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ABSTRACT BOOK

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in lymphocytes subsets, according to age and diseases; 3.To Study the impact of these factors on therapeutic responses and overall survival.
Methods: Healthy donors belonging to different ages groups and patients with lymphoproliferative diseases (LPD) are recruited for immune status evaluation after signed informed consent forms accepted by our ERB. Lymphocytes subsets were analysed by flow cytometry (CD4, CD4RA, CD4RO, CD4/CD25/FOXP3, CD8, CD19, CD16, CD56, CD197, CD27) before any treatment. Each lymphocytes subset was isolated by the MASC isolation technology for further molecular investigations. Tumor suppressor genes (TP53, PRDM1 and others) were quantified by RT-PCR on each purified lymphocytes subset.
Results: 21 healthy donors and 17 LPD (CLL and NHL) are currently prospectively investigated and stratified according to ages and diseases. Absolute lymphocytes count was not significantly different among the different groups. In terms of innate immunity, we found a significant lower number of NK celles (CD56+) betwenn younger (<50y) and older (p=0 ,002) and between healthy donors and patients (p=0,001). The functional tests are still on going. The CD4+/CD8+T cells ratio was significantly increased in older patients (p=0,008). Among CD4+T cells, CD4 memory and particularly T cenral memory lymphocytes were significantly increased. CD3-/CD4+was also increaded. Quantitative RT PCR analysis demonstrated a significant reduction in the TP53 gen expression in all pruified lympocytes subgroups (CD4+, CD8+, CD19+, CD57+) when matched healthy donors (>50yr) was compared with LPD patients (p=0,02). Thiese TP53 values were inversely correlated with the expression of RPDM1 gene. Patients recruitment is still on going and follow-up is too short to answer the tihrd objective.
Summary and Conclusions: our preliminary observations in a small series of donors confirm that 1) both innate and adoptive immunities are affected by aging: 2) Immuno-senescence is even more pronounced in patients compared to matched healthy donors. The reason remains to be investigated. 3) down-regulation of tumor suppressor gene such as TP53 is present in all lymphocytes subsets and is correlated with aging.

E1131

CHRONIC EXPOSURE TO INTERFERON-ALPHA DRIVES MEDULLAR LYMPHOPOIESIS TOWARDS T CELL DIFFERENTIATION IN MICE
M. Di Scala[1,*] I. Gil-Fariña[1], L. Vanrell[1], R. Sánchez-Bayona[1], D. Alignani[2], C. Olagüe[1], A. Vales[1], P. Berraondo[1], J. Prieto[1], G. Gonzalez-Aseguinolaza[1]
[1]Gene Therapy, [2]Department of Instrumental Techniques-Cytometry Unit, CIMA, Pamplona, Spain

Background: Interferon-α (IFNα) is an antiviral, immunomodulatory and antiproliferative cytokine which is produced in response to a variety of infectious agents including viruses and bacteria. It constitutes a key component of natural immunity linking innate and adaptive immune responses. In line with these functions, IFNα has been utilized in the treatment of chronic viral infections and diverse neoplastic conditions including hematological malignancies and solid tumors. However, these activities are counterbalanced by the induction of peripheral pancytopenia, which frequently limits its clinical use.
Aims: Although a large amount of information exits about the beneficial and deleterious effects of IFNα the modulation of hematopoiesis by IFNα still remains poorly understood. This study aimed to investigate the consequences of long-term IFNα treatment on blood cell homeostasis using a gene therapy vector expressing this cytokine.
Methods: In this work, we analyzed the hematopoietic changes occurring in mice subjected to chronic IFNα exposure. This was achieved by transducing the liver of C57/BL6 mice with an intravenous injection of an adenoassociated vector encoding IFNα (AAV-IFNα) to obtain sustained high serum levels of the cytokine. Furthermore, the consequences of chronic IFNα exposure on lymphoid differentiation was assessed by transferring bone marrow cells from IFNα-treated mice to Rag-/- mice. To understand the way IFNα modulates the commitment of HSCs, we analysed by quantitative real time-PCR the expression levels of key transcription factors (TFs) involved in multipotent progenitors (MPPs) lineage specification in total bone marrow (BM) cells, in purify Lin-cKit+(LK) cells and in differentiated cells (Lin+).
Results: Chronic IFNα exposure by AAV-IFNα injection induces a dramatic change in the composition of the leukocyte population in the peripheral blood and in the bone marrow. Here we found that (long-term hematopoietic stem cells) LT-HSCs and (short-term) ST-HSCs are dramatically reduced in IFNα treated animals causing a progressive and lethal pancytopenia indicative of the exhaustion of the HSCs compartment. Moreover, long term IFNα treatment guides multipotent hematopoietic progenitor cells toward a T cell fate. IFNα directly downregulates both *in vivo* and *in vitro* the expression of B cell TFs in lymphocyte precursor cells and this effect alters the differentiation of these cells driving them to the production of T lymphocytes.
Summary and Conclusions: In conclusion, our results demonstrate that long-term exposure to IFNα exerts a complex impact on hematopoiesis, it compromises the stemness of hematopoietic stem cells (HSCs) but also redirects the function of the hematopoietic precursors cells triggering an unique genetic program in these cells favoring the generation of T cells while blocking the development of B cells.

E1132

Abstract withdrawn

Hematopoiesis, stem cells and microenvironment

E1133

REQUIREMENT FOR PHOSPHOLIPASE C GAMMA 1 (PLCG1) IN DEVELOPMENT AND MAINTENANCE OF HEMATOPOIETIC STEM- AND PROGENITOR CELLS
P. Arreba-Tutusaus[1,*] T. Schnoeder[1], T. Fischer[1], F.H. Heidel[1]
[1]Department of Hematology and Oncology, Otto-von-Guericke University, Magdeburg, Germany

Background: Hematopoietic stem cells (HSC) play a crucial role in the maintenance of hematopoiesis, balancing self-renewal capacity and differentiation potential to form more committed progenitor cells. HSC homeostasis is highly regulated by a complex network of signaling pathways and transcription factors. Phospholipase C gamma 1 (Plcg1) is known as a key regulator of calcium signaling which plays an important role in proliferation and differentiation of immune. Upon activation by receptor and non-receptor tyrosine kinases, such as T-cell receptor or JAK2, Plcg1 regulates the hydrolysis of phosphatidylinositol 4,5-biphosphate, leading to the activation of various downstream pathways. Several studies have reported Plcg1 to be essential for erythropoiesis during murine embryonic development as well as for granulopoiesis in zebrafish models. Recently, our group provided first evidence for Plcg1 regulating maturation of adult erythropoiesis. However, no previous study has investigated whether Plcg1 is required for development or maintenance of hematopoietic stem cells.
Aims: In this study we aim to investigate the functional role of Plcg1 signaling in fetal liver cells and adult hematopoietic stem- and progenitor cells using RNA interference technology.
Methods: Fetal liver cells (FLC) were isolated from embryos at stage E13.5 while adult immature hematopoietic cells (Lin-Sca1+KIT+CD48-CD150+) were sorted from 6-8 weeks C57/BL6 mice. Cells were transduced with either (non-targeting) control shRNA or two different validated shRNAs targeting Plcg1. All shRNAs were either GFP-labeled or selectable by puromycin. Colony-forming potential was measured in methylcellulose medium and immunephenotype was analyzed by flow cytometry. Functional potential of HSPCs was measured *in vivo* using a short-term colony-forming unit spleen assay (CFU-S12) as well as a long-term competitive repopulation assays. Engraftment capacity was measured by a homing assay.
Results: Following Plcg1 knockdown, colony-forming capacity was significantly reduced in fetal liver cells and adult HSC (when compared to non-targeting control). While fetal liver cells showed a significant erythroid maturation defect upon Plcg1 knockdown in our previously published data, hematopoietic stem cells revealed no maturation defect or lineage bias following inactivation of Plcg1. Immunophenotypic analysis of colonies confirmed presence of all lineages at reduced total numbers. To assess for HSC function we performed colony-forming spleen assay and competitive repopulation studies *in vivo*. Here, loss of Plcg1 affected the functional potential of HSCs and fetal liver cells with significant impairment of their colony-forming potential and repopulation capacity. This was indicated by a drop in whole bone marrow chimerism below 10% at week 16-20 post-transplantation. Again, lineage commitment was not affected, while a reduction in HSPC abundance was observed. Inactivation of Plcg1 did not affect homing of transplanted HSCs but effectively reduced proliferative potential.
Summary and Conclusions: Taken together, our data provide first evidence that Plcg1 is required for HSPC homeostasis, since its genetic inactivation negatively affects the functional capacity of HSCs. Ongoing experiments investigate the effects of Plcg1 on cell cycle activity and induction of apoptosis and, aim to establish a mechanistic model to explain the observed phenotype.

E1134

THE UTILITY OF FLUORESCENCE LIFETIME IMAGING IN ROUTINE BONE MARROW SMEARS
I. Lorand-Metze[1,*] A.P. Racanelli[2,3], C. Lenz Cesar[4,5], M.A. Falconi[6], K. Metze[2,7,8]
[1]Internal Medicine, [2]Laboratory of Analytical Cellular Pathology, [3]National Institute of Photonics applied to Cell Biology ,(INFABIC), [4]Institute of Physics, [5]National Institute of Photonics applied to Cell Biology ,(INFABIC, [6]Hematology/Hemotherapy Center, [7]Pathology, [8]National Intitute of Photonics applied to Cell biology (INFABIC), University of Campinas, Campinas, Brazil

Background: After excitation by a photon, a fluorophore will drop to the ground state with some delay, according to exponential decay rates. This delay is called lifetime. The new technique "fluorescence-lifetime imaging microscopy (FLIM)" creates the image contrast with the help of the fluorescence lifetime values (transformed in pseudo-colors) at each pixel of the two-dimensional microscopic image and does not use the local concentration of the fluorophores or the emitted spectrum. Now we can visualize microscopic structures based on differences of their fluorescence decay rates, which depend on the physicochemical properties of the molecules, even when fluorescence is emitted at the same wavelength. FLIM is a non-invasive technique, using low-potency lasers. Therefore

it has been widely used in experimental settings with cell cultures or small organisms. There are only very few reports on its application for diagnostic purposes in routinely collected samples, such as bone marrow smears.
Aims: The aim of this pilot study was to investigate the utility of the FLIM technique for diagnostic purposes in routinely collected bone marrow smears.
Methods: We used non-stained routine bone marrow smears of 15 patients after fixation with formaldehyde vapor. Images were obtained with a confocal Zeiss Upright LSM780-NLO microscope equipped with a 63x oil immersion objective and a HPM-100-40 Hybrid detector (Becker & Hickl), Image size was 512 x 512 pixels. The specimens were excited by a 405 nm pulsed diode laser (80 MHz). In order to create equivalent images of the cytologic smears, pseudo-colors were attributed to different lifetime ranges. Images were compared with the standard May-Grünwald-Giemsa (MGG) stained smears.
Results: In every case we obtained highly contrasted FLIM images, with clearly distinguishable cellular elements. Photobleaching was rare. The obtained chromatin textures were somehow similar to that of the MGG images and permitted to recognize the different types of hemopoietic cells. Erythrocytes were characterized by the short lifetimes of their hemoglobin component. Cytoplasm and the protein background showed intermediate lifetime values. Fluorescence lifetime of granulopoetic nuclei was considerably longer than in nuclei of erythroblasts. Leukemic blasts of several types of acute leukemia showed considerable variation, generally long lifetime values. In one case of Chediak-Higachi disease, the pathologic cytoplasmic granula were clearly distinguishable. Besides, in that case, intracytoplasmic bacteria could be identified, which were not well visible in the MGG stained smears.
Summary and Conclusions: The FLIM technique can be applied in routinely acquired diagnostic bone marrow smears. No staining is needed. The images are well contrasted, and permit proper identification of the cellular elements. Different lifetime values of nuclear chromatin distinguish erythropoetic and granulopoetic lineage, thus suggesting relevant physicochemical differences of the nuclear organization. Supported: FAPESP, CNPq

E1135

CORD BLOOD STEM CELLS BUT NOT ADULT STEM CELLS AFTER TRANSPLANTATION OVEREXPRESS STEMNESS AND REPROGRAMMING GENES PARTIALLY OVERLAPPING THE SIGNATURE OF INDUCED PLURIPOTENT STEM CELLS (IPS)

D. Cilloni[1,*] M. Podestà[2], J. Petiti[1], S. Carturan[1], A. Bertaina[3], F. Sabatini[2], V. Campia[1], V. Gaidano[1], P. Nicoli[1], M. Berger[4], F. Saglio[4], G. Bandini[5], F. Bonifazi[5], F. Fagioli[4], L. Moretta[6], F. Locatelli[3], F. Frassoni[2]
[1]Dept Of Clinical and Biological Sciences, University of Turin, Orbassano, [2]Stem Cell Laboratory, G.Gaslini Institute, Genova, [3]Ospedale Bambino Gesù, Rome, [4]Ospedale Regina Margherita, Turin, [5]Seragnoli Institute, Bologna, [6]G.Gaslini Institute, Genova, Italy

Background: Hematopoietic stem cells (HCT) undergo tremendous expansion and amplification during Hematopoietic Cell Transplant (HTC). To cope with this challenge, HSC must activate several genes including those responsible for self-renewal.
Aims: To prove or disprove whether HSC decline or not in their proliferative potentiality after HCT, we analyzed the expression of genes involved in self-renewal and reprogramming in CD34+cells obtained from bone marrow cells after the engraftment has been achieved.
Methods: Ninety-three genes, mainly involved in HSC regulation plus 30 gene involved in epigenetic regulation, were analyzed in CD34+cells isolated from: (i) Cord Blood (CB), (ii) normal Bone Marrow (BM), (iii) BM taken after umbilical CB Transplant (UCBT), BM taken from (iv) adult or (v) children transplanted with adult HSC. Expression data were compared among the five groups and further with those obtained from iPS (induced pluripotent stem cells).
Results: Among the 93 genes analyzed, the following genes: *DPPA2, LIN28, NANOG, NESTIN, OCT4, SOX1* and *SOX2* were found highly over-expressed in CD34+cells isolated after UCBT with respect to CB or BM CD34+cells. The level of expression of the above mentioned genes found in CD34+cells after UCBT was similar to iPS cells. However, relevant differences in the expression of several other genes were found between CD34+cells post-UCBT and iPS. For instance, PTEN expression was 2 logs higher in UCBT CD34+cells than in iPS. Protein analysis on CD34+cells confirmed the RNA data. Remarkably, over-expression of genes overexpressed in CD34+cells after UCBT was not observed in CD34+taken from BM after any other type of adult cell transplantation. Moreover, we found about 2 logs over-expression of genes involved in epigenetic control in CD34+cells taken from BM after UBCT when compared to native CB CD34+cells.
Summary and Conclusions: CD34+cells taken from BM after UCBT overexpress fundamental genes involved in self-renewal and somatic cell reprogramming thus, partially acquiring the signature of iPS. These features are unique since no other CD34+cell taken either from CB or adult BM or after any hematopoietic adult cell transplantation shows similar pattern of gene expression. These findings open new perspectives: (i) toward a better understanding of transplantation biology, (ii) toward governing gene expression in iPS to render them safer for therapeutic purposes; (iii) in designing new methods to expand HSC in more efficient and consistent manner.

E1136

STROMAL CELL-DERIVED FACTOR-1 PLAYS IMPORTANT ROLES IN THE REGULATION OF HUMAN EARLY B- AND T/NK-LINEAGE LYMPHOID DIFFERENTIATION IN DIFFERENT MANNERS

H. Minami[1,*] K. Ohishi[2], M. Masuya[1], N. Katayama[1]
[1]Hematology and Oncology, Mie University Graduate School of Medicine, [2]Blood Transfusion Service, Mie University Hospital, Mie University, Tsu city, Mie prefecture, Japan

Background: Stromal cell-derived factor-1 (SDF-1) is shown to be essential for B-lymphoid differentiation in mice. However, role for SDF-1 in human lymphopoiesis remains undefined. We previously reported that telomerized human stromal cells support the differentiation of human hematopoietic progenitors to $CD45RA^+CD7^+CD10^-$ T/NK- and $CD45RA^+CD7^-CD10^+CD19^+$B-lineage lymphoid precursors (Br J Haematol. 157:674-86, 2012). Because the stromal cells produced SDF-1, we examined a role of SDF-1 in human early lymphoid differentiation, using our coculture system.
Aims: In this study, we investigated whether and how SDF-1 regulates early lymphoid differentiation from human hematopoietic progenitors to $CD45RA^+CD7^+CD10^-$ and $CD45RA^+CD10^+CD19^+$lymphoid precursors in the presence of stromal cells.
Methods: $CD34^+lin^-CD45RA^-CD38^{lo}$ hematopoietic progenitors were purified from cord blood and cultured for 21 days either on *hTERT*-transduced human bone marrow stromal cells or with conditioned medium (CM) collected from cultures with stromal cells, in the presence of SCF, Flt3L, and TPO. To block the binding of SDF-1 to CXCR4 receptor, anti-CXCR4 blocking antibody (Ab) was added to the cultures. In some experiments, $CD34^+lin^-CD45RA^-CD38^{lo}$ cells were cultured with CM for 14 days and $CD45RA^+CD7^+CD10^-$ or $CD45RA^+CD7^-CD10^+$cells were isolated and incubated on stromal cells with anti-CXCR4 Ab or isotype control.
Results: In the cultures on stromal cells, anti-CXCR4 Ab significantly inhibited the generation of $CD45RA^+CD7^+$as well as $CD45RA^+CD10^+$lymphoid precursors from $CD34^+lin^-CD45RA^-CD38^{lo}$ hematopoietic progenitors. Anti-CXCR4 Ab predominantly inhibited B-lineage differentiation in the cultures with CM. We next examined the effect of anti-CXCR4 Ab on $CD45RA^+CD7^+CD10^-$ lymphoid precursors by culture on stromal cells. In control cultures, the number of $CD45RA^+CD14^-$ lymphoid cells including $CD10^+$cells increased during 10 days of cultures. However, in the presence of anti-CXCR4 Ab, the generation of $CD45RA^+CD14^-$ lymphoid cells including $CD10^+$cells from $CD45RA^+CD7^+CD10^-$ cells was suppressed, and few or no $CD45RA^+CD14^-$ cells were detected at day 10 after culture. Significant numbers of $CD14^+$monocytic cells were generated in both cultures. In the culture of $CD45RA^+CD7^-CD10^+$cells on stromal cells, anti-CXCR4 Ab inhibited their differentiation to $CD45RA^+CD10^+CD19^+$proB cells.
Summary and Conclusions: These data suggest that SDF-1 is important for B-lineage differentiation regardless of presence of stromal cells but critical for lymphoid differentiation from human early hematopoietic and $CD7^+$lymphoid precursors in contact with stromal cells. These findings indicate that SDF-1 plays key roles in early B- and T/NK-lineage lymphoid differentiation in different ways.

E1137

COMPARISON OF SELF-RENEWAL EXPRESSION IN STROMAL IN VITRO AND IN VIVO MICROENVIRONMENT MODELS – CRITICAL DIFFERENCES AND THEIR IMPACT ON HEMATOPOIETIC SUPPORT FUNCTIONS

G. Horne[1,*] V. Campbell[1], H. Wheadon[1], T. Holyoake[1], M. Copland[1]
[1]Paul O'Gorman Leukaemia Research Centre, University of Glasgow, Glasgow, United Kingdom

Background: The intricate relationship between the bone marrow (BM) microenvironment and hematopoietic stem cells (HSCs), both normal and malignant, is well established. Recent evidence highlights the importance of the BM niche in governing stem cell behavior. Translational research is dependent on understanding this relationship, utilizing both 2D and 3D *in vitro* co-culture experiments aiming to recapitulate this environment prior to *in vivo* studies. The use of radiation and anti-proliferative agents on stromal cells is essential in long-term co-culture. The effects of these treatments on hematopoietic supportive function remain poorly understood.
Aims: (1) To define patterns of gene and protein expression of self-renewal pathway components in *in vitro* co-culture models, using mesenchymal stem cells (MSCs) and stromal cell lines; (2) To compare *in vitro* and *in vivo* models, using CD34+CML-engrafted NSG mice. We hypothesized that radiation, anti-proliferative agents and confluency changes would be integral in altering hematopoietic supportive function leading to incomparable results across models.
Methods: Human BM samples were collected from healthy donors and CML patients (n=13) following informed consent. An unselected population of mononuclear cells was isolated to allow expansion of MSCs by plastic adherence. MSC immunophenotype was confirmed as described (Mennan et al, 2013). Primary CD34+CML cells were transplanted into sublethally irradiated (2.5Gy) 8week old NSG mice (n=6). Mice were euthanized after 16weeks. Engraftment was assessed using anti-human CD45 FACS analysis. Sorted CD45+cells were evaluated for BCR-ABL translocation by FISH. *In vitro*, M210B4, SLSL and HS5