Pleural effusion of patients with tuberculosis is characterized by accumulation of γδ T lymphocytes that expresses distinct surface markers

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Background: Tuberculosis (TB) causes about 3 million of death per year. It is very important to elucidate the precise mechanism of defense against *Mycobacterium tuberculosis*. The role of gamma-delta (/9) T lymphocytes in adaptative immunity in tuberculosis remains uncertain. We addressed the distribution of these T subsets at diagnosis of tuberculosis patients and suggest they must be involved in the pathogenesis of the disease.

Methods: The frequency of /9 T lymphocytes was evaluated in both pleural effusion and peripheral blood of 33 patients with pleural tuberculosis before treatment. Frozen PBMCs were surface stained for CD3, V91, and V92. Analyze of memory and naive subsets were performed by expression of CD11a and CD27 on /9 T cell. Also, the percentage of IFN-/- producing V91 and V92 cells after IPP stimulation was evaluated. Latently infected contacts and healthy subjects were used as controls.

Results: The latent infection group presented the highest proportion of V91 T cells (12.9%, $p < 0.05$) compared to healthy control (4.29%, $p < 0.05$) and tuberculosis patients (6.2%, $p < 0.05$). It was observed higher percentage of memory cells in latent infection than the other groups ($p < 0.05$). Pleural effusion was characterized by higher frequency of memory phenotype cells than blood ($p = 0.0342$). These subsets produced more IFNg (7.05% in memory versus 3.62% in naïve cells, $p < 0.05$) confirming that they are memory cells. We found that in tuberculosis the frequency of V91T cells are higher when compared with V92 (5.07% versus 1.1%, $p < 0.05$). These numbers reflected an inverted V91:Vd2 ratio associated with tuberculosis. The latent infection group presented higher frequency of V92 T cells than healthy control (2.76% versus 1.29%, $p = 0.03$), and than TB ($p < 0.001$).

Conclusion: Marked accumulation of V91 and V92 in pleural effusion is seen in active tuberculosis, and /9 T lymphocytes are expanded in pleural effusion, and this site is mainly composed by memory subsets. Our date further indicate that /9 T cells participate in the immune response against *Mycobacterium tuberculosis*.

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Diagnostics of resistance mycobacterium tuberculosis (MR MT) to isoniazid (H) and rifampicin (R) by simultaneous identification of mutations in rpoB, katG, inhA and ahpC genes with use biological microchips (TB-biochip)

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Background: TB-biochip can simultaneously identify mutations in four MBT genes associated with MR to (R) and (H): rpoB, katG, inhA, ahpC.

Methods: We have studied opportunities of use of the TB-biochip for express diagnostic of genetic characteristic of MR MT in clinical samples (sputum) from 186 patients with disseminated tuberculosis.

Results: Mutations in rpoB gene were revealed in 125(67.2%) cases. Thus the replacement of codon 531 in 63(50.4%), including (Ser531-Leu) in 26(41.3%) was most often. This mutation is mostly distributed among R resistance strains in the world and commonly connected to Beijing genotype. This replacement causes resistance to high concentration R in vitro and does not affect MBT viability. Other variants of mutations in this codon were very seldom: Ser531-Cys(10), Ser531-Gln(19), Ser531-Trp(8) patients. Replacements in other codons: 511(12), 512(13), 513(9), 516(8), 526(15) and 533(5) cases. Drug resistance to H, caused by mutations in katG gene, was revealed in 95(51.0%) cases. Replacement in codon prevailed: isolated variant - in 41(43.2%) patients, combination with 328 codon replacement - 8(8.4%) or combination with 335 codon - 2(2.1%). We have revealed the replacement of nucleotides of inhA gene in 43(23.1%) patients. The ahpC gene has lowest number of mutations - 11(5.9%). Among the studied MBT we have revealed only 3(1.6%) with mutations in all studied gene. In 3 gene the mutations were - 16(8.6%) strains, in 2 gene - 59(31.7%), in 1 gene - 28(15.1%). The strains without any mutations revealed were only 37(19.9%). Our results had a good correlation with microbiological investigation of the same samples.

Conclusion: The prevalence of the strains with the combination of mutations of rpoB531 and katG315 once again confirms the presence of pool of drug resistant and multidrug resistant strains of Beijing genotype at territory of North-West region of Russia in conditions of wide application of R and H. This the method of biological microchips is high sensitive and specific for express detection of MR MT. It allows detection of resistance MT to R and H with in 24 hours that is very important for choosing the adequate anti-tuberculosis chemotherapy.

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