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suggest that the regulation of both genes may be coordinated through a bi - directional promoter. However the STORP gene is not transcriptionally induced by Interferon alpha (IFN α) and thus does not share this pattern of regulation with the PML gene. Its homologies to the EPB72 gene, which has been implicated in hereditary stomatocytosis, as well, as its overexpression in K562 cell line raises the questions of its possible role in red cells disorders or function.

Abstract# 2996

HEREDITARY SPHEROCYTOSIS DEFICIENT IN PROTEIN 4.2. ASSOCIATED TO MULTIPLE MYELOMA. ONE CASE REPORT. E. Granjo, M.L. Ribeiro, H. Almeida, A. Nóvoa, J. Candeias, M.M. Ribeiro. *Hospital S. João, Centro de Citologia Experimental, Porto, Centro Hospitalar de Coimbra, Coimbra, Portugal.*

Hereditary spherocytosis (HS) is an inherited hemolytic anemia characterized by anemia, intermittent jaundice and splenomegaly, it is due to mutations in the genes encoding ankyrin, the anion exchanger, spectrin, protein 4.1 or protein 4.2. Hemolytic crises are frequent, presumably secondary to the reticuloendothelial hyperplasia that accompanies many infections and other oxidative stress situations. HS patients have a mild, polyclonal hypergamaglobulinemia. The authors report a 66 years old Caucasian female referred to hematology out-patients clinics of Hospital de São João in May 1994, because of long standing history of anemia, severely aggravated by pregnancies and infections. One month before she had an emergency cholecystectomy due to lithiasic cholecystitis. Physical examination disclosed icterus and slight splenomegaly. The hematological and biochemical parameters were compatible with spherocytic hemolytic anemia. The diagnosis of HS was confirmed by an increased osmotic fragility test and the finding of 12% decrease in protein 4.2 in the red cell membrane protein electrophoresis by Laemmli and Fairbanks techniques. Serum immunoglobulins had a slight polyclonal increase. The patient refused therapeutic splenectomy and follow-up documented occasional hemolytic crises secondary to infections. In March 1996 she complained of fatigue, asthenia and bone pain. Laboratory investigations were compatible with multiple myeloma (MM) Ig A/l stage I, according to the criteria of Durie and Salmon. Protein 4.2 is a major component of the erythrocyte plasma membrane which binds to the cytoplasmic domain of band 3 and plays an important role in maintaining erythrocyte stability and integrity. A complete or partial deficiency of this protein results in HS with different degrees of severity. Protein 4.2 deficiency has been described in patients of Japanese descent and cases were identified in Tunisia, Portugal and Italy. Patients with HS and MM had been reported in the literature. Some had gallbladder disease, splenectomy had never been performed. The association may be due to chronic reticuloendothelial stimulation since splenic clearance of abnormal red cells induces proliferation of lymphocytes and plasma cells as well as macrophages.

Abstract# 2997

BAND 3 PROFILE AS A MARKER OF OXIDATIVE STRESS IN HEREDITARY SPHEROCYTOSIS. E. Granjo*, A. Santos-Silva*, I. Rebelo*, A. Nóvoa*, E. Costa*, J. Barbot*, M.M. Ribeiro, A. Quintanilha*. *Dep. Bioquímica Fac. Farmácia Univ. Porto, Instituto Biologia Molecular Celular Univ. Porto, Hosp. S. João Porto, Hosp. Crianças Maria Pia, Portugal.*

The RBC presents a particular sensitivity to oxidative and proteolytic stress on account of its limited biosynthetic capacity, which precludes the repair or replacement of damaged proteins. Therefore, any normal RBC during its life span undergoes physical and chemical changes, which become more pronounced with cell age. RBC senescence includes several modifications, namely a reduction in the metabolic activity resulting from a decreased enzymatic activity. The pathway for removal of senescent or damaged RBC involves the development of a senescent cell antigen immunologically related to band 3 protein, which marks the RBC for death, by triggering the binding of specific auto-antiband 3 antibody and complement activation. In the case of spherocytes, presenting a destabilized membrane structure, with loss of membrane components and with an abnormally reduced life span, the modifications in band 3 profile are probably enhanced. In this perspective we studied a healthy control (n = 29) and two groups of patients with hereditary spherocytosis. One included patients who undergone splenectomy (n = 11) and the other patients who did not undergo splenectomy (n = 10). We studied hematological parameters: concentration of WBC and of the several WBC types, concentration of RBC, Ht, Hb concentration, hematimetric indexes, RBC glucose-6-phosphate dehydrogenase activity (G6PD), band 3 profile (high molecular weight aggregates, monomer and total proteolytic fragments), % membrane bound hemoglobin (%MBH). The changes in band 3 profile, %MBH and in G6PD activity are enhanced in spherocytic patients who did not undergo splenectomy when compared to the control, denoting an accelerated oxidative and proteolytic membrane damage and metabolic degradation. These results provide evidence of premature senescence and removal of RBC in these patients. The removal of the spleen imposed an improvement in hematological profile. However, the changes in band 3 profile, in %MBH and in G6PD activity are even more pronounced after splenectomy. In the absence of the organ responsible for the removal of the old or damaged RBC, the RBCs in these patients can circulate for longer periods of time although presenting more oxidative and proteolytic lesions. Our data suggests that spherocytes from a patient who underwent splenectomy are more sensitive to an oxidative and proteolytic stress and, therefore, the development of that stress may trigger an hemolytic event. Actually these patients often present an hemolytic event during infectious or inflammatory processes.

Abstract# 2998

B, T, NK CELL POPULATION AND SERUM IMMUNOGLOBULINS IN HEREDITARY SPHEROCYTOSIS – PRE AND POST SPLENECTOMY AND UNDER OXIDATIVE STRESS. E. Granjo, A. Nóvoa, M. Fraga, M. Lima, A Santos Silva, I. Rebelo, L. Rodrigues, L. Ribeiro, A. Quintanilha, M.M. Ribeiro*, J Candeias. *H.S. João, H. Sto. Antonio, Pedro Hispano, Instituto de Biologia Molecular e Celular, Porto, Centro Hospitalar de Coimbra, Coimbra, Portugal.*

Hereditary Spherocytosis (HS) is the most common congenital hemolytic anemia. Its main clinical features are hemolytic episodes triggered by infection, pregnancy and other stresses and responsiveness to splenectomy (SPL). In order to clarify immunological disturbances associated to HS, the authors analyzed B-, T- and natural killer (NK)-lymphocyte (Lymph) populations and immunoglobulins (Igs) in the peripheral blood of 18 patients (pts) with HS (age 12-68 years; 10 males, 8 females), before and/or at different times after SPL. When non-SPL and SPL pts were compared the following results were obtained: adult non-SPL pts (n = 9) showed a tendency to a slight decrease of absolute numbers of Lymph compared to controls. Non-SPL children (n = 2) showed no differences compared to age-matched controls. Adults pts that have been studied 1 year or more after SPL (n = 6) showed a tendency to have increased absolute numbers of Lymph T- and NK-cells, compared to both non-SPL pts and normal controls. Three of these pts had absolute lymphocytosis (3.7-6.8 × 10^9/L) with increased T-, B- and NK-cells (2 pts) or T- and NK-cells (1pt). The highest NK-cell lymphocytosis (2.4 × 10^9/L) was observed in a 23 years old male with long standing recurrent chronic leg ulcers who had transitional neutropenia. This pt showed a minor abnormal NK-cell population (10%). TCR vβ and TCR β and δ rearrangement studies showed a germ-line pattern. None of these pts have been studied before. Four pts were analyzed sequentially before and after SPL (1 day-9 months). The day after SPL there was absolute leukocytosis with neutrophilia and slight lymphopenia, mainly due to a decrease of T- and NK-cells. After that there was a progressive normalization of absolute number of lymphocytes with tendency to increase above control values. No alterations were found in serum Igs (IgG, IgA and IgM) before SPL. In 3 out of 4 pts in the 1st day after SPL all Igs were decreased, with a tendency to increase above normal levels. SPL pts that were studied sequentially 1 year or more after SPL (n = 3) did not show relevant changes in Lymph populations during the follow-up period. These preliminary results seem to indicate that immunological abnormalities found in HS may be of multifactorial origin: disease itself; continuous stimulation of immune system (infections and/or hemolytic episodes); therapeutic SPL. Changes occurring immediately after SPL are probably associated to surgical stress. This is followed by a variable period of time characterized by peripheral blood changes that probably result from alteration in Lymph circulation and homing. After that period a new state of equilibrium is achieved in asplenic state, some pts developing lymphocytosis post SPL. Evaluation of serum levels of various cytokines and growth factors may clarify the mechanisms underlying the immunological changes associated to HS and SPL.

Abstract# 2999

URINARY COPPER AS A MARKER OF HEMOLYTIC ANEMIA. G.C. Harewood*, S.N. Markovic, R.L. Phyliky (Intr. by R.L. Phyliky). *Div. of Hematology, Mayo Clinic, Rochester MN, USA.*

Erythrocytes contain 5-10% of the body's copper stores as part of superoxide dismutase. It is conceivable that during hemolysis, copper is released and eliminated via the urine, resulting in increased urinary copper levels in a 24-hour collection (UCu). Herein, we present data to suggest the potential utility of urinary copper levels as a marker of hemolysis.

Using an electronic diagnostic database, cases of acquired hemolytic anemia (Coomb's positive or negative) and evidence of measured UCu at Mayo Clinic between 1987 and 1997, were identified. Patients with Wilson's disease were excluded.

Twenty-eight cases were identified, of which 16 had demonstrable hemolysis at the time of UCu collection (8 Coomb's positive). No patients had documented evidence of pyuria or hematuria. The rest of the patients (controls: 12/28) had a history of hemolytic anemia at some time during their life as well as had a UCu collection unrelated to the time of hemolysis. Hemolysis was defined as anemia associated with no evidence of blood loss and at least two of the following: elevated bilirubin, elevated LDH, decreasing hemoglobin, positive Coomb's test or decreased haptoglobin. Of the 16 patients that had UCu at the time of hemolysis: 14 had elevated urinary copper (normal: 15-60 ug/24 hr; patient's mean: 319.6, range 70-2430 ug/24 hr); 13 had elevated indirect bilirubin (mean 3.2); 6 had elevated LDH (mean 973); 9 had elevated reticulocyte count (mean 9.6); 6 had reduced haptoglobin (mean 7.2) and all 16 had anemia. In the control group of patients in which UCu was obtained in the absence of hemolysis: 1 patient had an elevated UCu (116, unexplained); 3 had elevated indirect bilirubin (2.6); 1 had raised LDH (655); 2 had raised reticulocyte count (mean 8.1%); 1 had reduced haptoglobin (19); and 2 had anemia.

Based on a limited data set, UCu potentially appears to be a useful marker of hemolysis. It is a "liver independent" marker of hemolysis which may be useful in the setting of patients with underline liver dysfunction. Further studies are necessary to more closely delineate the specificity and sensitivity of this test in hemolytic anemia.