ASBMT American Society for Blood and Marrow Transplantation

Anti-T Cell Antibodies as Part of the Preparative Regimen in Hematopoietic Cell Transplantation—A Debate

Frederick R. Appelbaum,[1] Andrea Bacigalupo,[2] Robert Soiffer[3]

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) often offers the best, and sometimes the only, chance for cure for thousands of patients with hematopoietic malignancies. The effectiveness of allogeneic HCT is, however, limited by the development of graft-versus-host disease (GVHD), by opportunistic infections, and by disease recurrence. Anti-T cell antibodies are often used as a component of the preparative regimen before allogeneic HCT in an effort to reduce the incidence and severity of GVHD. However, whether or not it is appropriate to include such reagents is unclear. The following article includes a brief summary of arguments for the inclusion of anti-T cell antibodies provided by Dr. Bacigalupo, a summary of arguments against their inclusion provided by Dr. Soiffer, and finally a summary of some of the major unanswered questions.

THE CASE FOR ANTI-T CELL ANTIBODIES IN ALLOGENEIC HCT

Anti-T cell antibodies have been explored as part of the preparative regimen because of the early days of allogeneic HCT. In some of the earliest experiments, antilymphocyte serum was the sole preparation for transplantation. The IgG fraction of antilymphocyte serum was later purified to produce antilymphocyte globulin (ALG) or antithymocyte globulin (ATG), the name depending on the immunizing cell population. The immunized animal was either a horse or rabbit. The advent of monoclonal antibodies in the early 1980s brought CAMPATH, which has been used predominantly, but not exclusively in Great Britain. ALG, ATG, and CAMPATH have been extensively tested in the clinic both for prevention as well as for treatment of acute GVHD (aGVHD). However, despite their widespread use and thousands of patients who have received these agents, the question still remains open today: Are anti-T cell antibodies useful as part of the transplant preparative regimen? The uncertainty is reflected in the fact that some centers include anti-T cell antibodies in the preparative regimen for almost all of their patients, while others never use them. To answer this question, this section will examine retrospective studies and the few prospective trials published in the past 2 decades.

ATG

Retrospective studies

In a retrospective study conducted by the International Bone Marrow Transplant Registry, 386 patients receiving ATG in their conditioning regimens were compared with 474 not receiving ATG as GVHD prophylaxis: grade II-IV aGVHD was 19% versus 51% ($P < .001$) and GVHD grade III-IV was 10% versus 22% ($P < .001$) [1]. In smaller single-center trials, the Hamburg group compared the outcome of good risk myeloid leukemia patients who received a conditioning regimen with (n = 45) or without ATG (n = 57) [2]. Acute GVHD II-IV was seen in 20% of ATG patients versus 47% of non-ATG patients ($P = .004$) and grade III-IV was seen in 7% versus 32% ($P = .002$). The Alberta, Canada, group completed a matched pair analysis of unrelated donor transplants receiving ATG in the conditioning regimen with HLA identical sibling transplants not receiving ATG [3]. In this study, all patients received the FLUBUP regimen (fludarabine, intravenous busulfan, peripheral blood allogeneic stem cells). Acute GVHD grade II was 19% versus 36% for patients receiving or not receiving ATG; grade III-IV was 10% versus 18%. This study suggests that patients receiving unrelated donor peripheral blood transplants with ATG have less aGVHD when compared with patients receiving HLA identical sibling peripheral blood transplants without ATG.

From the [1]Fred Hutchinson Cancer Research Center, Seattle, WA; [2]S. Martino's Hospital, Genova, Italy; and [3]Dana-Farber Cancer Inst., Boston, MA.

Financial disclosure: See Acknowledgments on page S115.

Correspondence and reprint requests: Frederick R. Appelbaum, MD, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N., D5-310, Seattle, WA, 98109.

1083-8791/$36.00
doi:10.1016/j.bbmt.2011.11.002

Randomized trials

There have been 3 randomized trials to test the hypothesis that ATG can prevent aGVHD [4-6]. These 3 studies included a total of 166 patients: In the 88 receiving ATG in the conditioning, the risk of aGVHD grade II-IV was 37% versus 72% ($P < .001$) for 88 patients not receiving ATG. The risk of grade III-IV GVHD was 11% versus 36% ($P < .001$). All 3 studies came to the same conclusion: Patients randomized to receive ALG/ATG have a significantly lower risk of aGVHD.

There have been 2 trials now available with longer follow that test the hypothesis that chronic GVHD (cGVHD) can largely be prevented when ATG is added to the standard GVHD prophylaxis combination of cyclosporine (CyA) and methotrexate (MTX) [5,7]. The first of these, the Italian Group for Marrow Transplantation trial, involved 109 patients who underwent an unrelated donor transplantation and randomized patients who received or did not receive ATG (Thymoglobulin, Genzyme, Cambridge, MA) (7.5-15 mg/kg) in the conditioning regimen. All patients were prepared with cyclophosphamide and total body irradiation, followed by CyA/MTX as GVHD prophylaxis, and all received unmanipulated bone marrow as the stem cell source [5]. The study was updated in 2006, looking specifically at cGVHD in the 75 patients who survived 100 days after bone marrow transplantation with a median follow-up of over 2000 days. Patients randomized to the non-ATG (n = 37) or ATG group (n = 38) were matched for age, disease, and disease phase. Each patient was updated for survival, cGVHD, chronic lung dysfunction (CLD), relapse of the original disease, and quality of life assessed by Karnofsky score.

At last follow-up, cGVHD developed in 60% versus 37% respectively, for non-ATG and ATG patients ($P = .05$) and extensive cGVHD in 41% versus 15% ($P = .01$). The cumulative incidence of CLD was 51% for non-ATG versus 19% for ATG patients ($P = .005$). In the non-ATG group, there was a significant decrease of FEV_1 beyond 2 years (average delta of −23%, $P = .02$) and the same was true for FVC (average delta of −20%, $P = .005$). This was not the case for patients receiving ATG (ΔFEV_1: −3%, $P = .2$; and ΔFVC: +3%, $P = .3$). The median FEV_1/FVC ratio was 1.02 and 1.04 for non-ATG and ATG patients, respectively. Bidimensional plots of FVC in the 2 groups, expressed as percent of predicted values, showed ATG patients with stable FVC with time, whereas non-ATG patients exhibited progressive worsening of FVC, with average FVC of just over 50%, 2500 days posttransplantation.

The proportion of patients with a Karnofsky score of ≥90% in the non-ATG versus ATG patients was 28% versus 44% at 1 year ($P = .2$), 62% versus 63% at 2 years ($P = .9$), 56% versus 95% at 4 years ($P = .005$), and 57% versus 89% beyond 4 years ($P = .03$) [8].

This study shows that ATG given pretransplantation provides (1) significant protection against cGVHD in patients undergoing unrelated donor bone marrow transplantation with CyA plus MTX prophylaxis, (2) reduces the risk of chronic lung dysfunction, (3) improves quality of life, and (4) reduces late transplant-related mortality. The latter point became evident only after a median follow-up of 7 years, because mortality because of cGVHD and CLD may take years to become manifest.

Potential drawbacks of this study, performed in the 1990s, are the small number of patients; the possibility of mismatched unrelated donors, according to current typing techniques for HLA typing; the high number of chronic myeloid leukemia patients; and the fact that 2 different doses of ATG were used (7.5 and 15 mg/kg). However, in the update analysis [8], both doses of ATG protected against cGVHD and CLD.

The second study to evaluate the effect of adding ATG to conventional CyA + MTX was published more recently [7]. In this study, 201 patients with hematologic malignancies were randomized to receive CyA and MTX with or without additional ATG-fresenius (ATG-F) and were evaluable. The stem cell source was peripheral blood (n = 164; 82%) or bone marrow (n = 37; 18%), the donor type was unrelated, and the conditioning regimen was myeloablative. The analysis comprised 103 ATG-F patients and 98 controls. The cumulative incidence of aGVHD grade III–IV was 11.7% in the ATG-F group versus 24.5% in the control group ($P = .054$), and the cumulative incidence of aGVHD grade II–IV was 33% in the ATG-F group versus 51% in the control group ($P = .011$). The 2-year cumulative incidence of extensive cGVHD was 12.2% versus 42.6% ($P = .0001$).

This study has been updated and extensive cGVHD at last follow-up was 45% for control patients and 12% for ATG patients [9]. The authors conclude that the addition of ATG-F to GVHD prophylaxis with CyA and MTX decreases cGVHD significantly and increases the proportion of patients alive without immunosuppressive therapy.

It is striking that 2 separate prospective studies, 1 performed in the 1990s and 1 in the last decade, using 2 different ATG products (Thymoglobulin and ATG-F), have produced nearly the same figures: The addition of ATG to a combination of CyA + MTX reduces the risk of extensive cGVHD from 41% to 45% to 12% to 15%.

An additional randomized trial examined the effect of marrow T cell depletion on the outcome of transplantation. Although this study does not directly address the question of whether anti-T cell antibodies should be added to the transplant preparative regimen, the results are nonetheless informative. In this study,

which included over twice as many patients as in the German trial, patients were randomized in the United States to receive a stem cell graft depleted of T cells (TCD) or not [1]. The study showed a reduced risk of aGVHD for patients receiving TCD grafts, but no difference in cGVHD. This trial has several problems that may explain the lack of efficacy on cGVHD. In the first place, it compared CyA + MTX versus TCD + CyA: So instead of adding TCD to a standard regimen (CyA + MTX), the study arm was devoid of MTX. Second, the TCD methods were different and included elutriation, the use of a monoclonal antibody, or horse ATG. Third, the conditioning regimens used for controls and TCD patients were different, with increased intensity of the regimens administered to TCD patients [10]. Therefore, no firm conclusion as to the effect of ATG added to CyA + MTX can be drawn from this study. The U.S. study did show that T cell depletion, as provided by the study protocol, did not reduce cGVHD. The question as to whether ATG added to CyA + MTX reduces cGVHD was not directly asked.

The Italian and German trial instead show that ATG (no matter what brand) added to a conventional CyA + MTX regimen significantly reduces cGVHD, and in the Italian trial, CLD. Also, the German trial shows that control patients had more "respiratory insufficiency" than in the ATG arm, although it is not specified whether this was acute or chronic respiratory insufficiency.

CAMPATH

Although CAMPATH has been extensively used in thousands of patients, there are no prospective randomized trials comparing patients receiving or not receiving CAMPATH.

In single-arm studies, CAMPATH has been shown to prevent aGVHD and cGVHD, although some of these studies have also shown an increased risk of relapse.

Conclusions

T cell antibodies, and specifically ATG, reduce aGVHD and cGVHD, as demonstrated in prospective randomized trials. An increased risk of infections has been documented in patients receiving ATG, in particular, Epstein-Barr virus-related infections; this problem can be addressed by using prophylactic or preemptive rituximab.

Reduction of aGVHD and cGVHD does not necessarily translate into a survival advantage in the short/medium term (3-5 years), but may result in less cGVHD-related deaths in the long term (beyond 5-10 years). Also, if survival is equivalent for patients receiving or not receiving ATG, quality of life would be expected to be superior in ATG patients because of the lower rate of cGVHD. So the answer to the question, "Are T cell antibodies beneficial if used as part of the preparative regimen in allogeneic HCT?" is yes in terms of GVHD, quality of life, and possibly life expectancy.

THE CASE AGAINST ANTI-T CELL ANTIBODIES IN ALLOGENEIC HCT

For over 30 years, in vivo T cell depletion with ATG preparations has been used by some groups with hopes of reducing GVHD, enabling engraftment, and limiting transplant-related mortality. Unfortunately, after this long period of time, the benefits of ATG have yet to be clearly established. Although case series of transplantations that included ATG suggested it may decrease GVHD, few adequately powered prospective randomized studies have been conducted to address the true effect of ATG on outcome.

One of the challenges in interpreting clinical reports is the variety of ATG preparations in use. ATG may be derived from horse or rabbit serum and can be raised against distinct cellular targets. The difference in derivation of these products as well as the various dose schedules employed in clinical trials impact both the extent and specificity of T cell depletion and immune reconstitution, consequently influencing GVHD, infectious complications, relapse, and survival.

Two small randomized trials reported from Italy suggested that in patients undergoing myeloablative transplantation from unrelated donors, a dose of 7.5 mg/kg thymoglobulin did not reduce rates of grade III-IV aGVHD, whereas a significant reduction was noted at a dose of 15 mg/kg. However, at the higher dose, there was an increase in fatal infections, negating any survival benefit for patients receiving thymoglobulin. In addition, platelet recovery was impaired at the higher dose level. Chronic GVHD incidence was significantly lower in patients receiving thymoglobulin [5]. A retrospective analysis of the use of ATG in myeloablative transplantation from France demonstrated no significant reduction in grades III-IV aGVHD, but a significant reduction in cGVHD [11]. In this report, there was no benefit in terms of nonrelapse mortality or overall survival (OS). A recent prospective randomized trial from Europe in the myeloablative setting compared no in vivo depletion versus in vivo T cell depletion using an ATG preparation (Fresenius) in 202 patients with hematologic malignancies [7]. In this study, aGVHD and cGVHD were lower with ATG. Relapse and nonrelapse mortality rates were similar in the 2 cohorts, and there were no significant differences in OS rates between ATG recipients and those who did not receive ATG. Although these studies do suggest that ATG can reduce cGVHD, which

can be disabling for some patients, there is no indication that ATG preparations will improve survival.

In the past decade, there has been a dramatic increase in the use of reduced-intensity conditioning (RIC) for allogeneic transplantation. Based on experience in the myeloablative setting, ATG preparations have been incorporated into RIC regimens by many groups in the absence of any substantial prospective data [12,13]. The success of RIC transplantation relies on the integrity of graft-versus-tumor activity because the cytoreductive effects of RIC are usually insufficient to eradicate malignancy. As such, immune manipulations that might weaken allo-immunity might compromise the therapeutic effect of transplantation. Recently, the Center for International Blood and Marrow Transplant Research studied adult patients undergoing RIC transplantation for hematologic malignancies from 7-8/8 related and unrelated donors [14]. This analysis included 584 patients in whom ATG was administered and 879 in whom it was not. Grade II-IV aGVHD was not impacted by ATG (38% versus 40%), although cGVHD, as noted in the ablative studies above, was lower in ATG recipients (40% versus 52%). However, relapse was more frequent with ATG compared with T cell–replete regimens (51% versus 38%, respectively, $P < .001$). Moreover, nonrelapse mortality and Epstein-Barr virus lymphoproliferative disease was higher with ATG. Consequently, disease-free survival was lower in ATG recipients (25% versus 39%, $P < .001$) as was OS (38% versus 46%). These results suggest that in the RIC setting, although ATG may be associated with a lower risk of cGVHD, decreased immune surveillance might negatively impact overall outcome.

At this point in time, there is insufficient evidence to advocate for the routine use of ATG in allotransplantation, particularly in the reduced-intensity study. However, transplant physicians are encouraged to enroll their patients on well-designed prospective randomized trials to definitively address the question of ATG and its role in improving quality of life and survival.

DISCUSSION

Any good debate should begin with a definition of terms. One of the difficulties in deciding the role of anti-T cell antibodies as part of a preparative regimen is the variability in the studies so far performed, particularly with regard to the form of antibody, the type of preparative regimen, and the source of stem cells. At least 4 different preparations of anti-T cell globulins are now commercially available: ATG-F, which is produced by immunizing rabbits with the Jurkat human T-lymphoblastic cell line; thymoglobulin, which is produced by immunizing rabbits with human thymocytes; and ATGAM and lymphoglobulin, which are produced by immunizing horses with human thymocytes. Because they are produced in different ways, it is not surprising that these different types of ATG contain variable specificities and amounts of antibodies. The cytotoxicities of these 4 ATGs on T cells are roughly similar; however, the clinically achievable concentrations of these agents vary considerably from about 75 μg/mL for thymoglobulin to up to 1000 μg/mL for ATG-F. However, it is becoming clear that T cell depletion is only part of the effect of these preparations. The recent report showing that horse-derived ATGAM is more effective than the rabbit ATG thymoglobulin in the treatment of aplastic anemia, despite the fact that the rabbit product produces more profound and prolonged lymphopenia, brings home the fact that we do not completely understand the biologic effects of these complex antibody mixtures [15]. Until we do, we should probably refrain from extrapolating the results of a study involving 1 ATG preparation to results with others, no matter how intuitively appealing it might be.

The transplant setting in which the reagent is tested almost certainly makes a difference as well. Dr. Bacigalupo's compelling arguments for the use of ATG were mostly based on clinical trials involving myeloablative conditioning regimens. It is reassuring that in that setting, the decreased incidence of grade III-IV aGVHD and cGVHD was not offset by an increase in relapse rates. The majority of patients (64%) in the treatment group had early-stage disease, and so it is uncertain if an impact on relapse would emerge among higher-risk patients. In contrast, Dr. Soiffer's analysis of the impact of anti-T cell antibodies in transplant recipients receiving RIC found a striking increase in relapse with CAMPATH or ATG versus T-replete transplants (relapse rates 49%, 51%, and 38%, respectively, $P < .001$). The increase in relapse rates and opportunistic infections when anti-T cell antibodies were included in reduced-intensity regimens resulted in inferior OS and suggests that such antibodies should not be routinely used in this setting.

Finally, the source of stem cells also likely affects the impact of the inclusion of anti-T cell antibodies. The majority of patients entered into the studies cited by Bacigalupo and Soiffer above received transplants from unrelated donors. In analyses available to date, no large difference in the effects of ATG depending on donor source (related versus unrelated) were noted, but the studies were not designed or sized to detect possible differences. In the myeloablative studies cited by Bacigalupo, there was variability in the use of either bone marrow or peripheral blood stem cells. A recently completed Bone and Marrow Transplant Clinical Trials Network randomized trial has shown increased cGVHD and no survival advantage with the use of peripheral blood versus bone marrow for unrelated donor transplants. ATG was not part of this trial.

In the setting of myeloablative transplants, whether peripheral blood with ATG is equivalent or better than marrow with or without ATG is untested.

ACKNOWLEDGMENTS

Financial disclosure: Dr. Bacigalupo has received small consulting fees from Fresenius and Genzyme. The authors have nothing to disclose.

REFERENCES

1. Bacigalupo A. Antithymocyte globulin for prevention of graft-versus-host disease. *Curr Opin Hematol*. 2005;12:457-462.
2. Kroger N, Zabelina T, Kruger W, et al. In vivo T cell depletion with pretransplant anti-thymocyte globulin reduces graft-versus-host disease without increasing relapse in good risk myeloid leukemia patients after stem cell transplantation from matched related donors. *Bone Marrow Transplant*. 2002;29:683-689.
3. Duggan P, Booth K, Chaudhry A, et al. Unrelated donor BMT recipients given pretransplant low-dose antithymocyte globulin have outcomes equivalent to matched sibling BMT: a matched pair analysis. *Bone Marrow Transplant*. 2002;30:681-686.
4. Ramsay NKC, Kersey JH, Robison LL, et al. A randomized study of the prevention of acute graft-versus-host disease. *N Engl J Med*. 1982;306:392-397.
5. Bacigalupo A, Lamparelli T, Bruzzi P, et al. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood*. 2001;98: 2942-2947.
6. Weiden PL, Doney K, Storb R, Thomas ED. Antihuman thymocyte globulin for prophylaxis of graft-versus-host disease. A randomized trial in patients with leukemia treated with HLA-identical sibling marrow grafts. *Transplantation*. 1979;27: 227-230.
7. Finke J, Bethge WA, Schmoor C, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol*. 2009;10:855-864.
8. Bacigalupo A, Lamparelli T, Barisione G, et al. Thymoglobulin prevents chronic graft-versus-host disease, chronic lung dysfunction, and late transplant-related mortality: Long-term follow-up of a randomized trial in patients undergoing unrelated donor transplantation. *Biol Blood Marrow Transplant*. 2006;12: 560-565.
9. Socié G, Schmoor C, Bethge WA, et al. Chronic graft-versus-host disease: long-term results from a randomized trial on graft-versus-host disease prophylaxis with or without anti-T-cell globulin ATG-Fresenius. *Blood*. 2011;117:6375-6382.
10. Pavletic SZ, Carter SL, Kernan NA, et al. Influence of T-cell depletion on chronic graft-versus-host disease: results of a multicenter randomized trial in unrelated marrow donor transplantation. *Blood*. 2005;106:3308-3313.
11. Mohty M, Labopin M, Balère ML, et al. Antithymocyte globulins and chronic graft-vs-host disease after myeloablative allogeneic stem cell transplantation from HLA-matched unrelated donors: a report from the Sociéte Française de Greffe de Moelle et de Thérapie Cellulaire. *Leukemia*. 2010;24:1867-1874.
12. Hamadani M, Blum W, Phillips G, et al. Improved nonrelapse mortality and infection rate with lower dose antithymocyte globulin in patients undergoing reduced-intensity conditioning allogeneic transplantation for hematological malignancies. *Biol Blood and Marrow Transplant*. 2009;15:1422-1430.
13. Johnston L. Acute graft-versus-host disease: differing risk with differing graft sources and conditioning intensity. *Best Pract Res Clin Haematol*. 2008;21:177-192.
14. Soiffer RJ, Lerademacher J, Ho V, et al. Impact of immune modulation with anti-T-cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. *Blood*. 2011;117:6963-6970.
15. Scheinberg P, Nunez O, Weinstein B, et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. *N Engl J Med*. 2011;365:430-438.