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The Characteristic of Expression of BCL-2 and BAX Apoptosis Regulatory Proteins in Lungs with the Development of HIV/TB Co-infection

ABSTRACT

In this study increase of expression Bcl-2 in zones of granulomatous inflammation and in lung parenchyma is revealed as well as an increasing rate of Bax-positive cells with change in the ratio of expression Bcl-2/Bax (coefficient of apoptosis). The balance pro– and antiapoptotic proteins of Bcl-2 family defines sensitivity of the cell to proapoptotic incentives that indicates activation of anti-apoptotic mechanisms in infected cells at VICh/TB coinfecton.

INTRODUCTION

Global medico-social problems related to HIV infection and tuberculosis remain unresolved and are considered as a threat to all humanity. The incidence of tuberculosis among HIV-infected individuals has increased significantly in the last few years, suggesting a number of common social and pathogenetic patterns and mechanisms of development co-infection HIV/TB, as well as mutual influence of infectious agents on each other [2,8]. The main characteristic of the clinical and morphological patterns of combined co-infection HIV/TB can be called the generalization of tuberculosis with multiple hematogenic dissemination of Mycobacterium tuberculosis (MBT) with the formation of caseous necrosis and infiltrates in various organs, these changes in lungs being observed in 100% of cases [1]. One of the major mechanisms of cell death in combined TB and HIV infection is apoptosis [5]. Despite numerous studies on the regulation of apoptosis [5,6,7], the role of protein BC1-2 and Bax in co-infection HIV/TB remains understudied.

The aim: to assess a level of expression of BC1-2 and Bax in the foci of granulomatorus inflammation in lungs at patients who died of generalized tuberculosis in co-infection HIV/TB.

MATERIALS AND METHODS

Autopsy material from patients (n=30) with generalized co-infection HIV/TB, stage 4 - group 1 was investigated. In the comparison group – the group 2 - autopsy cases (n=30) with mono-infection, with acute progressive pulmonary tuberculosis were investigated. Lung samples were extracted from corpses according to standard techniques in early stages after the death of the patient. Specimens were fixed in 10 % solution of buffered formalin over night at room temperature, washed and subjected to standard histological processing. From paraffin blocks histological preparations were made, stained with hematoxylin and eosin. For immunohistochemical investigation of biomarkers of Bcl-2 and Bax rabbit monoclonal antibodies (Spring Bioscience) were used. Visualization was performed with using indirect immunoperoxidase method with high temperature antigen retrieval, the study of specimens was carried out on microscope "Micros" (Austria), photomicrographs were made with the digital camera Olympus (Japan), the severity of expression of biomarkers was evaluated with using the Image Analysis program with subsequent morphometric analysis. Statistical processing of materials was performed using an application program Statistica 6.0, developed for biomedical research, the magnitude of the level of statistical significance was accepted for $p<0,05$.

RESULTS AND DISCUSSION

In lung specimens, stained with hematoxylin and eosin of the 1st group polymorphic changes were observed. In the lung parenchyma areas of emphysema with atelectasis were noted. Thickening of interalveolar septae was observed. In alveoli desquamated epithelial cells, macrophages and different amount of serous, hemorrhagic, and mixed exudate were present, most of macrophages was with pale cytoplasm. In addition, in lumina of alveoli caseous masses and tissue debris were observed.

The 2nd group (mono-infection TB) is characterized by the presence of pulmonary epithelioid cell granulomas type with giant multinuclear cells of Pirogov and Langhans, a significant number of small lymphocytes and different size foci of caseous necrosis. In group 1 productive inflammation, represented by foci of lymphohistiocytic infiltration, more localized in perivascular areas were detected. Foci of mild fibrosis and productive inflammation in subpleural departments with minor histiocytic infiltration and areas of emerging pneumoniogeic cavernas with different severity of inflammation were noted.

Outside areas of inflammatory infiltrate severe cytoplasmic expression (3-4 points) of immunoreactive material (IRM) in lymphocytes, alveolar macrophages, granulocytes, epithelial and endothelial cells were noted with using monoclonal antibodies to Bcl-2 in 1st group (HIV/TB). In areas of specific inflammation severe by mild (1 point) expression of the IRM was found.

In the 1st study group (HIV/TB) outside areas of inflammatory infiltrate moderate and severe (2-3 points) cytoplasmic expression of IRM was noted with using monoclonal antibody to Bax. In the same group in foci of specific inflammation severe and pronounced expression of the IRM (3-4 points) was detected.

In 2^{nd} group with using monoclonal antibodies to Bcl-2 (mono-infection TB) outside areas of inflammation in most cases mild and moderate (1-2 points) cytoplasmic expression of the IRM was found and weak (0-1 point) expression - in granulomatous infiltrates. With using monoclonal antibody to Bax moderate and severe (2-3 points) expression of the IRM was observed outside to specific areas of inflammation, and in areas of caseous-pneumonic lesions mild (1 point) expression of IRM was observed.

There were significant differences in morphometric parameters of the lung with using monoclonal antibodies to Bcl-2: outside areas of inflammation in 1^{st} group relative area of immunopositive objects was in 2 times higher ($p<0.001$) compared with the comparison group (2^{nd} group), and in foci of inflammation, this parameter increased in 5-fold ($p<0.001$) compared to mono-infection TB (Table 1, 2).

There is increase of the relative area of immunopositive objects in lung with using monoclonal antibody to Bax in foci of inflammation in 4 times ($p<0.001$) in the 1st group (co-infection HIV/TB), but at the same time there is reduction of the average area of immunopositive objects almost 3 times ($p<0.001$).

Thus, we identified increased levels of BC1-2 in different zones of the lung in co-infection HIV/TB with maximum expression in foci of inflammation, as well as increase the level of expression of Bax in caseous necrotic foci. Different protein expression Bcl-2 and Bax in mono-infection TB and co-infection HIV/TB, perhaps indicates a change in regulatory

mechanisms of cell death in persistence of co-infection, which leads to greater initiation of apoptosis and is most pronounced in areas of productive inflammation.

The marked increase Bax expression in macrophages, containing nuclei and dying epithelioid cells in foci of caseous necrosis is considered as a factor contributing to the expansion of tissue destruction. Alveolar macrophages are referred as fastly dying monocytic in origin cells, and with the absence of they activation by T lymphocytes, the number of which is reduced in later stages of HIV infection, their apoptosis occurs earlier [4,5,6], which possibly involves immature macrophages in the formation of caseous necrosis. This hypothesis confirms our earlier results, showing the expansion of foci of caseous necrosis in areas of productive inflammation in HIV/TB persons with drug dependence and low CD 4(+) lymphocytes according to morphometric studies [3].

CONCLUSION

Thus, in our study we revealed the increased expression of Bcl-2 in areas of granulomatous inflammation in the lung parenchyma in co-infection HIV/TB and increasing the change in the ratio of expression of Bcl-2/Bax (coefficient of apoptosis) indicates activation in infected cells anti-apoptotic mechanisms [5].

The intensity and number of Bcl-2 and Bax-positive cells in co-infection HIV/TB has been found out. The severity of apoptosis, dependent on the balance of pro- and anti-apoptotic proteins of the Bcl-2 family determined the sensitivity of cells to pro-apoptotic stimuli, was larger in foci of productive inflammation in lungs in co-infection HIV/TB .

Table 1

Morphometric parameters in the study of Bcl-2 expression in the lungs of the dead with TB mono-infection and HIV/TB co-infection

mono-infection TB (group 2)				co-infection HIV/TB (group 1)			
Sq., mkm^2	P, mkm	Perc. sq., %	CI, RVU	Sq., mkm2	P, mkm	Perc. sq., %	CI, RVU
outside areas of inflammation							
0,71 ±0,05	1,97 ±0,09	5,4 ±0,79	101,6 [107;128]	0,49 ±0,07	1,56 ±0,1	11,45 ±0,55	89,55 [79;105,5]
in focus of inflammation							
0,42±0,14	1,78±0,41	0,26±0, 01	100 [52;125]	1,06±0,35	2,0±0,37	1,29±0,07	106,8 [60;128]

Table 2

Morphometric parameters in the study of Bax expression in the lungs of the dead with the mono-infection TB and co-infection HIV/TB

Mono-infection TB (Group 2)				Co-infection HIV/TB (Group 1)			
Sq., mkm^2	P, mkm	Perc. sq., %	CI, RVU	Sq., mkm2	P, mkm	Perc. sq., %	CI, RVU
outside areas of inflammation							
0,56±0,05	1,99±0,11	11,5±0,76	141 [104;152]	0,6±0,05	2,24±0,16	11,5±0,7	86,8 [88;109]
in focus of inflammation							
0,64±0,05	2,4±0,14	3,18±0,13	133,4 [97;151]	0,26±0,02	1,27±0,04	12,1±0,9	152 [196;133]

S – average area of immunopositive areas mkm^2

P – average perimeter of immunopositive areas mkm,

Perc. sq. - Percent square of immunopositive areas, %,

RVU – relative value units

CI, – color intensity

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