Antihistone Activity of Bacteria

V. Yu. Sokolov

UDC 576.809.7:576.315.4

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 2, pp. 180–183, February, 1993
Original article submitted September 25, 1992

Key Words: *bacteria; antihistone activity; cell nucleus*

Histones are major nuclear proteins present in all eukaryotic organisms, playing a primary role in the structural organization of chromatin as well as in gene expression and activation [5-7]. It used to be believed that this was the major function of these evolutionarily ancient proteins of chromatin *in situ*. The bactericidic activity of histones reported in numerous publications was attributed to exogenous histones and was considered atypical for histones *in situ* [1-3]. Such a concept correlated with the core position of histones inside the DNA helix [8].

The present study was undertaken to examine the antihistone activity of bacteria (AHA) and the concomitant bacterial infection of the cell nucleus of the human organism.

MATERIALS AND METHODS

As material for the investigation we used epitheliocytes from the nasal mucosa of school-age children (100 children were examined). Epitheliocytes were taken with a cotton swab and transported to the laboratory in medium 199. The cells obtained were subjected to bacteriological and cytoscopic analysis. The bacterial strains were identified at species level and their AHA was determined according to the following methodology. The strains to be tested were cultured locally (on a area of 5-15 mm^2) on the surface of 1.5% meat-peptone agar (MPA) containing histones in a concentration of 0.8-8 mg/ml with an interval of 0.4 mg/ml (total histone fraction of Sigma was used). After a 20-24 h incubation at 37°C (1st stage) the bacterial strains were inactivated with chloroform for 20 min and the

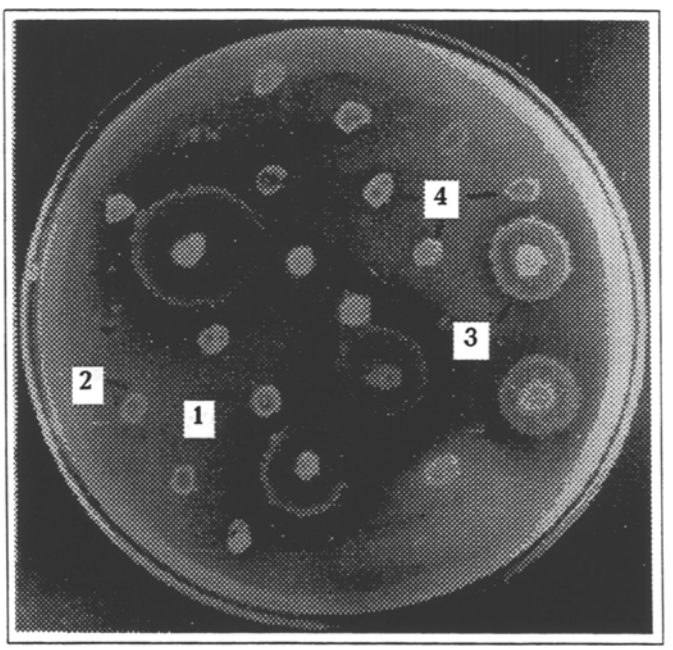

Fig. 1. Determination of antihistone activity of bacteria. Antihistone–active bacterial species (AHA+) inactivate the bactericidic activity of histones in the surrounding medium. As a result, a growth of the test culture is registered around the bacterial colonies. In case of antihistone–inactive (AHA–) strains, growth of the test culture is absent. 1) histone–containing nutrient agar (8 mg/ml); 2) local cultures of bacterial species; 3) antihistone–active bacterial species; 4) antihistone–inactive bacterial species.

Institute of Ecology and Genetics of Microorganisms, Russian Academy of Sciences

(Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

0007–4888/93/0002–0182$12.50

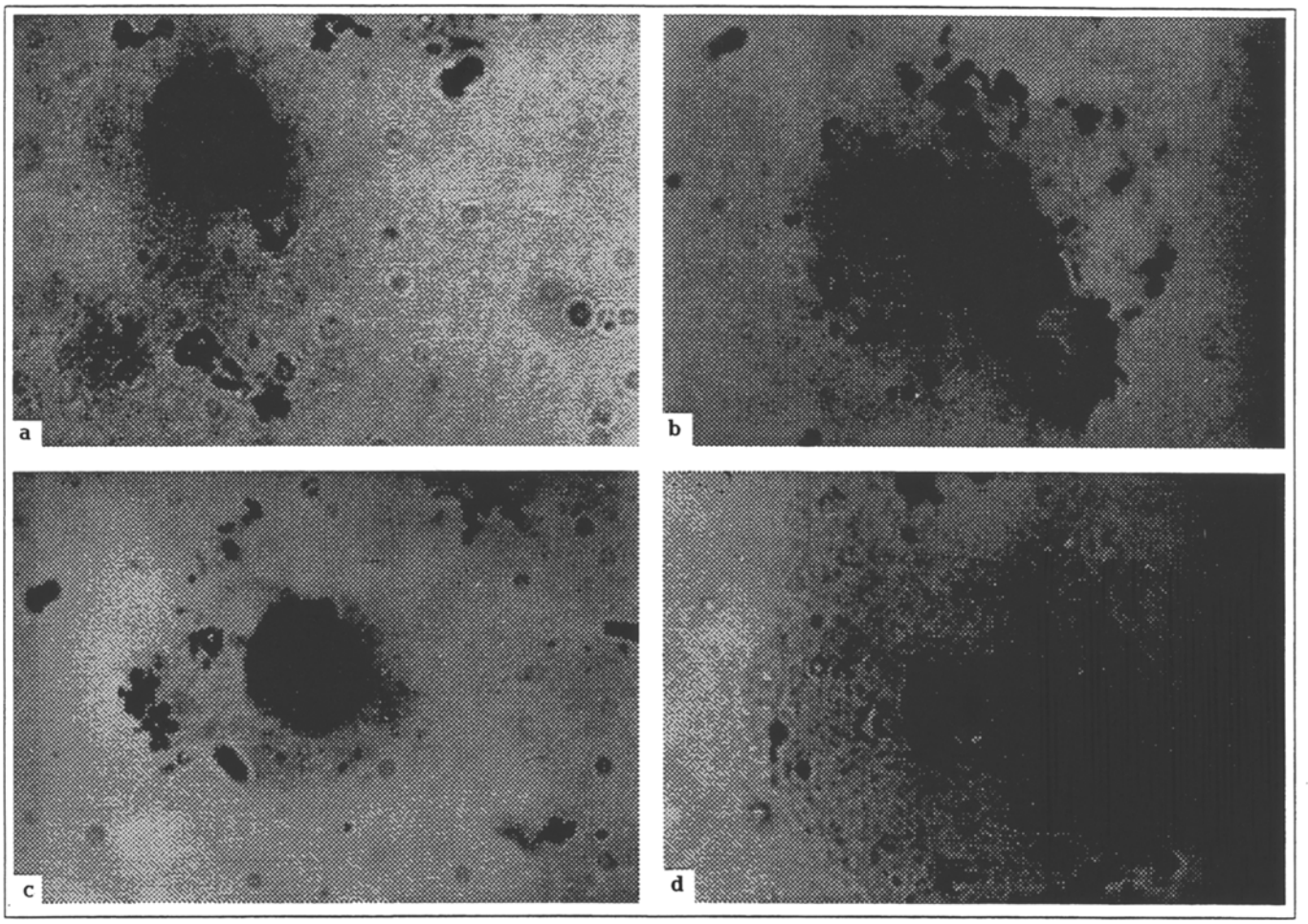

Fig. 2. Interaction of bacteria with nuclei of epitheliocytes from anterior nasal mucosa of children (cytoscopic method). *a*) invasion – *Corynebacterium*; *b*) adhesion and invasion – *Micrococcus*; *c*) intranuclear reproduction – *Neisseria*; *d*) intranuclear reproduction – *Staphylococcus*. An arrow indicates karyolemma; bacteria out of the limits of the optical section (image depth) are in parentheses. Staining with Manson Blue. Magnification ×700.

inactivated cultures were then placed onto 0.7% MPA containing a suspension of *Bacillus cereus* test culture.*

After a 16-24 h incubation the AHA of the bacteria was assessed from the growth behavior of the test culture around the colonies of the culture to be tested (2nd stage). The above method is illustrated in Fig. 1. In addition, we used an express modification of the method, enabling the investigation time to be reduced by one day provided that a sufficient quantity of the culture to be tested was available. In this case, we omitted a 24 h culturing of strains on histone-containing agar. Instead, their initial biomasses (1-2 ml) were incubated on the surface of the nutrient medium containing the same concentrations of histones (0.8-8 mg/ml with an interval of 0.4 mg/ml) for 30 min and then the second stage of the investigation was performed. For cytoscopic analysis, epitheliocytes were fixed on a cover glass with methanol for 5 min and stained with Manson Blue for 15 sec. To prepare the dye, 5 g borax (of chemical purity) were dissolved in 100 ml boiling distilled water. The dye was diluted 40 times directly before the experiment. The bacterial samples were examined under a light microscope under immersion.

* Any histone-sensitive bacterial strain can be used as a test culture: *Bacillus megaterium, Staphylococcus saprophyticus, Micrococcus lysodeicticus.*

RESULTS

The bacteriological analysis made it possible to identify 125 cultures. Their species spectrum is presented in Table 1. As follows from the table, the bacterial flora was represented by cocci of the genera *Micrococcus, Neisseria,* and *Staphylococcus* and bacilli of the genera *Corynebacterium* and *Acinetobacter.* In *C. pseudotuberculosis, M. luteus, N. sicca, S. lentus,* and *S. warneri* AHA was revealed with a 100% frequency and reached the highest level. Among the species *A.calcoaceticus, S.hominis, S.epidermidis, S.xylosus,* and *S.aureus* AHA was encountered with a lower frequency or was not detected at all and its value was much reduced. As is seen from the table, the AHA of the

TABLE 1. Characteristics of Bacterial Species (n = 125) Isolated from Anterior Nasal Mucosa of Children (n = 100) in Terms of their Species Affiliation and Antihistone Activity Compared by Different Methods

Bacterial species	Number of isolated strains		Number of AH-active strains				AHA range, mg/ml	
			method of determination				AHA in 24 h	
	abs.	%	2		1		2	1
			abs.	%	abs.	%		
Acinetobacter calcoaceticus	4	3.2	0	0	0	0	0	0
Corynebacterium pseudotuberculosis	4	3.2	4	100	4	100	8	8
Corynebacterium spp.	5	4.0	0	0	0	0	0	0
Micrococcus luteus	9	7.2	9	100	9	100	8	8
Neisseria sicca	4	3.2	4	100	4	100	8	8
Staphylococcus								
aureus	55	44.0	21	38.2	21	38.2	0–4	0–4
capitis	4	3.2	2	50	2	50	0–8	0–8
cohnii	8	6.4	5	62.5	5	62.5	0–8	0–8
epidermidis	8	6.4	2	25	2	25	0–3.2	0–3.2
hominis	8	6.4	0	0	0	0	0	0
lentus	2	1.6	2	100	2	100	8	8
sciuri	2	1.6	1	50	1	50	0–8	0–8
simulans	3	2.4	0	0	0	0	0	0
warneri	4	3.2	4	100	4	100	8	8
xylosus	5	4.0	0	0	0	0	0	0

Note: 0 – the method proposed failed to register any AHA, 8 – the highest level tested in determination of AHA of bacterial strains.

bacteria was equally determined by both the basic (two-day) and the express (one-day) method. Since both methods proved identical in terms of sign distribution and the AHA level, the express method is quite applicable for AHA determination in cases where there is a sufficient biomass of the culture.

Admittedly, the characterization of the bacteria according to their AHA does not correleate with the classical concepts on the taxonomic distribution of pathogenic properties among microorganisms, and therefore further in-depth research into the biological role of bacterial AHA should be performed. If we define AHA as a property of intranuclear bacteria, then it should be anticipated that microorganisms with a high AHA level such as *Micrococcus*, *Neisseria* and some species of *Staphylococcus* and *Corynebacterium* will be found primarily in epitheliocyte nuclei. This supposition was confirmed with the use of light microscopy: the cells with infected nuclei are shown in Fig. 2. The intranuclear bacteria are morphologically comparable to bacteria of the genera *Micrococcus* (Fig. 2, *b*) and *Neisseria* (Fig. 2, *c*); bacteria of the genera *Corynebacterium* and *Staphylococcus* were also detected (Fig. 2, *a*, *d*). Thus, all bacterial species with the highest AHA level were registered by light microscopy. Karyolemma is detectable on all micrographs and is in the same focus with the intranuclear bacteria. In addition, it is confirmed by the same image depth (0.3 μ at an objective aperture A=1.30) [4].

The following results should be emphasized. In the course of the study performed a new, earlier unknown, property of bacteria - their antihistone activity - was registered and investigation of this phenomenon was begun. The intranuclear distribution of the antihistone-active species was studied by light microscopy. Proceeding from the biological role of histones in the structural and functional organization of chromatin, we deem it appropriate to investigate this phenomenon on the biochemical, clinical, and ecological level it being essential to evaluate the effect of bacterial AHA on the genetic apparatus of the host cell.

REFERENCES

1. S. A. Anatolii, V. N. Kokryakov, M. I. Yunusova, *et al.*, *Zh. Mikrobiol.*, № 3, 71-74 (1977).
2. I. P. Ashmarin and S. M. Zhdan-Pushkina, *Zh. Mikrobiol.*, № 9, 103-108 (1966).
3. S. M. Zhdan-Pushkina, E. L. Avenirova, I. A. Arkhipchenko, *et al.*, *Antibiotiki*, **17**, № 12, 1080-1085 (1972).
4. K. Mikhel', *Principles of Microscope Theory* [in Russian], Moscow (1955).
5. V. L. Karpov, O. V. Preobrazhenskaya, and A. D. Mirzabekov, *Cell*, **36**, 423-431 (1984).
6. R. D. Kornberg, *Science*, **184**, 868-871 (1974).
7. V. V. Kozlov and G. P. Georgiev, *Nature*, **288**, 245-247 (1970).
8. A. Mirzabekov, V. Shick, A. Belyavsky, *et al.*, *Proc. Nat. Acad. Sci. USA*, **75**, 4184-4188 (1978).