection episodes.[13] In a small trial at the

TABLE 3
PROPHYLAXIS WITH OKT3 WITHIN 1 MONTH AFTER RENAL TRANSPLANTATION*

Treatment	Patients	Patients with rejection episodes	Mean rejection episodes/patient
OKT3	15	2	0.32
Control			
Low-dose steroid	18	18	1.11
High-dose steroid	19	11	–

*From Kreis.[10]

TABLE 4
EFFICACY OF OKT3 *v* ALG FOR STEROID-RESISTANT REJECTION EPISODES

	OKT3 N = 21 (%)	ALG N = 12 (%)
Rejections reversed	15 (72)	9 (75)
Subsequent rejections	5 (24)	5 (56)
Graft survival	13 (62)	7 (58)
Major infections	12 (57)	5 (42)
Deaths	3 (14)	1 (8)

Cleveland Clinic,[5] monotherapy with polyclonal ALG was almost universally effective in reversing first acute rejection episodes in renal transplant patients maintained on azathioprine and prednisone. Of 17 ALG patients, 100% experienced reversal of their first acute rejection episodes, as compared with 93% of 27 intravenous methylprednisolone (IVMP) patients; subsequent rejection episodes occurred in 35% of ALG patients and 48% of IVMP patients. Prophylactic administration of ALG for 2 weeks after transplantation may have accounted for the high percentage of patients who responded to steroid therapy for their initial rejection episode.

In the first large multicenter trial[14] in which OKT3 was compared with high-dose steroid therapy to treat acute rejection in patients maintained with azathioprine and prednisone immunosuppressive therapy, no antilymphocyte preparations were used prophylactically. A significantly greater number of patients responded to OKT3 therapy than those who were treated with high-dose steroids: 94% (58/62) *v* 75% (45/60), respectively.[14] From these studies it appears that polyclonal antilymphocyte preparations and OKT3 are equally effective (and more effective than high-dose corticosteroids) in reversing nearly 100% of early acute rejection episodes.

Treatment of steroid-resistant rejection

Patients with steroid-resistant rejection are more difficult to treat, but they may also respond to polyclonal ALG or to monoclonal OKT3 therapy. Initial trials with OKT3[15] showed a 65% reversal of steroid- or ALG-resistant rejection episodes, and similar results have been reported with polyclonal antilymphocyte preparations.[7]

Since cyclosporine has been available as maintenance immunosuppressive therapy at the Cleveland Clinic, both polyclonal Minnesota ALG and Orthoclone OKT3 have been used to treat steroid-resistant rejection episodes. The efficacy of both antibody preparations was found to be similar (*Table 4*). However, the incidence of infectious complications in both groups was significant, although this finding probably represents the cumulative effects of immunosuppressive therapy in this refractory group of patients. All patients received polyclonal ALG prophylactically after transplantation, maintenance immunosuppression with cyclosporine after ALG was discontinued, maintenance therapy with a low dosage of corticosteroids and azathioprine, and intravenous methylprednisolone as initial therapy for the refractory rejection episode.

As with any potent immunosuppressive therapy, both polyclonal and monoclonal antibody preparations carry the risk of serious infectious complications, especially when combined with other potent immunosuppressive agents.

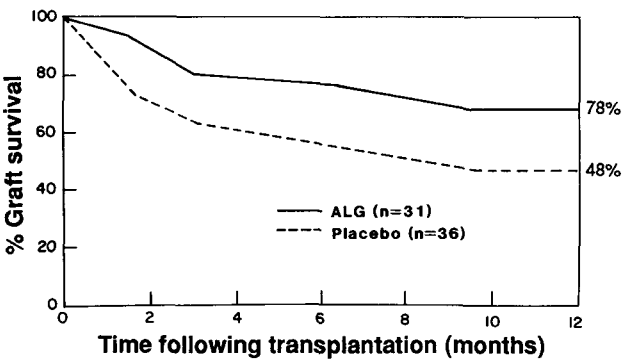

FIGURE 4. Allograft survival in antilymphocyte (ALG) *v* placebo groups by 12 months post-transplantation. From Novick et al.[2]

CLINICAL RECOMMENDATIONS

Antilymphocyte antibody preparations are attractive immunosuppressive agents because they attack the cell primarily responsible for acute cellular rejection in solid organ transplants. The monoclonal antibody Orthoclone OKT3 has been proven to be as effective as the polyclonal preparation ALG to prevent or treat acute rejection episodes in the early post-transplant period in renal transplant patients. Although the development of anti-mouse antibodies in the recipient may limit the number of times this monoclonal antibody can be effectively administered, techniques to prevent the development of these antibodies, or to inactivate them, may enable the preparation to be used repeatedly.[12,16,17] The specific activity of the OKT3 antibody against the CD_3 (T_3) antigen makes this drug very effective for the T cell-mediated immune response. Antibodies in the polyclonal preparation against undetermined antigens on other cells (such as B cells) might improve its therapeutic efficacy compared with a monoclonal preparation under certain clinical circumstances, although it remains to be determined whether such clinical circumstances exist.

At present, OKT3 and polyclonal ALG preparations are the most effective treatments of acute rejection available. OKT3 is probably the treatment of choice when corticosteroids have been or are anticipated to be therapeutically ineffective because of its unique characteristics—uniformity of the preparation, lack of contaminating antibodies, and peripheral intravenous administration. Both Orthoclone OKT3 and polyclonal ALG preparations are useful for prophylaxis of rejection early post-transplant.

REFERENCES

1. Najarian JS, Simmons RL, Condie RM, et al. Seven years' experience with antilymphoblast globulin for renal transplantation from cadaver donors. Ann Surg 1976; **184:**352.
2. Novick AC, Braun WE, Steinmuller DR, Buszta C, Greenstreet R, Kiser W. A controlled randomized double-blind study of antilymphoblast globulin in cadaver renal transplantation. Transplantation 1983; **35:**175–179.
3. Wechter WJ, Brodie JA, Morrell RM, Rafi M, Schultz JR. Antithymocyte globulin (ATGAM) in renal allograft recipients. Transplantation 1979; **28:**294–302.
4. Howard RJ, Condie RM, Sutherland DER, Simmons RL, Najarian JS. The effect of serum ALG concentrations on results following renal transplantation. Ann Surg 1977; **186:**619–624.
5. Novick AC, Knauli RB, Braun WE, et al. Improved results of cadaver renal transplantation with azathioprine, prednisone, and antilymphoblast globulin. J Urol 1984; **131:**636–640.
6. Filo RS, Smith EJ, Leapman SB. Therapy of acute cadaveric renal allograft rejection with adjunctive antithymocyte globulin. Transplantation 1980; **30:**445–449.
7. Shields CF, Cosimi AB, Tolkoff-Rubin N, Rubin RH, Herrin J, Russel PS. Use of antithymocyte globulin for reversal of acute allograft rejection. Transplantation 1979; **28:**461–464.
8. Hung PC, Goldstein G, Reinherz EL, Schlossman SF. Monoclonal antibodies defining distinctive human T cell surface antigens. Science 1979; **206:**347–349.
9. Kreis H, Chkoff N, Chatenoud L, Campos H, Bach JR, Goldstein G. Prolonged administration of a monoclonal anti-T3 cell antibody (Orthoclone OKT3) to kidney allograft recipients. Transplant Proc 1986; **18:**954–956.
10. Kreis H, Chkoff N, Chatenoud L, Campos H, Bach JR, Goldstein G. Prolonged administration of a monoclonal anti-T3 cell antibody (Orthoclone OKT3) to kidney allograft recipients. Transplant Proc 1985; **17:**2734–2736.
11. Jaffers GJ, Colvin RB, Cosimi AB, et al. The human immune response to murine OKT3 monoclonal antibody. Transplant Proc 1983; **15:**646–648.
12. Hricik DE, Zarconi J, Schulak JA. Influence of low-dose cyclosporine on the outcome of treatment with OKT3 for acute renal allograft rejection. Transplantation 1989; **47:**272–277.
13. Streem SB, Novick AC, Braun WE, Steinmuller DR. Antilymphoblast globulin for treatment of acute renal allograft rejection. Transplant Proc 1983; **15:**590–594.
14. Ortho Multicenter Transplant Study Group. A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. N Engl J Med 1985; **313:**337–342.
15. Goldstein G. An overview of Orthoclone OKT3. Transplant Proc 1986; **18:**927–930.
16. Goldstein G, Fucello AJ, Norman DJ, et al. OKT3 monoclonal antibody plasma levels during therapy and the subsequent development of host antibodies to OKT3. Transplantation 1986; **42:**507.
17. Thistlethwaite JR, Cosimi AB, Delmonico FL, et al. Evolving use of OKT3 monoclonal antibody for treatment of renal allograft rejection. Transplantation 1984; **38:**695.