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ABSTRACT BOOK

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P.E.03.12
metabolic requirements for neutrophil extracellular traps (NETs) formation

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Introduction: Neutrophils are the most abundant circulating cells of the immune system, have a short life-span, and are at the forefront of defence against infection. In addition to phagocytosis, the formation of NETs also contribute to their anti-microbial function. We aimed at analysing some of the metabolic requirements for NET formation. Materials and methods: Neutrophils were activated in vitro with PMA in culture medium with or without glucose and glutamine, or in the presence of metabolic inhibitors, NET formation was evaluated by fluorescence microscopy. Glut-1, glucose uptake, and glycolitic rate were also determined. Results: NET formation is dependent on glucose, and to a lesser extent on glutamine, that glucose in metabolized mainly by glycolysis, since the addition of the glycolysis inhibitor 2-deoxyglucose decreases the formation of NETs, while the ATP synthase inhibitor oligomycin has a minor effect. Upon PMA-activation, the amount of Glut-1, the glucose uptake and the glycolysis rate increase. PMA-stimulation, in the absense of glucose and glutamine allows for chromatin descondensation by 3 h post-stimuli, but not for NET release. The addition of glucose, but not of pyruvate, at this time point, induces NET release. Conclussion: we suggest that NET formation is an active process that requires energy from glycolisis and to a lesser extent from oxidative phosphorylation, and that it can be divided into two distinguishable metabolic phases: the first one, independent of exogenous glucose (chromatin decondensation) lasting 2-3 hours, and a second, highly dependent on exogenous glucose (expulsion of chromatin) that takes place within 5-10 minutes.

P.E.03.13
Role of Th17 cells-related cytokines in human obesity: inflammatory and metabolic impact in adipose tissue

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Recent findings in mice and humans revealed that obesity is tightly associated with chronic low-grade inflammation related to accumulation of immune cells in adipose tissue (AT). Alterations of AT immunological profile contribute to disrupt AT biology and, in turn, promote obesity-linked comorbidities, like type 2 diabetes. In obese human AT, we previously identified a specific enrichment in Th17 population which engages a paracrine proinflammatory cross-talk with AT macrophages, which is prominent in type 2 diabetic patients and reduced after surgery-induced weight loss.
Then, we hypothesized that Th17-related proinflammatory cytokines exert deleterious effects on AT structural and metabolic cellular components. To investigate this, we studied the impact of several Th17 cells-related cytokines and conditioned media from obese AT $CD4^+$ T cells on primary preadipocytes, adipocytes and endothelial cells obtained from human AT biopsies.
Recombinant IL-17 induced closely related proinflammatory and extracellular matrix remodeling transcriptional programs in preadipocytes and endothelial cells including overexpression and increased production of GRO cytokines, MCP-1, IL-8, IL-6, PAI-1, MMP-1 and MMP-3. Moreover, recombinant TGF-β and IFN-γ markedly enhanced the stimulatory effect of IL-17 on proinflammatory genes in preadipocytes and endothelial cells, respectively. In relevant way, conditioned media from AT $CD4^+$ T cells induce the same transcriptional pattern in both cell types. Furthermore, our preliminary data in mature adipocytes showed that IL-17 increases the release of some proinflammatory cytokines, like IL-6.
These results extend the involvement of Th17-related cytokines in intercellular communication complex network promoting inflammation, immune cells recruitment and extracellular matrix remodeling in AT during human obesity.

P.E.03.14
Serum levels of IgA and IgG antibodies against gliadin and glutenin in obese subjects

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Rationale: Unhealthy diet (type, composition and quantity of foods consumed) eventually leads to obesity and chronic metabolic disturbances. Emerging evidence indicate major role of unhealthy diet in the disturbance of gastrointestinal tract function (e.g. digestion, microbiota composition) and integrity (i.e. permeability), hypothetically leading to the impairment of oral tolerance and increased levels of antibodies against dietary proteins.
Methods: We investigated serum IgA and IgG immunoreactivity against commercial raw gliadin, and gliadin and gluteinin fractions from bread wheat genotype, by home-made ELISA, in 50 obese, 40 overweight and 50 lean controls. Subjects were recruited responders from the cohort of the study investigating self-assessed adverse reaction to food.
Results: The cohort (n=502) included 65 obese and 95 overweight subjects. The incidence of self-assessed adverse reaction to food was 23.36%, 19.17% and 40% in total, overweight and obese subjects, respectively. There was a lack of significant difference in IgA immunoreactivity to commercial gliadin between lean, overweight and obese subjects. However, IgG immunoreactivity to commercial gliadin in obese subjects was significantly higher than in overweight and lean subjects ($p < 0.04$). Additionally, 11/50 subjects in obese and 2/40 in overweight group had anti-gliadin antibodies level higher than mean±2SD value of the lean group. Levels of immunoreactivity to commercial gliadin and gliadin wheat fraction were similar, and only 2 of 11 obese subjects with high IgG levels to gliadin fraction had high IgG levels to glutenin fraction.
Conclusions: Factors associated with obesity putatively contribute to the immune disturbances related to systemic response to food antigens.

P.E.03.15
ST2 deletion attenuates high fat diet-induced steatosis, inflammatory cell infiltration and collagen deposition in liver

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Introduction: The role of IL-33/ST2 pathway in adiposity and obesity-associated liver pathology is incompletely defined. We aimed to investigate whether ST2 gene deletion affects liver steatosis, inflammation and collagen deposition in mice in response to high-fat feeding.
Materials and Methods: Male, 8-week old ST2 deficient (ST2-/-) and wild-type (WT) BALB/c mice were placed on high-fat diet (HFD; 60% kcal fat) or standard diet for 24 weeks and histological, immunophenotypic and gene expression analyses were performed.
Results: HFD-fed ST2-/- mice exhibited higher weight gain, amount of visceral adipose tissue (VAT) and higher percentages of adipose-tissue associated $CD11c^+$ dendritic cells, $IFNγ^+$, $IL\text{-}17^+$ and $CXCR3^+$ T cells compared to diet-matched WT mice. However, in the absence of ST2 markedly reduced HFD-induced hepatic steatosis was accompanied with lower expression of CD36, LXRα and PPAR-γ. Decreased inflammatory cell infiltration, number of $CD68^+$ macrophages and frequency of $CD11c^+$ dendritic cells in livers were also observed. Further, lower collagen deposition in livers of HFD-fed ST2-/- mice was associated with less numerous profibrotic $CD11b^+Ly6C^{low}$ monocytes and $CD4^+IL\text{-}17^+$ T cells and lower procollagen α1, IL-33 and IL-13 mRNA expression in livers, and lower serum levels of IL-33 and IL-13.
Conclusion: ST2 deletion enhanced high-fat diet induced adiposity, but attenuated hepatic steatosis, inflammation and fibrosis. The latter effect is due to the lower expression of lipid-related and profibrogenic molecules in liver, which is in agreement with the proposed profibrotic role of IL-33/ST2 axis.
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P.E.03.16
Differential regulation of TNF receptor signaling during viral infection of hepatocytes determines cell death

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Viral infections are detected by the innate immune system via pattern recognition receptors or by the adaptive immune system via (cross-)presentation of viral antigens. Many viruses have developed mechanism to escape such immune recognition. We identified a new CD8 T-Cell effector function against hepatotropic infections that overcomes immune escape by modulating MHC-I presentation and conventional innate immune recognition. CD8 T effector cells (CTLs) secrete TNF upon recognition of viral antigens on cross-presenting liver sinusoidal endothelial cells which selectively kills infected hepatocyte. Here we investigated the underlying mechanisms that render infected hepatocytes susceptible to TNF induced cell death. We infected mice with a hepatotropic virus and challenged these mice by injecting TNF. Using knockout mice, as well as biochemical and multiparametric histological methods we analyzed mitochondria from virus-infected mice and performed metabolomic investigations to unravel the role of mitochondria in virus recognition as well as their role in apoptosis execution. Here we could demonstrate that viral infections modulates metabolic processes, leads to increased levels of the pro-apoptotic BCL-2 family members Bax and Bad and inflicts damage to mitochondria. This leads to a downregulation of the anti-apoptotic protein XIAP.