ANTILYMPHOCYTE AND ANTITHYMOCYTE GLOBULIN

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Antilymphocyte globulin (ALG) and antithymocyte globulin (ATG) are purified, concentrated and sterile gammaglobulin, primarily monomeric IgG, prepared from hyperimmune serum of horses, rabbits and goats immunized with human thymus lymphocytes.

The potential usefulness of ALG prior to allogenic marrow infusion in treating aplastic anemia (AA) was first reported in 1970(1). Subsequently, several investigators have evaluated the therapeutic modalities of ALG/ATG to prevent graft rejection in organ transplant, skin grafting and treatment of bone marrow hypoplasia with varying success(2-6).

At present, it is by far the best available treatment for patients with severe aplastic anemia (SAA), who are not eligible for bone marrow transplantation (BMT), and is considered the first therapeutic option in patients of AA over 25 years(7,8). Clinical trials of this drug in India(6) and experience with children are very limited(9). With better availability, the drug is now being increasingly used.

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Mechanism of Action

Exact mechanism of action is not fully understood, but seems to be related to the abrogation of the number of function of some T cell subsets. Although immune suppression is the most likely mechanism of action, direct stimulation of hematopoietic stem cells cannot be excluded. Activation of accessary cells with the consecutive release of hematopoietic growth factors and an immune stimulatory action, could also be a possible mechanism(7,8,10).

Preparations

There is no Indian preparation of ALG/ATG. The various available preparations of antilymphocytic agents are summarized in *Table I*(10,11). They should be stored in the refrigerator at 2°C to 8°C (not to be frozen). The specific activity of Mexican ATG has been claimed to be at least 40 times greater than that of ATG of Upjohn Co (USA) and 10 times greater than that of Institute Merieux (France)(10).

Therapeutic Uses and Dosage

1. *As prophylactic immunosuppressant agent to prevent graft rejection:* ATG significantly, delays the onset of acute rejection and also reduces the need for high dose steroid therapy in the early post transplant period(2,4), when used with other immunosuppressive drugs.

TABLE I–*Various Antilymphocytic Agents*

	Manufacturer	Serum	Cells for immunization	Rosette inhibition titres
1.	ALG Behring Works, Germany	Horse	Cultured lymphoblasts	>1: 2000
2.	ATG Institute Merieux, France	Horse	Thymocyte and t.d. lymphocytes	>1: 2400
3.	ATG Upjohn Company, USA	Horse	Thymocytes	>1: 8000
4.	ATG Behring Works, Germany	Rabbit	Thymocytes	?
5.	ATG Institute Public Health, Netherlands	Rabbit	Thymocytes	1: 6000
6.	ATG Fresenius, Germany	Rabbit	Thymocytes	?
7.	ATG Mexican Society of Hematology, Mexico	Horse	–	1: 160,000

ATG is used in dose of 15 mg/kg daily for 14 days followed by 15 mg/kg every alternate day for another 14 days, thus giving a total of 21 doses in 28 days, administering the first dose within 24 hours before or after the transplant(9).

2. *In treatment for acute graft rejection:* When ATG is added at the time of diagnosis of initial rejection of episode, rather than prophylactic use, it has been found to be effective in reversing the rejection (3,5, 12-14).

ATG is started after the diagnosis of the first rejection episode, in dose of 10-15 mg/kg for 14 days. Additional alternate day therapy upto a total of 21 doses can be given(9).

3. *To prolong skin allograft survival:* When patients suffering from full thickness burns (70% or greater of the body surface), are treated by primary excision of the full thickness burn and complete immediate coverage with skin allografts, the allograft survival is prolonged by ATG suppression(4, 5).

4. *In treatment of bone marrow aplasia*

(A) *Red cell aplasia:* In a study of red cell aplasia, which were unresponsive to thymectomy, high dose steroids, cyclophosphamide and oxymetholone therapy, treatment with ATG without any other immunosuppressive agent except low dose prednisolone, showed dramatic hematologic response within 7 days and a normal hemogram resulted with no further therapy. It is able to maintain it during the succeeding 18 months with subsequent addition of cyclophosphamide 100 mg/day, a dose, interestingly even lower than that which was ineffective in producing a remission prior to ATG therapy. Treatment with ATG is usually given for 21 days(5).

(B) *Aplastic anemia:* This condition although rare, is often fatal. Although about 20% of all patients recover spontaneously(15), the various other modes of

therapy, *e.g.,* steroids, androgens, *etc.* have not proved much useful(16,17).

By now, the best treatment for patients of SAA rests upon bone marrow transplantation (BMT) and/or ATG infusion(7). The response and survival of patients with SAA, treated with BMT and ATG, BMT alone or ATG alone dose not seem to be very different(8,18-21). Varying dosage schedule of ATG has been recommended(9,16,21-23).

(*i*) *Using ATGAM* (Upjohn-USA)
(*a*) 20 mg/kg/day IV for 5 days, after premedication with antihistaminics(7).
(*b*) 10-20 mg/kg/day for 8-14 days with/without additional alternate day therapy upto a total of 21 doses.
(*ii*) *Using Mexican ATG*(10)
1-5 mg/kg/day for 5 days.

The clinical response has ranged from 14(24) to 85%(25). Responders to ATG therapy show a gradual improvement of hemopoiesis within the first 3 months, however, in majority of them blood counts do not return to normal. In some patients recovery may occur as late as 8-9 months after ATG. These late recoverers and lack of clear criteria to define response renders an inter study comparison very difficult. *Table II* depicts the criteria used by investigators(10,26) to label response to ATG therapy. Patients with improved peripheral counts are followed without further therapy. Long term follow-up is necessary because a minority of these responding patients have recurrence of their aplasia and require retreatment with a second or even a third course of ATG. However, a second course of ATG is usually not recommended for those patients who fail their first course. Long term survival of 50 to 75% can be achieved with ATG therapy. Results are best seen in cases, when treatment is given within 4 months of diagnosis(27,28).

TABLE II—*Criterias for Response to ATG Therapy*

	Category	Reticulocyte	Neutrophils	Platelets
A.	*Complete response* Increase without transfusion	$>20\times10^9$/L	$>1.5\times10^9$/L	$>150\times10^9$/L
B.	*Partial response* Increase in atleast two of these without transfusion	$>20\times10^9$/L	$>0.5\times10^9$/L	$>30\times10^9$/L
C.	*Improvement* Increase in the presence of transfusion in atleast 2 of these	$>20\times10^9$/L	$>0.5\times10^9$/L	$>10\times10^9$/L
D.	Failure	Any response not included in the above categories.		

Administration

Most of the clinical trials till date have used ATGAM (Upjohn, USA).

Skin testing: Before the first infusion of ATGAM, it is recommended that patients be tested with an intradermal injection of freshly prepared 0.1 ml of 1 : 1000 dilution (5 μg/horse IgG) in sodium chloride and a contralateral sodium chloride control. The patient and the skin test should be observed every 15-20 minutes, over the first hour after intradermal injection. A local reaction of 10 mm or greater with a wheal or erythema or both with or without pseudopod formation and itching should be considered a positive test. In such patients an alternative form of therapy should be considered. However, the predictive value of this skin test has not been proved clinically, thus therapy may still be considered on the basis of risk benefit ratio with adequate precautions(9).

Side Effects and Precautions

The side effects of xenogeneric sera can be severe, the various side effects using Upjohn ATGAM in one of the study(5) after administering 2,500 doses in 135 patients were chills and fever (15-20%), erythema/pruritis (18%), thrombocytopenia (10%), local phlebitis (2%), and serum sickness (1.5%). Long term hematological complications, include paroxysmal nocturnal hematuria, myelodysplasia, acute nonmyelogenous leukemia, or recurrent aplasia(8). *Table III* enumerates the various side effects, their occurrence and their treatment.

Dilution: ATGAM should always be diluted in sodium chloride solution. Dilution in dextrose injection is not recommended as low salt concentration may cause precipitation. While diluting ATGAM, bottle of sodium chloride is inverted before instilling the drug inside, so that the undiluted ATGAM does not contact the air inside. Diluted or undiluted ATGAM should not be shaken as excessive foaming and/or denaturation of protein may occur. Diluted solution should be gently rotated or swirled to effect thorough mixing prior to use. Concentration of ATGAM should not exceed 1 mg of ATGAM/m1 of sodium chloride solution. The diluted solution should not be kept for more than 12 hours, and a dose of ATGAM should not be infused in less than 4 hours(9).

Adjuvant Therapy

Azothiaprine and prednisolone or methyl prednisolone (MPN) have been used as adjuvant therapy with ALG in treating graft rejection during organ transplantation(5). Prednisolone 40 mg/m^2 has been given two days before starting of ATG and after completing 5 days schedule was tapered off during a two week period in treating AA(10).

Recently, ALG is being combined with high dose MPN in treating AA with the belief that the potential side effects of the steroids such as increased risk of infection, hyperglycemia and hypertension are more than offset by the improved tolerance of ATG infusion and decreased need for platelet transfusions. Furthermore, there are some indications that the combined therapy may result in faster bone marrow recovery than with ATG alone. MPN (Solumedrol) is administered 20 mg/kg/day IV for 3 days. Subsequently, prednisolone 2.5 mg/kg/day orally is given for 3 days, with halving the dose every 4th day.

TABLE III—*Side Effect of ATG Therapy*

	Side effects	Cause/Time of occurrence	Treatment
1.	Anaphylaxis	Though uncommon but serious & can occur at any time during therapy	Stop ALG/ATG infusion immediately Use of epinephrine, steroids & resuscitative measures Therapy should not be resumed
2.	Hemolysis	Any time	Erythrocyte transfusion/ IV mannitol, frusemide, sodium bicarbonate and IV fluids If severe and unremitting may require discontinuation of therapy
3.	Thrombocytopenia	Sequestration of platelets in the liver	Platelet transfusion
4.	Respiratory distress	Indication of anaphylactoid reaction	Discontinuities of infusion Administration of antihistaminics, epinephrine and/or corticosteroids
5.	Pain in chest, flank or back	May be an indication of anaphylaxis or hemolysis	Treatment of cause
6.	Hypotension	May indicate anaphylaxis	Stop infusion and stabilize BP
7.	Chills and fever	Probably due to release of endogenous leucocyte pyrogenes	Prophylactic and/or therapeutic administration of antihistaminics, antipyretics or corticosteroids
8.	Chemical phlebitis	Infusion in peripheral vein	Avoided by administering the infusion solution into a high flow vein. A subcutaneous arterialized vein produced by a Brescia fistula is also a useful site.
9.	Itching and erythema	Effect of ATG/ALG on blood elements	Antihistaminics
10.	Serum sickness like symptoms	May develop after about 10 days of start of ATG therapy, leads to severe arthralgia, vasculitis and fever.	Oral prednisolone or methyl prednisolone prophylactically

This way serum sickness becomes very rare because, the patient still has sufficient steroid therapy around that time to suppress the symptoms nearly completely(7). However, high dose corticosteroids have not been uniformly shown to have beneficial effects and methyl prednisolone in low dose (0.5-5 mg/kg/day IV) for 10 days is also considered equally effective(6,27).

BMT vs ALG in Aplastic Anemia

The cure rate after ALG therapy has been reported to be 40-70% as compared to 80% with BMT. However BMT is very costly and there is also a risk of acute and chornic graft versus host disease (GVHD). Moreover, it requires prolonged hospitalization and the results become poorer with increasing age. Thus ALG therapy is the first therapeutic option in older patients with aplastic anemia.

Cost

One vial of ATGAM (250 mg) costs about four thousand rupees presently. Though this cost is lower than that of BMT, it is still quite high and may not be affordable by many patients. In this regard Mexican Society of Hematology through its Mexican Co-operative group for the Study of Erythropoiesis and Hemopoiesis has started a programme to produce a batch of equine ATG which is low dose and high potency. Similar efforts are highly desirable. A standard protocol with dose of ATG, adjuvant therapy, duration of treatment, criteria for labelling responders with proper control is also required for international comparison.

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