УДК 616.24-002.5-097-07-085.28

I.V. Ieremenchuk,

L.D. Todoriko,

V.I. Slyvka,

T.I. Ilchyshyn

Higher State Educational Establishment of Ukraine "Bukovinian State Medical University", Chernivtsi

INDICATORS OF CYTOKINE REGULATION IN PATIENTS WITH MULTIDRUG-RESISTANT PULMONARY TUBERCULOSIS

Keywords: *tuberculosis, multidrug-resistant, interleukins.*

Abstract. *One of the reasons for worsening the epidemiological situation is the change of biological properties of bacilli, the failure of the first and the second courses of chemotherapy, interrupted treatment, relapse tuberculosis (TB), unadequit treatment, contact with patients drug resistant tuberculosis insufficient effectiveness of existing TB drugs [3,7]. Production of IL-6 and IL-10 in TB patients is independent of drug resistance, but increases in response to increased synthesis of endotoxins by MBT; the magnitude of endogenous intoxication and cytotoxic hypoxia creates prerequisites for the development of drug resistant strains. The IL-18/IL-10 ratio in these patients characterizes the increase in severity of the patient's state, the spread of inflammation processes in the lungs and the development of drug resistance; there is a significant bulk of the Tx-lymphocyte type 2 (CD4+), which indicates the development of deep gap in cell-mediated immune response and prevalence of an ineffective anti-inflammation immune activation.*

Introduction

According to WHO Ukraine ranks 1-st in the ratio multidrug-resistant tuberculosis (MDRTB) among patients receiving re-treatment (79.4 %) [7]. In Ukraine MDRTB diagnosed in 16 % of patients who first diagnosed TB and 44 % of patients with recurrent disease. In October 2013 surveillance WHO stated that all new TB cases in the world is 3.6 % MDRTB; 9.6 % (8,1-11,2 %) of all cases registered MDRTB - extensively drug resistant TB (XDR). 60.0 % of the world's MDRTB established in Brazil, China, India and South Africa [1, 3, 7].

Drug resistant TB - is the form in which the patient identifies Mycobacterium tuberculosis resistant to one or more anti-TB-drugs, as confirmed by a laboratory test drug susceptibility [3]. In patients with pulmonary TB drug resistance reaches 81 % [2]. Among the varieties the most concern is the stability MDRTB that may be the cause of XDR.

It remains unclear the role of cytokines in the development of resistance of mycobacteria to anti-TB-drugs. Also, are not yet identified markers that reflect the progression of pathological process in multidrug-resistant tuberculosis, and was not set their predictive role in assessing the success of anti-tuberculosis chemotherapy in standardized programs [4, 5, 6].

Aim

Determine the features of cytokine regulation in patients with multidrug-resistant pulmonary tuberculosis and their role in development of the systemic inflammatory response.

Material and methods

Were enrolled 116 patients with pulmonary TB. All subjects were randomized in 3 study cohorts: cohort 1 (41 subjects) were included patients with newly diagnosed pulmonary TB, with preserved sensibility to TB drugs; cohort 2 (63 subjects) were included MDR TB patients with confirmed resistance to at least 3 first line TB drugs (HRS), cohort 3 (12 subjects) were included patients with XDR TB, control group (20 subjects) were included healthy humans Clinical, radiological, biochemical, microscopic, microbiological, immune-enzymatic and statistical study (ANOVA and Pearson correlation) methods were used.

Discussion of the study

We carried out a comparative analysis of certain pro- and anti-inflammatory cytokines (Table) that shows a significant increase in the plasma concentration of cytokines in TB groups vs. control group, and we determined the probability of the dependence of these parameters upon the resistance profile of the MBT. So, the blood concentrations of IL-6 in all groups TB groups were significantly

Table

Plasma concentrations of certain cytokines in sensitive and resistant pulmonary tuberculosis patients

Cytokines	Control group (n=20)	Group 1 (n=41)	Group 2 (n=63)	Group 3 (n=12)
IL-6* (pg/ml)	1.708±0.015	18.92±14.17 p<0.001	23.70±13.39 p<0.001 p_1<0.01	6.84±5.4 p<0.001 p_2<0.01 p_3<0.001
IL-10 (pg/ml)	1.79±0.127	4.2±0.75 p<0.05	3.38±0.79 p<0.001 p_1>0.001	3.55±0.23 p<0.001 p_2<0.01 p_3>0.4
IL-18 (pg/ml)	268.34±101.74	537.67±276.67 p<0.001	329.32±148.10 p<0.1 p_1<0.001	194.11±81.89 p<0.05 p_2<0.001 p_3>0.05

Note: Data are presented as average and standard error (M±m). p – significance level related to control group; p_1 – significance level between group 1 and 2; p_2 – significance level between group 1 and 3; p_3 – significance level between group 2 and 3. * – interleukine

increased compared to control group, there was a 11.08 fold increase in group 1, 13.9 fold increase in group 2, and 4 fold increase in group 3 of IL-6 level (p<0.001). A significant intergroup difference was found of plasma concentration of IL-6 between patients with sensitive and resistant TB (Table). Thus, the level of IL-6 in group 2 was 1.7 fold increased, compared to group1 (p_1<0.01). However, in patients of group 3 marked reduction in IL-6 concentration was compared to group1 - 2.8 fold (p<0.001) and group 2 - 3.5 fold (p_3<0.001). Low values of IL-6 in patients XDR TB, in our opinion, can lead to chronic carrier of intracellular infection, rapidly progressive course of the inflammatory process, which poorly responds to anti-TB treatment and, probably, is one of the factors producing their own XDR TB forms due to prevalence of humoral immune responses [2, 3, 5].

A pronounced activation of all phases of the inflammatory niprocess in all study cohorts compared to control group, probably, is indicated by the increase in the level of anti-inflammatory IL-10 (Table 1). Thus, in group 1 level of IL-10 increased by 2.3 folds, in group 2 - 1.8 folds, in group 3 - by 1.9 folds (p<0.001), this indicate on inhibition of cellular immunity and perhaps the beginning of specific chronic inflammatory process. The plasma concentration of IL-10 in sensitive TB patients has increased by 1.2 folds compared to group 2 and 3 (p1<0.001, p_2<0.01). There were no statistically significant differences in concentration of IL-10 in group 2 and 3 (p_3>0.4).

The activity of IL-18, whose role is to improve the resistance to intracellular pathogens and is essential for the formation of anti-TB acquired immunity, significantly increased in sensitive and MDRTB patients compared to control group. For example, in gropu1 there is a 2 fold IL-18 increase (p<0.001), respectively, in group 2 - 1.2 fold (p<0.1). However, in patients XDR TB there is a tendency to reduce the plasma concentration of this cytokine below the level of control group. The level of IL-18 in group 3 decreased by 1.4 folds in comparison with control group (p<0.05) (Fig.). Intergroup difference of plasma concentration of IL-18 in sensitive and MDR TB patients was proved. Thus, in group 2 vs. group 1 there is a 1.6 folds decrease of IL-18 (p_1<0.001), IL-18 decrease in group 3 vs. group 1 was of 2.7 folds (p_2<0.001). Also, there is a decrease of IL-18 concentration in group 3 of 1.7 folds compared to group 2 (p_3<0.05). The difference in plasma concentration

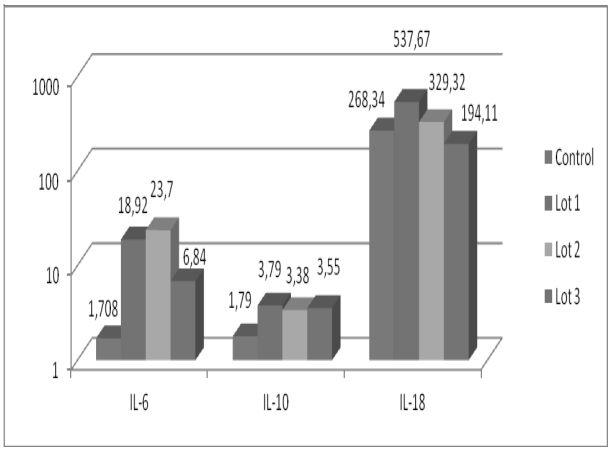

Fig. 1 Changes in the level of pro- and anti-inflammatory cytokines (pg/ml) in sensitive and MDR TB

of IL-18 in sensitive and MDRTB patients is on the ultimate level of statistical significance ($p_{1,2}$<0.001).

Discussion In our opinion, the production of IL-6 and IL-10 in TB patients, regardless of resistance, raise in response to increased synthesis of MBT endotoxins, increase of endogenous intoxication and cytotoxic hypoxia, all these create prerequisites for the development of resistance. What caused the lack of correlation between the IL is not yet clear, however, this fact is not crucial, as the most important prognostic criterion is the imbalance in the IL-18/IL-10 ratio; with an increase in the severity of the patient's condition, the spread of inflammation processes in the lungs and the development of drug resistance; there is a significant bulk of the Tx-lymphocyte type 2 (CD4+), which indicates the development of deep gap in cellmediated immune response and prevalence of an ineffective anti-inflammation immune activation. The increase in IL-6 plasma concentration, probably, indicates a high activity of systemic inflammatory response, which is maximally expressed in MDRTB patients (23.70± 13.39). This cytokine plays a key role in the development of inflam mation, immune response to infectious factor and lung tissue damage with the formation of massive destructive changes that were present in patients groups assessed by us. IL-6 plays a special role as "hepatocyte activating factor", which induce the synthesis of acute-phase proteins in the framework of systemic inflammatory response that leads to emerge of specific inflammation process outside the pulmonary tissue and activation of systemic inflammatory response syndrome.

High levels of IL-10 in patients with pulmonary TB have a favorable prognostic impact, because multifunctional properties of IL-10, ability to inhibit the synthesis of most proinflammatory cytokines and block apoptosis of macrophages and monocytes play an important role in the formation of a limited specific inflammation in the broncho-pulmonary parenchyma. Given the fact, those in MDR TB and XDR TB patients' levels of IL-10 is not too high and are efinitely lower than in newly diagnose TB patients, in such patients widespread, disseminated TB forms dominate over infiltrative forms (ratio 1:2).

Conclusions

1.Assessment of IL-6 plasma concentration in pulmonary MDR TB vs. sensible TB patients revealed a significant 1.7 folds increase (p_1<0.01), and, respectively, a significant 1.2 folds decrease in the level of IL-10 and IL-18 (p_1<0.001), these confirm the strengthening of endogenous intoxication, cytotoxic hypoxia and activation "systemic inflammatory response" syndrome.

2.Assessment of plasma concentration of certain pro- andanti-inflammatory cytokines in MDRTB patients showed that it is dependent on the profile of MBT resistance to anti-TB drugs. Plasma concentration of IL-10 in MDR and XDR TB patients is significant lower than in sensible TB patients and correlates with the prevalence in MDRTB patients of widespread/disseminate TB forms over infiltrative TB forms (1:2 ratio).

References. 1.Бялик И. Б. Актуальные вопросы химиотерапии больных мультирезистентным деструктивным туберкулезом легких / И. Б. Бялик // Туберкульоз. Легеневі хвороби. ВІЛ-інфекція. - 2011. - № 1. - С. 13-19. 2.Тодоріко Л.Д. Особливості еволюції імунопатогенезу лікарськостійкого туберкульозу / Л.Д. Тодоріко // Клінічна імунологія. Алергологія. Інфектологія. - 2014. - №3 (спецвипуск). - С.16-20. 3.Тодоріко Л.Д. Резистентність мікобактерій туберкульозу: міфи та реальність / Л.Д. Тодоріко, В.І. Петренко, М.М. Гришин // Туберкульоз. Легеневі хвороби. ВІЛ-інфекція. - 2014. - № 1(16). - С. 60-67. 4.Correlation between interleukin-10 and in situ necrosis and fibrosis suggests a role for interleukin-10 in the resolution of the granulomatous response of tuberculous pleurisy patients / T. Barbosa, S. Arruda, M. Chalhoubetal [et al.] // Microbes. Infect. - 2006. - Vol. 8, № 3. - P. 889 - 897. 5.Elevated serum level of interleukin (IL)-18, interferon (IFN)-gamma and soluble Fas in patients with pulmonary complications in tuberculosis / S. El-Masry, M. Lotfy, W.A. Nasif et al. // Acta. Microbiol. Immunol. Hung. - 2007. - Vol. 54, № 1. - P. 65 - 77. 6.Gamal A. Circulating Interleukin-18 and Osteopontin in Pulmonary Tuberculosis Patients and Their Correlation With Disease Activity / A. Gamal, Amer and Ahmed G. El-Gazzar // Egyptian Journal of Medical Microbiology. - 2008. - Vol. 17, №. 3. - P. 405-410. 7.Global tuberculosis report 2012 : WHO [Електронний ресурс]. - Режим доступу : http://www.who.int/tb/publications/global_report/en/.

ПОКАЗНИКИ ЦИТОКІНОВОЇ РЕГУЛЯЦІЇ У ХВОРИХ З МУЛЬТИРЕЗИСТЕНТНИМ ТУБЕРКУЛЬОЗОМ ЛЕГЕНЬ

І.В. Єременчук, Л.Д. Тодоріко, В.І. Сливка, Т.І. Ільчишин

Резюме. Однією з причин погіршення епідеміологічної ситуації є зміна біологічних властивостей мікобактерій, невдача першого та повторного курсів хіміотерапії, перерване лікування, рецидив туберкульозу (ТБ), безуспішне лікування, контакт з хворим на ХРТБ, недостатня ефективність існуючих протитуберкульозних препаратів.

Продукція ІЛ-6 та ІЛ-10 у групах хворих на ТБ незалежно від резистентності зростає у відповідь на підвищення синтезу ендотоксинів МБТ і наростання ендогенної інтоксикації та цитотоксичної гіпоксії, що створює передумови для розвитку їх резистентності. Дисбаланс співвідношення ІЛ-18/ІЛ-10 у цих хворих характеризує наростання тяжкості стану пацієнта, поширеність запального процесу в легенях і формування резистентності; відзначається істотна перевага лімфоцитів Тх-2 типу (CD4+), що вказує на розвиток глибокого дефекту клітинно-опосередкованого імунного захисту й перевагу протизапальної імунної активації.

Ключові слова: туберкульоз, мультирезистентний туберкульоз легень, інтерлейкіни.

ПОКАЗАТЕЛИ ЦИТОКИНОВОЙ РЕГУЛЯЦИИ У БОЛЬНЫХ С МУЛЬТИРЕЗИСТЕНТНЫМ ТУБЕРКУЛЕЗОМ ЛЕГКИХ

И.В. Еременчук, Л.Д. Тодорико, В.И. Слывка, Т.И. Ильчишин

Резюме. Одной из причин ухудшения эпидемиологической ситуации является изменение биологических

свойств микобактерий, неудача первого и повторного курсов химиотерапии, прерванное лечение, рецидив туберкулеза (ТБ), безуспешное лечение, контакт с больным ХРТБ, недостаточная эффективность существующих противотуберкулезных препаратов.

Продукция ИЛ-6 и ИЛ-10 в группах больных ТБ независимо от резистентности возрастает в ответ на повышение синтеза эндотоксинов МБТ и нарастание эндогенной интоксикации и цитотоксической гипоксии, что создает предпосылки для развития их резистентности. Дисбаланс соотношения ИЛ-18/ИЛ-10 у этих больных характеризует нарастание тяжести состояния пациента, распространенность воспалительного процесса в легких и формирование резистентности; отмечается существенное преимущество лимфоцитов Тх-2 типа (CD4+), что указывает на развитие глубокого дефекта клеточно-опосредованной иммунной защиты и преимущество противовоспалительной иммунной активации.

Ключевые слова: туберкулез, мультирезистентный туберкулез легких, интерлейкины.

Высшее государственное учебное заведение Украины "Буковинский государственный медицинский университет", г. Черновцы

Clin. and experim. pathol.- 2015.- Vol.14, №2 (52).-P.95-98.

Надійшла до редакції 02.06.2015
Рецензент – проф. І.Й. Сидорчук