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process against the background of the immune system disregulation, caused by intoxication with metal compounds, can serve as a model. Rapid development of research in the field of immunology has led to many scientific breakthroughs, and however, some aspects of immune-associate diseases remain under-explored. The goal of research was studying the functioning of individual immunity units in rats with aseptic inflammation and preliminary seeded with metal compounds. The research was carried out on not pedigree male rats weighting 180-220 grams. Animals were randomly divided into 4 groups of 30 animals each: control animals; the animals with aseptic inflammation; the animals, which had been orally fed on ammonium vanadate and potassium dichromate in a dose of 5 mg / kg bwt within two weeks; the animals in which, at the end of seeding with metal compounds, aseptic inflammation was modeled. In 1, 7 and 14 days after the modeling of aseptic inflammation in animals of all groups (10 individuals for each research period), blood was sampled under chloroform anesthesia, then euthanasia was followed by extraction of thymus, bone marrow, spleen and mesenteric lymph nodes. A two-week monitoring of the immune status of the rats under inflammation, after being preliminary seeded with ammonium vanadate and potassium dichromate, displayed prolonged course of inflammation and impaired wound healing. Haematologic state displayed a sharp inhibition of immune competent cells and the development of anemia, which is inherent in immune depression. The course of inflammation was aggravated by the intervention of anti-inflammatory activity of IL-10 in the first term, and TGF-β in the research remaining periods, which significantly influenced the development and outcome of the inflammatory process in experimental rats. Expressed structural changes in lymphatic organs throughout the experiment were represented by a decrease in the cortical-medullary index, dystrophically altered cells and their scarcity. Quite the opposite, evolving increase in cellularity was observed in the spleen, an increase in the cell populations with the MDSC phenotype having expansion toward G-MDSC, a significant reduction in the Th2 immune response, and a reduction in the cytotoxic CD3 + CD8 + T-lymphocyte content by the end of the experiment.

P1862

Board Number: B882

Effect of p53 activation on target gene expression and cytokine release in peripheral blood mononuclear cells.

A. Valiullina[1], M. Gomzikova[1], T. Khaibullin[1], E. Garanina[1], E. Zmievskaya[1], A. Rizvanov[1], E. Bulatov[1];
[1]Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia

Multiple sclerosis (MS) represents a common chronic autoimmune diseases with largely unknown etiology that is characterized by demyelination of axons in brain and spinal cord tissues. In the recent years it has become apparent that p53, a well-known tumor suppressor, has a primary role in regulation of innate immune responses. Data includes increased p53 expression in MS lesions, predisposition of p53-deficient animals to autoimmune diseases and high levels of inflammatory demyelination. Here we report the effect of Nutlin-3a-induced p53 activation on expression of various p53-dependent genes and release of proinflammatory cytokines. The results indicate that treatment with gradually increasing Nutlin-3a concentrations (5 - 40 uM) leads to correspondent gradual increase in expression levels of p21, Mdm2 and Puma target genes in peripheral blood mononuclear cells. In addition, upon treatment of cells with 10 uM Nutlin-3a we observed rise in IL-6, IL-10 and TNF-alpha levels. Gene expression data was obtained by TaqMan real-time PCR using CFX96 Touch Detection System (Bio-Rad). Quantivative cytokine detection and measurements were performed using Luminex 200 multiplexing system (EMD Millipore) and ELISA assay (Vector-Best). Our results could assist better understanding the p53-dependent regulation of immune cells and aid the development of novel immunotherapies. The study

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References 1. Bulatov E, Khaiboullina S, Reis dos HJ, Palotás A, Venkataraman K, Vijayalakshmi M, Rizvanov A. Ubiquitin-Proteasome System: Promising Therapeutic Targets in Autoimmune and Neurodegenerative Diseases. BioNanoSci. 2016 Aug 11;6(4):341–4.

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Cellular events mediating extracellular trap formation in HL60-derived and mouse neutrophils.

H.R. Thiam[1], S.L. Wong[2,3], D.D. Wagner[2,3,4], C.M. Waterman[1];
[1]National Heart, Lung, and Blood Institute, National Institute of Health, Bethesda, MD, [2]Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston, MA, [3]Department of Pediatrics, Harvard Medical School, Boston, MA, [4]Division of Hematology/Oncology, Boston Children's Hospital, Boston, MA

Extracellular trap (ET) release is a line of host defence during which activated immune cells such as granulocytes decondense and release their chromatin to the extracellular environment, leading to the formation of web-like DNA structures decorated with histones and cytotoxic proteins. This DNA “trap” participates in innate immunity by capturing and neutralizing bacteria, fungi and viruses. However, ETs also form in sterile inflammation. While a considerable amount of knowledge regarding the in vivo relevance of ET release has been cumulating, little is known about the cellular and biophysical mechanisms leading to ET formation. To determine the cellular events occurring during ET release, we performed high resolution live cell imaging of HL60-derived neutrophils stimulated to release NETs with ionomycin as well as mouse blood neutrophils (MBN) stimulated with ionomycin or LPS (bacteria component). MBN actin, microtubule and endoplasmic reticulum (ER) networks were visualized using vital dyes. HL60 cells stably expressing F-tractin-mApple were co-transfected with mEmerald-tagged ensconsin, lamin A/C, B1 and calreticulin-KDEL for visualizing respectively microtubules, the nuclear lamina and the ER. Cellular DNA was visualized with far-red Hoechst. Spinning disk confocal and DIC microscopy revealed that both ionomycin- and LPS-stimulated MBN as well as ionomycin-stimulated HL60-derived neutrophils undergo a stereotypical series of changes in cell morphology and cytoskeleton/endomembrane dynamics prior to NET release. Within minutes after stimulation, the actin cytoskeleton disassembles followed by drastic plasma membrane vesiculation and shedding coincident with ER vesiculation, but leaving the nuclear envelope (NE) intact. Subsequently, chromatin decondenses, the nucleus rounds up, and microtubules depolymerise. The NE ruptures, leading to rapid expulsion of decondensed chromatin into the cytosol. Finally, most cells rupture their cell membrane resulting in NET release. Peptidylarginine deiminase 4 (PADI4) has been shown to be required for NETs release. To determine its requirement for the cellular events leading to NETosis, we stimulated MBN from PADI4 deficient mice with ionomycin and visualized the cytoskeleton and endomembrane using vital dyes. We found that while actin and microtubule disassembly as well as ER vesiculation occur independently of PADI4, nuclear envelope rupture requires PAID4. Our data revealed that NET release proceeds via a well conserved succession of events that mediates the systematic disassembly of cytoskeletal and membranous components prior to chromatin release into the extracellular environment. The mechanism by which PADI4 mediates nuclear envelope rupture will be further studied in HL60-derived neutrophils.