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## **Anti-Thymocyte Globulin (ATG) for Treatment of T- Cell-mediated Allograft Rejection**

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### **Abstract**

Anti-thymocyte globulin (ATG) is a pivotal immunosuppressive therapy utilized in the management of T-cell mediated rejection and steroid-resistant rejection among renal transplant recipients. Commercially available as Thymoglobulin (rabbit-derived, Sanofi, USA), ATG-Fresenius S (rabbit-derived), and ATGAM (equine-derived, Pfizer, USA), these formulations share a common mechanism of action centered on their interaction with cell surface markers of immune cells, imparting immunosuppressive effects. Although the prevailing mechanism predominantly involves T-cell depletion *via* the complement-mediated pathway, alternate mechanisms have been elucidated. Optimal dosing and treatment duration of ATG have exhibited variance across randomized trials and clinical reports, rendering the establishment of standardized guidelines a challenge. The spectrum of risks associated with ATG administration spans from transient adverse effects such as fever, chills, and skin rash in the acute phase to long-term concerns related to immunosuppression, including susceptibility to infections and malignancies.

This comprehensive review aims to provide a thorough exploration of the current understanding of ATG, encompassing its mechanism of action, clinical utility in the treatment of acute renal graft rejections, specifically steroid-resistant cases, efficacy in rejection episode reversal, and a synthesis of findings from different eras of maintenance immunosuppression. Additionally, it delves into the adverse effects

associated with ATG therapy and its impact on long-term graft function. Furthermore, the review underscores the existing gaps in evidence, particularly in the context of the Banff classification of rejections, and highlights the challenges faced by clinicians when navigating the available literature to strike the optimal balance between the risks and benefits of ATG utilization in renal transplantation.

## **INTRODUCTION**

Rejection is one of the common complications after kidney transplantation. About 10-20% of kidney transplant recipients experience acute rejection (AR) in the first year post-transplant [1, 2]. AR can be defined clinically as a rise in serum creatinine in the absence of other pathology, and verified by allograft biopsy using the Banff classification system [3]. AR is associated with an increased risk of long-term graft loss, morbidity, and mortality [4]. Therefore, timely treatment of AR is crucial in improving long-term outcomes in kidney transplantation. A proportion of AR can be resistant to steroids (25-30% of AR episodes) [2]. Anti-thymocyte globulin (ATG) is a polyclonal antibody used as an induction agent to reduce rejection rates and treat rejections following a kidney transplant. It is available in rabbit-derived (rATG; Thymoglobulin), ATG-Fresenius, and equine-derived forms (eATG; ATGAM). During the early use of ATG, its role in treating steroid-resistant allograft rejection was established [5]. The Kidney Disease Improving Global Outcomes (KDIGO) and British Transplant Society guidelines advise using ATG at induction in high-risk individuals and as an option to manage steroid-resistant acute rejection [6, 7]. For this review, we studied peer-reviewed research articles published in PubMed-indexed journals. We reviewed the various clinical trials of ATG, its use in treatment of acute rejection, steroid-resistant rejection, and recurrent rejections and clinical studies published in similar journals. We excluded reports presented as conference abstracts and those published in languages other than English. We aimed to evaluate the risks and benefits of ATG treatment in rejections and its implications in clinical practice. We envisage that such analysis of the literature will

help clinicians and patients evaluate the role of ATG holistically in current transplantation protocols and aid in clinical decision-making at an individual patient level. Lastly, we identify gaps in evidence and outline potential strategies that could help bridge these gaps to improve post-transplant patient and allograft survival.

### **MECHANISM OF ACTION**

ATG predominantly targets T Cell antigens (although some of these antigens are present in other cell types) like TCR/CD3, CD2, CD4, CD5, CD6, CD8, CD25, CD28, CD45, and HLA (Human Leukocyte Antigen) class I to induce the immunosuppressive effect. The complement-dependent T cell lysis in the intravascular compartment i.e., blood and the phagocytosis of T cells by macrophages in peripheral and secondary lymph nodes is regarded as the primary mechanism of action of ATG. The pre-activated T cell present in blood or peripheral tissues is depleted through antibody-dependent cell-mediated cytotoxicity and Fas-ligand-dependent apoptosis pathways [5, 8, 9]. The pharmacokinetics of ATG depend on the dose and schedule of administration as well as the number of 'targeted' immune effectors [9]. A lower concentration of thymoglobulin in the 0.1-1µg/mL range induces lysis of preactivated T cells. A higher concentration (10-100µg/mL) triggers CD178 (CD95-L) expression by resting T cells and apoptosis of preactivated T cells through pathways mostly involving Fas/Fas-L interactions [10, 11]. ATG also modulates cell surface expression of adhesion molecules (ICAM-1, -2, and -3), integrins (LPAM-1 and VLA-4), and chemokine receptors (CXCR4, CCR5, and CCR7), thus interfering with leukocyte-endothelial interactions that play a role in ischemia/reperfusion injury, graft vs host disease and rejection [10, 12]. The modulation, particularly in this setting, is the process of internalization of the ATG-antigen complex by endothelial cells. This results in decreased surface antigen which ultimately decreases the interaction of leucocytes with endothelium and trans-migration into tissue.

ATG has been shown to contain antibodies against a few B-cell antigens, including B-cell-specific and non-specific surface proteins CD19, CD20, CD40, CD80, CD30, CD38,

CD95, and HLA-DR. ATG crosslinks with these surface proteins and induces apoptosis (in vitro) in naïve and activated B cells with clinically relevant concentrations (1–100 ng/mL). ATG can also bind with Syndecan-1 (CD138), a plasma-cell-specific molecule, however, *in vivo* ATG treatment is not associated with a reduction in either splenic or bone marrow plasma cells [5, 9]. ATG interferes with the functional properties of Dendritic cells (DC) including maturation and migration and influences the balance between solid organ rejection and tolerance. Several *in vitro* studies showed the tolerogenic effect of ATG. ATG attaches to toll-like receptors (TLR) present on DC's surface. The common mechanism is the induction of complement-mediated DC lysis hampering lymphocyte proliferation [13–15]. Finally, ATG is also known to produce dominant tolerance by the expansion of CD4+CD25+high Foxp3+ T-regulatory cells which inhibits the action of CD4+CD25- T Cells, CD8+ T cells, B cells, DC cells, and Natural Killer (NK) cells [16–18]. It also associates with the increase of NK-T cells (CD4-/CD8- subset of T cells) which seems to decrease the incidence and severity of acute rejection [19]. Figure 1 summarizes the five documented mechanisms of ATG.

### TREATMENT OF T CELL-MEDIATED REJECTION

TCMR is a process initiated by interaction of T-cells with donor antigens predominantly presented by macrophages. The interaction of these biomolecules leads to local inflammation (infiltration of T cells and macrophages) that further leads to recruitment of effector T cells, tubulitis, nephron response to injury including differentiation of epithelium, and if untreated, nephron loss that will be irreversible. Acute rejection is clinically suspected in patients experiencing an increase in serum creatinine, after the exclusion of other causes of graft dysfunction. Subclinical acute rejection is defined by the presence of histological changes specific for acute rejection on screening or protocol biopsy, in the absence of clinical symptoms or signs. Kidney Biopsy remains the gold standard test to diagnose acute rejection, with characteristic infiltration of donor tissue interstitium with host T cells, cells in monocyte-macrophage

lineage, and nephron injury <sup>[20]</sup>. <sup>8</sup> Treatment of T Cell-Mediated Rejection (TCMR) has changed little over time and sparse data exist comparing one strategy to another. <sup>4</sup> AR requires a short course of more intensive immunosuppression in addition to baseline immunosuppression therapy. Options include treatments with steroids, antibody preparations, alteration of maintenance immunosuppression, or a combination of these options. <sup>3</sup> Corticosteroid therapy is the most commonly used, first line treatment for acute cellular rejection episodes. Although most patients respond to corticosteroids, the dose and duration of treatment has not been well defined by RCTs. Table 1 Lists the published clinical trials and Table 2 Lists published cohort studies, majority of which were retrospective single centre studies. <sup>12</sup> Treatment of acute cellular rejection with T-cell depleting antibody can be more effective in improving kidney function and preventing graft loss than treatment with corticosteroids alone <sup>[21]</sup>. However, all these trials were published more than 20 years ago, with the majority between 1970s and 1990s, when Banff classification was yet to be incorporated into clinical practice or clinical trials / studies. In clinical practice, treatment is guided by biopsy features as longer-term graft survival varies with the type of TCMR <sup>[22]</sup>. The majority of Banff class I lesions respond to methylprednisolone alone; conventionally <sup>8</sup> pulse methylprednisolone at 250–500mg daily for 3–5 days is recommended by international guidelines <sup>[6]</sup>. <sup>8</sup> TCMR involving lymphocytic infiltrate of the vasculature (Banff II and III lesions) may necessitate T cell-depleting therapy. Polyclonal antibodies include horse-derived (anti-lymphocyte globulin, ALG) and rabbit-derived (Anti thymocyte globulin, ATG) antibodies against the human lymphocyte or thymocyte respectively. Most commonly rATG dosed at 1.5 mg/kg for 7-14 doses was used (Table 1 and Table 2) <sup>[8, 21-24]</sup>. Reversal of rejections was seen in 50-90% in clinical trials, Intravenous immunoglobulin (IVIG) and Anti thymocyte serum were also used in past <sup>[25]</sup>. Recently alemtuzumab had been put forward as a possible treatment option for rejection <sup>[26]</sup>.



## **STEROID RESISTANT REJECTIONS**

In approximately 25 to 30% of the patients, rejections are not reversed with steroid therapy alone. In these recipients, more intensive immunosuppressive therapy is required to reverse the AR episode. When serum creatinine levels do not recover to within 120% of the pre-rejection baseline value following corticosteroid pulse therapy within 14 days of the steroid medication's initiation, the episode is deemed steroid resistant [27]. Up until day 5, patients with steroid-responsive and steroid-resistant AR experienced similar changes in their serum creatinine levels. However, at that point, the responders' creatinine levels significantly decreased, while the non-responders' levels stayed high. Therefore, conventionally, physicians typically wait five days before classifying a rejection as steroid-resistant [28].

## **ADVERSE EFFECTS**

Infusion of ATG may be complicated by immediate toxicity in the form of fever, chills, or skin rash which are considered self-limiting and managed with symptomatic therapy (Paracetamol, antihistamines, bolus steroids) and reducing rates of infusion. Lymphopenia, neutropenia, and thrombocytopenia can occur, but these are amenable to dosage adjustment. Medium to longer-term effects include cytopenia, higher rates of infection, and malignancy. Serum sickness is a rare complication caused by the deposition of immune complexes in tissues. Characteristic symptoms include fever, jaw pain, arthralgia, lymphadenopathy, and rash [10, 22]. Registry studies have tried to determine whether ATG induction therapy is associated with a greater risk of developing post-transplant lymphoproliferative disease (PTLD), but results are mixed and remain inconclusive [10]. Tables 1 and 2 outline frequency of these adverse effects published in the randomised controlled studies and cohort studies respectively.

## **DISCUSSION**

Despite the advancement of immunosuppressant therapy, AR remains one of the major problems in the field of clinical renal transplantation. The current approach in the

management of acute kidney rejection in adults and children is based on the 2009 KDIGO guidelines [29]. These guidelines recommend corticosteroids for the initial treatment of acute cellular rejection. They advise adding or restoring maintenance prednisone in patients with rejection episodes who are not on steroids. They also recommend using lymphocyte depleting agent or muromonab-CD3 (OKT3) for TCMR that does not respond to corticosteroids and for recurrent acute cellular rejections. Lymphocyte-depleting agent, in particular, ATG has been used extensively for treating and preventing AR in kidney transplant recipients [21]. ATG has also been used as first-line therapy for those with severe acute TCMR including vascular lesions (Banff II or higher categories), and as rescue therapy for steroid-resistant acute TCMR (see Table 1 and Table 2).

It has been shown that steroid-resistant rejection can be a significant problem in patients immunosuppressed with triple therapy (combination of Tacrolimus, Mycophenolate mofetil, and Steroid) and 70% of such rejections can be reversed following ATG treatment [30]. A systemic review by Webster *et al* [23] was one of the comprehensive studies describing the advantage of using ATG over steroids for the treatment of steroid resistance rejection. They studied 21 trials (49 reports, 1394 randomized participants) and concluded that in treating first rejection, ATG was superior to steroids in reversing rejection (RR 0.57; CI 0.38-0.87) and preventing graft loss (death-censored RR 0.74; CI 0.58-0.95). However, there was no difference in preventing subsequent rejections (RR 0.67; CI 0.43-1.04) or death (RR 1.16; CI 0.57-2.33) at 1 year between ATG and steroids. Additionally, they also found no benefits with the use of muromonab-CD3 over ATG or ALG in reversing rejection, preventing subsequent rejection, or preventing graft loss or death. A decade later, in 2017 [21], Webster *et al* updated the review with 11 new trials (76 reports, 1680 participants). The updated meta-review concluded that antibody therapy was still better than steroid therapy (RR 0.50, 95%CI 0.30 to 0.82) for reversing the first acute rejection, preventing subsequent rejections (RR 0.70, 95%CI 0.50 to 0.99) and tended to help prevent graft loss (death censored: (RR 0.80, 95%CI 0.57 to 1.12). There was no benefit of muromonab-CD3 over ATG in reversing rejection,



preventing subsequent rejection, or preventing graft loss or death [29]. Muromonab-CD3 (Orthoclone, OKT3) is the first monoclonal antibody used clinically for immunosuppression. It eliminates CD3+ T cells from the peripheral circulation to produce the immunosuppressive effect. A few noticeable studies compared Muromonab-CD3 with monoclonal and polyclonal antibodies in the treatment of steroid-resistant rejection [31, 32]. Using clinical records stored in the Austrian Dialysis and Transplant Registry, Kainz *et al* [33] conducted a retrospective descriptive analysis of 399 (368 ATG treated *vs* 31 OKT3 treated) patients diagnosed with biopsy-confirmed acute rejection (BCAR) between 1990 to 2005. Their study suggested that ATG treatment for rejecting allograft exhibited longer graft survival over OKT3 treatment (median graft survival 9.5 years in ATG group *vs* 4.6 years in OKT3 group) and increased risk of graft loss in OKT3 group (HR = 1.73, CI = 1.09 – 2.74, *P* = 0.019). ATG was better tolerated compared to OKT3, with lesser frequency of cytokine release syndrome. Clinicians all around the world have backed studies to find a better alternative or newer, safer but more effective immunosuppressive regimen. Due to cost-effectiveness, adverse infusion reaction, prolonged duration of inpatient stay, and need for central venous access for ATG, Alemtuzumab (CD52-specific monoclonal antibody) which can be given subcutaneously in a single dose, has been put forward with some promising results. A propensity controlled study of 116 patients treated with Alemtuzumab, in comparison to 108 patients treated with ATG showed similar patient and allograft survival [26] whilst having superior infection free survival with Alemtuzumab. The authors suggest that Alemtuzumab therapy may therefore be an alternative therapy for glucocorticoid-resistant, recurrent, or severe acute kidney transplant rejection. Registry data shows that the incidence of AR has been steadily falling. The rate of AR used to be more than 50% in the 1970s, which has markedly dropped to 10-20% today as per the US, Australian, and New Zealand registries [2]. This can be attributed to the improvement of induction and maintenance of the immunosuppressive regimen. During the 80s, the triple therapy regimen which was the combination of low-dose cyclosporine, azathioprine, and prednisolone was prescribed for maintenance

immunosuppression [34,35]. Over the years, various combinations were tried to find the optimal regimes. As of today, the best results overall are achieved with Tacrolimus (Tac), Mycophenolate mofetil (MMF), and steroids. A randomized trial conducted by Gonwa *et al* demonstrated that this triple therapy regimen showed overall better outcomes in terms of graft and patient survival compared to other drug combinations. The study also showed that this combination provided particular benefits to kidney allograft recipients who develop delayed graft function/acute tubular necrosis [36]. The landmark Symphony trial consolidated evidence for reduced exposure to calcineurin inhibitors in kidney transplantation, in conjunction with induction with daclizumab, MMF and corticosteroids [37]. Van der Zwan *et al* recently showed the long-term outcome of the use of rATG with the combination of Tac + MMF + steroids for the treatment of AR [38]. They concluded that early detection of AR followed by Tac + MMF + steroids with ATG provides better allograft functioning and survival. Survival after rATG was comparable to the overall survival of all kidney transplantation patients ( $P = 0.10$ ).

However, there is paucity of studies using ATG in current immunosuppression era and contemporary classification of AR. Only few studies in Table 1 & 2 used Banff classification in the description of AR and when used, was from earlier classifications [39] and also at which point role of antibody mediated component was less well understood.

The ATG dosage and duration varied widely among randomised studies as well as cohort studies described in Table 1 & 2. The optimal dosing schedule in patients at high or low immunological risk has yet to be determined. Preville *et al* derived data from a non-human primate model (cynomolgus monkey) which suggested that T-cell depletion with rATG is dose-dependent and that the optimal total dose required to achieve lymphocyte depletion in both peripheral blood and secondary lymphoid tissues (spleen and lymph nodes) is approximately 6.4 mg/kg [40]. About 40% of patients treated with Thymoglobulin (mean of 6 doses at 1.5 mg/kg/day) recover >50% of initial lymphocyte count at 3 mo. Yet, time to immune reconstitution is characterized by not

only a high intra-individual variability in the immune cell subpopulations (T and B cells, NK cells, DCs) but also an interindividual variability leading to prolonged lymphopenia for some patients up to 5 years [8]. When used as induction agent, significant difference in infection rates were reported with rATG dose of <7 mg/kg compared to use of >7 mg/kg [41, 42]. Since then, other studies have attempted to use the lower dose while optimizing the immunosuppressive effects of ATG [43, 44]. However, in the context of AR treatment, guidance for use of ATG at 1.5 mg/kg remains broad at 7-14 days. It is difficult to pre-determine precise duration based on published studies. Variation in effects with intermittent dosing, and continuous dosing were also reported (included in Table 1 and 2). These included a) use of 4 mg/kg bolus dose followed by re-dosing after 4 days based on CD3 count (T-cells), if <200 [38], CD-3 count <50 [45], limitation to mean of 5 doses [46] and reduction in dose based on cytopenias. There is a need for further studies to unravel implications of ATG in treatment of rejections.

These

include:

- a) identifying patients most likely to benefit from ATG therapy. Clinical risk factors, kidney biopsy findings will need to be tested as a multivariate prediction model in determining outcomes that would enable choice of right patient.
- b) It is possible that some of the intra-graft mRNA expression profiles (immune and non-immune biomarkers) could predict response to pulse glucocorticoid therapy in transplant recipients and likewise additional therapy to ATG [47].
- c) Evaluating benefit of ATG in late rejections compared to its benefit in treating early rejections.
- d) finding the optimal balance of immunosuppression in renal allograft recipients. Suboptimal immunosuppression can lead to rejection while over-immunosuppression can lead to life-threatening post-transplant infections. There remains no precise way to monitor the intensity of immunosuppression to prevent infectious complications [21]. Reports of CMV infection (Tables 1 & 2) were considerably high in published studies and prophylactic treatment with Valganciclovir for 3-6 mo is common practice lately.
- e) role of Torque-Teno Virus (TTV) measurement (as a biomarker of

immunosuppression to predict over / under-immunosuppression) is still in an infantile state [48].

f) Role of ATG treatment in rejections due to non-compliance with maintenance immunosuppression medications. Currently outcomes of treatment of such rejections is unclear, and

g) Lastly, role of anti-ATG antibodies and their role in negating therapeutic potency of ATG needs to be established [49].

## **CONCLUSION**

In conclusion, Anti-thymocyte globulin (ATG) emerges as a valuable therapeutic option for managing acute T-cell mediated rejections, particularly in cases refractory to steroid treatment or characterized by higher grade rejections, such as Banff II or III. While the established standard dosing regimen recommends 1.5 mg/kg for a duration spanning 7 to 14 days, it is imperative to underscore the complexity of tailoring ATG therapy to individual patients, where striking the optimal balance between risks and benefits remains a formidable clinical challenge. To further advance our comprehension of this crucial treatment approach, it is imperative that we embark on comprehensive investigations. Large-scale studies, ideally based on registries, should be conducted with meticulous phenotyping of transplant recipients and thorough analysis of renal transplant biopsy characteristics. Such endeavours are indispensable in augmenting the existing body of scientific knowledge, ultimately enabling us to address the pertinent questions surrounding the precise use of ATG in the management of acute T-cell mediated rejections.

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