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Posters

Adriana Rodriguez-Cruz et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Phenotypic characterization of macrophages subpopulations CD3+ TCRαβ+/TCRαβ-

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Background: It was reported that 5-15% of macrophages isolated from peripheral blood mononuclear cells (PBMC) express the TCRαβ receptor. Using monocyte derived macrophages (MDM), from healthy donors, and infected *in vitro* with BCG, it was observed that the percentage of TCR+ macrophages increased in response to the infection. Preliminary results from our group, in mice, showed that after an intravenous BCG-infection there is an increase in the recruitment of two sub-populations of macrophages: CD11b+CD3+TCRαβ- and CD11b+CD3+TCRαβ+, at moment the characterization of these subpopulations has not been assessed.

Methodology: PBMC were obtained from healthy donors, CD14+ cells were obtained through immunomagnetic positive selection. After 7 days in culture MDM were obtained and characterized by flow cytometry. The MDM CD3+ TCRαβ+ and TCRαβ- subpopulations were evaluated for the coexpression of: CD80, CD86, CD11B, CD68, CD14, CD3, TCRαβ, TCRγδ, HLA-I, HLA-II, CD1a, CD1b, CD1c, CD1d, CCR4, CCR7, CXCR1, CD16, and tmTNF.

Findings: Our data shows that both of these sub-populations express efficiently the HLA-I, HLA-II. Moreover, the subpopulation CD3+TCRαβ+ showed an increase in the expression of CD1a, CD1b, CD1c and CD1d molecules, however in CD3+TCRαβ- this expression was absent. The expression of pro-inflammatory molecules CD16 and tmTNF had a stronger augment in the CD3+TCRαβ+ subpopulation. Chemokine receptors were measured and CCR4, CCR7 and CXCR1 were expressed 3 times more in CD3+TCRαβ+ compared to CD3+TCRαβ-.

Conclusion: The CD3+TCRαβ+ MDM are efficient cells to present peptide antigens; however the expression of CD1 family molecules suggests that CD3+TCRαβ+ could play an important role in the presentation of lipid antigens. Probably, overexpression of pro-inflammatory molecules and chemokines receptors on CD3+TCRαβ+ is used by the MDM to favor a pro-inflammatory function. The phenotypic characterization of CD3+TCRαβ+ provides evidence that this subpopulation could be crucial in pathologies where lipids and inflammatory environment trigger a signal, such as tuberculosis.

Biography

Adriana Rodríguez Cruz burned in Mexico, 1989. Awarded bachelor's degree in Biology with honors by Universidad Nacional Autónoma de México (UNAM), 2012. Education abroad in 2010 at University of California Berkeley, U.S.A. Research internships in 2013 at University of Arizona, U.S.A., and in 2014 at Charité Universitäts Medizin Berlin, Germany. Currently studying PhD in Biomedical Sciences at UNAM with the project "Evaluation of the signaling pathway of macrophages CD3+ TCRαβ+/TCRαβ- and their function as pro-inflammatory macrophages"; to identify the mechanisms of activation and cellular function of these macrophages, as well as their implication in the physiopathology of pulmonary tuberculosis.

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Anne Schink et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Identification of novel anti-inflammatory herbal extracts

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Acute and especially chronic inflammation underlies the development and progression of most severe diseases, such as inflammatory bowel disease (IBD), allergic asthma/chronic obstructive pulmonary disease (COPD), and organ fibrosis that lead to dysfunction and death. Regardless of the origin, inflammatory processes on the molecular level can be described as a cyclic events, involving innate immune activation, e.g. via Toll-like receptors (e.g. the TLR4-complex) and reactive oxygen species (ROS), and adaptive immunity directed to target tissue. Activation of TLR4 triggers secretion of pro-inflammatory cytokines and chemokines, which further activate these immune responses. To date, no effective orally active TLR4 antagonists are available for experimental or clinical application. The aim of our study was to identify anti-inflammatory herbal drugs and their active compounds that are stable in the gastrointestinal tract and preferably possess antagonistic TLR4 activity, or interfere with downstream inflammatory signaling pathways. To this aim, we screened more than 100 plant ethanolic extracts in TLR4-transfected reporter cells challenged with lipopolysaccharide (LPS) to identify their anti-inflammatory activity. 28 promising extracts were additionally confirmed in LPS-stimulated THP-1 monocytes for their dose-dependent anti-inflammatory activity. To detect TLR4 antagonistic activity or interference with the downstream inflammatory cascade, these extracts were also tested in TLR2- and TLR4-transfected HEK cell lines, which permit pathway differentiation, since both receptors share similar downstream signaling upon stimulation. Promising anti-inflammatory herbal extracts were fractionated by high-performance liquid chromatography-diode array detector (HPLC-DAD) technology and specific compounds in the active fractions were identified. Pure compounds were tested for TLR4 antagonistic activity in the cell culture systems which were described above. Selectively identified active compounds will be discussed.

Biography

Anne Schink earned her Master's degree in Toxicology in 2012 from the Technical University, Kaiserslautern, Germany. Her thesis title was "Biomarkers to characterize and monitor skeletal muscle toxicity in the rat". Afterwards, she held a position as Consultant at the a-tune software AG, Darmstadt, Germany. In August 2015, she enrolled herself into PhD and is now actively included in the research work at the Max Planck Institute for Chemistry, Mainz, Germany and the Institute of Translational Immunology, University Medicine, Mainz, Germany. Her research focus is on the identification of anti-inflammatory herbal extracts and their active compounds, which can alleviate different chronic diseases.

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Seong Kug EO et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Amelioration of Japanese encephalitis by blockage of 4-1BB signaling is coupled to divergent enhancement of type I/II IFN responses and Ly-6C^{hi} monocyte differentiation

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Japanese encephalitis (JE), a neuroinflammation caused by zoonotic JE virus, is the major cause of viral encephalitis worldwide, and poses an increasing threat to global health and welfare. To date, however, there has been no report describing the regulation of JE progression using immunomodulatory tools for developing therapeutic strategies. We tested whether blocking the 4-1BB signaling pathway would regulate JE progression using murine JE model. Blocking the 4-1BB signaling pathway significantly increased resistance to JE and reduced viral burden in extraneural tissues and the CNS, rather than causing a detrimental effect. In addition, treatment with 4-1BB agonistic antibody exacerbated JE. Furthermore, JE amelioration and reduction of viral burden by blocking the 4-1BB signaling pathway was associated with an increased frequency of IFN-II-producing NK and CD4^{+} Th1 cells as well as increased infiltration of mature Ly-6C^{hi} monocytes in the inflamed CNS. More interestingly, DCs and macrophages derived from 4-1BB KO mice showed potent and rapid IFN-I innate immune responses upon JEV infection, which was coupled to strong induction of PRRs (RIG-I, MDA5), transcription factors (IRF7), and antiviral ISG genes (*ISG49, ISG54, ISG56*). Further, the ablation of 4-1BB signaling enhanced IFN-I innate responses in neuron cells, which likely regulated viral spread in the CNS. Finally, we confirmed that blocking the 4-1BB signaling pathway in myeloid cells derived from hematopoietic stem cells (HSCs) played a dominant role in ameliorating JE. In support of this finding, HSC-derived leukocytes played a dominant role in generating the IFN-I innate responses in the host. Blocking the 4-1BB signaling pathway ameliorates JE via divergent enhancement of IFN-II-producing NK and CD4^{+} Th1 cells and mature Ly-6C^{hi} monocyte infiltration, as well as an IFN-I innate response of myeloid-derived cells. Therefore, regulation of the 4-1BB signaling pathway with antibodies or inhibitors could be a valuable therapeutic strategy for the treatment of JE.

Biography

Seong Kug EO's lab has focused on unveiling how hosts response to pathogen infection. They have used various infectious models to prove host responses upon pathogenic infection. In recent, EO's lab has found the detailed pathway that IFN-I signal pathway orchestrated environments to provide effective protection against mucosal viral infection (PLoS Pathog., 2016). Moreover, EO's lab is expert on viral acute encephalitis caused by flaviviral infection. They have got many reports to unveil how immune system works on viral encephalitis caused by Japanese encephalitis virus (J. Neuroinflammation, 2014 and 2016).

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Sánchez B Sandra et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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In silico study of lipids associated in non-bilayer phospholipid arrangements

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Statement of the Problem: Systemic lupus erythematous (SLE) is an autoimmune, chronic and multifactorial disease. For SLE study, some animal models had been developed, and our lab has one. Our model was obtained by administering a different lipid bilayer structure called non-bilayer phospholipid arrangements. These non-bilayer phospholipid arrangements can be stabilized with drugs such as chlorpromazine or procainamide and specifically in female mice, causing a disease very similar to human SLE. To help understand how these lipid structures in the development of the disease involved, we used bioinformatics tools to understand how they form, and now we'll try to extrapolate this to explain some of the symptoms faced by patients with SLE.

Methodology & Theoretical Orientation: A molecular simulation was established using all the conditions used in our murine model with chlorpromazine and was created with GROMACS software. Analyses of results were made with GROMACS utilities, proprietary Python programs and VMD tools.

Findings: With our simulation strategy, we were able to observe differences between diverse lipidic environments with one or various molecules of chlorpromazine. Also, we have started new simulations to improve our strategy and get more useful information for our molecular knowledge of the models and the etiology of the human disease.

Conclusion & Significance: These findings help us to understand how the models are triggered and give us clues about how to improve them for a further research of how SLE is initiated in humans. That probably will contribute to the improvement of the models and support our novel theory about the etiology of the SLE disease in humans.

Biography

Sánchez B Sandra has her expertise in Molecular Biology and Bioinformatics, and is passionate about Biochemistry and Science Communication. Her novel approximation to the function of a drug on a bilayer membrane can help to better understand our murine models and how this can impact our knowledge on the systemic lupus erythematosus disease. She has built her abilities after years of experience in research, project administration and protocols development in the Pharmaceutical Industry, teaches of Biochemistry and Genetics for physicians, and translates pharmaceutical documents and participates in science fairs for kids. This work was possible due to her willingness to collaborate with external researchers, as Laura Domínguez from FQ-UNAM and Cornelio Yañez from CIC-IPN, who gave her the tools and knowledge to use bioinformatics and a Mexican Supercomputer to realize this project.

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Eduardo Martins de Sousa et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Serodiagnosis of tuberculosis: Specific detection of antibodies against the Ag85C-MPT51-HspX fusion protein (CMX)

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Tuberculosis (TB) is a major cause of the global infectious disease-related morbidity and mortality. In 2014, 9.6 million new TB cases were estimated throughout the world, among which 1.5 million deaths occurred due to the infection. In the same year, the incidence of TB in Brazil was 44 cases per 100,000 inhabitants, placing Brazil 16th among the 22 countries comprising 80% of TB cases in the worldwide. The development of new tools for rapid and accurate diagnosis of active pulmonary TB is considered by many countries to be a strategy for controlling the disease. The aim of this study was to apply the enzyme-linked immunosorbent assay (ELISA) for measuring anti-CMX IgM antibodies in individuals with active pulmonary TB and healthy controls in an area endemic for TB. Analysis of the clinical signs and symptoms showed that most individuals with active pulmonary TB more frequently presented with a cough (94.7%), weight loss (83.4%), and, to a lesser extent, hemoptysis (37%). In addition, we evaluated the tests that were used to diagnose TB. The chest X-ray (100%) and sputum smear microscopy (80%) were the most frequently used tests for diagnosis; in contrast, only 33% of patients underwent computerized tomography, and microbiological culture was performed for 12%. Patients with active pulmonary TB had higher titers of anti-CMX IgM antibodies (optical density (OD=0.502±0.281; p=0.0001) than healthy controls (OD=0.200±0.125). The cut-off for IgM-ELISA was determined using ROC curve analysis (AUC=0.868) with a sensitivity of 80.1% and a specificity of 78.2%. These results suggest that the recombinant protein CMX can be used as a serological marker for screening individuals suspected of having active pulmonary TB.

Biography

Eduardo Martins de Sousa holds a Bachelor's degree in Biomedicine, a Master's degree in Tropical Medicine (Immunology) from the Institute of Tropical Pathology and Public Health of the Federal Goiás University. He has obtained his PhD degree in Tropical Medicine (Immunology) from the Institute of Tropical Pathology and Public Health of the Goiás Federal University, being part of a Sandwich Doctorate held at the Institute of Molecular and Cellular Biology of the University of Porto, Portugal. Currently, he is a Professor of the Post-graduate program in Parasite Biology (Master's degree) at the University Center of Maranhão (UNICEUMA). He is an Associate Professor of Post-Graduate program in Biodiversity and Biotechnology of the Bionorte Network (PPG-BIONORTE) (Doctoral level). He has experience in Immunology, with emphasis in Applied Immunology, working mainly on the following topics: *Mycobacterium tuberculosis*, *Mycobacterium massiliense*, ELISA, experimental infection, vaccine, flow cytometry, real-time PCR and mice.

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IL-10 polymorphism and IL-10 cytokine production in response to mite stimuli and its association with atopy and asthma in children living in a poor area in Latin America

Flávia de Araújo Sena[1], Alana Alcantara Galvão[1], Emília Mª M de Andrade Belitardo[1], Gustavo Nunes de Oliveira Costa[1], Mariese Conceição Alves dos Santos[2], Camila Alexandrina Viana de Figueirêdo[1], Maurício Lima Barreto[3] and **Neuza M Alcântara-Neves[1]**
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Rationale: IL-10 is an important regulatory cytokine with a protective role in allergies. This study aim to verify if IL-10 gene polymorphisms interferes in IL-10 cytokine production according to mite stimuli and atopy/asthma status.

Methods: 1119 subjects from Salvador, Brazil, were genotyped using 2.5 Human Omni Beadchip from Illumina. IL-10 production by whole blood culture with the following mite stimuli: Blomia tropicalis and Dermatophagoides pteronyssinus was measured by ELISA. Asthma status was defined by ISAAC questioner. Atopy was determined through skin prick test to regional aeroallergens and specific IgE levels to *B. tropicalis*, *D. pteronyssinus*, *Blatella germanica* and *Periplaneta americana*. Statistical analyses were done using PLINK 1.9 and SPSS 22.1.

Results: *B. tropicalis* was the mite with the higher frequency of sensitized individuals (34.26%) and the bigger frequency of IL-10 responders (93.1% against 21.3% to *D. pteronyssinus*). There was no association between asthma severity and *B. tropicalis* IL-10 induction. SPT ≥ 3mm for *B. tropicalis* had a positive correlation with IL-10 production in response to this stimulus (r=0.126, p=0.031). None genetic variant was associated with IL-10 production by *B. tropicalis*, furthermore one variant rs3024496 (C allele) was associated with greater skin reactivity (OR 1.33; 95% CI, 1.02-1.73).

Conclusion: The absence of association between IL-10 polymorphism and this cytokine production can be explained by the lack of the promoter region of the gene on these analyses. Our findings did not support the IL-10 regulatory role once we did not find differences between IL-10 cytokine productions according to atopy/asthma status; we otherwise found a positive correlation between IL-10 production and SPT positivity and a high frequency of IL-10 producers in *B. tropicalis* stimulus response. Now, we question what IL-10 function in allergy caused by *B. tropicalis*, once this mite stimulus IL-10 production and there is association with more allergic biomarkers.

Biography

Flávia de Araújo Sena, is graduated in Biomedicine from Bahiana School of Medicine and Public Health. Master in Immunology by the Postgraduate Program in Immunology (PPGIM) at the Federal University of Bahia (UFBA). Currently does a specialization in Microbiology and is a PhD student of Immunology at UFBA, working in the Laboratory of Allergy and Acarology (LAA). She collaborates with the following projects: An asthma cohort in children and adolescents of the city of Salvador – Bahia, SCAALA (2013), Immuno-intervention in experimental models of respiratory allergy and Chagas disease and study of the in vitro immunomodulatory effect of candidin fractions (2015).

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Alana Alcântara Galvão et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Asthma, allergy and polymorphisms in vitamin D pathway: A cross section study

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Introduction: Vitamin D presents a highlighted immune system activity. Its deficiency or insufficiency has been associated with low asthma control. Also, genetic determinants on the vitamin D pathway have been associated with asthma. In this study, we investigated associations between vitamin D serum levels with atopy and asthma, as well as polymorphism genes of the vitamin D pathway.

Methods: 25 (OH) vitamin D quantified from 970 of 11-17 years old Brazilians by ELISA. Asthma diagnosis was obtained by ISAAC, Phase III questionnaires. Specific IgE detection to allergens was performed by ImmunoCAP; DNA was extracted and genotyped using 2.5 Human Omni Biochip from Illumina. Statistical analyses were performed using PLINK 1.9 and SPSS 22.1 programs.

Results: The prevalence of vitamin D insufficiency was 64% in 76 asthmatic and 62.5% in 446 atopic individuals; however there was no significant association between vitamin D and this outcomes. Negative correlation was found between vitamin D and specific IgE levels to *Dermatophagoides pteronyssinus* on atopic subjects (r=-0.11, p=0.04). Genetic variants in *CYP2R1* gene, rs7935792 (C allele) (Beta 1.66; 95% CI 0.20-3.11) and rs7129781 (C allele) (Beta 1.55; 95% CI 0.07-2.96), were associated with vitamin D serum levels. In addition, the same variants had suggestive protection on asthma, but it was not significant (OR 0.74; 95% CI 0.39; 1.39; OR 0.73; 95% CI 0.38; 1.37, respectively). VDR variants rs7965397 (G allele) was positively associated with atopy (OR 1.43; 95% CI, 1.07-1.92); rs4328262 (G allele) (OR 1.44; 95% CI 1.09-1.90) and asthma rs2408876 (C allele) (OR 2.31; 95% CI; 1.18-4.53); rs2238317 (T allele) (OR 2.19; 95% CI 1.02-4.72).

Conclusions: Vitamin D can modulate the immune system and reduce allergic biomarkers. Genetic variants in *CYP2R1* regulated vitamin D levels, and must prevent asthma symptoms. Genetic variants in VDR were associated with asthma and atopy susceptibility may be by modifying VDR expression.

Biography

Alana Alcântara Galvão has completed her graduation in Pharmacy, Master's in interactive processes of the organs and systems by the Federal University of Bahia (UFBA) and currently is a PhD student of Immunology at UFBA. She is actually working in Laboratory of Allergy and Acarology (LAA). Her research covers immuno-epidemiology, immunomodulation and immunogenetics. She collaborated with projects like: Research on the prevalence of respiratory allergies and their risk factors in children from rural areas in the city of São Francisco do Conde, Bahia (2010); An asthma cohort in children and adolescents of the city of Salvador - Bahia, SCAALA (Social Change in Asthma and Allergy in Latin America) (2012); Immunogenetic study on possible associations between Vitamin D, atopy and asthma in children and adolescents of Salvador, Bahia (2014). The objective of her study is to identify the association of vitamin D with asthma and atopy, highlighting genetic variants in vitamin D pathway.

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Anti-inflammatory activity of *Bixa orellana* extract against *Mycobacterium abscessus subsp. bolletii*

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Infectious diseases continue to be one of the biggest health problems in the world, affecting millions of people annually *M. abscessus* and other species of rapidly growing mycobacteria (RGM) are naturally resistant to antimicrobial compounds and disinfectants because they have an impermeable cell wall composed by peptideoglycan and mycolic acids. These RGM are responsible for various hospital outbreaks worldwide, causing lung infections in patients with cystic fibrosis, chronic lung disease (bronchiectasis, nodules and cavitations), post-surgical infections and skin and soft tissue infections in immunocompromised patients. The resistance of *M. abscessus* (Mabs) to the medications used in current therapy challenges the search for new treatment strategies. Previous studies on the search for new natural compounds with antimicrobial action highlighted the potential of *Bixa orellana* (urucum). The seeds of this plant are already used in folk medicine for treating heart disease, gastrointestinal problems and respiratory infections. In this study, we evaluated potential anti-inflammatory activities of hydroalcoholic (BoEH) and ethyl acetate (BoEA) extracts of *B. orellana* leaves, using a murine model of peritonitis induced by heat killed *Mabs*. C57BL/6 mice were orally treated with different concentrations of BoEH or BoEA. After one hour, peritonitis was induced by inoculation of $1x10^8$ CFU of heat killed *Mabs*. BoEH and BoEA inhibited the migration of total leukocytes (Figure 1A-B), migration of polymorphonuclear cells (Figure 1C-D) and mononuclear cells (Figure 1E-F) into the peritoneum in the periods analyzed 4 and 24 hours after the induction of peritonitis. Our results suggest anti-inflammatory actions of the extracts tested, indicating this plant as natural source of compounds with potential for pharmacological and biotechnological applications.

Biography

Eduardo Martins de Sousa holds a Bachelor's degree in Biomedicine, a Master's degree in Tropical Medicine (Immunology) from the Institute of Tropical Pathology and Public Health of the Federal Goiás University. He has obtained his PhD degree in Tropical Medicine (Immunology) from the Institute of Tropical Pathology and Public Health of the Goiás Federal University, being part of a Sandwich Doctorate held at the Institute of Molecular and Cellular Biology of the University of Porto, Portugal. Currently, he is a Professor of the Post-graduate program in Parasite Biology (Master's degree) at the University Center of Maranhão (UNICEUMA). He is an Associate Professor of Post-Graduate program in Biodiversity and Biotechnology of the Bionorte Network (PPG-BIONORTE) (Doctoral level). He has experience in Immunology, with emphasis in Applied Immunology, working mainly on the following topics: *Mycobacterium tuberculosis*, *Mycobacterium massiliense*, ELISA, experimental infection, vaccine, flow cytometry, real-time PCR and mice.

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Substitute mesenchymal stromal cells therapy in graft versus host disease with a chemically defined cocktail

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Mesenchymal stromal cell (MSC) therapy has been shown to be effective in phase I/II clinical trials in the treatment of graft versus host disease (GVHD) after allogeneic hematopoietic cell transplantations. However, MSC trials still face major challenges, such as complex and time-consuming manipulation, requiring a good manufacturing practice facility, difficult and expensive to produce etc. In a screen of MSC-derived factors with serial factorial designs, we first time identified two MSC-derived factors, CXCL5 and CCL24 inhibitor (antibody), which exhibited synergistic immunomodulation effect in mixed lymphocyte reaction. This two-factor (2F) cocktail also showed excellent *in vivo* immunosuppressive effect in ameliorating GVHD symptoms and improving survival. A xenograft GVHD animal model was created by injecting 400×106 cells/kg of cryopreserved human PBMCs into NSG mice respectively. Four consecutive treatments were given on day-10, day-14, day-17 and day-21 post-transplantation. The 2F cocktail exhibited excellent immunosuppressive effect as it could improve mice 36-day survival from 19.0% with severe symptoms to 61.9% with mild symptoms ($p<0.01$). It was significantly better than BM-MSCs (8.3%, $p<0.001$) and Cyclosporine A (26.1%, $p<0.05$). Synergistic effect was again observed between those two factors (CXCL5, 18.2%; anti-CCL24, 9.1%; $p<0.05$). The 2F cocktail treatment reduced cytotoxic T lymphocytes (CTLs), T helper 1 (Th1) cells, Th17 cells, NK cells in the circulation and macrophages in the spleen, but did not affect human hematopoietic stem cells (HSCs) reconstitution in the bone marrow. Concurrently, it reduced pro-inflammatory cytokine IFN-γ, IL-1β, IL-6, IL-12, TNF-α, IL-17A, IL-8, MIP-1β and MCP-1 in the circulation. In conclusion, the efficacy of a novel identified 2F cocktail was validated in an *in vivo* xenograft GVHD model. It demonstrated a robust immunosuppressive effect and kept the development of GVHD under control. The 2F cocktail could be a potential chemically defined substitute for MSCs in GVHD therapy.

Biography

Yap Chui Sun joined the Department of Clinical Translational Research, Singapore General Hospital (SGH) in 2013, and has been working on reprogramming mesenchymal stem cells. Her first postdoctoral position was at Duke-NUS Medical School, where she was studying the contribution of micro RNAs on thyroid hormone function. She obtained her Master's degree in Molecular Immunology at the National University of Singapore and Ph.D. degree in Molecular Oncology at Brown University (U.S.A.).

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Emília M M de Andrade Belitardo et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Association between higher levels of pro-inflammatory cytokines (TNF, IL-6) and IL-5 in induced sputum and lower lung function among subjects with asthma

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Aim: Asthma is a chronic and heterogeneous disease presenting various phenotypes. The aim of this study was to assess airway inflammation among subjects with asthma by counting cells and measuring cytokines in the sputum to search for associations with clinical features of the disease.

Methods: We studied 66 subjects, divided into three asthma subgroups [16 with severe asthma resistant to treatment (SAR), 22 with severe asthma controlled with treatment (SAC), 19 with mild to moderate asthma (MMA)] and a group with no asthma (NA) including 9 subjects. Total cellularity of the sputum samples was counted using a hemocytometer and differential cytology was observed in cytospin. Measurements of cytokines were performed by Luminex (Upstate/Millipore system "Flex kit"). Statistical analysis was performed using nonparametric tests.

Results: Sputum of SAR had higher percentage of neutrophils as compared with MMA (p=0.05) and higher percentage of eosinophils compared with NA (p=0.02). TNF was increased in SAR compared to NA, MMA and SAC (p=0.001). Subjects with asthma, and treated with high doses of inhaled corticosteroids presented higher levels of TNF (p=0.02), IL-6 (p=0.01) and IL-5 (p=0.03). Increased TNF production was associated with reduced lung function before and after bronchodilator as measured by FEV1% [p=0.00 for both), FEV1/FVC% (p=0.03 and p=0.02 respectively) and FEF25-75% (p=0.00 for both).

Conclusion: Increased inflammatory cytokines (TNF, IL-6 and IL-5) and number of inflammatory cells (neutrophils and eosinophils), were associated with SAR, and high levels of TNF were associated with worse lung function. Our findings support the concept these subjects may have an end phenotype of asthma related to airway inflammation that goes beyond the Th2 type response, and that it is indeed resistant to high doses inhaled corticosteroids, requiring a different approach to treatment.

Biography

Emília M M de Andrade Belitardo is a Physiotherapist, Specialist in Respiratory Physiotherapy by the Federal University of São Paulo and Master in Immunology by the Federal University of Bahia (UFBA). She is currently a PhD scholar of Immunology at UFBA, working in the Laboratory of Allergy and Acarology (LAA). Her research interests include Immuno-Epidemiology, Clinical Immunology, Immunomodulation and Immunogenetics. She collaborates with two major research projects: (i) An asthma cohort in children and adolescents of the city of Salvador - Bahia, SCAALA (Social Change in Asthma and Allergy in Latin America); and (ii) A case-control study developed in partnership with the Center of Excellence for Asthma (UFBA).

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Memory T cells are significantly increased in rejecting liver allografts in rhesus monkeys

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Introduction: In kidney, heart and islet transplantation the rhesus monkey (*Macaca mulatta*, RM) has been shown to be an excellent preclinical model that can provide the basis for new immunosuppressive protocols for clinical studies. However, there remain relatively few liver transplant (LT) models in nonhuman primates. In this study, we analyzed the immune cell populations of PBMC and secondary lymphoid organs along with livers of normal rhesus monkeys and compared them to those of rejecting liver transplanted recipient's following withdrawal of immunosuppression.

Methods & Results: We undertook six allogeneic ABO compatible orthotopic LT in monkeys using six normal donor monkey livers. We collected tissues including lymph-node, spleen and blood from which we isolated immune cells for FACS analysis along with the liver from the recipient. We found that CD4 or CD8 naïve T cells were normally seen at low levels (13.89±8.67 or 1.50±1.44 respectively) and memory T cells were seen at high levels (76.12±11.40 or 98.0±1.60) in the liver rather than lymphoid organs or PBMC. However, regulatory cells such as CD4+FoxP-3+ T cells and CD8+CD28- cells remained in high numbers (0.77±0.54 and 34.99±6.40) in the liver but not in lymph node or PBMC. These results demonstrate that the liver has rather unique immunological properties compared to other organs. We also compared CD4/8 T sub-populations in normal or rejected livers and the various tissues showed that naïve cells were dramatically decreased in spleen, lymph node and PBMC of rejected transplanted monkeys but rather their memory cells were increased in all tissues and PBMC.

Conclusion: We have shown that the normal liver has large numbers of C4Tregs or CD8+CD28- or MDSC which are the known immune suppressive cells at much higher levels than other lymph node or peripheral blood. Memory T cell populations in rejected livers or lymphoid organs were expressed at significantly higher levels than those seen in normal tissues including as seen in the peripheral blood.

Biography

During the course of my Ph.D. I studied therapeutic anti-inflammatory effects of human mesenchymal stem cells on traumatic brain injury and studied the role of stem cells in human brain tumor development using SD-rat model. As a post Doc my focus was to study therapeutic strategies towards successful xenotransplantation. I was involved in two main projects related to the development of the first pre-clinical nonhuman primate study of solid organ xenotransplantation. This was done using genetically engineered pigs expressing multiple human complement and coagulation regulatory proteins in order to overcome the immunological and physiological barriers against successful xenotransplantation.

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Notes:

Manukyan G et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Impaired priming and increased ROS production by circulating neutrophils from patients with chronic lymphocytic leukemia

Manukyan G[1,3], Papajik T[1], Gajdos P[2], Mikulkova Z[1], Urbanova R[1], Gabcova G[1], Kudelka M[2], Turcsanyi P[1], Ryznarova P[1], Prochazka V[1] and **Kriegova E[1]**
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Besides a classical role in antimicrobial functions, emerging evidence indicates that neutrophils could have an effect on chronic and progressive diseases such as leukemia. Nowadays, there is limited information about the function of circulating neutrophils in the chronic lymphocytic leukemia (CLL). The aim of the present study was to study functional properties of circulating neutrophils in CLL. 18 CLL patients and 17 healthy controls were enrolled in the study. Priming of neutrophils with LPS as well as oxidative stress capacity was analyzed using flow cytometry. Spontaneous and induced with fMLP and PMA oxidative stress was measured using dihydrorhodamine 123. Despite fMLP and PMA significantly induced ROS production by neutrophils in both healthy ($P<0.01$) and CLL groups ($P<0.01$ and $P<0.001$, respectively), stimulation with both inducers has led to a maximum ROS-release in neutrophils from CLL patients compared to healthy ones ($P<0.05$ and $P<0.01$, respectively). Spontaneous production of ROS by CLL neutrophils was also increased ($P<0.05$) compared with healthy neutrophils. LPS up-regulated TLR2 in healthy cells ($P<0.05$), and TLR2 expression was down-regulated in CLL cells ($P<0.05$). LPS exposure of isolated neutrophils from healthy group induced production of IL-1β and TNF-α ($P<0.05$). In contrast, LPS-stimulated CLL neutrophils failed to induce releasing of both cytokines. LPS-induced production of IL-1β and TNF-α in CLL group was lower than those released by neutrophils from healthy group ($P<0.05$). Taken together, circulating neutrophils in CLL patients have altered functional properties of which may account for the heightened sensitivity to bacterial infection as well as influence the disease course. Future studies are needed to prove our observations.

Biography

Gayane Manukyan is researcher in the National Academy of Sciences of the Republic of Armenia and a group leader of a Group of Molecular and Cellular Immunology Institute of Molecular Biology. Currently she is postdoctoral fellow in the Department of Immunology, Medical Faculty, Palacky University, Olomouc, Czech Republic. She has strong expertise in flow cytometry and studies focused on innate immunity and imunology of inflamatory and infectious diseases.

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Gabcova G et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Expression profiling of chemokine receptors in peripheral blood mononuclear cells in chronic lymphocytic leukemia

Gabcova G[1], Kraiczova V[1], Manukyan G[1,2], Mikulkova Z[1], Fillerova R[1], Urbanova R[1], Turcsanyi P[1], Ryznarova P[1], Papajik T[1] and **Kriegova E[1]**
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Chronic lymphocytic leukemia (CLL) cell is characterized by a progressive accumulation of long-lived CD5+ B lymphocytes in bone marrow/lymph nodes, whose survival requires exogenous activation signals such as chemokines. Growing number of studies show that chemokines and their receptors, in addition to trafficking, have much broader influence on neoplastic cells, such as cell growth, differentiation and survival. To gain more insights into the chemokine receptor network in CLL, we characterized the expression pattern of 16 canonical and 4 atypical chemokine receptors in peripheral blood mononuclear cells (PBMC) of CLL patients (n=88) and healthy subjects (n=34) by using quantitative RT-PCR. The expression of CXCR3, CXCR4, CXCR5, CXCR7, and CCR7 was confirmed by 6-color flow cytometry. Among deregulated receptors, 5 receptors (CCR7, CCR10, CXCR3, CXCR4, CXCR5) were up-regulated and 9 receptors (CCR2-CCR6, CCR8, CCRL2, CXCR1, CXCR2) down-regulated in CLL; the expression of others did not differ between CLL and controls (P>0.05). We have also analyzed differences in expression pattern in CLL groups subdivided according to cytogenetics (13q and 17q deletions). In patients with del(17q) having worse prognosis, we observed higher mRNA levels of CXCR6, CXCR7 and CCR10 comparing to del(13q) associated with good prognosis. In conclusion, differential expression patterns of chemokine receptors suggest the relevance of the network of these receptors in CLL pathogenesis. The potential of chemokine/chemokine receptor network as determinant for clinical outcome and novel therapies is worth exploring.

Biography

Gabriela Gabcova is a PhD student of Immunology at Palacky University Olomouc, Czech Republic. Her main research activity includes immunophenotyping of cells in peripheral blood and synovial fluids by flow cytometry. She has experience with development of multi-colours panels for flow cytometry. She is primarily focused on disorders of connective tissue (osteoarthritis, rheumatoid arthritis) and hematological malignancies (chronic lymphocytic leukemia, Hodgkin lymphoma).

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Notes:

Guan-Ming Ke, J Clin Cell Immunol 2017, 8:3(Suppl)
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Development of genotype VII NDV inactivated vaccine

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Newcastle disease virus (NDV), also known as avian paramyxovirus serotype 1, is a member of the family Paramyxoviridae and causes a highly contagious respiratory, neurological, or enteric disease in chickens. Currently, genotype VII strains are the predominant virulent strains. Although the different genotypes of NDV all belong to one serotype, it is still difficult to confer cross-protection under stressful environmental conditions. The aim of this work is to generate an attenuated NDV strain and develop a cell culture-derived genotype VII ND inactivate vaccine against current virulent NDV strains. After the 40th passage, we found that the MDT of NDV strains is higher than 120 hours, the intracerebral pathogenicity indexes (ICPI) had decreased to 0-0.08, and TCID50 has reached 10^8. The attenuated strain, KGM-01, is then inactivated and mixed with oil adjuvants for vaccine evaluation. Hemagglutination inhibition test showed that the inactivated vaccine elicited high antibody titer two weeks after immunization and no virus shedding was detected using real-time PCR when challenged with the Sato strain and field type VII strains. Thus, KGM-01 is a low virulent, type VII genotype strain with high antigenicity suitable for inactivated NDV vaccine development

Biography

Guan-Ming Ke is a licensed veterinarian and teaches at the National Pingtung University of Science & Technology as an associate professor. He focuses on animal vaccine development and production, continuously applying new technologies to develop new types of vaccines and to *better* the manufacturing process. His work aims to turn research into applicable technologies that will aid in disease prevention and improve both animal and human welfare.

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Notes:

Chi-Chi Wen, J Clin Cell Immunol 2017, 8:3(Suppl)
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Efficient production of type O foot-and-mouth disease subunit vaccine using baculovirus expression system

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The foot and mouth disease (FMD) is a highly contagious viral disease of Artiodactyla, causing severe economy loss worldwide. In this study, the structural proteins VP1, VP2, VP3 and VP4 of FMD serotype O were produced with the concentrations between 250 μg/mL to 550 μg/mL by using baculovirus expression vector system (BEVS). This improved the low productivity problem of eukaryotic expression system. The concentration of the anti-FMD antibodies in animal sera can be distinguished by using purified VP1 proteins, which were coated on the ELISA plates, showing that the recombinant VP1 protein has good antigenicity. The virus like particles (VLPs) was observed under electric microscope by using the mixture of the four proteins and pH value adjustment. Presently, those four structural proteins were tested alone or in mixture in pig experiment. In conclusion, this subunit vaccine has the potential to provide protection against FMD.

Biography

Chi-Chi Wen holds a Master's degree in Veterinary Medicine from National Taiwan University. She is a Research Fellow at Graduate Institute of Animal Vaccine Technology of National Pingtung University of Science and Technology. Her research interest includes Molecular Biology and Biotechnology, continuing in the research of Veterinary Medicine. She focuses on development of animal vaccines, designing new type of vaccines with biotechnology, with the hope to help animals avoid suffering.

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Notes:

María Cristina Gutiérrez-Jiménez et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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PMNs as "Trojan horse" vehicles for *Brucella abortus* persistence in murine bone marrow

María Cristina Gutiérrez-Jiménez, Ricardo Mora-Cartín, Carlos Chacón-Díaz, Esteban Chaves-Olarte, Edgardo Moreno and **Elías Barquero-Calvo**
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Brucella abortus is a facultative intracellular pathogen that causes chronic infections. Neutrophils (PMNs) are the first cells that encounter *Brucella* after invasion, however, *Brucella* resist their killing action and induce premature cell death of these leukocytes. It has been described that *B. abortus* persist in bone marrow at chronic stages of infection. Nevertheless, the role of PMNs in bone marrow persistence has not been studied. Here we show that *B. abortus* organisms are able to persist in murine bone marrow even at the "declining stages of chronic infection". *B. abortus* were observed inside a PMN/monocyte cell type at very low rates. Additionally, we demonstrate that murine bone marrow PMNs phagocyte antibody-opsonized *B. abortus* and die quickly after infection. These dying infected PMNs show increased adhesion and are readily taken up by RAW 264.7 macrophages. When *ex vivo* macrophage infections were performed, *B. abortus* were more infective and replicated at higher rates when macrophages were infected through PMNs than when infected with *Brucella* only. In general, proinflammatory and anti-inflammatory cytokines showed increased values in macrophages infected through PMNs; but only after 24 hours of infection, when *Brucella* has already reached their replication niche inside the cell. Our results support the notion that infected bone marrow PMN might behave as vectors for *Brucella* persistence in bone marrow in a non-logistic way.

Biography

María Cristina Gutiérrez-Jiménez is a Master's student of the University of Costa Rica. She is developing a research project on different Immunology aspects, regarding the infectious disease called brucellosis. Her work's main objective is to study the role of polymorphonuclear neutrophils as "Trojan Horse" vehicles during brucellosis, using a bone marrow murine model and the chronicity and persistence of the disease. She has acquired expertise in ELISA, flow cytometry, bacterial infections, cell culture, cell differentiation, cell infection through cells and fluorescence microscopy. She has co-authored a publication regarding the role of neutrophils during brucellosis.

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Notes:

Nebal Morad Abdelhamid Mohamed et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Tissue echocardiography in early detection of myocardial dysfunction in RA

Nebal Morad Abdelhamid Mohamed, Dina Abdel- Halim Shahin, Asmaa Hegazy Aboelftoh, Medhat Abdel-Samad Sakrana and **Nagy Abdel-Hady Sayed Ahmed**
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Statement of the Problem: Rheumatoid arthritis (RA) is a multi-organ inflammatory disorder. A reduction in life expectancy in RA patients is primarily due to myocardial disease, which is clinically silent. There is increasing interest in autoimmune diseases, especially their relationship with cardiovascular disease. RA in particular has been considered an independent risk factor for coronary artery disease in recent years. Various studies have aimed to clarify important aspects of risk stratification and treatment options in patients with rheumatoid arthritis. TDE offers the promise of an objective measure to quantify regional and global LV function. So, it is important for using a non-invasive technique and can be serially followed over time for cardiac risk progression and detecting patients at the greatest risk of cardiac morbidity and mortality.

Methodology & Theoretical Orientation: Case control design has been carried out on rheumatoid arthritis (RA) patients and control. All patients evaluated clinically and echocardiographically (M mode, trans-mitral and tissue Doppler). And all echo-parameters were correlated with various clinical data.

Findings: Sensitivity of tissue echo compared to conventional echo in diagnosis of diastolic dysfunction in RA patients is weak. Tissue Doppler finding were not related to DAS28CRP. Tei index showed significant positive correlation with disease duration.

Conclusion & Significance: Diagnostic accuracy of Tie index by tissue echocardiography is weak diagnostic. Myocardial dysfunction in RA is a matter of time and not related to disease activity.

Biography

Nebal Morad Abdelhamid Mohamed is working as a Lecturer at the Rheumatology and Immunology Department in Mansoura University, Egypt.

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Notes:

SD Thakur et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Development of flow cytometry based adherence assay for *Nessieria gonorrhoeae* using 5′-carboxy-fluorosceinsuccidyl ester (CFSE) and ME-180 cells

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Statement of the Problem: The microorganism *Nessieria gonorrhoeae* is an obligate human pathogen and its adherence to host cells is essential for its pathogenesis. We devised a flow cytometry-based method to quantify the adherence of piliated *N. gonorrhoeae* strain F62 to human cervical ME-180 cells.

Methodology: Piliated *N. gonorrhoeae* F62 were collected after 10 to 12 hours of growth then stained with cell-permeable fluorescent dye 5'-carboxyfluoroscein succidyl ester (CFSE). The bacteria were incubated with 0.5 µl of 5 mM CFSE in 2.5 ml of PBS and incubated at 37°C for 15 min. ME-180 cells were incubated for 2 hours with fluorescent, piliated *N. gonorrhoeae* (multiplicity of the infection 1:100) then the ME-180 cells were washed with phosphate buffer saline to remove loosely adherent bacteria. Flow cytometry was used to quantify the percentage of ME-180 associated with $CFSE^+$ fluorescent bacteria and a minimum of 30,000 events were recorded.

Finding: Results indicated that 19.2% ± 0.99 (n=4) ME-180 cells were associated with the fluorescent, piliated bacteria. To assess whether antibodies specific for *N. gonorrhoeae* blocked their adherence to ME-180 cells, rabbit hyper-immune anti serum was raised against heat-killed piliated *N. gonorrhoeae* F62. Adherence efficiency, the percentage of cell-associated $CFSE^+$ bacteria divided by the total input $CFSE^+$ bacteria ranged between 37-47% (n=5). Heat-inactivated hyperimmune serum, at 1:10 to 1:80 dilutions, significantly inhibited gonococcal adherence by 6 and 3 fold, respectively. Heat-inactivated negative rabbit serum was significantly (3 to 5 folds) less effective at preventing bacterial adherence suggesting that antibody specificity and not a non-specific serum component were involved. Flow cytometric analysis was amenable to the quick, easy and high-throughput quantification of *N. gonorroheae* association with eukaryotic cells. These approaches may be adapted for use in *in vitro* and *in vivo* adherence studies related to gonococcal pathogenesis.

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Notes:

Kira Ziegler et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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The effect of nitration on the potential allergenicity of wheat derived alpha amylase trypsin inhibitors (ATIs)

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Over the past decades, environmental pollution and allergy incidence have been increasing on a global scale, implicating that they are interconnected. Air pollutants e.g. nitrogen dioxide and ozone are capable of chemically modifying airborne allergens, particularly under humid summer smog conditions. As demonstrated for birch pollen Betv1, these nitrated allergens are known to have enhanced allergic potential. We demonstrated that wheat derived alpha-amylase-trypsin inhibitors (ATIs), which previously were identified as major allergens of baker's asthma, are also strong activators of the intestinal innate immune system when ingested with wheat products. Moreover, via their innate immune stimulatory activity, these ATIs also promote experimental allergies. As air pollution and fertilizers can lead to nitration of these ATIs in the living grains, the aim of this project was to elucidate the effect of nitrated ATIs on innate and adaptive immunity. Therefore, a HeLa TLR4 dual reporter cell line was stimulated with untreated ATIs vs. nitrated ATIs. Furthermore, human monocyte-derived dendritic cells (DC) were exposed to ATIs or nitrated ATIs and changes in specific DC maturation markers and cytokine patterns were analyzed by flow cytometry or multiplex ELISA. Additionally, T cell proliferation after co-cultivation with different ATI-treated autologous DC was determined. In all these different *in vitro* systems we could demonstrate a stronger stimulatory capacity of nitrated ATIs in comparison to native ATIs, indicating that nitration of an antigen/allergen not only affects its allergenicity but also its immunogenicity.

Biography

Kira Ziegler has obtained her Diploma in Biology from the Johannes Gutenberg-University, Mainz, Germany. The topic of her thesis was "Expression and recovery of two recombinant proteins in *Escherichia coli* for cancer vaccination". Following this, she started her PhD at the Max Planck Institute for Chemistry, Mainz, Germany. Her research focusses on the allergenic effect of nitration on wheat derived alpha amylase trypsin inhibitors, bridging with this topic atmospheric science and fundamental medical research.

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Notes:

Landa S Carla et al., J Clin Cell Immunol 2017, 8:3(Suppl)
DOI: 10.4172/2155-9899-C1-037

Participation of plasmacytoid dendritic cells and NKT cells in the mouse model of lupus induced by non-bilayer phospholipid arrangements

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Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease, where animal models are used to study its pathogenesis. We have developed a mouse model of autoimmune disease resembling human lupus by the injection of liposomes with non-bilayer phospholipid arrangements (NPA). Also, we described the presence of IgG antibodies against NPA in the serum of mice with lupus and patients with SLE. In this work, we determined by citofluorometry the presence of plasmacytoid dendritic cells (pDC) the main producer of type I interferons, and of NKT cells in the secondary lymphoid organs of mice, 30 and 60 days after the injection of liposomes with or without NPA induced with 8 mM promazine. In both groups of mice injected with liposomes bearing NPA, a significant increase of pDC cells (5-fold) was found, which correlates with the high concentration of type I interferon previously detected in mice with lupus and in human SLE. A significant increase of NKT (3-fold) was detected at 30 days, specifically in the NKT subpopulation $CD4^+$ which is known to cooperate with B cells in response to lipid antigens; this increase suggests its probable involvement in adaptive immune responses, which lead to the production of anti-NPA IgG antibodies. The increase of pDC and NKT cells found by cytometry in secondary lymphoid organs in this work, suggests their involvement in the formation of anti-NPA IgG antibodies and the development of the disease resembling human lupus.

Biography

Landa S Carla is a PhD Scholar, and is currently working with murine lupus model induced by lipidic antigens. We are interested in the immune response, especially in the participation of NKT cells and plasmacytoid dendritic cells (pDC) in this autoimmune disease. We had found an increase in the percentage of NKT cells by pDC in secondary lymphoid organs at 10, 20 and 30 days after being injected in mice with non-bilayer phospholipid arrangements and we already know that these antibodies are produced via germinal centers in this murine model that resembles human lupus.

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Notes:

Jong-Yeup Kim et al., J Clin Cell Immunol 2017, 8:3(Suppl)
DOI: 10.4172/2155-9899-C1-037

Significance of DNA methylation to polyp formation of eosinophil and neutrophil in chronic rhinosinusitis

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Predicting which patients are at a higher risk for recurrent chronic rhinosinusitis with nasal polyps (CRSwNP) is one of the most challenging problems in clinical rhinology. A direct association between nasal polyp and eosinophil/neutrophil counts was reported. This study aimed to identify difference of eosinophils and neutrophils for formation of polyp by DNA methylation in CRS. We have previously shown that KRT 19, NR2F2, ADAMTS1, and ZNF222 levels are changed in nasal polyps (NPs) of patients with chronic rhinosinusitis (CRS) in patients. A study was performed from 30 patients with CRS with bilateral NP, examining the prognostic role of eosinophil and neutrophil levels. 30 patients with CRS were classified by the rate of eosinophils and neutrophils in tissue. The methylated genes detected by DNA methylation microarray were validated by methylation-specific polymerase chain reaction (PCR), bisulfite sequencing, and real-time PCR. DNA methylation microarray identified 43,674 CpG islands in 518 genes. Specific genes were found to have a hypermethylated signal, and some genes were significantly hypomethylated in the promoter region in eosinophils compared with neutrophils. Real-time PCR showed that the expression levels of genes were changed in eosinophils, when compared with neutrophils. We clearly demonstrated that the two subgroups of CRSwNP had characteristic differences in DNA methylation, which allows for pathophysiologically meaningful differentiations with likely therapeutic consequences. Further studies are needed to confirm the significance of these epigenetic factors in the mechanisms underlying NP formation.

Biography

Jong-Yeup Kim has his expertise in improving the Otorhinolaryngology (snoring, septic disease, sinusitis, tonsillitis, nasal molding and allergic rhinitis). His open and contextual evaluation model based on responsive constructivists creates new pathways for improving nose disease. He has built this model after many years of experience in research, evaluation, teaching and administration both in hospital and education institutions.

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Simona Valova et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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The NOD-like receptor (NLRP3) gene variability in patients with recurrent aphthous stomatitis

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Statement of the Problem: Recurrent aphthous stomatitis (RAS) is a multifactorial disease with an unclear etiopathogenesis, resulting from the interplay between genetic and environmental factors. As the dysregulation of the immune system can play a role in the RAS development, single nucleotide polymorphisms (SNPs) in the genes for immune and inflammatory molecules were studied. The NOD-like receptor *(NLRP3)* gene, encoding the component of the inflammasome, has been proposed as one of the candidate genes for RAS. The aim of our study was to investigate three SNPs (rs4612666, rs10754558, rs3806265) in *NLRP3* gene in patients with RAS and healthy controls in the Caucasian population.

Methodology: A total of 200 Czech subjects were enrolled in this case-control study. 143 healthy controls, 57 patients with RAS were genotyped by method based on polymerase chain reaction using 5' nuclease TaqMan® assays. Clinical parameters such as complete blood count, levels of immunoglobulins including allergen-specific immunoglobulin E or presence of antibodies against cytomegalovirus, Epstein-Barr virus were determined in RAS patients.

Findings: Although no significant differences in the *NLRP3* (rs10754558, rs3806265) allele and genotype frequencies between patients with RAS and controls were observed, statistically significant differences in *NLRP3* rs4612666 genotype frequencies between subjects with RAS and controls were found. Carriers of *NLRP3* rs4612666 TT genotype had a higher risk of developing RAS in comparison to subjects with CT + CC genotypes (OR=16.71, 95% CI=1.96-142.14, P=0.0024). No association between *NLRP3* haplotypes and RAS was detected.

Conclusion & Significance: In contrast to the previous study, associations between *NLRP3* (rs10754558, rs3806265) polymorphisms and RAS were not confirmed. However, we suggest that *NLRP3* rs4612666 polymorphism can strongly influence the risk of developing RAS in the Czech population.

Biography

Simona Valova studied Molecular Biology and Genetics at Faculty of Science, Masaryk University, Brno, Czech Republic. She is currently in her third year of PhD in Physiology and Pathological Physiology at Faculty of Medicine, Masaryk University. She works in the team of Professor Lydie Izakovicova Holla that focuses on variability in candidate genes for multifactorial diseases, including periodontitis, recurrent aphthous stomatitis, diabetes mellitus or gastroesophageal reflux disease.

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Seung-Heon Hong et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Lipin-2 mRNA inhibition aggravates TLR ligands induced inflammation

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Lipins are phosphatidic acid phosphatases involved in synthesis of phospholipids and triglycerides, although they regulate cellular levels of important signaling lipids. Lipin-1 contributes positively to macrophage stimulation through TLR4, and other TLRs, by affecting MAPKs and AP-1 activation and, as a consequence, the generation of pro-inflammatory factors. Lipin-2 reduces pro-inflammatory signaling induced by saturated fatty acids in macrophages. Here we examined whether LPIN-2 mRNA inhibition affects TLR mediated inflammatory signaling in HT29, a colon cancer cell line. The LPIN-2 siRNA pre-treatment reduced the up-regulated defensins stimulated by TLR ligands, LPS and flagellin. And the increased level of IL-8 mRNA by LPS and R848 were more increased by LPIN-2 mRNA inhibition. And LPS and R848 induced JNK and ERK phosphorylation whose expressions were more elevated by Lipin-2 inhibition. On the other hand, the lipid transcription factors like PPARγ and PGC1α did not change by LPIN-2 siRNA pre-treatment. Taken together, LPIN-2 inhibition aggravates TLR ligands induced inflammatory signaling through ERK and JNK phosphorylation.

Biography

Seung-Heon Hong works as a Professor at the Department of Oriental Pharmacy, College of Pharmacy, Wonkwang University, Iksan, Korea. Since 2005, he has been an Editor of *Oriental Pharmacy and Experimental Medicine* and an Editorial Board Member of *Evidence-based Complementary and Alternative Medicine*. His research interest is to investigate pharmacological effect of herbal medicine on cancer, allergic inflammation and obesity.

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Notes:

Sun Park et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Regulation of TIM-3 expression in T cells by tumor-conditioned media

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Tcell immunoglobulin- and mucin-domain-containing molecule-3 (TIM-3) is well known as one of the immune check point molecules. TIM-3 expression is increased on exhausted T cells and senescent T cells in numerous immune diseases including cancers. However, the regulatory mechanisms of TIM-3 expression in cancers have not been well studied. Using Jurkat T cells, we examined TIM-3 regulatory mechanisms in condition similar to tumor microenvironment. TIM-3 mRNA and protein levels were increased by co-culture of Jurkat T cells with tumor cell lines and by incubation of them in tumor cell conditioned media. Given that cyclic adenosine monophosphate (cAMP) can be transferred from tumor cells to T cells, we examined the effect of cAMP signaling on TIM-3 expression. It was promoted by intracellular elevation of cAMP concentration and activation of cAMP downstream pathways. Further, inhibition of cAMP downstream pathway attenuated TIM-3 expression in Jurkat T cells cultured in tumor-CM as well as in Jurkat T cells stimulated with a cAMP elevating agent. Conclusively, this study suggests that TIM-3 expression in Jurkat T cells may be induced by tumor CM through activation of cAMP pathway.

Biography

Immune regulation has important roles in various immune diseases. The authors have studied the regulatory mechanisms and function of TIM-3 in various cells and in an *in vivo* tumor model. The authors revealed the involvement of MEK and c-jun in TIM-3 expression by CD4+ T cells. Additionally, they reported that the efficacy of tumor vaccine can be up-regulated by TIM-3 pathway blockade and the IL-2 production is decreased in CD4+ T cells expressing TIM-3 through NFAT dephosphorylation and AP-1 transcription.

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Notes:

Young Sang Kim, J Clin Cell Immunol 2017, 8:3(Suppl)
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Ectopic expression of the membrane-bound form of IL-17A promotes the growth and tumorigenicity of cancer cells

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Interleukin-17A is a member of the IL-17 family, and is known as CTLA8 in the mouse. It is produced by T lymphocytes and NK cells and has proinflammatory roles, inducing cytokine and chemokine production. However, its role in tumor biology remains controversial. We investigated the effects of locally produced IL-17A by transferring the gene, encoding it into mouse tumor cells including B16 melanoma, and MethA fibrosarcoma, either in a secretory or a membrane-bound form. Expression of the membrane-bound form on CT26 colon cancer cells dramatically enhanced their proliferation in *in vitro*. The enhanced growth was shown to be due to an increased rate of cell cycle progression. After synchronizing cells by adding and withdrawing colcemid, the rate of cell cycle progression in the cells expressing the membrane-bound form of IL-17A was much faster than that of the control cells. Both secretory and membrane-bound IL-17A induced the expression of Sca-1 on the cancer cells, which is commonly associated with aggressive phenotype of cancer cells. When tumor clones were grafted into syngeneic BALB/c mice, the tumor clones expressing the membrane-bound form IL-17A grew rapidly; those expressing the secretory form also grew faster than the wild type CT26 cells, but slower than the clones expressing the membrane-bound form. These results indicate that IL-17A promotes tumorigenicity, in part, by enhancing cell cycle progression. This finding should be considered in treating tumors and immune-related diseases.

Biography

Young Sang Kim is a Professor in Biochemistry Department in Chungnam National University, finished his PhD at University of Illinois at Chicago and continued Post-doctoral Research at Yale University for 2 years. His research interests focus is to develop a strategy for selective activation of tumor associated antigen (TAA)-specific cytotoxic T lymphocytes. He evaluates anti-tumor effect of tumor cell vaccines engineered to express cytokines on tumor cell surface as a membrane-bound form instead of the secretory form. In this way, he expects that the membrane-bound form of cytokine on tumor cells may function as a co-stimulatory molecule to TAA-specific cytotoxic T lymphocytes. He has published more than 70 scientific papers in the last 20 years.

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Notes:

Sung-Joo Park et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Anti-inflammatory effect of guggulsterone on inflammatory responses through ROS-HO-l axis

Sung-Joo Park, **Gi-Sang Bae** and **Seung-Heon Hong**
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Guggulsterone (GS) is a phytosterol that has been used to treat inflammatory diseases such as colitis, obesity and thrombosis. Although many previous studies have examined the anti-inflammatory activities of GS, the specific effect and mechanism of GS on lipopolysaccharide (LPS)-induced inflammation and endotoxemia have not been examined. Therefore, we investigated the anti-inflammatory action of GS on LPS-induced inflammatory responses on murine peritoneal macrophages and in septic mice. In the mouse endotoxemia model, GS prolonged survival and inhibited inflammatory mediators such as interleukin (IL)-1β, IL-6 and tumor necrosis factor-α (TNF-α) and also inhibited the organ injury. In murine peritoneal macrophages, GS significantly inhibited production of nitric oxide (NOS) and COX-derived prostaglandin PGE2, as well as IL-1β and IL-6 and TNF-α. Furthermore, the anti-inflammatory activities of GS were mainly through heme oxygenase-1 (HO-1) induction, which was mediated by GSH depletion and ROS production from endoplasmic reticulum (ER). The ROS mediated by GS caused the phosphorylation of GSK3β (ser9/21) and p38 and leads to translocation of nuclear factor (erythroid-derived 2)-like 2 (NRF2) which ultimately could induce HO-1. These results suggest that GS exerts anti-inflammatory responses through ROS -HO-1 axis.

Biography

Sung-Joo Park is a Professor of Wonkwang University in South Korea, and majored in Korean Traditional Medicines and Immunology. He has many focuses on inflammatory diseases such as pancreatitis, sepsis, obesity and asthma. He has many experimental models and molecule detection techniques to examine the pathophysiology and possible drug of the diseases. Recently, he mostly studied about acute pancreatitis and sepsis and reported many papers about them.

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Notes:

Amare Aregay et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Lack of full re-constitution of exhausted HCV-specific CD8+ T cells following IFN-free DAA therapy is partially reversed upon immune check-point inhibitions during chronic HCV

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Hepatitis C virus (HCV) persists and sets-up chronicity in majority of infected patients. Fortunately, new IFN-free direct-acting antiviral (DAA) therapies resulted in rapid and sustained clearance of HCV from infected patients. However, the impact of HCV clearance on HCV-specific CD8+ T cell responses remain yet to be understood. Owing to the rapid cessation of HCV replication and ensuing abrupt clearance of viral antigens mediated by IFN-free DAAs, we aimed at investigating the possible repercussions thereof on exhausted HCV-specific CD8+ T cells during chronic hepatitis C. We could show, by employing multimer-based magnetic bead enrichment technique that unlike activation markers that increased, *ex-vivo* surface expressions of co-regulatory markers remain unaffected following HCV clearance. Upon 10 day peptide stimulation *in-vitro*, the overall frequency of dextramer positive CD8+ T cells increased from baseline to 24 weeks after treatment in patients without advanced liver disease despite the fact that majority (55%) of patients did not show increase in proliferation. Meanwhile, HCV-specific CD8+ T cells proliferative capacity was not restored in patients with advanced liver disease. In addition, cytokines secretion and degranulation of HCV-specific CD8+ T cells remain unaffected following HCV clearance. Importantly, however, blockade of PDL1 pathway as well as PDL1/TIM3 double blockade resulted in enhanced proliferation and cytokine secretion by HCV-specific CD8+ T cells after IFN-free DAA therapy. Interestingly, HCV-specific CD8+ T cells that did not show increase in proliferation upon peptide stimulation alone could preferentially increase their proliferation and cytokine secretion upon blockade of PDL1 pathway. Taken together, our data implies that despite rapid HCV clearance, IFN-free DAA therapy does not fully re-constitute the altered phenotype and function of HCV-specific CD8+ T cells in chronic HCV. However, combining PDL1 or PDL1/TIM3 blocking therapy with IFN-free DAA therapy might possibly confer a functional and protective virus-specific CD8+ T cell response against re-infection.

Biography

Amare Aregay is currently pursuing his PhD at the Department of Gasteroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany under supervision of Professor Dr. Heiner Wedemeyer. He completed his Master's degree from Wageningen University Research Center. His current PhD work focuses on Cellular Immune Response (specifically T and NK cell response) towards chronic HCV infection in the context of IFN-free DAA therapy and liver transplantation.

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Notes:

Gerard Pasternak et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Analysis of factors affecting the differences between total IgG and sum of the IgG subclasses in children with suspected immunodeficiency

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Statement of the Problem: Deficits in disorders of humoral immunity associated with a deficit of antibodies are the most common primary immunodeficiency. In the case of patients with primary and secondary immunodeficiencies, the total IgG and IgG subclasses measurements are used in patients diagnosis. Results of measurements show the discrepancy between the sum of IgG subclasses (IgGsum) and total IgG The purpose of this study is to analyze the influence of gender, age and IgG abnormality on the value of this discrepancy.

Methodology & Theoretical Orientation: The group of patients was 648 children (aged 6 months to 18 years) referred to the Department of Clinical Immunology and Pediatrics for the purpose of diagnosis of immune disorders. For all children, measurements of the total IgG and the IgG subclasses (IgG1, IgG2, IgG3, IgG4) were conducted. The group of patients was divided into subgroups according to gender, age (under 6 years of age, in the age range between 6.5 and 12 years and in the age range between 12.5 and 18 years) and IgG abnormality (below the normal range, normal and above the normal range).

Findings: Statistical analysis (Kruskal–Wallis test) indicated that the all three parameters (age, gender and IgG abnormality) have a statistically significant effect on discrepancy between the IgGsum and total IgG. The higher average value of discrepancy between the IgGsum and total IgG was recognized in female group. The percentage of patients with IgG greater than IgGsum decreases as the age increases. The average value of discrepancy between the IgGsum and total IgG is highest for the group of age between 12.5 and 18 years. In the group of patients with normal IgG, the average value of discrepancy between the IgGsum and total IgG is lower than for children with IgG abnormality.

Biography

Doctor Gerard Pasternak graduated from the Medical University Wroclaw in 2008. Since 2015 he has been the assistant 3rd Department and Clinic of Paediatrics, Immunology and Rheumatology of Developmental Age, Wroclaw Mediacal University and the Department of Immunology and Pediatrics, Provincial Hospital J. Gromkowski, Wroclaw (since 2010). He serves as a didactic assistant professor in the Department. Participates in conducting activities in the field of primary immunodeficiency for students of III-VI of the year, and takes part in the preparation and conducting of workshops for patients with PID and their families. Professional interests and research are concentrated on the diagnosis and treatment of primary and secondary immunodeficiencies, with particular emphasis on deficit of the IgG subclasses.

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Notes:

Seong Kug EO et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Distinct upstream role of type I IFN signaling in hematopoietic stem cell-derived and epithelial resident cells for concerted recruitment of Ly-6C^{hi} monocytes and NK cells via CCL2-CCL3 cascade

Seong Kug Eo, Jin Hyoung Kim, Ajit Mahadev Patil, Jin Young Choi, Seong Bum Kim and **Erdenebileg Uyangaa**
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Type I interferon (IFN-I)-dependent orchestrated mobilization of innate cells in inflamed tissues is believed to play a critical role in controlling replication and CNS-invasion of herpes simplex virus (HSV). However, the crucial regulators and cell populations that are affected by IFN-I to establish the early environment of innate cells in HSV-infected mucosal tissues are largely unknown. Here, we found that IFN-I signaling promoted the differentiation of CCL2-producing Ly-6C^{hi} monocytes and IFN-γ/granzyme B-producing NK cells, whereas deficiency of IFN-I signaling induced Ly-6C^{lo} monocytes producing CXCL1 and CXCL2. More interestingly, recruitment of Ly-6C^{hi} monocytes preceded that of NK cells with the levels peaked at 24 h post-infection in IFN-I–dependent manner, which was kinetically associated with the CCL2-CCL3 cascade response. Early Ly-6C^{hi} monocyte recruitment was governed by CCL2 produced from hematopoietic stem cell (HSC)-derived leukocytes, whereas NK cell recruitment predominantly depended on CC chemokines produced by resident epithelial cells. Also, IFN-I signaling in HSC-derived leukocytes appeared to suppress Ly-6G^{hi} neutrophil recruitment to ameliorate immunopathology. Finally, tissue resident CD11b^{hi}F4/80^{hi} macrophages and CD11c^{hi}EpCAM^{+} dendritic cells appeared to produce initial CCL2 for migration-based self-amplification of early infiltrated Ly-6C^{hi} monocytes upon stimulation by IFN-I produced from infected epithelial cells. Ultimately, these results decipher a detailed IFN-I-dependent pathway that establishes orchestrated mobilization of Ly-6C^{hi} monocytes and NK cells through CCL2-CCL3 cascade response of HSC-derived leukocytes and epithelium-resident cells. Therefore, this cascade response of resident-to-hematopoietic-to-resident cells that drives cytokine-to-chemokine-to-cytokine production to recruit orchestrated innate cells is critical for attenuation of HSV replication in inflamed tissues.

Biography

Seong Kug EO's lab has focused on unveiling how hosts response to pathogen infection. They have used various infectious models to prove host responses upon pathogenic infection. In recent, EO's lab has found the detailed pathway that IFN-I signal pathway orchestrated environments to provide effective protection against mucosal viral infection (PLoS Pathog., 2016). Moreover, EO's lab is expert on viral acute encephalitis caused by flaviviral infection. They have got many reports to unveil how immune system works on viral encephalitis caused by Japanese encephalitis virus (J. Neuroinflammation, 2014 and 2016).

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Notes:

Kriegova E et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Serum protein pattern associated with severe manifestations of SLE: Organ damage and lupus nephritis

Kriegova E, Petrackova A, Smrzova A, Schubertova M, Schneiderova P, Dyskova T, Skacelova M, Mrazek F and **Horak P**
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Statement of the Problem: Systemic lupus erythematosus (SLE) is a remarkably heterogeneous autoimmune disease. The greatest challenges remain in management of organ damage and lupus nephritis (LN), one of the most feared phenotypes in SLE. Despite tremendous effort, our knowledge on serum protein pattern in this severe SLE manifestation is still limited. The purpose of this study was to investigate the serum protein pattern of organ damage and LN in SLE using a highly sensitive multiplex Proximity Extension Immunoassay (PEA) on 92 inflammation-related proteins.

Methodology & Theoretical Orientation: We enrolled 75 Czech patients with SLE, subgroups were formed according to the organ damage (assessed by the SLICC/ACR damage index, SDI) (SDI≥1, n=42; SDI=0, n=33) and biopsy-proven presence of LN (no LN, n=48; LN, n=27). The serum levels of 92 inflammation-related proteins were assessed by PEA (Proseek Multiplex, Olink Bioscience, Sweden). Statistical tests (Student t-test, Benjamini-Hochberg correction) were performed using GenEx (Sweden), p-value≤0.05 was considered as significant.

Findings: SLE patients with organ damage had elevated serum levels of IL-8, CCL2, IL-6, CCL11, FGF21, MMP10, IL-18, CCL3, FGF5 and FGF23 comparing to those without organ damage. Of these, enhanced levels of CCL11, MMP10s and CCL2 were informative for identification of patients with organ damage. Importantly, IL-8 and MMP10 also correlated with disease activity ($r \leq 0.355$, $p \leq 0.002$). Comparing patients with/without LN, elevated levels of CSF1, sCX3CL and GDNF, sCD40 ($P_{corr} < 0.05$) were detected and showed usefulness in prediction of this severe SLE manifestation. Although all upregulated proteins correlated with disease activity, the best correlation was observed for sCX3CL1 and GDNF ($r \geq 0.403$, $p \leq 0.0003$).

Conclusion & Significance: This highly sensitive PEA analysis identified serum pattern of organ damage and LN, with many novel candidate proteins detected. Their exact role and suitability as biomarkers in SLE deserves further investigation.

Biography

Dr. Eva Kriegova is an immunologist at the Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic. She is leader of Molecular Immunology group and her research interest is focused on molecular mechanisms of immune processes ongoing in inflammatory lung disorders and autoimmune diseases, hematological malignancies and orthopaedic disorders.

Anna Petrackova is a PhD student of Immunology at Palacky University Olomouc, Czech Republic. Her research is focused on gene expression studies of inflammatory mediators in autoimmune disorders and hematological malignancies.

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Notes:

Hwajung Kim et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Significantly increase of memory T-cells in rejected liver allografts in rhesus monkeys

Hwajung Kim[1], Kyung-Suk Suh[1], Hyeyoung Kim[1], Sun-Kyung Lee[1], Xue-Li Jin[1], Tae Jin Kim[2], Jae-Il Lee[1], Hyo-Sin Kim[1], Suk Kyun Hong[1], Kyoung-Bun Lee[1], Wayne Hawthorne[3], Nam-Joon Yi[1], Jaeseok Yang[1] and **Kwang-Woong Lee[1]**

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Introduction: In kidney, heart and islet transplantation the rhesus monkey (*Macaca mulatta*, RM) has been shown to be an excellent preclinical model that can provide the basis for new immunosuppressive protocols for clinical studies. However, there remain relatively few liver transplant (LT) models in nonhuman primates. In this study, we analyzed the immune cell populations of PBMC and secondary lymphoid organs along with livers of normal rhesus monkeys and compared them to those of rejecting liver transplanted recipient's following withdrawal of immunosuppression.

Methods & Results: We undertook six allogeneic ABO compatible orthotopic LT in monkeys using six normal donor monkey livers. We collected tissues including lymph-node, spleen and blood from which we isolated immune cells for FACS analysis along with the liver from the recipient. We found that CD4 or CD8 naïve T cells were normally seen at low levels (13.89±8.67 or 1.50±1.44 respectively) and memory T cells were seen at high levels (76.12±11.40 or 98.0±1.60) in the liver rather than lymphoid organs or PBMC. However, regulatory cells such as CD4+FoxP-3+ T cells and CD8+CD28- cells remained in high numbers (0.77±0.54 and 34.99±6.40) in the liver but not in lymph node or PBMC. These results demonstrate that the liver has rather unique immunological properties compared to other organs. We also compared CD4/8 T sub-populations in normal or rejected livers and the various tissues showed that naïve cells were dramatically decreased in spleen, lymph node and PBMC of rejected transplanted monkeys but rather their memory cells were increased in all tissues and PBMC.

Conclusion: We have shown that the normal liver has large numbers of C4Tregs or CD8+CD28- or MDSC which are the known immune suppressive cells at much higher levels than other lymph node or peripheral blood. Memory T cell populations in rejected livers or lymphoid organs were expressed at significantly higher levels than those seen in normal tissues including as seen in the peripheral blood.

Biography

During the course of my Ph.D. I studied therapeutic anti-inflammatory effects of human mesenchymal stem cells on traumatic brain injury and studied the role of stem cells in human brain tumor development using SD-rat model. As a post Doc my focus was to study therapeutic strategies towards successful xenotransplantation. I was involved in two main projects related to the development of the first pre-clinical nonhuman primate study of solid organ xenotransplantation. This was done using genetically engineered pigs expressing multiple human complement and coagulation regulatory proteins in order to overcome the immunological and physiological barriers against successful xenotransplantation.

Kyung-Suk Suh is a Post-doctoral Fellow and focuses on the study of therapeutic strategies towards successful xenotransplantation. He was involved in two main projects (supported by the government) related to the development of the first pre-clinical nonhuman primate study of solid organ xenotransplantation in Korea. This was done using genetically engineered pigs expressing multiple human complement and coagulation regulatory proteins in order to overcome the immunological and physiological barriers against successful xenotransplantation.

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Notes:

Wenming Zhao et al., J Clin Cell Immunol 2017, 8:3(Suppl)
DOI: 10.4172/2155-9899-C1-037

The humanized antibody h8G12 prevented arthritis through targeting both TNF-α and RANKL in DBA/1 monoarthritic mice

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Statement of the Problem: The TNF-α and RANKL are the key pathogenic factors in the onset and progression of rheumatoid arthritis (RA). TNF-α inhibitors have shown good clinical therapeutic effects of relief inflammation and joint swelling, but single application of TNF-α agents have a relatively weak protection from cartilage and bone destruction. RANKL are largely produced from inflamed synovium and cause activation of osteoclasts during the bone remodeling cycle, and an anti-RANKL antibody denosumab possesses a potential to inhibit joint destruction as well as systemic osteoporosis. The purpose of this study is to prepare humanized bispecific antibody (h8G12) targeting both TNF-α and RANKL and to evaluate its therapeutic effects on arthritis.

Methodology & Theoretical Orientation: The h8G12 was produced from co-transfected Chinese hamster ovary (CHO) cells. The identification, purification and characterization of h8G12 were detected by SDS-PAGE, Western blot and indirect ELISA. To evaluate its therapeutic effects, the monoarthritis model mice were prepared though intra-articular injection of rhTNF-α and rhRANKL.

Findings: The confluence rate of co-transfected CHO cells reached 80% at about 48 h after resuscitation. The concentration of h8G12 antibody in supernatant was kept at a steady state at 96 h after cell passaging. HE staining showed that h8G12 significantly inhibited more than 50% inflammatory cell infiltration in the joint cavity, peripheral soft tissue and bone marrow. Destruction of cartilage in h8G12-treated mice was significantly lower than that in positive control group. Interestingly, the joint structure and the thickness of articular cartilage of the mice in treated group had no significant difference with those in normal ones. The h8G12 inhibited the differentiation of osteoclasts and significantly decreased the number of osteoclast-like cells.

Conclusion & Significance: The h8G12 ameliorated inflammation and bone destruction through targeting TNF-α and RANKL. The h8G12 may be a good candidate for inflammatory bone diseases.

Biography

Wenming Zhao has focused on researching the therapeutic strategies and mechanisms in rheumatoid arthritis (RA). His major findings are biological therapies for collagen-induced arthritis (CIA) including OPG recombinant protein, RANKL-TNF homologous vaccine and humanized antibody. He also devotes to discover the mechanisms of Ahr signal pathway in joint bone destruction. This study investigated the effects of humanized antibodies h8G12 on relief of bone destruction.

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Notes:

Xulong Zhang et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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NK cells aggravate acute lung injury via up-regulation of NKG2D during early stage of H1N1 influenza infection

Xulong Zhang, Bo Liu, Yunqing An, Wei Deng, Bin Cao and **Chen Wang**
Capital Medical University, China

Acute lung injury was considered as the major pathological contribution of 2009 pandemic H1N1 influenza virus infection. NK cells were the first line to defend against virus infection, but their roles in the lung pathogenesis and virus elimination were not fully elucidated. The influenza infection model was established with Balb/c mice. The flow cytometry was used to detect the expression of extracellular and intracellular molecular. H&E straining was done to evaluate the pathological lession of lung. Severe Balb/c mice infection model was intranasally inoculated with influenza A virus strain A/California/07/2009. Following virus challenge, the body weight was lost and survival rate decreased. The infected lung showed severe lung injury, including pulmonary edema and capillary leak, and a large number of infiltrating lymphocytes were recruited to perivascular and parenchyma areas in mice model and patients. Total lymphocytes in lung and bronchoalveolar lavage fluid (BALF) were increased as the infection progressed, and the ratio and number of NK cells was significantly increased. But the ratio of T cells in lung had no change. Furthermore, NK cells were rapidly activated, and secreted a large amount of IFN-γ and increased high level of perforin and granzyme B. H1N1 infection induced significant high expression of NKG2D, but not NKG2A, on NK cells. NKp46, which can recognize virus HA, was also improved. Meanwhile, H1N1 infection induced significantly high expression of NKG2D ligands (RAE-1) and low expression of NKG2A ligands (Qa-1α). Depletion of NK cells with AsGM1 show lighter lung damage and weight loss, but higher virus titer compared with PBS control for the first three days after infection, accompanying with reduced secretion of IFN-γ. Our data demonstrated NK cells played dual roles in lung injury and virus elimination during the early stage of H1N1 virus infection.

Biography

Xulong Zhang received his MD degree from Shandong University and completed his Post-doctoral Training at USTC. Now, he is focusing on the innate immune cells or molecular medicated protection or damage after influenza virus infection.

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Notes:

8TH EUROPEAN IMMUNOLOGY CONFERENCE

June 29-July 01, 2017 Madrid, Spain

e-Posters

Pushkala Subramanian, J Clin Cell Immunol 2017, 8:3(Suppl)
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Immunological and molecular characterization of hepatitis B virus in asymptomatic voluntary blood donors

Pushkala Subramanian
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It is mandatory to test each donor's blood for syphilis by a VDRL, and for HBsAg, anti-HCV, and anti-HIV. In July 1989, consequent to the reports of high seroprevalence in commercial blood donors, mandatory screening of blood and blood products for HIV antibodies was initiated by Indian NACO. The first objective of this study is to estimate the seroprevalence of TTIs among voluntary blood donors at Chennai. This knowledge might give us the idea of disease burden of the society and the basic epidemiology of these diseases in the rural community. Hence in the current study , more importance has been given to explore more on the role of the other serological markers as well as molecular HBV markers that could serve as an surrogate marker in detecting the hidden HBV so that transfusion transmitted HBV is lowered considerably

Objectives of the Study: The first objective of this study is to explore the current sero-prevalence of HBsAg, anti-HCV and anti-HIV among the voluntary blood donors by the routine rapid assays (card test) and by the ELISA methods. The second objective is to explore the real efficiency of NAT tests in terms of increased sensitivity in identifying the potential pathogens in voluntary blood donors who are found negative by the regular serological assays used by the blood banks for the routine screening. The third objective of this study is to compare the positivity of HBV, HCV and HIV by both the serological markers (carried out by the routine card test and ELISA method tests) and by the NAT (using the molecular markers) in voluntary blood donors. The role of HBV seromarkers in identifying the HBV disease status in HBsAg positive cases – to study the pattern of HBV serological profile for identifying the stage of HBV stage in cheronic HBV infected subjects among voluntary blood donors who are asymptomatic and also identify the cryptic cases. To identify the nonresponders in vaccinated population amongst the blood donors who are HBsAg positive. To study the relevance of the seromarkers and HBV viral load to establish the fact that inclusion of one or more seromarkers in routine blood screening would be beneficial. To estimate the liver function test to determine the extent of damage of liver. To study the HLA pattern in hosts affected by the virus to study the occurrence of the protective gene that helps host to remain asymptomatic but they would be carriers.

Results: Out of 3160 voluntary blood donors 126 were found to be positive for HBsAg (3.9%), 2 were found positive for HCV and 2 were found positive for HIV by the routine card test. For the detection of HCV and HIV, NAT was employed and it was able to pick-up the cases that were picked up by the routine card test, it was not able to pick-up any new additional positives. Through the routine card test only126 samples were found to be positive for HBsAg, but from the same group ELISA was able to pick up 6 more HBsAg positive cases. In addition NAT picked up two more positives from the same group of 3160 voluntary blood donors. So through the ELISA the HBsAg prevalence was found to 4.1% (by picking up 6 additional positive cases). And with the help of the NAT the HBV prevalence was found to be 4.2% (by picking up two more HBV positives). And by employing the NAT for the detection of HBV-DNA the prevalence was found to be 4.2% (by picking up two more HBV positive cases which were missed by the regular Card test and ELISA). 134 voluntary blood donors were found positive for HBsAg, Out of the 134 HBsAg positive, 79 were in the age group of 18 to 38 years, and the rest 55 were in the age group of 39-59 years. The HBsAg positive group consisted of 89% male and 10.5% females. 71.7% of them were graduates and 28.3 % were illiterates.

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Aliya N. Tokusheva et al., J Clin Cell Immunol 2017, 8:3(Suppl)
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Vanadium and chromium mediated impairments in the immunological reactivity of rats with aseptic inflammation

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Chronic inflammation is a consequence of the immune system dysfunction. Such dysregulation of the immune response may induce chronic diseases, such as autoimmune diseases, diabetes, and malignant transformation of cells. Xenobiotics, including vanadium and chromium, were shown to induce inflammatory changes leading to chronic inflamation. The purpose of this research was to study the process of aseptic inflammation accompanied with intoxication with salts of heavy metals. In our study, sexually mature rats were administered aseptic inflammation (AI) alone or ammonium vanadate and potassium dichromate (AV/PD) at a dose of 5 mg/kg of BW for two weeks and after that aseptic inflammation was modeled. Lymphatic organs were studied on day 1, 7, and 14 after the onset of aseptic inflammation. Administration of AV/PD and AI resulted in structural changes in lymphatic organs and anemia observed throughout the experiment. We observed a decrease in the cellularity of the bone marrow and thymus, ratio of thymic cortex to medulla, and dystrophic changes in thymic cells and their scarcity. Also, we detected increased levels of anti-inflammatory cytokines in serum: IL-10 on day 1 and TGF-β on day 7 and 14 after the beginning of the experiment in the group AI+AV/PD comparing to intact rats and AI group. Phenotypical analysis demonstrated that by the end of experiment freshly obtained splenocytes of AI and AI+AV/PD rats contained increased percentage of $His48^{+}CD11b/c^{+}$ and $His48^{high}CD11b/c^{+}$ cells, and decreased number of induced $CD3^{+}CD4^{+}IFN\gamma^{+}$, $CD3^{+}CD4^{+}IL\text{-}4^{+}$ and $CD3^{+}CD8^{+}$ cells, comparing to control animals. Interestingly, during the next period of the experiment, we observed significant decrease of induced $CD3^{+}CD4^{+}IFN\gamma^{+}$, $CD3^{+}CD4^{+}IL\text{-}4^{+}$ and $CD3^{+}CD8^{+}$ cells in the AI+AV/PD group comparing to AI group.

Thus, it is possible that an elevated sera IL-10 and TGF-β1 observed in mice with chronic inflammatory processes administered with AV/PD result of abundant accumulation of $His48^{+}CD11b/c^{+}$ and $His48^{high}CD11b/c^{+}$ cells myeloid cells in the periphery, and, in turn, it could participate in the maintaining of the immunosuppressive environment that supports persistence of chronic inflammatory conditions. Therefore, intoxication with vanadium and chromium salts may support immunosuppressive environment, contributing to chronic inflammation development.

Biography

Aliya Tokusheva is a PhD student at the Asfendiyarov Kazakh National Medical University. She studies molecular mechanisms of epigenetic regulation of tissue-specific expression of genes, revealing the effect of heavy metal compounds on the variability of the genome of immunogenesis organs and the mutual regulatory influence of important links of these mechanisms on the course of the inflammatory process.

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Notes:

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Exogenous truncated IK protein ameliorates inflammatory arthritis by HIF-1α induced A20

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IK protein was first isolated from the cultured media of K562, leukemia cell line. It is known as an inhibitory regulator of constitutive and interferon-γ-induced major histocompatibility complex (MHC) class II expression. Previously, we found the reduction of pathogenic Th17 cells that have been known to be involved in the development of rheumatoid arthritis (RA), in polarizing condition in the truncated IK (tIK)-transgenic (Tg) mice as compared with that in the wild type (WT) Balb/c mice. Based on this finding, we investigated the therapeutic effect and protection mechanism of exogenous tIK protein produced by insect expression system for the RA mouse disease model (collagen antibody-induced arthritis, CAIA). Injection of tIK protein alleviated the symptoms of RA and reduced Th17 cell population in the CAIA model. Interestingly, the computer modeling structure of IK protein is similar to IL-10 structure. It can be speculated that tIK protein may belong to the IL-10 protein family. In addition, treatment of tIK protein on cultured T cells induced A20, as a negative regulator in NFκB pathway, through hypoxia-inducible factor-1α (HIF-1α) and reduced several transcriptional factors related to T cell activation. Based on these results, we suggest that tIK protein has a potential to act as a new therapeutic agent for RA patients, because it has a different mode of action as compared with the currently used biologics for RA, such as monoclonal antibody drugs.

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Investigation of IL-12B gene polymorphism (rs3212227) in Iranian patients with Alopecia areata

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Objective: Alopecia areata (AA) is an autoimmune disease characterized by patchy hair loss affecting both scalp and body hair. Although the etiology and pathogenesis of this disease is still unknown, a polymorphism within IL-12B gene have been described in few studies to be associated with AA susceptibility. Yet, these findings had so far not been independently replicated, and no data on a possible association of IL-12B mutation and AA in Iranian population were available.

Methods: This study contains 30 AA patients and 15 healthy controls. Genomic DNA was isolated using DNG-plus and PCR-RFLP analysis was performed to detect IL-12B rs3212227 polymorphism. Several relevant information such as demographic data (age, gender, …) or clinical characteristics were analyzed for a possible effect of these factors on susceptibility to AA in patients who carry CC, AC, and AA genotypes.

Results: No association between the IL-12B rs3212227 mutation and susceptibility to AA was observed in our Iranian cohort. PCR-RFLP results showed that frequency of CC genotype (13.3% vs. 6.6%) are similar in both patient and control groups. AC genotype was detected in 46.6% and 6.6% of patients and controls, respectively. The AA genotype which is wild genotype had higher frequency in healthy individuals. Statistical analysis indicate that there no significant difference in distribution of genotypes between patients and controls (P= 0.12). Although the C allele frequency of IL-12B was higher in the patients than control subjects (36.6% vs. 10% respectively) but there is no significant difference (P= 0.12).

Conclusion: We here demonstrate that the IL-12B rs3212227 polymorphism is not associated with the risk to develop AA in our Iranian cohort. Therefore, this study failed to confirm reported association between gene mutation and susceptibility to AA. Hence, the genetic predisposition to develop AA greatly varies among different ethnic groups.

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Validation of *Candida* colonization associated with anamnestic response of anti-enolase IgG at early stage of invasive candidosis by memory B-cell ELISPOT

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Objective: The rapid elevation of IgG antibody against *Candida* enolase has been observed in the patients at early stage of invasive candidosis. The aim of the study was to build a comprehension of the rapid increase of specific IgG titers against dominant antigens (such as enolase) at the early stage of invasive *Candida* infection resulted from humoral immune anamnestic response associated with previous *Candida* colonization.

Methods: The oral *Candida* colonization mouse model was established, and the immuno-competent and immuno-compromised *Candida* colonized mice were challenged by intraperitoneal injection of *Candida* spore. The numbers of enolase-specific memory B-cells in the spleen were measured by ELISPOT and compared with the levels of specific IgG, IgM and IgA antibodies in the peripheral blood.

Results: The burst of enolase-specific memory B cells was detected in both immuno-competent and immuno-compromised mice at day 7 post-invasive infection; which was followed by a strong increase in specific antibody titre in the *Candida* colonized mice. The Eno-IgG antibody was positively correlated with the antigen specific Bm ($r=0.737$, $P<0.01$).

Conclusion: It was confirmed that the *Candida* colonization associated with anamnestic response of IgG against dominant antigen at early stage of invasive candidosis and the rapid elevation of specific-IgG would suggest a diagnosis of invasive infection.

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The quinoline-3-carboxamide paquinimod prevents development of diabetes in the non-obese diabetic (NOD) mouse

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The NOD mouse spontaneously developed type 1 diabetes (T1D). At the age of 3-4 weeks, there was detectable infiltration of mononuclear cells in the pancreatic islets of Langerhans of these mice. This process known as insulitis, causes selective cell death of the insulin producing β-cells in the islets. Female NOD mice displayed severe insulitis at about 15 weeks of age and developed hyper-glycaemia at around 15-30 weeks of age. We have previously shown that the immunomodulatory compound paquinimod can reduce the influx of monocytes to sites of inflammation. Since monocyte-derived macrophages are known to be involved in pathogenesis in NOD pancreas, we have in here investigated the impact of paquinimod treatment on the development of T1D in the NOD mouse. In cohorts of mice treated between weeks 10 to 20 of age and followed up until 40 weeks of age, we observed dose-dependent reduction of incidence of disease as well as delayed onset of disease. Further, in mice treated with paquinimod from 15 weeks of age, most of the treated mice had not developed glycosuria at 30 weeks of age and displayed strongly reduced insulitis. Importantly, in these treated mice there were significantly more non-infiltrated islets than in untreated controls. Collectively, these data indicate that paquinimod treatment inhibits progression of insulitis to overt diabetes in the NOD mouse.

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Activation of skin and lymph nodes antigen-presenting cells induced by *Salmonella typhi* porins

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Introduction: *Salmonella typhi* (S. *typhi*) porins are important targets of the mice and human's immune protective immune response, and are also potent immunogens capable of generating life-lasting bactericidal antibodies in mice. Mechanisms involved in this atypical antibody response remain understood. We report the activation, migration and T cell activation induction capacity of antigen-presenting cells (APC) in skin and lymph nodes in mice.

Methods: Mice were immunized intradermally with porins. Epidermis of the skin was obtained 12 h post-immunization and stained with MHC-II, CD86, CD40 and PD-L1. Tissue sections were analysed by confocal microscopy. Cervical lymph nodes were obtained and prepared for a flow cytometry staining to identify dendritic cell subsets (resident and migratory) and its activation. The capacity of porin-activated APC to activate T cell responses was evaluated by co-immunising porins with inactivated *Sporothrix schenckii* conidia. Conidia specific memory T CD4+ cells in lymph nodes were analysed by flow cytometry and in skin by a delayed-type hypersensivity test.

Results: *S. typhi* porins induced a higher expression of MHC-II and CD40 in skin, in contrast, CD86 and PD-L1 expression were not increased. Porins induced an increased number of CD86+ cells in skin despite CD40+ and PD-L1+ cells were not increased. Porins induced an increased number of migratory dendritic cells in lymph nodes which had an activated phenotype. Conidia specific total T CD4+ cells, central memory T CD4+ cells and effector memory T CD4+ cells, were increased in lymph nodes by porins co-immunization. The cellular response in skin induced by conidia-porins was higher.

Conclusion: Intradermal immunization with *S. typhi* porins induced early activation of epidermal dendritic cells and recruitment of antigen-presenting cells to skin, also promoted migration of skin dendritic cells that are able to generate memory T CD4+ cells in lymph nodes and skin, inducing systemic immune responses.

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Majority of T cells, including Treg cells, NKT and γδ T cells are developed from $CD4^-CD8^-$ T progenitor cells without the involvement of $CD4^+$ $CD8^+$ stage in thymus

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We recently have detected higher levels of Foxp3 in DN cells from mice by developing a new method of flow cytometry. In this study, we examined the expression levels of cell markers in thymocytes that exhibited an obvious change during different developmental stages of T cells. We found many cells that expressed intracellular CD4, intracellular CD8 and intracellular $CD4^+$ $CD8^+$ in $CD4^-CD8$- DN cells. The highest expression level of CD25 was observed in $CD4^-CD8^-$DN cells, followed by $CD4^+CD8^-$SP, $CD4^+CD8^+$DP and $CD4^-CD8^+$ SP cells. The expression level of CD44 in DP cells was much lower than that in the DN cells, and also recorded for $CD4^+CD8^-$ and $CD4^-CD8^+$ cells. NKT cells and γδT cells were found in DN and SP cells, but not in DP cells. The highest expression level of Notch and CD117 were observed in DN cells, followed by SP and DP cells. Unexpectedly, intracellular CD3 was not only expressed in SP and DP thymocytes, but also in most of DN thymocytes at various stages. Contaminated cells in DN thymocytes could be removed by the intracellular CD3 gated, replaced with specific blocking antibodies. Our results suggested that T cells classification has been completed in the DN thymocytes stage. T cells, including γδ T cells, NKT and Treg cells may develop from DN T progenitor cells, but without the involvement of the $CD4^+CD8^+$ stage in the thymus. We present an effective, easy and accurate method that avoids interference of contaminated cells and does not require the use of blocking antibodies to remove contaminated cells.

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The effect of nano-particle size and chemistry on human dendritic cells

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Dendritic cells (DCs) are professional antigen presenting cells that play a key role in initiating immune responses and maintaining tolerance in response to different stimuli and under steady-state conditions. Given the crucial role of DCs in orchestrating immune responses, better understanding of mechanisms and conditions that control DCs function could provide opportunities for developing new treatment for infectious and autoimmune diseases. There is a growing interest in the use of nanoparticles (NPs) in drug delivery, vaccination and imaging; however, the impact of NPs on the immune system particularly the function and phenotype of DCs has remained elusive. The possibility to control size, shape, and other properties of NPs provides the opportunity to achieve immune modulation for immunotherapeutic applications through stimulation or suppression of immune responses. Herein, we hypothesized that NPs with the same size but different chemistries will differentially influence DC phenotype and function. To examine this, NPs with the similar size but different chemistries i.e. PLGA, Silica and polystyrene (PS) were fabricated or commercially sourced. DCs were then exposed to defined concentrations of NPs to study the effect of different NPs on DC phenotype, cytokine profile and endocytic ability. The data show that while spherical Silica and PLGA NPs in 100 nm and 160 nm size range respectively do not change any aspects of DC function, PS NPs of similar size significantly suppress the expression of mannose receptor (MR or CD206) on DCs by around 90% without affecting their viability, maturation status or cytokine profile. Not surprisingly the reduction in MR expression in these cells was also accompanied by reduced endocytic ability. Our data indicates that MR suppression is likely due to enhanced MR shedding in response to PS NPs. None of the NPs induced DCs maturation as evidenced by low CD83 expression. Future work will focus on better understanding of the mechanism underpinning such NP induced phenotypical and functional changes on human DC.

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Chorea and aphasia as a manifestation of SLE in an adolescent female: Case report

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Background: Systemic Lupus Erythematosus (SLE) is a chronic, inflammatory and autoimmune disease with multi-systemic involvement. Children represent 10-20% in the general population and neuropsychiatric symptoms have a prevalence of 17-70%.

Objective: Objective of the study was to describe the clinical presentation and neurological manifestations in a pediatric patient with SLE.

Methods: We present the case of a 15 year female with 3 week history of movement disorder, dysarthria and emotional liability. Laboratory and imaging studies were performed and a diagnosis of Sydenham's chorea was established. Following 4 weeks of treatment, she developed arthritis and aphasia.

Results: Radiologic evaluation did not show any disturbances, general laboratories were normal, immunological profile reported anti-nuclear antibodies with a title of 1:640, fine speckled pattern, and lupus anti-coagulant reported 2.18.

Conclusion: Diagnostic evaluation of SLE in children represents a challenge due to its clinical heterogeneity; the time related to adding signs during life and the wide severity of symptoms among pediatric population. Neurologic and psychiatric manifestations represent one common and severe evolution in SLE. Choreic movements occur in less than 5% of patients and may precede the accurate diagnosis for months and years, before the development of the whole clinical picture.

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Case with hypersensitivity pneumonia

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Introduction: Hypersensitivity pneumonia is an immunology based lung disease caused by antigens that are inhaled, characterized by peripheric airway lymphocytic infiltration and granulomatous lesions that surround interstitium. It is an allergic lung disease that develops as a result of the inhalation of organic dust. The most common form of this lung disease is pigeon fancier disease and it develops as a result of the inhalation of some kinds of organic antigens. Pigeon fancier disease is a hypersensitivity pneumonia that develops as a result of the stool of winged animals, serum and feather antigens. Not only pigeons in the first instance, but also the other winged animals cause hypersensitivity pneumonia. The most important thing is not only to diagnose the clinical findings and radiologic symptoms, but also to detect the exposure to such animals. Here, we are presenting the case that develops the pigeon fancier hypersensitivity pneumonia and the treatment carried out.

Case: 65 year male patient, a farmer consulted the clinic as a result of having cough and difficulty in breathing. He had suffered from these problems for two months. In physical examination, saturation via pulse oxymeter was %90. Bilateral rales were diagnosed in respiration. Blood analysis was non-specific and in lung graphy, rise in nonhomogenous density on bilateral lower and middle zone was detected. Respiration function test was seen as FVC 1.52 lt. %41 FEV1 1.52 %50 FEV1/FVC %129. Thoracic CT was carried out. It was seen that there were areas with distinct common ground glass density, in bilateral lower lobes in CT (Picture 1). It was seen that the patient had been a pigeon fancier for two months. The patient was diagnosed as having hypersensitivity pneumonia and 40 mg methyl prednisolone was given to him for treatment. He kept himself away from pigeons from that day on. The dose of steroid was reduced and finally at the end of the 4th month the patient was no longer given it. Via the control of thoracic CT of the patient, distinct recovery was detected on ground glass density areas. (Picture 2) After the treatment, distinct clinic recovery was seen.

Discussion: Hypersensitivity pneumonia develops as a result of the inhalation of organic dusts. Though the help of radiologic symptoms cannot be ignored, the exposure of the patient to such animals has an important role in diagnosing. It is suggested that the patients who are compatible with clinic and radiologic symptoms of hypersensitivity pneumonia, should also be inspected in terms of exposure to such animals.

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ERAP1-ERAP2 dimers trim MHC I-bound precursor peptides: Understanding peptide editing

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Statement of the Problem: The human endoplasmic reticulum amino peptidase 1 (ERAP1) and ERAP2 are critically important in the final processing of MHC class I antigens in the endoplasmic reticulum. To date, the molecular context of peptide trimming by ERAPs and how ERAPs shape antigen repertoires remains open questions.

Methodology: Using a cell-free system composed of ERAP1 and ERAP2 heterodimers (ERAP1/2), MHC class I molecules and N-terminally extended model and natural peptides, we characterized the function of ERAP1/2.

Findings: We provide evidence that ERAP1/2 trims MHC I-bound precursor peptides to the final lengths, albeit more slowly than the corresponding free precursors. We show that trimming of MHC I-bound precursors by ERAP1/2 increases the conformational stability of MHC I/peptide complexes.

Conclusion & Significance: Our study provides new findings on ERAP1/2 as a key antigen processing complex. From our data, we propose a molecular mechanistic model of ERAP1/2 as an editor of class I antigens. Understanding class I antigen processing is significant given the role that class I antigens play in the normal recognition of virally infected and transformed cells by CD8+ T cells.

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Controlling TH17 cells in inflammation and carcinogenesis

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Statement of the Problem: The incidence and prevalence of immune mediated inflammatory diseases (IMIDs) are steadily increasing. Unfortunately, most therapies used against these diseases have as of yet palliative character and mostly do not offer a cure. Thus, life-long treatment using immune-suppressive agents is required in the majority of cases. As a consequence, patients with IMID must live with the side effects of these treatments, such as increased risk of opportunistic infections and of relapsing flares of the disease itself. Furthermore, chronic inflammation, another possible side effect, can promote the development of certain forms of cancer. Therefore, there is major need for new therapies, which can modulate the immune response more specifically.

Theoretical Orientation: TH17 cells and their associated cytokines play an important but ambiguous role in these diseases. On one hand they play a key role in chronic inflammatory diseases and carcinogenesis. On the other hand, however TH17 cells and their cytokine products, such as IL-22, also have beneficial properties such as promotion of wound healing and defence against pathogens. This obviously reveals that it is crucial to find out the molecular and cellular mechanisms which physiologically control TH17 cells in order to not just simply deplete them, but rather reset them to their beneficial state. We have indeed already identified several mechanisms, which explain how TH17 cells and their cytokines can be physiologically controlled (Figure 1). Of note, the intestine plays a key role during this regulation.

Conclusion & Significance: Our data indicated that TH17 cells can be controlled in the intestine. Our aim is to furthermore understand the involved mechanism.

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Investigation of a T cell subset that constitutively signals via STAT1 activation and is unresponsive to JAK1 inhibition: A potential mechanism of T cell subset autoimmune activation

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Introduction: JAK-STAT (Janus kinase-signal transducer and activator of transcription) is a conserved cell signalling pathway responsible for transduction of signal induced by receptors for a diverse range of interferons, cytokines and growth factors. Polymorphisms of JAKs and STATs are functionally and clinically relevant to a variety of human diseases, particularly cancer and immune-related, but also common multigenic diseases.

Statement of the Problem: A population of CD4+ T cells demonstrating constitutive STAT1 phosphorylation were identified both in systemic lupus erythmatosus (SLE) patients and healthy volunteers. STAT1 phosphorylation in these cells is both independent of IL-6 stimulation and resistant to JAK1 inhibition. These cells may represent a novel paradigm of JAK-independent STAT activation, giving rise to a pathogenic population that is aberrantly regulated during activation in autoimmune disorders. The aim of this preliminary research was to confirm the existence of this population and begin characterisation of an extracellular phenotype in the hope of identifying population-specific markers.

Methods: The expression of various surface (CD3, CD4, CD25, CD127, CD45RA, CD197, CXCR3 and CCR6) and intracellular (pSTAT1, pSTAT5, FOXP3) markers in whole blood or isolated lymphocytes from 106 healthy volunteers were analysed by flow cytometry.

Results: The constitutively signalling population was demonstrated to persist for up to 48 h in un-stimulated samples and in the presence of a selective JAK1 inhibitor. The population demonstrated a CD25 low/intermediate and CD127+ phenotype, with FOXP3 expression in some cells. The population was shown to be CD45RA-, indicative of a T-memory cell phenotype. Chemokine receptor analysis demonstrated the population to be CXCR3- and CCR6+, indicative of a Th17-like phenotype.

Conclusion: The constitutive STAT1 signalling population may represent a terminally activated group of CD4+ cells that can no longer regulate STAT activation through potential loss of regulatory mechanisms (SOCS) or constitutive kinase activation, that may be driving autoimmune disease.

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Case series: Association between elevated circulating plasma blasts and their gated flocytometric picture in a cohort of diversity of clinical presentations of IGG4-RD cases

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Background: Immunoglobulin G4-related disease (IgG4-RD), is an immune-mediated disorder with certain clinical, serological, and histopathological features. The affected tissues and organs show sclerosis with dense lymphoplasmacytic infiltrate rich in IgG4-positive plasma cells usually associated with high level of serum IgG4 and elevated circulating plasmablasts.

Objectives: To report a retrospective analysis of case series of patients diagnosed as IgG4-RD who were seen in Kobri El-Kobba military complex, Cairo, Egypt since January 2015 till June 2016. Reporting includes the relationship between elevated circulating plasmablasts with their gated flow cytometric picture and the presented diversity of clinical presentation of patients

Methods: 22 patients of IgG4-RD with different clinical presentations are included. The diagnosis was made based on the clinical manifestations, detecting elevated circulating plasmablasts, imaging studies, flow cytometry by gating with CD138, CD38, CD19LOW, CD20-, and CD27, and the appropriate tissue biopsy characteristic to the diagnosis of the disease.

Results: The presented manifestations were as follows: biliary diseases (4) 18.1%, orbital diseases (6) 20.7%, interstitial lung disease (4) 18.1%, thyroid disease (4) 18.1% and salivary gland disease (4) 18.1%. Elevated circulating plasmablasts were found in all cases (100%) irrespective of their count. All patients had imaging studies related to diagnosis of the disease corresponding to the affected organ. All patients had immunophenotyping on peripheral blood by flow cytometry with gating to CD138, CD38, CD19LOW, CD20 -, and CD27 all of them are indicative to the disease. All the results were same for different clinical presentations of the IgG4-RD cases included.

Conclusions: Our retrospective case study provides data on a variety of clinical presentation of a cohort of cases of IgG4-RD. All the presented cases with their diversity show common results in having a high level of circulating plasmablasts and a picture of flow cytometry consistent with the disease especially positive CD138, CD38, Dim CD20 which support the previous results of the criteria of the diagnosis of the disease. In addition, combination of elevated circulating plasmablasts and the above results of flow cytometry are present in all varieties of clinical presentations.

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Combinatorial chemo/immunotherapy for soft tissue sarcoma

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Background: Trabectedin has direct cytotoxic activity in tumor cells and has been shown to deplete pro-tumor macrophages in the tumor microenvironment. Nivolumab inhibits the immune checkpoint molecule, PD-1, which restores anti-tumor activity in tumor-infiltrating T cells.

Purpose: To assess the safety/toxicity and efficacy of sequential administration of trabectedin and nivolumab in patients with advanced soft tissue sarcoma (STS).

Methodology: 14 patients with metastatic STS were evaluated. Each patient received one dose of single-agent trabectedin (1.5 mg/m2 continuous intravenous infusion, CIV, for 24 hours), followed by trabectedin CIV every 3 weeks, and nivolumab 3 mg/kg IV every 2 weeks. Safety/toxicity was analyzed using the NIH/NCI CTCAE v.4.03. Tumor responses were assessed by RECIST v1.1 and immune-related response criteria (irRECIST).

Findings: Histologic subtypes include undifferentiated pleomorphic sarcoma, leiomyosarcoma, synovial sarcoma, myxoid liposarcoma and chondrosarcoma. All patients had metastatic disease and a median of 4 lines of prior chemotherapy.

Safety Analysis: Grade 3 treatment emergent adverse events include anemia, fatigue, decreased platelet count, decreased granulocyte count and increased creatine kinase.

Efficacy Analysis: 13 patients received at least 2 cycles of sequential chemo-/immuno-therapy, had follow-up CT scan/MRI, and were evaluated for objective response (OR), best overall response rate (BORR), disease control rate (DCR), progression-free survival (PFS) and overall survival (OS). There were 3 partial responses, 7 stable disease and 3 progressive disease, with BORR of 23.1%, DCR of 76.9%, median PFS >7.8 months (range: 3.5->10.4 months), median OS >8.4 months (3.6->10.4 months), 6 month PFS rate, 69.2%, and 6 month OS rate, 92%. Six-month OS rate for all 14 patients was 86%. In a phase 3 study, the median PFS was 4.2 months using trabectedin alone.

Conclusion: Taken together, the data suggest that paired administration of trabectedin and nivolumab is safe, and that this chemo-/immuno-therapy approach has synergistic activity.

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New prospects of human C4 complement component system in recognizing diagnostics and analyses of autoimmune diseases

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Aim: The aim of this study was to summarize our data on human complement C4 subcomponent system involving in co-functioning between complement and other human organism innate protective elements against both infectious and autoimmunity diseases.

Methods: Components of the patient serum complement (CPSC) were registered by quantitative immunochemical methods in microplates (variants of functional analyses of isotypes C4A and C4B including their simultaneous evaluation, and C1-inhibitor upon supramolecular assembling on well bottom) and on the blot (preliminary isoelectrofocusing of patient sera in the plate of polyacrylamide gel was performed to separate isotypes C4A and C4B complexes including sub-isotypes diagnostic forms). Rabbit and goat polyclonal antibodies against purified human complement components were used. Activity of antibodies conjugated peroxidase bound to the CPSC was detected in the presence of TMB (for analyses in microplates) or chemiluminescent substrate BioWest (Pierce) in a real time.

Results: 1. Sera of patients possessing autoimmune diseases were characterized on the blot by appearance of complexed (covalently aggregated) C4B and C4A in more acidic region (pI 4.0-4.7) compared to that for free isotypes. Functional abilities of isotypes were confirmed by analyses in microplate. Absolute amounts of isotypes and their subisotypes as well as ratio of isotypes characterized prognostic-diagnostic patient groups of autoimmune diseases (SLE, antiphospholipid syndrome, rheumatoid arthritis). Appearance and relative intensities of the system of aggregated isotypes and sub-isotypes of C4 indicated the presence of disease, its initiation, reached phase of disease and disease character. 2. Similar localization on the blot for the complex C4B and C1-inhibitor of patients was registered.

Conclusions: Results indicate possible co-functioning C4B and C1-inhibitor in protection complement network upon development of autoimmune diseases. New mechanisms of cascade protection involving new combinations of CPSC may be revealed. Results open new practical possibilities in deeper (at the level of C4 sub-isotypes) diagnostics of early, progressive and chronic autoimmune diseases.

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Viruses in female breast cancer

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Japanese encephalitis (JE), a neuroinflammation caused by zoonotic JE virus, is the major cause of viral encephalitis worldwide, and poses an increasing threat to global health and welfare. To date, however, there has been no report describing the regulation of JE progression using immunomodulatory tools for developing therapeutic strategies. We tested whether blocking the 4-1BB signaling pathway would regulate JE progression using murine JE model. Blocking the 4-1BB signaling pathway significantly increased resistance to JE and reduced viral burden in extraneural tissues and the CNS, rather than causing a detrimental effect. In addition, treatment with 4-1BB agonistic antibody exacerbated JE. Furthermore, JE amelioration and reduction of viral burden by blocking the 4-1BB signaling pathway was associated with an increased frequency of IFN-II-producing NK and $CD4^+$ Th1 cells as well as increased infiltration of mature Ly-$6C^{hi}$ monocytes in the inflamed CNS. More interestingly, DCs and macrophages derived from 4-1BB KO mice showed potent and rapid IFN-I innate immune responses upon JEV infection, which was coupled to strong induction of PRRs (RIG-I, MDA5), transcription factors (IRF7), and antiviral ISG genes (*ISG49, ISG54, ISG56*). Further, the ablation of 4-1BB signaling enhanced IFN-I innate responses in neuron cells, which likely regulated viral spread in the CNS. Finally, we confirmed that blocking the 4-1BB signaling pathway in myeloid cells derived from hematopoietic stem cells (HSCs) played a dominant role in ameliorating JE. In support of this finding, HSC-derived leukocytes played a dominant role in generating the IFN-I innate responses in the host. Blocking the 4-1BB signaling pathway ameliorates JE via divergent enhancement of IFN-II-producing NK and $CD4^+$ Th1 cells and mature Ly-$6C^{hi}$ monocyte infiltration, as well as an IFN-I innate response of myeloid-derived cells. Therefore, regulation of the 4-1BB signaling pathway with antibodies or inhibitors could be a valuable therapeutic strategy for the treatment of JE.

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NLRP3-inflammassome participates in the inflammatory response induced by *Paracoccidioides brasiliensis*

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Several studies have shown that the inflammatory response is crucial for the control of paracoccidioidomycosis (PCM); however, exacerbation of inflammation leads to tissue damage and imbalance of the acquired immune response. The inflammatory response initiates after the recognition of pathogens by receptors expressed by innate immune cells. Among these receptors, the NLRP3 was associated with the recognition of pathogenic fungi in experimental models. NLRP3 operates forming a multi-proteic complex called inflammasome, which actives caspase-1, responsible for the production of the inflammatory cytokines IL-1beta and IL-18. In this study, we aimed to investigate the involvement of NLRP3 in the inflammatory response elicited in macrophages against *Paracoccidioides brasiliensis* (Pb), the etiologic agent of PCM. Macrophages were differentiated from THP-1 cells by treatment with phorbol-myristate-acetate. Following differentiation, macrophages were stimulated by Pb yeast cells for 24 hours, after previous treatment with specific NLRP3 (3, 4-methylenedioxy-beta-nitrostyrene) and/or caspase-1 (VX-765) inhibitors, or specific inhibitors of pathways involved in NLRP3 activation such as: Reactive Oxygen Species (ROS) production (N-Acetyl-L-cysteine), K+ efflux (Glibenclamide) or phagosome acidification (Bafilomycin). Quantification of IL-1beta and IL-18 in supernatants was performed by ELISA. Our results showed that the production of IL-1beta and IL-18 by THP-1-derived-macrophages stimulated with Pb yeast cells was dependent on NLRP3 and caspase-1 activation, once the presence of their specific inhibitors diminished the production of these cytokines. Furthermore, we found that the major pathways involved in NLRP3 activation, after Pb recognition, were dependent on ROS production and K+ efflux. In conclusion our results showed that NLRP3 participates in the recognition of Pb yeast cells by macrophages, leading to the activation of the NLRP3-inflammasome and production of IL-1beta and IL-18. Together, these cytokines can induce an inflammatory response against *P. brasiliensis*, essential for the establishment of the initial inflammatory response and for the development of the subsequent acquired immune response.

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