Changes in Chromatin Structure under the Effect of Intranuclear Bacteria with Antihistone Activity

V. Yu. Sokolov

UDC 576.809.7:576.315.4

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol.116, № 11, pp. 536-538, November, 1993
Original article submitted June 10, 1993

Key Words: *bacterial antihistone activity; cell nucleus*

To cope with host defense potential, microorganisms are known to select pecular immunodeficient sites, for instance, nonprofessional phagocytes or blood vessel walls [4,6]. The cell nucleus, however, is the most favorable strategic niche for bacteria. And though the nuclear genome possesses its own nonspecific means of protection from infection histones it is inferior to the defense potential of the cell. Detection of antihistone activity in bacteria has postulated the presence of such microorganisms in the cell nucleus, as was demonstrated at the light microscopy level [1]. Evidently, bacterial survival in chromatin has to influence its structure, particularly if the microorganisms possess genetically active "instruments."

The purpose of this study was to examine all the possible changes in chromatin structure under the effect of antihistone-active intranuclear bacteria.

MATERIALS AND METHODS

Human nasal mucosa epitheliocytes were examined which were bacteriologically analyzed and prepared for cytoscopy as described previously [1]. Cytoscopy was carried out by computer-aided television morphodensitometry [7]. The strains isolated during bacteriological analysis were identified to species and their antihistone activity to the total histone fraction (Sigma) and to fractions H1 and H2A+H4 was assessed as described previously [1]. H1 and H2A+H4 histones were obtrained as described previously [5]. The host cell nucleus was infected by inoculating a Hep-2 cell culture with a *Micrococcus luteus* strain with a total antihistone activity of 10/ml with respect to the total histone fraction and fractions H1 and H2A+H4. The Hep-2 cell monolayer grown on slides was washed free

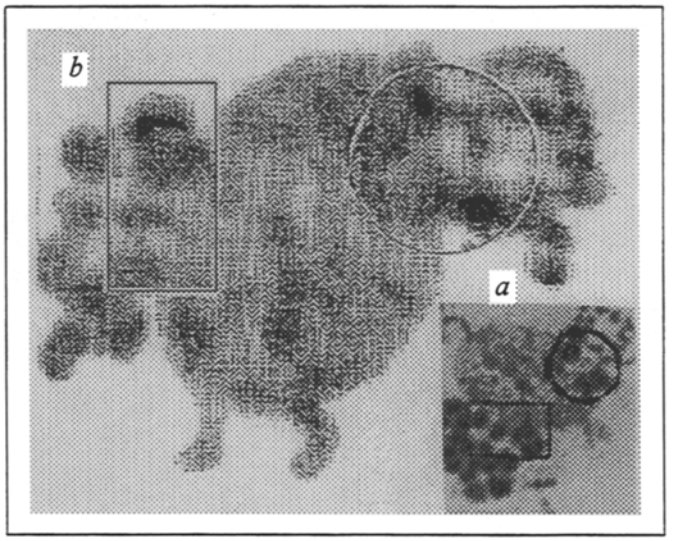

Fig. 1. Computer preparation of epitheliocyte nucleus by coding. *a*) initial image of epitheliocyte; *b*) computer image of epitheliocyte nucleus: bacteria marked with continuous black crosses are outside nuclear plane, those with intermittent crosses in interphase chromatin plane. Figures show analyzed sites of infected nucleus: *M.luteus* invasion (large cocci) in the circle, *M.luteus* adhesion in the rectangle. Manson blue staining. Magnification: *a*) objective 100, ocular 7; *b*) ×7500.

Department of Microorganism Persistence, Institute of Microorganism Ecology and Genetics, Urals Branch of the Russian Academy of Medical Sciences, Orenburg. (Presented by I. P. Ashmarin, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0011-1426$12.50

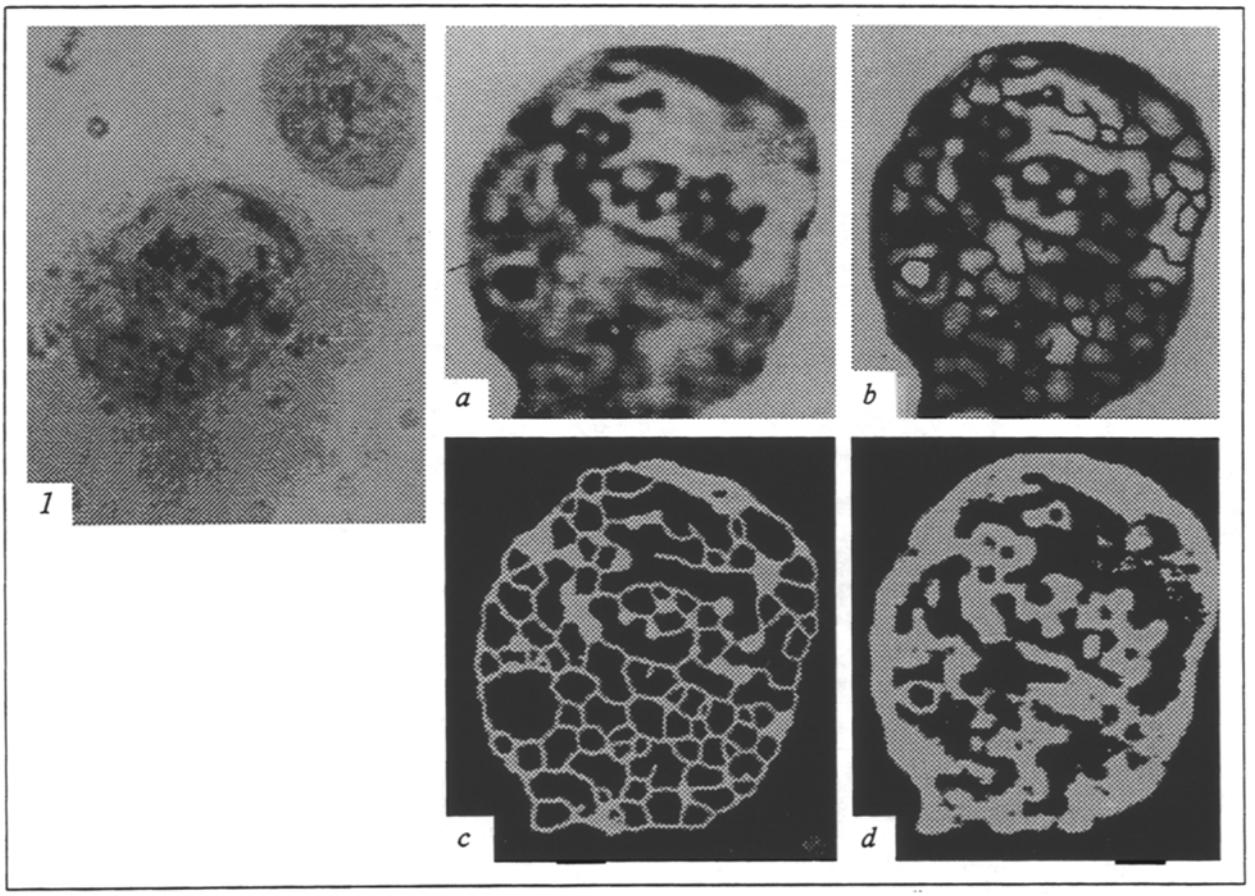

Fig. 2. Computer preparation of epitheliocyte nucleus by mathematical morphology. *1*) initial image of epitheliocyte; *2*) computer image of epitheliocyte nucleus: *a*) contrast image of a nucleus freed from the object background; *b*) nuclear pattern (initial image superposition; compact sites of chromatin and bacteria are colored black; the presence of bacteria (black) in chromatin texture nodes proves their integration in the nuclear genome); *c*) rough computer image of chromatin texture and intranuclear bacteria; *d*) fine computer image of chromatin texture (compact sites of chromatin and bacteria are colored white); the presence of bacteria in nuclear texture nodes proves their integration in the nuclear genome). Manson blue staining. Magnification: *1*) objective 100, ocular 7; *2*, *a*–*d*) ×7500.

of antibiotics before infection, placed in medium 199, and infected with the *M.luteus* strain in a dose of 1×10^8 CFU/ml. After a one-hour incubation at 37°C the cell layer was repeatedly washed in Hanks solution, the preparations were transferred to fresh maintenance medium, incubated for one more hour under the same conditions, and then fixed and stained as described above for epitheliocytes. The preparations were examined under a microscope with immersion using manual scanning from top to bottom at 1.5 μ intervals.

RESULTS

Bacteriological analysis of epitheliocytes detected *M.luteus* and *Corynebacterium pseudotuberculosis*. Antihistone activity of both strains was manifested both vis-a-vis the total histone fraction and with respect to fractions H1 and H2A+H4, its concentration being the maximal one used in the method, 10 mg/ml.

The integration of these microorganisms into the nuclear genome and incorporation in the chromatin structure are shown in Figs. 1 and 2. Figure 1 shows micrococci fusing with the interphase chromatin plane. This fusion occurs in the course of micrococcus invasion of the nucleus from top to bottom. It is clearly seen because the upper unintegrated portion of the bacterial cell is stained with continuous crosses (Fig. 1, *b*), whereas the portion embedded in the genome acquires the density of the chromatin and fuses with it. Figure 2, in which computer preparation of the cell is performed by the mathematical morphology method, shows the reticular structure of the chromatin and bacterial incorporation in the cell nuclear genome. Note the drastic enlargement of the nucleus (Fig. 2, *1*) with disordered reticular chromatin and a zone of nuclear hypocromasia in the infected portion of the nucleus. But the nuclear and the large size of the nucleus suggest an increase of the genetic material, possibly due to genome activation [2]. The mecha-

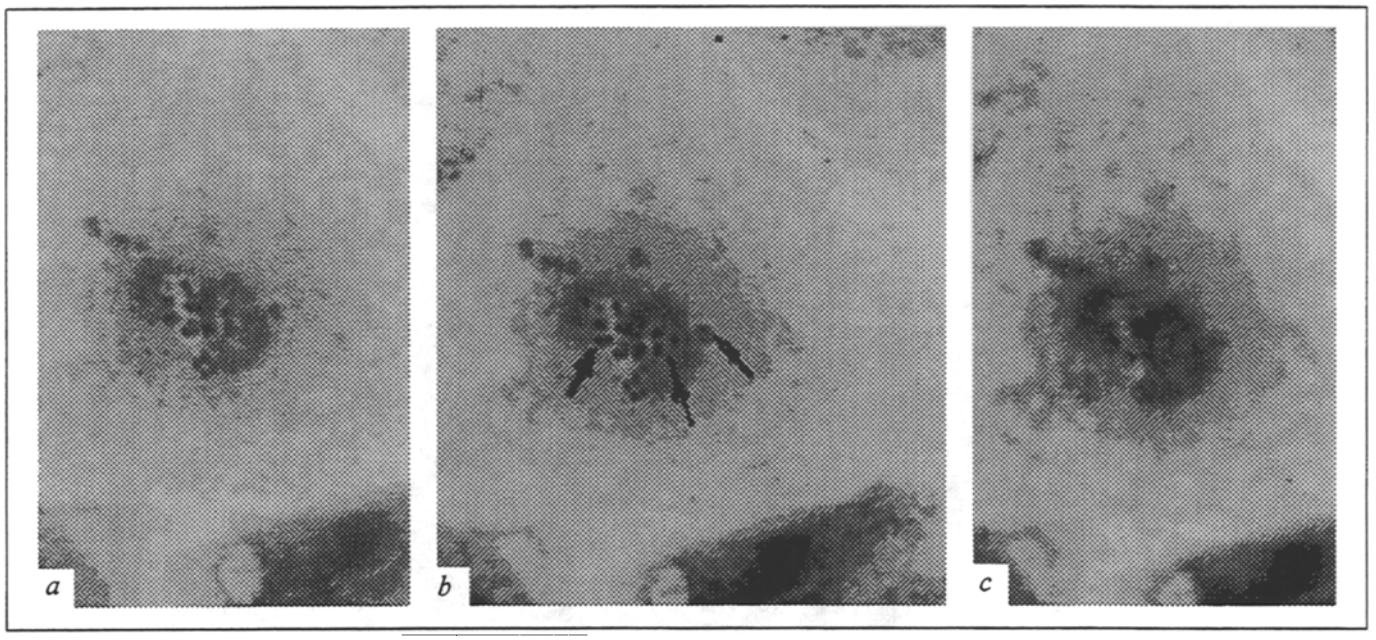

Fig. 3. Experimental infection of host cell nucleus (Hep–2) with antihistone–active *M.luteus* strain (manual scanning, 1.5 μ interval). *a*) top section of nucleus: group of bacteria with pronounced polymorphism clearly demarcated against background of nucleus; *b*) middle section of nucleus: individual colonies stand out more clearly (shown by arrows); c) bottom section of nucleus: bacteria not focused, as they are dispersed in the top–middle portion of the nucleus. Manson blue staining. Magnification: objective 100, ocular 7.

nism of this phenomenon may derive primarily from inactivation of linker H1 histone [3], in this case possibly due to antihistone-active intranuclear bacteria.

During experimental infection of host cell, nucleus disorders are seen not only in the nucleus, but also in the nucleolus, manifested by a zone of hypochromasia in its infected portion (Fig. 3). Manual scanning shows that the bacteria are mainly located in the central upper portion of the nucleus.

These results suggest necessitate further studies of interactions between genetically active bacteria and host cell nucleus.

REFERENCES

1. V. Yu. Sokolov, *Byull. Eksp. Biol. Med.*, **115**, № 2, 180-183 (1993).
2. E. A. Erenpreisa, *Chromatin Arrangement in the Interphase Cell Nucleus* [in Russian], Riga (1990).
3. V. G. Allfrey, V. C. Littau, and A. E. Mirsky, *Proc. Nat. Acad. Sci. USA*, **49**, 414-421 (1963).
4. A. I. Braude, *Microbial Perturbation of Host Defences*, London (1981), p. 13.
5. E. W. Johns. *Biochem J.*, **92**, 55-59 (1964).
6. H. Smith, in: *Bacterial and Viral Inhibition and Modulation of Host Defences*, London (1984), pp. 171-190.
7. A. V. Zhykotsky and E. M. Kogan, *Sov. Med. Rev. B. Physicochem. Asp. Med.*, **2**, 25-77 (1989).