Lipid Peroxidation in the Liver of Mice during Development of Granulomatous Inflammation after Combined Infection with *C. albicans* and *M. tuberculosis*

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Activity of LPO in the liver of CBA mice during the development of granulomatous inflammation after combined infection with *C. albicans* and *M. tuberculosis* was evaluated by the levels of conjugated dienes, ketodienes, conjugated trienes, and products of interactions between intermediate LPO products and 2-TBA. The content of primary and secondary LPO products peaked on days 3 and 10 after infection by *C. albicans*, respectively. After *M. tuberculosis* infection, the maximum accumulation of TBA-active LPO products in the liver was noted on day 3, while the level of primary lipid peroxides virtually did not increase. The dynamics of primary and secondary LPO products in the liver during granulomatous inflammation development was similar to that during infection of mice with *C. albicans* alone. Hence, the development of granulomatous inflammation induced by *C. albicans* or *M. tuberculosis* or both these agents, was associated with increased LPO activity in the liver. The dynamics of primary and secondary LPO products depended on the infectious agent or combination of agents.

Key Words: *granulomatous inflammation; combined infection; C. albicans; lipid peroxidation; liver*

Opportunistic *C. albicans* fungi (eukaryotes) and BCG vaccine mycobacteria (prokaryotes) are capable of similarly long persistence in the vacuolar system of macrophages, inducing the development of granulomatous inflammation (GI) [4,5]. The level of destruction of the involved organ in successive infection by these microorganisms varies depending on the initial function of macrophages, modulated by one of the agents. It is known that *C. albicans* belong to the main type of agents, inducing opportunistic mycotic infections in immunocompromised hosts [8]. At early stages of infection, the liver is the main barrier regulating the development of systemic mycosis [9]. The capacity of this organ to limit the growth of *C. albicans* and determine the outcome of fungal infection largely depends on reactivity of organotypical macrophges (Kupffer cells) [3]. Stimulated by microorganisms, liver macrophages produce a wide spectrum of proinflammatory mediators (oxygen and nitrogen radicals), destroying not only infectious agents, but initiating LPO processes in the involved organ [7,11]. Activity of LPO makes a certain contribution to liver injury [10]. Pre-infection of animals with *C. albicans* suggests modification of liver macrophage reactivity [5] and development of GI induced by another infectious agent, for example, BCG mycobacteria, and can essentially modify LPO activity.

We studied specific features of LPO processes in the liver during the development of GI induced by *C. albicans* or BCG mycobacteria or combined

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infection of animals by these granulomogenic factors.

MATERIALS AND METHODS

Experiments were carried out on male CBA mice (18-20 g; age 2.0-2.5 months). The animals were kept on standard diets with free access to water and food. All manipulations were carried out during the morning hours. Group 1 consisted of animals injected intraperitoneally with 0.5 ml 0.85% aqueous solution of NaCl. Group 2 mice were intraperitoneally injected with a 24-h culture of *C. albicans* (strain ATCC 24433; L. A. Tarasevich Institute of Standardization and Control of Medical Biological Preparations) in a dose of 0.5×10^9 bacteria in 0.5 ml 0.85% aqueous solution of NaCl. Group 3 animals were intraperitoneally injected with 0.5 mg BCG vaccine (Microgen Firm) in 0.5 ml 0.85% aqueous solution of NaCl. Group 4 animals were injected with BCG vaccine in the same dose by the same method on day 10 after infection by *C. albicans*.

The material for the study was collected on days 3, 10, and 120 after infection. Liver samples (200 mg) were homogenized on cold in Potter homogenizer in 1 ml 0.85% aqueous solution of NaCl with 0.1% EDTA, the homogenate was centrifuged for 15 min at 4000 rpm. The supernatants were frozen at -18°C and analyzed on the next day. Activity of LPO processes in the liver was evaluated by the percentage of lipid peroxides in a heptane—isopropanol system and by the concentration of 2-TBA-reactive products [1]. The levels of lipid peroxides were measured in each phase of the lipid extract at three wavelengths (220, 232, and 278 nm) on a Hitachi-611 spectrophotometer. The results were expressed in oxidation index units. Oxidation index $E_{232/220}$ reflected the level of conjugated dienes (CD), while $E_{278/220}$ reflected the levels of ketodienes and conjugated trienes. The concentration of TBA-reactive substances (TBARS) was measured spectrophotometrically at λ=532 nm and expressed in μmol/kg (molar extinction coefficient 1.56×10^{-5} mol^{-1} cm^{-1}).

The differences between the groups were evaluated by analysis of dispersions using the Mann—Whitney test with correction for multiple comparisons.

RESULTS

Intensification of LPO in the liver was observed in group 2 rats after *C. albicans* infection. It is known that accumulation of secondary lipid peroxides is preceded by the appearance of earlier intermediate products (CD, ketodienes, and conjugated trienes) [2]. On day 3 after infection, the content of heptane-soluble CD, ketodienes, and conjugated trienes in liver homogenates increased by 1.6 times (Table 1) and the concentration of TBARS increased by 5.7 times (Fig. 1) in comparison with group 1 animals (control). On day 10, the concentrations of TBARS in the liver were still increasing in group 2, their content being 7.5 times higher than in group

TABLE 1. Content of Primary LPO Products in Lipid Extracts of the Liver in Hepatitis Resultant from Combined Infection by *C. albicans* and BCG (*M*±*m*)

Group	Day after infection	Oxidation index			
		heptane phase		isopropanol phase	
		CD	ketodienes and conjugated trienes	CD	ketodienes and conjugated trienes
1 (18)		0.441±0.058	0.293±0.046	0.864±0.046	0.451±0.087
2	3 (8)	0.697±0.084**	0.492±0.088**	0.920±0.047	0.380±0.104
	10 (8)	0.358±0.102	0.228±0.104	0.782±0.025	0.330±0.049
	120 (7)	0.547±0.024	0.318±0.074	0.571±0.012***	0.375±0.022
3	3 (5)	0.404±0.169	0.143±0.064	0.792±0.027	0.368±0.157
	10 (6)	0.319±0.028	0.234±0.026	0.751±0.06	0.156±0.03**
	120 (4)	0.726±0.121	0.386±0.078	0.525±0.028**	0.389±0.035
4	3 (8)	0.861±0.186*	0.316±0.079	0.826±0.035	0.321±0.087
	10 (7)	0.539±0.121	0.335±0.123	0.773±0.027	0.260±0.026+
	120 (7)	0.555±0.019+	0.258±0.019	0.594±0.015***+	0.362±0.025

Note. The number of animals in experimental group is shown in parentheses. *p<0.05, **p<0.01, ***p<0.001 compared to group 1; +<0.05 compared to group 3.

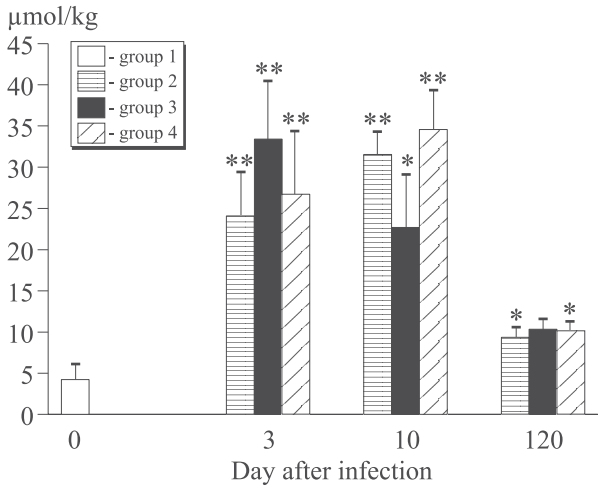

Fig. 1. Dynamics of TBARS accumulation in rat liver after co-infection with *C. albicans* and BCG mycobacteria. $p<0.05$, $^{**}p<0.01$ compared to group 1.

1 and 1.3 times higher than on day 3. On day 120 after infection, activity of LPO decreased, which was seen from reduced (by 1.5 times) content of isopropanol-soluble CD in liver homogenates in comparison with group 1 (Table 1). The content of TBARS decreased by 3.4 times, but still surpassed the control level (Fig. 1).

In group 3, the peak of LPO products was observed on day 3 of BCG-induced GI. During this period, the concentration of TBARS in the liver of group 3 animals increased 7.9 times in comparison with group 1 (Fig. 1), while the level of primary LPO products remained unchanged (Table 1), which presumably could be explained by active utilization of these products. On day 10 after BCG infection, activity of LPO in the liver decreased, the content of isopropanol-soluble ketodienes and conjugated trienes and TBARS decreased by 2.4 and 1.5 times, respectively, in comparison with the values on day 3. On day 120, the level of secondary LPO products approached the normal (Fig. 1).

In group 4, the content of CD in the heptane phase of lipid extracts of the liver increased 2.1 times (Table 1) and of TBARS 6.3 times (Fig. 1) on day 3 of GI development in comparison with group 1. On day 10, the concentration of TBARS was still increasing and surpassed the level observed on day 3 and in control mice by 1.3 and 8.2 times, respectively (Fig. 1). The content of isopropanol-soluble ketodienes and conjugated trienes in group 4 animals was 1.7 times higher than in group 3. On day 120 after infection, LPO activity in the liver decreased and approached the normal. The content of isopropanol-soluble CD during this period was 1.5 times lower than in group 1 mice. The concentration of TBARS was reduced 3.4 times in comparison with day 10, but remained significantly higher than in the control (Fig. 1). It is noteworthy that the reduction of LPO intensity in the liver of group 4 animals by day 120 after infection was more pronounced in comparison with group 3. For example, in group 4 the percentage of primary LPO products (CD) in the heptane phase of lipid extracts of the liver was 1.3 times lower during this period than in group 3.

Analysis of the results showed that the development of GI after infection with *C. albicans* was associated with activation of LPO processes and accumulation of primary and secondary LPO products in the liver. The maximum levels of primary and secondary products were observed on days 3 and 10, respectively. LPO activation in the liver after *C. albicans* infection could be caused by increased production of reactive oxygen species. It was previously shown that *C. albicans* infection was associated with reduced production of glucocorticoids [6] inhibiting generation of reactive oxygen species by liver phagocytes [12]. LPO activation was also observed after infection of animals with BCG mycobacteria. However, maximum accumulation of TBARS in the liver was observed on day 3 without appreciable increase in the content of primary products during this period. The dynamics of primary and secondary LPO products in the liver during the development of inflammatory process induced by combined infection by fungi and BCG mycobacteria was similar to that in *C. albicans* mononfection. It seems that opportunistic *C. albicans* fungi were the main etiological factor inducing LPO processes in the liver under conditions of combined infection. Activation of LPO as the main pathogenetic factor seemed to cause the development of destructive processes in the liver in massive infection of animals with both microorganisms [4,6].

Hence, the study showed that infection of animals with *C. albicans*, BCG mycobacteria, or their combination was associated with activation of LPO processes in the liver. The dynamics of primary and secondary LPO products depended on the infection agent or their combination.

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