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# Antitumor effects of polyclonal antithymocyte globulins: focus on B-cell malignancies and multiple myeloma

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**Abstract** Polyclonal antithymocyte globulins (ATGs) are used in organ and allogeneic stem cell transplantation mainly due to their immunomodulatory potential. Since ATGs contain antibodies against antigens expressed on various hematopoietic cells, it is not surprising that they induce cell death not only in healthy T-, B-, NK, and dendritic cells but also in malignant cells of lymphatic and to a lesser extent of myeloid lineage. The cytotoxic and antiproliferative effects of ATGs in malignant B-cells have been known for many years, without attracting clinical attention due to the advent of monoclonal antibodies like rituximab. Recent data indicate a potential role of ATGs in the therapy of multiple myeloma, a disease in which monoclonal serotherapy has been rather unsuccessful. This review discusses available data demonstrating the cytotoxic effects of ATGs and envisages a possible new role of these polyclonal antibodies in the therapy of hematological malignancies.

**Keywords** ATG · Antitumor · Hematological malignancies

## Introduction

Polyclonal antithymocyte globulins (ATGs) are widely used for induction and treatment of acute rejection in solid organ transplantation, as part of conditioning regimens in allogeneic hematopoietic stem cell transplantation (HSCT) and in the treatment of graft-versus-host disease (GvHD). ATGs

are a subgroup of anti-lymphocyte globulins which are produced by immunization of rabbits or horses with human thymocytes or the Jurkat T-lymphoblastic cell line.

Inclusion of ATG in conditioning regimens is known to reduce the risk of acute and chronic GvHD [1–4]. The potent immunosuppression induced leads to a delay in immune recovery and increases the risk of infections [5–8], however so far without being accompanied by a theoretically expected increase in relapse rates [9, 10].

By virtue of their mode of production ATGs do contain antibodies targeting a wide range of antigens expressed on various normal and malignant hematopoietic cells including T-, B-, NK, dendritic, and plasma cells. Over the last years, there has been growing evidence that ATGs have potent cytotoxic effects particularly on lymphatic and to a lesser extent on myeloid malignancies [11]. This may at least in part explain the relatively low relapse rates observed in patients receiving ATG as part of the conditioning regimen for hematological malignancies.

The mechanisms involved in the cytotoxic effects of ATG include complement-dependent cytolysis, cell-mediated antibody-dependent cytotoxicity, opsonization and subsequent phagocytosis by macrophages, activation-induced cell death as well as apoptosis [12–14].

This paper aims at reviewing *in vitro*, xenograft, and clinical data on the antitumor effects of ATG with focus on B-cell malignancies and multiple myeloma and discussing future perspectives.

## Types of ATG and dosing

ATGs contain purified IgG sera produced by immunization of animals with human cells. Currently, there are three commercially available preparations of ATG (Table 1).

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**Table 1** Commercially available ATG preparations

Trade name	Animal immunized	Cells used
Thymoglobulin®	Rabbit	Human thymocytes
ATG-Fresenius®	Rabbit	Jurkat cell line
ATGAM®	Horse	Human thymocytes

ATG-Fresenius® (Fresenius-Biotech GmbH, Gräfelfing) is produced by immunization of rabbits with the Jurkat human T-lymphoblastic cell line. Thymoglobulin® (Genzyme) is produced by immunizing rabbits with human thymocytes while ATGAM® (Pharmacia Upjohn) is produced by immunizing horses with human thymocytes. Of the three ATG preparations, Thymoglobulin is best investigated. The human thymus is known to contain T-cells, dendritic cells, stroma cells as well as B- and plasma cells [12]. Antibodies contained in Thymoglobulin may thus target T-cell antigens like CD2 and CD3, B-cell antigens like CD19, CD20, and CD80, plasma cell antigens like CD126 and CD138 as well as antigens found on various leukocyte populations like CD29 and CD45 [15–20]. Due to differences in manufacturing procedures, the different types of ATGs contain variable specificities and amounts of antibodies [16, 17, 21], explaining the wide variability in doses used in the clinical setting. For Thymoglobulin, a total dose of 4.5 to 8 mg/kg BW has been recommended for matched unrelated donor allogeneic HSCT [22–24]. Meanwhile, the recommended dose for ATG-Fresenius is about ten times as high [25, 26]. Consequently, the concentrations of ATG detected in patients' sera vary broadly and may be as high as 1,000 µg/ml in patients treated with ATG-Fresenius [27]. It is very important to consider clinically achievable concentrations when interpreting *in vitro* data.

#### Antitumor effects of ATGs in T-cell malignancies

Though produced using thymocytes and T-cell lines, data on cytotoxic activity of ATGs in T-cells are scanty. A recent *in vitro* study reported complement-dependent cytotoxicity (CDC) and apoptosis of horse ATG in T-lymphoblastic leukemia samples from five patients [28]. Other *in vitro* data show potent cytotoxic effects of ATG preparations in malignant T-cell lines [11].

#### Antitumor effects of ATGs in myeloid malignancies

As would be expected, ATGs have very limited cytotoxic effects in myeloid cells. They only exhibit weak pro-apoptotic effects in myeloid leukemia cell lines and complement-dependent cytotoxicity varies strongly from one cell line to the other [11, authors' unpublished data].

#### Antitumor effects of ATGs in B-cell malignancies

ATG-induced cell cytotoxicity appears to be most pronounced in B-cells. Initial studies on EBV-transformed and non-transformed cell lines showed that various ATGs could potentially induce apoptosis and inhibit proliferation of malignant B-cells. These effects were irrespective of the immunogens and animals used for preparation of the ATGs [11, 29]. ATG-induced CDC and apoptosis in primary CLL samples has recently been reported [30]. In this study, the cytotoxic effect of Thymoglobulin was significantly stronger than that of ATG-Fresenius when the same concentration was used for both preparations. However, there was no significant difference between ATG-Fresenius 100 µg/ml compared to Thymoglobulin 50 µg/ml. Both ATG-Fresenius and Thymoglobulin showed synergistic cytotoxic effects in CLL in combination with fludarabine. The cytotoxicity of ATG in CLL cells *in vitro* is comparable to that observed using clinically relevant and thus comparable concentrations of alemtuzumab. In contrast to alemtuzumab, ATG on its own could directly induce apoptosis in CLL samples [30]. Case reports of patients with CLL and pure red cell anemia indicate a possible clinical anti-CLL effect of ATGs [31, 32].

In a recent report on reduced intensity conditioning (RIC) allogeneic stem cell transplantation with 800 cGy total lymphoid irradiation (TLI) and Thymoglobulin (5 × 1.5 mg/kg BW), seven out of 16 patients with lymphoid malignancies showed tumor clearing outside of the TLI field. The authors attributed this to a graft-versus-lymphoma effect, although the incidence of graft-versus-host disease was exceptionally low in these patients [33]. The tumor clearing may in part have been due to a direct effect of Thymoglobulin on the lymphoma cells. Considering the excellent effects of monoclonal antibodies like rituximab and alemtuzumab in B-cell malignancies, it is not conceivable that ATGs will be included in standard treatment regimens. In the light of the above reports, it may however be of interest to consider the potential effect of ATGs in B-cell malignancies when designing conditioning regimens for stem cell transplantation.

#### Antitumor effects of ATGs in multiple myeloma

Cytotoxic effects of ATG (Fresenius) in myeloma cell lines and primary myeloma samples have been reported using ATG concentrations of 100 to 1,000 µg/ml [10]. Zand and colleagues later reported similar data using Thymoglobulin in about the same concentration range [34]. Moreover, they could demonstrate that Thymoglobulin could overcome the stroma-mediated resistance of myeloma cells which may play an important role in resistance to conventional

anti-myeloma agents. In another study, Timm et al. showed that ATG (Thymoglobulin) kills myeloma cell lines that are resistant to conventional anti-myeloma agents and delays tumor growth in a xenograft plasmocytoma mouse model of multiple myeloma in a dose-dependent manner. More so, the reduction in tumor growth resulted in better survival for mice treated with Thymoglobulin compared to controls [35]. The pathways involved in ATG-induced myeloma cell death include complement-dependent cytotoxicity, caspase-dependent apoptosis, and the genistein-sensitive tyrosine kinase pathway [10, 34, 35]. A possible role of antibody-dependent cellular cytotoxicity has not yet been investigated but may require even lower concentrations of ATG than needed for apoptosis and CDC.

In patients undergoing allogeneic stem cell transplantation for multiple myeloma, response and disease-free survival have been reported to positively correlate with the incidence of chronic graft-versus-host disease [36, 37], which on the other hand, may have a negative impact on quality of life. A comparison of ATG and alemtuzumab in myeloma patients (who had not relapsed prior to transplant) undergoing RIC allogeneic stem cell transplantation showed a better progression-free survival and lower relapse rate for the ATG group. These results may have been due to the higher rate of GvHD observed in patients who received ATG compared to alemtuzumab [9]. In a more recent study, inclusion of ATG-Fresenius in a fludarabine/melphalan conditioning regimen for myeloma led to reduced incidence and severity of chronic GvHD and at the same time higher rates of complete remission as determined on day 100 after allografting [10]. These reports emphasize a possible clinical relevance of the above in vitro and xenograft data.

Experience from lymphoma therapy indicates that serotherapy can strongly improve the fate of lymphoid malignancies. Monoclonal antibody-based strategies in myeloma have however been of limited success due to the heterogeneous phenotype of myeloma cells which may vary even in the same patient depending on the maturation stage of the malignant cells [38, 39]. It may thus be worthwhile targeting various surface antigens with a combination of monoclonal antibodies or with polyclonal antibody preparations like ATGs.

There has been increasing evidence that mature B lymphocytes may constitute a “myeloma stem cell pool”, a probable source of relapsing disease in patients who apparently attain complete remission after chemotherapy [40]. Since ATGs can potently kill mature B-cells as discussed above, these polyclonals may not only target the mature myeloma cells but also the clonogenic B-cell-like “myeloma stem cells”. The potential of ATG to induce myeloma cell death by multiple mechanisms may also offer another advantage over monoclonal serotherapy and conventional anti-myeloma agents.

## Conclusion and future perspectives

Polyclonal antibodies may widen the spectrum of treatment options available for patients with hematological malignancies, especially B-cell lymphoma and multiple myeloma. A limitation of ATGs is surely the potent immunosuppression and high rate of infections that may be associated with their application. A further limitation of ATGs would be the development of antiglobulin (antirabbit) antibodies, which would hamper repeated application. In the context of autologous or allogeneic stem cell transplantation, however, ATGs could be incorporated in the conditioning regimen to enhance the effect of chemotherapy. Phase I/II trials are underway examining the clinical effects of ATG in patients with myeloma.

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