272. Tuberculous and non-tuberculous mycobacterial infections: diagnostic tools

P2632

FcγR and CR on blood monocytes in differentiation between sarcoidosis and tuberculosis

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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 FcγRI-III 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 frequencies of FcγRI+ and FcγRII+ monocytes in both SA and TB but in contrast to TB, sarcoid monocytes had increased FcγRIII 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

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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 level 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 tuberculous lesions were immunostained with rabbit polyclonal antibody to thymosin β4 (1:1,000 dilution; ALPCO Diagnostics, Windham, NH, USA), HIF-1 α (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 by the 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
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Objective: Multidrug-resistant tuberculousis(MDR-TB), a lethal global threat today, requires prolonged and expensive second-line drugs of heightened toxicity. A dysregulation of $CD4^+$ 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.
Methods: We phenotypically examined circulating T-helper(Th)17 cells, regulatory T cells(Tregs),Th1,Th2 cells by flow cytometric detection 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-TBs compared to HCs and DS-TBs. More remarkble 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($P<0.05$).
Conclusions: 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 immunities during the evolution of MDR-TB. Further study on the immune homeostasis restoration of MDR-TB patients may aid in improving adjuvant immunotherapies and developing potential therapeutic strategies.

P2635
Phagocytosis by blood monocytes in differentiation between sarcoidosis and tuberculosis
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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 (FcγR) FcγRI+ and FcγRII+ monocytes in both SA and TB but in contrast to TB, sarcoid monocytes had increased FcγRIII occurrence with receptors for fragment of complement (CR) CR1 and CR4 deficiency. Abnormal expression of FcγR and CR may cause of a disorder of the phagocytosis by monocytes and clearance of immune complexes (CIs) with following immunocomlexemia, which concentration was higher in SA than in TB. Therefore, we have evaluated the percentage of phagocitizing 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 phagocitizing 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 FcγR and CR deficiency on sarcoid than on tuberculoid monocytes. The increased phagocytosis of CIs 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
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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-g and TNF-a, play important roles 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 ELISAin pg/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 were studied. In serum IFN-g and TNF-a concentrations were elevated in patients compared with their contacts and controls(24.51±10.60, 20.45±4.93, 14.14±8.22 at $p< 0.002$), (13.62±8.14, 11.34±5.30, 6.39±4.10 at $p<0.003$). In contrast, r32kd–stimulated PBMCs from patients produced less IFN-g and TNF-a (36.21±16.9, 45.57±41.96, 56.37±56.37at $P<0.002$), (59.74±46.51, 87.28±53.40, 109.2±87.56 at $p<0.007$), whereas IL-6 concentrations were elevated in controls both in serum and stimulated PBMCs(11.61±6.09, 4.11±3.03, 1.44±1.20 at $p<0.0004$) (74.49±15.18, 61.21±11.79, 68.66±69.36 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 Ag85B-stimulated T cells for IFN-g and TNF-a mRNA was significantly depressed in TB patients.On the other hand, Expression of IL-6 was high in patients. Therefore, in conclusion, IFN-γ, TNF-α and IL-6 production to 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
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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 93 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 rates 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
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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 opacities in upper lobes. Tuberculin test was positive in one case and not performed in others two. The 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. Adding the patients expected for final evaluation is necessary for a more apropiate evaluation of the utility of this rapid TB test.

P2639
Impact of immunogenetic factors to tuberculosis of intrathoracic lymph nodes in children in northwestern Russia
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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 North-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 MBT 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 North-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 tuberculosis) have the frequency of HLA- DRB1*04 significantly higher (36,7% vs. 21,1%, 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 North-western region of Russia.

P2640
A change in blood transcriptional signatures accompanies successful tuberculosis therapy
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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 individuals 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 (Illumina). Changes in transcriptional 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 comparing patients before and after treatment. Both signatures showed significant changes in response to treatment and could be quantified for individual patients.
Conclusion: These results provide evidence that blood transcriptional signatures could be used as biomarkers of a successful treatment response. These potential biomarkers, measured in whole blood, could assess treatment response in patients more consistently than currently available tests.

P2641
Survival of mycobacterium tuberculosis (MBT) on model surfaces
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Goal is determine the presence of viable MBT in same days after contamination of glass and metal surfaces.
Material and methods: Use has been made of 3 MBT suspensions the concentration of which was 1*109 colony-forming units (CFU) in 1 ml. Two strains taken from the patients: drug susceptibility strain and multi-drug resistance (MDR). H37Rv was also studied. 12 glass and 12 metal plates were covered with suspension of each strain. Then in 1, 7, 14 and 21 days after contamination 0.85% NaCl was poured on the surface. The rest of the washout was inoculated of solid and liquid mediums. Besides, the rest of the washout obtained on the 14 and 21 days was used to infect guinea pigs and PCR was studied.
Results: The growth of all strain MBT in cultural media has been found, after inoculated washout obtained (from glass surfaces) on 1 and 7 days and on 14 days, on the 21 day there growth in liquid medium was found only MDR strain. As for metal surfaces obtained on 1 and 7 days the growths of each MBT strain in cultural medium. In washouts after 14 and 21 days MBT growth was not found. DNA of MBT in all tests were found. Histologic research of visceral organs guinea pigs infected by washout made on the 14 day from model surfaces made it possible to reveal lung and liver TB, and guinea pigs infected by washout made on the 14 day did not have any signs of TB.
Conclusion: 1)MDR strain can save survival in 21 day after contamination on glass surfaces and can cause guinea pigs to get TB infected after staying on glass and metal surfaces during 14 days.
2)The most sensitive methods for reveling MBT on the surfaces were PCR technique and inoculation method. It has revealed MBT after staying on glass and metal surfaces during 21 days.

P2642
Drug resistance patterns in patients with multi-drug resistant tuberculosis (MDR – TB)
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Introduction: Incidence of drug resistance to Mycobaterium tuberculosis is increasing with with the emergence of Multi Drug Resistant (MDR) and Extensively Drug Resistant (XDR) strains[1].
Aim: To study drug resistance patterns in patients with MDR tuberculosis attending the outpatients' clinic at a tertiary care center.
Methodology: Sputum of 32 patients, previously diagnosed as MDR tuberculosis was tested for culture and drug sensitivity to first and second line anti-tubercular drugs by Bactec MGIT 960 TB System [2].
Results: Resistance patterns obtained.

	Pyrazinamide	Ethambutol	Streptomycin	Amikacin
No. of resistant/Total tested	16/20	20/30	18/24	2/12
% Resistant	80%	66.66%	75%	16.67%

	Kanamycin	Ofloxacin	Ciprofloxacin	Moxifloxacin
No. of resistant/Total tested	6/31	11/24	7/8	6/9
% Resistant	19.35%	50%	87.5%	66.66%

	PAS	Cycloserine	Capreomycin	Ethionamide	Clofazamine
No. of resistant/Total tested	8/34	11/17	3/12	12/27	1/8
% Resistant	23.53%	64.71%	25%	44.44%	12.5%

A high incidence of drug resistance to first line drugs was noted. Amongst the second line drugs, high percentage of resistance to quinolones, cycloserine and ethionamide was observed. 6/32 (18.75%) patients had XDR TB.
Conclusion: The study highlights a very high percentage of drug resistance to first and second line anti tubercular drugs amongst patients with MDR TB.
References:
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P2643

Expression of P-gP in patients with resistant tuberculosis

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Background: Resistant tuberculosis (TB) is one important cause of treatment failure. One of the MDR resistance mechanisms is MDR1 gene expression, as P-glycoprotein (P-gP) expressed on cell surface, its related with output of drugs and could modify their biodisponibility.
Objectives: To evaluate P-gP expression in patients with monoresitance (MR) or multi-drug resistance (MDR) tuberculosis.
Methods: A prospective study was performed analyzing blood samples of patients with confirmed resistant TB in treatment at Evandro Chagas Research Institute (IPEC) in Rio de Janeiro, Brazil, since 2010. Flowcytometric analyses of P-gP Activity - For detection of P-gP function as a transporter, Rhd 123, by Sigma-Germany (SG) was used as a fluorescent. Ciclosporin-A (SG) was used in this study to reverse P-gP mediated drug resistance and Rifampicin (R) as inductor. Detection P-gP expression - The monocytes P-gP expression was determined using a murine anti-P-gP monoclonal antibody (eBioscience-USA) and anti-CD14, and analyzed using flowcytometer (EPICS XL-MCL System II; Beckmam Coulter, USA).
Results: The samples of 12 patients were analysed. Seven men and five women, with ages from 23 to 73 years old.The panel of resistance showed: R(2); Rifampicin more isoniazide (RI) (4); RI more Streptomycin (S) (3); RI more Ethambutol (E) (2) RIS more Pirazinamide (Z) (1).The efflux activity was identified in 53.8% of patients.The Rifampicin was able to efflux induce in majority of patients.Unexpected the monocytes P-gPexpression was found in 57, 7% and 74, 1%, respectively in patients with efflux activity and no efflux activity.
Conclusion: These findings can be involved with resistant TB and future relation with response of treatment must be evaluated.

P2644

Influence of pre analytical and analytical variables in extraction, amplification and detection of *M. tuberculosis* in pleural fluid

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Introduction: Pleural tuberculosis (PT) is paucibacillary difficulting *M. tuberculosis* identification in pleural fluid (PF) cultures. PCR- based tests have been shown to improve and speed up the diagnosis of tuberculosis. Because these tests are validated for respiratory specimens, their use in PF samples remains controversial, since hemorrhage, cells and protein content may influence its performance.
Objectives: To evaluate if pre analytical and analytical factors may influence the sensitivity of PT diagnosis using real time PCR.
Materials and methods: From a PF transudate sample, we simulated *in vitro* samples with increasing concentrations of cells, erythrocytes and protein in which were inoculated *M. tuberculosis* in concentrations ranging from 1.5 x 10^6 to 1.5 x 10^1 CFU/mL.Two extraction (Qiagen and Roche) and two detection techniques (Roche and Nanogen) were tested in real time PCR.
Results:

	D = Roche and E = Roche (Mtb detection)	D = Roche and E = Qiagen (Mtb detection)	D = Nanogen and E = Roche (Mtb detection)	D = Nanogen and E = Qiagen (Mtb detection)
Erytrocytes				
5–10,0000/mm^3	10^3	10^3	10^2	10^2
10,000–50,000/mm^3	10^3	10^3	10^2	10^1
>50,000/mm^3	10^3	10^3	10^2	10^1
Cells				
<3,000/mm^3	10^2	10^2	10^2	10^2
3,000–10,000/mm^3	10^2	10^2	10^2	10^2
>10,000/mm^3	10^3	10^2	10^2	10^2
Protein				
<3.0 g/dL	10^3	10^3	10^2	10^2
3–4.5 g/dL	10^3	10^3	10^2	10^2
>4.5 g/dL	10^3	10^3	10^2	10^2

D: detection; E: extraction; Mtb: Mycobacterium tuberculosis.

Conclusion: PCR tests were capable to detect mycobacteria in PF samples even at low concentrations. Hemorrhage, cells and high protein content did not influence the results. The lower mycobacteria detection was obtained with Nanogen kit, independently of the extraction method.

P2645

In vitro susceptibility of non-tuberculous mycobacterial strains against moxifloxacin

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Introduction: Pulmonary infections due to non-tuberculous mycobacteria (NTM) are a clinical challenge. There are to date no recommendations for the use of potential second line drugs, when first line treatment failures. For Moxifloxacin, in vitro susceptibility could be shown for M. avium-komplex (MAC), but almost no data available on its in vitro effectivity against various other NTM species.
Methods: From 100 NTM-positive cultures other than MAC isolated at our center during 2003-2010 (9 M. fortuitum, 2 M. chelonae, 6 M. gordonae, 5 M. marinum and 78 M. kansasii), we tested the minimal inhibitory concentrations (MIC) of moxifloxacin and thus the sensitivity(breakpoint $\leq$ 2 µg/ml). Culturing and resistance testing was performed on solid Middlebrook agar plates (7H11) by agar dilution.
Results: Out of 100 tested NTM-positive cultures, 98 (98%) showed sensitivity to moxifloxacin at. Only two strains (M. fortuitum and M. chelonae) showed higher MIC.
Conclusions: Our study revealed a high sensitivity rate of moxifloxacin against several NTM strains other than MAC in vitro. Despite the absence of clinical treatment studies, we see a potential use of moxifloxacin as a second line drug.

P2646

Molecular characterization of mycobacterium tuberculosis resistant to fluoroquinolones, distributed in Saratov region

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Aim: To study the spectrum of mutations of gena gyrA M. tuberculosis, coding for drug-resistance to fluoroquinolones, in the Saratov region.
Methods: We examined 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 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 (ACC) due to the natural polymorphism of the gene and do not lead to the development of drug resistance. In 9 (41%) samples identified mutations in 91 (33,3%), 94 (44,4%), 90 (22,2%) codons that encode resistance to fluoroquinolones. Primary drug resistance is set in 5 (12,8%) patients. In 4 cases revealed secondary drug resistance in patients, which received 90-120 doses antibacterial drugs. Of these, 3 cases have double mutations in gyrA (Asp94->Gly + Ala90->Val); (Asp94->Ala + Ala90->Gly_a; Asp94->Gly + Ala90>Val) and in 1 patient was found a combination of four mutations.
Conclusion: In the Saratov region primary resistance to fluoroquinolones at the level of genetic mutations was set in 12,8% cases. Was found the increase in the number of mutations among MBT strains isolated from patients receiving prolonged treatment with anti-TB drugs.

P2647

Interpretations of three sputum smear examinations at the first diagnosis in our hospital’s tuberculosis patients

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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-Nielsen 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 Specimens were evaluated using Gaffky’s number and sputum properties 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 specimen 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 (67.7%). With an M1 or M2 sputum property, 114 among 168 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%).

Conclusion: TB patients with weakly positive smears or with a not purulent sputum property should undergo three sputum smear tests.

P2648
Role of the macrophage-inducible C-type lectin Mincle in the lung host defense against mycobacterial infections in mice
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The macrophage-inducible C-type lectin Mincle has been identified as receptor for the mycobacterial cell wall component trehalose dimycolate (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-a, 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
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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 technology 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/HIV 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: 7.6pg/ml vs 6.0pg/ml). We noted moderate inhibition of IL4 production in the MTB/HIV cohort (11.8pg/ml vs 13.8pg/ml). Interestingly, we report lower levels of TNF-α in the pleural compartment of co-infected patients (21.0pg/ml) relative to MTB infection alone (33.2pg/ml), despite stable systemic levels of the cytokine among all groups investigated. Taken together, our data suggests that co-infection with HIV may alter Th1/Th2 polarization dynamics at pleural sites. The high level of TNF-α at MTB pleural sites, in the absence of HIV, is suggestive of conventional pro-inflammatory, innate responses usually associated with tuberculosis.

P2650
Regulatory T-cells and high levels of FOXP3 mRNA lead to decreased immune responses during HIV-TB co-infection
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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 analysed 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 response to p24 was higher in the HIV+ group than the HIV-TB participants. The FOXP3 gene was significantly upregulated in HIV/TB co-infected participants. Participants with HIV/TB co-infection have significantly more regulatory T-cells than those infected with either HIV or TB which leads to a dampened immune response to both HIV and TB. Differential gene expression and increased frequencies of regulatory 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
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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 biochip assay.
Results: We have demonstrated that in the rpoB gene of MDR M.tb strains the prevalent point mutation was Ser531Leu (59%). Among strains resistant to isoniasid the mutations of katG gene were found in 91% (inhA gene – 7% and ahpC gene -2%, respectively). The most frequent mutations in katG was Ser315Thr (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 Ser531Leu mutation in rpoB gene, to isoniazid – Ser315Thr mutation of katG gene. The cause of resistance to fluoroquinolone are gyrA gene – Asp94Gly and Ala90Val.