MORPHOLOGY AND PATHOMORPHOLOGY

Effects of Specific Antibodies on the Ultrastructure of Mouse Oocytes

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The effects of antiovarian antiserum and monoclonal antibodies to the oolemma antigens on the ultrastructure of mouse oocytes and their microenvironment are studied. The antioolemma monoclonal antibodies cause more pronounced degenerative changes in the oocyte that in its microenvironment. Antiovarian antiserum induces greater changes in the microenvironment than in the oocyte. Changes induced in the oocyte by the antiserum are secondary relative to changes occurring in the microenvironment, while changes observed in the oocyte treated with monoclonal antibodies are primary.

Key Words: *oocyte; pellucid zone; monoclonal antibodies*

Since immune mechanisms play an important role in reproduction, considerable attention has been focused on the relationships between the immune and reproductive systems [3,14-16]. Autoantibodies to ovarian [6,12], pellucid zone [1,2], and oolemmal [7,8] antigens have been identified in blood serum of infertile women. The effects of antibodies on the reproductive system were studied under conditions of active and passive immunization [5,9,11]. It was found that antibodies reacting with the oolemma change ion transport and excitability of the oocyte [4]. In the present study we examined some morphological manifestations of the effects of antioolemmal monoclonal antibodies (MAb) in comparison with those of antiovarian antiserum (AOA) during the entire estrus cycle.

MATERIALS AND METHODS

Adult (8-10 weeks) female CBA mice were used. Both AOA and MAb were injected intraperitoneally during diestrus (vaginal smear control). The antiserum was administered in a dose of 200 μg/ml, and the MAb (antioolemmal monoclonal antibodies I_{11}) in a dose of 100 μg/ml peripheral blood (the volume of peripheral blood in mice is 2 ml). The antiserum was prepared from cytotoxic serum of rabbits immunized with a water-salt extract from mouse ovaries. Monoclonal antibodies I_{11} were shown to react with the oolemma by the indirect immunofluorescence method. The ovaries were incised under ether anesthesia 96 h after administration of MAb or AOA. They were fixed in cold (4°C) 4% glutaraldehyde in cacodylate buffer and postfixed with 1% osmium tetroxide in the same buffer, dehydrated in ethanols, and embedded in Epon-Araldite. Ultrathin sections were cut in an LKB ultratome, stained by the method of Reynolds, and viewed under a JEM-100 electron microscope.

RESULTS

Both ultra- and histostructure of the follicle are determined by the stage of its development. According to the classification [13], stage 3b-5b mouse follicles (the preantral follicle by the International

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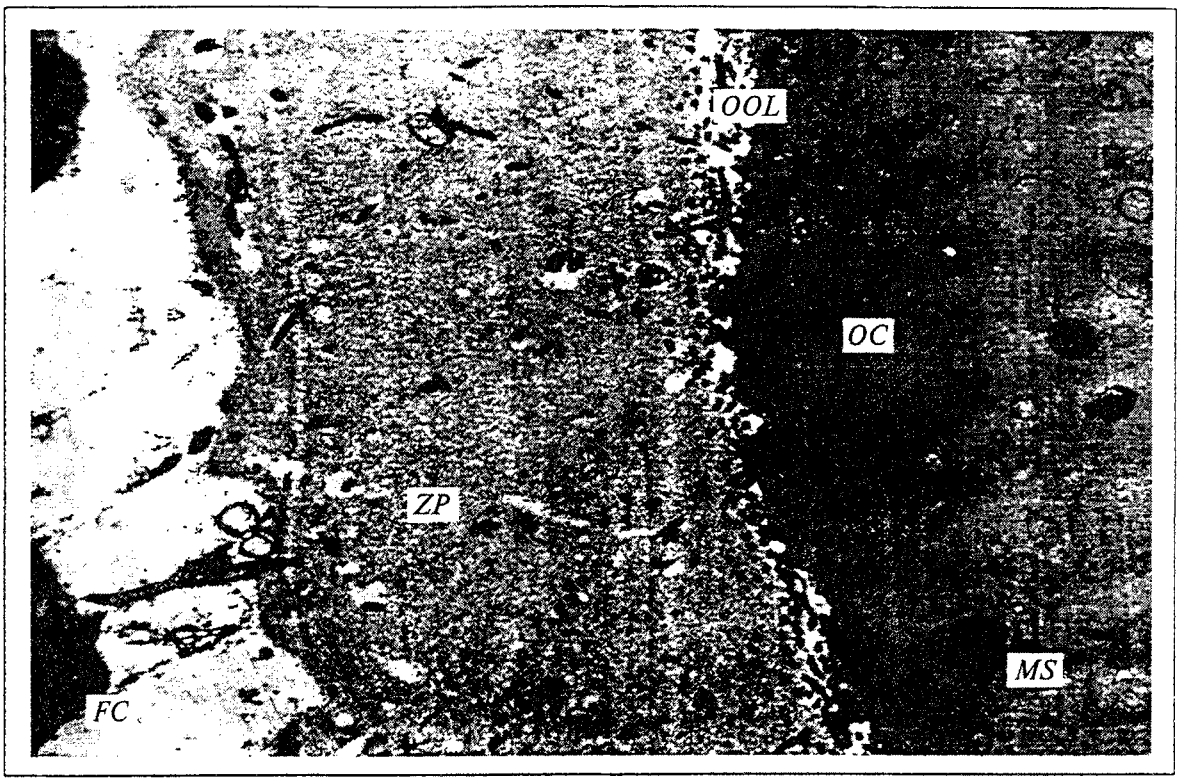

Fig. 1. Oocyte, follicular epithelium, and pellucid zone in the follicle after administration of antiovarian antiserum (200 μg/ml peripheral blood). Magnification 2500. *OC*) oocyte; *OOL*) oolemma; *FC*) follicular cell; *ZP*) pellucid zone; *MS*) myelin-like structure.

Fig. 2. Destruction of the plasma membrane of follicular cells after administration of antiovarian antiserum in a dose of 200 μg/ml peripheral blood. Magnification 2500.

Nomenclature) are resistant to atresia [10]. In order to differentiate pathological changes caused by the antibodies from physiological atresia we examined only stage 3b-5b follicles.

The antibodies induced minor changes in the oolemma; however, the number of contacts between oocyte and follicle cells decreased. The oocyte cytoplasm contained normal mitochondria, mitochondria with a clear matrix and fewer cristae, and mitochondria without cristae. Occasional mitochondria had vacuolized matrix. The number of ribosomes and terminal vesicles of the Golgi complex as well as the number of cortical granules in the juxtamembrane zone dropped (Fig. 1).

The plasma membrane of follicular cells changed considerably: the number of cytoplasmic processes and well as that of processes penetrating pellucid zone decreased. Follicular cells were separated from pellucid zone by an electron-transparent space. The intercellular space of the follicular epithelium was widened considerably. The number of cell-to-cell contacts dropped; they were represented by desmosomes, hemidesmosomes, and occasional gap junctions. The ratio between "dark" and "light" epithelial cells shifted toward "light" cells. Degenerative changes occurred both in "light" and "dark" epithelial cells. The mitochondria in "light" cells were swollen, their matrix contained election-transparent foci, and the cristae were destroyed. Some mitochondria contained myelin-like structures. The rough endoplasmic reticulum cisternae were widened, and their granularity decreased. The nuclei of "light" cells contained lumps of condensed chromatin. The perinuclear space was widened. In some epithelial cells, the cytoplasm was vacuolized, the plasma membrane was disrupted, and the organelles were lying in the intercellular space. The "dark" cells were smaller, round, oval, or elongated; their nuclear membrane was scalloped. The number of mitochondria decreased, and their cristae were partially destroyed. Some mitochondria had a clear matrix or myelin-like structures. The number of pinocytic vesicles and ribosomes decreased. The plasma membrane of some cells was destroyed (Fig. 2). The basal membrane of the follicular epithelium was loose and had deep creases. Some areas of the plasma membrane lost contact with the follicle cells and contacted with the widened intercellular space. Degenerative changes were observed in the theca. The capillaries were widened and plethoric; their endothelium was thin, and luminal and basal surfaces were smooth. Large vacuoles and exoplasmic vesicles were seen. The intercellular spaces were widened. The basal membrane had a low electron density and vague contours. Erythrocyte diapedesis was observed.

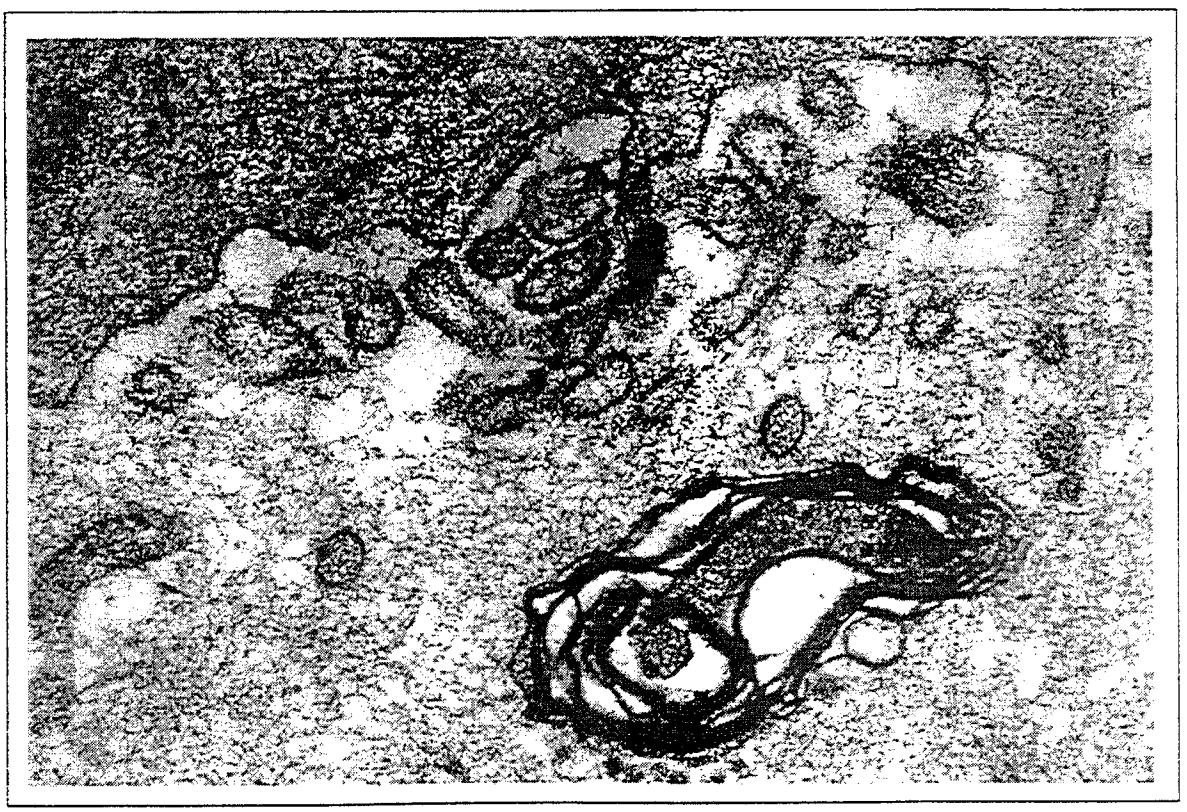

Fig. 3. Myelin-like structures in the pellucid zone near the oolemma after administration of MAb I_{11} (100 μg/ml peripheral blood). Magnification 20,000.

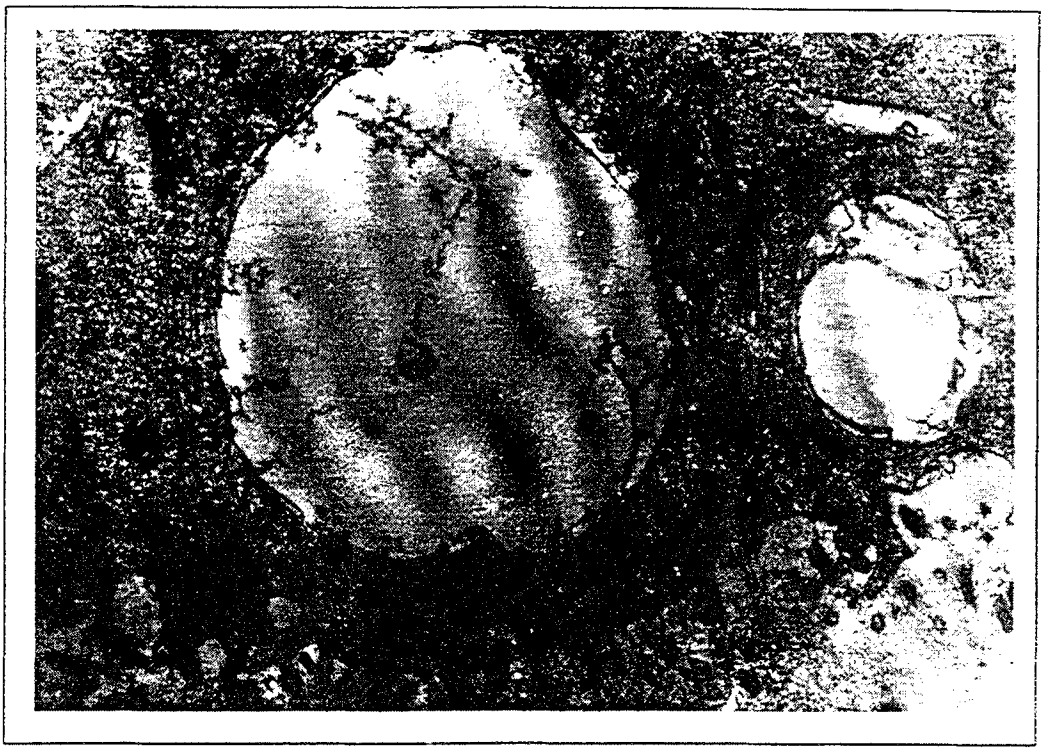

Fig. 4. Large vesicles formed in the cortical zone of an oocyte after administration of MAb I_{11} (100 μg/ml peripheral blood). Magnification 10,000.

Loosening of the follicular cell layer and formation of pseudocavities were observed after administration of MAb. Polar location of the basal cells was preserved; however, in some cells the cytoplasm basophility decreased. The thickness of the pellucid zone varied. The oocyte cytoplasm was granular and contained numerous vacuoles in the cortical zone. Changes it the theca were not pronounced; the microvessels were slightly widened. The number of microvilli on the oocyte surface decreased compared with the control. The fibrillar structures of the glycocalyx were less developed than in the control. Pellucid zone was separated from the oocyte surface. Occasional contacts between the follicular cells and oocyte were seen; the nexus were absent. The mitochondria had a clear matrix, several or no cristae, and myelin-like structures. Similar myelin-like structures were found in the pellucid zone near the oocyte plasma membrane (Fig. 3). Lipid granules and secondary lysosomes were diffusely distributed over the cytoplasm. Large vesicles at the oocyte periphery contained electron-transparent material with some electron-dense granules and filaments (Fig. 4). Cortical granules and micropinocytic vesicles were not seen. The lamellar complex was fragmented.

The follicular epithelium was edematous: the intercellular space was widened and contained electron-dense membrane "vacuoles". Cell-to-cell contacts between the granulosa cells were badly developed. Cytoplasmic processes of follicular cells were short. The number of processes penetrating pellucid zone and oriented toward the oocyte decreased considerably. Along the entire border with the oocyte pellucid zone lost fibrillar and granular components, which led to the formation of an electron-transparent area. The ratio between "light" and "dark" follicular cells was practically the same as in the control. Basal epithelial cells preserved their polarity; however, widened gap junction contacted with the basal membrane. The intensity of degenerative changes varied in a wide range: from slight alterations in the mitochondria and accumulation of lipid granules to obvious destruction of the oocyte accompanied by swelling of the cytoplasm and disruption of the plasma membrane. Reduplications of the follicular basal membrane, local widenings of subfollicular space, and loosening of the basal lamina were observed. Pronounced changes occurred in thecal microvessels. The capillaries were widened and their wall was thin. Basal and luminal surfaces of capillary endotheliocytes were smooth; the number of pinocytic vesicles in their cytoplasm dropped. The basal layer had vague contours.

Ultrastructural changes occurring in mouse follicles indicate that MAb I_{11} and antiovarian antiserum damage all components of the ovary. These changes

are consistent with atretic processes. It should be noted that MAb I_{11} induce more pronounced changes in the oocyte, while changes in its microenvironment are less pronounced compared with those induced by AOA. Thus, in the ovaries of AOA-treated mice changes in the oocyte are secondary relative to changes in the microenvironment, while in MAb I_{11}-treated mice changes occuring in the oocyte are primary.

REFERENCES

1. M. Sh. Verbitskii, *Vestn. Akad. Med. Nauk SSSR*, No. 1, 28-36 (1984).
2. M. Sh. Verbitskii, I. P. Papazov, V. N. Shoshev, *et al.*, in: *Immunological Aspects of Developmental Biology*, N. G. Khruchshev (Ed.) [in Russian], Moscow (1984), pp. 219-234.
3. V. I. Govallo, *Reproductive Immunology* [in Russian], Moscow (1987).
4. Ya. M. Gotsulyak, R. I. Yanchii, M. V. Il'chevich, and G. Yu. Yurakh, *Fiziol. Zh.*, **37**, No. 1, 106-108 (1991).
5. G. B. Mardanova, I. P. Papazov, V. N. Shoshev, and M. Sh. Verbitskii, *Arkh. Anat.*, **92**, No. 2, 74-82 (1987).
6. M. P. Barbarino, M. C. Bene, B. Gobert, *et al.*, *Fertil. Steril.*, **56**, No. 5, 928-932 (1991).
7. R. A. Bronson and G. M. Cooper, *Fertil. Steril.*, **49**, No. 3, 493-496 (1988).
8. R. Bronson, G. Cooper, and D. Rosenfeld, *J. Reprod. Immunol.*, Suppl., 56 (1983).
9. M. Damjaanovic and B. D. Jankovic, *Am. J. Reprod. Immunol. Microbiol.*, **20**, No. 1, 1-8 (1989).
10. D. L. Ingram, in: *The Ovary*, S. Zuckerman *et al.*, (Eds.), New York - London (1962), pp. 247-273.
11. B. D. Jankovic, K. Markovic, S. Petrovic, and K. Isakovic, *Eur. J. Immunol.*, **3**, No. 6, 375-377 (1973).
12. W. R. Jones, C. Chen, T. Mudge, and R. Ing, *Gynecol. Obstet. Invest.*, **35**, No. 2, 239-240 (1981).
13. T. Pedersen and H. Peters, *J. Reprod. Fertil.*, **17**, 555-557 (1967).
14. H. Serrano, *Invest. Clin.*, **27**, No. 4, 259-284 (1986).
15. S. Shulman, *Concepts Immunopathol.*, **2**, 189-227 (1985).
16. G. P. Talwar and S. K. Gaur, in: *Recent Advances in Clinical Immunology*, R. A. Thompson (Ed.), Vol. 4, Edinburgh - London - Melbourne - New York (1987), pp. 183-200.