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Notch-signaling has a crucial role in T cell development. This has led to the development of a bone marrow stromal cell line, transduced with the Notch ligand delta-like 1 (OP9-DL1), which supports differentiation of hematopoietic stem cells to mature T cells. However, the mechanism of final maturation and positive selection of T cells *in vitro* remains to be elucidated. The system lacks thymic epithelial cells that present peptide-MHC complexes to the maturing T cell *in vivo*. We have shown previously that induction of human HLA-A2 on murine OP9-DL1 cells does not augment *in vitro* maturation efficiency. This suggests that MHC complexes, and consequently TCR signaling, might not be involved in maturation of T cells on OP9-DL1. To confirm these data, we explored the role of TCR signaling in the final maturation of T cells on OP9-DL1 by performing conditional knockdown experiments of linker for activation of T cells (LAT), a linker protein in proximal TCR signaling, that is essential for beta-selection as well as positive selection in murine knockout models. To validate the LAT shRNA, transcription of shRNA was induced at a stage before the first TCR 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 sponsorised 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 HSCT (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). These results suggest that ATG may be responsible for the delay of immune 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.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 leukaemia 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.

P.69 Identification of biomarkers of hemostatic, 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 antibiotherapy, 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 numeration of regulatory T cells and monocyte subpopulations, i.e., 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. 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<.05). ROTEM assays revealed a delayed initiation of clot formation, enhanced clot propagation and hypofibrinolysis (all P<.05). As previously described by Monneret *et al.*, the proportion of Treg was increased in sepsis (P<.05). All monocyte subsets were increased in sepsis patients, mostly the intermediate fraction (P<.05). MFI of HLA-DR was downregulated while expression of CD163 was higher in all fractions (P<.05). Expression of CX3CR1 was lower in classical and intermediate monocytes (P<.05) but higher in non-classical monocytes (NS). CEC were largely decreased in sepsis patients (P<.05) and EPC were slightly increased (NS).

A large array of haemostatic, vascular and immune abnormalities are identified in sepsis patients. Work is in progress to establish correlations with clinical scores and outcomes.

P.70 Immune Reconstitution After Alternative Hematopoietic Stem Cell Transplantation: Comparison of Unrelated Cord Blood (CB) and Mismatched Unrelated Donor (mmUD) Stem Cell Transplantation (SCT)

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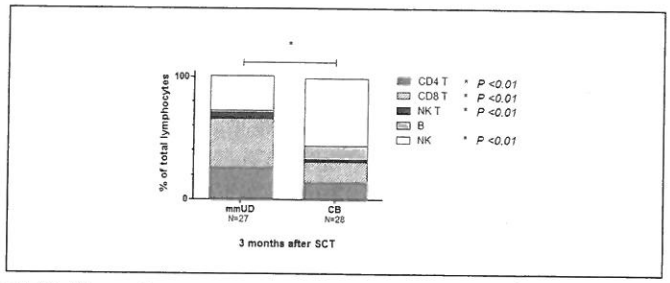
There is no consensus about the best alternative stem cell source when no suitable HLA-matched (un)related donor is identified. Data about immune recovery and infections risk after alternative SCT are limited. Here, we compared immune reconstitution after CB- *vs* mmUD-SCT.

Methods

Sixty-six patients who underwent SCT from either CB (n=30) or 9/10 HLA-mmUD (n= 36) at Saint-Louis Hospital (Paris) from 01/2005 to 12/2010 were evaluated. Immune reconstitution was prospectively assessed by flow cytometry on fresh blood samples collected at one month before and then at 3, 6, 12, 18, 24 and 30 months after SCT. The following phenotypes were studied: NK cells (CD3-CD56+); B cells (CD19+) and their nave (CD27-) and memory (CD27+) subsets; CD4+ and CD8+T cells and their nave (CD45RA+CCR7+), central memory (CM:CD45RA-CCR7+), effector memory (EM:CD45RA-CCR7-) and late effector memory (LEM:CD45RA+CCR7-) subsets as well as regulatory T cells (Treg:CD4+CD25+CD127low) and NKT cells (CD3+CD56+).

Results

Reconstitution of T cells was delayed in CB cohort compared with mmUD during the first 12months post-SCT (P<0.05), particularly for CD8+T cells subset (P<0.01). In opposite, NK cells recovered more rapidly during the first 6months after CB-SCT (P<0.01). B cells counts were also higher in CB recipients till 24months post-SCT (*P*=0.005). This resulted in significant differences in the pattern of immune circulating cells after SCT in CB and mmUD recipients, particularly at 3 months post-transplant (Fig.1). Concerning CD4+ and CD8+ T cells, the distribution between nave and memory subsets was different in CB and mmUD cohorts as T cells from CB were characterized by smaller proportion of nave and higher



P.70 *Figure 1.*

proportion of EM cells during early post-transplant period (P<0.01). B cells reconstitution was characterized by predominance of nave subsets in both cohorts.

Conclusion

In comparison with mmUD, SCT from CB was characterized by faster NK and B cells reconstitution but delayed T cells recovery, mainly for CD8+ and nave compartments. We are currently assessing if these patterns of immune reconstitution translated in different susceptibility for severe infections.

P.71 Swachman-Diamond Syndrome: Frequent misdiagnosis as Jeune Syndrome and other peculiarities

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Background

Shwachman-Diamond Syndrome (SDS) is a rare inherited disorder. The typical diagnostic triad (neutropenia, skeletal dysplasia and exocrine pancreatic insufficiency) is not always present at diagnosis. Aims: to review mutations and initial presentation in a Belgian cohort of patients with genetically proven Shwachman-Diamond Syndrome (SDS).

Methods

A retrospective study in eleven patients with SBDS mutations.

Results

In ten patients an SBDS mutation was identified in both alleles, patient eleven was heterozygous. The mean age at diagnosis was 2.9 years. All patients had exocrine pancreatic insufficiency. Radiological evidence of skeletal dysplasia was present in 9/10 studied. Neutropenia was present in 8/11 patients. Failure to thrive was demonstrated for all but P8. 2/3 patients experiencing cholestatic hepatitis required admission to ICU. Both had blood CMV PCR(+). The 3rd patient suffers from chronic liver failure due to liver fibrosis. 10/11 experienced recurrent infections (septicemia, respiratory tract infections, skin infections). Two patients had an episode of symptomatic (convulsions) hypoglycemia without satisfying explanation despite extensive metabolic analysis.

Three patients received a diagnosis of Jeune syndrome (one patient died of respiratory insufficiency) and 1/11 of hypobetalipoproteinemia prior to diagnosis of SDS. A metabolic disorder was first suspected in patient 11 because of hypertrophic cardiomyopathy. Two couples of siblings in our cohort showed an entirely different course.

Conclusion

SDS triad was present at diagnosis in only 6/9. A high index of suspicion is crucial. The peculiar misdiagnoses as Jeune syndrome is striking as are the episodes of symptomatic hypoglycemia and the suspected increased susceptibility to severe CMV disease.