Posterior Session III

**Infectious Agents and Cytokine Induction**

**PS3-01**

Multiplex profiling of cytokines released by A549 human lung epithelial cells after infection with encephalomyocarditis virus and vesicular stomatitis virus

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The model cell-virus systems of the human lung epithelial cell line A549:Encephalomyocarditis Virus (EMCV) and A549:Vesicular Stomatitis Virus (VSV) have been used routinely in standard cytopathic effect inhibition (CPE) assays for measuring the bioactivity of interferons (IFN) and neutralizing titers of anti-IFN antibodies. In this study, we have used solid phase single- and multiplex ELISAs to examine a panel of cytokines including IFN-α, IFN-β, IFN-γ, IFN-α/IL-25, IFN-γ/IL-28A, IFN-α, selected interleukins (IL), and TNF in media collected 12, 24, 48, and 72 hr after exposure to EMCV or VSV. Infection of A549 cells with EMCV over a near five log range of MOI elicited substantial increases (vs. non-infected cells) in IFN-α, IFN-β, and IFN-γ in A549 media in an MOI- and time-dependent manner. EMCV also increased induction of IL-6 and markedly elevated both IFN-α and IFN-β. In contrast, infection with similar MOI of VSV modestly increased A549 6 mL of IL-6 but failed to elicit increased induction of IFN-α, IFN-β, IFN-γ, and IL-2. Neither virus induced detectable levels of IFN-α, IFN-β, IFN-γ, IL-1x, IL-1γ, IL-12, IL-13, IL-17, or IL-23 at any time point examined. Notably, A549 cells were substantially more sensitive to VSV than to EMCV at similar MOIs with cell death occurring as early as 12 hr post-infection at the highest VSV MOI of 0.1. Cell death with EMCV was seen only at the highest MOIs at 48 and 72 hr. To provide more clarity regarding early cytokine profiles in response to VSV infection, additional time course studies are underway examining cytokine induction prior to 12 hr. In conclusion, this study provides unique insight into the cytokine (protein) production profiles of A549 cells treated with VSV and EMCV allowing a view of both the similarities and major differences in cytokine induction generated by these hallmark negative- and positive-strand RNA viruses (respectively) in culture.


**PS3-02**

Neutralization of endogenous IL-12 inhibits the Th-helper Type 1 immune response in experimental Sporothrix Schenckii infection

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Background. Sporothrix schenckii, etiological agent of sporotrichosis, is a widespread dimorphic fungus that usually causes cutaneous infection after local implantation; in patients with Acquired immunodeficiency syndrome (AIDS), the infection may become systemic and potentially fatal. Since endogenous IL-12 is an efficient inductor of Th1 type immune response, it is important for resistance to most bacteria, intracellular protozoa, and fungal pathogens. However, previous studies have not analyzed the effect of neutralization of endogenous IL-12 on the Th1 immune response during S. schenckii infection. Objective. To analyze whether administration of neutralizing anti-IL-12 antibodies exerts an effect on the Th1 immune response in gerbils infected with S. schenckii yeast cells. Methods: Twenty male gerbils were infected subcutaneously with 6 x 107 S. schenckii yeast cells (SsY) in the left hind footpad. Ten of these infected gerbils were then immediately sacrificed and the fungal burden in spleen and liver tissue was measured by colony-forming units (CFU) counts. Results: Anti-IL-12-treated infected gerbils showed a decrease in serum levels of Th1 cytokines and an increase in the Th2 ones, compared with anti-IL-12-un-treated infected gerbils (p < 0.0001); accompanied by a higher tissue fungal burden in spleen and liver. Concomitant with these effects, neutralization of endogenous IL-12 increased the severity of cutaneous lesions from anti-IL-12-treated-infected gerbils. Conclusion. These results suggest that endogenous IL-12 plays a key role in host resistance to S. schenckii infection by promoting a Th1 immune response.


**PS3-03**

Effect of Hepatitis C virus core and core+1 proteins on pro- and anti-inflammatory cytokine and chemokine gene expression Emmanouil Koczios1, Pelagia Foka1, Polyxeni P. Doumba2,3, John Koskinas1, Penelope Mavromara1, 1MoVes Modern Virology Laboratory, Hellenic Pasteur Institute, Athens, Greece, 2Laboratory of Surgical Research, Medical School of Athens, Hippokration Hospital, Athens, Greece, 32nd Department of Internal Medicine, Medical School of Athens, Hippokration Hospital, Athens, Greece

Hepatitis C virus (HCV) infection is a major public health problem, with more than 170 million people infected worldwide. Chronicity and persistence of infection constitute the hallmark of the disease. Certain pro- (IL-6, TNFs, MIP-1α), IL-8, MCP-1) and anti-inflammatory (IL-10) cytokines and chemokines may play a pivotal role in the development of inflammation that characterises chronic HCV infection, as reflected by their altered serum levels in patients. The HCV nucleocapsid protein (core) has been implicated in modulating host immune response, nevertheless this may be attributed partly to the newly discovered core+1 protein, produced by an alternative reading frame overlapping the core sequence. A putative effect of core and core+1 on cytokine and chemokine gene expression in both immune and hepatic cells, could account for the establishment of chronic inflammation observed in HCV infection. To investigate this hypothesis, hepatoma Huh-7 cells or human monocyte-derived macrophages (HMDMs) were transfected separately with core, core+1 and empty vector expression plasmids or infected with herpes ampiclon vectors expressing these viral proteins, respectively. Total RNA was extracted and subjected to semi-quantitative RT-PCR. Huh-7 cells transfected for 48h, showed elevated levels of MCP-1, TNFs, IL-6 and IL-10 by more than 2-fold in the presence of core, whereas only TNFs, IL-6 and IL-10 levels were affected by core+1/S. No significant change in cytokine mRNA was observed at 24h post-transfection. A similar expression profile was observed in HMDMs, where MIP-1α mRNA was also found up-regulated by the viral proteins. Expression of selected viral proteins in primary hepatocytes from healthy donors using a baculovirus-based system is in progress in order to validate our data ex vivo. In conclusion, our results suggest the differential action of HCV core and the novel core+1/S proteins on the intrahepatic and macrophage transcriptional profile of important cytokines involved in inflammation and the host immune response.

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**PS3-04**

Cytokine induction during coronavirus entry

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Infections with human coronaviruses (H-CoVs) result in varying clinical manifestations from mild upper respiratory symptoms to lethal pneumonia. The differing severity of diseases between the H-CoVs may be a result of dysregulated immune responses to certain H-CoVs. While the immune system can be stimulated at multiple
PS3-05
The chemokine network in the formation of tertiary lymphoid structures in colorectal cancer
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Tertiary lymphoid structure (TLS) formation in non lymphoid organs is associated to many chronic inflammatory conditions, including infections and autoimmune diseases, with the lymphoid chemokine CXCL13 playing an important role in the organization of ectopic lymphoid tissues. Although colorectal cancer (CRC) represents a paradigm of cancer-related inflammation, the presence of tertiary lymphoid structures in CRC has been poorly investigated. In a previous study we have investigated the role of the chemokine CXCL13 in the formation of isolated lymphoid follicles in the gut. Our results suggested that overexpression of CXCL13 in the intestine during inflammatory conditions favors mobilization of B cells and lymphoid tissue inducer cells (LTi) with immunomodulatory and reparative functions and formation of tertiary lymphoid follicles. LTi cells produced IL-22, a cytokine implicated in epithelial repair and the IL-23 receptor, a key regulator of IL-22 production. In the present study we analyzed the process of TLS formation in the intestinal mucosa in neoplastic conditions. Our preliminary results show that B cell aggregate formation increases in the context of the chronic inflammation associated to intestinal neoplasia, in the AOM/DSS mouse model. Moreover, staining with an antibody recognizing CD121, a marker selectively expressed on follicular dendritic cells (FDC) evidenced the presence of lymphoid aggregates in the mucosa of CRC patients. Future task is to determine whether tertiary lymphoid structures contribute to the persistence of the tumor-associated inflammatory reaction, rather than represent functional immune structures, actively participating to the anti tumor response.

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PS3-06
Differences in magnitude and timing of human monocyte-derived dendritic cell innate immune responses to two strains of respiratory syncytial virus
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Respiratory syncytial virus (RSV) is the primary cause of infant bronchiolitis and pneumonia. Comparing RSV strains that were isolated from severely affected infants and induce severe disease in mice with those associated with mild disease in infants and mice enables us to identify immune mechanisms associated with RSV disease and its sequelae. RSV strain A19 was isolated from a severely affected infant and elicits severe disease in mice characterized by enhanced mucous secretion, expression of IL-13, and low expression of IFN-alpha. RSV strain A2 is associated with mild disease in humans and mice. We compared the differences in expression by human monocyte derived dendritic cells (MDDC) of inflammatory, IFN response genes (IRG) and, using a novel assay developed in our laboratory, the twelve subtypes of IFN-alpha, IFN-beta and three types of IFN-lambda. At 24 hours, we found similar responses to each virus, but the response to strain 19 was attenuated for many IRG and inflammatory genes, including CXCL10, CCL2 and genes associated with the RIG-1 pathway. At eight hours, however, the pattern is reversed with higher expression of IRG in response to strain 19 than A2. At 24 hours, IFN-alpha was detectable in supernatants from 9 of 10 donors in response to A2, but only 1 of 10 donors in response to strain 19. Among the IFN subtypes, MDDC expressed IFN-alpha1, IFN-beta, IFN-lambda1, and -lambda2 in response to either strain; levels of expression in response to strain 19 were up to 200-fold lower than expression in response to A2. Taken together, these results suggest that a faster, but shorter host innate immune response results in more severe disease.

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PS3-07
Differential patterns of response to toll-like receptor 9 agonist and exogenous interferon-z in the mouse liver
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With over 200 million individuals infected worldwide and high rates of progression to chronic liver disease, hepatitis C virus (HCV) infection is a considerable health burden. Since 1990 recombinant IFNα has been used for the treatment of chronic hepatitis C (CHC). It is injected once daily or every second day for several months. Pegylated IFNα (pegIFNα), a longer acting form of IFNα, has replaced standard IFNα in therapies of chronic hepatitis C because it is more effective, supposing by inducing a toll-like activation of IFN signaling pathways. However, we have recently published that tyrosine phosphorylation of STAT1 and STAT2 becomes refractory to further stimulation within hours after the first injection for up to two days due to an upregulation of USP18. IMO-2125 is a toll-like receptor 9 (TLR9) agonist that is in clinical development for the treatment of CHC. IMO-2125 induces a Th-1 type response including the production of endogenous interferons. The aim of the present study was to compare the pharmacodynamic effects of IMO-2252, a novel TLR9 agonist, and IFNα in the mouse liver. After single or repeated injections of IMO-2252, mice were sacrificed at different time points, and Jak-STAT signaling and gene induction was assessed. IMO-2252 potentely induced STAT1 phosphorylation with bimodal kinetics. Contrary to repeated injections of IFNα, IMO-2252 did not induce a refractory state in the mouse liver, despite an upregulation of USP18. Whole transcriptome comparative microarray analysis of mice injected with IFNαs and IMO-2252 revealed differential gene induction profiles, with IMO-2252 showing a broader spectrum of transcripts at various time points assayed. The lack of refractoriness to IMO-2252 and the long-lasting and strong induction of IFNα stimulated genes hold promise for TLR9 agonists for the treatment of CHC.


Pattern Recognition
PS3-08
Role of IL-1 and TNF in human monocyte-derived dendritic cell responses following beta-glucan and LPS stimulation
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Beta1,3-linked glucans (beta-glcan) and lipopolysaccharide (LPS) are pattern-associated molecular patterns (PAMPs) recognized by Dectin-1 and Toll-like receptor 4 (TLR4), respectively. Beta-glcan is one of major components of fungal cell walls and yeast, whereas LPS is found in most gram-negative bacteria. We previously described that human monocyte-derived dendritic cells (mo-DCs) produce high levels of IL-1beta and IL-23 in response to beta-glcan. Both LPS- and beta-glcan treated mo-DCs produced comparable levels of TNF with similar kinetics. Consistently, beta-glcan-treated mono-DCs induced the development of Th17 cells, and IL-1beta was found to play a central role in this process.

In addition, we observed that IL-1 and TNF are produced by mo-DCs stimulated with beta-glcan at earlier time points compared to IL-23. We examined the possibility whether these early cytokines could drive the late responses of activated DCs. Using microarray analysis, qrt-PCR and Nanosting μCooled μArray analysis system, we identified a subset of genes (e.g. IL-23 subunit genes) regulated by endogenous IL-1 in mono-DCs activated by beta-glcan. The neutralization of endogenous TNF and IL-1 revealed that both cytokines play a role in gene expression and cytokine production induced by beta-glcan, whereas only TNF is sufficient for the responses to LPS. We are currently analyzing at the molecular level the specific contribution of IL-1 and TNF signaling pathways in these processes.