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IRRADIATION INFLUENCES ENDOTHELIAL CELL FUNCTION IN VITRO AND IN VIVO: A POSSIBLE ROLE FOR IP10

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The cytokine storm induced by the pre-transplant conditioning regimen, plays a major role in the development of graft-versus-host disease (GVHD) by enhancing antigen presentation and recruiting donor T cells to the tissues. Some of the cytokines implicated in GVHD also activate vascular endothelium and might influence lymphocyte trafficking. We hypothesised that in addition, endothelial cells (EC) irradiation contributes to their activation thus enhancing leukocyte trafficking. T cell migration through irradiated or non irradiated lung-derived endothelial cell (EC) monolayer was measured at various intervals. We observed that, independently of the dose used, irradiation significantly increased the proportion of migrating T cells at each time point. This effect could either be due to cytoskeletal rearrangements and/or to soluble factors induced by irradiation. Irradiation induced in the EC a dose-dependent re-organization of actin stress fibres. In addition, supernatants obtained from irradiated endothelium induced T cell chemotaxis in vitro. The chemotactic effect was significantly reduced (51 to 63% inhibition) by the addition of an antibody neutralizing the chemokine IP10. The migration pattern of T cells was also investigated in an in vivo model in which CFSE labelled donor T cells were infused in irradiated (1000 cGy) or untreated (control) syngeneic recipients. Although the proportion of injected T cells in the lymphoid organs was much higher in the irradiated than in control hosts, the overall pattern of migration was similar. The distribution of donor infiltrating cells was homogeneously diffuse in the control group whilst clustered in follicles in irradiated mice. We conclude that irradiation influences the migration of donor T cells possibly via an increased production of IP10 by EC.

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TWICE-DAILY INTRAVENOUS BOLUS TACROLIMUS INFUSION FOR ACUTE GRAFT-VS-HOST DISEASE PROPHYLAXIS

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Background: Tacrolimus (FK) is usually given as a continuous 24-hour infusion for acute graft-vs-host disease (GVH) prophylaxis in the setting of allogeneic hematopoietic stem cell transplantation (HSCT). Often, this schedule becomes logistically difficult and requires dedicated line and monitoring. We report our experience with the use of twice-daily intravenous (IV) bolus injection. **Patients and Methods:** Between 01/00–06/04, 59 patients (pts) with hematologic indication for allogeneic HSCT received twice-daily FK for GVH prophylaxis. Patients were given FK at initial dose of 0.015 mg/kg IV bolus over 3 hours on day T −1 then every 12 hours. First trough level was drawn before the seventh dose on day T +2 and then twice weekly unless clinically indicated otherwise. Doses were adjusted for a target level of 10 ng/ml (range 5–20 ng/ml). Patients were switched to oral form when were clinically able to tolerate it. **Results:** Median age was 49 years (range 19–64 y). Donors were transplanted for hematologic disorder indications. Donor compatibility status was as follows: matched-related 38 (64.4%), matched-unrelated 10 (17%), and mismatched-related 11 (18.6%). FK was used in 2 GVH prophylaxis protocols: with methotrexate or in combination with mycophenolate mofetil and daclizumab. Median first trough level was 9 ng/ml (range 2.6–22.5). Rate of grade I or II acute GVH was 16.9%. Only one pt developed grade III (1.7%) and no pt had grade IV. Significant nephrotoxicity (peak creatinine level ≥2× baseline or ≥2 mg/dl) occurred in 16 patients (27.1%). Five of these pts (31.25%) had at least 1 FK trough level ≥20 ng/ml whereas 15 of 44 patients with normal renal function (34.1%) had such elevated levels. Severe nephrotoxicity requiring dialysis occurred in 4 pts and only 1 of these had elevated FK level. One pt developed HUS/TTP and had all FK trough levels <20 ng/ml. There were no grade 3 or 4 seizures or tremors. Median discharge day was T +19 (range 12–34). Two pts relapsed, but were alive, by day 100. Day-100 relapse-free mortality was 18.6%. **Conclusion:** Results of twice-daily bolus tacrolimus compare favorably to historical safety and efficacy data of continuous infusion of FK in allogeneic HSCT. Bolus infusion was easy to administer and adjust and did not correlate with increased risk of nephrotoxicity. These results should be further investigated in a prospective clinical trial.

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STEM CELL MOBILIZATION WITH NOVEL G-CSF ANALOGUES AUGMENT NKT CELL RESTRICTED GRAFT-VERSUS-LEUKAEMIA EFFECTS

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We have studied the effect of stem cell mobilization with the potent G-CSF and Flt-3 receptor agonist progenipoietin-1 (ProGP-1) on graft-versus-leukaemia effects (GVL) in the B6 → B6D2F1 murine model of allogeneic stem cell transplantation (SCT). Donor pre-treatment with ProGP-1 significantly improved leukaemia free survival compared to G-CSF or control diluent pre-treatment (73% versus 31% and 0% respectively; $P<0.05$) due to both reduced graft-versus-host disease (GVHD) mortality (23% versus 55% and 100% respectively; $P<0.05$) and prevention of leukaemic progression (4% versus 30%, ProGP-1 versus G-CSF; $P=0.01$). To dissect the mechanism responsible for the paradoxical enhancement of GVL we developed an in vivo cytotoxicity assay that reflects the killing of CD45.1 disparate donor versus CFSE labelled host splenocytes after SCT. Anti-host cytotoxicity was significantly enhanced when donors were mobilised by ProGP-1 rather than G-CSF (cytotoxicity index 6.6 ± 1.0 vs 3.1 ± 0.3, $P=0.01$). We then utilized wild-type, perforin, FasL, TNFα or TRAIL deficient donor grafts which were depleted of $CD4^+$ and/or $CD8^+$ T cell subsets to study the molecular pathways involved. The reduction in leukaemic progression in recipients of ProGP-1 treated grafts was a consequence of a 2-fold increase in anti-host cytotoxicity by donor $CD8^+$ T cells and was perforin-restricted ($P<0.01$). ProGP-1 also prevented GVHD directed to multiple minor histocompatibility antigens in the B6 → Balb/B model despite enhancing anti-host cytotoxicity. The addition of Flt-3L to G-CSF during stem cell mobilization or the addition of purified $CD11c^{hi}$ dendritic cells to G-CSF mobilized grafts failed to augment donor anti-host cytotoxicity or further separate GVHD and GVL. Conversely, stem cell mobilization with pegylated-G-CSF to prolong G-CSF receptor stimulation prevented GVHD and reproduced the augmentation of cytotoxicity seen in recipients of ProGP-1 mobilized grafts. Mobilization with ProGP-1 significantly increased splenic and hepatic invariant NKT cell numbers and increased IFN-γ secretion following in vitro stimulation with α-GalCer analogues. Strikingly, the augmentation of donor $CD8^+$ T cell cytotoxicity following mobilization with ProGP-1 was lost when NKT deficient ($J\alpha18^{-/-}$) mice were utilized as donors. Thus, enhanced G-CSF signalling by potent G-CSF receptor agonists during stem cell mobilization augments NKT cell dependent $CD8^+$ cytotoxicity following SCT and profoundly separates GVHD and GVL.

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SUICIDE GENE THERAPY FOR HUMAN T CELL MEDIATED GRAFT VERSUS HOST DISEASE IN A MURINE XENOGRAFT MODEL

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Graft-versus-host disease (GvHD) remains a major cause of morbidity and mortality following allogeneic bone marrow transplantation. We developed a novel chimeric suicide gene in which the