GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

LPO and Apoptosis during Pulmonary Tuberculosis

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LPO and apoptosis in blood mononuclear cells were studied in patients with pulmonary tuberculosis before and during treatment with standard chemotherapeutics. Pulmonary tuberculosis was accompanied by LPO activation and intensification of apoptosis in lymphocytes and monocytes. These changes were observed before and after the course of intensive care. The intensity of lipid peroxidation retuned to normal, while activity of apoptosis remained high after therapy.

Key Words: *tuberculosis; lymphocytes; monocytes; apoptosis; lipid peroxidation*

Monocytes and lymphocytes play a major role in antituberculous protection of the macroorganism. Structural and functional destabilization of membranes plays a role in the development of immune dysfunction during tuberculosis and is associated with free radical lipid oxidation. Hyperactivation of this process is an apoptogenic factor. *M. tuberculosis* initiate free radical reactions, which results in cell death due to activation of lipid-mediated apoptosis [3]. However, little is known about the mechanisms of oxidative damage to blood immunocompetent cells. The role of blood immunocompetent cells in apoptosis during pulmonary tuberculosis (PT) and involvement of these cells in the pathogenesis of tuberculous infection are poorly understood.

Here we studied lipid peroxidation (LPO) and apoptosis in peripheral blood lymphocytes and monocytes taken from patients with PT before and after antituberculous treatment.

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MATERIALS AND METHODS

We examined 70 patients (men, 20-55 years) with drug-sensitive and drug-resistant infiltrative PT. The diagnosis of PT was made according to the results of microscopic examination of sputum and X-ray examination of the lungs. Drug-resistance of mycobacteria was estimated by the method of absolute concentrations. The patients were examined before and after the course of intensive care, as well as after completion of therapy (by the end of maintenance therapy).

The control group included 11 healthy volunteers (men, 22-55 years) and 11 patients (men, 22-55 years) with chronic nonspecific diseases of the lungs (chronic obstructive bronchitis and community-acquired pneumonia).

Peripheral blood was taken from the cubital vein after overnight fast.

Blood lymphocytes and monocytes were isolated on Ficoll-Urografin density gradient (1077 and 1083 kg/m^3, respectively) [2]. The concentra-

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tions of malonic dialdehyde (MDA) and conjugated dienes in leukocytes were measured spectrophotometrically. The results were expressed in μmol/mg protein. The intensity of apoptosis was determined by means of spectral photometry using Annexin V (BD Biosciences). The percentage of Annexin V-positive cells was determined.

The results were analyzed by Statistica 6.0 software (StatSoft Inc.). Normal type of distribution in the samples was assessed by calculating the coefficients of asymmetry and excess. Statistical treatment included χ^2 and Kolmogorov—Smirnov test. Student's *t* test was used for testing the hypothesis about equality of the means drawn from a normally distributed population. When the data did not have a normal distribution, the differences were assessed by means of Mann—Whitney *U* test. The differences were significant at $p<0.05$.

RESULTS

The intensity of LPO in peripheral blood mononuclear cells increased in patients with drug-sensitive PT before the start of chemotherapy (compared to healthy donors). In patients with drug-resistant PT before therapy, the intensity of LPO in blood lymphocytes and monocytes did not differ from normal (except for the concentration of conjugated dienes in monocytes, Table 1). The intensity of apoptosis in lymphocytes and monocytes significantly increased in patients with drug-sensitive and drug-resistant PT (Table 1).

Respiratory burst resulting in the formation of considerable amounts of reactive oxygen species is an important mechanisms of antibacterial protection. A similar effect is typical of lymphocytes with high killer activity. Reactive radicals damage mycobacteria by activation of LPO in membranes. However, high-reactivity compounds formed during free radical oxidation can cause a strong destructive effect not only on bacteria, but also on membrane lipids in host cells. This effect is associated with activation of LPO and lipid-induced apoptosis. The observed changes probably contribute to activation of these processes in blood mononuclear cells from patients with drug-sensitive PT [1,3]. Activation of apoptosis in lymphocytes and monocytes from patients with drugs-resistant PT was not accompanied by an increase in the intensity of LPO. It was probably associated with the ability of mycobacteria resistant to antituberculous drugs to adapt to the internal environment in macrophages [4].

These data suggest that activation of apoptosis in leukocytes from patients with this form of PT is mediated by a non-lipid mechanism [6].

The intensity of apoptosis in lymphocytes and monocytes from patients with both forms of PT remained high by the end of intensive chemotherapy (Table 1). It was probably related to the toxic effect of standard antituberculous drugs [5,7]. Under these conditions the intensity of LPO decreased in patients with drug-sensitive PT (normal concentrations of conjugated dienes in lymphocytes and monocytes), but increased in patients with drug-resistant

TABLE 1. Intensity of LPO and Apoptosis in Blood Mononuclear Cells from Healthy Donors and Patients with Chronic Nonspecific Diseases of the Lungs (CNDL) and Drug-Sensitive (DSPT) or Drug-Resistant (DRPT) Infiltrative PT (IPT; $X\pm m$)

Group	MDA, μmol/mg protein		Conjugated dienes, $\times10^3$ μmol/mg protein		Apoptotic cells, %	
	lymphocytes	monocytes	lymphocytes	monocytes	lymphocytes	monocytes
Healthy donors (n=11)	1.80±0.14	1.66±0.09	1.86±0.31	1.95±0.18	17.00±1.52	14.33±2.60
Patients with CNDL (n=11)	3.31±0.42**	2.90±0.35**	3.77±0.53**	2.85±0.47**	23.20±1.49	19.80±0.96
Patients with IPT (n=70)						
before therapy						
DSPT (n=15)	4.09±0.56***	6.43±1.18***+	2.34±0.38+	3.61±0.82**	34.43±1.62*+	29.43±0.89*+
DRPT (n=13)	1.53±0.19++	2.51±0.43	2.58±0.52	3.83±0.48*	37.71±1.59*+	36.71±2.81*+
after intensive chemotherapy						
DSPT (n=11)	2.84±0.58**	4.36±0.34**	1.52±0.30	1.94±0.35	40.50±1.82*+	33.00±1.75*+
DRPT (n=11)	3.95±0.45*	3.51±0.43*	5.92±0.42**	6.60±0.39**	37.17±1.85*+	35.8±2.54*+
after completion of therapy						
DSPT (n=11)	2.17±0.19	2.22±0.34	2.48±0.34	2.61±0.48	29.33±1.76*+	23.00±2.52*
DRPT (n=9)	4.31±0.76*	3.10±0.32*	1.33±0.33	1.03±0.03	24.00±3.18	31.33±1.85*+

Note. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to healthy donors; +$p<0.05$ and ++$p<0.01$ compared to patients with CNDL.

PT (compared to the level observed before therapy, Table 1).

After completion of therapy, the intensity of apoptosis in blood mononuclear cells decreased compared to the previous stage and LPO returned to normal in patients with drug-sensitive PT (Table 1). The concentration of conjugated dienes in lymphocytes and monocytes and intensity of apoptosis in lymphocytes from patients with drug-resistant PT did not differ from normal. However, MDA concentration in mononuclear cells and number of apoptotic monocytes remained high in these patients (Table 1). These changes were probably associated with elimination of mycobacteria and reduction of drug load during maintenance therapy (compared to that observed upon intensive therapy).

Our results indicate that PT is accompanied by activation of LPO and increase in the intensity of apoptosis in peripheral blood cells. These changes were observed before and after the course of intensive care. The intensity of LPO in blood mononuclear cells from patients with drug-sensitive and drug-resistant PT was highest before therapy and after the course of intensive chemotherapy, respectively. The intensity of LPO in lymphocytes and monocytes returned to normal after completion of therapy. Apoptotic activity of cells tended to decrease during treatment with specific drugs, but remained above normal.

REFERENCES

1. Yu. A. Vladimirov and A. K. Archakov, *Lipid Peroxidation in Biological Membranes* [in Russian], Moscow (1972).
2. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture Methods in Hematology* [in Russian], Tomsk (1992).
3. V. V. Eremeev and K. B. Maiorov, *Probl. Tuberkuleza*, No. 3, 54-57 (2002).
4. A. N. Mayanskii, *Immunologiya*, No. 2, 53-63 (2001).
5. F. K. Tashpulatova, *Probl. Tuberkuleza*, No. 6, 17-20 (2003).
6. S. R. Umanskii, *Mol. Biol*., **30**, No. 3, 487-498 (1996).
7. O. G. Chelnokova, *Probl. Tuberkuleza*, No. 5, 16-19 (2003).