assessed. VIP-KO mice, WT littermates, and recipients were infected with 5×10^4 PFU murine cytomegalovirus (mCMV) and T cell response to viral antigen was measured by flow cytometry for mCMV peptide-MHC class I-tetramer$^+$ CD8$^+$ T-cells at day 0, 3, 7, 10, and day 15 post infection or 80, 83, 87 and 101 days post-transplant (infection at day 80 post-transplant). Day 15 post mCMV challenge, VIP-KO and WT mice were euthanized. DC and T-cells were purified from BM and SP by FACS and MACS, respectively. 2×10^5/mL DC treated with 1×10^4 PFU mCMV peptide-expressing Listeria-CMV construct and incubated with 2×10^6/mL T-cells at 37 °C. Cultured 3 days and 7 days, cells were harvested and analyzed with DC and T-cell surface marker, tetramer, and intracellular cytokines by flow cytometry.

Results: allogeneic recipients of VIP-KO BM and VIP-KO SP developed more GvHD than recipients of WT grafts using a lower dose of donor SP (1×10^6), while there was no difference in survival. The GvHD scores and the percentage of survival showed no difference among other syngeneic or allogeneic BMT settings.The specific anti-viral immunity was similar among the non-transplanted VIP-KO mice, and allogeneic and syngeneic transplant recipients of VIP-KO donor cells. 3 and 7 days post culture, VIP-KO DC expressed higher-level of CD80, MHC-II and lower-level of PD-L1, VIP-KO T-cells had higher-level of tetramer$^+$ CD8$^+$ T-cells and intracellular IFN-γ, lower-level of IL-4 and IL-5, PD1, and ICOS. Taken together, these observations suggest that VIP expressed on immune cells suppresses anti-viral immune responses and Th1 polarization.

Conclusion: The anti-viral immune responses of VIP-KO immune cells were independent allogeneic immunity; VIP expressed by neurocrine cells in WT recipients did not compensate for the lack of VIP in mice transplanted with VIP-KO cells. Modulation of the VIP pathway is a novel method to regulate post-transplant immunity allogeneic transplant recipients.

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THE LIVER MAINTAINS STRONG POPULATIONS OF INNATE IMMUNE CELLS THAT CONTRIBUTE TO HOST PROTECTION AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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The liver is a critical immunocompetent organ armed with lymphocytes, natural killer (NK) cells, and a variety of antigen-presenting cells (APC), including resident macrophages, called Kupffer cells (KC). Exposed to large amounts of both destructive and harmless toxins and antigens, the liver must provide immunogenic and tolerogenic immunity. Here, we studied the role of the liver after hematopoietic cell transplantation (HCT). Lethally irradiated BALB.K mice received MHC-matched, purified hematopoietic stem cells (HSC; cKit + Sca1 + Thy1.1loLin-) +/- splenocytes (SP) from AKR/J donors. Ficoll-separated mononuclear cells (MNC) from PBS flushed livers were FACS analyzed post-HCT (pTX). In recipients of HSC + SP the liver was a major target organ of acute graft-vs-host disease (GVHD) with prominent donor T cell (TC) expansion, while NK cell (DX5 + CD122+) and KC (CD11b + F4/80+) levels were severely decreased. HSC-derived donor cells were rare. In contrast, mice given pure HSC showed no signs of GVHD, and early pTX high proportions of NK cells and KC were present within the livers. NK cells comprised up to ~30% of cells and were mixed donor/host type, while KC were donor derived at 6w pTX. We hypothesized that rapid regeneration of KC may shield against the pathogen and toxin load entering the circulation from irradiation-damaged intestines. In fact, when KC reconstitution was suppressed by silica administration mice displayed severe weight loss, hunched posture, ruffled fur, diarrhea, and a >50% mortality. Survivors stabilized ~d12, presumably with gut recovery. To test if regenerating APC could protect against GVHD, a lethal dose of SP was given at 0, 4, 7, or 10d pTX, time points at which control livers contained 0, 11, 25, 32% KC, respectively. All mice receiving SP on d0 died, but death occurred in only 50%, 17% and 0% of mice when SP were given on d4, 7, and 10, respectively. Although donor chimerism decreased with delayed SP injection, lymphocyte reconstitution was improved. In conclusion, the role of the liver as an immunologically active organ after 'conventional' HCT is often masked by donor TC expansion and GVHD. Rapid recovery of innate liver immunity may protect the host from endotoxemia and mediate tolerance between donor and host. Elevated proportions of NK cells, which are lacking in GVHD affected mice suggest another beneficial mechanism. Immunohistochemical studies for a better quantitative assessment of resident liver immune cells are underway.

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A TREND FOR BETTER IMMUNE RECONSTITUTION AND LOWER INCIDENCE OF INFECTIONS AFTER UNRELATED CORD BLOOD TRANSPLANTATION IN CHILDREN COMPARED TO ADULTS

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In umbilical cord blood transplantation (UCBT), the lower infused cell dose might lead to an increased infectious risk. To get further insights on this issue, we retrospectively analyzed infectious events and immune restoration in 35 consecutive patients (pts) treated by UCBT from Jan 2005 to Dec 2008 in the University Hospital of Besançon.

There were 7 children and 28 adults aged 6 to 62 y (median 35y). All pts suffered from malignant diseases. Nine pts were in CR1, 10 in CR2 and 16 in ≥CR3 or in PR. Nine pts were CMV$^+$ and 29 EBV$^+$. A myeloablative conditioning regimen with 12 GY TBI (16pts) or Busulfan (2 pts) + 120 mg/kg cyclophosphamide + 75 mg/m^2 fludarabine was given to 18 pts, one child received ALG instead of fludarabine. A fludarabine based reduced intensity conditioning (RIC) was given to 17 pts. There were 29 double and 6 single unit transplants. The median follow-up is 638 days (259-1449).

All pts engrafted except 3 after RIC. Fifteen pts died (43%), 10 of relapse, 4 of infection (1 ARDS,1 zygomycosis, 1 fusariosis and 1 HHV6 encephalitis) and 1 died of post-conditioning toxicity. There were 8 infectious events in 7 children, (mean 1.14/pt) with 1 death in the pt who received ALG and 86 infectious events in 28 adults (mean 3.07/pt) causing 3 deaths.

Viral infections occurred in 27 pts (77%) in majority before D100 (73%). BKV$^+$ hemorrhagic cystitis (HC) occurred in 13 pts, VZV (6), CMV (5), HHV6 (1), HSV (1), and RSV (1). We recorded 22 documented bacterial infections caused by 13 Gram negative and 9 Gram positive agents. Invasive fungal infections were diagnosed in 9 pts (26%) with 6 proven or probable aspergillosis, 2 fungal septicaemias and 1 zygomycosis. Fungal infections seemed more frequent in pts experiencing chronic GVHD (35% versus 21.4%, hazard ratio = 1.6, p = 0.91).

The immune reconstitution appeared quicker in children, the median CD4 T cell count at 3-6-12 months post UCBT was 224-1008-1333/mm^3 in children and 119-216-364 in adults. Median B cell count at 6 mo post UCBT was 868 in children versus 106 in adults. Among the 20 survivors at 1 year post transplant, the vaccine response was complete in 15 pts, incomplete in 2, non available in 3.

The lower incidence of infectious events in children might be due to a better immune reconstitution. The overall infectious mortality rate is relatively low (11%). The high prevalence of HC advocates for a prospective follow-up of BK virus and the high rate of Zoster warrants preventive strategies.

LATE EFFECTS/QUALITY OF LIFE

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A PROSPECTIVE STUDY OF IRON-OVERLOAD (IO) MANAGEMENT IN ALLOGENEIC HEMATOPOIETIC-CELL TRANSPLANT (ALLO HCT) SURVIVORS

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While transfusional IO occurs in 30-60% of allo HCT survivors, the treatment of post-HCT IO is not well described. We

report the results of a single-center, prospective evaluation for IO and its subsequent treatment in 147 allo HCT survivors. Adult (>18 years) allo HCT recipients were eligible for this study if (1) they had survived for ≥1 year post-HCT, (2) were in remission from their primary disease, (3) had no uncontrolled infection or GVHD, (4) had no contraindication for MRI, (5) had adequate blood counts and renal and hepatic function, and (6) had significant IO. Patients were screened with serum ferritin, and those with ferritin >1000 ng/mL underwent liver R2 MRI to estimate liver iron concentration (LIC, normal ≤1.8 mg/g). Significant IO was defined as LIC ≥5 mg/g. Based on physician and patient preference, patients with significant IO were offered observation only, phlebotomy, or were enrolled on a pilot study of deferasirox (if phlebotomy was not feasible [hemoglobin <11 g/dL] or was refused). 23 patients had IO (LIC >1.8) and 16 had significant IO (LIC ≥5). Among patients with significant IO, 69% had acute leukemia and 56% had received myeloablative conditioning. The median baseline LIC was 10.0 (range, 5.1-43). All patients were transfusion independent at enrollment. Five patients received no treatment (median LIC 6.4), 8 underwent phlebotomy (median LIC 13.1) and 3 received daily deferasirox 20 mg/kg/day for 6 months (median LIC 6.3). Two patients had abnormal liver function tests and one patient each had cirrhosis and unexplained heart failure; all four received phlebotomy. Followup serum ferritin decreased spontaneously in 4 patients on the observation arm (median ferritin, 1606 at baseline to 1264 at followup); 1 also had followup MRI (LIC decreased from 6.4 to 2.3). Phlebotomy was well tolerated and no patient needed ESA's to facilitate phlebotomy. Deferasirox was well tolerated and led to a decrease in LIC in all 3 patients (baseline to 6 mo LIC: 6.3 to 2.8, 9.0 to 6.8, 19.9 to 8.8). Mild adverse events were observed that did not require discontinuation of drug therapy. Phlebotomy is feasible in the majority of allo HCT recipients who have survived for ≥1 year after HCT and have significant IO. Iron levels may decrease with time without any therapy in selected allo HCT survivors. Although the numbers are small, deferasirox may be a safe and effective alternative for allo HCT survivors with IO who cannot undergo phlebotomy.

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CLINICAL RELEVANCE OF LARGE GRANULAR LYMPHOCYTE EXPANSION FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH HLA IDENTICAL SIBLING DONORS

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Background: Large granular lymphocytes (LGLs) are a morphologically distinct but an immunophenotypically heterogeneous set of lymphocytes of activated T- or NK cells that mediate non-MHC-restricted cytotoxicity. LGL expansion following allogeneic hematopoietic stem cell transplantation (HSCT) has been reported although its clinical relevance on transplant outcomes is not clear. Moreover, precipitating factor evoking LGL expansion is unknown.

Methods: A total of 323 consecutive patients who received allogeneic HSCTs from HLA identical sibling donors between January 2000 and October 2007 at the Princess Margaret Hospital, Toronto, Canada, were included in the retrospective study. The patients' characteristics were: gender (male/female = 196/127); median age 49.5 yrs (range 17-71); stem cell source (PBSC/BM = 25172); conditioning (myeloablative/reduced intensity = 228/95). The definition of LGL expansion is as follows: 1) increasing number of peripheral blood lymphocyte counts $\geq 3.0\times10^9$/L for at least 3 months, and 2) the predominance of LGLs in the peripheral blood smears.

Results: Out of 323 recipients, 64 cases (19.8%) showed LGL expansion after allogeneic hematopoietic stem cell transplantation (HSCT). The median onset of LGL expansion was 306 days (95% C.I. 205-407 days). The 1- and 2-year(s) incidence of LGL expansion was 14.4 ± 2.2% and 22.0 ± 2.8%.

Compared to the patients without LGL expansion, improved transplant outcomes were observed in patients with LGL expansion: better overall survival (2 years OS; 94.8% vs 60.3%, $p < 0.001$), lower non-relapse mortality (2 years NRM; 2.6% vs 23.4%, $p < 0.001$) and lower relapse incidence (5.6% vs 29.5%, $p < 0.001$).

Three risk factors were identified for the development of LGL expansion such as CMV serostatus of recipient, CMV reactivation, and occurrence of chronic GVHD. Higher incidence of LGL expansion was noted 1) in recipient CMV IgG (+) group (CMV-R^+) compared to CMV-R^- group regardless of CMV serostatus of donor (52/193 [27%] vs 11/128 [9%]; $p < 0.001$); 2) in patients experiencing CMV reactivation (43/140 [31%] vs 21/183 [11%]; $p < 0.001$); 3) in patients developing chronic GVHD (61/252 [24%] vs 2/55 [4%]; $p = 0.02$).

Conclusion: LGL expansion is not uncommon following allogeneic HSCT with HLA-identical sibling donors, and strongly associates with favorable transplant outcomes esp. in terms of non-relapse mortality. Its association with chronic GVHD suggested that expanded LGLs may mediate GVL effect after allogeneic HSCT.

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PERIPHERAL AIRWAY FUNCTION DECLINES FOLLOWING ALLOGENEIC TRANSPLANTATION AND IS ASSOCIATED WITH THE DEVELOPMENT OF CHRONIC GVHD

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Bronchiolits Obliterans (BO) is the most frequent non-infectious pulmonary complication post allogeneic stem cell transplant (SCT) and is strongly correlated with cGVHD. BO remains a major cause of late post SCT morbidity and mortality. Airflow obstruction as detected by spirometry occurs late in BO, only when widespread small airways disease is well established and the process is more likely to be irreversible. The Multiple Breath Nitrogen Washout (MBNW) test is a sensitive and reproducible measure of peripheral airway function that is highly sensitive to bronchiolitis in smokers with otherwise normal spirometry. Its role in assessing peripheral airway dysfunction and early BO post SCT has not been assessed. In a cross sectional study, 30 pts post SCT (mean age 47, 19-68 yrs) underwent standard spirometric and lung volume testing together with measurements of acinar (Sacin) and conductive (Scond) peripheral airway ventilatory heterogeneity and Lung Clearance Index (LCI) as assessed by MBNW. Median time post SCT was 12 (3-73) mths, with most pts receiving reduced intensity conditioning (17/30) for acute leukemia (15/30). 50% had prior aGVHD, though no pts had active aGVHD at the time of testing. 20/30 pts had cGVHD, with 18 pts having active disease at the time of testing. 40% had moderate/severe cGVHD by NIH Consensus Criteria. 27% cohort had evidence of airway obstruction (FEV1/FVC <70% predicted), while abnormal Sacin ($>0.3L^{-1}$) and Scond ($>0.04L^{-1}$) affected 73% and 80% respectively. Sacin, Scond and LCI were inversely correlated to FEV1% predicted while Scond and LCI were correlated with RV/TLC% predicted. 1 pt with evidence of obstruction had normal Sacin, while 13 pts with normal spirometry had abnormal Sacin. On univariate analysis, poorer FEV1/FVC%, Sacin, Scond and LCI measures were strongly associated with days post transplant, but only Sacin ($p = 0.016$) and Scond ($p = 0.014$) were significantly associated with a diagnosis of cGVHD. Poorer Sacin was also correlated with increasing severity of cGVHD ($R^2 = 0.48$, $p = 0.002$). On multivariate analysis only cGVHD ($p = 0.01$) remained an independent predictor of abnormal Sacin. Peripheral airway function appears to decline following SCT, and is strongly associated with the development of cGVHD. Prospective studies are being