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P01. Presence of anti-nuclear antibodies (ANA) in patients with positive anti-CCP antibodies during 2019-2020 in general hospital of Ioannina

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P02. Presence of elevated levels of antithyroid antibodies in thyroid disease during 2019-2020 in general hospital of Ioannina

Marina Gerasimou[1], **Panagiotis Christodoulou[2]**, Stavros Mantzoukis[2]
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P03. Different expression patterns of transforming growth factor-β (TGF-β) during rabbit hemorrhagic diseases virus (RHDV) infection

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P04. Doxorubicin retention and degradation define resumed growth in vivo

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P05. An interferon-stimulated transcriptionally independent isoform of ACE2 inhibits SARS-CoV-2 infection

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P06. Autoreactive B cells escape peripheral checkpoint in Sjögren's Syndrome

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P07. Assembly of a large clinically diverse COVID-19 cohort offers multiple prospective study designs

Vasiliki Triantafyllia[1*], Vicky Lampropoulou[1*], Eleni Siouti[1*], Maria Salagianni[1], Maria Papadaki[1], Ioanna-Evdokia Galani[1], Eleftherios Pavlos[1], Evangelia Koukaki[2], Garyphallia Poulakou[3], Nikoletta Rovina[2], Antonia Koutsoukou[2], Evangelos Andreakos[1]
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P08. Uncovering the transcriptional regulation of IFN-λ production

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P09. Deep immunophenotyping of asymptomatic and very mild COVID-19 patients

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P10. CD40 rs1883832 polymorphism as a predictor of anti-SARS-CoV-2 vaccine-induced antibody responses

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P01. Presence of anti-nuclear antibodies (ANA) in patients with positive anti-CCP antibodies during 2019-2020 in general hospital of Ioannina

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Objectives: Aim of this study is to record the incidence of positive ANA in patients with diagnosed rheumatoid arthritis (RA), who did not have collagen disease and had positive antibodies against cyclic citrullinated peptide (anti-CCP) (a new laboratory marker of RA) during one year.

Materials and Methods: The material of our study consisted of 76 patients with diagnosed RA and positive anti-CCP antibodies. Enzyme-linked immunosorbent assay (ELISA) was used to analyze antinuclear antibodies (ANA) screening and indirect immune fluorescence, using cells Hep-2. Titre ≥1 / 80 is considered positive. All patients were tested for anti-ds-DNA, anti-ENA (anti-RNP, anti-sm, anti-ro, anti-La, anti-SCL-70, anti-CEN, anti-Jo-1).

Results: 33/76 patients (43,4%) were positive by ELISA method, 34/76 (44,7%) were positive by the method of indirect immune fluorescence. 82.3% of the immune fluorescence pattern was homogeneous (82,3%).

Conclusions: None of the patients with positive ANA had positive sub-specific antibodies (anti-ds-DNA, anti-RNP, anti-sm, anti-ro, anti-La, anti-SCL-70, anti-CEN, anti-Jo-1). The frequency of the presence of positive ANA was higher in patients with advanced RA than in patients with early disease. The presence of ANA and other autoantibodies non-specific for the disease, advocates for the polyclonal activation of B lymphocytes.

P02. Presence of elevated levels of antithyroid antibodies in thyroid disease during 2019-2020 in general hospital of Ioannina

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Objectives: Autoimmune diseases have a high prevalence in the population, and autoimmune thyroid disease (AITD) is one of the most common representatives. Thyroid autoantibodies are not only frequently detected in patients with AITD but also in subjects without manifest thyroid dysfunction. Aim of this study is to record the incidence of elevated levels of anti-Tg and anti-TPO antithyroid antibodies in patients with thyroid disorders.

Materials and Methods: 120 patients with hyperthyroidism, 84 patients with Hashimoto's chronic thyroiditis and 51 patients with hypothyroidism were tested for one year. The method used was Microparticle Enzyme Immunoassay.

Results:45/120 (37.5%) patients with hyperthyroidism had high levels either anti-Tg or anti-TPO or both. 62/84 (73.8%) patients with Hashimoto's thyroiditis and 16/51 (31.4%) with hypothyroidism had high levels either anti-Tg or anti-TPO or both.

Conclusions: Increased levels of anti-thyroid antibodies usually accompany AITDs, and their detection may support its diagnosis**.** High levels of anti-thyroid antibodies appear in patients suffering from either hyperthyroidism, Hashimoto's thyroiditis or hypothyroidism. Patients suffering from hypothyroidism exhibit the lowest rate of high levels of anti-Tg and anti-TPO antibodies while patients with Hashimoto the highest.

P03. Different expression patterns of transforming growth factor-β (TGF-β) during rabbit hemorrhagic diseases virus (RHDV) infection

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Objectives: TGF-β belongs to the cytokines with suppressive effects on the immune response. The biological role of TGF-β in the pathogenesis of viral diseases aetiology, associated with liver and other organs dysfunction is not fully understood. The objective of present study was quantify *TGF-β1* expression patterns in liver, lung, spleen and kidney tissue of rabbits experimentally infected with RHDV.

Material and methods: The specimen of liver, lung, spleen and kidney tissue were collected from rabbits infected with RHDV, Erfurt strain (n=10) and healthy controls (n=10). The expressions of *TGFβ1* was analysed by TaqMan® Real-Time PCR Assays.

Results: Intrahepatic expression of *TGFβ1* was enhanced after RHDV infection (2.8-fold increase vs. controls, $p = 0.0016$) what is accompanied by an elevated virus copy number in livers. Meanwhile, in spleen tissue *TGFβ1* expression was significantly reduced (40% reduction, $p = 0.004$). Nevertheless, in lung and kidney tissue expression of *TGFβ1* was comparable to controls ($p = 0.2$ and $p = 0.8$, respectively).

Conclusion: In our study, the intrahepatic expression of *TGFβ1* was approximately three times higher in animals infected with RHDV than in controls. Moreover, a significant increase in this gene was associated with progressed apoptosis and viral organ destruction, demonstrating that in acute liver failure, there is a persistent recruitment of mononuclear inflammatory infiltrate, leading to chronic inflammation with sustained liver damage that is modulated by *TGFβ1*. In kidney and lung tissues this mechanism seems to be dysregulated, with unchanged levels of *TGFβ1,* which needs to be investigated.

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P04. Doxorubicin retention and degradation define resumed growth in vivo

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Background: Tumor recurrence, common obstacle for successful treatment outcome, is caused by residual tumor cells which might remain dormant for years before resuming proliferation[1–4]. These cells might be associated with long-term presence of tissue bound cytotoxic drugs and somehow contribute to dormancy or recurrence of a tumor.

Objectives: The purpose of this study was to design two step dormancy/recurrence (TSDR) murine model demonstrating the role of cellular drug doxorubicin internalization on tumor recurrence rates.

Materials and Methods: SL2 lymphoma cells preloaded with various doxorubicin concentrations were implanted intraperitoneally into DBA/2 mice. The survival rates of the mice were used to demonstrate the relationship between resumed tumor growth and drug internalization patterns. To simulate long-term tissue indwelling of dox, the drug was incubated up to 365 days at 37°C (dox-dgr). Then HPLC, flow cytometry and confocal microscopy and spectral analyses were performed. Myelotoxic capacity was evaluated by hematological nadir after drug iv injection.

Results: Although low doxorubicin concentrations (0.01-1.0 μg/ml) resulted in tumor recurrence and worse survival outcome, SL2 cells exposed to 10 μg/ml of DOX were unable to recur. Dox-dgr showed reduced fluorescence and a loss of nuclear selectivity. The drug also lost its capability of inducing a myelotoxic and tumoricidal effect.

Conclusions: Our TSDR model might be a convenient tool to study the effect of tissue-bound drugs to the dormancy and remission. Although one-year body temperature exposure dramatically affected both cytotoxic and myelosuppressive capacity of the free doxorubicin, further drug extrusion analysis of drug tissue indwelling role in dormancy is needed.

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P05. An interferon-stimulated transcriptionally independent isoform of ACE2 inhibits SARS-CoV-2 infection

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Objectives: Regulation of ACE2, the SARS-CoV-2 receptor, could be important for susceptibility to COVID-19 or its outcomes. We previously reported the discovery of a transcriptionally independent truncated isoform of *ACE2*, designated as *deltaACE2* (*dACE2*). *dACE2* starts from a new exon in intron 9 of *ACE2* and is highly conserved in primates. *dACE2*, but not *ACE2*, is induced by interferons (IFNs) and viruses, including SARS-CoV-2. Here, we evaluated the functional consequences of *dACE2* expression for SARS-CoV-2 infection.

Materials and Methods: We used custom generated antibodies to explore the expression of dACE2 in tumor microarrays by immunohistochemistry. Cell surface localization of dACE2 was evaluated by surface biotinylation, followed by streptavidin precipitation. Flow cytometry was used to examine the internalization of the SARS-CoV-2 spike protein receptor-binding domain (spike-RBD) in T24 cell lines transiently transfected with expression constructs for ACE2 or dACE2.

Results: We detected endogenous dACE2 expression in squamous head and neck tumors. When overexpressed in T24 cells, dACE2 was detected on the cell surface but did not bind SARS-CoV-2 or spike-RBD. However, dACE2 appeared to inhibit the internalization of spike-RBD by ACE2.

Conclusion: Induction of *dACE2* by IFNs or viral infection is unlikely to increase SARS-CoV-2 infection. However, the induced dACE2 may be able to reduce SARS-CoV-2 infection by inhibiting the internalization of SARS-CoV-2 by ACE2. Our results suggest that dACE2 might be part of an antiviral mechanism that evolved in primates to defend against certain viruses that utilize the ACE2 receptor for entry.

P06. Autoreactive B cells escape peripheral checkpoint in Sjögren's Syndrome

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Abstract

Although it is widely acknowledged that B cells play a central role in many autoimmune diseases (AID) including primary Sjögren's syndrome (pSS), a full understanding of their characteristics has not been elicited. This study aims at characterizing circulating autoantigen-specific B-cells in patients with pSS compared to healthy subjects.

We developed a new flow-cytometry method to detect circulating auto-reactive B cell based on the specificity of their B-cell receptor (BCR). Phenotype analysis showed that circulating B cells that reacted to SSA (SSA+ B cells) in patients were enriched in the memory B cell compartment compared with healthy controls. It suggests that in AID, theses auto-reactive cells are able to differentiate into IgG isotype-switched cells and escape peripheral tolerance checkpoint but not in healthy subjects. Interestingly, Natural auto-reactive B cells present in healthy subjects, are able to secrete only IgM isotype autoantibodies upon *in vitro* stimulation but not IgG class switched antibodies. A genomic analysis of the antibody repertoire as well as a transcriptional profiling of these cells by single-cell RNA seq is ongoing to understand further the differences of these autoreactive B cells between healthy subjects and patients with AIDs.

Our results suggest that autoreactive B cells escape peripheral tolerance checkpoint and are able to differentiate into IgG isotype-switched cells in patients with AIDs but not in healthy subjects.

P07/ Assembly of a large clinically diverse COVID-19 cohort offers multiple prospective study designs

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Objectives: COVID-19 is a heterogeneous infectious disease with a strong immunoinflammatory component and an unpredictable clinical course. A broad spectrum of comorbidities and treatments, potential genetic defects and the emergence of SARS-COV-2 mutants amplify interpatient variability. We therefore set out to assemble a large clinically diverse COVID-19 patient cohort and establish a "biobank" of longitudinally sampled specimens that allow multifaceted analyses and various study designs.
Materials and methods: Blood and nasopharyngeal specimens are sampled from COVID-19 patients upon admission and at specific intervals throughout hospitalization at Sotiria Thoracic Diseases Hospital of Athens. Plasma, serum, DNA, RNA, PBMC, whole and white blood cells are isolated and stored for downstream assays, including multiplex cytokine quantification, deep immunophenotyping, RNA-Seq, metabolomics, GWAS and virome studies. Results combined with clinical parameters are subjected to bioinformatic interrogations and data mining.
Results: Our expanding COVID-19 cohort comprises currently >900 patients who vary in severity, ranging from asymptomatic to ICU admitted, in comorbidities and treatments and, therefore, allows for diverse patient stratification. Our cohort contains patients with the most prominent comorbidities associated with disease progression. Moreover, clinical and immunological parameter analyses reveal major differences among subgroups and indicate factors of paramount importance to untangle this complex disease.
Conclusions: Our COVID-19 cohort has some of the most comprehensive clinical and laboratory information available, including demographic data, comorbidities, treatments, hospitalization and ICU times, extensive respiratory measurements, standard clinical biomarkers as well as immunological and genetic information that will facilitate studies on disease mechanisms, severity prediction and the development of novel personalized therapies.

P08. Uncovering the transcriptional regulation of IFN-λ production

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Objective: Type III IFNs or IFN-λ are anti-viral cytokines that mediate front-line defense at epithelial barriers. The purpose of this study is the characterization of transcription factors that act as regulators of IFN-λ expression, based on binding site analysis of IFN-λ genes promoter regions, in combination with transcriptomic data of IFN-λ expressing cells.

Materials and methods: Murine $CD8a^+$ and human $BDCA3^+$ DCs are major producers of IFN-λ in response to poly(I:C). Here, we have used the functional equivalent in vitro generated $CD24^{high}$BMDC subset to examine the transcriptional regulation of eGFP+/IFN-λ-producing cells isolated from the *IFNλ*^{eGFP} reporter mouse. Two experimental approaches were followed. First, transcription factor expression was examined by RNA-Seq analysis. Second, transcription factor-DNA interactions were evaluated by chromatin accessibility assays. To this end, DNase I hypersensitive sites (DHS) and Chromatin immunoprecipitation (ChIP) assays were performed. In DHS assay, DNase I digests chromatin only to open regions identifying the location of putative regulatory regions. In parallel, ChIP-seq was performed against sites residing in an accessible "active" chromatin environment, such as H3K4ac, H3K27ac, and H3K4me1, identifying chemically cross-linked proteins to these DNA sequences.

Results: RNA-Seq analysis of eGFP+/IFN-λ-producing cells vs eGFP- cells has provided us with a list of transcription factors that may play a role in IFN-λ production. This list is analysed in combination with chromatin accessibility assays to examine the binding of these transcription factors in the promoter regions of IFN-λ genes.

Conclusion: Our data support the hypothesis that IFN-λ expression is regulated by unique molecular pathways.

P09. Deep immunophenotyping of asymptomatic and very mild COVID-19 patients

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Objectives: Coronavirus disease-19 (COVID-19) is caused by SARS-CoV2. Since the beginning of 2020, it has become one of the main challenges of our times. Symptoms vary in severity and include acute respiratory distress syndrome, thrombosis and organ failure. Several studies have highlighted a complex network of peripheral blood immune responses in COVID-19 infection. A distinct phenotype is observed in severe and critical patients, consisting of a highly impaired interferon type I response, characterized by low IFN-α production and activity, which is associated with a persistent blood viral load and an exacerbated inflammatory response. However, little is known about the immunological features and the molecular mechanisms involved in COVID19 asymptomatic/mild infection.

Material/Methods: We performed an in-depth phenotypical analysis of immune cells with CyTOF technology, transcriptomic analysis, and cytokine measurements on a group of 65 COVID-19 patients with variable severity from asymptomatic to critical. Patients were assessed to fit in one of the categories of the WHO's Eight-category scale for clinical improvement, based on oxygen support requirements. Uninfected individuals aged-matched were used as control group.

Results: Unsupervised clustering of samples revealed a distinguishable immune profile between severe/critical cases and mild/very mild cases. Cell population abundance analysis revealed neutrophilia and low DCs in the most severe group, whereas high DCs levels in the asymptomatic/very mild group were clearly observed. Correlation analysis with cytokines revealed higher IFNa levels in the milder disease patient groups. RNAseq data will shed light into the mechanistic role of DCs in asymptomatic patients.

Conclusion: Studies suggested that type I IFN deficiency in the blood could be a hallmark of severe COVID19. Our study presents distinct immunological features of asymptomatic/ mild groups of patients, pointing to an important role of DCs in preventing severity of COVID19 infection.

P10. CD40 rs1883832 polymorphism as a predictor of anti-SARS-CoV-2 vaccine-induced antibody responses

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Objectives: BNT162b2 mRNA represents a promising vaccine candidate against SARS-CoV-2. Several factors, including the genetic background of vaccinated individuals, may affect the antibody and cellular responses after vaccination. CD40 (TNFRSF5) is a member of the TNFR superfamily and its activation on B cells is crucial for germinal center formation and antibody secretion. A functional polymorphism (c.-1C>T, rs1883832) in the 5' UTR of CD40 modifies mRNA translation and immunoglobulin production and has been associated with susceptibility to immune-mediated diseases. The aim of our study was to evaluate whether this polymorphism affects antibody responses after BNT162b2 mRNA vaccination.

Materials & Methods: Two-hundred and one (201) individuals (male/female 64/137, mean age: 55.4 years, rage: 27-105), who received two doses of BNT162b2 mRNA vaccine (Pfizer/BioNTech), were enrolled in the study. Anti-SARS-CoV-2 IgG and IgA antibody responses on the 21st and 42nd day after the first vaccination were evaluated by ELISA, using commercial kits (IgG GA Generic and IgA Serion, respectively), according to manufacturers' instructions. Genomic DNA was extracted from peripheral blood and the analysis of CD40 rs1883832 polymorphism was performed by PCR-RFLP.

Results: Eighty-two individuals carried the c.-1C>T polymorphism, with 20 being homozygotes; the polymorphism was in Hardy-Weinberg Equilibrium ($p=0.619$). Interestingly, carriers of the T allele displayed significantly lower levels of IgA antibodies on both the 21st and 42nd day after vaccination ($p=0.027$ and $p= 0.017$, respectively). On the other hand, the polymorphism did not affect the levels of IgG antibodies.

Conclusions: The functional rs1883832 polymorphism may be considered as a useful genetic marker to predict BNT162b2 mRNA vaccination efficacy.