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ATG overdose in a kidney-grafted patient

Clinical course and possible implications

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Abstract. During antirejection treatment with antithymocyte globulin (ATG), a 48-year-old male kidney graft recipient was erroneously given 30 mg/kg ATG on day 5 of the treatment course. Clinically, the patient presented with abdominal pain, profuse diarrhea, and fever. Initially, the platelet count dropped to $94 \times 10^3/\mu l$ and showed a further decrease to $65 \times 10^3/\mu l$ 2 days thereafter. Plasmapheresis carried out about 12 h after the end of ATG infusion resulted in a decrease in serum levels of total rabbit immunoglobulin as well as the antibody fraction reactive with human T lymphocytes. Within 10 days after the discontinuation of ATG treatment, the relative and absolute number of the T-cell subpopulations, which had disappeared from the circulation at the time of antirejection therapy, returned to normal values. In addition, at this time both platelet and lymphocyte counts were again found to be in the normal range. Kidney function had recovered from the rejection crisis such that the patient could be discharged 26 days posttransplantation.

Key words: Kidney transplantation - Antithymocyte globulin - Overdose - Side effects - Immunologic monitoring.

The prophylactic [10, 11] or therapeutic [2, 5, 7] administration of antilymphocyte-globulin preparations to patients after organ transplantation is well established. The use of polyclonal rabbit antithymocyte globulin (ATG) at the recommended dose over a period of 10-14 days can result in side effects such as leukopenia and thrombocytopenia [6, 7] or infectious episodes [9] sometimes necessitating reduction of the dose or - in rare instances - discontinuation of the treatment.

To our knowledge, there are no records except experimental data [1, 14] of iatrogenic ATG overdose in the literature. Therefore, we report the case of a patient who, after undergoing a cadaveric kidney graft, was erroneously given 30 mg/kg ATG on day 5 of antirejection therapy.

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Methods

Measurement of serum ATG levels

Serum ATG levels were measured by a sandwich ELISA. Donkey anti-rabbit Ig (Amersham, UK) was used as the first layer. After incubation with serial dilutions of the sera or the ATG preparation, alkaline phosphatase-conjugated goat anti-rabbit IgG (Bio Makor, Rehovot, Israel) was added. Absorbances were read at 405 nm after a 30-min incubation period with *p*-nitrophenyl phosphate. The amount of ATG present in the respective sera was determined by interpolation into the standard curve set up with serial dilutions of ATG (Fresenius, Bad Homburg, FRG).

Determination of the T-lymphocyte-specific proportion of ATG

The proportion of the ATG preparation reactive with human T lymphocytes was determined by flow cytometry. The human T-cell line HPB-ALL [8] was reacted with serial dilutions of the sera followed by a fluorescein-conjugated goat anti-rabbit Ig (Behring Institut GmbH, Marburg, FRG) and the fluorescence intensity was determined. Similarily, serial dilutions of ATG were incubated with the human T-cel line to obtain a standard curve of the fluorescence intensity caused by the T-lymphocyte-specific component of ATG. The results are expressed as μg/ml ATG equivalents.

Determination of human antibodies (IgG and IgM) to ATG

Microtiter plates coated with ATG were blocked with swine skin gelatin (Sigma Chemical Co., St. Louis, Mo.) and bovine serum albumin (Sigma) in PBS and then incubated with 1:20 dilutions of the sera. The amount of bound antibodies to ATG was assessed using alkaline phosphatase-conjugated rabbit anti-human IgM (Fc5μ) or anti-human IgG (Fcgamma), respectively (Dianova GmbH, Hamburg, FRG). The results were expressed by the following index:

$$\frac{\text{mean absorbance of test sample}}{\text{mean absorbance of the sera before ATG treatment}}$$

A test sample was considered positive if the index was higher than 1.8. Absorbances obtained from pretreatment sera did not differ significantly from the blank.

Clinical course

In June 1987, patient P. I. (male, 48 years old, 67 kg) received a cadaveric kidney graft (one mismatch in the HLA-B and one in the HLA-DR locus). Initial immunosuppressive therapy consisted of cyclosporin A and low-dose steroids. Because of postoperative anuria, hemodialysis was continued posttransplantation. Rejection was suspected on clinical grounds and could be confirmed on day 9 by a percutaneous transplant biopsy, which showed a severe interstitial and vascular rejection. Consequently, antirejection treatment was started with steroid bolus injection (500 mg methylprednisolone) for 3 days, followed by ATG (Fresenius). On the 5th day of ATG treatment, 30 mg/kg ATG was erroneously given instead of the prescribed 3 mg/kg per day. Within 3 h after the administration of the high dose of ATG, the patient presented with abdominal pain and profuse diarrhea as well as fever up to 39°C. About 5 h later this symptomatology had ceased. In an attempt to remove circulating ATG, plasmapheresis was carried out approximately 12 h after the end of ATG infusion. During plasmapheresis, 95% of the calculated plasma volume was exchanged by a 5% human albumin solution (Immuno AG, Vienna, Austria), and 5 g immunoglobulin (Endobolin, Immuno AG) was substituted at the end of the session. Prophylactic treatment with antibiotics, cytomegalovirus hyperimmunoglobulin, and acyclovir was started immediately thereafter and antirejection treatment with ATG was discontinued.

The most striking laboratory finding was a decrease in platelet count from $150 \times 10^3/\mu l$ to $94 \times 10^3/\mu l$ (Fig. 1). The lowest levels in platelet and lymphocyte counts were determined 2 days after discontinuation of ATG treatment: $65 \times 10^3/\mu l$ and $164/\mu l$, respectively. ATG serum levels increased from an average 6-h through level of about 100 μg/ml on days 2–5 of the ATG antirejection therapy to a peak level of 643 μg/ml 1 day after the overdose. Plasmapheresis led to a drop in the serum level of ATG to 329 μg/ml, which significantly exceeded the plasma half-life of ATG calculated from

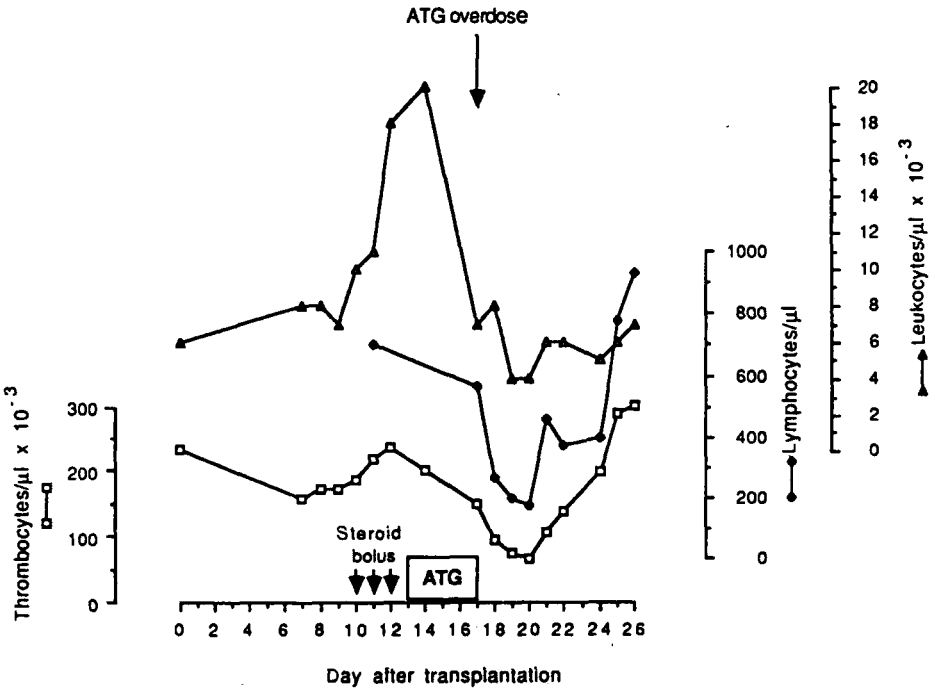

Fig. 1. White cell and thrombocyte profiles after transplantation

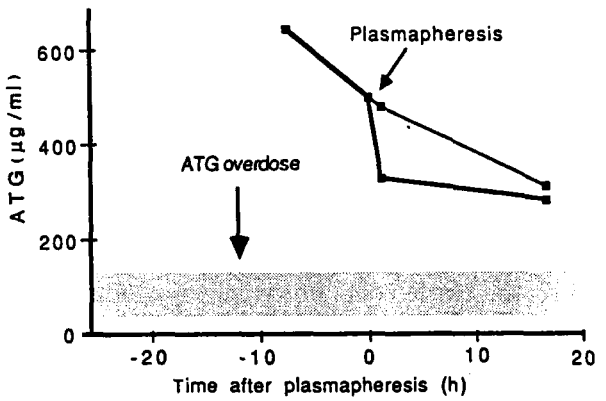

Fig. 2. Influence of plasmapheresis on serum levels of ATG. ▭ Range of ATG levels, when given at the recommended dose, — measured ATG levels, — expected ATG levels without plasmapheresis

the spontaneous decrease observed between the ATG peak level and the level before the initiation of plasmapheresis ($P<0.05$; Fig. 2). In the removed plasma (3000 ml), 413 µg/ml ATG were found. After 1 week, levels of ATG had returned to values similar to those observed prior to the overdose. Concomitantly, serum levels of the T-lymphocyte-specific proportion of the ATG preparation peaked 1 day after the overdose, followed by a sharp drop due to plasmapheresis and a continuous decrease thereafter. The ratio of specific ATG/total ATG fell from the time of ATG treatment at the recommended dose until 1 day after the overdose, followed by a further decrease thereafter. Within 8 days after discontinuation of ATG treatment, the relative and absolute number of T cells as well as T-cell subpopulations returned to normal values. In addition, recovery from the ATG overdose could be concluded from an increase in both platelet and lymphocyte counts (Fig. 1). The development of human antibodies (both IgG and IgM) to ATG was observed 20 days after the initiation of antirejection therapy with ATG.

The ATG overdose did not seem to be advantageous to the treatment of this rejection crisis because kidney function improved during antirejection therapy at the recommended dose of ATG. Serum creatinine as well as BUN levels showed a continuous decrease during ATG treatment and thereafter, and the patient could be discharged with excellent kidney function 26 days posttransplantation. Further follow-up over a 6-month period on an outpatient basis revealed a stable graft function, with no signs of complications due to the deposition of immune complexes, such as increased proteinuria or the development of nephritic sediment. Therefore, no additional biopsy was carried out until this time. Throughout the observation period, no signs of infection were observed.

Discussion

Discontinuation of treatment using rabbit ATG at the recommended daily dose is necessary only in rare instances due to side effects such as leukopenia and/or thrombocytopenia [6, 7]. Our patient, who was erroneously given an overdose of ATG (Fresenius) developed neither severe nor irreversible leukopenia nor thrombocytopenia. Although he had already received four doses of ATG, whose side effects are commonly observed only after the first dose [5, 7], administration of the tenfold higher dose led to fever up to 39°C and profuse diarrhea. These complications were even more pronounced than early side effects [5] and seemed to reflect a renewed massive release of mediators during lympholysis caused by the overdose. Sensitization [7, 13] to rabbit immunoglobulin seemed unlikely, as pretreatment sera showed no reactivity with ATG and the development of host anti-ATG antibodies was first observed 3 weeks after ATG therapy. Even then, no significant symptoms of hypersensitivity, anaphylaxis, or serum sickness were observed. Throughout the observation period, urinary analysis provided no indication of complications due to the deposition of immune complexes in the transplanted kidney.

Plasmapheresis succeeded in decreasing serum levels of both total ATG and the specific anti-T-lymphocyte component of ATG. Calculating the amount of ATG removed, we found that with 1.24 g ATG/3000 ml filtrate, 62% of the overdose had been eliminated. However, from the kinetic data (Fig. 2) it could be concluded that ATG levels would have reached similar values within 1 day without therapeutic intervention. The ratio of specific ATG/total ATG fell from the time of ATG treatment at the recommended dose until 1 day after the overdose, followed by a further decrease thereafter. Therefore, one might speculate that the specific component of ATG had selectively been removed from the circulation by rapid adsorption to T lymphocytes, such that only early plasmapheresis would be effective in removing specific components of the ATG preparation. Repeated plasmapheresis would probably have resulted in a faster, more effective reduction of serum ATG levels; however, since hematologic parameters improved within a few days after the discontinuation of ATG treatment, single plasmapheresis seemed to be sufficient to reduce high ATG levels. The actual

contribution of plasmapheresis to the recovery of the patient, however, remains unclear because, as can be seen from the kinetic data, plasmapheresis resulted in an acceleration of the decrease in serum ATG levels of only 1 day. Whether prolonged high serum levels of ATG without plasmapheresis would have been harmful, especially because of the rapid selective removal of the specific components of the ATG preparation from the cirulation, cannot be excluded by these observations.

Thus, in the patient described, a single tenfold higher dose of a particular ATG preparation (Fresenius) was well tolerated without irreversible side effects on the hematopoietic system. However, if given at high-dose levels, ATG preparations from other manufacturers, might have resulted in a less favorable outcome, because different physiologic derangements may be triggered both qualitatively and quantitatively by other preparations [4, 12]. The immediate adverse reactions were similar to those observed after the administration of monoclonal anti-T-cell preparations [5] but were completely reversible and did not affect the outcome of either the graft or patient. Since the dose as well as the different pharmacokinetics of ATG have been reported to influence the incidence of immunologic complications in transplanted patients [3, 7], it might be worth considering whether the administration of a higher dose of ATG could exert beneficial effects on the treatment of rejection episodes that cannot be reversed by the commonly used triple or quadruple immunosuppressive therapy. This immunosuppressive strategy could especially benefit patients after heart or liver transplantation, where an immediate retransplantation is not possible and graft loss due to rejection is life-threatening.

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