IMMUNE RECONSTITUTION

390

CYCLOSPORINE A (CSA) SIGNIFICANTLY REDUCES THE CYTOTOXIC EFFECTS OF IN VITRO EXPANDED NK CELLS: IMPLICATIONS FOR ADOPTIVE NK CELL THERAPY IN THE SETTING OF ALLOGENEIC HEMATOPOIETIC STEM CELL (HCT) TRANSPLANTATION

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Recently, clinical trials have evaluated the impact of infusing natural killer (NK) cells following HCT). The impact of CSA, commonly used to prevent GVHD after HCT, on adoptive NK cell function is unkown Hong et al reported the phenotype and function of fresh NK cells was altered by CSA, showing CSA treated NK cells elicited enhanced cytotoxicity against tumor targets. However, the impact of CSA on in vitro expanded NK cell function and phenotype has not been explored. We analyzed the impact of culturing fresh and in vitro expanded NK cells in physiological doses of CSA (40, 200, 1000 ng/ml for 18 hrs)Fresh NK cells were obtained from peripheral blood mononuclear cells of healthy donors using immunomagnetic beads to isolate $CD56^+/CD3^-$ cells. NK cell expansion in vitro was maximized by using irradiated EBV transformed B cells as feeder cells in media containing IL-2 [500U/ml] for 12–14 days. Compared to controls, CSA treated fresh NK cells had a significant reduction in IL2-induced proliferation (stimulation index 0.51 ± 0.1) and decreased TRAIL expression (MFI 17.3 vs 10.9, p = 0.023). Furthermore, an ELISA assay showed fresh NK cells treated with CSA had a significant reduction in IL-2 induced IFN-g production (median 231 vs 57 pg/ml, p = 0.025). In vitro expanded NK cells cultured in CSA also showed a reduction in IL-2-induced TRAIL expression (MFI 26.2 vs 16.3). Cytotoxicity assays showed that at the highest dose of CSA (1000 ng/ml), freshly isolated NK cells maintained their ability to kill K562 targets. In contrast, expanded NK cells cultured in CSA for 18 hours with or without IL-2 compared to controls had a significant reduction in the killing of K562 cells (E:T = 10:1, median 66 vs 43% lysis, p = 0.011) and RCC cells (E:T = 20:1, 78.5 vs 38.5% lysis). These data show CSA alters the phenotype and function of both fresh and expanded NK cells. More importantly, these experiments show NK cell TRAIL mediated killing of tumor targets is significantly reduced when expanded NK are exposed to physiologic doses of CSA in vitro. These findings suggest the anti-tumor effects of in vitro expanded NK cells could be hindered when adoptively infused in HCT patients receiving CSA.

391

RITUXIMAB INFUSION AFTER ALLOGENEIC HCT PREVENTS DONOR B CELL RECONSTITUTION AND ALLOIMMUNITY ONE YEAR POST TRANSPLANT

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B cells are implicated in chronic graft-vs-host disease (cGVHD) and anti-B cell rituximab is effective cGVHD therapy. We hypothesize post allo-HCT rituximab therapy will prevent chronic GVHD. Thirty-five MCL and CLL patients conditioned with nonmyeloablative total lymphoid irradiation and anti-thymocyte globulin (TLI-ATG) conditioning received rituximab 375 mg/m^2 56, 63, 70, and 77 after allogeneic HCT.

Peripheral B cells are normal in number and of donor origin 3 months after TLI-ATG allo- HCT. With rituximab addition, peripheral CD19+ B cells remained undetectable 9 months after transplant; were first detected in 6/11 patients at 1 year and 8/8 patients at 1.5 years. These cells predominately expressed a memory phenotype (CD19+CD27+, n = 6). Hi-Dimension 12-color FACS analysis of bone marrow aspirates collected day 56 (pre-rituximab), 90, 180 and 365 days post-HCT showed lymphoid progenitors were prevalent days 56 and 90 (20–40%) and decline to 3–8% of the lymphocytes, 365 days after HCT. Second, mature IgD+ CD19+ B cells are present day 56 pre-rituximab. Plasma cells are unchanged throughout.

Plasma IgG levels decreased to 60% pre transplant levels at 6 months and 78% at one year. At 6, 9, and 12 months, EBV titer was 76%, 104%, and 103% relative to pretransplant patient titers demonstrating protective antibodies are maintained despite rituximab therapy presumably secreted from long-lived recipient CD20 negative plasma cells. Similar results are seen for tetanus. Our strongest support for persistent recipient plasma cell immunity is allotype discrimination of donor and recipient IgG2 showing the absence of donor antibody development 1.5 years post-HCT. Further supporting this, no allogeneic antibody responses have developed in the five male with female donors against H-Y antigens.

In summary, rituximab therapy 2 months after allo-HCT prevents donor B cell reconstitution one year after HCT, but protective recipient humoral immunity persists. Chronic GVHD assessment requires further follow-up, but is promising.

Days after HCT	Lymphoid Progenitors (CD34+ CD117+)	Pro B Cell (CD19+CD34+ CD10-)	Pre B Cells (CD34-CD10+ CD19+)	Mature B Cells (CD19+ IgD+IgM+)	Plasma Cells (CD38+ CD138+)	Total IgG
56, n = 6 (Pre-ritux)	22–25%	2–6%	0.1–4%	0.2–1%	0.7–1%	655 ug/dl 81% pre
90, n = 4	20–40%	2–9%	0.5–1.7%	Not detected	0.5–3%	910 ug/dl 101% pre
180, n = 5	13–20%	5–12%	0.7%	1%	0.5–2%	507 ug/dl 60% pre
365, n = 5	3–8%	2–10%	0–0.5%	1–5%	0.5–3.7%	642 ug/dl 78%

% range of cells gated on total lymphocytes; Not detected: within the range of error for the measurement.

392

DELAYED RECOVERY OF MYELOID (mDC) AND PLASMACYTOID (pDC) DENDRITIC CELLS AT 3 MONTHS AFTER ALLOGENEIC HSC TRANSPLANTATION CORRELATES WITH AN INCREASED TRANSPLANT-RELATED AND OVERALL MORTALITY INDEPENDENTLY OF GVHD

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Background: A low number of antigen presenting cells (APC) in the peripheral blood 1 month after allogeneic HSC transplantation has been previously associated with increased mortality. However, the impact of APC recovery at later time points (3 through 12 months) after transplant has not been thoroughly investigated. **Aim:** In this study we analyzed the factors affecting APC recovery between 3 and 12 months after allogeneic HSC transplant and determined its impact on the clinical outcome. **Patients and Methods:** Blood samples from all patients undergoing an allogeneic HSC transplant in our institution from June 1999 through June 2006 were analyzed. PB numbers of CD11c+ mDC, CD123+ pDC and CD14+ monocytes were determined based on flow cytometry at 3 (n = 161), 6 (n = 127) and 12 months (n = 80) after transplant. **Results:** Median numbers of PB APC at 3 months after transplant were ($x10^6$/L): mDC (7.4), pDC (2.5) and monocytes (254), and then increased gradually at 6 (mDC 9.2, pDC 3.4, monocytes 272) and 12 months (mDC 11.3, pDC 4.6, monocytes 335). mDC recovery was delayed both at 3 and 6 months in patients with advanced disease at transplant (p < 0.00001 and 0.003, respectively), and acute GVHD grade II-IV (p = 0.00009 and 0.01). Similarly, pDC recovery was delayed at 3 and 6 months in patients with advanced disease (p = 0.0001 and p = 0.003) and acute GVHD (p = 0.0004 and p = 0.02). Moreover, both mDC and pDC levels at 6 months were reduced in patients with extensive chronic GVHD (p = 0.001 and p = 0.0007 respectively). All other analysed factors did not correlate with mDC and pDC recovery. Also, mDC and pDC numbers at 12 months, as well as monocyte

numbers at any time point, did not correlate with any of the analysed factors. Lower numbers of mDC and pDC in the PB at 3 months after transplant significantly correlated with increased overall and transplant-related mortality in univariate analysis. In multivariate analysis low mDC counts correlated with increased overall mortality ($p = 0.008$) whereas low pDC counts correlated with increased TRM ($p = 0.07$). PB Monocyte numbers instead did not correlate with any of the analysed end points. **Conclusion:** Recovery of mDC and pDC 3–12 months after transplant was reduced in patients with advanced disease at transplant, or developing acute or chronic extensive GVHD after transplant. Nevertheless, a delayed recovery of mDC and pDC within 3 months after transplant was an independent factor contributing to a greater mortality.

393

PRESENCE OF ACTIVATING KIR GENOTYPES INFLUENCES CYTOMEGALOVIRUS (CMV) REACTIVATION AS DETECTED BY Q-PCR IN HCT RECIPIENTS

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Killer Ig-like receptor (KIR) is a cluster of natural killer cell receptors presented by T cells and natural killer (NK) cells. The compatibility of these receptors between the donor and the recipient plays a role in hematopoietic cell transplantation (HCT) for either activating (aKIR) or inhibitory KIRs. Some aKIRs bind only weakly to HLA class I and the natural ligands remain undetermined whereas the inhibitory KIRs bind HLA class I molecules with specificity for defined alleles of HLA-C, HLA-B, or HLA-A. It has been suggested that the donor KIR genotype influences the rate of CMV infection and, more particularly, that aKIR genes are inversely associated with CMV reactivation (Cook et al Blood 2006; 107: 1230–32; Chen et al. BMT 2006; 38: 437–44). In this study, a cohort of 92 non T-cell depleted subjects, who had received a matched unrelated or HLA identical related donor, were analyzed for inhibitory KIR ligands and KIR genotypes obtained using a multiplex PCR-SSP method. All recipients were at risk for CMV reactivation which was monitored bi-weekly by Q-PCR on plasma DNA.

Results: Two groups, those with CMV reactivation ($n = 68$) vs No CMV ($n = 24$), were compared for the presence of KIR determinants and analyzed using a contingency table with Chi^2 test for probability values. An increased number of aKIR genes in the donor, either involving an A haplotype, carrying a single aKIR gene, or the AB and BB haplotypes carrying more than one aKIRs, was significantly associated with a reduction of CMV reactivation ($p = 0.0438$). In addition, using a previously described algorithm for scoring KIR determinants in donor and recipient (Sun et al. BMT 2005; 56: 525–530), values >0, associated with more aKIRs in the donor but not in the recipient, were associated with significantly less CMV reactivation (62% VS 85%, $p = 0.0372$). When the recipients were analyzed for HLA-Cw KIR ligand group1 or 2, there was no significant association with CMV reactivation. This was still true when HLA class I mismatched donor-recipient pairs were excluded. **Conclusion:** In the HCT setting, aKIR genes in donor cells protect the recipient from CMV reactivation but the HLA-Cw allotype of the recipient does not predict CMV infection.

394

VACCINATION WITH CARNARYPOX PP65 CMV VACCINE INDUCES RELIABLE CD4+ AND CD8+ T CELL RESPONSES ONLY IN INDIVIDUALS WHO LACK BASELINE RESPONSES. IMPLICATIONS FOR DONOR VACCINATION TO BOOST CMV IMMUNITY IN STEM CELL TRANSPLANT RECIPIENTS

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CMV reactivation after allogeniec stem cell transplant (SCT) remains a major cause of post-transplant morbidity but may be mitigated by strong T-cell responses in the transplanted donor T-cell repertoire. We vaccinated 25 subjects with "ALVAC pp-65" (sanofi pasteur, Lyon, France) to boost CMV responses in seropositive subjects and induce CMV responses in seronegative subjects. An accelerated regimen giving 1.0 ml ALVAC pp-65 on days 0 and 5 to seropositive subjects and days 0, 5, and 10 to seronegative individuals was used. CD4+ and CD8+ T-cell responses to a CMV pp65 peptide library were measured at serial timepoints post vaccination using flow cytometry to identify interferon-gamma producing T-cells. Positive responses were defined as a twofold increase of interferon-gamma production compared to unstimulated cells. Of the 25 subjects analyzed, 13 were seropositive and 12 were seronegative. As previously reported, serostatus did not correlate with baseline CD4+ and CD8+ CMV response. Indeed there were no differences in T-cell response patterns according to serostatus. We therefore segregated subjects into baseline CD4+ or CD8+ responders or non-responders irrespective of serostatus. Six of 9 CD4+ non-responders had a 2–8 fold (median 4) increase in response by day 5. In contrast, 13 of 16 subjects with a baseline CD4 response (identified by a stimulation index of ≥ 2 at baseline) showed decreased or undetectable responses at day 5 with variable responses thereafter. Nine of 11 CD8+ non-responders had a 2–9 fold (median 3) response by day 5. In contrast, 10 of 14 CD8+ responders showed decreased response by day 5 with variable responses thereafter. Apart from pain at the injection site, vaccine was well tolerated. These results show that ALVAC is a well tolerated vaccine with the potential to induce pp65 responses in subjects who lack baseline responses. In subjects that have baseline responses, vaccination appears to dampen immediate immune response. It is unclear if the vaccine is inducing tolerance in these individuals and if this is related to dose of the vaccine. ALVAC pp65 can elicit quick responses of varying duration in subjects that lack CD4 or CD8+ T cell responses to CMV and could therefore be used to improve the transfer of CMV immunity to SCT recipients using such donors. However, in donors that have a baseline CMV response, vaccination may be detrimental to the recipients' ability to respond to CMV.

395

IMPACT OF DONOR T CELLS ON B CELL DEVELOPMENT AFTER HEMATOPOIETIC CELL TRANSPLANTATION: LESSONS FROM B CELL DEFICIENT MICE

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Allogeneic hematopoietic cell transplant (HCT) recipients often exhibit B cell (BC) lymphopenia due, in part, to graft-versus-host-disease (GVHD). Here, we studied the influence of donor T cells (TC) on BC deficiency post-HCT. Following lethal irradiation, BALB. B mice were given FACS purified hematopoietic stem cells (HSC: cKIT+Thy1.1loLin-Sca-1+) alone, or with co-transfer of whole splenocytes (SP), CD4 or CD8 TCs from minor antigen-mismatched C57BL/6 (B6) mice. When pure HSC were transplanted, BC reconstituted promptly (median 33% of lymphocytes [d30]; 61% [d60]; 74% [d90]), whereas TC engraftment was retarded and did not achieve full donor chimerism. In contrast, addition of SP or CD4 TCs substantially suppressed BC reconstitution, correlating with the degree of acute GVHD, and TC transfer advanced TC engraftment and resulted in early conversion to full donor chimerism. To test if previous events in the donor sensitize TCs against BC structures, thereby promoting anti-BC cytotoxicity post-HCT, TCs from B cell deficient μMT B6 mice were co-transplanted with wild type (WT) HSC. Remarkably, BC engraftment was completely prevented through d90. TCs regenerated faster, but the vast majority originated from spleen and not HSC. To differentiate this lack of BC engraftment from GVHD-associated alloreactive BC lymphopenia, syngenic B6 recipients were used. Again, initially complete blockade of BC engraftment was observed, although this suppression was overcome earlier post-HCT as