IMMUNOLOGY AND MICROBIOLOGY

Effects of NF-κB Blocker Curcumin on Oogenesis and Immunocompetent Organ Cells in Immune Ovarian Injury in Mice

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Immunization of CBA mice with extracts from the ovaries of outbred albino mouse led to disorders in meiotic maturation of oocytes, enhanced death of immunocompetent cells in the thymus, spleen, and lymph nodes mainly by necrosis, and promoted the development of inflammatory reaction, as was shown by complete blood count. Treatment with activation inhibitor NF-κB curcumin against the background of immunization significantly reduced disorders in meiotic maturation of oocytes, decreased the number of cells dying by necrosis in immunocompetent organs, and attenuated the inflammatory reaction.

Key Words: *immune disorders of the ovaries; curcumin; apoptosis; necrosis; immunocompetent cells*

Immune mechanisms play an important role in diseases of the female reproductive system. Autoimmune involvement of the ovaries is a separate nosological entity. The immune component is a part of inflammatory diseases of the ovaries [1,4,13,14]; inflammation and immune reactions are closely related to each other. According to modern concepts, natural immunity cells (macrophages, neutrophils, dendritic cells, *etc*.) through Toll-like receptors recognizing bacteria and cell degradation products initiate all signs of inflammation. In addition, immune reaction to cell degradation antigens develops in inflammation of any kind (including aseptic). Inflammation is an important component in the pathogenesis of allergic and autoimmune processes. An important mechanism of inflammation is activity of proinflammatory factors, including cytokines (IL-1, IL-6, IL-8, TNF-α), oxygen radicals, adhesion molecules, NO, eicosanoids, *etc.* The nuclear transcription factors, primarily NF-κB (also an important regulator of cell survival, proliferation, and apoptosis), plays the key role in regulation of proinflammatory genes expression [3,8,9,15]. Activation of NF-κB was found in many pathologies, including autoimmune diseases [3,9,15]. The possibility of inhibiting NF-κB stimulation opens new vistas to modulation of inflammation development. One of well-known inhibitors of NF-κB stimulation is curcumin, a substance of plant origin (*Curcuma longa*) with anti-inflammatory and antioxidant effects. A positive effect of curcumin was detected on models of systemic and local inflammation and in some diseases, including autoimmune pathologies (rheumatoid arthritis, Crohn's disease, multiple sclerosis) [3,5,6,9]. Intense studies of curcumin efficiency are unfolding. It remains unclear whether

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curcumin is always effective and whether blockade of NF-κB modulating a variety of processes in the body aggravates the situation. Therefore, the efficiency of anti-NF-κB therapy in diseases of various organs deserves further studies.

We studied the effects of curcumin on meiotic maturation of oocytes and cell death in immunocompetent organs (thymus, spleen, lymph nodes) by apoptosis and necrosis in immune involvement of the ovaries in mice.

MATERIALS AND METHODS

The study was carried out on adult CBA females (18-22 g). Immune disease of the ovaries was induced by immunization with an extract from the ovaries of outbred albino mice. At stage 1, the mice were immunized by subcutaneous injection of water-salt extract of allogenic ovary (1.5-2.0 mg protein per mouse) in complete Freund's adjuvant. Remmunizations with ascending doses of the antigen intravenously (0.5, 0.75, and 1.0 mg protein) were carried out every 3rd day. Six days after the last immunization, the material (ovaries, spleen, lymph nodes, thymus) was collected for the study.

The status of the ovaries was evaluated by meiotic maturation of oocytes in culture [2]; the percentage of oocytes in the metaphase I (dissolving of germinal vesicle) and metaphase II (formation of the first polar body) was evaluated.

The levels of living and dying via apoptosis and necrosis cells were evaluated in suspensions of cells isolated from the thymus, spleen, and lymph nodes after their life-time staining with fluorescent stains Hoechst and propidium iodide [2].

Curcumin (Sigma) suspended in sunflower oil was administered intragastrically (100 μg/g) 4 times a week throughout the entire experiment; if the day of its administration coincided with the day of antigen injection, it was administered 1 h before the injection. The animals were divided into 3 groups: group 1 (control), injection of saline (instead of the antigen) by the immunization protocol and of sunflower oil (curcumin solvent); group 2: immunization with ovarian antigen and sunflower oil (curcumin solvent); and group 3: immunization with ovarian antigen and treatment with curcumin suspended in sunflower oil.

The results were statistically processed by one-way dispersion ANOVA; the differences between the groups were evaluated by Newman–Keuls test and Statistica 6.0 software; the results were considered significant at $p<0.05$. All data expressed in percent were subjected to Fisher's arc sine transformation before statistical analysis. Relationships between the parameters were detected using the Spearman correlation test.

TABLE 1. Number of Oocytes in Metaphases I and II (% Total Content) after Immunization with Allogenic Ovarian Antigen and Curcumin Treatment

Experiment conditions	Metaphase I	Metaphase II
Control	79.5	57.3
Immunization	57.7**	15.5***
Immunization+curcumin	78.2^{++}	35.5^{*++}

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to the control; $^{++}p<0.01$ compared to immunization.

RESULTS

The data on meiotic maturation of oocytes in culture indicated that immunization of mice with allogenic ovarian antigen significantly disordered oogenesis (Table 1). The numbers of oocytes with dissolved germinal vesicle (metaphase I) and with formed polar body (metaphase II) decreased. Oogenesis disorders were paralleled by more intense death of cells of immunocompetent organs (thymus, spleen, lymph nodes) mainly by necrosis (Table 2). This led to a reduction of the counts of living cells in these organs. Blood picture

TABLE 2. Number of Apoptotic and Necrotic Cells (% of Total Count) in the Thymus, Spleen, and Lymph Nodes during Immunization with Allogenic Ovarian Antigen and Curcumin Treatment

Experiment conditions	Spleen		Lymph nodes		Thymus	
	apoptosis	necrosis	apoptosis	necrosis	apoptosis	necrosis
Control	7.1	3.8	6.8	5.5	3.4	5.5
Immunization	9.3	6.3**	9.6	16.6***	5.2	11.4**
Immunization+curcumin	9.4	3.7^{++}	7.0	7.8^{+++}	6.1	6.0^{++}

Note. **$p<0.01$, ***$p<0.001$ compared to the control; $^{++}p<0.01$, $^{+++}p<0.001$ compared to immunization.

changed: the percentage of stab and segmented neutrophils increased, while lymphocyte content decreased.

Curcumin treatment in parallel with immunization promoted recovery of disordered meiotic maturation of oocytes. The level of oocytes in metaphase I reached the control level (no immunization), the level of cells in metaphase II increased significantly in comparison with that after immunization (Table 1).

In animals receiving curcumin, the decrease in the count of living cells in the thymus was less pronounced than after immunization without treatment (control: 91.1±1.6%; immunization: 83.4±1.9%; immunization+curcumin: 87.4±0.6%; $p<0.05$ *vs.* immunization). The increase in the level of living thymocytes was due to intensification of their necrotic death (Table 2). Similar changes were observed in the peripheral organs of immunity (spleen and lymph nodes) during curcumin treatment: the levels of living cells reduced less intensely than after immunization without treatment, the levels of cells dying by necrosis decreased significantly. The levels of cells dying by apoptosis virtually did not change (Table 2). Curcumin treatment during immunization reduced the percentage of stab neutrophils in the blood to the control level (control: 3.2±0.4%; immunization: 7.4±0.8%; $p<0.001$ compared to the control; immunization+curcumin: 3.6±0.5%, $p<0.001$ *vs.* immunization).

Analysis of correlations of oogenesis parameters (percentage of oocytes resuming meiosis) and immunocompetent cell mortality showed a negative correlation between immunocompetent cell necrosis and percentage of oocytes in metaphase I (Table 3). Importantly that this correlation was more pronounced for oogenesis parameters and cells necrosis in the peripheral immunocompetent organs (spleen and lymph nodes).

Immunization of mice with allogenic ovarian antigen can be regarded as a model of autoimmune disease in women. Like any other model, this one is not free from limitations. Using this model, we showed disorders in oogenesis and more intense death of immunocompetent cells, mainly by necrosis. A negative correlation between cell necrosis in immunocompetent organs, primarily peripheral (spleen and lymph nodes), and meiotic maturation of oocytes indicates the cause-and-effect relationships between more intense necrosis of immunocompetent cells and oogenesis disorders. This conclusion is important for understanding the mechanism of pathological process development in the ovaries. Autoimmune oophoritis is characterized by the presence of lymphocytic infiltration in the ovaries. Necrotic death of any cells, including lymphocytes infiltrating the ovary, is the source of immune reactions to cell degradation products and hence, leads to more intense and prolonged immune inflammation.

TABLE 3. Correlation between Parameters of Death of Immunocompetent Cells and Number of Oocytes Resuming Meiosis; Spearman's Rank Correlation Coefficients (*R*)

Cell death type		Cells	
		R	*P*
Necrosis	spleen	-0.52	0.011*
	lymph nodes	-0.43	0.038*
	thymus	-0.40	0.195
Apoptosis	spleen	-0.19	0.395
	lymph nodes	-0.13	0.556
	thymus	-0.02	0.940

Note. *Significant values.

Changes in complete blood count indicate the development of inflammatory reaction in our studies. Our data indicate that curcumin treatment of immunized animals reduced necrotic death of immunocompetent cells, attenuated inflammatory reaction, and eventually improved oogenesis, this suggesting an important role of NF-κB in the development of immune disorders in the ovaries and the efficiency of its stimulation blockers for normalization of ovarian function. Reduction of cell necrosis was primarily due to inhibition of TNF-α formation under the effect of curcumin, this cytokine being characterized by a significant pronecrotic effect [7,10,12]. Importantly that inhibition of proinflammatory factors cannot be considered *a priori* useful for correction of inflammatory processes, as many of them play an important role in the realization of physiological functions of the organism, including ovarian functions [1,11]. Cytokines, including TNF-α, modulate the folliculogenesis, ovulation, and corpus luteum formation.

Our data indicate that blocking of NF-κB, the key factor of inflammation, in immune involvement of the ovaries, under conditions of hyperexpression of the proinflammatory factor genes, is an important mechanism preventing cell necrosis, inflammation, and ovarian dysfunction.

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