

## Polyclonal Anti-T Cell Antibodies in Renal Transplantation

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Polyclonal anti-T cell antibodies or antilymphocyte serum (ALS) are xenogeneic antihuman polyclonal antibodies, more precisely antithymocyte globulins (ATG). They are prepared by immunizing rabbits or horses with human lymphoid cells derived from the thymus. They were first prepared for clinical use in horses (1) and followed by a more potent preparation in rabbits. Despite some problems of using polyclonal ATG, they are used in organ transplantation for induction and particularly to reverse acute rejection.

### Mechanism of ATG immunosuppressive activity

The ATG bind to a variety of lymphocyte cell surface receptors inducing profound depletion of T- and/or B- cells. Lymphocyte depletion may result from complement-dependent and Fc-dependent opsonization and lysis or activation associated apoptosis. Activated T-cells may undergo Fas-mediated apoptosis. (2,3) Furthermore T-cells escaping depletion in vivo are hyporesponsive in mixed lymphocyte reactions possibly associated with down regulation of their surface receptors targeted by ATG, such as TCR/CD3, CD4, CD8, CD2, CD5, CD7 etc. Lymphocyte depletion is dose dependent. Within 24 hrs of administration, peripheral blood lymphocyte drop below 100 to 200  $\text{mm}^3$ . ATG have a narrow therapeutic window between 50 to 150 cells/  $\text{mm}^3$ . The therapeutic effect is achieved when T-cells count is reduced to below 150 cells/  $\text{mm}^3$  while reduction to no more than 50 cells/  $\text{mm}^3$  is accompanied by adverse effects associated with severe immunosuppression. In non human primates rabbit ATG was shown to induce a dose dependent T-cell depletion not only in peripheral blood, but also in peripheral lymph nodes and spleen at a short course high dose equivalent to 3mg/kg in the human. At these high doses there was induced a decrease of B-cells and Natural Killer lymphocytes (NK) counts in peripheral lymphoid organs, along with a transient decrease in blood neutrophil counts (2) In renal transplant recipients a short-course, 3-day treatment with rabbit ATG at high dose, 3mg/kg/d, followed by 1.5 mg/kg/d for 2 days, induced on even more profound lymphopenia than a 7-day course of 1.5mg/kg/d (4). In very recent experiments (5) the mechanisms of action of a rabbit ATG extend beyond lymphocyte depletion to include leukocyte antigen modulation, inhibition of leukocyte-chemotaxis and inhibition of leukocyte tracking. Rabbit ATG may interfere with the main leukocyte surface molecules involved in chemotactic signals but mainly inhibit the expression of integrins required for firm cellular adhesion. Rabbit ATG contain functional antibodies to  $\text{b}_2$ ,  $\text{b}_1$ , and  $\text{b}_7$  integrins, to the three  $\text{b}_2$  integrin LFA-1 ligands ICAM-1, ICAM-2 and

ICAM-3 and to the chemokine receptors CCR5, CCR7 and CXCR4 (4).

$\text{b}_1$  and  $\text{b}_7$  integrins bind to VCAM-1 on endothelium and to the mucosal address Mad CAM-1 respectively (6,7).

Experiments with ATG monotherapy in cynomolgus monkey demonstrate that ATG-coated lymphocytes did not migrate to the spleen or lymph nodes (2).

Antibodies, anti- $\text{b}_1$  integrin, that interfere with leukocyte-endothelium interactions may contribute to the rapid reversal of steroid-resistant acute the rapid rejection by ATG treatment.

In addition, interference with leukocyte adhesion to the endothelium and leukocyte tracking might account for the decreases incidence of acute rejection, graft loss and delayed graft function, when used for induction therapy, when the first infusion is initiated intraoperatively before reperfusion of the allograft (8).

T-cell depletion by ATG at the time of reperfusion may further decrease ischemic injury and acute renal failure in view of the important role of CD4+ cells in such processes (9).

### ATG for induction treatment

Induction therapy with ATG, mycophenolate mofetil or azathioprine, calcineurin inhibitors, cyclosporine or tacrolimus, and corticosteroids, was popular between 1985 and 1995, but has widely been abandoned due to its toxicity and high cost.

The use of ATG for induction therapy initiated intraoperatively or in the immediate postoperative period targeted to prevent acute rejection was based upon the belief that accelerated or early acute rejection episodes have more pernicious outcome than the late ones. A second reason for the use of ATG in induction therapy was to avoid calcineurin inhibitors during the first post transplantation days, because their potent nephrotoxicity may delay more the recovery of the allograft from the ischemia reperfusion events. The use of more moderate calcineurin inhibitors dosage regimens has diminished the problem of nephrotoxicity. In addition to the T-and/or B-cells and NK cells depletion induced by ATG, the recent evidence of additional mechanisms for prevention of acute rejection and ischemia reperfusion injury, renewed the use of ATG for induction therapy targeting in reducing the immunosuppression (10). ATG dose for induction treatment is 1-1.5mg/kg/day for 2 to 9 days post renal transplantation. The first dose is more preferable to be given during 24 hrs before operation.

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**ATG for acute rejection treatment**

ATG are mainly used for the treatment of acute rejection and mostly as rescue therapy of corticosteroid resistant acute rejection (8,11). The efficacy of ATG in reversing acute rejection varies according to the animal source of ATG. A direct comparison between a rabbit and a horse ATG was performed in a multicenter, double blind, randomized trial in which 163 renal transplant recipients with acute rejection were treated. Rabbit ATG compared to horse ATG, resulted in a higher rate of reversal of acute rejection (88 vs 76%,  $p=0.027$ ) and a lower rate of recurrent rejection at 90 days after ATG treatment (17 vs 36%,  $p=0.011$ ) (12).

During ATG administration the dose of conventional immunotherapeutic agents, cyclosporine, azathioprine, and mycophenolate mofetil are reduced by 50% to decrease the total immunosuppression. Cyclosporine or tacrolimus should be restarted, using the prerejection dose, three days before stopping ATG, while azathioprine and mycophenolate mofetil, should be started in prerejection doses after stopping ATG.

ATG dose for acute rejection treatment is 1.5mg/kg/ day for 3 to 14 days.

**Adverse effects: Prevention**

The majority of pts reveal fever and chills during the initial ATG infusion. In order to minimize this allergic reaction to animal antibodies, a cocktail of corticosteroids, antihistamines and antipyretics is administered prior to ATG infusion.

Anaphylactic reactions are rare. Late in the course, a pruritic rash (20%) and a presumed antiplatelet antibody induced thrombocytopenia (50%) can occur. Cytomegalovirus and Herpes infection can also occur, but usually are mild and rarely life threatening due to the simultaneously administered prophylactic antiviral therapy. ATG do not induce host antibody response to the rabbit or horse globulins. So ATG can be readministered successfully and safely and can be used instead of OKT3. In patients previously treated with OKT3 with high titers of antimouse antibodies (12). ATG can cause phlebitis and should be administered via a central venous access.

ATG increase the incidence and severity of opportunistic infections, particularly cytomegalovirus (CMV) infections and lymphomas far beyond those observed under maintenance drug regimens.

In conclusion ATG may be regarded as a mixture of monoclonal antibodies with polyspecific antigen targets and have been used efficaciously for over 30 years in transplantation despite their adverse reactions. For many years, the CD3 antibody OKT3 was the only monoclonal antibody used for induction therapy or acute rejection treatment. Over the past 10 years, chimeric and humanized antibodies, basiliximab and daclizumab respectively, to the a chain of interleukin-2 receptor (CD25) are effective in prevention of acute rejection

in renal transplantation and are extensively used. ATG do not include anti-CD25 antibodies. Consequently in patients on the reduction therapy with basiliximab or daclizumab and acute rejection, ATG administration may be expected to be additive with complementary mechanisms of action.

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