Mimiviruses: Giant viruses with novel and intriguing features (Review)

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Received December 2, 2021; Accepted January 26, 2022

DOI: 10.3892/mmr.2022.12723

Abstract. The Mimivirus is a giant virus that infects amoebae and was long considered to be a bacterium due to its size. The viral particles are composed of a protein capsid of ~500 nm in diameter, which is enclosed in a polysaccharide layer in which ~120-140 nm long fibers are embedded, resulting in an overall diameter of 700 nm. The virus has a genome size of 1.2 Mb DNA, and surprisingly, replicates only in the cytoplasm of the infected cells without entering the nucleus, which is a unique characteristic among DNA viruses. Their existence is undeniable; however, as with any novel discovery, there is still uncertainty concerning their pathogenicity mechanisms in humans and the nature of the Mimivirus virophage resistance element system (MIMIVIRE), a term given to describe the immune network of the Mimivirus, which closely resembles the CRISPR-Cas system. The scope of the present review is to discuss the recent developments derived from structural and functional studies performed on the distinctive characteristics of the Mimivirus, and from studies concerning their putative clinical relevance in humans.

Contents

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Key words: Mimivirus, *Acanthamoeba polyphaga*, Mimivirus virophage resistance element, virophages, Marseilleviridae

1. Introduction

In 1892, Ivanowski reported that extracts from infected tobacco plants remained infectious following filtration through a Chamberland filter candle (1). As bacteria did not pass through such filters, a novel group of filterable pathogens were discovered. Several years after this discovery, Beijerinck was the first to refer to the causative agent of the tobacco mosaic as a 'virus'. He revealed that the 'cause of illness' was able to migrate in an agar gel, which indicated that it was an infectious soluble agent, and not fixed in place as would be the case for bacteria. Discoveries made on the tobacco mosaic virus triggered the beginning of virology (1), while the subsequent discovery of bacteriophages by Felix d'Herelle and of numerous other viruses by the early 20th century further advanced the field (2). In 1940, the first electron micrograph of a bacteriophage was published, which convinced sceptics who had argued that bacteriophages were relatively simple enzymes and not viruses (3). At present, the current concept of a virus refers to an ultramicroscopic (20-300 nm in diameter), metabolically inert, infectious agent that replicates only within the cells of living hosts, primarily bacteria, plants and animals, is composed of either RNA or DNA as its genetic material, and is enclosed by a protein coat or capsid and, in more complex types, by a surrounding envelope (4,5). Viruses are common pathogens in humans, causing a number of diseases such as hepatitis, measles, poliomyelitis and smallpox (6). However, during the last twenty years, viruses have emerged as powerful tools in gene therapy, as they have been extensively used as vehicles of therapeutic genes for the treatment of several monogenic diseases such as immunodeficiencies (X-SCID, ADA-SCID), β-thalassemia, sickle cell disease or hemophilia, but also for complex diseases such as cancer (7). The advent of CAR-T cell gene therapy by utilization of lentiviral vectors has further revolutionized cancer treatment (8) while T-VEC, an engineered herpes simplex virus-1, has shown great promise in melanoma (9).

In 2003, La Scola *et al* (10) described the Mimivirus, which is a parasite of amoebae and was long considered to be a bacterium due to its size. Specifically, this particular virus has a diameter of 500 nm and a 1.2 Mb DNA genome. However, the Mimivirus is not the only type of giant virus. Philippe *et al* (11) later discovered two Pandoraviruses with 1.9 and 2.5 Mb DNA genomes, respectively.

The Mimivirus was observed for the first time in 1992, when a case of nosocomial pneumonia was investigated for amoeba-associated microorganisms; however, at the time it was considered to be an intracellular bacterium based on its appearance under the light microscope. Furthermore, *Acanthamoeba polyphaga* isolated from a water-cooling tower were reported to contain this organism, which years later was characterized as a giant virus and termed *Acanthamoeba polyphaga* Mimivirus (APMV). This was the origin of the identification of the novel family of Mimiviridae (10,12). Results from transmission electron microscopy combined with relevant genomic data confirmed that the organism was in fact a virus (10).

The Mimivirus has unique characteristics. Its genome is larger than the majority of other viruses, and certain bacteria and archaea, and it is comparable to the genome of certain eukaryotes. The genome of the Mimivirus contains 1,262 genes, which is three times higher than the number of genes contained in any other virus (10). Furthermore, genomic analysis has indicated that the Mimivirus may be a chimera, due to their ability to exchange genetic material with their hosts and also with other parasites that exist within the same host cell. Additionally, these viruses exchange genes with other large DNA viruses of amoeba, such as the Marseillevirus (13). The virions of the Marseilleviruses enclose a genome ranging from 348 to 404 kb that encodes for 386-545 predicted proteins, while their overall genome organization resembles that of the Mimivirus (13). These data prompted virologists to establish Megavirales, a novel virus order composed of Mimivirus, Marseillevirus and other similar viruses, as well as members of the Poxviridae, Iridoviridae, Ascoviridae, Phycodnaviridae and Asfarviridae families (14). However, this order remains under discussion among virologists.

2. Evolution of the Mimivirus

A central characteristic of other large DNA viruses, such as Poxviridae, is that their genome encompasses a stable region that is unique to the virus and a variable region that is frequently composed of, or is similar to, host genes. Specifically, for the Mimivirus, the large diversity observed in the variable regions prompted scientists to propose that these viruses are as old as the three traditional domains on Earth, consisting of Archaea, Bacteria and Eukarya, as proposed by Woese *et al* (15). Notably, Raoult (16) have proposed that phylogenesis should be performed not based on the classical ribosomal RNA genes sequences, but on transfer RNA (tRNA) and RNA polymerase-encoding genes. Such clustering resulted in four groups with different genetic repertoires: Giant viruses, Archaea, Bacteria and Eukarya. Thus, giant viruses may be considered as a fourth branch in the tree of life (16-18). Interestingly, in 2017 Marcelino *et al* (19) demonstrated that Mimiviruses are a sister group of Eukarya using analysis of evolutionary relationship of proteins involved in the translation system.

Furthermore, Forterre (20) argues that giant viruses lie at the origin of the eukaryotic nucleus and, according to the theory of viral eukaryogenesis, the large DNA viruses were important in the formation of the nucleus. Forterre (20) and others (21-23) also propose that DNA may have been 'devised' by viruses in order to convert a world of RNA-based organisms to one where DNA is the major hereditary material. According to the 'RNA world' hypothesis, RNA is considered to be the molecular basis of the origin of life on Earth (24), primarily due to its catalytic potential. Therefore, the theory proposed by Forterre hypothesizes that early RNA cells and ancient RNA viruses coexisted, and that early RNA cells were parasitized by these viruses. However, the introduction of a DNA virus into such primitive cells may have been facilitated by the fact that these hosts may have begun to develop RNA-specific defense mechanisms to protect them against an RNA viral infection. During that process, viruses may have shared or exchanged gene sequences with the host cells, leading to the introduction of DNA membrane-surrounded vacuoles that later evolved into nuclei, which contained DNA molecules that are steadier as genetic material compared to RNA molecules in terms of resistance to degradation, and thus were favored by natural selection (17).

3. Genome organization and replication cycle of the Mimivirus

The Mimivirus is considered to be a unique type of virus. Although their genome does not contain any ribosomal RNA-encoding genes, it does include genes responsible for cellular processes, such as protein translation and metabolism, with genes including amino-acyl tRNA synthase, tRNA and translation factors. The presence of these genes within the Mimivirus genome confers a degree of independence in terms of viral replication, as the protein machinery of the host is not an absolute necessity. The Mimiviruses and Marseilleviruses possess numerous chimeric genes with sequences that are derived from other viruses, bacteria or eukaryotic organisms, suggesting the occurrence of lateral gene transfer (25). The genome of the Mimivirus comprises four primary groups of open reading frames (ORFs), including Megavirales core genes, genes involved in lateral gene transfer, duplicated genes and ORFans, which represents genes with limited or no homology with any other characterized or mapped nucleotide sequence (26). Their genome also contains a component that is termed transpoviron, which is equivalent to a transposon and is a mobile genetic element of ~7 kb that encompasses 6-8 protein-encoding genes (27,28). Transpovirons encode a superfamily 1 helicase, which includes an inactivated family B DNA polymerase domain. Based on the phylogenetic analysis of the helicase domain, it has been concluded that transpovirons evolved from polinton-like viruses via the deletion of several genes (27).

Mimivirus viral particles are composed of a protein capsid of ~500 nm in diameter, which is enclosed within a polysaccharide layer in which multiple fibers are embedded. These fibers are ~120-140 nm long and 1.4 nm thick, which contributes to an overall diameter of ~700 nm, as shown in

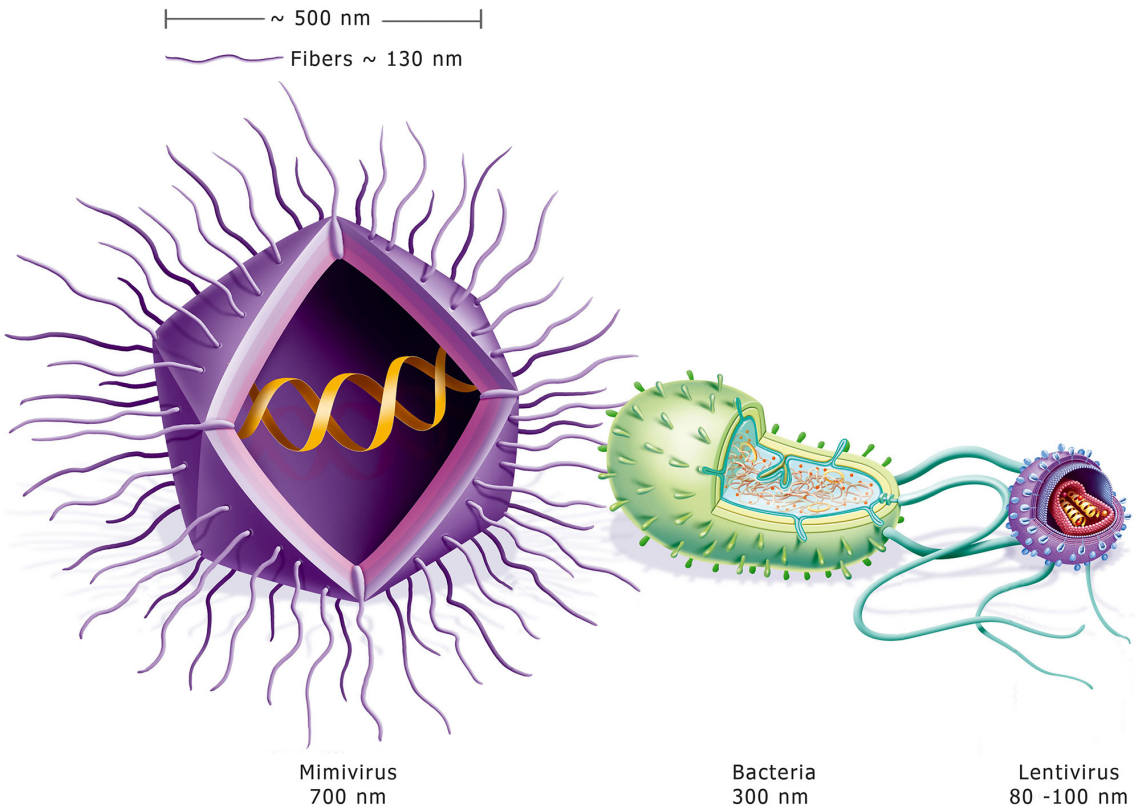

Figure 1. Size comparison among different microorganisms. The uniquely large size of the Mimivirus (~700 nm) is depicted and compared to the size of a bacterium and of a lentivirus, such as the human immunodeficiency virus. Modified with permission and license from BSIP SA/Alamy Stock Photo (https://www.alamy.com/stock-photo/bsip.html).

Fig. 1. Another important characteristic of the Mimivirus is their capacity to propagate exclusively within the cytoplasm of the infected cell. Notably, although they are DNA viruses that infect eukaryotic cells, Mimiviruses never enter the nucleus of the host cell. Initially, Mimivirus particles are internalized by endocytosis and are surrounded by a membrane vesicle until the viral 'star-gate' channels open, which leads to membrane fusion and results in the release of the genome-containing capsids into the cytoplasm of the host cell (26). However, recent data using electron microscopy, indicate that all giant viruses studied so far including Mimivirus enter cells by phagocytosis. Fusion of lysosomes surrounding the virion-containing phagosome has been observed and suggested to trigger the virus uncoating (29). Recent studies attempt to characterize proteins released from Giant viruses including Samba virus, a member of Mimivirus lineage A, during infection elucidating the molecular forces that trigger it. Remarkably, not only the expected protein types are released, such as those involved in genome translocation, blocking host replication and hijack cell machinery, but also proteins that play a role in virus protection from oxidative stress and chemotaxis (30). Mimivirus DNA replication occurs exclusively in the cytoplasm and it is dependent on the host nucleus. A vast number of nuclear factors that originate from the endoplasmic reticulum or the outer nuclear membrane, which are necessary for replication, are transferred through vesicle transportation from the nucleus to the cytoplasm. The vesicles fuse together in the cytoplasm to create what is termed a 'viral factory', which is a transcriptional and translational mechanism that copies the viral genome and ensures viral replication by utilizing nucleotides of the host cell. The host cell dies within ~16 h following infection, a process which leads to the production of ~10,000 new viruses (26).

4. The Mimivirus immune system

While examining the Mamavirus, a strain of Mimivirus, La Scola *et al* (31) identified a virus that infects other giant viruses, which was termed Sputnik. These small viruses were detected using transmission electron microscopy and were later termed virophages, in accordance with the name given to bacteriophages. Virophage replication utilizes the viral mechanism that the Mamavirus manufactures within its amoeba host. Sputnik, which is not the only characterized virophage, has a genome size of 18 kb and carries genes from various host types. Another virus that is similar to the Mimivirus, termed *Cafeteria roenbergensis* virus (CroV), is parasitized by a virophage that is termed 'Mavirus' (32). CroV is a giant virus that infects the marine bicosoecid flagellate, *Cafeteria roenbergensis*, and has one of the largest genomes of all established marine viruses (32,33). In 2014, another virophage was characterized and was termed Zamilon, originating from the Arabic word 'xamilon', which means 'the neighbor'. Surprisingly, Zamilon was not able to infect all strains of Mimivirus (34). In 2019, a novel virophage named Guarani was described. Guarani has a 19 kb double-stranded DNA genome encoding 22 genes, quite similar to Sputnik genes, despite the fact that Guarani seems to be more related to Zamilon. As

all Sputnik strains, Guarani is capable of infecting the three lineages of the Mimiviridae family (35).

Zamilon was used to infect different Mimiviruses strains that were derived from the three characterized lineages, A, B and C, of the Mimiviridae family (36). The results demonstrated that Zamilon was able to infect B and C lineages, but strains from the A lineage were resistant to Zamilon infection. All resistant strains, including all strains from the A lineage and one strain from the C lineage (*Megavirus chilensis*), were demonstrated to have a 28 bp Zamilon sequence incorporated in their genome. This sequence is encoded by the ORF4 in the genome of Zamilon and results in the expression of a protein similar to transposase A; the sequence is integrated within the Mimivirus gene R349 and the corresponding orthologous genes in APMV A Mimiviridae strains. Notably, all APMV A genomes contained a 15 nucleotide-long sequence in four copies, which was derived from the Zamilon 28 bp sequence. However, the respective sequence was not detectable in group B and C genomes. Furthermore, a significant association between Zamilon resistance and the presence of the repeated Zamilon sequence in Mimiviruses was observed (36). A recently isolated Mimivirus that belongs to lineage A, lacking three of four repeats of R349 gene, exhibited susceptibility to Zamilon (37). A more detailed analysis of this 15 bp repeat sequence and its vicinity within the Mimivirus genome revealed that, downstream of the 15 bp repeats, there is a putative phage-type endonuclease, which is encoded by the APMV A ORF R354 and is associated with a lamda exonuclease protein belonging to the Cas4 nuclease family. Adjacent to the R349 gene, there is a putative helicase domain associated with a SNF2 domain, encoded by the APMV A ORF R350, which contains motifs characteristic of the Cas3 protein. In addition, a putative RNase III gene, encoded by the APMV A ORF R343, is localized upstream of the 15 bp sequence repeats (36). In order to determine the role of the aforementioned system in Zamilon infection, Levasseur *et al* (36) employed RNA interference technology to silence all associated gene sequences, 27 genes in total, in the Mimivirus genome. The results demonstrated that the silencing of R354, R350 and R349 significantly inhibited Zamilon infection. Therefore, this network of ORFs was termed the 'Mimivirus virophage resistance element' or MIMIVIRE (36).

5. MIMIVIRE: The Mimivirus CRISPR-Cas system

The above findings prompted scientists to associate MIMIVIRE with the recently characterized CRISPR-Cas system (38,39). In prokaryotes, the CRISPR-Cas system has a key role in defense and is detected in ~48% of bacteria and 80% of archaea (38). The bacterial type II clustered, regularly interspaced, short palindromic repeats (CRISPR) system, comprises three minimal components (40): A CRISPR-associated effector nuclease Cas9, a specificity-determining CRISPR RNA (crRNA), and an auxiliary trans-activating crRNA (tracrRNA). The hybridization of crRNA and tracrRNA leads Cas9 to target genomic loci that match a 20-nucleotide guide sequence (gRNA) contained at the 5' end of crRNA, and residing upstream of an essential 5'-NGG protospacer adjacent motif (PAM). In addition, crRNA and tracrRNA duplexes may also be fused to form chimeric single guide RNA (sgRNA), which mimics the natural crRNA-tracrRNA hybrid. Thus, crRNA-tracrRNA duplexes and sgRNAs may be used to bind to Cas9 and target specific loci. Furthermore, this system allows bacteria to retain a memory of viruses that have infected them previously, and allows them to respond more efficiently during subsequent infections. Specifically, during the first viral infection, a fragment of the viral DNA is incorporated into the CRISPR locus. When bacteria encounter the same virus strain again, they recognize the viral DNA and digest it via the Cas9 nuclease, which is directed to cleave the exogenous DNA via the crRNA-tracrRNA duplexes transcribed from the respective region of the CRISPR locus. As crRNA-tracrRNA duplexes and sgRNAs may be used to target Cas9 for multiplexed genome editing in eukaryotic cells, the CRISPR system has revolutionized the field of genome editing (41).

Comparison of the MIMIVIRE system to the CRISPR-Cas system revealed that nuclease R354 may cleave the invading nucleic acid and lead to unspecific cleavage and partial degradation of the double-stranded DNA, being more efficient on low content (28-38%) GC templates. Therefore, Mimivirus and virophage genes with ~29% GC content are readily degraded, while the *Acanthamoeba polyphaga* genome, with 59% GC-rich content, is not. Thus, the components of the MIMIVIRE system that correspond to those of the CRISPR-Cas system are the R354 protein, which cleaves the DNA, the R350 gene with the proposed helicase activity and the 15 bp sequence in the R349 ORF (36,42). However, it should be noted that the similarity between the MIMIVIRE and the CRISPR system remains controversial (43).

6. Mimiviruses and human health

At present, the importance of Mimiviruses in human health is an area that has not been investigated extensively. However, antibodies against Mimiviruses have been detected in a technician who developed pneumonia after working with Mimiviruses, while in a small number of reported cases, strains of Mimivirus were detected in the lung of patients who developed pneumonia (44-46), compatible with features fulfilling several of the criteria for viral disease causation (47). Furthermore, antibodies against Mimivirus-encoded collagen, have been implicated in rheumatoid arthritis (48). Interestingly, immunofluorescence studies of young asymptomatic adults revealed that humans are frequently exposed to Marseillevirus (49). Marseillevirus has also been detected in a lymph node of a patient with Hodgkin's lymphoma, associated with IgG antibodies against the virus (50). However, the hypothesis suggesting Marseillevirus as a potential additional viral causative agent of Hodgkin's lymphoma requires further investigation. Another case report documented the detection of Marseillevirus in the lymph node of a child with adenitis of unknown etiology, suggesting that this virus can cause symptomatic infection on a background of a defective immune system (49). Additionally, APMV has been reported to grow in human peripheral blood mononuclear cells and induce immune reaction via the production of type I interferons (IFNs) (51). This observation is the first convincing evidence of a host-pathogen interaction between APMV and humans in terms of immunity. Moreover, APMV is able to replicate on IFN-α pretreated cells, but not on IFN-β pretreated cells, as they are sensitive to the antiviral action of IFN-β in a dose-dependent manner (52). However, APMV infection is

not able to induce the expression of IFN-stimulated genes, and infected peripheral blood mononuclear cells do not express viroceptors for IFN-α_2 and IFN-β (52).

The IFN-α and β receptor subunit 1 complex controls an exclusive group of genes by a signaling pathway that remains unknown and is thought to be mediated by IFN regulatory factor 1. One of these genes is the immune responsive gene 1 (IRG1) that codes an enzyme responsible for producing itaconic acid, which is an organic compound that inhibits isocitrate lyase. Isocitrate lyase is an enzyme that has an important function in the glyoxylate shunt, a process that is required for bacterial growth (53). IRG1 is expressed in macrophages thus leading to an association between metabolism with immune defense. Therefore, itaconic acid functions as an immune-supportive metabolite in mammalian immune cells by exhibiting antibacterial action (53). Previous studies (51,54) have investigated itaconic acid as a virucidal agent and reported its ability to inactivate APMV *in vitro* in a dose-dependent manner. However, experimental data clarifying whether the action of itaconic acid against APMV is direct or indirect are still missing. Thus, as the production of itaconic acid is mediated by IFN, this novel mechanism may also be implicated in viral infections, and IFN-β antiviral activity may be responsible for inhibiting APMV infections in human cells. Such studies for the experimental validation of this working hypothesis need to be performed.

7. Conclusions

Undoubtedly, Mimiviruses represent a novel concept in biology and medicine, and constitute a novel and intriguing field for future research, since their discovery has challenged our traditional perception about viruses and the definition of life in general. Their existence is undeniable; however, as with any novel discovery, uncertainty exists concerning their pathogenicity in humans and the importance of the MIMIVIRE system, which comprises an immune network of ORFs that resembles the CRISPR-Cas system.

Thus, although numerous scientific issues are yet to be addressed, research concerning giant viruses may provide important opportunities for future experimentation and clinical investigations in all aspects of biomedicine, including immunology, molecular biology, oncology and internal medicine.

Acknowledgements

Not applicable.

Funding

The present review was co-funded by the European Union's European Social Fund and Greek National Funds through the Program THALIS, under the *Operational Program Education and Lifelong Learning* of the National Strategic Reference Framework (project no. 383418; grant no. 70-3-11830) to Professor Kalliopi I. Pappa.

Availability of data and materials

Not applicable.

Authors' contributions

EK searched the literature and wrote the manuscript. EP and EM reviewed the manuscript. KIP and NPA conceived and designed the study, and edited the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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