Personalized prognosis of sarcoidosis based on the complex analysis of possible aetiological agents and mechanisms of immunopathogenesis

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The current concept of immunopathogenesis of sarcoidosis is based on an exaggerated immune response to a specific unidentified antigen. In recent years, the high Th17-cell plasticity has been shown to play an important role in the pathogenesis of sarcoidosis, along with contribution of Th1. In this study an analysis of Tfh subpopulation composition in peripheral blood of patients with chronic sarcoidosis debut was performed. Electron microscopic analysis of the microbiological component of bronchoalveolar lavage fluid was conducted to identify infectious agents, in order to determine their aetiological significance in patients in the early stages of sarcoidosis. The data obtained indicate a shift in the balance of Tfh cells towards cells with proinflammatory phenotype, which may indicate their active participation in the immunopathogenesis of sarcoidosis. Commensal bacteria representatives of the normal microbiota were observed in bronchoalveolar lavage fluid. Morphological properties of macrophages witnessed for the active manifestation of their phagocytic function.

Keywords: sarcoidosis, cytokines, chemokines, immunopathogenesis, granuloma, electron microscopy.

Sarcoidosis is a complex systemic disease, heterogeneous in clinical presentation, the course of disease, prognosis and the efficacy of the treatment, in this regard, the development of new personalized approaches to the therapy of sarcoidosis and the development of new immunopathogenetic methods of treatment, e.g. those using anticytokine and antichemokine drugs [1; 2].

The current concept of immunopathogenesis of sarcoidosis is based on an exaggerated immune response to a specific unidentified antigen. Among the list of possible causative factors of sarcoidosis were mentioned antigens, derived from M. tuberculosis (catalase peroxidase (mKatG), superoxide dismutase A (sodA), the 6 kDa early secretory antigenic target (ESAT-6), as well as heat-shock proteins; and several disease triggering antigens, like protein RP35 from Proprionibacterium acnes — and others [3; 4]. The hallmark pathological sign of sarcoidosis is the non-necrotizing granuloma, a compact

aggregate of migrated immune cells. In its core activated macrophages (in pulmonary form — alveolar ones) are contained, converting into epithelioid cells and multinucleated giant cells in response to stimulation with T helper (Th)-cells cytokines; and its shell comprised of activated T-cells, a few B-cells, and fibroblasts.

The main Th-cell subsets in the granulomas are Th1-cells mainly producing interferon gamma (IFN-γ) and tumor necrosis factor-alpha (TNF- α) and expressing the transcription factor T-bet. In recent years, the paradigm of the pathogenesis of sarcoidosis has also taken into account the role of Th17-cells producing interleukins-17A and -22 (IL-17A and IL-22) and controlled by transcription factor RORγT.

An important part in controlling exaggerated immune responses is known to be played by regulatory T-cells (Tregs) expressing the transcription factor FoxP3 and secreting immune-regulatory cytokines IL-10 and transforming growth factor (TGF)-β. They can also dampen inflammatory responses through cell-cell interaction.

The clinical course of sarcoidosis is individually variative and ambiguous. In the chronic form of sarcoidosis, the probability of disease progression, with the initiation of fibrotic processes is high, and the prescription of immunosuppressive steroid therapy is included in the complex treatment of such cases. On the contrary, in the acute form of sarcoidosis — Löfgren's syndrome, which is characterized by typically favorable outcome and a high probability of spontaneous remission, a patient may recover even without treatment [5].

In recent years, the high Th17-cell plasticity has been shown to play an important role in the pathogenesis of sarcoidosis, along with contribution of Th1. The Th-cell subsets including so-called "non-classical" Th can be discriminated basing on their chemokine receptor expression CCR6 (Th17- lineage) as well as CXCR3 (Th1-lineage).

Probably, the preferential activation of certain Th-cell subsets in the peripheral blood and in the affected organs, the expression of chemokine receptors, and the degree of activation of Tregs, largely determine the course of disease, efficacy of the treatment and the prognosis of the disease.

Aim

Analysis of Tfh subpopulation composition based on chemokine receptor expression in peripheral blood of patients with chronic sarcoidosis debút.

Electron microscopic analysis of the microbiological component of bronchoalveolar lavage fluid (BAL) and bronchial biopsy (BB) to identify infectious agents, in order to determine their aetiological significance in patients in the early stages of sarcoidosis.

Materials and methods

Samples from 46 patients chronic onset of sarcoidosis (CS) with histologically confirmed diagnosis of sarcoidosis (first identified, untreated, on the background of the natural course without the use of immunosuppressive therapy and plasmapheresis) and 26 healthy volunteers (HV). Age of patients — from 23 to 65 years. For electron microscopy study — the samples of BAL and BB from 5 patients were taken.

Using multicolor flow cytometry we conducted an analysis of the relative shares of Tfh1, Tfh2, Tfh17, Tfh17/ Tfh22 within the general Th population from patients with onset of newly diagnosed untreated chronic sarcoidosis (CS) (n = 46) and from healthy volunteers (HV) (n = 26).

The results were given in the form of Me (Q25; Q75), the significance of the differences was assessed using the nonparametric Mann-Whitney test. Electron microscopic examination of ultrathin sections of BAL and BB (Transmission electron microscope JEM-100 C, JEOL, Japan) and histological analysis of preparations by light microscopy were performed.

Results

Analysis of Tfh distribution by subpopulations showed that CS-patients Tfh1 content was reduced to 14.23 %, compared to 18.49 % HV ($p < 0.001$).

Tfh2 in CS-patients reached values of 12.38 %, which exceeded the results of HV 8.34 % ($p < 0.001$).

The blood serum of CS patients exceeded the performance of the group HV in content Tfh17/Tfh22 — 26.03 % as against 20.21 % ($p < 0.002$).

Serum of CS-patients was characterized by a reduced content of Tfh17 (7.38 %) compared to HV — 11.38 % ($p < 0.005$).

The analysis of the material of BAL from 5 patients in the early stages of sarcoidosis was made.

Electron microscopic examination of ultrathin sections of BAL in a transmission electron microscope allowed establishing the structure of this clinical material. Along with the inclusion of organic components in the composition of BAL, the presence of microorganisms of bacterial nature and cells of the immune system, mainly macrophages was noted. The samples contained single bacterial cells belonging to gram-positive cocci (Streptococci), clusters of streptococcal cells and its microcolonies. Also, the high-quality microbial communities similar to biofilms were detected. Morphological properties of macrophages witnessed for the active manifestation of their phagocytic function.

Discussion and conclusion

The current concept of immunopathogenesis of sarcoidosis indicates excessive activation of the immune system in response to unspecified antigenic stimulation.

The data obtained indicate a shift in the balance of follicular Th cells towards cells with proinflammatory phenotype, which may indicate their active participation, along with the B-lymphocytes — in the immunopathogenesis of sarcoidosis.

The probable causal factors in sarcoidosis include antigens from tuberculosis (catalase, peroxidase, (mKatG), superoxide dismutase A. (sodA), heat shock proteins; and protein RP35 of Proprionibacterium acnes. Quite often in patients with sarcoidosis the investigations reveal commensal bacteria representatives of the normal microbiota. But to date, none of the microorganisms has been identified as an aetiological factor of sarcoidosis. Microorganisms, providing constant antigenic stimulation, serve as a trigger mechanism in the development of sarcoidosis. Perhaps the analysis of BAL is an important step for differential diagnosis and further treatment of patients with sensitivity to detected microorganisms.

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