Purpose: Thymoglobulin (ATG) given before allogeneic hematopoietic stem cell transplantation (HSCT) with unrelated donors reduces acute graft-versus-host disease (GVHD). However, the possible role of serum concentration of rabbit ATG for the subsequent development of acute GVHD after HSCT is unknown. **Methods:** The serum concentration of rabbit IgG was analyzed by enzyme-linked immunosorbent assay in 61 patients after unrelated donor HSCT. Doses of ATG as part of the conditioning ranged between 4 and 10 mg/kg. Stem cell source was bone marrow (BM) in 28 cases and peripheral blood (PBSC) in 33. Conditioning was mainly cyclophosphamide combined with total body irradiation (TBI) or busulfan. Most patients received GVHD prophylaxis with cyclosporine and methotrexate. **Results:** Even though we found a good correlation between given ATG dose and serum concentration of rabbit IgG after transplant ($r = 0.67$), there was a wide variation of rabbit IgG levels within each dose group. After administration, levels of rabbit IgG decreased slowly and could still be detected up to 5 weeks after HSCT. We found a correlation between grade of acute GVHD and concentration of rabbit IgG in serum obtained before transplantation ($P = .017$). Patients with serum levels of rabbit IgG > 70 mg/ml before HSCT had very low risk for developing acute GVHD grades II-IV compared with those with < 70 mg/ml (11% vs 53%; $P < .001$). **Conclusion:** Measuring rabbit IgG levels in patients receiving ATG as prophylaxis against GVHD after HSCT may be a helpful tool in decreasing the risk of severe GVHD.

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GENERATION OF TOLEROGENIC DENDRITIC CELLS AND REGULATORY T CELLS THROUGH INTRAVENOUS DELIVERY OF AUTOLOGOUS PHOTOPHERESIS-INDUCED APOPTOTIC CELLS

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Phagocytosis of apoptotic cells by macrophages or dendritic cells has been shown to regulate immune responses both in vivo and in vitro. Extracorporeal photopheresis (ECP) involves the clinical reinfusion of peripheral blood leukocytes that are undergoing apoptosis following exposure ex vivo to 8-methoxypsoralen (8-MOP) and UVA light. ECP is approved for the palliative treatment of cutaneous T-cell lymphoma and has been reported to have utility in immune-mediated inflammatory diseases, such as graft-versus-host disease (GvHD) and solid-organ transplant rejection, and autoimmune diseases, such as rheumatoid arthritis and Crohn's disease. We have evidence to suggest that ECP therapy may modulate host dendritic cell function and induce regulatory T-cell generation. When coincubated with ECP-treated cells, activated dendritic cells produce reduced levels of proinflammatory cytokines, such as interleukin-12, whereas transforming growth factor-β levels are modestly increased. Activation of CD4+ T cells in the presence of allogeneic dendritic cells and ECP-treated cells promotes generation of a population of T cells that can suppress proliferation of naive syngeneic T cells, as well as suppress interferon-γ production. To confirm these findings in vivo, we used a murine contact hypersensitivity model. ECP-treated or control leukocytes from mice sensitized with the hapten dinitrofluorobenzene (DNFB) were injected intravenously into naive recipients. Compared with controls, mice that received ECP-treated cells demonstrated significantly less ear swelling after sensitization and challenge with DNFB. Suppression of ear swelling was specific for DNFB and was cell-mediated, as demonstrated by the ability to transfer DNFB tolerance to naive mice, which could appropriately respond to the unrelated hapten oxazalone. Transfer of this tolerance was abrogated by depletion of either CD4+ or CD25+ T-cell populations. Collectively, these results suggest that the delivery of ECP-treated cells promotes generation of regulatory T cells that are capable of modulating immune responses. Regulatory T cells have been implicated in the control of GvHD, and as previously reported, ECP has demonstrated beneficial activity in GvHD. As a result, international phase II clinical trials are currently underway to assess the efficacy of photopheresis in GvHD patients.

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PRETRANSPLANT RECIPIENT BLOOD CD14+ PREDC LEVELS CORRELATE WITH INCREASED ACUTE GVHD AFTER ALLOGENEIC PBSC TRANSPLANTATION

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Host dendritic cells (DCs) present host alloantigens to donor T lymphocytes. Human peripheral blood contains various circulating DC precursors including CD11c+ myeloid preDC (mDC), CD14+ monocytic DC precursors (CD14+ preDC) and plasmacytoid preDC (pDC). We used flow cytometry to enumerate both mDC (lin-, HLA-DR+, and CD11c+), mono-DC (CD14+), and pDC (lin-, HLA-DR+, and CD123+) numbers in the blood of patients receiving an allogeneic HSCT. Fifty consecutive patients undergoing HSCT from HLA-matched related (n = 28) or unrelated (n = 22) donors were enrolled in the study. The stem cell source was bone marrow in all unrelated donors, and branulocyte colony-stimulating factor (G-CSF) mobilized PBSC in related donors. All patients received CsA and MTX as GVHD prophylaxis. Moreover, 26 patients (52%) received ATG before transplant. mDC and pDC PB counts were significantly lower in patients than in 28 age-matched healthy controls (8.8 cells/μl [25th-75th percentile, 3.5–14.5] mDC, and 2.8 [1.3–5.5] pDC, versus 15.5 [12.1–25.1] and 8.6 [5.6–13.1], respectively; $P < .001$). However the mDC/pDC ratio was significantly higher in the patient group (3.5 [1.6–6.2] vs 1.7 [1.3–2.6]; $P = .002$). CD14+ preDC counts were not significantly different. Among the 46 patients who were evaluable, 12 (26%) developed acute GVHD grade II-IV. Risk factors significantly associated with acute GVHD were older age ($P = .01$), PBSC transplantation ($P = .02$), and the absence of ATG in the conditioning regimen ($P = .01$). Patients with acute GVHD had significantly higher pretransplantation mDC:pDC ratio (5.7 [3.3–16.4] vs 3.1 [1.6–5.5]; $P = .03$) and CD14+ preDC counts (395 [326–625] vs 284 [187–395]; $P = .02$). A subset analysis was performed in PBSC patients, only 3 of whom had received ATG before transplant. Among 26 evaluable patients, 10 (38%) developed acute GVHD grade II-IV. Besides older age ($P = .02$), the only risk factors significantly associated with acute GVHD in PBSC patients were the pretransplantation mDC:pDC ratio (5.7 [4.1–13.1] vs 1.7 [1.2–2.9]; $P = .008$) and CD14+ preDC counts (395 [352–710] vs 259 [199–314]; $P = .004$). In multivariate analysis, only older age ($P = .04$) and pretransplantation circulating CD14+ preDC numbers ($P = 0.04$) were significantly associated with acute GVHD in PBSC transplants. These findings demonstrate that blood levels of DC precursors may correlate with a higher risk of developing aGVHD. Future studies will be aimed at depleting host preDC before allotransplant as a means of GVHD prophylaxis.

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ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD) IN PATIENTS UNDERGOING ALLOGENEIC BMT FOR THALASSAEMIA MAJOR

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Methods: Between October 1991 and June 2004, 152 thalassemic patients who underwent allogeneic BMT and survived more than 2 weeks were evaluated for graft-versus-host disease (GVHD). Conditioning regimens included cusulfan (Bu) 16 mg/kg, cyclophosphamide (Cy) 200 mg/kg, and antilymphocyte globulin (ALG) 120 mg/kg (in 91 patients); Bu 600 mg/m^2 and Cy 200 mg/kg (in 51 patients), Bu 14 mg/kg, Cy 10 mg/kg, and ALG 120 mg/kg (in 8 patients), and others (in 2 patients). GVHD prophylaxis consisted of cyclosporine (CSA) alone in 11 patients, CSA and methotrexate (MTx) (15 mg/m^2 on day 1, 10 mg/m^2 on days 3, 6, and 11) in 40 patients and CSA and MTx (10 mg/m^2 on day 1, 7 mg/m^2 on days 3, 6, and 11) in 99 patients. All donors were 6 antigen HLA-matched sibling (91.4%) or family (8.6%) donors. **Results:** There were 103 males and 49 females, with mean age of