checkpoint (beta-selection) and the effect was measured by the generation of DP cells. Ten days after induction of the LAT shRNA, only 14% of induced cells showed a DP phenotype, compared to 36% of uninduced control cells. We then evaluated the role of TCR signaling at the T cell selection checkpoint: LAT knockdown was induced when cells had reached the DP CD3+ stage. For TCRgd+ cells, we observed fewer mature T cells when LAT was downregulated versus control (17% vs 35%). Few TCRab+ mature cells were present in both control and LAT-downregulated populations, but a similar trend was observed. Our data suggest that acquisition of a mature phenotype in OP9-DL1 cocultures is TCR mediated, at least for the TCRgd+ population.

P.67 Comparison of immune reconstitution after hematopoietic stem cell transplantation with FLU-TBI vs. TLI-ATG conditioning

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The impact of the type of reduced intensity conditioning regimen used on immune recovery after allogeneic hematopoietic cell transplantation (allo-HCT) is poorly determined. We analyzed immune reconstitution in patients enrolled in a BHS-HCT sponsored randomized study comparing two non-myeloablative conditioning regimens for allo-HCT for which cell samples were prospectively collected. The conditioning regimen consisted of either 2 Gy TBI with 90 mg/m fludarabine (=TBI arm, n=21), or 8 Gy TLI plus thymoglobulin (ATG) 7.5 mg/kg (=TLI arm, n=19). Median ages at HCT were 59 yrs and 61 yrs in the TBI and TLI arms, respectively. Written informed consent has been obtained for each patient included. Absolute T cell counts were lower in the TLI arm than in the TBI arm on day 28 after HCT (P=0.04) but not thereafter. Further, B cells, as well as CD4+, CD4+CD45RA+ and CD4+CD45RO+ T cell reconstitution lagged behind in the TLI arm compared to the TBI arm the first year after HCT (B cells: p=0.0295 and others: p>0.0001). In contrast, reconstitution of CD8+ T cells, NK cells, Tregs and INKT cells were similar in both groups. For the thymic function, while sjTREC levels were higher in the TBI arm than in the TLI arm on day 100 (P=0.002) and on day 365 (not significant) after HCT, the increase in sjTREC levels from day 100 to day 365 was similar in the 2 groups. The diversity of the TCR repertoire was similar in the 2 groups of patients on day 100 after HCT. Finally, we found that ATG persists in patients up to 17 days after allo-HCT in TLI patients (median of [ATG] at day 17=0.62 mg/l and for one patient at day 20=0.53). In contrast, reconstitution of CD4+ T cells in the TLI arm and probably destroyed grafted sjTREC+ T cells. Finally, TLI conditioning has no impact on immune regulatory populations (Treg and INKT) after the transplantation.

P.69 Identification of biomarkers of hematostatic, endothelial and immune function in sepsis

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The pathophysiology of sepsis is still poorly understood. Recent evidence indicate that after an initial hyperinflammatory and procoagulant state, a protracted phase of consumptive coagulopathy, endothelial cell dysfunction and immune suppression is ultimately responsible for mortality. Most patients survive the initial phase with antibiotic, but may later need targeted treatment of hypocoagulability and immune stimulation. The identification of biomarkers of haemostasis, microvascular status and immune function is thus needed for patient stratification and tailored therapy.

In this study, eight patients with documented sepsis were tested at inclusion and after one, two and three days together with 21 normal individuals. Platelet function was assayed using the Multiplate instrument under ADP, arachidonic acid, ristocetin, collagen and thrombin stimulation. Clot formation was monitored by rotational thromboelastometry (ROTEM) using 1:1000 Innovin dilution (Srensen protocol). Immune competence was evaluated by measurement of regulatory T cells and monocyte subpopulations, i.e., CD14+CD16++ (non-classical), CD14++CD16- (classical), CD14++CD16+ (intermediate) and CD14+CD16++ (non-classical) monocytes. Expression of HLA-DR, CD163 and CX3CR1 was quantified in each monocyte subset.

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normal individuals. Platelet function was assayed using the Multiplate instrument under ADP, arachidonic acid, ristocetin, collagen and thrombin stimulation. Clot formation was monitored by rotational thromboelastometry (ROTEM) using 1:1000 Innovin dilution (Srensen protocol). Immune competence was evaluated by measurement of regulatory T cells and monocyte subpopulations, i.e., CD14+CD16++ (non-classical), CD14+CD16+ (intermediate) and CD14+CD16+++ (non-classical) monocytes. Expression of HLA-DR, CD163 and CX3CR1 was quantified in each monocyte subset. Circulating endothelial cells (CEC) and endothelial progenitor cells (EPC) were identified using a stringent protocol proposed by Case and colleagues (Curr. Protoc. Cytom., 52:9.33.1, 2010) with slight modifications. With all agonists, platelet activation was amplified in septic patients compared to controls (P<0.05). ROTEM assay revealed a delayed initiation of clot formation, enhanced clot propagation and hypofibrinolysis (all P<0.05). As previously described by Monneret et al., the proportion of Treg was increased in sepsis (P<0.05). All monocyte subsets were increased in sepsis patients, mostly the intermediate fraction (P<0.05). MFI of HLA-DR was downregulated while expression of CD163 was higher in all fractions (P<0.05).

P.68 Heterosexual HIV-1 Transmission is Associated with Allogeneic KIR/HLA Ligand Combinations Governing Natural Killer Cell Alloreactivity

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Killer-immunoglobulin-like receptors (KIR) regulate natural killer (NK) cells in a human leukocyte antigen (HLA)-dependent manner. KIR/HLA gene combinations at the level of the individual influence susceptibility to HIV-1 acquisition and disease progression. Allogeneic KIR/HLA mismatches improve survival of leukemia patients after hematopoietic stem cell transplantation. In this study, we analysed the effect of allogeneic KIR/HLA mismatches on HIV-1 transmission in a West African population of HIV-1 discordant and concordant couples. HIV-1 discordant couples were characterised by recipient partners with homozygous KIR2DL2, and by a mismatched recipient partner KIR2DL1/HLA-C2 index partner HLA-C1/C1 combination expected to allow licensed missing self NK cell killing of index partners’ cells. HIV-1 concordant couples on the other hand were characterised by KIR2DL3 homozygous recipient partners with HLA-C1/C2 bearing index partners, resulting in a matched KIR/HLA combination expected to inhibit NK cell killing. In vitro co-cultures of healthy donor-derived NK cells and HIV-1 patient-derived CD4+ T-cells confirmed the involvement of these allogeneic KIR/HLA combinations in NK cell-mediated CD4+ T-cell killing. Our data suggest that KIR/HLA incompatibility between sexual partners confers protection against HIV-1 transmission and that this may be due to recipient NK cell-mediated killing of the HIV-1 infected partner’s cells.