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ABSTRACT BOOK

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P.B.32 Diseases - Inflammation - Part 3

P.B.32.01
The impact of fibrinogen carbamylation on blood coagulation in inflammatory diseases

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Introduction: The body responds to injury with a temporary inflammatory reaction that remains persistent in certain diseases including chronic kidney disease and rheumatoid arthritis. Interestingly, these pathological conditions are associated with a pro-thrombotic tendency.
Conversion of lysine to homocitrulline (carbamylation) is a post-translational modification that occurs in the inflammatory milieu. Carbamylated proteins have been detected both in chronic kidney disease and rheumatoid arthritis.
We hypothesize that carbamylation has an impact on fibrinogen processing and contributes to the pro-thrombotic state observed in various inflammatory diseases. Moreover, we believe that the cleavage peptides from carbamylated fibrinogen differ from their unmodified counterparts regarding their inflammatory potential.
Methods: Modification patterns of fibrinogen were defined by mass spectrometry. Clot formation was measured based on turbidity changes, while scanning electron microscopy was used to visualize structural features of the fibrin clots. Cytokine release in response to the fibrinopeptides was tested in cell culture and quantified by ELISA.
Results: Our experiments show that carbamylation has no impact on fibrinogen cleavage, but affects fibrin polymerization and clot structure. Moreover, treatment of epithelial cells with FCPs from carbamylated fibrinogen resulted in higher cytokine secretion compared to the control suggesting that carbamylation increases the FCPs-mediated inflammatory response.
Conclusion: Fibrinogen carbamylation seems to be linked to aberrant blood clot formation in chronic inflammatory diseases. The present project aims at identifying correlations between disease specific carbamylation patterns of fibrinogen and malfunction of the coagulation cascade. The use of these modifications as biomarkers could help to identify patients at increased risk for thrombosis.

P.B.32.02
Effects of chronic fluoride exposure on the CGRP myenteric varicosities of the small intestine

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Introduction: The calcitonin gene-related peptide (CGRP) is a neuropeptide expressed in the Enteric Nervous System (ENS), influencing intestinal motility and regulating inflammation in the gastrointestinal tract (GIT), especially as anti-inflammatory molecule, inhibiting edema caused by inflammatory mediators. Although the GIT is the main rout of fluoride (F) exposure, there is no information about F effects on enteric neurons, even with the report of intestinal symptomatology as result of excessive F intake. Therefore, we decide to evaluate the effects of a chronic F exposure on CGRP neurons, through the morphological analysis of their varicosities, which are the axonal portion that concentrates CGRP.
Material and methods: 18 male rats (*Rattus norvegicus)* were divided into 3 groups: 0 (Control), 10, and 50 ppm F. The small intestine (duodenum, jejunum, and ileum) was collected, and processed for immunohistochemical technique for the CGRP identification. Morphometric analyses were carried out in 2400 myenteric varicosities per group. The groups were compared by Tukey's t-test ($p < 5\%$).
Results: The duodenum presented a statistically significant increase in the mean area values for the 10 ppm F, and a decrease for the 50 pppm F group in relation to the control group. The ileum presented a significant increase for the 10 and 50 ppm F groups.
Conclusion: The doses of 10 and 50 ppm F can alter the morphology of CGRP myenteric varicosities, which can be due to an attempt to control the intestinal motility altered by F ingestion, or an anti-inflammatory effect against F toxicity.
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P.B.32.03
Galectin-3 deletion attenuates inflammation and IL-33 dependent fibrosis in mouse model of nonalcoholic steatohepatitis

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Introduction: The importance of Galectin-3 (Gal-3) in obesity-associated liver pathology is incompletely defined.
Material and methods: Gal-3-deficient (LGALS3-/-) and wild-type (WT) C57Bl/6 male mice were placed on obesogenic high-fat diet (HFD, 60% kcal fat) for 12 and 24 weeks and metabolic, gene expression, histological and immunophenotypical analyses in liver, peripheral blood and bone marrow cells were performed.
Results: Compared to WT mice, HFD-fed LGALS3-/- mice developed, in addition to increased visceral adiposity and diabetes, marked liver steatosis which was accompanied with higher expression of hepatic PPAR-γ, Cd36, Abca-1 and FAS. However, hepatocellular damage, inflammation and fibrosis were more extensive in WT mice which had elevated number of mature myeloid dendritic cells, proinflammatory CD11b+Ly6Chi monocytes/macrophages in liver, peripheral blood and bone marrow, and increased hepatic CCL2, F4/80, CD11c, TLR4, CD14, NLRP3 inflammasome, IL-1β and NADPH-oxidase enzymes mRNA expression. HFD-fed WT mice had higher number of hepatocytes that strongly expressed IL-33 and hepatic CD11b+IL-13+ cells, increased levels of IL-33 and IL-13 and upregulated IL-33, ST2 and IL-13 mRNA in liver compared to LGALS3-/- mice. Additionaly, IL-33 failed to induce ST2 upregulation and IL-13 production by LGALS3-/- peritoneal macrophages in vitro. In vivo administration of IL-33 enhanced liver fibrosis in HFD-fed mice in both genotypes, albeit to a significantly lower extent in LGALS3-/- mice which was associated with less numerous hepatic IL-13 expressing CD11b+ cells.
Conclusion: We provide evidence that Gal-3 attenuates steatosis, but promotes liver injury, inflammation and IL-33 dependent liver fibrosis.
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P.B.32.04
Inhibition of epidermal growth factor receptor tyrosine kinase by erlotinib prevents sclerodermatous graft versus host disease in a mouse model

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Introduction: Chronic graft-versus-host disease (GVHD) follows allogeneic hematopoietic stem cell transplantation. It results from alloreactive processes induced by minor MHC incompatibilities leading to the activation of $CD4$ T cells and the development of fibrosis and inflammation of the skin and visceral organs and autoimmunity that resemble systemic sclerosis. EGFR, a ubiquitous cell receptor deeply involved in cell proliferation, differentiation, and motility, has recently been implicated in autoimmune and fibrotic diseases. We tested whether Erlotinib, an EGFR tyrosine kinase inhibitor can prevent clinical and biological features of sclerodermatous GVHD.
Materials and methods: Sclerodermatous GVHD was induced in BALB/c mice by B10.D2 bone marrow and spleen cell transplantation. Mice were treated *per os* 5 days a week for 4 weeks with Erlotinib (50mg/kg/day) beginning on day 7 post-transplantation. Disease severity score, skin thickness, histological and immunological analysis were assessed after bone marrow transplantation.
Results: Transplanted mice displayed severe clinical symptoms including alopecia, fibrosis of the skin and visceral organs, vasculitis, and diarrhea. The symptoms were reversed in mice treated with Erlotinib. These beneficial effects were mediated by the decreased production of effector memory $CD4+$ T cells and the reduction of T cell infiltration of the skin and visceral organs along with a decrease in IFNγ and IL-13 production and autoimmune B cell activation.
Conclusions: The improvement provided by Erlotinib in the mouse model of sclerodermatous GVHD supplies a rationale for the evaluation of Erlotinib in the management of patients affected by chronic GVHD.

P.B.32.05
Epidemiology of hepatitis B,C and HIV in pregnant women of a secondary care hospital in Greece

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The purpose of our study was to summarize information on the epidemiology of hepatitis B (HBV), hepatitis C (HCV) and HIV in pregnant outpatient women of a secondary care hospital in Greece.
Methods: During a period of 9 months, 96 women were included in the study, 17 (17.70%) of which were Albanians. Blood was collected during the first trimester of pregnancy. The samples were analyzed for HBV (HBsAg, HBeAg, anti-HBs, anti-HBc and anti-HBe)., HCV and HIV. Blood analyses were performed in the Roche Elecsys 2010 and in Abbott Axcym immunoenzymic analyzer.
Results: Among 96 women: 1 (1.04%) woman from Albania was determined to be chronic HBsAg carrier.
14 samples were further analyzed for HBeAg, anti-HBs, anti-HBc and anti-HBe. 8 women (7 Greek and 1 Albanian) have been vaccinated (anti-HBs(+), anti-HBc(-) and anti-HBe(-)).