

# Induction of Immunosuppression with Polyclonal Antithymocyte Globulins: An Overview

*A Beiras-Fernandez, E Thein, C Hammer*

**The induction of immunological tolerance to solid organ allograft is currently a subject of major investigation due to the morbidity and mortality related to immunosuppressive therapy. Immunosuppression induction by recipient treatment may allow to tailoring the timing and dosage of standard therapy not only reducing adverse reactions but also improving the graft outcome. Depletion of recipient T cells with polyclonal antithymocyte globulins is one of the methods nowadays investigated both in experimental and clinical procedures, demonstrating a better outcome of organ engraftment. Our intention is to give an overview of the literature about the mechanisms of action of polyclonal ATGs, the status of induction treatment in clinical and experimental transplantation as well as of the possible pathophysiological relationships with acquired tolerance, delayed graft failure and ischemia-reperfusion injury.**

**Keywords:** *Immunosuppression, Induction therapy, ATG, Transplantation, Tolerance*

## **Polyclonal ATGs**

Antileukocyte sera (ALS) were first described in 1899 by Metchnikoff. He proposed that xenoantibodies coat foreign cells, leading to their destruction [1,2], a proposal that led to the application of animal antihuman polyclonal antibodies to reduce rejection reactions in the clinical practice decades later. Several species were tested to find the best source of ALS for its application

on human beings. Horse would be "a priori" the first choice due to the large quantity of product available in an individual animal. However, the efficacy of horse antibodies varies considerably additionally causing undesirable adverse reactions. Sheep and goats were also dismissed due to their immunological unreliability. Rabbits were identified as the best antibody producers despite the small quantity of serum available per animal. The antigens used for ALS-production were total leukocyte preparations. They were soon replaced by pure lymphocytes, predominantly T-cell lymphocytes [3]. The new preparations were called polyclonal antithymocyte or antilymphocyte globulins (ATGs) depending on the source of the antigen, whether human thymocytes or cultured human lymphocytes.

These polyclonal antibodies present a broad spectrum of immunological properties. They bind to cell surface receptors, thereby opsonizing lymphocytes for complement-mediated lysis or reticuloendothelial cell-dependant phagocytosis [3-6]. ATGs recognize most of the molecules involved in the T-cell activation cascade such as CD2, CD3, CD4, CD8, CD11a, CD18, CD25, HLA DR and HLA class I [7]. Although lymphocyte depletion constitutes the primary mechanism of the immunosuppressive effects of ATGs, other mechanisms such as blocking of adhesion molecules and apoptosis induction are involved. T-cell depletion involves active cell death, demonstrated by annexin V binding [8] and TUNEL analysis [9]. A second mechanism of T-cell depletion would be an activation-associated apoptosis, being independent from Fas and Tumor Necrosis Factor-alpha (TNF-alpha) [10]. Profound immunosuppression achieved is evidenced by a peripheral blood T-cell lymphocyte count of less than 150 T-cells/ml [11]. Monitoring of the lymphocyte subsets (CD2, CD3, CD4, CD5, CD7, CD8, CD14, CD19 and CD25) confirmed the broad range of T-cell specificities of ATG [12-15]. Over 85% depletion of

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*Institute of Surgical Research, LMU-Munich, Germany*

*Adress reprint requests to: Andres Beiras-Fernandez, MD, Institute of Surgical Research, University of Munich, Marchioninstr. 7, 81377 Munich, Germany, E-mail: abeiras@icf.med.uni-muenchen.de*

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CD2, CD3, CD4, CD8, CD25, CD56 and CD57 lymphocytes was shown after the first two weeks of treatment while monocytes underwent less marked depletion and B-cells were almost unaffected [16]. ATGs present also functional effects on preactivated T-cells, which may be relevant in their activity on acute cellular rejection. ATG induce Fas-Ligand (Fas-L) (CD95L) and TNF $\alpha$  expression on resting T-cells [17]. OKT3 shares this property although it is more active on preactivated than on resting T-cells [18]. Induction of Fas-L expression on resting cells is completely inhibited by Cyclosporine, decreased by corticosteroids but only marginally affected by Rapamycin. Preactivated T-cell blasts as well as NK-cells may also be attacked by ATG by a mechanism of Antibody-Dependent Cellular Cytotoxicity (ADCC) [19]. In addition to depletion and apoptosis induction, some major functional effects are also achieved by ATG such as modulation of leukocyte surface antigens and adhesion molecules such as CD11a, CD18, CD102 or CD45 [20,21] being these effects relevant in transplantation. Further studies are followed on to exactly assess the influence of polyclonal ATGs on the expression of integrins and adhesion molecules in vivo as well as the mechanisms of interaction of these molecules with endothelial cells.

Modulation by polyclonal ATGs applies to molecules that control T-cell activation (T-cell receptors CD2, CD3, CD4, CD5, CD6, CD8) and also to molecules involved in leukocyte endothelium interaction such as the  $\beta$ 2 integrins, especially LFA1 (CD11a). Even low concentrations of ATGs induce a nearly complete disappearance of LFA1 on monocytes, granulocytes and lymphocytes [22]. Important lymphocyte activation molecules such as  $\beta$ 1 and  $\beta$ 2 integrins and even endothelial inflammatory and adhesion molecules such as ICAM1 are efficiently blocked by ATGs [20]. This property may reduce one of the most important features of IRI, reducing the deleterious effects of the reperfusion in the microvasculature of tissues and solid organs.

These unique properties of polyclonal ATGs, not achieved by other immunosuppressive agents, make them an interesting subject of study as leukocyte antigens and adhesion molecules play a crucial role in IRI, delayed organ failure and chronic rejection.

### Clinical Trials

ATGs immunological properties have an outstanding

clinical relevance. Clinical studies have been performed in the last years with ATGs not only as pre-induction therapy but also as post-transplant immunosuppression [23-26] in spite of their side effects [27-30]. Induction of immunosuppression with ATGs as cyto-ablative drug has proved to ameliorate the transplantation process and the organ outcome in many clinical protocols. *Szczzech et al.* performed a meta-analysis, which showed a benefit of induction therapy at 2 years, particularly among presensitized patients in renal transplantation after ATG pre-treatment [31]. These results are concordant with new clinical trials [32-34]. *Charpentier et al.* performed a three-arm study comparing standard tacrolimus therapy with induction therapy with ATGs followed by tacrolimus or Cyclosporine A (CsA) in an adult renal transplantation. He concluded that although more hematologic and infectious adverse events were observed in both ATG induction groups, acute rejection was significantly lower in the ATG-Tacrolimus group in comparison to CsA and to standard monotherapy with Tacrolimus [32]. Furthermore, Swanson has communicated excellent results after induction with ATGs and monotherapy with Syrolimus in renal transplantation, allowing the withdrawal of multi-drug treatment [33]. There are some authors providing evidence of a better outcome of renal engraftment after induction with Basiliximab, a chimeric monoclonal antibody binding to the IL-2 receptor [35]. However, other authors show comparable effectiveness to ATGs only with early immunosuppression with CsA thus increasing the adverse reactions due to this drug [28]. Furthermore, *Lebranchu et al.* have discussed that both immunosuppressive strategies provide good results, despite the differences in patterns, in patients receiving their first cadaveric renal allograft. However, ATG-treated patients show higher rates of survival and lower tendency to dialysis [24].

Induction of immunosuppression with ATGs has shown to be beneficial in other solid organ transplantations. *Eason et al.* presented a lower rejection rate in orthotopic liver transplantation (OLT) enabling avoidance of steroids. In addition, they found a decreased incidence of post-OLT diabetes, recurrent hepatitis C and CMV infections rate [36]. *Schulack et al.* [37] as well as *Tschervenkov et al.* [38] demonstrated that ATG-mediated induction therapy reduced the incidence of early rejection episodes and helped to pre-

vent organ failure in adult OLT. However, Neuhaus *et al.* studies did not show a significant better outcome after ATGs induction when compared to tacrolimus and steroid induction. Both treatments yielded similar safety and effectiveness after liver transplantation [39]. ATGs have proved to be useful in pancreas and combined kidney-pancreas transplantation as induction therapy. Stratta *et al.* have documented an improved rate of survival, lower rate of rejection and better outcome after inducing immunosuppression with polyclonal ATGs in pancreas transplantation [40]. Land, on behalf of the EUROSPK group showed a low rate of rejections and fewer pancreatic graft losses in the simultaneous kidney-pancreas (SKP) transplantation [41]. Rigotti *et al.* quote, as advantages of ATG in SKP, the lower rejection rate as well as the delayed onset and lower severity of rejection episodes [42].

Induction with ATGs has been extensively applied in heart, lung and combined heart-lung transplantation. Zuckermann *et al.* demonstrated a low rate of rejection accompanied by a good tolerance in first-heart transplant patients treated with two different rabbit polyclonal antibodies [43], results confirmed two years later by Schnetzler *et al.* [27]. Several different groups have reported good outcome of grafted organs after induction with pATGs in heart transplantation single-center experiences [44,45]. Carrier *et al.* induced immunosuppression with an initial 3-day course of ATG in a cyclosporine based protocol, demonstrating a lower rate of acute rejection. Moreover, the risk of infection and of developing cancer was not increased and after 10 years follow-up, a trend towards a lower incidence of coronary atherosclerosis was observed [46]. Concordant results were obtained by Barlow *et al.* in combined heart-lung transplantation. The rejection and survival rate after induction with polyclonal ATGs was significantly improved in comparison to OKT3 [47]. Palmer *et al.* found that ATGs induction in addition to conventional immunosuppression decreased the biopsy-proven rejection in lung transplant recipients [48]. Although the use of polyclonal ATGs in lung transplantation remains controversial due to the higher incidence of cytomegalovirus infections, these authors report no significant differences of CMV infection or invasive disease between the patients pre-treated with ATGs and those treated with standard therapy [48]. These results are consistent with the previous retrospective

study by Griffith *et al.* [49].

Induction therapy with ATGs is performed nowadays with two different protocols. ATGs can be given as a single intra-operative bolus, which has demonstrated an increase of the survival rates of transplanted organs. Dosage of these intra-operative bolus, administered before opening of the anastomoses, ranged from 9 mg/kg [11] to 4 mg/kg [50]. ATGs can also be administered at intervals pre-operatively. There is no consensus either in the number of the days in which ATGs should be administered or the adequate dosage. Starzl *et al.* [51] e.g. injected 5mg/kg of a broadly reacting ATG several hours before the operation in a wide protocol including pancreas, kidney and liver. Palmer *et al.* [48] induced immunosuppression before lung transplantation during three days with a dosage of 1.5 mg/kg/day. Knight *et al.* used the same dosage (1.5 mg) during seven days to achieve immunosuppression in pancreas transplantation [52]. Although Nampoory *et al.* [34] reported a better outcome and reduction of side effects with bolus induction, there is however, no gold standard protocol for induction of immunosuppression with ATGs, being the dosage and time of administration dependent of the transplanted organ and the medical staff's experience.

Starzl *et al.* [51] have recently published a clinical trial relating induction of immunosuppression with broad reacting rabbit ATGs and low dose maintenance treatment to acquisition of tolerance. This study was performed in kidney, kidney-pancreas, liver, pancreas and intestine transplantations. Patients survival was of 95% and graft survival at one year was 89%, showing induction with ATG a beneficial effect in preventing acute cellular rejection and its further consequences.

In spite of the presented results, no clinical trial to investigate the effect of ATGs upon ischemia/reperfusion injury has been performed at the present date.

### Experimental Studies

Polyclonal ATGs immunological properties have been extensively studied, as previously described. In vitro experiments have been performed to investigate the antigen specificity of these preparates against leukocyte surface antigens and adhesion molecules [53] as well as the toxicity to other blood components [54]. Induction of immunosuppression with ATGs has been related to their direct lymphocytotoxic properties,

their ability to block adhesion molecules as well as leukocyte surface antigens [9] and their induction of lymphocyte apoptosis [55]. Bourdage *et al.* investigated the activity of ATGs against CD2, CD3, CD4, CD5, CD7, CD8, CD11a, CD18, CD28, CD44, CD45, and TCR-alpha/beta antigens, showing specificity of positive reactions to these leukocyte antigens [7]. Potency of these immunosuppressive agents was tested by Conrad *et al.* by in vitro methods: cytotoxic assay and rosette inhibition assay, both of which were evaluated by microscopy, showing ATG activity against functional molecules on T-cells, B-cells, NK-cells and macrophages [56]. Merion *et al.* investigated the hypothesis that polyclonal ATGs-mediated effect was immunologically specific consisting on simultaneous engagement of multiple T-cell receptors [57]. These authors showed not only a significant dose-dependent inhibition of in vitro proliferation of pre-treated T-cells, but also a significant reduction of inflammatory cytokines, such as IL-2. These engagements of T-cell receptors and co-stimulator molecules could be leading to partial T-cell activation and anergy. Oettinger *et al.* studied in an in vitro whole blood model the induction of cytokine release by ATGs and OKT3, concluding that the release of cytokines after ATGs treatment was significantly lower than after OKT3 [58]. Antithymocyte and antilymphocyte globulins, which contain antibodies directed against CD3 and other functional molecules on the surface of T and B cells, generate various transduction signals to the target cells, which can affect their functions in different ways. Recent in vitro studies suggest that these antibodies interfere with activation signals [59]. Indeed, ATGs, at low concentrations inhibit T-cell activation induced by alloantigens, whereas they induce polyclonal T-cell activation at higher concentrations. ATGs induce in vitro a state of specific unresponsiveness (clonal anergy) which may contribute to their long-lasting immunosuppressive effect. Bonnefoy-Berard *et al.* hypothesized from their in vitro data that in spite of their nonspecific immunosuppressive effects, polyclonal ATGs may also act on stimulated alloreactive T-cell clones and therefore contribute to donor-specific graft adaptation [60].

In vivo experiments have also been performed to elucidate the mechanisms of action of ATGs in immunosuppression induction. Most of the experiments have been performed in non-human primates due to the human nature of these pharmacological

preparations. These antibodies are raised from human thymocytes or cultured human lymphocytes, which does not allow experimentation in murine or swine models.

Preville *et al.* studied the mechanisms of polyclonal ATGs immunosuppressive activity in non-human primates, demonstrating a dose-dependent lymphocytopenia in blood as well as a dose-dependent T-cell depletion in spleen and lymph nodes but not in the thymus, indicating a limited access of ATG to this organ [9]. Remaining T cells in peripheral lymphoid organs were coated by antibodies and had down-modulated surface expression of CD2, CD3, CD4, and CD8 molecules. The results of this author indicate that T-cell depletion is achieved rapidly and primarily in peripheral lymphoid tissues at high ATG dosage. T-cell apoptosis in peripheral lymphoid tissues is the main mechanism of depletion of ATGs according to the same group, since ATG can trigger Fas (CD95) mediated T cell apoptosis [17]. These authors conclude that induction treatments with ATGs in solid organ transplantation may enhance the efficacy of maintenance immunosuppressive therapy, thus having beneficial effects on the organ outcome.

ATGs have been employed as induction therapy in many concordant transplantation and xenotransplantation protocols. Asano *et al.* observed in a concordant non-human primate model that an immunosuppressive regimen with ATG induction led to long-term survival with fewer rejection and infection episodes by means of suppression of the interleukin 2 pathway and xeno-antibody production [61]. Other authors have obtained partial tolerance in concordant models after induction with ATGs. Bartholomew *et al.* presented donor specific T-cell hyporesponsiveness in a concordant rhesus-cynomolgus monkey renal transplantation, showing an extended graft survival of more than six months without chronic immunosuppression [62].

## Summary

Classical induction with polyclonal ATGs administered during or shortly after the perioperative period does not always improve graft survival. However, it has demonstrated both in clinical and experimental protocols a high rate of success in preventing acute cellular rejection and ameliorating graft function. ATGs applied to the recipient blood before reperfu-

sion of the grafted organ, react immediately with the recipients leukocytes and platelets, reducing or blocking recognition events, cell activation and thus expression of adhesion and inflammation molecules. Hammer suggested that applying polyclonal ATGs two or three hours before the operation as induction therapy may have a beneficial influence on the early mechanisms of reperfusion injury occurring after ischemia. Among them, downmodulation of the cell-surface selectins (P- and L-selectin) and integrins such as ICAM-1 or LFA [63]. The experimental studies performed show a modulation of T-cell surface antigens and co-stimulatory mediators that lead to partial tolerance and clonal anergy. The clinical trials performed presented a high rate of graft and patient survival and low rates of rejection after induction of immunosuppression with polyclonal ATGs. However, the mechanisms by which ATGs are leading to tolerance remain unclear. A beneficial influence on ischemia/reperfusion injury (IRI) may be a valid hypothesis to perform further investigations. Blocking the adhesion molecules, the T-cell receptors responsible for the antigen-cell interactions and the molecules responsible for endothelial cells activations may reduce the early graft dysfunction rate and the appearance of late graft dysfunction, chronic rejection.

These beneficial effects on the early onset of IRI could enlighten the mechanisms by which polyclonal ATGs pre-treatment in solid organ transplantation improves the outcome of the graft. Previous induction of immunosuppression with polyclonal ATGs would, in our opinion, benefit the recipient by lessening the deleterious effect of the reperfusion injury in a physiological rather than immunological manner.

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