Role of Sexually-Transmitted Infections in the Structural and Functional Reorganization of the Prostate

E. L. Lushnikova, L. M. Nepomnyashchikh, N. A. Abdullaev, I. I. Klepikova, O. P. Molodykh, and A. I. Neimark

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Intracellular reorganization of secretory epitheliocytes in the main, intermediate, and periurethral prostatic glands was studied in chronic prostatitis under conditions of sexually-transmitted infections. The destructive and autophagic processes in the secretory epitheliocytes were stimulated by persistence of microorganisms, *Mycoplasmataceae* (mainly mycoplasmas and ureaplasmas) and *Chlamydia trachomatis*, in the prostatic terminal compartments, epithelial layer, and epitheliocytes. Significant intracellular reorganization of smooth-muscle cells was found: focal destruction of ultrastructures (mainly in the perinuclear zone) and lythic changes in the myofilaments (focal and diffuse).

Key Words: *urethroprostatitis; sexually-transmitted infections; prostate; ultrastructure*

The etiological role of sexually-transmitted infections (STI), specifically, *Ureaplasma urealyticum*, *Mycoplasma genitalium*, and *Chlamydia trachomatis*, *etc*., in the development of chronic prostatic and urethral diseases is still discussed [3]. Some authors think that this specific flora can cause chronic urethroprostatitis, including the so-called nongonococcal or postgonococcal urethritis [5,11,12,14], others think the role of this microflora is disputable [4]. Study of the contribution of the most prevalent opportunistic microorganisms (*U. urealyticum*, *M. genitalium*, and *C. trachomatis*) to the development of chronic urethroprostatitis remains a pressing problem, as they can cause changes in the male reproductive system, most often asthenozoospermia [1,15] and microcirculatory disorders in the prostate. Sexually-transmitted mixed infection is clinically the most incident.

There still many questions about the pattern and severity of lesions of the prostatic glandular epithelium and smooth-muscle stroma under conditions of STI. Up to the present time it remains unclear whether the infectious agents can reach the terminal compartments of the prostatic glands and persist in epitheliocytes and other cell populations. Studies of pathomorphogenesis of chronic urethroprostatitis caused by STI are essential for the development of the optimal methods for the treatment of this disease.

We studied the probable role of STI in lesions of the prostatic secretory epithelium and smooth-muscle cells.

MATERIALS AND METHODS

Morphological studies were carried out on prostatic biopsy specimens collected for diagnosis by pistol diagnostic transrectal biopsy under ultrasonic control in 16 patients (mean age 40.7±2.3 years) with uretheroprostatitis caused by STI. Urethral discharge from all patients was analyzed by the bacteriological method and PCR. Specimens of the prostate (0.5-1.0 cm) were fixed in 10% neutral formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin, after van Gieson, and after Gram.

In order to prepare semithin and ultrathin sections, small fragments of prostatic biopsy specimens (1 mm^3) were fixed in 4% paraformaldehyde, post-

Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** pathol@soramn.ru. E. L. Lushnikova

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fixed in 1% OsO_4, and after dehydration embedded in epon and araldite mixture. Semithin and ultrathin sections were sliced on LKB III and Leica ULTRACUT EM UC7 (Leica) ultratomes. Semithin sections were stained with azur II, ultrathin were contrasted with uranyl acetate and lead citrate. The preparations were examined under a JEM-1400 electron microscope (Jeol) at accelerating voltage of 80 kV and photographed using Veleta digital camera and iTEM software (Olympus).

RESULTS

Clinical laboratory (bacteriological) data and PCR results showed sexually-transmitted mixed infections in the urethral discharge of all patients. The main agents were *U. urealyticum*, *C. trachomatis*, *M. genitalium*, and *G. vaginalis*, detected in various combinations. The most prevalent agents were *U. urealyticum* and *C. trachomatis*. Combinations of *M. genitalium* and *G. vaginalis*, *U. urealyticum* and *G. vaginalis* were detected in 25% cases, *M. genitalium*, *U. urealyticum*, and *G. vaginalis* in 37.5%, *C. trachomatis*, *M. genitalium*, and *G. vaginalis* in 12.5% cases. Gram-positive rod-shaped bacteria and cocci were found in the terminal compartments of the main prostatic glands in 25% cases (according to results of histological analysis of semithin sections). Bacteriological studies of smears from the urethra showed significant growth of this microflora in all patients, with the bacterial cell concentration above 10^4 CFU.

The severity of pathomorphological changes in the prostate depended not so much on the species and combination of microorganisms infecting the urogenital system, but mainly on the incidence and duration of infection. All biopsy specimens collected from the prostatic peripheral zones contained the terminal compartments of the main prostatic glands, their lumens were moderately plicated. Desquamated epithelial cells, small-vesicular formations (including those with secretory granules), and prostatic concrements ("stones") were sometimes found in the lumens of the terminal compartments (Fig. 1, *a*). The epithelial layer was always thickened in such cases, while epitheliocytes had no secretory granules. These changes in the terminal portions of the prostatic glands indicated disorders of the prostatic secretion evacuation.

The main secretory cells lining the terminal compartments of the prostatic glands contained numerous secretory granules in the apical part. A characteristic feature of their structure was the presence of numerous large secondary lysosomes (autophagosomes) with lipofuchsin incorporations (Fig. 1, *b*). These formations were clearly visualized not only in semithin sections, but in paraffin ones as well, particularly by van Gieson staining. In addition, large lipid incorporations were found in many epitheliocytes.

The prostatic peripheral stroma was represented by scattered smooth-muscle cells and their small bundles. Pronounced degenerative changes in the smooth-muscle cells (cytoplasm rarefaction and vacuolation, lipid incorporations) were found in all studied biopsy specimens. Significant phenotypical heterogeneity of these cells (atrophic and hypertrophic) and their reduction were worthy of note. These changes were paralleled by the development of significant periglandular fibrosis (Fig. 1, *c*). Just solitary smooth-muscle cells were retained near some terminal compartments of the main glands, while the entire stroma was formed by collagen fibers. Blood vessels were plethoric, with leukocyte stasis. Moderate perivascular edema was seen.

Inflammatory cell infiltration was found in 50% cases. By location it was mainly periglandular, by composition lymphocytic and plasmocytic with an admixture of neutrophils and eosinophils, and by intensity it was mainly scanty. Significant diffuse lymphoplasmocytic infiltration of the periglandular zones and partially of the fibromuscular layer was detected in only one patient (Fig. 1, *d*). It is noteworthy that mononuclear infiltration was more pronounced in the central zones than at the periphery. Inflammatory reaction in the prostatic central and peripheral zones indicated necrotic death of the secretory epitheliocytes in STI. *M. genitalium* can induce inflammatory reaction in infected tissues by stimulation of NF-kB transcription factor in epithelial cells through TLR2/6 [10]. Interactions of mycoplasmas with various associations of viruses and other infectious agents with induction of cell apoptosis or necrosis are also probable [8].

Gram staining of the microflora colonizing the epithelium in the prostatic terminal compartments showed gram-positive and coccal microorganisms on the surface of secretory epitheliocytes of the central glands just in solitary cases. Presumably, the greater part of micro-organisms could be removed from the epithelial surface as a result of histological processing, and just small colonies remained. It was impossible to differentiate the microorganisms in paraffin sections.

Studies of semithin sections stained with azur II proved to be the most informative. It showed rod-shaped and coccal microflora on the surface of the main secretory cells and in secretion leftover in the glandular lumen in 25% cases. The microorganisms often colonized desquamated epitheliocytes. Abundant microflora was often found in the semithin sections in the lumens of the terminal compartments of the main (peripheral) prostatic glands.

Electron-microscopic analysis showed that the apical zones of the main prostatic cells contained numerous polymorphic vacuole- and lacune-shaped

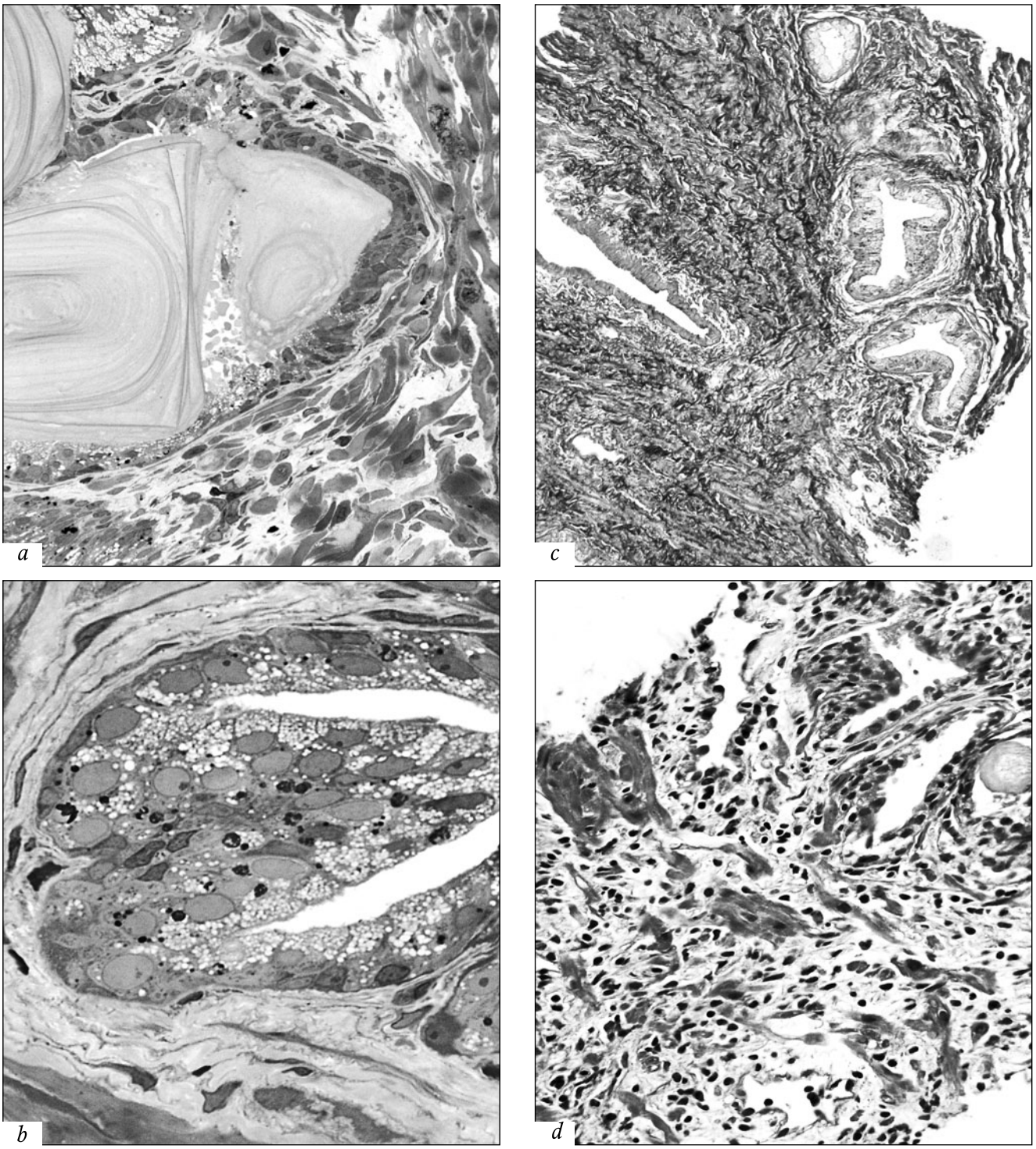

Fig. 1. Morphogenesis of chronic prostatitis in sexually-transmitted infections. Hematoxylin and eosin staining (*d*), van Gieson (*c*), azur II staining, semithin section (*a*, *b*); ×400 (*a*, *c*, *d*), ×1000 (*b*). *a*) formation of lamellar prostatic concrements in lumens of prostatic terminal compartments, flattening of cylindrical epithelium; *b*) numerous large autophagosomes in the main secretory cells; periglandular sclerosis; *c*) pronounced fibrosis of prostatic central zone; *d*) pronounced diffuse lymphocytic and plasmocytic infiltration of the prostatic gland.

structures containing floccular substance and heterogeneous secretory granules. These structures very often fused to form large electron-transparent conglomerations. Large fragments of the cytoplasm stemmed from the apical zones of some cells; they were seen in the lumens of terminal portions – visualization of the apocrine secretion process. The apical plasmalemma integrity was violated in some cells. Numerous vesicles and profiles of agranular and granular cytoplasmic reticulum, solitary small mitochondria were located

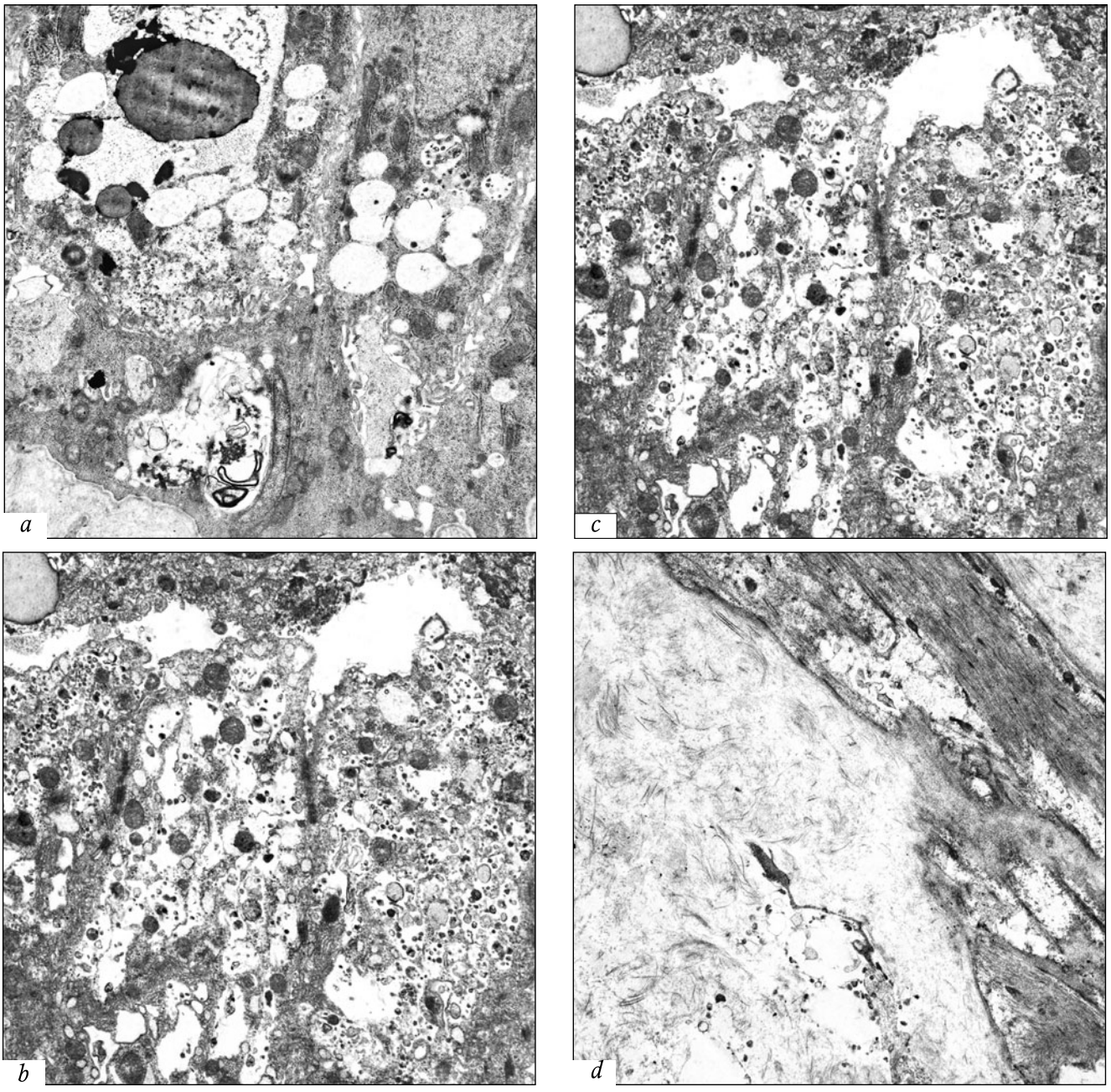

Fig. 2. Ultrastructural changes in the prostate in sexually-transmitted infections. *a*) large autophagic vacuoles in the basal zones of the main prostatic cells; mycoplasma penetration along lateral cell surfaces to basal membrane, ×12,000; *b*) accumulation of myco- and ureaplasmas heterogeneous by size and structure on the apical surface of main prostatic cell, ×15,000; *c*) secretory granules with heterogeneous electron-dense vesicular formations and membranous structures in apical zones of secretory epitheliocytes of the central prostatic glands, ×8000; *d*) focal lysis of sarcoplasma in prostatic smooth-muscle cells, ×8000.

under the secretory granules. A well-developed Golgi complex with unevenly dilated dictiosomes was as a rule found in the apical zone.

Large nuclei, containing mainly euchromatin, were located in the basal zones of the main secretory cells. Numerous small mitochondria were located round the nuclei. The cytoplasm contained numerous glycogen granules. Giant secondary lysosomes with osmiophilic incorporations and osmiophilic myelin-like structures formed in the basal zones of secretory epitheliocytes (Fig. 2, *a*).

Mycoplasmataceae family microorganisms (polymorphic globules of different size) were detected on the surface of secretory epitheliocytes. These microorganisms were enveloped in plasma membrane and contained finely dispersed floccular substance, some-

times few electron-dense granules (the size of ribosomes) and paired vacuoles of different sizes (Fig. 2, *b*). These microorganisms were detected in the patients whose secretion contained (according to PCR) mycoplasmas and ureaplasmas. *Mycoplasmataceae* microorganisms in the prostatic specimens were located on the surface of secretory epitheliocytes and contacted with them and also penetrated along the lateral membranes, reaching the epithelial layer basal membrane, where they were located between two neighboring cells (Fig. 2, *a*). These data indicated that mycoplasmas and ureaplasmas could penetrate in a retrograde mode via the ductal system to the main prostatic glands and persist there, contact with the secretory epitheliocytes, and penetrate into the depth of the epithelial layer, violating its integrity and promoting its destruction.

The structure of the secretory epitheliocytes of the intermediate and central (periurethral) glands was similar to that of the main prostatic cells, but their apical zones were smaller. Numerous polymorphic vacuole- and lacune-like structures (secretory granules) with heterogeneous electron-dense vesicular formations and membranous structures, morphologically corresponding to *C. trachomatis*, were located in these zones (Fig. 2, *c*). The secretory granules often fused to form large conglomerations. Osmiophilic lipid incorporations, dilated profiles of the granular cytoplasmic reticulum, small mitochondria, often subjected to myelin-like transformations, were often found between the secretory granules.

Large lipid incorporations and giant autophagosomes with lipofuchsin incorporations, in some cases released through the apical membrane, were often seen in the basal zones. Numerous small mitochondria and dilated profiles of the granular cytoplasmic reticulum were located round the nuclei.

The formation of giant autophagosomes and their accumulations in the secretory epitheliocytes of the main and central prostatic glands can be regarded as a specific feature of intracellular reorganization of these cells in chronic urethroprostatitis in the presence of STI. Autophagic processes started round the secretory granules located in the median and basal zones of the secretory epitheliocytes. Electron-dense (osmiophilic) depositions formed at the periphery of the secretory granules. Gradually they filled the entire volume of the granules, which eventually fused. Presumably, more autophagocytosis of epitheliocytes was stimulated by invasion of microorganisms, disorders in secretion synthesis and release.

According to modern concepts, chlamydia and myco/ureaplasmas penetrate into host cells for maintaining their vital activity. After penetrating into the cells chlamydia exhibit specific activity towards lysosomes, providing themselves the possibility of further multiplication in the cytoplasm. It is noteworthy that in parallel with the direct destructive effect of the agent on infected cells, toxicity intrinsic of all chlamydias is essential in the pathogenesis of urogenital chlamydiasis. On the other hand, penetration of mycoplasmas into eukaryotic cells, including epithelial cells, is still disputed. This possibility has been denied and is proven only for some mycoplasma species (for example, *M. penetrans*) [9].

Unfortunately, the compact osmiophilic bodies or incorporations in the autophagosomes could not be definitely characterized as chlamydia. But presumably, chlamydia and myco/ureaplasmas directly or indirectly (through disordering of secretion process) stimulated the formation of giant autophagic vacuoles which could be considered as one of the morphological criteria of a persistent infection. Transposition of autophagic conglomerations towards the apical surface and their release were noted in some cells. Presumably, intensification of epitheliocyte autophagocytosis was stimulated by microorganism invasion, cytoskeleton damage, and the resultant disorders in secretion synthesis and release.

Significant ultrastructural changes were found in smooth-muscle cells forming the prostatic stroma. Pronounced diffuse or focal lysis of myofilaments, focal degradation of organelles (mainly in the perinuclear zone) with formation of osmiophilic structures were seen in many leiomyocytes. Dilatation of granular cytoplasmic reticulum profiles, particularly in the subsarcolemmal zones, were often seen. Pronounced polymorphism of smooth-muscle cells (presence of atrophic and hypertrophic forms, new cells) was worthy of note.

We described significant impairment of the prostatic smooth-muscle carcass of this kind in other diseases – chronic bacterial prostatitis, benign hyperplasia, vibration prostapathy [2]. Stereotypical lesions and intracellular reorganization of the smooth-muscle cells in various diseases indicate common molecular mechanisms of destruction of the ultrastructures and involvement of the leiomyocytes in pathological processes. Smooth-muscle cells constitute the stroma for prostatic glandular epithelium and through paracrine regulation mechanisms modulate the epitheliocyte proliferation, differentiation, and apoptosis and the glandular structures morphogenesis [6,7,13]. The epithelial-stromal interactions play the key role in realization of the hormonal and cytokine regulation of morphogenetic processes in health and neoplastic transformations. Hence, not only phenotype changes (atrophy, hypertrophy) and injuries to smooth-muscle cells promote modification of the paracrine regulation of glandular epitheliocyte proliferation and differentiation, but epitheliocyte damage, death, and elimination

promote disorders in smooth-muscle cell proliferation and differentiation.

Hence, STI of the prostatic gland is associated with significant structural and functional reorganization of the two main compartments – prostatic glands and smooth-muscle carcass. Persistence of *Mycoplasmataceae* microorganisms in the terminal compartments of the prostatic glands and of *C. trachomatis* in epitheliocytes lead to destruction of the epithelial layer and manifest damage to secretory cells (destruction of organelles, stimulation of autophagocytosis). These events are paralleled by significant destructive changes in smooth muscle cells. The ultrastructural changes in epitheliocytes and smooth-muscle cells could be caused by direct interactions of microorganisms with the cells and by induction of inflammatory processes.

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