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REPRODUCTION OF CORONAVIRUSES IN CELL CULTURES

The authors discuss the data available concerning coronaviruses replication in vitro, the difficulties of virus reproduction due to some biotope factors as well as to strain and host cells properties

Coronaviridae family includes a lot of viruses responsible for some human and animal diseases causing in particular gastrointestinal and respiratory systems disorders as well as multiple damages of sensitive cardiovascular and nervous systems injuries, the list of such agent becoming meanwhile longer [1—3]. *Coronaviridae* are generally spherical-shaped glycoprotein-enveloped RNA-containing viruses carrying club-shaped surface projections forming so-called «corona» appearance of virus particles.

Until a certain time an opinion existed [1] each coronavirus to be able to infect only some susceptible cell types originated from vertebrate species being natural coronavirus hosts. Little by little, however, such a conception has been proved to be erroneous because of new established facts described by several authors. At the same time, some *Coronaviridae* representatives have been failed to be cultivated in homologous cell cultures infected by virus suspensions containing, beyond all question, complete virus particles visualized under electron microscope. Such a property of some coronaviruses arises many difficulties for research workers investigating these agents; so there are some attempts to adapt intestinal and other coronaviruses and to obtain virus reproduction in cell cultures. In this review we discuss some approaches, failures, and successful results of such experiments as well as conclusions following from comprehension of all the data concerning *Coronaviridae* cultivation *in vitro*.

It is well known from general virology [4] the successful virus infection is due to virus adsorbtion on the sensitive cell and further virus penetration into the cell in the process of viropexis or cells fusion accompanied by syncytia formation and primary cytopathogenic effect of virus infection. Cell susceptibility to a given virus strain is predetermined by the presence of virus-specific receptors on the cell surface. The absence of such structures is often a real cause of unsuccessful infection experiments despite of all the machinery necessary for intracellular virus replication being proved to be present in the infected cells. Such a situation has been demonstrated in the experiments with monkey kidney cells (COS) resistant to mouse hepatitis virus and giving a productive virus infection after cells transfection by viral RNA extracted from virions [1].

Boyle et al. [5] have shown *Coronaviridae*-sensitive gastroenteric tract cells to possess a membrane-located virus-binding receptor protein, its absence or presence being correlated with cell virus-resistance or virus-sensitivity. Monoclonal antireceptor antibodies are able to prevent virus binding to the susceptible cell and to save it in such a way from destruction by this virus.

It has been shown that a protein usually binding to cell receptors is the *S*-peplomer protein (designed earlier as *E2* or *gp180*) located on virion envelope [6]. Lai [1] has summerized the data concerning the fact

that some monoclonal antibodies against mouse hepatitis virus *S*-protein are able to neutralize virus infectivity; these data, however, do not prove direct interaction between virus-specific receptors and *S*-protein. Besides, the *HE*-glycoprotein (earlier named *E3* or *gp65*) interacts also with cell receptors; this component is not present in all the members of *Coronaviridae* family. According to the Lai's review, it is not yet known whether receptors interacting with *S*- and *HE*-proteins are identical or different ones. If these receptors are different the viral strains possessing both *S*- and *HE*-proteins are able to infect more different cell populations or cells of more quantity of vertebrate species.

It should be also noted that *S*-peplomer ability to interact with cell receptors depends also on its conformational state [6] due to temperature and pH values. The experiments have demonstrated *S*-protein aggregative capacity to be strongly increased at pH 8,0 (37 °C), such a process being accompanied by a loss of virions infectivity.

Working with non-detective coronarivions and with virus-sensitive cells it is not always possible to obtain any cell infection without preliminary cultures and virions treatments; it is especially true for intestinal *Coronaviridae* strains. Sturman and Holmes [6] demonstrated many years ago that proteolytic treatment of mouse hepatitis virions using trypsin preparations increased virions ability to cause cell fusion, *S*-protein being responsible for this process [7]. It has become evident that intestinal coronavirus strains, similar to intestinal rotaviruses [8], increase strongly their infectivity after limited proteolytic cleavage of envelope proteins. It is of special interest that continuous existance in the same biotope leads to similar characters development in non-relative viruses, preliminary proteolytic treatment of surface virion components having become a stage necessary for following successful host cell infection [8]. No such infection with intestinal virus strains and isolates has been obtained until research workers have understood the virions to be previously protease-treated, adaptation to intestinal conditions having been accompanied with strains selection requiring such treatment. We shall show below the different strains dependence on trypsin treatment is not the same. Having understood the necessity of limited virus proteins cleavage, we cannot, however, contend that such a cleavage causes no changes of cell surface and especially of cell receptors. To obtain a successful cell infection by some transmissible gastroenteritis virus strains adapted to gnotobiotic piglets and by wild strains of this virus it is important, according to the data of Komaniwa et al. [9], to assure both limited virus proteolysis and proteolysis inhibitors elimination. The animal sera are known to contain trypsin inhibiting substances, α-1-antitrypsin being the most important one [10]; besides, there is a direct evidence [11] fetal calf serum to contain a substance or several substances inhibiting coronavirus attachment to cell membrane receptors. So CPK cells monolayer has been twice washed by culture medium before virus inoculation in order to wash away the serum. The virus-containing suspension has been incubated with trypsin (final enzyme concentration was usually 10 μg/ml) at 37 °C during 30 min and then with serum-free cell monolayer (1 h at 37 °C); the infected cells have been washed with the Earle medium and cultivatived in the serum-free Eagle medium, the medium containing also trypsin (0,5 μg/ml).

Sierguieyev et al. [12] have also shown the trypsin pretreatment of infective material to increase the yield of infective particles even in the virus-cell system producting attenuated swine gastroenteritis virus progeny also without such a pretreatment, the virus titers obtained being 1,5—2 lg higher comparing with control virus inocula.

It is of great interest that porcine epidemic diarrhea virus replication in Vero cells (established line of *Cecropithecs aethiops* monkey kidney cells) becomes impossible if trypsin is absent in the cell medium; it should be pointed out that even the best adapted virus strains do not simply decrease their infective titers in such conditions but stop comple-

tely their reproduction [13]. It is evident that adaptation process of intestinal viruses to cell cultures has caused no selection of virus clones having trypsin-independent reproduction.

Another evident example of foreign coronavirus adaptation is turkey intestinal virus reproduction in established human rectal neoplasm cells HRT-18 [14] incubated in trypsin-containing medium; these cells assure productive infections also after inoculations of bovine, canine, and human coronaviruses. So enzyme pretreatment of coronavirus particles seems to be a necessary step for a lot of virus-cell systems (although not for all the systems described) permitting virus peplomers interaction with cell repectors impossible or difficult without such incubation [15].

Besides, the coronavirus reproduction is found to be also sometimes limited both *in vivo* and *in vitro* by other mechanisms which are not yet completely understood. For example, OC43, a respiratory human coronavirus, adapted to suckling mice brain has been demonstrated to penetrate into spine radix cells, astrocytes, fibroblasts, and olygodendrons. After virus penetration, radix neuron cells have been shown to produce complete infective virions, astrocytes and fibroblasts have been proved to synthesize virus-specific antigen; at the same time, no virions or virus antigens have been detected in infected olygodendrons [16]. The infection-sensitive brain cells of human fetuses produce no virions. Perhaps a lot of cells possesses some barriers preventing viral RNAs trancription or/and viral proteins translation from viral mRNAs. Investigations carried out with mutant mice hepatitis strains [17] show a non-structural protein *ns2* to be not important for virus replication in transformed cells (perhaps this protein or its structural analogue is synthesized by these cells) being at the same time necessary for virus progeny production both in primary mice cells and in mice organism. Sturman and Holmes [18] discuss in detail many problems concerning coronaviruses cultivation in malignant established cell lines; their opinion is that intracellular events due to *Papova*-and/or *Retroviridae* activities are favourable for *Coronaviridae* strains reproduction in these cells.

Some results of the experiments having the goal to obtain coronaviral infection *in vitro* seem to be paradoxical. Hofmann and Wyler [13], for example, have had a successful porcine epidemic diarrhea virus reproduction only in Vero cells but not in any natural host cells of this vigus, a lot of primary and secondary cultures from different swine organs as well as PD5 cell line (originated from swine thyroid gland) and PK15 cell line (of porcine kidney origin) having been tested. The authors themself suppose such results are due to higher Vero cells resistance against trypsin degradation comparing with porcine cells; it is very probable that trypsin-damaged cells are not more able to remain permissive systems for their own coronaviruses replication. The same conclusion has been drawn by Kusanagi et al. [19]; working with a lot of cultures of primate, porcine, and hamster origin they have failed to adapt porcine epidemic diarrhea virus to these cells with the only exception of Vero.

The data cited above confirm the *in vitro* infection of homologous and foreign primary and established cells by *Coronaviridae* agents are quite possible; some cells are fully permissive giving a productive coronavirus infection and complete virions formation. The situation seems to be paradoxical because of host range out of natural host organism becoming both more extensive and more narrow, the investigators being unable to obtain viruses reproduction in their own «suitable» and «usual» biotopes [9, 19]. The fact that some isolates of coronaviruses can be grown *in vitro* without preliminary treatment of virions by trypsin proves that sometimes mutant particles arise among intestinal viruses progeny which are able to reproduce even without proteolysis realized in host intestine and necessary for «normal» viruses life cycle.

Adaptation of viruses to out of organism growth during laboratory experiments and also during biotope changes in the organisms of the same or of the foreign host may be accompanied by phenotype variations

of the cultivated strain as well as by virus genetic material changes. It is well known [20] that natural virus populations are usually heterogeneous and contain several mutant viruses possessing different tissue tropism. Coronaviruses tissue tropism is found to be predetermined at least by *S*-protein [21] being of marked variability [22]. The fact especially important from the epidemiological point of view is the selection of antigenically different virus strains during out of organism cultivation and also in the process of virus growth in non-usual cells of the natural hosts; such a selection being non-immune in *in vitro* systems depends on cells characters and sometimes on some other factors. It must be also noted an important prerequisite of increased strain variability is the cell inoculations by high virus doses (high moieties of infection) being favourable for mutants selection present in virus preparations and having selective advantages in new reproductive conditions. So it is necessary to use the lowest possible moieties of infection in order to preserve as fully as possible original characters of virus strains [18]. While coronaviruses cultivation in new environmental conditions host predetermined glycosylation of virion proteins is of great importance [1]. In *Coronaviridae* family, in particular in porcine transmissible gastroenteritis virus, it is namely *S*-protein known to be highly glycosylated; it has been shown in Laude's laboratory this antigen to possess four antigenic epitopes, C-epitope being of the highest variability [22]. Properties of envelope proteins due to their glycosylation degree determine further such a viral marker as their host range, so virus particles adaptability in new cells depends mostly on their glycosylation patterns. The inhibition of virus assembly and some defects of this process are known to be correlated with excessive glycosylation.

It is evident that it is necessary to cultivate virus populations in homologous cell cultures using virus inocula of the lowest moieties of infection in order to preserve some original virus characters, the last ones being controlled as fully as possible.

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РОЗМНОЖЕННЯ КОРОНАВІРУСІВ У КУЛЬТУРНИХ КЛІТИН

Резюме

Розглянуто літературні дані стосовно розмноження коронавірусів у культурах клітин, труднощі адаптації вірусів, обумовлені особливостями нових біотопів, а також властивостями вірусних штамів та клітин хазяїна.

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