P2632
FcγR and CR on blood monocytes in differentiation between sarcoidosis and tuberculosis
Anna Dubaniewicz1, Marta Gaszynska1, Monika Wybieralska1, Katarzyna Rogoza1, Jan M. Slominski1, Piotr Konieczny1, Piotr Tzonkowksi2, Adam Sternau1, 1Department of Pneumonology, Medical University of Gdansk, Poland; 2Department of Clinical Immunology and Transplantology, Medical University of Gdansk, Poland; 2Department of Thoracic Surgery, Medical University of Gdansk, Poland; 2Department of Endocrinology and Internal Diseases, Medical University of Gdansk, Poland

Genetically different patients with sarcoidosis (SA) and tuberculosis (TB) induce dissimilar immune responses to the same mycobacterial heat shock proteins, which are implicated in forming of immune complexes (CIs). The complexemia in both diseases may result from a different function disorder of receptors for Fc of immunoglobulin G (FcγR) and complement receptors (CR) on monocytes in the phagocytosis and clearance of CIs with following persistent antigenemia and granuloma formation. Therefore, we analyzed the occurrence of FcγRI, FcγRII, FcγRIII and CR1, CR3, CR4 on blood CD14+ monocytes in 24 SA patients, 20 TB patients and 20 healthy volunteers using flow cytometry. Our results revealed significantly increased monocytes’ presence with FcγRII-I and decreased with CR1 and CR4 in SA than controls. Analysis of monocytes’ phenotypes revealed increased FcγRIII-CR1+ and decreased FcγRII-CR1, FcγRII-CR3+, FcγRII-CR4+ occurrence in SA vs controls. In TB, higher presence of monocytes with particular FcγRI+, FcγRII+, CR1+ and CR3+ than in controls was detected. In SA vs TB, the occurrence of monocytes with FcγRIII+ receptor was significantly higher and with CR1+ was less frequent. The monocytes’ phenotype FcγRIII+CR1+ was increased in SA vs TB. In summary, there are increased FcγRI+ and FcγRII+ monocytes in both SA and TB but in contrast to TB, sarcoid monocytes had increased FcγRII+ occurrence with CR1 and CR4 deficiency. In SA, increased FcγR presence but CR deficiency on surface of blood monocytes may explain persistent antigenemia and complexemia in our patients with SA. This study may be useful for differentiation of both diseases.

P2633
Over-expression of thymosin beta4 in granulomatous lung lesions in active pulmonary tuberculosis
Chul Ho Oak1, Tae Won Jang1, Bong Guen Chyun2, Hee Jae Cha3, Maan Hong Jung1, 1Internal Medicine, Kosin University Gospel Hospital, Busan, Korea; 2Pathology, Kosin University Gospel Hospital, Busan, Korea; 3Genetics, College of Medicine, Kosin University, Busan, Korea

Background: Recent studies have shown that thymosin β4 stimulates angiogenesis by induction of vascular endothelial growth factor (VEGF) expression and stabilization of hypoxia inducible factor (HIF)-1α protein.
Purpose: We investigated the expression of thymosin β4 in the various stages of pulmonary tuberculosis. We also examined the expression pattern of VEGF and HIF-1α and compared the expression pattern of thymosin β4 with VEGF and HIF-1α.

Material and method: Ten surgical samples of active pulmonary tuberculosis lesions were immunostained with rabbit polyclonal antibody to thymosin β4 (1:1,000 dilution, AbCys Diagnostics, Windham, NH, USA), HIF-1α (1:1,100 dilution, Novus Biologicals, Littleton, CO, or VEGF (1:2,000 dilution, Abcam Inc., Cambridge, MA, USA) at 4°C for overnight. The expression levels were analyzed in pathological stages of pulmonary tuberculosis.

Result: Thymosin β4 was highly expressed in both alveolar macrophages in granuloma and surrounding lymphocytes in early stage of granulomatous tissues but not expressed in late stages of fibrous tissues. The expression pattern of HIF-1α was similar with that of thymosin β4. VEGF was weakly expressed in alveolar macrophages in granuloma but highly expressed blood vessels surrounding granuloma. The expression pattern of VEGF was co-localized with CD31 (Platelet endothelial cell adhesion molecule, PECAM).

Conclusion: These data suggested that thymosin β4 is highly expressed and associated with HIF-1α and VEGF-mediated inflammation and angiogenesis in the granulomatous lesions of active pulmonary tuberculosis.

P2634
Altered imbalance between Th17 and regulatory T-cells and impaired Th1 response in the recovery of multidrug-resistant tuberculosis
Qi Fan1, Rui Min1, Hong Wang1, Weiping Xue1, Zhuhai Xu2, Weiguo Xu1, Mao Huang1, Chunyan Shi1, Hongguo Pan1, 1Department of Respiratory Medicine, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China; 2Department of Pulmonary Medicine, Shanghai Pulmonary Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.

Objective: Multidrug-resistant tuberculosis (MDR-TB), a lethal global threat today, requires prolonged and expensive second-line drugs of heightened toxicity. A dysregulation of Th1/Th17 T cell subsets, found in the pathogenesis of TB, is a crucial question still unsolved in MDR-TB. Insights into MDR-TB immune responses are urgent for developing new solutions.

Method: Phenotypically examined circulating T helper/Th17 cells, regulatory T cells (Tregs), Th1, Th2 cells by flow cytometry in 26 MDR-TB patients, 26 drug-sensitive TB patients (DS-TBs) and 26 healthy subjects (HCs). The levels of circulating T cell subsets were further analyzed during before/post-treatment phases in MDR-TB patients.

Results: We found upregulation of circulating Th17 expression (7.45±1.54 and decreased ratio of Treg/Th17(0.42±0.01) in MDR-TB patients compared to HCs and DS-TBs. More remarkable suppression of Th1 cell activation was detected in MDR-TBs (11.99±0.87) than that in DS-TBs compared to HCs. Although clinical signs of MDR-TB patients did not show obvious recovery after 7 month-chemotherapy, the circulating ratio of Treg/Th17(0.88±0.13) and level of Th1(15.1±0.90) in MDR-TB patients tended to normal compared to their previous level before treatment (P<0.05).

Conclusion: These data provided evidence for an unbalanced immune status of Treg/Th17 and inhibition of Th1 type immunity in MDR-TB infection, and suggested a specific role of these T cell-induced immunity during the evolution of MDR-TB. Further study on the immune homeostasis restoration of MDR-TB patients may aid in improving adjunct immunotherapies and developing potential therapeutic strategies.

P2635
Phagocytosis by blood monocytes in differentiation between sarcoidosis and tuberculosis
Anna Dahanicewicz1, Monika Wyberska1, Katarzyna Rogoz1, Marlena Tylia1, Adam Sternal1, Jan M. Smolinski1, Piotr Trzonkowski2, 1Department of Pneumology, Medical University of Gdańsk, Poland; 2Department of Clinical Immunology and Transplantology, Medical University of Gdańsk, Poland; 3Department of Thoracic Surgery, Medical University of Gdańsk, Poland.

Due to clinical and histopathological similarities between sarcoidosis (SA) and tuberculosis (TB), we try to find some biomarker(s), which allow to a differential diagnosis between these disorders. We recently revealed increased frequencies of receptors for Fc fragment of IgG (FcγRIIa and FcγRIIb) monocytes in SA and TB but in contrast to TB, sarcoïd monocytes had increased FcγRIIb expression with receptors for fragment of complement (CR) CR1 and CR4 deficiency. Abnormal expression of FcγRI and CR may cause of a disorder of the phagocytosis by monocytes and clearance of immune complexes (CrIg) with following immunocomplexia, which concentration was higher in SA than in TB. Therefore, we have measured the pathological phagocytizing blood monocytes from 22 patients with SA, 20 patients with TB, and 20 healthy volunteers using the PHAGOTEST® kit by flow cytometry. Our study revealed increased percentage of monocytes in SA than in TB and the controls (p=0.003, p=0.002, respectively), but there was no difference between TB and healthy individuals. The percentage of phagocytosing monocytes was increased in SA than in the controls (p=0.03) and it was slightly elevated compared to TB. There was no difference between TB and the controls.

In summary, current study revealed increased of phagocytic activity of monocytes in SA than TB and explained previously obtained results regarding higher frequency of FcγRI and CR deficiency on sarcoid on tuberculosis monocytes. The increased phagocytosing of CrIg and high antigen load with following persistent antigenemia may explain the presence persistent complexemia in our patients with SA. This study may be useful for differentiation of both diseases.

P2636
Serum and r32kd induced cytokine levels and expression in tuberculosis patients and contacts
Lavanya Jothi1, P.S. Raje1, Vijayakaliamah Valluri1,2, Suman Itha Gaddam1, 1Department of Immunology, Bhagwan Mahavir Medical Research Centre, Hyderabad, Andhra Pradesh, India; 2Immunology and Molecular Biology, LEPRA Society-Blue Peter Research Centre, Hyderabad, AP, India.

Tuberculosis is a disease caused by Mycobacterium tuberculosis (MTB) whose interaction with the host may lead to a cell-mediated protective immune response. Several cytokines, including IL-6, IFN-γ and TNF-a, play an important role in mediating resistance against MTB. The aim was to investigate the candidate cytokines in active pulmonary tuberculosis patients of younger age (15 to 25yrs), their Household Contacts and controls. Levels were estimated by ELISA in pm/ml in serum(n=30) and PBMCs stimulated with r32-kd antigen of M bovis BCG (n=30). Expression by qRT-PCR in 5 individuals from each group in culture supernatants was studied. In serum IFN-γ and TNF-a concentrations were elevated in patients compared with their contacts and controls (24.51±10.60, 20.45±4.93, 14.3±4.82 at p<0.002). (13.62±8.14, 11.34±5.30, 6.39±4.10 at p<0.003). In contrast, r32-kd stimulated PBMCs from patients produced less IFN-γ and TNF-a (36.21±16.9, 45.57±4.19, 56.37±5.37 at P<0.002), (15.76±4.31, 15.28±4.50, 10.9±2.85, 7.56 at p<0.007), whereas IL-6 concentrations were elevated in controls both in serum and stimulated PBMCs (Ct16.1±6.0, C11.4±1.3, 1.44±1.20 at p<0.0004) (74.99±18.15, 61.21±17.11, 68.76±6.93 at p<0.01). Out of the 30 cases studied 6 contacts behaved similar to the patients in their clinical and Immunological aspects. The cell-associated mRNA in AgSSB stimulated T cells was significantly less as compared to controls. IFN-γ, TNF-a and IL-6 production were decreased in r32-kd antigen could be used as biomarkers for the clinical status of TB patients and early diagnosis of their contacts.

P2637
Serum amyloid A is a sensitive marker of activity of the process in patients with pulmonary tuberculosis
Abhijayi Kirug, Kasinravana Galina, Oksana Komissarova.-Biochemistry Laboratory, Central TB Research Institute of RAMS, Moscow, Russian Federation

Aim: Study level of serum amyloid A (SAA) in blood serum of patients with active pulmonary tuberculosis (TB) and its comparison with level of C-reactive protein (CRP).

Materials and methods: We studied level of SAA and CRP in blood serum of 39 M TB patients in the age from 18 till 55 years (male – 38 and female – 55). Concentrations of SAA were determined by ELISA and CRP by immunoturbidimetric method.

Results: It was established that the level of SAA increased in 99% of patients, whereas ratio of CRP was increased in 80.7% of patients. The degree of increase SAA also was much higher: the levels of SAA over 100 mg/l were observed in 68.8% of patients, but CRP – only 11.1% of cases. The degree of increase SAA was directly related with the manifestation of tuberculosis intoxication, the quantity of M tuberculosis (MTB) in sputum, the spectrum of drug resistance of MTB, the extension of the lung process and the presence of destruction in lung tissue. A maximum value of SAA in patients with pulmonary tuberculosis was 247 mg/l. The level of SAA after 3 months of chemotherapy decreased by about half in patients with effective treatment, but remained significantly increased in comparison to the norm. In patients with inefficient of the treatment the level of SAA was not substantially changed.

Conclusion: SAA is a useful marker of activity of the process in patients with pulmonary tuberculosis and its sensitivity is higher than that CRP.

P2638
The usefulness of antimycobacterial antibodies detection in TB diagnosis
Elena Copicani1, Codrin Popa2, Irina Luciana Dumitriu1, Bogdan Garzu1, Cristian Copicani1, 1Physiology, University of Medicine and Pharmacy “GrT Popa”, Iasi, Romania; 2Pneumology, Clinic of Pulmonary Diseases, Iasi, Romania.

Aim: Due to the high number of cases, it could be useful a rapid and cost effective test for early diagnosis of tuberculosis. We planned to evaluate if a test based on detection of anti-lipoarabinomannan antibodies in serum is suitable for diagnose the tuberculosis (TB)

Methods: The test used is based on detection of anti-lipoarabinomannan antibodies
from the sample using plastic combs. The test is positive if a colored spot appears on plastic comb. We used a randomized lot of patients addressed to Clinic of Pulmonary Diseases, Iasi, Romania. Results: We have tested 46 patients from June 2011 to January 2012 from Iasi County. The final bacteriological results are available for 27 patients in present (February 2012). All the data presented below are just for patients with final evaluations. The average age was 45 years. Based on bacteriological tests 10 cases (37%) were diagnosed with TB. From patients with TB, the rapid serum test was positive in 8 cases (80%); 2 cases were negative. The other 17 cases were culture negative for M. tuberculosis. From negative cases 14 (82.4%) were also negative for rapid TB test, and 3 cases were positive. For all 3 cases negative in bacteriology but positive in rapid TB test were observed opacity in upper lobes. Tuberculin test was positive in one case and not performed in others. The two producer revealed that this test has a sensitivity of 70.2% in patients with pulmonary tuberculosis and a specificity of 95.1%. Conclusion: In the study population, this rapid TB test seems to be interesting for a rapid TB screening. The patient expected for final evaluation is necessary for a more appropriate evaluation of the utility of this rapid TB test.

P2630
Impact of immunogenetic factors to tuberculosis of intrathoracic lymph nodes in children in northernwestern Russia
Anna Nistsinger1, Maria Pavlova1, Irina Dovgaluk1, Irina Pavlova1, Ludmila Bobnova1. 1. Department of Children Phthisiopulmonology, 1. Petersburg Institute of Phthisiopulmonology, St. Petersburg, Russian Federation; 2. Department of Haematology and Transfusiology, Russian Institute of Haematology and Transfusiology, St. Petersburg, Russian Federation
Severity of the disease depends not only on peculiarities of causative agent but also on genetic profile of patients. The aim of the study: to study the distribution of HLA-DRB1* alleles in children with tuberculosis of intrathoracic lymph nodes in Northern-western region of Russia. Totally 188 children from 1 to 15 years old were included in this study. They were divided into two groups. First group of children (n=90) was infected MBD according to tuberculin test, but without clinical signs. The second group (n=98) have tuberculosis of intrathoracic lymphatic nodes. Comparative group (control) consisted of 346 healthy residents of Northern-western region of Russia. HLA-DRB1* alleles were studied using PCR-SSP method. Statistical analysis was performed using chi-square criterion with Yates correction. The comparative analysis revealed statistically significant difference in HLA-DRB1* alleles’ distribution between both investigated groups and control group, at the same time no difference between two investigated groups was revealed. Children of the second group (with tuberculosis of intrathoracic lymphatic nodes) have 4.6 times higher frequency of HLA-DRB1*15 allele. Moreover the chi-square 10,08; p=0,01) in comparison with control group, while frequency of HLA-DRB1*15 was significantly lower (18,4% vs 28,3%, chi-square 3,9; p=0,05) in this group in comparison with the control group. The results suggest that HLA-DRB1*04 may be considered as immunogenetic marker of susceptibility to tuberculosis of intrathoracic lymph nodes, while HLA-DRB1*15 may be associated with resistance to this disease in children of Northern-western region of Russia.

P2640
A change in blood transcriptional signatures accompanies successful tuberculosis therapy
Chloe Bloom1, Christine Graham1, Matthew Berry2, Katalin Wilkinson1, Tom Tohill1, Zhaohui Xu3, Jose Rossello-Urry1, Damien Chassabel1, Jacques Banchereau4, Virginia Pascual1, Marc Lipman1, Robert Wilkinson1, Anne O’Garra1. 1. Division of Immunoregulation, MRC National Institute for Medical Research, London, United Kingdom; 2. Respiratory Medicine, St. Mary’s Hospital, London, United Kingdom; 3. Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, South Africa; 4. BJIR, Baylor Research Institute, Dallas, United States; 5. Respiratory Medicine, Royal Free Hospital NHS Trust, London, United Kingdom; 6. Division of Mycobacterial Research, MRC NIMR, London, United Kingdom
Introduction: Inadequate treatment of patients with active TB leads to worsening disease, infection transmission and drug resistance. Effective anti-TB therapy monitoring is difficult as the best accepted method is the 2-month sputum culture conversion. However this has low sensitivity for predicting an individual’s response, monitoring is difficult as the best accepted method is the 2-month sputum culture conversion. However this has low sensitivity for predicting an individual’s response, and difficult to implement since culture results are not available in 30-50% of patients. No recognised biomarkers of treatment response earlier than 2 months exist.
Aims: Determine if blood transcriptional profiles can be used in early TB treatment monitoring.
Methods: Mtb culture-positive, HIV-uninfected, pulmonary TB patients were recruited in Cape Town, South Africa and London. Patients with latent TB infection were also recruited. Whole blood samples were taken before, during, and after, 6 months of standard first line TB drug therapy. Samples were processed for microarray analysis. Changes in transcript expression were analysed. An algorithm to quantify the changes was also devised.
Results: All patients responded successfully to therapy. An active TB transcriptional signature was derived by comparing the untreated active and latent TB patients. A specific treatment response transcriptional signature was derived compar-
P2644
Influence of pre analytical and analytical variables in extraction, amplification and detection of M. tuberculosis in pleural fluid
Gabriela Carnevale 1, Roberta Sales 1, Francisco Vargas 1, Milena Acenicio 1,*

e1: Pulmonary Division, Hospital das clinicas de Sao Paulo, SP, Brazil.

Introduction: Pleural tuberculosis (PT) is paucibacillary difficulting diagnosis. In our hospital’s tuberculosis (TB) diagnosis.

Methods: We evaluated 47 samples of sputum patients with active pulmonary tuberculosis. Detection of Mycobacterium tuberculosis (MBT) and determine their drug susceptibility to fluoroquinolones was performed using biological microchips. The results of the reaction were evaluated with the use of hardware-software complex “Chipdetektor-01”. Technology research, a set of reagents and equipment were developed by the staff of the Institute of Molecular Biology (“Chipdetektor-01”, Moscow).

Results: DNA M. tuberculosis were found in 39 (83%) patients. Mutations in the gene gyrA were identified in 22 (56.4%) samples, of which - in 13 (59%) found the mutation in codon 95 Ser (AGC) - Thr (ACG), in 9 (41%) - in codon 90 Ala (GCA) - Val (GTA).

Conclusions: We studied the spectrum of mutations of gene gyrA M. tuberculosis, coding for drug-resistance to fluoroquinolones. In some patients with efflux activity and no efflux activity.

Patients. The Rifampicin was able to efflux induce in majority of patients. Unexpected findings can be involved with resistant TB and future relation with response of treatment must be evaluated.

P2647
Interpretations of three sputum smear examinations at the first diagnosis in our hospital’s tuberculosis patients
Katouko Kobayashi, Shuichi Yano, Toshikazu Ikeda. Pulmonary Medicine, National Hospital Organization Matsue Medical Center, Matue, Shimane, Japan

Purpose: Toman et al. (1979) suggested that three serial sputum smear examinations were recommended as a standard method for tuberculosis (TB) diagnosis. However, this was based on direct smear methods with Ziehl-Nielson staining. Thus, we investigated if three sputum examinations were necessary for our hospital using homogenization/digestion, concentration, and fluorochrome staining.

Methods: We evaluated TB patients admitted to our hospital between April 1, 2005 and December 31, 2009. A retrospective study was used to assess each test's positivity. The samples were evaluated using Gaffky’s number and sputum smears were used by Miller and Jones classification. Sputum was transported promptly, and N-acetyl-L-cysteine (NALC)-2% NaOH was added. After sputum underwent homogenization/digestion and decontamination, a sample was immediately spun down in a refrigerated centrifuge. Fluorochrome staining of smears was used.

Results: Of 268 patients who met the screening criteria, 204 were positive on their first sputum smear (76.1%). Nineteen patients whose first sputum smear was negative became positive with the second test (7.1%). Eight patients whose first and second tests were negative became positive with the third test (3.0%). After excluding strongly positive smear patients, 134 among 198 patients were positive on their first sputum smear (67.9%). In contrast, with P1-P3, 81 among 89 patients were positive on the first sputum smear (91.0%).
P2648
Role of the macrophage-inducible C-type lectin Mincle in the lung host defense against mycobacterial infections in mice

Friederike Behling1, Kathrin Steineuwel1, Regina Mauß1, Jennifer Behling1, Tobias Wehr2, Ulrich Mauß3, Department of Experimental Pathology, Hannover Medical School, Hannover, Germany; 2Clinic for Pulmology, Hannover Medical School, Hannover, Germany

The macrophage-inducible C-type lectin Mincle has been identified as receptor for the mycobacterial cell wall component trehalose dimycocytone (TDM) of M. tuberculosis. We here examined the role of Mincle in lung protective immunity against mycobacterial pathogens in mice. We found that mice infected with M. bovis BCG responded with a delayed expression of Mincle on alveolar macrophages by days 14-21 post-challenge. In line with this finding, we observed that Mincle KO mice showed significantly reduced proinflammatory cytokine release and alveolar leukocyte recruitment as well as increased mycobacterial loads particularly in lung draining lymph nodes and spleens relative to wild-type mice infected with M. bovis BCG. Importantly, flow-sorted alveolar macrophages of wild-type mice responded with substantially greater proinflammatory TNF-α, KC, CCL2 and CCL5 mRNA levels to infection with M. bovis BCG relative to alveolar macrophages of BCG-infected Mincle KO mice. Together, the current study shows that Mincle exhibits delayed cell surface expression kinetics on alveolar macrophages upon M. bovis BCG challenge, thus acting as a ‘delayed-type’ regulator of proinflammatory macrophage activation during mycobacterial infections.

P2649
Pleural effusion cytokine profiles in HIV/MTB co-infection

Anil Chamarangue, Umesh Lallos, Alina Phulakdaree, Devapregasan Moodley, Kennedy Nyamunde, Aneesh Ambaram, Biomedical Sciences, University of KwaZulu-Natal, Durban, South Africa Pulmonology, University of KwaZulu-Natal, Durban, South Africa

Pleural effusion is a common presentation in HIV/MTB co-infection. During infection, it is composed primarily of an extensive inflammatory cell infiltrate and an as yet poorly characterized cytokine milieu. Previous reports have shown significant expansion of CD4 T cells in pleural compartments of patients with active MTB infection. Both the immune infiltrate, and to a large degree, the pleural cytokine milieu, may augment viral dynamics and as a result, promote HIV replication in co-infected patients. This may have significant implications for clinical management of dually infected patients. To characterize the pleural effluent in HIV/MTB co-infected patients, we used cytokine array-based technologies to profile key cytokines. Our data showed significantly elevated levels of IL6 and IFN-γ in pleural fluid from HIV/MTB co-infection compared to MTB alone (MTB/HH vs MTB: IL6: 90.6pg/ml vs 85.6pg/ml; IFN-γ: 91.9pg/ml vs 85.4pg/ml). This observation may have reflected increases in systemic levels of these cytokines in dually infected patients (IL6: 32.1pg/ml vs 29.5pg/ml; IFN-γ: 85.4pg/ml). This observation may have reflected increases in systemic levels of these cytokines in dually infected patients (IL6: 32.1pg/ml vs 29.5pg/ml; IFN-γ: 85.4pg/ml). This observation may have reflected increases in systemic levels of these cytokines in dually infected patients (IL6: 32.1pg/ml vs 29.5pg/ml; IFN-γ: 85.4pg/ml). This observation may have reflected increases in systemic levels of these cytokines in dually infected patients (IL6: 32.1pg/ml vs 29.5pg/ml; IFN-γ: 85.4pg/ml). This observation may have reflected increases in systemic levels of these cytokines in dually infected patients (IL6: 32.1pg/ml vs 29.5pg/ml; IFN-γ: 85.4pg/ml). This observation may have reflected increases in systemic levels of these cytokines in dually infected patients (IL6: 32.1pg/ml vs 29.5pg/ml; IFN-γ: 85.4pg/ml).

P2650
Regulatory T-cells and high levels of FOXP3 mRNA lead to decreased immune responses during HIV-TB co-infection

André Loxton, Teri Roberts, Gillian Black, Gerhard Walzl. Biomedical Sciences, University of Stellenbosch, Cape Town, Western Cape, South Africa

Tuberculosis causes 2 million deaths per year and is the most important opportunistic infection in patients infected with HIV. During the co-infection of HIV/TB, natural regulatory T cells down regulate Th1/Th2 responses. We performed direct ex vivo phenotyping of whole blood with antibodies to CD4, CD25, FOXP3, CD38 and PD-1. In a 7-day whole blood assay, diluted blood was incubated with M.tb proteins. The supernatant was removed and analyzed for interferon-gamma production by ELISA. The Multiplexed Ligation dependent Probe Amplification technique was used to amplify ex vivo RNA and compare gene expression of 45 genes. We found an increase in the ratio and frequency of regulatory T-cells in HIV/TB co-infected participants. PD-1 expression on highly activated T-cells was increased in participants infected with HIV or TB alone. The median interferon-gamma responses to control and DOS-R M.tb antigens (ESAT-6/CFP10, TB10.4, Ag85A and TB10.3) were the highest in the control group. The median level of IFN-γ at MTB and TB, pleural sites, in the absence of HIV, is suggestive of conventional pro-inflammatory, innate responses usually associated with tuberculosis.

T-cells in the HIV/TB co-infected participants may have important implications for future vaccine designs. A more precise characterization of the gene and cellular factors are needed in our attempt to unravel the mechanisms of immune failure which is present during HIV/TB co-infection.

P2651
Genetic markers of multi-drug and extensive drug resistant M. tuberculosis in Kyrgyz Republic

Maimajul Isakova, Nurimah Sovhuzova, Almaz Altashay. Molecular and Cell Biology, Institute of Molecular Biology and Medicine, Bishkek, Kyrgyzstan

Tuberculosis (TB) is still a major cause of morbidity and mortality worldwide and also in Kyrgyz Republic. Decades of tuberculosis treatment failures in Kyrgyz Republic led to acquired resistance to the first-line antituberculous drugs, isoniazid (INH) and rifampicin (RIF), resulting in multidrug resistant tuberculosis (MDR-TB) (300-400 cases per year). Switch to second line drugs lead to acquisition of resistance to them and spread of XDR-TB. XDR tuberculosis cases represent about 3 to 10% of MDR cases in the countries like Kyrgyz Republic.

Aim: The aim of this study is the identification of mutation in rpoB, katG, inhA, ahpC and gyrA genes in MDR-Tb cases in Kyrgyz Republic.

Materials and methods: DNA samples of M. tuberculosis (M.Tb) were collected from 99 sputum samples from adult patients with primary MDR-TB. Mutations associated with resistance to rifampicin, isoniazid and fluoroquinolone were analyzed by biopch assay.

Results: We have demonstrated that in the rpoB gene of MDR M.tb strains the prevalent point mutation was Ser513Leu (59%). Among strains resistant to isoniazid the mutations of katG gene were found in 91% (inhA gene – 7% and ahpC gene -2%, respectively). The most frequent mutations in katG was Ser513Thr (91% of cases). The frequent mutations of gyrA gene were Asp94Gly and Ala90Val.

Conclusion: In Kyrgyz Republic the main cases of M.Tb resistance to rifampicin is Ser513Leu mutation in rpoB gene, to isoniazid – Ser315Thr mutation of katG gene. The cause of resistance to fluoroquinolone are gyrA gene – Asp94Gly and Ala90Val.

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