proliferate, express KIR and NKG2A receptors and develop cytotoxic and cytokine-producing potential. Umbilical cord blood, a site of developing hematopoiesis, contained a significantly higher percentage of $CD56^{+dim}$ $NKG2A^{-}KIR^{-}$ cells (59 ± 11%) supporting the notion that these cells are developmentally immature. We conclude that lineage-committed NK cells in the blood do not require inhibitory self-tolerance mechanisms until they reach a late stage of differentiation.

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PRE-CLINICAL STUDY OF THE EFFECT OF THE AS-SIG-TAA/ECDCD40L VECTOR PRIME-TAA/ECDCD40L PROTEIN BOOST VACCINE IN ELDERLY RECIPIENTS FOR SUPPRESSION OF RECURRENT CANCER FOLLOWING ALLOGRAFTING AND DONOR LYMPHOCYTE INFUSIONS (DLI)

Tang, Y.[1], Akbulut, H.[1], Maynard, J.[1], Petersen, L.[1], Deisseroth, A.[1]
[1]Sidney Kimmel Cancer Center, San Diego, CA.

The mini-allogeneic transplant has opened the door to treating individuals in the fifth and sixth decades of life. Unfortunately, matched related donors for these individuals usually have an aged immune system in which the immune response has been impaired by a reduction in the ratio of antigen naïve/memory CD4 and CD8 T cells and acquired functional defects in activated "helper" CD4 T cells (eg diminished CD40 ligand (CD40L) expression). This has limited the applicability of mini-allografts to older individuals and to the use of post allograft vaccines to expand specific populations of CD8 effector cells to bolster the anti-cancer and anti-viral immune response. Our laboratory has developed an adenoviral vector (Ad-sig-TAA/ecdCD40L) vaccine which is designed for the in vivo target associated antigen (TAA) loading and activation of dendritic cells (DCs), and to overcome the absence of CD40L expression in activated CD4 helper T cells in older individuals. The subcutaneous (sc) injection of this vector leads to the release of a fusion protein composed of a TAA linked to the extracellular domain (ecd) of the CD40 ligand (CD40L), which binds to the CD40 receptor on DCs, activates the DCs, and leads to the presentation of TAA fragments on Class I MHC. VPP vaccine overcomes anergy in TAA.Tg transgenic mouse, and induces TAA specific memory cells. Two sc injections of the TAA/ecdCD40L protein as a booster following the sc administration of the Ad-sig-TAA/ecdCD40L vector (VPP) expands the magnitude of the cellular and humoral immune response induced by the vector in 18 month old aged mice as well as in younger mice. This vaccine decreased levels of negative regulatory CD4 FOXP3 T cells in tumor nodules. We administered TBI and an allogeneic stem cell transplant 7 days post sc injection of the E7 positive TC-1 cells. DLI from an Ad-sig-E7/ecdCD40L vector prime-E7/ecdCD40L protein boost vaccinated donor were injected iv 3 days post transplant, and a single E7/ecdCD40L protein boost sc vaccination one week thereafter. We found that the growth rate of the E7 positive TC-1 tumor cells post allograft was less in the vaccinated than in the control (injection of tumor cells followed in 7 days by TBI), or the animals in which the allograft recipient was vaccinated without DLI. Thus, the use of DLI from VPP vaccinated allodonor decreased tumor cell growth post allograft.

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DIRECT ISOLATION AND INFUSION OF DONOR-DERIVED CMV-SPECIFIC T CELLS FOR TREATMENT OR PROPHYLAXIS OF CMV INFECTION FOLLOWING ALLOGENEIC TRANSPLANTATION

Lowdell, M.W.[1], Thomson, K.[1], Julie, A.[1], Pang, K.[1], Samuel, E.[1], Desborough, M.[1], Mackinnon, S.[1] [1]Dept of Haematology, Royal Free & UCL Med Sch, London, United Kingdom.

Reactivation of CMV is common following allogeneic HSCT and virus-specific T lymphocytes are necessary for control. CMV-specific donor T cell infusions have been used but most methods involve several weeks of ex-vivo culture. The current method involves a 20hr incubation of donor peripheral blood mononuclear cells with rCMV-pp65 protein. Isolation of interferon-gamma positive T cells by CliniMACS using IFNγ capture microbeads (Miltenyi Biotec) provides a CMV-reactive T cell product which is cryopreserved in dosed aliquots for subsequent infusion. A single arm phase I study is underway, with CMV-T cells given pre-emptively at first detection (by qPCR) of CMV DNA in peripheral blood, or at day +40-50 as prophylaxis. A dose of 1×10^4 $CD3^+$/kg recipient weight is infused and CMV monitored by weekly PCR. Antiviral drug therapy commences if the viral load rises above our institutional threshold. 12 patients have received CMV-T cells at a median of 4 weeks post HSCT and 9 are alive and well. None experienced infusion-related toxicity and no deaths were associated with CMV-T cell treatment or CMV disease. Incidence and severity of GvHD was no different from historical controls. The median yield of CMV-T cells following enrichment was 5.2×10^6 (range 0.29-26.7) of which 24.7% were $CD4^+/IFN\gamma^+$ and 11.2% were $CD8^+/IFN\gamma^+$; a total mean CMV-reactive cell yield of 2.2×10^6 per donor. Following infusion, in vivo expansion of CMV-T cells was seen in all patients. CMV-T cells averaged 9.8% of $CD4^+$ cells and 8.1% of $CD8^+$ cells by 2-4 weeks post-infusion; the result of in vivo expansions of CMV-reactive cells of up to 5000-fold. 9 patients received antiviral therapy for CMV reactivation but in 5 patients this was required for a significantly shorter period than in historical controls (11-14 days). 3 patients had a second CMV reactivation. 1 patient showed substantially delayed expansion of CMV-T and required prolonged anti-viral treatment (33 days). 1 required antiviral drug treatment, the second received no treatment and cleared virus after a further in vivo expansion of CMV-T cells, suggesting the presence of a functional memory population. 3 patients were treated prophylactically at day 40-50 and expansions of CMV-reactive T cells were seen in all 3 despite lack of detectable viral DNA in peripheral blood. This technique rapidly provides clinical-grade CMV-reactive $CD4^+$ and $CD8^+$ T cells which appear to provide effective antiviral immunity.

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THYMIC SHIELDING (TS) IN RECIPIENTS OF TOTAL BODY IRRADIATION (TBI) AND ALTERNATIVE DONOR HEMATOPOIETIC STEM CELL TRANSPLANT (AD-HSCT): REDUCED RISK OF OPPORTUNISTIC INFECTION IN PATIENTS WITH FANCONI ANEMIA (FA)

MacMillan, M.L.[1], Blazar, B.R.[1], DeFor, T.E.[1], Dusenbery, K.R.[1], Wagner, J.E.[1] [1]University of Minnesota, Minneapolis, MN.

Delayed immune reconstitution and consequent opportunistic infections remain major obstacles to the successful application of HSCT, particularly in older patients, those with HLA mismatched donors and in selected diseases, such as FA. Based on preclinical work suggesting that TS may improve immune reconstitution in recipients of TBI and allogeneic HSCT (J Immunol 1987:139:358), we evaluated the safety and potential efficacy of TS in FA patients. After CT localization of the thymus, 5HVL cerrubend blocks were fabricated and used to shield the thymus. Otherwise all patients received the standard preparative regimen consisting of fludarabine 175 mg/m2, cyclosphosphamide 40 mg/kg, single fraction TBI 450 cGy, and antithymocyte globulin, with cyclosporine and short course methylprednisolone as GVHD immunoprophylaxis. In order to assess the potential risks and benefits of TS, we compared transplant outcomes of these FA patients who received TS to FA patients treated with the exact same preparative regimen without TS. Between April 1999-June 2006, 59 FA patients underwent AD-HSCT at the University of Minnesota; 16 patients had TBI with TS and 43 had TBI without TS. For those with and without TS, donors were HLA matched (n=42) or mismatched (n=17), and stem cell sources were T cell depleted bone marrow (n=9 vs n=38) or umbilical cord blood (n=7 vs n=5). While excess graft failure was considered to be the principal toxicity risk in recipients of TS, incidence of engraftment was similar in those with and without TS (94% vs 97% respectively, p=.46). Although not statistically significant, survival at one year was higher in FA patients with TS (67% vs 53% respectively, p=.46). However, as shown, TS was associated with a significantly lower risk of all three categories of opportunistic infection after HSCT (Table 1).

In conclusion, TS in recipients of TBI is associated with significantly lower risk of opportunistic infections without any deleterious effect on hematopoietic recovery in recipients of AD-HSCT. While these results indicate that TS reduces the infection rate and potentially improves survival in patients with FA, they also suggests that TS should be considered for other high risk populations (e.g. adults) and in those with other non malignant disorders. While it appears that immune reconstitution is improved based on the reduced incidence of opportunistic infections, correlative laboratory assessments (TREC, CD4 recovery) are currently being performed.

Impact of TS on HSCT Outcomes

Preparative Therapy	Probability of Neutrophil Engraftment (95% CI)	Probability of Survival at 1 Year (95% CI)	Total # Infections	#Bacterial Infections	#Viral Infections	#Fungal Infections
TBI with TS (n=16 patients)	94(82-100)	67(23-91)	9	4	3	2
TBI Without TS (n=43 patients)	97(92-100)	53(38-68)	126	68	37	21
P value	NS	NS	<.01	<.01	<.01	<.01

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CD4+CD25+ REGULATORY T CELLS ENHANCE IMMUNE RECONSTITUTION FOLLOWING ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION BY PROTECTING THYMIC AND LYMPHOID COMPARTMENTS FROM GRAFT-VERSUS-HOST DISEASE DAMAGE WITHOUT IMPACTING T CELL REPERTOIRE DEVELOPMENT

Nguyen, V.H.[1], Chang, D.[1], Shashidhar, S.[1], Bachmann, M.[1], Contag, C.H.[1], Brown, J.M.Y.[1], Negrin, R.S.[1] [1]Stanford University School of Medicine, Stanford, CA.

We previously showed that Treg improved the quantitative and functional lymphoid reconstitution in a murine model of bone marrow transplantation. We hypothesize that Treg prevent thymic and lymphoid damage from graft-versus-host disease (GvHD), leading to enhanced lymphoid reconstitution. Lethally-irradiated adult thymectomized Balb/c ($H2^d$) recipients received FVB ($H2^q$) T cell depleted bone marrow (TCD-BM) cells and CD4+/CD8+ cells (Tcon), the latter to induce GvHD, with or without donor Treg. At day 30, when all groups had reached full donor chimerism, transplant recipients were challenged with murine CMV (5×10^5 pfu/mouse). Day 90 survival for thymectomized groups with TCD-BM alone, with Tcon, or with Tcon+Treg was 78%, 0%, and 45%, respectively, compared to 100%, 0%, and 86% survival in their respective euthymic infected counterparts ($p<0.05$ for thymectomized vs euthymic Treg groups). Elispot showed CMV specific donor responses in infected groups. Viral titers in the liver and kidney 2 weeks after infection was lower in Treg recipients compared to animals that received Tcon alone. Thymectomized animals had higher viral titers compared to euthymic controls. Uninfected thymectomized controls in the respective groups separate the effect of CMV infection from GvHD on survival. Treg recipients, infected or uninfected, had no clinical GvHD compared to animals that received Tcon alone. In euthymic recipients, gross and histologic examination confirmed the general preservation of thymic integrity and architecture in Treg recipients compared with smaller involuted thymuses partially replaced by adipose tissue in animals that received Tcon alone. V-beta TCR screening with FACS showed a polyclonal TCR repertoire in animals with or without Treg. Spectratyping 30 days post-transplantation showed that Treg had no significant impact on the TCR repertoire diversity in animals which received Tcon. Survival of a subset of infected thymectomized animals that received Treg lead to the evaluation of the impact of Treg on secondary lymphoid organs following HCT. Animals without Treg had significant splenic and lymph node fibrosis and hypoplasia with a reduction in T cell numbers due to GvHD. Our findings indicate that Treg indirectly enhance immune reconstitution by protecting the thymic and secondary lymphoid compartments from GvHD damage, allowing the generation and peripheral expansion of lymphoid cells without impacting the diversity of T cell repertoire.

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TRANSPLANT CONDITIONS DETERMINE THE CONTRIBUTION OF HOMEOSTATICALLY EXPANDED DONOR CD8 CELLS TO HOST LYMPHOID RECONSTITUTION FOLLOWING HEMATOPOIETIC CELL TRANSPLANTATION

Roskos, M.[1], Levy, R.B.[1] [1]University of Miami, Miami, FL.

Patients undergoing hematopoietic cell transplantation (HCT) typically experience a period of immune deficiency commensurate with levels of pre-transplant conditioning. Accordingly, developing approaches to facilitate the reconstitution of the host T cell compartment and restore host immune function post-HCT is an important clinical challenge. The capacity of T cells to expand under conditions of lymphopenia is well recognized. To determine the contribution of homeostatically expanded donor memory CD8 cells (TM) on the reconstitution of the host CD8 compartment post-HCT, a homogeneous TCR-transgenic (tg) TM population was monitored following transplantation with T cell-depleted C57BL/6 bone marrow into 9.0Gy-conditioned syngeneic recipients. Homeostatic expansion was assessed by $CD8^+/V\beta5^+/V\alpha2^+$ numbers in recipient spleens beginning 3 days post-HCT. Infused TM expanded for 2 wks then reached a steady-state splenic number ($1.5x\uparrow$) that was maintained >7 months post-transplant. Similar kinetics and expansion were observed with a non-tg TM population. To determine how transplant parameters affect OT.I homeostatic expansion, reduced intensity conditioning, delayed infusion, and varying TM doses were examined. OT.I TM transplanted after 4.5Gy TBI v 9.0Gy, underwent a shorter period of expansion (1 v 2 wks) and resulted in lower overall homeostatic cell numbers ($3x\downarrow$). OT.I TM infused into 9.0Gy-condtioned recipients a week post-transplant displayed similar kinetics and reached the same homeostatic number as OT.I cells infused at the time of transplant. However, when the infusion was delayed for 3 wks, there was marked reduction in expansion and lower homeostatic numbers ($6x\downarrow$). Transplantation of 0.1×10^6 OT.I TM v 1.5×10^6 TM, after 9.0Gy, TBI resulted in a shorter period of expansion (1 v 2 wks) and lower set-point ($4x\downarrow$). Interestingly, transplantation of 10×10^6 TM resulted in a rapid expansion the first week and steep contraction the second week followed by a rebound expansion and contraction. Notably, the ultimate set-point was greater than that achieved with 1.5×10^6 TM ($1.5x\uparrow$). In total, these results demonstrate the period of expansion and ultimate homeostataic numbers depend on the level of conditioning, timing of infusion, and number of TM transplanted. We conclude these parameters are important when engineering new strategies designed to enhance immune function post-transplant, including both immediate anti-tumor immunity and long-term anti-viral protection.

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HAPLOTYPE MISMATCHED MYELOABLATIVE STEM CELL TRANSPLANTATION: PHASE I CLINICAL TRIAL OF DONOR LYMPHOCYTE INFUSION DEPLETED OF ALLOREACTIVE T CELLS TO LIMIT INFECTIONS AND MALIGNANT RELAPSE WITHOUT CAUSING GVHD

Roy, D.C.[1], Cohen, S.[1], Busque, L.[1], Fish, D.[1], Kiss, T.[1], Lachance, S.[1], Sauvageau, G.[1], Caudrelier, P.[2], Roy, J.[1] [1]Hopital Maisonneuve-Rosemont, Montreal, QC, Canada; [2]Celmed BioSciences, Montreal, QC, Canada.

Patients with very high risk hematologic malignancies who cannot find an HLA-matched related or unrelated donor can benefit from haplo-mismatched transplantation. The latter is, however, complicated by frequent and severe infectious complications and disease relapse due to delayed immune reconstitution. We have previously reported that photodynamic therapy (PDT) could selectively deplete donor alloreactive populations while preserving lymphocytes for im-