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Immunobiology

The impact of antithymocyte globulin on short-term toxicity after allogeneic stem cell transplantation

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Summary:

Antithymocyte globulin (ATG) is commonly used in allogeneic haematopoietic stem cell transplantation (HSCT). Little information is available, however, as to the optimal protocol for use and the side-effects occurring if ATG is administered in high daily doses (10–30 mg/kg). We report our experience with ATG Fresenius (ATG-F) in conditioning for allogeneic HSCT. During a period of 3 days, 47 patients received doses between 10 and 30 mg/kg either over 4 h preceded by 1–1.5 mg/kg prednisolone 30 min before the start of ATG-F (protocol A) or alternatively, over 12 h with 3–4 mg/kg prednisolone being administered before and 6 h after start of ATG (protocol B). During treatment with ATG-F, the side-effects observed included inflammation, disseminated intravascular coagulation, hyperdynamic circulation and renal dysfunction. Although these complications caused substantial morbidity, they were reversible within a few days. Side-effects were significantly more severe in patients treated according to protocol A than in those treated according to protocol B. As prolonged infusion of ATG-F does not reduce T cell clearance due to the long half-life of ATG-F, and since less cytokine release during conditioning might have beneficial long-term effects, we recommend administering ATG-F over 12 h preceded by high-dose steroid treatment.
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Graft-versus-host disease (GVHD) is a frequently encountered, life-threatening complication of haematopoietic stem cell transplantation (HSCT), particularly if the donor is not an HLA-identical sibling. In the past, the efficacy of antithymocyte globulin (ATG) in prophylaxis and treatment of acute GVHD has been reported in numerous studies. In animal models, treatment of the recipients of haematopoietic cell grafts with antithymocyte antibodies improved engraftment[1] and modified GVHD.[2] In clinical studies it was shown that the use of ATG not only prevented rejection, but also resulted in a low incidence of GVHD.[3–5] Based on these observations, ATG-Fresenius (ATG-F) was included as prophylaxis for GVHD in the conditioning regimen in our transplantation unit.

Side-effects of ATG treatment are common. Toxicity results from ATG cross-reacting with lymphocytes and macrophages, thereby producing symptoms such as chills, fever, leukopenia, thrombocytopenia and skin rashes. Within weeks serum sickness and nephritis may occur in immunocompetent patients. To investigate the spectrum and frequency of side effects, we studied patients being treated prophylactically with ATG-F, using two different protocols.

Patients and methods

Demographic and transplantation data

All patients in our transplant unit receiving ATG-F (Fresenius HemoCare Immune Therapy, Gräfelfing, Germany) for at least 3 days between January 2001 and August 2001 were included in this retrospective analysis of our institutional policy. Demographic and transplantation characteristics and details of conditioning regimens are given in Table 1. There was a time interval of at least 24 h between the end of TBI or chemotherapy (busulfan, fludarabine, FlAmsA) and the beginning of ATG-F therapy. Although this was a sequential study, the groups were comparable in all demographic and transplantation data. Written informed consent was obtained from every patient before the start of transplantation and allowed retrospective analysis of the transplantation course.

Treatment protocol

Of the 47 patients, 25 received ATG-F according to protocol A and 22 according to protocol B.

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Table 1 Demographic, transplantation and ATG characteristics and the transplantation-associated complications of the patients under study

	Protocol A	*P value*	*Protocol B*
n	25		22
Patient characteristics			
Age (years)	43 (18–61)	NS	52 (19–66)
Sex (M/F)	15/10	NS	12/10
Underlying disease			
CML	36.0% (9)	NS	36.4% (8)
AML	24.0% (6)	NS	13.6% (3)
ALL	16.0% (4)	NS	9.1% (2)
NHL	16.0% (4)	NS	13.6% (3)
CLL	4.0% (1)	NS	4.5% (1)
SAA	4.0% (1)	NS	9.1% (2)
MDS	0% (0)	NS	13.6% (3)
Transplant characteristics			
Donor type			
allogeneic RD	44.0% (11)	NS	54.5% (12)
allogeneic URD	56.0% (14)	NS	45.5% (10)
Conditioning regimen			
reduced intensity	64.0% (16)	NS	72.7% (16)
standard intensity	36.0% (9)	NS	27.3% (6)
ATG-F therapy			
Blood count at start of ATG-F			
Leucocytes $<$1.0 g/l	60.0% (15)	NS	59.1% (13)
platelets $<$50 g/l	52.0% (13)	NS	54.5% (12)
Daily ATG-F dose			
10 mg/kg BW	44.0% (11)	NS	45.4% (10)
20 mg/kg BW	52.0% (13)	NS	50.0% (11)
30 mg/kg BW	4.0% (1)	NS	4.5% (1)
Transplant-associated complications ($<$day 100)			
Acute GVHD	55.6% (20)	NS	44.4% (16)
acute GVHD score (median)	3.0 (0–4)	NS	2.5 (0–4)
Infection	88.0% (22)	NS	72.2% (16)
Death rate	36.0% (9)	NS	36.4% (8)
infection-related death	16.0% (4)	NS	13.6% (3)
Engraftment (days)	17 (8–67)	NS	18 (9–54)

All values values are medians (range) and percentage (numbers), respectively.
NS = not significant; *P* value = protocol A compared to protocol B, determined by the Mann–Whitney *U*-test; reduced intensity = conditioning regimen with either fludarabine (intravenous 30 mg/m^2 for 4 days) or FIAmsA (intravenous fludarabine 30 mg/m^2 for 4 days in parallel with cytarabine 2000 mg/m^2 for 4 days and amsacrine 100 mg/m^2 for 4 days) combined with 2–4 Gy TBI; standard intensity = either 12 Gy TBI (fractionated with 4 Gy on 3 consecutive days at a dose rate of 4.5 cGy/min) or busulfan (orally administered 4 mg/kg for 4 consecutive days); RD = related donor, HLA-identical; URD = unrelated donor, HLA-identical; acute GVHD = score according to Glucksberg;[4] engraftment = days after transplantation until neutrophil count was $>$0.5 g/l.

Protocol A: ATG-F was given at an increasing rate over 4 h, as stated in the manufacturer's instruction leaflet,[3] following premedication with Dimetinden and 100 mg prednisolone (1–1.5 mg/kg) immediately before the start of ATG-F.

Protocol B: ATG-F was given at a continuous rate over 12 h following premedication with Dimetinden and 250 mg prednisolone (3–4 mg/kg) immediately before the start of the drug. After 6 h, a second dose of 250 mg prednisolone was administered. After completion of the ATG-F infusion steroid treatment was stopped.

Two to 4 h after the end of the ATG-F infusion, cyclophosphamide was started on the same days that ATG-F was given (40 mg/kg body weight per day intravenously over 6 h). In eight patients (five in protocol A and three in protocol B), cyclophosphamide was started 12 h before beginning the ATG-F infusion.

Heart rate, blood pressure and peripheral oxygen saturation were continuously monitored in all patients during the treatment period. In cases of fever ($>$38.5ºC) blood cultures were taken. Before starting ATG-F treatment, the CVP was raised above 7 mm H_2O, the urine was alkalized by intravenous sodium bicarbonate (10 mval/h) and hydration was increased to 150 ml/h. In all patients the presence of human anti-rabbit antibodies (HARAS) was excluded before administering ATG-F and a test dose of ATG-F was tolerated without problems in all patients. Table 1 gives the dose of ATG-F used. As the majority of patients was treated for at least 3 days (3 days: 89.4%, 4 days: 6.4%, 5 days: 4.3%), we confined our observations to this period.

Recordings

Body temperature, heart rate and blood pressure were monitored on an hourly basis. Maximum and minimum were used for analysis. Single donor platelet concentrates were given if the platelet count dropped to less than 20 g/l. Furosemide (20 mg) was administered if the weight of the patient increased by more than 2 kg compared to the starting weight. Therapeutic data (number of transfused platelet

concentrates, furosemide dose) and the following laboratory parameters were recorded: platelets (H6000 analyser, Coulter, Hialeah, FL, USA), creatinine (Jaffe Method, Roche, Mannheim, Germany), prothrombin time (PT; STA Neoplastin Plus, Roche), antithrombin (AT; chromogenic assay, STA Antithrombin III, Roche), D-Dimer (latex agglutination test, STA LIA D-Dimer, Roche), C-reactive protein (CRP; turbidimetry, Roche), interleukin 6 (IL-6; IL-6-EASIA-CB, Biosource Europe, Nivelles, Belgium), procalcitonin (PCT; luminescence immunoassay; Brahms, Berlin, Germany), bilirubin (DPD method, Roche), glutamine-oxalacetic transaminase (GOT; Opt Standard Method of the DGKC, Roche, GOT), glutamate-pyruvate-transaminase (GPT; Opt Standard Method of the DGKC, Roche, GPT). To detect changes from the baseline values, clinical and laboratory data were recorded beginning from the day before ATG treatment was initiated up until day 4. Thus, three complete treatment cycles of ATG-F were completely documented.

Transplant-associated complications and engraftment

Up to day 100 after transplantation the following complications were recorded: infection (defined as fever >38.5ºC), acute GVHD (score according to Glucksberg *et al*[4]) and transplant-related mortality. Engraftment was defined as a neutrophil count over 0.5 g/l. Analysis of chronic GVHD was not undertaken, because due to the sequential structure of the study, the longer observation time of protocol A patients would bias the results.

Statistical analyses

All analyses were performed with SPSS 10.0 for Windows software (SPSS, Chicago, IL, USA). All results were expressed as medians (range). Comparisons between groups were carried out using the Mann–Whitney test and the Kruskal–Wallis test. All P values are given as two-sided values.

Results

Clinical parameters

Cardiovascular system: After the start of ATG-F treatment a decline in the minimum systolic blood pressure below the critical value of 100 mm Hg was seen in 52% of patients on protocol A and in 22.7% of patients on protocol B ($P = 0.038$). The shock index (heart rate/blood pressure) was significantly higher for the entire period of treatment in patients receiving protocol A (Figure 1b). A positive shock index (shock index >1.0) as a consequence of hyperdynamic circulation was seen in all patients in the protocol A group and in 59.1% of protocol B patients ($P = 0.001$).

Furosemide needs: Weight was controlled by treatment with furosemide. Compared to the low furosemide doses needed before ATG-F treatment, all patients required significantly higher daily furosemide doses during ATG-F therapy as a consequence of the intensive hydration (Table 1). However, on day 1 the furosemide needs were significantly higher in protocol A patients than in those treated according to protocol B. Retention of fluid may result from either inadequate secretion of ADH, a known complication of cyclophosphamide therapy, or from decreased renal function.

Body temperature: Following initiation of ATG-F treatment, the median temperature rose to a maximum on day 1 in both groups. However, by day 2 the median temperature had returned to the baseline value in protocol B patients, whereas temperatures in protocol A patients stayed elevated during the whole treatment period (Figure 1c). Daily temperature and maximum temperature were significantly higher in group A (39.4ºC) than in group B (38.2ºC). 96.0% of protocol A patients had a body temperature higher than 38.5ºC (101.3ºF), whereas in the protocol B group the percentage was only 36.4% ($P < 0.001$). Blood cultures were taken and found to be negative in all patients.

Platelet transfusions: The need for platelet transfusions following ATG-F treatment increased significantly in both groups. No significant difference was detectable between the two groups; however, the protocol A group showed a tendency towards higher transfusion requirements (0.918 units compared to 0.736 in group B).

Course of transplantation: No significant differences were found between the two groups with respect to infection rate, incidence of acute GVHD, score of acute GVHD, early death rate and time to engraftment (Table 1). However, a tendency toward a lower incidence of acute GVHD and less severe acute GVHD was noted in patients receiving protocol B.

Laboratory parameters

Inflammatory response: All patients had a significant increase in median CRP and PCT values after the start of ATG-F treatment. However, daily median values were significantly higher in patients receiving protocol A than in those receiving protocol B (Table 2). 76% of the patients on protocol A and 31.8% on protocol B group ($P = 0.003$) had PCT values higher than 5 mg/ml, which are thought to be indicative of serious bacterial or fungal infection.[5] The distinctly elevated interleukin-6 values in protocol A patients were almost completely suppressed in protocol B patients. No correlation of inflammatory parameters was found with regard to ATG-F dosage, age, conditioning regimen, leucocyte count or underlying disease.

Coagulation system: We observed a significant lengthening of PT on day 1 in all patients. On day 3 the PT normalized. However, 87.5% of protocol A patients had maximum values beyond the normal upper limit, whereas in protocol B patients the percentage was only 12.5% (Figure 1d; $P < 0.001$). The plasma antithrombin levels were significantly lowered on day 1 only in patients receiving protocol A, whereas in patients receiving protocol B

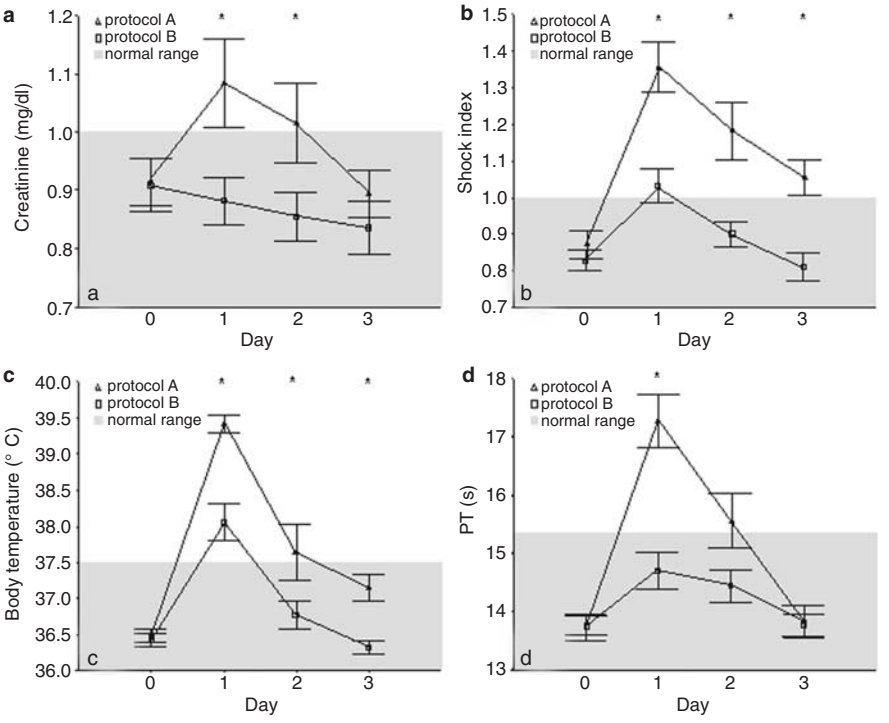

Figure 1 Laboratory and clinical parameters. (**a**) Creatinine serum levels; (**b**) shock index (heart rate/systolic blood pressure); (**c**) body temperature; (**d**) prothrombin time. Data are given as means ± s.e.m. * = Significant difference between protocol A when compared to protocol B ($P < 0.05$). All values indicate the maximum of the day.

values stayed normal. 75% of the patients on protocol A had minimal values below the lower limit of 80%, indicating disseminated intravascular coagulation (DIC), whereas in patients on protocol B the percentage was only 25% ($P = 0.003$). As a consequence of DIC, a significant increase of D-dimer was found beginning 24 h after the start of ATG-F in protocol A patients, whereas in protocol B patients the increase was significant only on day 2 and much lower than in the other group (Table 2). Maximum D-dimer values were significantly higher in protocol A patients (8.1 mg/ml) compared to those on protocol B (2.1 ng/ml; $P = 0.049$). Most patients had already had chemotherapy and/or irradiation before the start of ATG-F therapy and the platelet counts were kept above 20 g/l by transfusion. Platelet numbers were thus not significantly different between the two groups. No patient had clinical signs of disseminated intravascular coagulation, such as local or diffuse haemorrhage.

Kidney function: Patients receiving protocol A had significantly higher creatinine values on day 1 compared to the baseline levels and significantly higher creatinine values on days 1 and 2 compared to patients receiving protocol B (Figure 1a). In 75.0% (A) *vs* 36.4% (B) of the patients, ATG-F induced a rise in creatinine ($P = 0.001$). The increase was smaller in protocol B patients (0.174 mg/dl *vs* 0.004 mg/dl, $P = 0.002$). No correlation between the rise in creatinine and ATG-F dosage, age, conditioning regimen, leucocyte count, underlying disease or creatinine value before the beginning of ATG-F treatment was found. Creatinine levels remained constant in those eight patients receiving cyclophosphamide 1 day prior to ATG-F. Renal impairment normalized after the end of ATG-F treatment and did not predict later renal impairment in the course of transplantation. Cases of serum sickness or nephritis were not observed.

Liver parameters: The mean bilirubin level increased continuously during ATG-F therapy in 90.9% of patients, irrespective of the treatment protocol. Bilirubin levels beyond the normal range were newly observed in 22.7% of the patients. In most patients we found no change in the level of transaminases (data not shown). However, one patient on protocol A had a significant elevation of liver enzymes during treatment with ATG-F, indicating parenchymal damage. The values had normalized again by day 3. In those eight patients receiving cyclophosphamide the day before ATG-F therapy was initiated no significant rise in bilirubin values was noted 24 h prior to the ATG-F infusion. Intravascular hemolysis was excluded in all patients with elevated bilirubins, as lactate dehydrogenase and haptoglobin levels were normal.

Figure 2 summarizes the organ toxicities of the two treatment protocols. The graph gives the frequency of organ-specific side-effects of ATG-F.

Discussion

Acute side-effects of ATG may be serious and produce much discomfort for the patient. This study reports our experiences after including ATG-F in the conditioning regimens for allogeneic haematopoietic transplantation. If administered over 4 h following premedication with daily

Table 2 Cllinical and laboratory parameters

	Protocol A	*P value*	*Protocol B*
Furosemide requirement (mgl)			
day 0	5 (0–60)	NS	20 (0–80)
day 1	60 (0–80)*	0.048	35 (0–120)*
day 2	80 (20–300)*	NS	80 (0–300)*
day 3	60 (0–280)*	NS	45 (20–310)*
Platelet transfusions			
day 0	0 (0–3)*	NS	0 (0–2)
day 1	0 (0–3)	NS	0 (0–2)*
day 2	2 (0–4)*	NS	1 (0–3)*
day 3	1 (0–4)*	NS	1 (0–3)*
procalcitonin (ng/ml)			
day 0	0.1 (0.1–45.9)	NS	0.1 (0.1–0.4)
day 1	19.5 (1.5–132.0)*	<0.001	1.7 (0.1–48.7)*
day 2	12.4 (0.5–95.7)*	<0.001	0.9 (0.1–31.8)*
day 3	5.9 (0.3–61.0)*	<0.001	0.7 (0.1–16.3)*
CRP (mg/dl)			
day 0	0.8 (0.5–4.2)	NS	0.7 (0.5–6.6)
day 1	9.7 (5.4–18.5)*	<0.001	5.3 (1.3–10.0)*
day 2	12.0 (1.2–27.3)*	<0.001	5.9 (1.2–15.1)*
day 3	9.0 (2.8–18.4)*	<0.001	3.4 (0.7–11.5)*
Interleukin 6 (ng/ml)			
day 0	22.9 (1.5–436.0)	NS	12.5 (2.2–179)
day 1	397.0 (72–14161)*	<0.001	23.4 (2–2339)
day 2	146.5 (22–4386)*	<0.001	12.5 (2–633)
day 3	56.7 (14–2062)*	<0.001	14.0 (2–237)
Antithrombin (%)			
day 0	88 (57–114)	NS	96 (70–125)
day 1	76 (44–103)*	0.002	90 (68–124)
day 2	86 (65–107)	0.05	97 (72–115)
day 3	90 (59–117)	NS	97 (78–125)
D-dimer (μg/ml)			
day 0	1.2 (0.5–9.4)	NS	1.3 (0.5–8.2)
day 1	10.5 (1.1–40.0)*	0.006	2.1 (0.6–32.5)*
day 2	4.1 (0.8–40.0)*	0.046	1.8 (0.5–15.8)
day 3	2.8 (0.5–16.0)	NS	1.1 (0.5–8.0)
Platelets (g/l)			
day 0	43 (15–180)	NS	40 (9–388)
day 1	20 (4–263)	NS	25 (3–243)
day 2	16 (9–120)*	NS	22 (5–129)*
day 3	17 (4–71)*	NS	15 (3–124)*
Bilirubin (mg/dl)			
day 0	0.58 (0.2–2.2)	NS	0.50 (0.3–1.2)
day 1	0.60 (0.3–2.3)	NS	0.63 (0.3–1.2)
day 2	0.80 (0.4–2.6)*	NS	0.72 (0.4–1.5)*
day 3	1.10 (0.6–3.2)*	NS	1.05 (0.5–1.6)*

All values are medians (range) and percentage (numbers) and give the maximal (CRP, procalcitonin, interleukin 6, D-dimer, bilirubin)/minimal (all others) value of the day,
NS = not significant. * = significant when compared to day 0 ($P < 0.05$). *P* value = protocol A compared to protocol B, determined by the Mann–Whitney *U* test; furosemide requirement = daily furosemide dose needed to balance the patient's fluid; body temperature = maximum body temperature of the day; platelet transfusions = transfused platelet units per day.

dosages of 100 mg prednisolone, ATG-F caused a high rate of serious complications. In spite of intense monitoring and preparative measures taken to raise the CVP in the patients, 87.5% of patients showed laboratory signs of disseminated intravascular coagulation (DIC), 52% experienced critical hypotension and 96% suffered from intense inflammatory reactions with fever and chills. Impaired renal function was observed in 75% of cases, an increase of bilirubin occurred in 90% of patients and in one patient toxic hepatocellular damage occurred. Although side-effects were most severe on day 1, and toxic side-effects resolved within a few days in all patients without sequelae, these complications have a substantial impact on patient morbidity. In contrast, prolonged treatment with ATG-F over 12 h with 250 mg prednisolone given every 6 h in parallel with ATG-F highly reduced the rate of serious complications, making ATG-F a well-tolerated drug. Cases of serum sickness or nephritis were observed with neither of the protocols, possibly due to the steroid premedication and the long aplastic period following ATG-F use.

ATG-F is a purified polyclonal immunoglobulin G (IgG) prepared from the plasma of healthy rabbits hyperimmunized with a human T cell line (Jurkat).[6] ATG-F binds to the surface of circulating T lymphocytes, resulting in

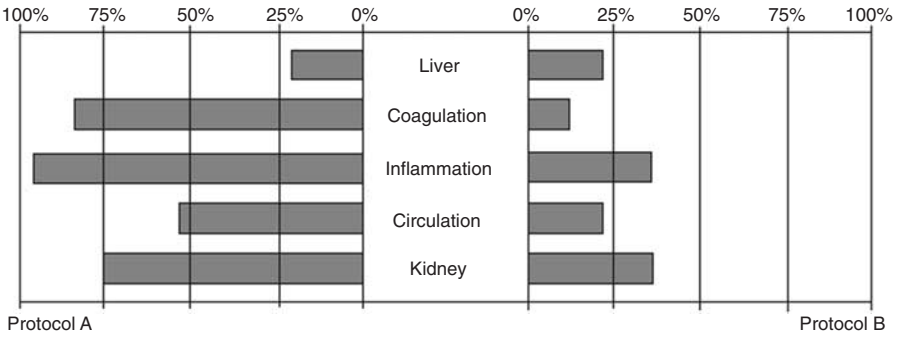

Figure 2 Frequency of side-effects. The graph gives the frequency of organ-specific side-effects of ATG-F. Liver = pathologic bilirubin; coagulation = PT beyond 15.4 s; inflammation = body temperature >38.5ºC; circulation = systolic blood pressure below 100 mm Hg; kidney = increase of creatinine. Side-effects of protocol B are significantly lower for all organ systems, except liver.

lymphopenia and impaired T cell immune responses and persists *in vivo* for up to 3 weeks after administration.[7] Documented information on the side-effects of ATG-F is rare, although it is widely used in treatment of SAA, rejection after organ transplantation and in HSCT. Published reports observed only very mild symptoms.[8–14] However, in most cases only low doses of ATG-F (2–5 mg/kg daily) were given. The much higher doses used during conditioning before HSCT may cause an accelerated activation and disintegration of T-lymphocytes, possibly inducing an intense cytokine release syndrome leading to the serious side-effects observed in our study. Titiz *et al*[9] reported an accidental injection of 20 mg/kg in one patient, who suffered serious side-effects with fever, disseminated intravascular coagulation and retroperitoneal haematoma due to the lympholysis.

The observed clinical symptoms may be accounted for by a systemic inflammatory response syndrome (SIRS) triggered by the antibody, which may be comparable to that described following OKT-3 treatment.[10] ATG-F, possibly combined with products of generalized T cell lysis, might induce monocytes and macrophages to synthesize tumour necrosis factor α (TNFα) and interleukin-1 (IL-1), both playing a central role in the pathogenesis of SIRS. Indeed, release of cytokines, especially of TNFα, has been proven to be the main mediator of SIRS following ATG in studies using monoclonal anti-TNFα antibodies during pretransplant conditioning. In a recently evaluated randomized trial from our group,[11] patients receiving anti-TNF-antibody and ATG had significantly less fever, less increases in CRP levels, as well as reduced lengthening of the PT and drop in platelets as compared to those receiving placebo and ATG. As IL-6 levels in blood have been demonstrated to be proportional to the level of immune effector cell activation,[12] the exorbitantly high IL-6 values in the patients receiving protocol A indicate a generalized secondary activation of all circulating leucocytes, which may lead to disturbance of the microcirculation and ischemic injury of end organs. Combined with significantly lowered blood pressure, this may explain the renal dysfunction in our patients. As it is well known that the cytokine response activates coagulation and subsequently inhibits the fibrinolytic system,[13,14] the observed impairment of the coagulation system and the initiation of disseminated intravascular coagulation (DIC) may also be regarded as a consequence of the cytokine storm and may contribute to the development of organ failure.

The data in this study show that high-dose prednisolone and a prolonged infusion time substantially attenuate the undesirable side-effects of ATG-F. Due to the design of the study, it is not clear whether repeated treatment with high-dose corticosteroids, or prolonged infusion time, or both, contribute to the beneficial effects in protocol B. Prolonged infusion times may lead to a delayed liberation of cytokines with lower peak values and less intense stimulation of the immune system. In addition, prolonged infusion might allow better induction of counterregulatory cytokines such as interleukin-10. As previously shown for the first dose effects of OKT3,[15] high-dose corticosteroids may also be beneficial. Although the pure genomic action of prednisolone via cytosolic steroid receptors is limited to doses up to 200–300 mg per day, higher doses of steroids have specific non-genomic effects on membranous steroid receptors rather than non-specific biophysical effects which subsequently enhance the immunosupressive effect.[16] Although it is known that steroid treatment of patients with SIRS has no beneficial effects,[17–19] SIRS following ATG-F differs in several ways from SIRS associated with clinical infectious complications. It is noticeable that the side-effects of ATG-F are most severe after 1 day of treatment and then recede within a few days, whereas in septic patients there is ongoing and prolonged activation, which has been shown to be crucial for multiple organ dysfunction.[18,19] Furthermore, in ATG-related SIRS, corticosteroids are used in a preemptive way, and preemptive cytokine neutralisation has also been effective in models of shock induced by bolus application of endotoxin. Interestingly, high-dose steroids did not increase the infection and infection-related death rate within a 100-day period, indicating that the intensified immunosuppression over a period of only a few days has no impact on the pathogenesis of these complications.

As all patients also received cyclophosphamide starting 2 h after the end of the daily ATG-infusion, an influence of cyclophosphamide on the observed side-effects cannot be completely excluded. Indeed, it is well known that cyclophosphamide may cause SIADH syndrome,[20] which may explain the increased doses of furosemide required to control weight. However, coagulation impairment and high inflammation activity were already observed in most

patients even before cyclophosphamide was administered. Since modification of ATG-F treatment protocol crucially tempered most of the side-effects, an influence of cyclophosphamide is rather unlikely. In contrast, rising bilirubin levels were observed in 90% of the patients, and one patient also had elevated transaminases. As this side-effect was irrespective of the treatment protocol of ATG-F, it might have been due to direct toxic effects by cyclophosphamide or ATG-F.

Procalcitonin (PCT), a precursor peptide of calcitonin, has recently been reported to be a very useful and reliable marker for diagnosing and monitoring systemic bacterial and fungal infections. As it is much more specific for inflammatory processes due to bacterial or fungal infections than the commonly used parameters such as C-reactive protein (CRP) and white blood cell count,[21,22] it has proven helpful in distinguishing between infectious and noninfectious causes in clinical situations, such as ARDS,[23] burn patients[24] and autoimmune diseases.[25] However, this study demonstrates a massive elevation of procalcitonin plasma levels in the absence of infection with ATG-F treatment. Similar observations were made by Sabat *et al*[26] who found high PCT values in patients having antibody treatment. This indicates that PCT is a parameter of massive cytokine activation, secondary to various causes.

Due to the long half-life of ATG,[7] duration of ATG treatment should have no impact on the effectiveness of ATG, especially if *in vivo* T cell depletion is thought to be the relevant mechanism of ATG prophylaxis. Indeed, analysis of acute GVHD rate indicates no impact of the application on this complication. In addition, since altered immunoregulation via blockade of adhesion molecules or via secondary induction of anti-inflammatory cytokines, such as interleukin-10 are thought to contribute to ATG effects, and as high TNFα levels before transplantation even predict transplant complications,[27] it may be speculated that ATG treatment over 12 h preceded by high-dose prednisolone even may have beneficial long-term effects. Indeed, complication rates are similar with both protocols with a beneficial tendency for the protocol with high-dose steroids and a long infusion time. However, recognising the limitations of the small number of patients and the heterogeneity of the study population, these results have to be confirmed in further investigations.

In conclusion, use of ATG-F with a prolonged infusion time along with high-dose steroid premedication significantly reduces the severe acute toxicity without undesired long-term side-effects, making ATG-F a well-tolerated drug for prophylaxis of acute GVHD.

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