exposed to the CM (6 h), and Hamp levels were quantified by real-time PCR. CM of Epo-treated K562 cells (CM-E) significantly decreased Hamp mRNA compared to CM of control K562 cells (CM-C= 1.00; *CM-E= 0.33 ± 0.12; *P<0.05, n= 5). Since Epo directly suppresses Hamp in HepG2 cells, we studied its production by the K562 cell line. Epo mRNA was undetectable in both Control and Epo-treated K562 cells (RT-PCR). Similarly, protein levels of Epo were scarce in lysates and in CM of control and Epo-treated K562 cultures (Western blotting), thus excluding the possibility that Hamp suppression by CM is due to the release of Epo by these cells. This indirect activity of Epo led us to investigate ERFE expression in K562 cells exposed to the cytokine for different times. ERFE mRNA was detected at basal levels in this cell line, and increased 2-fold on average after 1 h of Epo exposure (n= 3). Our results suggest that Epo-treated erythroid cells release a factor that reduces Hamp levels in HepG2 cells, consistently with the expression of ERFE in the K562 cell line. Further research using this experimental model may help increase knowledge about Fe regulation.

0568 - ASYMMETRIC DIMETHYLARGININE (ADMA) INHIBITS THE EFFECT OF ERYTHROPOIETIN ON ENDOTHELIAL CELL MIGRATION IN A PROINFLAMMATORY ENVIRONMENT

María Eugenia CHAMORRO | Romina MALTANERI | Agustina SCHIAPPACASSE | Daniela VITTORI | Alcira NESSE

DEPARTAMENTO DE QUÍMICA BIOLÓGICA-IQUIBICEN, FCEN, UBA

Abstract/Resumen: Hyperhomocysteinemia induces vascular endothelial dysfunction, an early hallmark of atherogenesis. Previously, we found a sensitizing effect of the inflammatory cytokine TNF-alpha on a promigratory action of erythropoietin (Epo), which was not observed when the molecule was modified by N-homocysteinylation with homocysteine thiolactone, the reactive derivative of homocysteine. The strong correlation found between hyperhomocysteinemia and levels of asymmetric dimethylarginine (ADMA), the amino acid formed during methylation of proteins, has been considered of interest in endothelial dysfunction. Therefore, the aim of this work was to study whether ADMA accumulation could affect the ability of different factors to induce endothelial cell migration. Wound healing assays of EA.hy926 endothelial cells stimulated by 10 % fetal bovine serum (FBS), 20 ng/mL VEGF or Epo+TNF-alpha (80 ng/mL and 30 ng/mL, respectively) were performed in the presence or absence of ADMA (30 or 100 µM). Results are expressed as a percentage of the cell migration obtained in the presence of FBS. FBS: 100; Control: 22.0 ± 2.4; ADMA: 20.1 ± 3.4; *FBS+ADMA30: 66.6 ± 3.5; **FBS+ADMA100: 43.3 ± 3.9; Epo+TNF-alpha: 56.4 ± 1.2; *Epo+TNF-alpha+ADMA30: 37.4 ± 2.9; **Epo+TNF-alpha+ADMA100: 31.1 ± 1.6; VEGF: 44.5 ± 2.9; *VEGF+ADMA30: 30.3 ± 2.1; **VEGF+ADMA100: 19.6 ± 2.8; *p<0.05 and **p<0.01 vs. respective controls, n= 4. It can be seen that the inhibition of the promigratory effects of all the factors analyzed was dependent on ADMA concentration. The results of cell viability and MTT assays confirmed that the effects of ADMA on the promigratory action of Epo+TNF-alpha and VEGF cannot be attributed to cytotoxicity or cell proliferation. These results underline the important role of homocysteine in cardiovascular pathophysiology, since increased homocysteine is involved in a direct inhibition of the enzymes responsible for ADMA degradation.

0638 - BCL2 AS A MARKER TO IDENTIFY REFRACTORY AND AT RISK OF RELAPSE DISEASE IN CLASSIC HODGKIN LYMPHOMA PATIENTS. ITS BLOKEADGE AS A POTENTIAL DIRECTED-TEHRAPY.

Angélica GAMBOA-CEDEÑO (1) | Mariángeles DÍAZ(2) | Nancy CRISTALDO(3) | Victoria OTERO(3) | Natalia SCHUTZ(3) | Dorotea FANTL(3) | Federico JAUK(3) | Hernán GARCÍA-RIVELLO(3) | Nuñez MYRIAM(2) | Stella Maris RANUNCOLO(3)

IMTIB CONICET-IUHI-HIBA (1); FACULTAD DE FARMACIA Y BIOQUÍMICA UBA (2); HIBA (3)

Abstract/Resumen: Refractory and relapsed disease (RRD) is currently the challenge when treating classic Hodgkin Lymphoma (cHL) patients. There is no specific therapy rather than rescue chemotherapy which fails in 50% of the cases and associates with high toxicity. We have previously reported that the alternative NFkB pathway, mediated by Rel-B and NIK, plays a key role in cHL survival through high BCL2 expression levels. We aimed to analyze if mediators of this pathway and BCL2 could be useful as prognosis markers and would represent targetable factors in RRD. We analyzed NIK and BCL2 expression in Hodgkin Reed-Sternberg cells in the lymph node biopsies of 113 cHL naïve of therapy patients by IHQ [52 female Md age (range) 36 (6-88), 61 male 40.7 (9-78)]. The univariate analysis showed no correlation between NIK or BCL2 expression and the clinical and pathological parameters, including the PET scan indicated at the end of the first line treatment. The statistical significance was maintained in multivariate analysis (Cox Regression, p= 0.01). NIK expression did not associate with prognosis but the BCL2 expression level correlated with lack of response to conventional therapy and both early and late disease progression. The survival analysis, using the Kaplan-Meir curves, showed that patients with ≥60 % positive HRS cells had a shorter disease-free survival (DFS) [log rank test, p= 0.002] and a reduced overall survival (OS) [log rank test, p=0.02]. Human cHL cell lines that express BCL2 protein, were sensitive to venetoclax, a specific BCL2 inhibitor. The drug induced a citostatic effect with cell arrest in S-Phase. We found that the alternative NFkB pathway plays an important role in the refractory and relapsed Hodgkin disease, being BCL2 a key downstream target. BCL2 performed well as a prognosis marker identifying refractory patients and those that relapsed. We believe BCL2 directed-therapy should be explored in cHL patients that express this protein in the biopsy performed at diagnosis.

0656 - ACTIVATION OF TOLL-LIKE RECEPTORS 2 AND 4 ON CD34+ CELLS INCREASES HUMAN MEGAKARYO/THROMBOPOIESIS INDUCED BY THROMBOPOIETIN

Camila Sofía RODRÍGUEZ(1) | Carolina Paula MIGUEL(1) | Roberto Gabriel POZNER(1) | Juan Manuel ORTIZ WILCZYÑSKI(1) | Soledad NEGROTTO(1) | Eugenio Antonio CARRERA-SILVA(1) | Paula Graciela HELLER(2) | Mirta SCHATTNER(1) | **Lina Paola D'ATRI** (1)

INSTITUTO DE MEDICINA EXPERIMENTAL (IMEX), CONICET-ACADEMIA NACIONAL DE MEDICINA (ANM) (1); INSTITUTO DE INVESTIGACIONES MÉDICAS ALFREDO LANARI - UNIVERSIDAD DE BUENOS AIRES (2)

Abstract/Resumen: Platelet (PLT) toll-like receptors (TLR) 2 and 4 are key players in amplifying the host immune response. However, its role in human megakaryo/thrombopoiesis has not yet been defined. Our aim was to evaluated whether Pam3CSK4 (Pam) or lipopolysaccharide (LPS), TLR2/4 ligands respectively, modulate human megakaryocyte (MK) development and PLT production. CD34+ cells from human umbilical cord were stimulated with LPS or Pam3CSK4 with or without thrombopoietin (TPO). TLRs expression was determined by RT-PCR and flow cytometry (FC). Differentiation, maturation, PLT generation, c-MPL (TPO receptor) expression and IL-6 production was evaluated by FC and proPLTs and NF-E2 migration by confocal microscopy. CD34+ cells and MK express TLR2 and TLR4 at both RNA and protein level. Direct stimulation of CD34+ cells with LPS or Pam had no effect in cell growth. Interestingly, both TLR ligands markedly increased TPO-induced CD34+ cell proliferation, MK number and maturity, proPLT and PLT production when added at day 0 (n= 7, p<0.05), without increases of c-MPL surface expression. In contrast, this synergism was not observed when LPS or Pam were added 7 days after TPO addition. IL-6 release was observed upon CD34+ or MK stimulation with LPS or Pam but no with TPO and this effect was potentiated in combination with TPO (n= 4, p<0.05).