The revival of the therapy with use of bacteriophages (an option in opportunistic infections)

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Bacteriophages are small viruses 40 times smaller than bacteria. These widespread in nature creatures aren’t pathogenic neither to humans nor to animals. They are built of deoxyribonucleic acid DNA – they can multiply on bacteria. When they replicate on bacteria they lead to bacteria’s death. Therefore they can be an alternative option in treatment for bacterial infections in opposition to antibiotics [1–13]. The first reference to bacteriophages dates back to 1915 when a British researcher Twort mentioned it, but not before a research of Canadian Felix d’Herelle in the Pasteur’s Institute in Paris had the name bacteriophage been introduced into literature – for the bacteria infected with phages – lytic phages.

The first boom concerning the role of bacteriophages was in the 1930s and 1940s before the age of antibiotics in Grusia, in Tbilisi in the still existing Elias Institute. These were the Stalin’s times. The first medical usage was for contagious illnesses of soldiers, e.g. in dysentery treatment. Afterwards the antibiotics’ revolution has led to forget about the lethal function of bacteriophages. Apart from that the Cold War with the Soviet Union that framed the credibility of the Soviet achievements was unfavourable to development of knowledge about phages in the West. In the East, in Poland [10, 12, 13] the research on phages has been sustained due to such personages of the Wroclaw Institute as Hirszfeld, Slopek, Górski et al. At present the achievements of the Wroclaw Institute are appraised the best of all and cited with the name of the supervisor Professor Górski in publications on therapy with use of phages. The role of phages is enjoying its revival in the battle against bacteria. The WHO report of the year 2000 warns that the bacterial resistance against antibiotics approaches its critical point. If we don’t work out any alternative, nothing will be possible to be done against the spread of infections resistant to antibiotics. For example the Staphylococcus aureus exists globally in a resistant form. The development of resistance depends on overuse or misuse of antibiotics. Many authors in the Western countries mention the underestimation of the importance of research done behind the Iron Curtain in the times of so-called Stalinism.

That concerns in particular the use of bacteriophages in the battle against bacteria. The fact that in the Eastern Europe phages had been used successfully in treatment of resistant infections of alimentary tract, in sepsis, osteomyelitis or in lungs inflammation had been undervalued [12]. Contemporary achievements on animals have confirmed the effectiveness in different, sometimes lethal infections, e.g. meningitis meningococcica in transplantology, in burns, abscesses of mediastinum, liver etc. Another report of The National Institute of Health (NIH) in Bethesda speaks out about a 60-years delay in the science of bacteriophages in the Western Europe caused by underestimation of the research works of the Eastern Europe [3, 6]. Thus, facing the increasing resistance, bacteriophages may prove to be an important alternative in the antibacterial battle.

For a few years an intensive preclinical research with hope to introduce bacteriophages widely in the therapy of resistant infections has been conducted outside Poland. The costs of the bacterial resistance to antibiotics are immense e.g. the deaths caused by infections took the 5th place in the classification of all causes of deaths in the 1980s. At present they

Fig. 1 The body schema of a phage resembles a lunar vehicle. There are receptors on its legs to recognize and attack specific bacteria. In the opening there is a syringe which function is to pierce the bacterial capsule and to inject one copy of phage’s DNA into the bacteria. In a few minutes bacteriolysis occurs as a result of replication of millions of copies of phages. A phage is 40 times smaller than bacteria
are the 3rd most frequent cause. For example Staphylococcus aureus seems to be absolutely resistant.

Bacteriophages, similarly to other viruses, have been identified in the 30’s due to the electron microscopy. Unlike other viruses they are built of nucleic acids DNA or RNA or both threads, which have linear or circular structure. The reproduction and metabolism of the bacteriophages can only occur on living organisms. To multiply and to conduct metabolism, a bacteriophage needs a host. Bacteria are the only hosts suitable for bacteriophages.

Until now about 4000 kinds of phages have been identified [2, 4, 7]. A phage consists of two parts: a head and a long tubular tail, where 6 pairs of legs and a syringe for injection of DNA copy are situated. As a whole a bacteriophage is compared to a spider or even a space craft (40 times smaller than that of other viruses (so-called circular) or bacteria. During the second minute there is 200 replications of its DNA (1st generation). This replication is 200 times faster than that of other viruses or bacteria. The legs enable to attach to the bacterial membrane and to inject a characteristic copy of bacteriophage’s DNA genome through the tubular tail into the bacteria afterwards. A receptor at the end of the mentioned syringe recognizes and attacks a specific type of bacteria. In one minute’s time it kills bacteria, makes the first 200 copies of its DNA (1st generation). This replication is 200 times faster than that of other viruses (so-called circular) or bacteria. During the second minute there are 200–200 replications which gives 40000 copies (2nd generation). In the 3rd generation we obtain 8 million phages and in the 4th- 1,6 billion copies (2nd generation). In the 3rd generation we obtain 8 million phages in one droplet of clean sea water and only one million in a droplet of contaminated water. Probably the healing properties of the Ganges River observed in the year 1896 depend on the presence of phages. It is important to underline that a popular observation that sauerkraut has a beneficial influence on health is connected with the presence of phages in it. Contrary to phages, the other viruses or bacteria need to penetrate into the cell, e.g. a liver cell, with whole their body and they replicate much slower (one cell division per minute). The estimated weight of all phages on the Earth is 1000 times larger than that of the whole number of elephants.

Nowadays the research on phages concerns learning their genotype, classification, banking and occurrence of mutations. The Polish school of researchers from the Hirszfeld’s Institute in Wroclaw takes part in these tasks together with the Institute of Microbiology in Budapest and the Elias Institute in Tbilisi. Also in the Western countries many preclinical research works are conducted at present. The particular interest is in selection of the strains and learning the mutations in phages as an alternative option in treatment of opportunistic infections. Contrary to antibiotics, bacteriophages never stop acting. Antibacterial effect is immediate and takes 12–24 hours. Usually 1–3 courses are administered locally, orally or intravenously. Bacteriophages do not exert any side effects or the minimal effect derives from the bursting bacteria and release of their exo- and endotoxins. Bacteriophages do not get into nuclear cells. However, an exception may occur if there is a conformity of the epitope of cell membrane with the epitope of the phage. The elimination of phages takes place in the reticuloendothelial system of spleen.

References
Clear cell colitis in children: pathology and clinical manifestation

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Abstract

Non-inflammatory bowel disease of children represents a variety of chronic intestinal dysfunctions, majority of them with no bacterial or viral basis. There are several morphological patterns observed in microscopic examination: superficial colitis, microscopic colitis, lymphocytic, and plasma cellular, eosinophilic, follicular colitis. We carried out an observation of 81 children with colon dysfunction characterized by giant, foamy clear cells in lamina propria of the mucous membrane of large intestine in the years 1994–2003. These cells were characterized by clear cytoplasm. Their number ranged from a few to a dozen in the field of vision. Their presence in routine histology suggested the preliminary systemic storage diseases of Niemann-Pick type, Gaucher type ganglioside lipidosis or Whipple’s disease. In repeated biopsies performed within 10 year’s observation the occurrence of foamy cells were still persistent. Some patients exhibited symptoms of systemic allergy and/or asthma. The usage of an electron microscope and immunocytochemical methods made it possible to exclude decisively the diseases mentioned above in the examined group. We decided to use a new diagnostic name for this non-specific inflammatory disease – a benign chronic foamy clear cell colitis (CCC). Since the cells represent expression of CD68 which is typical for macrophages thus we suggest that in the pathogenesis of this disease possible inheritance of macrophages’ failure plays an essential role.

Key words: allergy, children, clear cell colitis, foamy cell colitis, non-IBD colitis, obstipation

Introduction

The non-inflammatory bowel disease (IBD) colitis makes a group of diseases, which should be precisely differentiated from the IBD, such as an ulcerating colon inflammation or Leśniowski-Crohn syndrome. Despite some clinical manifestations may be similar the microscopic examination enables the differentiation. The non-specific colitis is usually superficial, it doesn’t cause destruction nor rebuilding of glands, it doesn’t disturb the mucous and submucous membranes’ structure. The non-specific microscopic colitis includes lymphocytic, eosinocytic, plasma-cellular inflammation. Until now it was rare to find the gigantoecellular, histiocytic or phagocytic form, which would involve CD68+ cells, indicating the mobilisation of the foamy non-nuclear phagocytic cells line. The presence of these cells in large amount may suggest the Whipple’s syndrome, the neuronal intestines’ dysplasia, AIDS, Salmonella derived diseases [4, 8, 15, 18, 19] or even classic forms of colitis ulcerosa [19].

The aim of study

The aim of study was the clinical and endoscopic evaluation and the elaboration of microscopic criteria of recognition of the colon disease of children with a relatively mild but chronic course, where giant cells with foamy cytoplasm are present in the specimens of the large intestine.
Material and methods

Patients

The clinical and morphological research included 81 children aged between 17 months and 17 years who have undergone treatment in pediatric centers in South-Eastern Poland in the Subcarpathian County between 1994 and 2004. There were 63% boys in the group and 68% of all were inhabitants of urban areas, whilst the rest (32%) lived in the countryside. The reason of diagnostic investigation was colon dysfunction. The diagnosis was set on the base of conscientiously collected interview and a precise physical and endoscopic examination. Each child had undergone endoscopy of the final part of the alimentary tract at least two times (23 had rectoscopy and 58 had colonoscopy), followed by histopathological and immunocytochemical examination of obtained specimens.

Clinical and endoscopic evaluation was based upon the Rachmilewitz classification (modified by Ryzka and Woynarowski) [12, 13, 18, 19]. Clinical diagnosis was established with regard to the number of stools per week, the presence of mucus and blood in the stools, the general comfort of the child, the intensity of stomachaches, the presence of fever, OB, hemoglobin concentration, the state of nutrition and the presence of parenteral manifestation. This kind of evaluation lets us distinguish three degrees of illness severity: mild, moderate or serious. In the evaluation of rate of changes in the endoscopic picture the following features were taken into consideration: the vascular drawing, the granulation of mucous membrane, the presence of mucus, pus, superficial ulcerations and erosions, hemorrhages. The evaluation of endoscopic activity makes it possible to distinguish mild and moderate intensity of changes.

In all patients the examination of intestinal pathogens (Salmonella, Shigella, Campylobacter, Escherichia coli, Yersinia, Clostridium difficile) and Giardia infection was done. In order to obtain a full virological evaluation a research into presence of retrovirus and enteroviruses was done including Hep-2 and RD cell lines and latex reactions with the usage of Slidex Rota-Kit 2 set and the ELISA serological examination.

Morphological examination

Separate and multiple sections were subjected to the basic microscopic examination with use of the paraffin technique. Serial sections were stained with hematoxylin and eosin. PAS reaction with and without diastase digestion and mucicarmarin staining were done. Additional paraffin sections on the sialinised slides were used in the immunocytochemical examination, in which the monoclonal DAKO antibodies were used (anti-CD3, -CD4, -CD68, -TNF-alpha, -IL-1-beta, -neurophilament, -synaptophyzine, -chromogranine, Ki67, PCNA). In some cases also an examination under an electron microscope Jeol 100CX was performed. Preparation of the intestinal mucosa segments were fixed in the 4% glutaraldehyde in cacodylic buffer, then in the OsO4 and afterwards a typical Epon procedure was performed. The ultrathin sections were restained with lead citrate and uranyl acetate.

Table 1

<table>
<thead>
<tr>
<th>Marker</th>
<th>CCC colitis</th>
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<tbody>
<tr>
<td>CD3 (T cells)</td>
<td>Negative</td>
</tr>
<tr>
<td>CD4 (T cells)</td>
<td>Negative</td>
</tr>
<tr>
<td>CD22 (B cells)</td>
<td>Negative</td>
</tr>
<tr>
<td>CD34 (precursor cells)</td>
<td>Negative</td>
</tr>
<tr>
<td>CD31 (endothelial)</td>
<td>Negative</td>
</tr>
<tr>
<td>CD68 (macrophages)</td>
<td>+++ Strongly positive</td>
</tr>
<tr>
<td>PCNA (proliferation antigen)</td>
<td>Negative**</td>
</tr>
<tr>
<td>Ki67 (mitotic activity)</td>
<td>Negative*</td>
</tr>
<tr>
<td>Chromogranin</td>
<td>Negative</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>Negative</td>
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<tr>
<td>Naurofilament</td>
<td>Negative</td>
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<tr>
<td>Actin</td>
<td>Negative</td>
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<tr>
<td>P53 mutations</td>
<td>Negative</td>
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<tr>
<td>GFAP glial marker</td>
<td>Negative</td>
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<tr>
<td>Mucicarmarin</td>
<td>Negative/or weak</td>
</tr>
<tr>
<td>PAS</td>
<td>Strongly positive</td>
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<tr>
<td>PAS diastase</td>
<td>Digested</td>
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<tr>
<td>Osmium tetraoxide</td>
<td>Positive</td>
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</table>

* 1–3 per crypt
** Positive in the cryptal epithelium

Results

Clinical observations

In the analysed group all children had OB < 100 mm and MHC/haemoglobin concentration > 8.5 g/dl. Treatment with sulphosazine or mesalazine for a few months had no clinical effects and didn’t result in regression of endoscopic nor histological changes. A small but clinical perceptible amelioration occurred with time and it was independent from the applied treatment. Preliminary findings indicated that there was a beneficial clinical effect of a probiotic Saccharomyces bulardi, but the observation period was too short for statistical evaluation.

The dominant symptoms in both girls and boys were: recurrent stomachaches, stiff and (or) loose stools, even diarrhoeal stools with mucus additive. Some patients were sent to hospital mainly because of the suspected digestive intolerance. They showed atopic skin inflammation and/or recurring upper respiratory system inflammations. Five children were treated because of bronchial asthma. The laboratory examination of peripheral blood and urine and microbiological analyses hadn’t reveal any pathology. Stomachaches appeared in each case except for one boy. They were mild or moderate. Less than 18 stools per week were counted in
7 children, 18–35 stools in 59 and 36–60 stools per week in the remaining 15 children.

Subjective evaluation of general feeling of the ill children was good only in 5 of them. The subfebrile status up to 38°C occurred in majority of children.

Parenteral symptoms were noted in 19 out of 81 children. These were mainly the upper respiratory tract infections. Forty three children had blood sedimentation under 40 mm; the rest had 50–100 mm after an hour. The haemoglobin concentration in blood in g/dl above 10 was noted in 65 cases, 16 children having Hb concentration above 10 g/dl.

The state of nutrition (Cole and Stanfield) was over 85% in 27 children, 80–85% in 46 and under 80% in 8.

**Endoscopic examination**

The endoscopy was done with use of stiff rectoscopes. Eighty two percentages had also a supplementary colonoscopy done. Macroscopically, all patients appeared to have a distinctly granulated, mucosa with sharply outlined vascularisation and a thin layer of mucus in the rectum and sigmoid colon. In children who had also colonoscopy done there was granulation and increased sharpness of vascular outline in descending and transverse colon too (22%), 6 children (11%) presented also changes in the ascending colon. The endoscopic examination usually revealed the presence of multiple granulations of the mucus membrane of the large intestine together with hyperaemia and congestion. The vascular markings of the mucosa were blurred in 44 cases, absent in 36 cases and normal in 1 case. The willingness to bleed was increased in 49 children. No polyps, ulcerations, erosions or cicatrization were noted. Endoscopic investigation of the upper part of alimentary tract didn’t show any inflammatory changes in 17 children.

**Histological and immunocytochemical examinations**

The microscopic picture of the routine stained sections with HE was characteristic. In the lamina propria of mucosa there were large, bright, foamy cells appearing either separately or in groups. They occupied the entire lamina propria breadth (from the covering epithelium to the basis of the cryptal glands and the level of muscularis mucosae). An uneven distribution of the foamy cells allowed the separation of 3 degrees of changes’ intensity: 1° – single cells on different levels of lamina propria; 2° – presence of aggregates made of 3–10 cells; 3° – foamy cells uniformly in all lamina propria. It needs to be underlined that the presence of foamy cells was constantly observed during the long years of treatment of the patients, notwithstanding with the evident amelioration of the general state of the patient.

Immunocytochemically the clear cells presented a positive reaction with the anti-CD68 antibody, which is typical for the macrophages. The cells were negative in the reactions characteristic for B and T lymphocytes. They were also negative for chromogranine, synaptophysine and neurofilaments. The PCNA reaction was positive in the proliferative zone cells of the crypt epithelium, which indicates an excessive activity of nucleus without mitotic activity. The mitotic activity was typical for the zone where a positive expression of Ki67 in some cells was disclosed. TNF-alpha and IL-1-beta expression was negative in foamy clear cells. Clear cells presented a positive reaction with neutral mucopolisachari-
Foamy rotaviruses inducing diarrhoea, which can be suspected in the differentiation of changes in the phagosomes, usually don’t exist in the human population. In our group of patients we considered the possibility of persistent changes, e.g. after the rotaviral diarrhea, but the presence of enteroviruses were ruled out on the ground of the negative examination of blood and stools.

Contrary to our results, Bajerano et al. ascertained the presence of foamy histiocytes far more often (up to 40% in the small intestine) in IBD patients suffering from AIDS, that imitate the Whipple’s disease. Egan et al. [3] revealed the presence of foamy histiocytes in different organs in the Erheim-Chester’s illness (osteosclerosis symmetria). Contrary to the presence of isolated macrophages of the large intestine, these were the neoplasmatic histiocytes. In the microscopic examination the typical Langerhans-Birbeck figures were found. Yao et al. [19] presented that microaggregates of the foamy cells were very important, because they suggested the diagnosis of the Leśniowski-Crohn illness. They described aggregates coexistent in the mucosa of duodenum with the granulomas characteristic for the Leśniowski-Crohn disease. However, we didn’t confirm this observation in our series of Leśniowski-Crohn disease.

Ribardo et al. [11] basing on the fact that the prosta
glandins are present in intestinal macrophages suggested that the large bowel macrophages contain macrophages stimulated in the process of inflammation by lipopolysaccharides (IL-1-beta and TNF-alpha positive).

Contrary to the IBD, in CCC the macrophages do not contain TNF-alpha or IL-1 [4]. Microscopic colitis [6, 7, 10, 14, 17] is the term suggested by some authors for the alternating diarrhoeas with obstructions and the endoscopic picture close to normal, but with a characteristic microscopic picture: thickenings of the basal lamina, the presence of a zone of lymphocytes’ concentration underneath the surface epithelium. In such chronic cases even a spontaneous recovery of the patient during a few years is possible.

The cause of microscopic colitis may be the abuse of medications and an increased concentration of antigens (con
tipitations) in the colon’s lumen [6, 10, 16, 17].

In our opinion CCC colitis can be distinguished as a distinct group of nonspecific large intestine diseases, manifesting a characteristic clinical picture and morphological exponents of the colon dysfunction.

**Discussion**

The microscopic diagnosis of the non-specific colitis with presence of clear cells colitis is relatively easy [5]. This illness doesn’t manifest the destruction nor rebuilding of glands. The basal membranes are not damaged by the lym

phoplastomocytic infiltration, characteristic for the IBD. The foamy clear cells are not characteristic for ulcerous colitis or Crohn disease. The mononuclear cell population in IBD exhibits positive expression with TNF-alpha and IL-1 beta [1, 9, 16].

The Whipple’s illness, rare in children, is a systemic disease. Foamy macrophages are present not only in the intestine, but also parenterally. The parenteral characteristic feature is the presence of Troponema Whippelli in the cyto

plasm. In the PAS reaction the cytoplasmic material is not digested by diastase. The group of 81 children examined by us presented foamy cells only in the mucous membrane of the large intestine without any parenteral occurrence (e.g. in the mucus of stomach, small intestine, liver, lymph nodes).

Contrary to the patients suffering from AIDS, our pa
tients didn’t show the inclusion bodies in the lyso
somes in the cytoplasm of the foamy cells [18].

Differential diagnosis should also consider the neuro
al dysplasia of intestine where large, bright ganglion cells can be very similar to the foamy cells. In HE staining attention should pay to the fact that contrary to the foamy-clear histiocytes, the ganglionic cells contain a typical nucleolus. The positive reaction for neuronfilaments, synaptophysine or (and) chromogranine is conclusive in these cases [8]. Some authors suggest that Salmonella typhimurium can mobilize phagocytosis in the macrophages of the large intestine. We haven’t found bacterial and viral particles in the phagosomes of foamy cells, and Langerhans figures seen in histiocytosis.
References


