Congenital Immune Defense of the Hair

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Studies of two recent decades provided ample data on antibacterial peptides protecting animal and human epithelium of different types; however, no reports about the presence of these compounds in hair keratinocytes appeared up to the present time. Peptides were extracted from specimens of normal intact hair with citric acid solution in 50% ethanol. Antibacterial activity of the resultant extracts was evaluated on *Candida albicans* test culture by staining and microscopy, by evaluation of growth inhibition zones, and by inoculations. Direct contact with the extract destroyed the greater part of *Candida albicans* cells up to complete destruction of membranes. Application of the extract to dishes with agar with *Candida albicans* led in the formation of apparent zones of growth inhibition. The percentage of killed cells increased with prolongation of incubation with the extract. Electrophoresis of hair extract showed bands characteristic of antibacterial peptides RNase, psoriasin, and β-defensins. Removal of the peptides from the extract by filtration through membrane filter led to loss of its activity. The results indicate that human hair keratinocytes possess congenital antibacterial immunity.

Key Words: *hair; keratinocytes; antibacterial peptides*

Antibacterial peptides (ABP), a family of more than 500 compounds protecting various epithelial tissues, are an important factors of congenital local immunity [2,7]. We previously discussed the structure and function of ABP secreted on the skin surface [1]. However, we failed to find data on the chemical defense of the skin appendages hair and nails. On the other hand, they are liable to be infected by numerous microorganisms with keratinase activity, including representatives of normal skin microflora, *e.g.* staphylococci and propionic bacteria. Obviously, the role of fatty acids and chlorine ions in protection of skin appendages is minimum, and we have to hypothesize that endogenous ABP play the key role here.

We tested the presence of ABP in normal hair cells and evaluated their activity.

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MATERIALS AND METHODS

Hair specimens were collected from 5 women aged 7-50 years using no chemical dyes and cold waving. Freshly washed hairs were cut at a distance of no less than 10 cm from the roots. The hairs (360 mg) were fragmented with scissors and grinded in a mortar to complete homogeneity with 8 ml of 0.1 M citric acid solution in 50% ethanol, which was added by drops [4]. The resultant cell-free homogenate was centrifuged (7 min, 10,000 rpm), the supernatant (3 ml) was dried in a Petri dish at 27°C overnight. Dry extract was washed with 0.5 ml potassium phosphate buffer (pH 8.2) and again centrifuged; the final pH of the solution was 6.5 (this solution is further referred to as the experimental solution). The initial solution of citric acid, processed according to the same protocol, served as the control.

Candida albicans strain No. 927 from the collection of I. I. Mechnikov Institute of Vaccines and Sera

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served as the test culture. The culture was grown in glucose/peptone/yeast medium at 27°C for 48 h.

Antibacterial activity (ABA) was studied by 3 methods: detection of viable cells by microscopic examination and by inoculation and evaluation of growth inhibition zone (GIZ). For microscopic examination, 1 loop (1 mm in diameter) of the test culture was diluted in 1 ml potassium phosphate buffer (pH 4.6) and 100 µl suspension was mixed with 80 µl experimental solution. The mixture was incubated (2 h, 32°C), bromocresyl purple (800 µl of 2 mM solution in the same buffer) [6] was added, and the mixture was incubated (1 h, 32°C), after which the cells were separated by centrifugation at 5000 rpm (5 min), examined under a microscope (×1750), and photographs were made with a Sony DSC-W7 camera.

For evaluation the ABA by the inoculation method, one loop of the test culture was diluted in 1 ml sterile distilled water and 10 µl of cell suspension was mixed with 3 ml potassium phosphate buffer (pH 5.5); the final concentration of cells was 10^3 PFU/ml. The resultant suspension (20 µl) was then mixed with 80 µl experimental solution and 20 µ mixture was inoculated in 6-cm Petri dishes with glucose/peptone/yeast medium directly and after a certain time intervals. The dishes were incubated for 48 h at 27°C, after which the colonies were counted.

In order to evaluate GIZ, the test culture was inoculated (50 PFU/ml) into warm melted medium, poured into 2 Petri dishes (6 cm), and left to solidify and dry. Experimental or control solution (20 µl) was then applied to the center of the dishes. The dishes were incubated at ambient temperature for 4 days, after which photographs were made.

The proteins in the experimental sample were separated by electrophoresis with sodium dodecylsulfate in 5-20% PAAG gradient. The sample was prepared under non-denaturing conditions; 1 volume of the sample was mixed with 2 volumes of the buffer and the resultant mixture (40 µl) was applied to the track. Silver nitrate staining was carried out. The LMW mixture (Amersham) served as the molecular weight reference.

RESULTS

The ABP were extracted from hair cells in a solution used for the same purpose with skin scales [4]. The resultant acid extract contained an admixture of keratin (high molecular weight protein, the main protein of keratinocytes) even after centrifugation. After neutralization of this extract with alkaline buffer, the preparation was repeatedly centrifuged for elimination of keratin admixture.

Microscopic examination of the test culture treated with hair extract showed that almost all cells remained viable after treatment with control solution, *vs.* almost no living cells in experimental sample (Fig. 1). Most cells were killed; we observed not only membrane destruction (a typical ABP effect) [1], but also cell walls were destroyed, which made impossible to determine the percentage of dead cells.

Treatment of the test culture with hair extract and subsequent inoculation showed that the suspension

TABLE 1. Dynamics of Cell Death during Incubation of the Test Culture with Hair Extract ($M\pm m$; n=5)

Exposure, h	% of dead cells
0	0
0.5	20.7±8.3
1	32.6±7.3
3	46.7±5.3

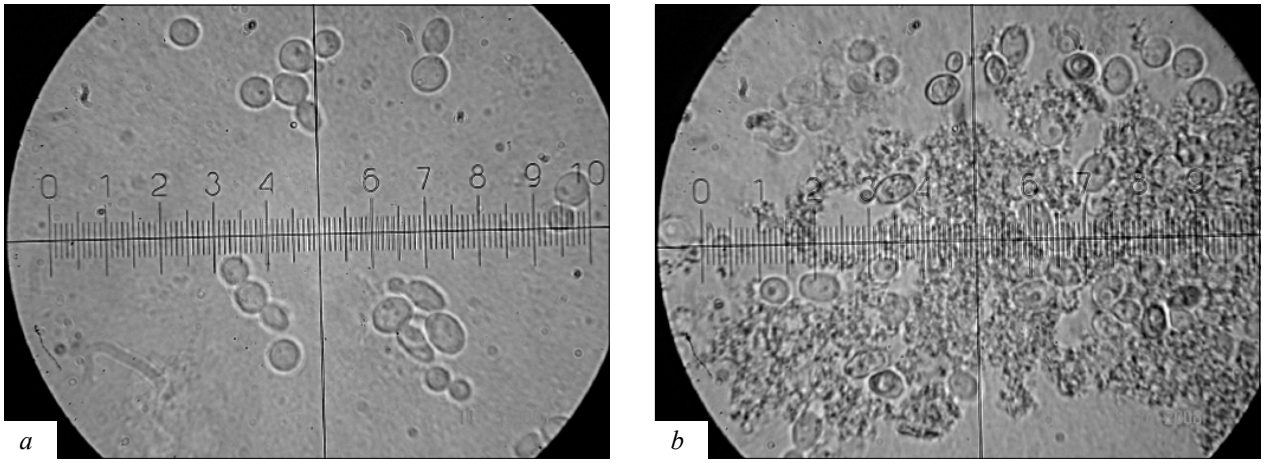

Fig. 1. Treatment of *C. albicans* test culture with hair extract. *a*) intact cells; *b*) destroyed cells.

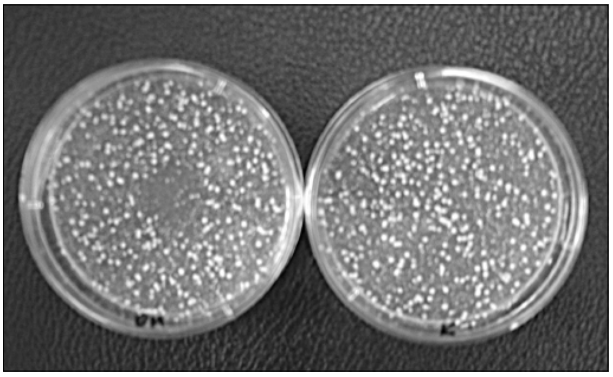

Fig. 2. Evaluation of ABA in hair extract by GIZ method on *C. albicans* test culture.

initially containing 200-300 living cells/20 μ lost up to 52% cells within 3 h (Table 1). Cells treated with control solution completely retained their viability.

Studies by the GIZ method showed clear zones 15 mm in diameter in the experimental, but not in control dishes (Fig. 2).

Hence, ABA of hair extract was proven by three different methods. Since the extraction method implies the extraction of peptides, it was just logical to expect a relationship between their presence and ABA. We therefore separated the hair extract in PAAG gradient (Fig. 3). The extract really contained low-molecular proteins (Fig. 3). The most clear-cut band corresponded to a molecular weight of 14.4 kDa. Judging from this parameter, it seemed most likely to be RNase (ABP constitutively expressed in skin keratinocytes) [5]. The band next by intensity corresponded to molecular a weight of about 23 kDa, but there seemed to be no peptides with this weight among the known skin ABP. Numerous bands were seen from 12 to 3 kDa, which could correspond to psoriasin (11.4 kDa) [3] and β-defensins (3.5-4.5 kDa) [2].

Filtration of the hair extract through membrane filter with pores corresponding to 3 kDa showed the absence of anticandidal activity of the resultant filtrate.

Hence, normal hair extracts are characterized by antibacterial activity inducing lysis of microorganism membranes. Antibacterial effect of hair extracts is due to the presence of intracellular ABP. Hair keratinocytes, similarly as skin keratinocytes, contain endogenous peptides playing an important role in congenital protection of the hair from keratinophilic microorganisms.

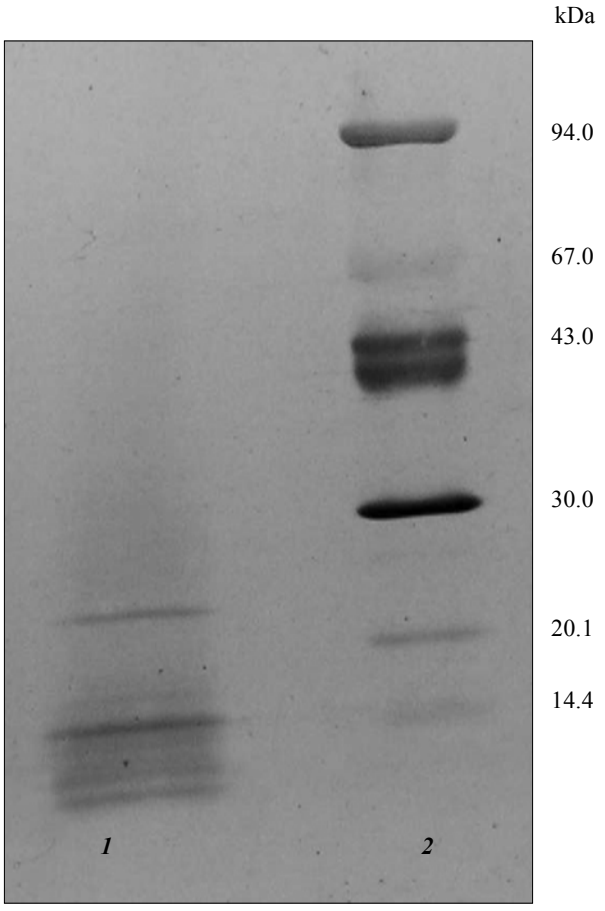

Fig. 3. Separation of hair extract peptides in PAAG gradient. *1*) hair extract; *2*) low-molecular weight markers.

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