Interaction of *Paramecium caudatum* and Picornaviruses

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Abstract In our paper we have researched the relationship between picornaviruses (poliovirus, foot-and-mouth disease virus and encephalomyocarditis virus) and Ciliata (*Paramecium caudatum*). We show that the number of *Paramecium* in medium sharply increased during coincubation with picornaviruses within 2–5 days. This cannot be explained only by the fact that viruses were nutrient source for *Paramecium* because in case of inactivated viruses the number of infusorians in medium increased a little. At the same time the titer of viruses harshly decreased whereas in the control group, which is free of *Paramecium*, the fall of titer was little. Picornaviruses were eliminated from medium if only living *Parameciums* were present in medium. After 7–9 days of coincubation only a few number of viruses were liberated from destroyed *Parameciums*. These results will be especially useful for management of reservoirs of picornaviruses in water and prevention of diseases.

Keywords Ciliate · *Paramecium caudatum* · Picornavirus

Compared extensive information in the biology and genetics of viruses shows that there is only a limited understanding of the occurrence and distribution of viruses in protozoan ecosystems, and of the relationships between viral and host communities in the natural environment. Free-living protozoans generally and ciliates particularly, widely occur in the

biosphere in both temperate and arid regions and are thought to be globally ubiquitous [7]. They populate soils and sediments, and suspend aggregates in fresh and marine waters, roots and surfaces of plants, and bodies of animals. Moreover, they are dispersed by the movement of water and wind [15].

Epidemiological investigations suggest that only in rare cases of animal movements were directly involved in the transmission of picornaviruses. Therefore, a key issue in the design of control measures for multi-host viruses is defining reservoirs of infection and developing guidelines for their identification.

Many infectious agents, especially those that cause emerging diseases, infect more than one host species. The management of reservoirs of multi-host pathogens often plays a crucial role in effective disease control. As mentioned above, both foot-and-mouth disease virus (FMDV) and encephalomyocarditis virus Columbia-SK strain (EMCV-CSK) have a wide host range and the not vertebrate's reservoirs of these viruses are poorly investigated [2, 13]. Currently polio virus (PV) (Sabin) has an extremely high ratio of presence; this is due to the worldwide eradication of poliomyelitis by massive oral polio vaccine (Sabin) immunization carried out by the World Health Organization [17, 20]. Although the spreading pathways of PV in nature were described enough but there is a big lack concerning their distribution by not vertebrate's.

Protozoa could have an important role in viral epidemiology and such actions are beginning to be understood. Protozoa may affect on virus levels by grazing virus particles. In the marine environment, some protozoans were found to ingest viruses [9]. The ciliates are interesting because they can be found in most freshwater habitats, including engineered ones [6].

Current research is useful for understanding the ecology of widely distributed ciliate-virus systems in nature. The

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most interesting aspect of the free-living ciliate-virus system is the viral ecology. The fast replication of free-living infusorians, their non-durable generation, and their ability to produce stable forms are epidemiologically important factors that can aid the long-term preservation of viruses in the environment. Lastly, another aim of our research is to study the possibility of infection of free-living ciliate by the viruses from the family of picornaviridae, and to reveal the role of infusorians as a reservoir for viral infections.

Encephalomyocarditis virus Columbia-SK strain, PV-1 (Sabin) and foot-and-mouth disease virus-O (FMDV-O) were used at multiplicity of infection 5.0 log TCD₅₀/ml. Viral titers in TCD₅₀/ml were calculated by the method of Kärber. The titers of the both control group of viruses in free Losina-Losinsky solution and the coincubated viruses with *Paramecium* in the same solution were calculated on cell lines (RD, HeLa and BHK-21) at 24, 48, 72, 96, 120, 144, 168, 192, 216 h of incubation at 22 ± 2 °C.

RD, HeLa and BHK-21 cells were cultured in Eagle's medium supplemented with 10 % bovine serum and antibiotics at 36–36.5 °C.

Parameciums were grown at 22 ± 2 °C in physiological, in standard inorganic salt solution (Losina-Losinsky solution).

Three different samples of viable *P. caudatum* (10 cells/ml)-EMCV-CSK (10⁵ TCD/ml), *P. caudatum* (10 cells/ml)-FMDV-O (10⁵ TCD/ml) and *P. caudatum* (10 cells/ml)-PV-1 (Sabin) (10⁵ TCD/ml) mixtures in Losina-Losinsky solution were prepared for incubation at 22 ± 2 °C. Paralleled a protozoan-free controls were also prepared. Samples were assayed at 0, 24, 48, 72, 96, 120, 144, 168, 192 and 216 h after coincubation.

Inactivation of the viruses was done by the incubation at 60 °C during 75 min. The doses of inactivated viruses were the same as described above. The samples of inactivated viruses with *P. caudatum* (10 cells/ml) were assayed at the same times as previously.

The inactivation and destruction of *P. caudatum* were took place by three times by freeze/defreeze procedures. The number of destroyed infusoria was used starting from the average number of *P. caudatum* after receiving plateau in control group—25–27 cells/ml.

All experiments were repeated six times and the summarized mean data is presented. All statistical analyses were performed with Student's *t* test using the SPSS version 13.0 software package (SPSS Inc., Chicago, IL, USA).

As background for investigating any direct effects of *Paramecium* on the picornaviruses, the maintenance of virus titers, or infectivity, in solutions used to grow and manipulate *Paramecium* was tested. Investigated picornaviruses were still infective during all investigation period in the medium which was used for the grow or maintenance of the ciliates (data shown on Fig. 1). Titers of EMCV,

FMDV and PV-1 decreased respectively up to 4–4.75 log, 4.0 log and 4–4.25 log in medium at 144 h. Moreover, at 216 h titers of EMCV, FMDV and PV-1 decreased up to 4.25 log, 3.5 log, and 4.0 log respectively.

Fig. 1 Removal of picornaviruses from medium by live *Paramecium caudatum* as the coincubation time with the viruses increased. Coincubations of viruses were begun in Losina-Losinsky solution. **a** PV-1 (Sabin) titers at coincubation with *Paramecium*, **b** EMCV-CSK titers at coincubation with *Paramecium*, **c** FMDV-O titers at coincubation with *Paramecium*. Y axis, viral titers in log₁₀; X axis, hours of experiment

The infectivity of EMCV, PV and FMDV declined as the time of incubation with *Paramecium* increased (Fig. 1). When the mixture of virus and *Paramecium* was centrifuged and the top layer was assayed for infectivity of picornaviruses, the virus titer declined steadily with incubation time (Fig. 1). Picornaviruses were inactivated in the free medium in which *Paramecium* had been grown with low speed. This suggests that the elimination of viruses was not a sequence of the activity of antiviral components of medium. The decrease of viral titers began at 24 h after coincubation with *Paramecium*. The most decrease of viral titers was to be observed in PV (2 log and more). EMCV and FMDV are more stable than PV (decreased about 1.5 log). As shown in figure significant difference in titers was observed in control group and in virus titers after coincubation with *Parameciums*.

The infected *Parameciums* were collected by centrifugation, and after three freeze-thaw cycles the viruses were cumulated for investigation. When all *Paramecium* were lysed, a few infective viruses were released in all cases (FMDV, PV-1 and EMCV) (Fig. 2) and difference with control groups was significant.

When the ciliate from 144 to 216 h coincubations were collected by centrifugation and then resuspended in medium without viruses and incubated for further 48 h, the titer became even lower or the viruses totally disappear. Overall, the results suggested that *Paramecium* actively removed and inactivated picornaviruses from the coincubation medium.

Figure 3 shows that *Paramecium*, in a mixed with picornaviruses cultures attain higher levels than separately. The amount of infusorians shows the difference with control group beginning at the second day of coincubation until fifth day. At 48 h coincubation of FMDV-O with infusorians, the amount of *Parameciums* in control group was 10.5 ± 0.71 and in test group was 25.3 ± 4.8 ($t = 3.05$, $p < 0.05$). At 72 h coincubation of EMCV-CSK with infusorians, the amount of *Parameciums* in control group was 11.0 ± 1.8 and in test group was 74.6 ± 15.4 ($t = 4.10$, $p < 0.01$). At 120 h coincubation of PV-1 with infusorians, the amount of *Parameciums* in control group was 18.1 ± 2.3 and in test group was 33.2 ± 6.1 ($t = 2.32$, $p < 0.1$). Moreover, in the case of 168 h coincubation the amount was 17.4 ± 1.9 and 48.1 ± 7.7 ($t = 5.83$, $p < 0.005$) respectively. Such an abrupt increase in number of infusorians continued at least for 2–4 weeks (unpublished data).

Picornaviruses were eliminated from medium if only alive *Parameciums* were present in medium. When *Parameciums* were destroyed by freeze/defreeze process, a low titers (compared with control group) of picornaviruses remained in the supernatant.

Confirmation of picornavirus ability persist in a protozoan organism has been proved in a number of works in

Fig. 2 Liberation of picornaviruses from destroyed *Paramecium caudatum* as the coincubation time with the viruses increased. Control coincubations of viruses were begun in free Losina-Losinsky solution. Y axis, viral titers in log₁₀; X axis, hours of experiment

60–70 years of twentieth century [14, 18]. However, interactions between ciliate and are not well understood. The data of Brutsaert et al. [4] suggested the neutral type of interactions between *Tetrahymena pyriformis* and PV, but in fact PV can multiply in *T. pyriformis* as Kovacs and Bucz [14] showed it. The possibility of interaction of EMCV with infusorians for the first time has been shown on example *Tetrahymena* [14]. Furthermore, there is many controversial data about the future of ingested viruses. Some bacteriophages like PhiX174 and MS2 were resistant to an inactivation by *T. thermophila* [1]. Another bacteriophage—T4 was ingested and inactivated by the same *T. thermophila* [10]. Thus lead to conclusion that there is few and uncompleted information about relationship and coexistence of the viruses (especially picornaviruses) and protozoans.

Most studies on the survival and fate of picornaviruses in natural waters have been focused directly on enteroviruses [16], but role of protozoa in these processes was poorly described. O'Brien and Newman [16] proved that enterovirus inactivation rates are primarily affected by the water temperature. The data of Fujioka et al. [8] indicated that the time for 90 % reduction of PV-1 at 24 ± 1 °C in seawater samples ranged from 24 to 48 h, and complete inactivation occurred within 72–96 h. Antiviral activity of the seawater was related to the growth activities of microorganisms. Some bacteria have been found to reduce

Fig. 3 Changes of number of the *Paramecium caudatum* after coinubation without and with live and inactivated picornaviruses. **a** *Paramecium* with PV-1 (Sabin). **b** *Paramecium* with EMCV-CSK. **c** *Paramecium* with FMDV-O. Changes of number of the *Paramecium caudatum* after coinubation with and without picornaviruses. **a** *Paramecium* with PV-1 (Sabin). **b** *Paramecium* with EMCV-CSK. **c** *Paramecium* with FMDV-O. Y axis, number of *Paramecium*; X axis, days of experiment

the number of viral pathogens. Kim and Unno [12] reported that *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* can inactivate polioviruses through predation. Our data shows that the levels of picornaviruses decreased in the infusorians free medium at $22 \pm 2^\circ\text{C}$ approximately on 0.75–1.0 log in 5 days and about 1.0–1.75 log in 9 days. Therefore, we described more than 10 times less inactivation of the viruses compared with results of O'Brien, and Newman [16]. It can be result of

decreased activity of microorganisms with antiviral properties and low temperature. Picornaviruses lost their activity in free medium and in medium with destroyed *Parameciums* at low speed wherewith in case of alive *Parameciums*.

The growth of the number of infusorians in viral environment cannot be explained only by the fact that viruses were nutrient source for the infusorians, and a little increase of the number of infusorians in medium with inactivated viruses confirms that. As the number of infusorians begin to increase, the titer of viruses decreases. It is well known that viruses usually demonstrate less persistence in natural waters and other environmental media and matrices compared to the same media that has been sterilized or pasteurized. Studies generally have shown that viruses better survive in autoclaved or filter-sterilized waters, than in their unsterilized counterparts [16]. Enzymatic activity of natural waters, in particular proteolytic enzymes, has also been implicated in the inactivation of picornaviruses [5, 11]. However, the involvement of enzymes, derived from destroyed infusoria, to the role of main virus-inactivating factor is improbable, because of unredused number of viruses presented after coinubation with destroyed *Paramecium*.

Ciliated grazers (*Paramecium* in our case) can play a role in viral inactivation, either through production of metabolites that adversely affect the virus particles, or by direct use of the viruses as a nutrient source.

Data of Benyahya et al. [3], has shown that a mammalian rotavirus was found to adsorb to *Tetrahymena* and over time the virus was released again. Our data indicate that *Parameciums* not only adsorb virus, but also remarkable inactivated them.

Our data shows no long-term survival of picornaviruses in the presence of *P. caudatum*. *Parameciums* were able to remove picornaviruses from solutions in which the infusorians and viruses had been coinubated. At the same time the issue of the long term persistence of picornaviruses in *Paramecium* with minor release of viruses in the medium remains open. The information about existence of picornaviruses as above is mentioned in scientific literature before [19]. One of the main conclusions of our research is that the presence of picornaviruses impacts on population of infusorians leading to sharp increase of their number in the medium.

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