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IMMUNITY OF TICKS

ИММУНИТЕТ ИКСОДОИДНЫХ КЛЕЩЕЙ

V.M.Podboronov*, A.M.Podboronov**
В.М.Подборонов*, А.М.Подборонов**

*Gamaleya Research Institute for Epidemiology and Microbiology of RAMS, Gamaleya st., 18, Moscow 123098 Russia
**Pensa Pedagogical University, Pensa 440000 Russia
*Институт эпидемиологии и микробиологии им.Гамалеи РАМН, Москва, ул.Гамалеи, 123098 Россия
**Пензенский педагогический университет, г.Пенза, 440000 Россия

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ABSTRACT

A concept of availability in ticks a complex of protective mechanisms enabling their existence in nature as biological systems, was put forward and grounded. In this connection there were studied both specific and non-specific antibacterial factors in tick organism. The role of tick lysozyme and other protective factors in the system «microorganism-tick» was demonstrated.

РЕЗЮМЕ

В настоящей статье выдвигается и обосновывается идея наличия у иксодоидных клещей комплекса защитных мезанизмов, обеспечивающих их существование как биологических систем. С этой целью изучены специфические и неспецифические антибактериальные факторы в организме клещей. Показана роль лизоцима и других защитных факторов в системе микроорганизм-клещ.

INTRODUCTION

The knowledge of protective reactions of blood-sucking ticks is necessary for study of their interactions with pathogenic microorganisms. Development of new biological methods of control, application of biological preparations for the control of ticks is only possible if there is a deep and comprehensive understanding with regard to what protective reactions are inherent in the blood-sucking ticks and against what bacteria they are aimed, and what is the mechanism of their immunity. There are just a few papers devoted to the protective reactions of ticks. The major investigations with these insects were conducted by Alekseyev [1971], Alekseyev and Kondrashova [1985]. The attempts to determine antibodies in hemolymph were not successful [Bernheimer, 1952; Krieg, 1957; Stephens, 1963].

Up to now there were no data concerning the detection of some cellular structures which might bear the function of the specific antibody producers.

The purpose of the present investigation was to reveal immune reactions of tick organism to the secondary introduction of phage FX-174, to study the role of pH in the development of microorganisms, to reveal bactericidal effect of lysozyme on microorganisms and the phenomenon of phagocytosis.

MATERIALS AND METHODS

In the experiments there were used 18-24 h agar cultures of *Micrococcus lysodeikticus* 2665, *Staphylococcus aureus* 209, *Streptococcus pyogenes* T 1M, *Corynebacterium diphtheriae* 2841, *Escherichia coli* WP-2, *Salmonella typhimurium*, strains LT-2, *Listeria monocytogenes* 9-127, *Yersinia pseudotuberculosis* 114, *Francisella tularensis* B-15 (vaccine strain), egg cultures of *Rickettsia prowazekii* (strain Breinl), *Rickettsia canada* (strain 2678), and *Rickettsia sibirica* (strain Netsvetaev). Viral group was represented by phage FX-174.

The following species of tick were taken: *Alveonasus lahorensis* (Neumann, 1908), *Ornithodoros moubata* (Murrey, 1877), *Ornithodoros papillipes* (Birula, 1895), *Hyalomma asiaticum* P.Sch. et Schl., 1929, and *Ixodes persulcatus* Schulze, 1930.

Lysozymes were isolated from ticks by method of specific sorption on chitine [Revina, Podboronov et al., 1977]. Study of the influence of pH on growth and development of microorganisms, of the phenomenon of phagocytosis, bactericidal effect of lysozyme on microorganisms, presence of antibodies in tick organism was performed according to the methods carlier described by us [Podboronov et al., 1975, 1982, 1988, 1993].

RESULTS

It was found that after single introduction of phge into hemolymph of ticks *Alveonasus lahorensis* it remained detectable there for the long term of observation. By the end of day 6 after phage introduction into ticks the number of phage pertricles was reduced in their organism to 2×10^4, on day 12 — to 1.1×10^2. The number of phage particles was 460 and 25 respectively on day 18 and 24, and it was only on the elapse of day 30 that phage was not detectable in tick organism. The dynamics of phage FX-174 persistence was practically the same when

these ticks were repeatedly infected with the phage 3-4 weeks after the first infection. The population of phage particles by the end of day 6 went down to 2.5×10^4. On days 12, 18, 24 the numbers of phage particles from these ticks made respectively 1350, 510, and 46, whereas on day 30 the phage could not be passaged. In control group of ticks not infected with virus, phage plagues were not defectable. Thus we have not detected in ticks the antibodies to the secondarily introduced phage. This fact may be regarded as an evidence of the lack in arthropods of humoral immune responce which is characteristic of warm-blooded animals. It is likely that responce in ticks is not connected with specific humoral factors of defence and are determined by non-specific factors (phagocytosis, fermentative degradation, medium pH, etc.) [Podboronov et al., 1982, 1991, 1993].

In the course of study of the role of hydrogen ions concentration in gut and hemolymph of ticks in the process of their growing and development it was found that pH value of gut, hemolymph and homogenates of engorged non-infected ticks grows on the average by 0.1, and by 2 and more in ticks *A.lahorensis, O.moubata, O.papillipes* released from staphylococcus 48–72 h after their infection. Tularemia agents as well as listerias turned out to be more resistant to pH changes. They were preserved in tick organism up to 25–30 days when ticks *Hyalomma asiaticum* were infected with these microbe, the response was less similar. Staphylococci persisted in the tick organisms up to 5 days, whereas tularemia bacteria and listeria were inoculable within the whole term of observation, though their amount was significantly reduced, not reaching the initial level. Thus the increased alkalinity of the medium of tick gut and hemolymph may cause teir active bactericidal effect [Podboronov V., Podboronov M., 1993].

One of the factors of natural resistance of ticks is lysozyme, the bactericidal effect of which has been demonstrated with regard to a number of microorganisms. Comparative study of fermentative activity of tick lysozyme by turbodimetric method and by diffusion in agar has revealed the highest activity of lysozyme of *O.moubata* — 29200 U/mg, the activity of lysozyme ticks *A.lahorensis, O.papillipes,* and *H.asiaticum* being respectively, 25000, 19400, and 11200 U/mg.

While studying the mechanism of lysozyme action on models *M.lysodeikticus, E.coli* WP-2 by turbodimetric technique it was found that lysozyme showed lytic action on *M.lysodeikticus*, not showing it on *E.coli* WP-2. Lytic action with regard to *E.coli* WP-2 was only noticed in combination of lysozyme and EDTA.

Comparison of antibacterial activity of tick lysozymes on microorganisms (*M.lysodeikticus* 2665, *St.aureus* 209, *St.pyogenes* T1M, *E.coli* WP-2, *Cor.diphtheriae* 2841, *S.typhymurium* LT-2, *L.monocytogenes* 9-127, *Fr.tularensis* B-15, *Y.pseudotuberculosis* 114) revealed that antimicrobial activity of argasid ticks (*A.lahorensis, O.papillipes, O.moubata*) 2–4 times exceeds that of egg lysozyme and lysozyme of ticks *H.asiaticum, I.persulcatus.*

Study of antirickettsial activity of tick lysozyme in concentration of 15 mg/ml on *R.prowazekii, R.canada,* revealed a clear antirickettsial activity of *O.moubata* lysizyme with regard to *R.prowazekii, R.canada* and none — on *R.sibirica.* Lysozyme of ticks *A.lahorensis, O.papillipes, H.asiaticum* did not shaw any effect on quantitative values of infectivity of rickettsial suspensions [Podboronov et al., 1978].

Thus, we were the first to have determined the role of lysozyme in tick-microbe interactions. For the first time, based on biological action of lysozyme of ticks on microorganisms, there were demonstrated both specific and non-specific interactions agents. Being one of the factors of natural resistance of arthropods, lysozyme plays a certain role in regulation of tick-microorganism interactions.

In the course of studies of biological relations of ticks and microorganisms in invivo tests we managed to observe the same regularities as in invitro experiments. Bacteria of facultative-transmissive group (*Fr.tularensis, L.monocytogenes*) survived in argasid tick organism for 15–30 days, whereas in *H.asiaticum* — for the whole period of obsrvation. Purification of ticks *A.lahorensis, O.papillipes, O.moubata* from micricoccus, staphylococcus, diphtheria agent and colibacillus occured after 24-72 h. In ticks of the above species infected with bacteria lysozyme production increased by 10–16 fold, in ticks *H.asiaticum* there was 5–6 times increase observed.

We observed the phenomenon of phagocytosis in hemolymph of *O.papillipes* infected with salmonellas. The amount of prohemocytes, immature and mature plasmatocytes and spherular cells was noticed to grow. Basing on morphologic study data we considered these cells as mature plasmatocytes.

The phenomenon of phagocytosis was observed as soon as 2 hours after the bacteria introduction. In the smears there were found some cells that injested salmonellas. The destruction of salmonella bacilli was clearly seen by the increase of their size, washed out and lightened character of their cytoplasm. In paralel with phagocytosis there occured a process of increase of lysozyme concentration in hemolymph.

DISCUSSION

We studied antibacterial factors being formed in organism of ixodoid ticks and mechanisms of their action on some microorganism species: rickettsia, salmonella, listeria as well as agents of pseudotuberculosis, diphteria and tularemia (vaccine strain B-15, colibacillus, staphylococci and streptococci). Reliably vast experimental material was sufficient for thorough study of a problem of specific and non — specific factors regulating

interactions of ixodoid ticks and bacteria. For the first time antibacterial mechanisms of ixodoid ticks, regarding various microorganism species, were revealed and studied. Interrelations between ixodoid ticks and microorganisms belonging to taxonomically distant species, such as rickettsias-abligate intracellular parasites, agents of transmissive and nontransmissive infections, were studied. From various tick species lysozyme was first isolated, and fermentative activity of this substance as well as the spectrum of its action was studied. On the basis of the obtained data the role of lysozyme as a leading factor of tick organism protection in case of invasion was cleared up. Other factors of tick organism protection were also discovered, such as phagocytosis and changing concentration of hydrogen ions in the contents of gut, hemolymph and their role in growth and development of microorganisms infecting tick organism was shown. A concept of the availability in ticks of a complex of non-specific factors of antibacterial protection was formulated.

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