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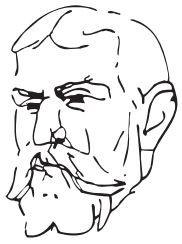

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ORIGINAL ARTICLE

EXPERIMENTAL ANALYSIS OF WAYS OF VIRAL INFECTIONS INTO THE HUMAN BODY

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ABSTRACT

The aim: The aim of the study is to experimentally test the process of viral infection and determine the ways of its penetration into the human body.

Materials and methods: This experimental analysis is based on systematic research, published peer-reviewed articles, books, textbooks, monographs. It should also be noted that in order to identify some immunocompetent lymph node cells and the ability to visualize certain sites in the lymphoid nodes of Peyer's patches, where the initial processes are presented below, we resorted to sampling anatomical material. The study involved 10 adult albino rats weighing 200.0 ± 20.0 g. The search period covered the period from 2010 to 2021, but the experimental analysis contains some valuable data from previous years, as these literature sources have significant scientific value.

Results: According to immunohistochemical analysis of the epithelium associated with the dome of the lymph nodes of the small intestine of white rats, the bulk was B-lymphocytes (about 47%) and T-lymphocytes (about 35%), while plasma cells, macrophages and dendritic cells accounted for approximately 5% for each of them.

Conclusions: Process of development of viral infection can be represented in the form of the following targeted steps: 1) massive invasion of viruses into the body; 2) the pathway of viruses to the intended target (target cells) is carried out by the blood flow; 3) achieving the target by viruses and their penetration into target cells. In the pathogenesis of viral diseases, the role is played by the preparedness of the particular body, which directly depends on the functional state of its immune system, which determines the possibility, severity and outcome of the disease.

KEY WORDS: virus, Coronavirus infection, COVID-19, entrance gate of infection, immune system

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INTRODUCTION

The paper has been written in response to the ongoing spread of the coronavirus infectious disease (COVID-19) pandemic caused by the novel coronavirus (SARS-CoV-2), causing severe acute respiratory syndrome [1, 2]. Considering that the questions raised in the present paper are common to all types of human viral infections, we can limit ourselves only to a general characteristic of their pathogens.

Coronaviruses (Coronaviridae, CoVs) are a large family of RNA viruses that can cause mild to severe acute respiratory infections in humans such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (Severe Acute Respiratory Syndrome, SARS). Coronaviruses are capable of infecting the respiratory, gastrointestinal tract, liver and central nervous system of humans and many other species of vertebrates, including domestic animals and livestock, birds, bats, etc. [3]. Before the epidemic outbreaks of SARS in 2002 and MERS in 2012, coronaviruses were not considered highly pathogenic for humans, since viruses previously circulating in the human population in immunocompetent individuals mainly caused only mild forms of the disease.

THE AIM

The aim of the study is to experimentally test the process of viral infection and determine the ways of its penetration into the human body.

MATERIALS AND METHODS

This experimental analysis is based on systematic research, published peer-reviewed articles, books, textbooks, monographs. For the purposes of this experimental analysis, a literature search was conducted (to study the development process and identify possible ways of viral infection) using the Internet, domestic and foreign literature, scientific and electronic library of Poltava State Medical University by the following keywords: "virus", "Coronavirus" infection "," COVID-19 "," entrance gate of infection "," mucociliary barrier "," mucous membrane "," follicle-associated epithelium "," immune system ". The search period covered the period from 2010 to 2021, but the experimental analysis contains some valuable data from previous years, as these literature sources have significant scientific value.

The following inclusion and exclusion criteria have been used:
- inclusion criteria: original articles published in journals and conference proceedings, books, study guides, monographs; language of publication: Ukrainian, Russian, English;
- exclusion criteria: reviews, case studies, editorials, letters, etc., not peer-reviewed; language of publication: others.

Also, it should be noted that in order to identify some immunocompetent cells of lymphoid nodules and the possibility of visualizing certain places in the lymphoid nodules of Peyer's patches, where the initial processes presented below occur, we resorted to sampling anatomical material. 10 mature albino male rats weighted 200,0±20,0 g were involved into the study. Before the experiment, all animals were kept in standard conditions of the experimental biological clinic (vivarium) at the Poltava State Medical University in compliance with the regulations on keeping experimental animals, adopted by the European Parliament and Council Directive (2010/63/EU), the Order of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, No. 249 "On approval of the procedure for conducting tests, experiments on animals by research institutions" and "General ethical principles of experiments on animals", adopted by the V National Congress on Bioethics (Kiev, 2013), (Minutes No. 178 as of 24.12.2019 of meeting the Commission on Biomedical Ethics at Poltava State Medical University).

After vivisection made by thiopental anesthesia overdose (75 mg/kg of animal body weight intramuscularly in the upper third of the thigh of the hind paw) in compliance with the requirements for dissection of the abdominal cavity, the entire complex of the gastrointestinal tract was removed, which was fixed in 10% formalin solution for two days. Following a week (after pre-washing in running water) the murine gastrointestinal tract was examined and sections of the small intestine, containing Peyer's patches, were selectively excised. The set of Peyer's patches were clearly visualized along its length beneath the serous membrane (on the side opposite to the mesentery attachment site) in the form of whitish spots, different in shape and size.

The specimens, after washing from formalin and dehydration in alcohol of increasing concentration, were embedded into paraffin blocks, from which serial sections of 4 μm thick (Microm HM 325) were obtained in the cross-section of the small intestine and, subsequently, stained with hematoxylin-eosin. Their study and documentation was carried out using the "Konus" light microscope equipped with the Sigeta DCM-900 9.0MP digital microphoto attachment and the Biorex 3 program (serial number 5604) adapted for these studies. The morphometric characteristics of the tissue structures of the corresponding specimens were obtained using a system of visual analysis of histological specimens, as well as using the Sigeta X 1 mm/100 Div.x0.01mm stage micrometer, the scale of which (equal to 1 mm, where a small step corresponds to 10 μm) was applied to the corresponding microimage obtained in the same magnification.

The Ethics Commission of Poltava State Medical University has no comments on the methods used in this study.

RESULTS

Viruses are known to be non-cellular life forms with their own genome, but not capable of self-reproduction. Therefore, they are able to carry out the process of reproduction of their own kind in generations only due to the synthetic apparatus of cells of all, without exception, plant and animal organisms (including bacteria). According to their structural organization, they are divided into simple and complex forms. The first consists of an outer protein coating called the capsid, which protects the enclosing RNA or DNA nucleic acids. Coronaviruses are single-stranded RNA-containing forms. Such a viral particle is clearly visualized only in an electron microscope. In contrast to them, complex viruses, being larger (some of them are distinguishable in a light microscope), have a complex layer-by-layer structure of the outer shell, a supercapsid, which contains carbohydrates (glycoproteins) and lipids (lipoproteins) in combination with proteins, which have separate antigenic properties [4-6].

But in terms of understanding the development of viral infection, it is more important to know that viruses in general are characterized by two forms of existence in the host body: extracellular, or dormant, and intracellular, multiplying (reproductive). The synonyms of the first form are «viral particle», «viral corpuscle», «virion», and the second – «virus-cell complex» [7, 8].

The intracellular form appears as a result of the penetration of virions into the target cell and reproduction of viruses in it. In this process, the following stages are distinguished: adsorption, penetration of virions into the cell, transcription, translation, replication, assembly of viral particles and release of viral particles from the cell [9].

Most of all we are interested in the first, initial stage. It is believed that at the very beginning, the adsorption process is facilitated by the forces of electrostatic interaction of positively and negatively charged groupings located on the surface of the virus on the one hand, and the cell surface, on the other hand. This nonspecific stage of adsorption is followed by a highly specific one, when the proteins of the surface of the virus combine with matching receptors on the plasma membrane of the cell. It is this local site of interaction of the virus with the cell surface that is currently understood as the gateway to infection [10, 11]. This view is supported by the findings of morphological studies, mainly of cell cultures infected with the virus [12]. However, it should be noted that cells are infected by the nutrient medium in which they are grown, that is, this model is expressed by a simple formula: virus ⟶ nutrient medium ⟶ cell.

This, extremely simplified, model, in principle, can be likened to an animal organism, in the understanding that enclosed diverse numerous cellular structures receive nutrition from its internal (humoral) environment, which is understood as a complex system of recirculating fluids,

Table I. The main immunocompetent cells in group lymphoid nodules small intestine of white rats is normal

Cellular elements	Localization area	n=10
B-lymphocytes	B-zone	40,0-45,0%
	T-zone	<1%
	Dome	before 2,0%
T- lymphocytes	B-zone	<1%
	T-zone	27,6-30,7%
	Dome	3,7-4,1%
Plasmocytes	B-zone	<1%
	T-zone	1,0-1,6%
	Dome	2,8-3,2%
Macrophages	B-zone	1,4-2,2%
	T-zone	1,1-1,8%
	Dome	1,3-1,9%
Dendritic cells	B-zone	5-6,7%
Phagocytic enterocytes	Epithelium	–

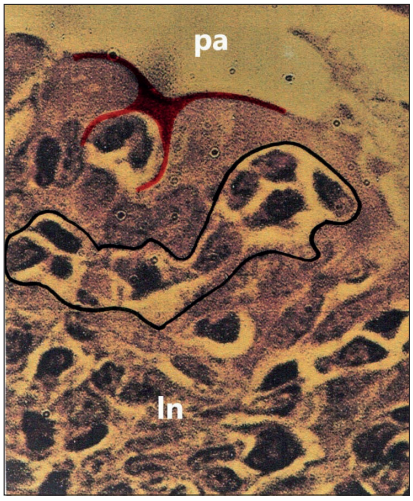

Fig. 1. Microscopic structure of lymphoid-associated epithelium of lymphoid nodule of Peyer's patch of small intestine of rat. Paraffin sections; H&E stain; Lens: 100×magnification (author's figure).
ln – lymphoid nodule; pa – parietal area of the small intestine. The border of the intraepithelial lymphoid cell is indicated by a black outline, the presumed M-cell is colored in red.

namely, blood, intercellular (interstitial) fluid, lymph, as well as cerebrospinal and peritoneal fluids. Naturally, the infection of these cellular structures with viruses can be carried out only from the abovementioned humoral environment, where they should not normally be, but they can appear in it, having entered during infection from the external environment. Here, just the key question that interests us arises: how can pathogenic microorganisms enter the internal environment of the body from the outside, if it is known that on their way they will inevitably encounter an insurmountable obstacle in the form of the so-called morphophysiological barrier of the mucous membranes of hollow organs.

Among them, the most important place in this regard belongs to the intestinal tract and the tracheobronchial tree, the mucous membranes of which differ from each other by the covering epithelium, bordering on the external environment, which constitutes the structural base of the morphophysiological barriers. If in the intestinal mucosa it is represented by the polarized monolayer of enterocytes of different specialization, closely conjoined and interconnected by means of intercellular contacts, among which

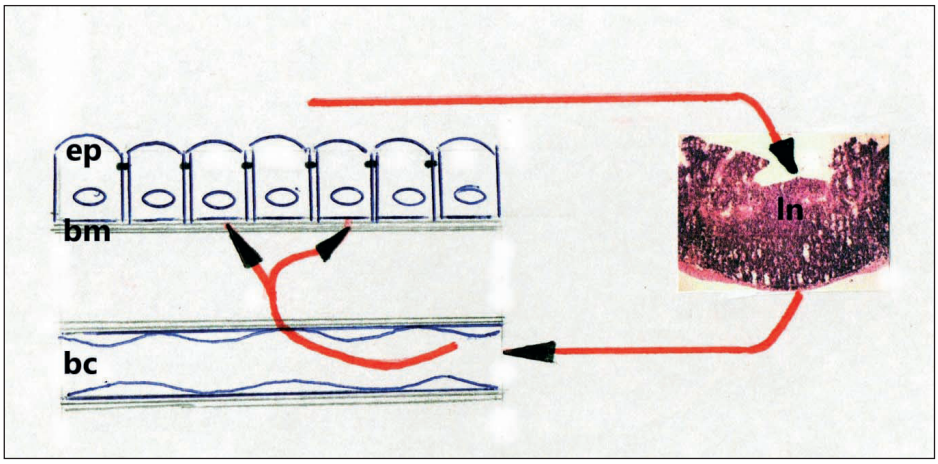

Fig. 2. Scheme of the pathways of viral infection of the epithelium of the mucous membranes (author's figure).
ep – epithelium (target cells); bm – basal membrane; ln – lymphoid nodule; bc – blood capillary. Red lines show the paths of movement of viruses.

absorbing cells predominate, then in the tracheobronchial tree there is pseudostratified (a kind of simple) epithelium with more complex structure, with a quantitative predominance of ciliated cells. Due to the targeted synchronized motor activity of the apically located cilia, a fairly effective continuous translation of a thin layer of mucus along the surface of the mucous membrane towards the pharynx is carried out, in the viscous base of which various dust particles and microorganisms that enter during respiration are stuck. Consequently, the mucous membranes of the respiratory tract, compared to those of the intestinal tract, have an additional active host defense mechanism, which is called the mucociliary barrier [13-16].

The second component of the morphophysiological barrier of the mucous membranes are secretory structures in the form of goblet cells and numerous small (intramural) glands that cover the outer (apical) surface of the covering epithelium with a thin layer of mucus. Notably, in its composition (together with mucins of the corresponding type) there is a bactericidal enzyme lysozyme (mureinase) in the proper concentration, which belongs to humoral factors of innate (nonspecific) immunity, as well as the class A secretory immunoglobulin, which appears as a result of the humoral response of the specific (adaptive) immunity [17, 18]. But all this still needs to be supplemented by the fact that this antiviral active mucin layer, which protects the epithelial layer of the mucous membranes, is, on the one hand, in close connection with the epithelial glycocalyx, and on the other hand, it is associated with the so-called biofilm, which is a specific form of colonization of mucous membranes, mainly by obligate (useful for the body) microflora, in the environment of which pathogenic bacteria and viruses reside in a small amount (about 5 %). Normally, the latter are usually depressed by the obligate microflora, which thus perform a protective function in relation to mucous membranes [19-21].

Therefore, if we add together all the abovementioned protection factors, it becomes obvious that this eliminates not only the possibility of adhesion (sticking) of viruses to the apical surface of epithelial cells, but also their penetration through the epithelium into the mucous membranes, i.e., into the internal environment of the body.

But we paid special attention to immunohistochemical analysis of the epithelium associated with the dome of lymph nodes. According to modern ideas, the intestinal epithelium covering the apical surface of group lymphoid nodules is endowed with the ability to selectively react with the antigenic composition of the contents of the small intestine as an initial link in the formation of immune responses in the gastrointestinal tract (table I).

According to these data, the bulk of them were B-lymphocytes (about 47%) and T-lymphocytes (about 35%), while the share of plasma cells, macrophages and dendritic cells accounted for about 5% of each.

Currently, we can clearly visualize those places in the lymphoid nodules of Peyer's patches, where these initial processes occur (Fig. 1).

It is noteworthy that due to them, according to the literature, in the intestine, slow inflammatory processes constantly take place, which is a physiological norm [27]. Apparently, the respiratory pathways are not an exception in this respect, because in their mucous membrane, as noted above, there are lymphoid nodules. Recently, interest in these formations has increased, because it is believed that they mediate the mechanism of the formation of acquired immunity in oral use of a live vaccine, which is considered more effective compared to other forms of vaccination. In this regard, it is reasonable to recall the history of the creation of the vaccine against poliomyelitis [28, 29].

Generally, the issue on structured lymphoepithelial formations in the form of single and grouped lymphoid nodules arises, which perform the function of immune surveillance of the parietal microbiota of hollow organs, thus being the outposts and the so called «portals» of the immune system of mucous membranes. However, in some extreme situations, for example, in an excessive load of viral infection on the mucous membranes of the intestine or respiratory tract, these formations become the weakest places for the massive entry of viruses into the internal environment of the body. From here, that is, from this source of invasion, they will be carried by the bloodstream throughout the body, reaching their «selected» target cells. For example, in COVID-19 they are mainly alveolocytes of the first and second type, in which coronaviruses penetrate after preliminary adhesion with their basement plasma membrane. But to do this, they have to overcome the basement membrane that underlies these cells. We do not know how this happens, because this issue is not elucidated in the literature. But in general, the whole process of infection of the target cells and reproduction of viruses in them is carried out in the sequential order presented at the beginning of our paper. We only specify that for the strictly polarized cells, which are part of epithelial layers, the entrance gate of an infection is not any of their surface, but only that by which this cell is adhered to in the internal environment of the body (Fig. 2).

DISCUSSION

There must be some special places in this continuum of the morphophysiological barrier of the mucous membranes, potentially accessible for infection, ie some gaps. This point of view is not new. For example, back in the middle of the last century, some authors postulated the presence of special tubules in the mucous membrane of the intestinal and respiratory tracts, through which various microorganisms can enter it [22]. Later, in the process of targeted search for information, our attention was attracted by some reports in the literature that relate to the study of the functional purpose of the well-known structured lymphoepithelial formations related to peripheral organs of the immune system, such as lymphoid nodules. Some of them, individual (solitary) nodules, occur both in the tracheobronchial tree and in the intestinal tract, while the others, grouped nodules (Peyer's patches), are found only in the intestine. But it should be noted that the tonsils, which are located at the very beginning of the digestive and respiratory systems, can be considered similar to the latter in morphology and function. These formations, by

their nature, are normally intended to notify the immune system of the mucous membranes about newly appeared pathogens in their proximity (parietally) [23, 24].

According to the current dogma, the transporters of all antigens from the cavity, for example, from the intestine to its mucous membrane, are the so-called M-cells, which are localized among the polarized monolayer of enterocytes covering the apical sections of the lymphoid nodules, in connection with which it received the name of follicle-associated epithelium [25, 26]. Obviously, M-cells are capable to transcytotic transfer and delivery intact pathogens to dendritic macrophages localized beneath them (subepithelially), which, after processing, present this pathogen to T-lymphocytes, initiating host immune responses, transferring them to the systemic level.

CONCLUSIONS

Thus, based on the above, we believe that the process of development of viral infection can be represented in the form of the following targeted steps:

1. Massive invasion of viruses into the body, that is, into its internal environment. The follicle-associated epithelium of the lymphoid nodules of the mucous membranes serves as a gap for this invasion.
2. The pathway of viruses to the intended target (target cells) is carried out by the blood flow.
3. Achieving the target by viruses and their penetration into target cells.

But in the very pathogenesis of viral diseases, the role is played not so much by the viruses themselves, but by the preparedness of the particular body, which directly depends on the functional state of its immune system, which determines the possibility, severity and outcome of the disease.

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Conflict of interest:
The Authors declare no conflict of interest.

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