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The role of giant viruses of amoebas in humans

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Since 2003, dozens of giant viruses that infect amoebas (GVA), including mimiviruses and marseilleviruses, have been discovered. These giants appear to be common in our biosphere. From the onset, their presence and possible pathogenic role in humans have been serendipitously observed or investigated using a broad range of technological approaches, including culture, electron microscopy, serology and various techniques based on molecular biology. The link between amoebal mimiviruses and pneumonia has been the most documented, with findings that fulfill several of the criteria considered as proof of viral disease causation. Regarding marseilleviruses, they have been mostly described in asymptomatic persons, and in a lymph node adenitis. The presence and impact of GVA in humans undoubtedly deserve further investigation in medicine.

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The emergence of giant viruses of amoebas

The story of giant viruses that infect amoebas (GVA) began with the isolation of the Mimivirus in 1992 [1,2]. This was made possible by using a strategy that consisted of inoculating samples on an axenic culture of *Acanthamoeba* spp. and was implemented to isolate amoeba-resisting microorganisms such as *Legionella* spp. [2]. The first mimivirus isolate was obtained from cooling tower water while investigating a pneumonia outbreak in England. It took a decade to identify that one of the amoeba-resistant microbes was a giant virus, which was visible on light microscopy and looked like a Gram-positive coccus. This was eventually revealed in 2003 in Marseille by using electron microscopy [1,2]. Thus, the investigation triggered in 1992 by pneumonia cases serendipitously led to the discovery of the largest viruses known so far, which strongly challenge the concept and definition of viruses [1,3,4]. Moreover, it suggested the link between these GVA and humans and their possible pathogenicity.

Dozens of additional mimiviruses, which were classified in the family *Mimiviridae*, were isolated in amoebas from environmental water samples collected in various geographical areas worldwide [5,6]. In addition, these studies led to the discovery of the first viruses of viruses, named 'virophages', which replicate in the viral factories of mimiviral hosts and can impair their replicative cycle and morphogenesis [7,8]. Moreover, other GVA have been discovered since 2008 [4,9]. Some were classified in the family *Marseilleviridae* and others include pandoraviruses [10,11], *Pithovirus sibericum* [12], faustoviruses [13] and *Mollivirus sibericum* [14], which represent new putative virus families [9]. All these GVA cultured in amoebas display many unique characteristics that put them on the edge of the virus definition, and warrant proposing their reclassification as representatives of a fourth 'TRUC' (an acronym for Things Resisting Uncompleted Classifications) of microbes [15] (reviewed by Sharma *et al.* [4]). They have been proposed for classification in a new viral order, *Megavirales*, alongside other double-stranded DNA viruses [16].

GVA appear to be common in our biosphere; they have been isolated from marine water, freshwater and soil samples collected in several countries worldwide (https://drive.google.com/open?id=1TmFZ3DBnD3jNl3lwjyS6TOa741M&usp=sharing) [5,9,17,18]. This has been corroborated by metagenomic studies that detected sequences matching these viruses in similar environmental samples collected in highly diverse geographical areas [19,20] (reviewed by Halary *et al.* [21•]). In addition, their hosts, *Acanthamoeba* spp. (for most of these viruses) or *Vermamoeba vermiformis* (for faustoviruses) are ubiquitous organisms that are common in human environments, very resistant and described as 'Trojan horses' for their parasitic pathogens [22,23]. Moreover, GVA prevalence was probably underestimated because 'viral' fractions analyzed were most often obtained by filtration through a 0.2 μm-large pore size, which neglects gigantic virions [20]. Taken together, these findings strongly suggest that humans are exposed to GVA. Noteworthy, 12% of 242 samples collected from inanimate surfaces in a Brazilian hospital were

positive for Mimivirus DNA by PCR, the incidence being significantly greater in respiratory isolation facilities, and amoebal lysis was obtained from 83% of these samples [24].

Other studies have reported the isolation of mimiviruses from oysters [25] and a leech [26], and their detection by PCR in monkeys and cattle [27]. In addition, a Marseillevirus was isolated from a diptera [26] and a faustovirus was cultured from culicoides [28•]. Moreover, mimivirus-like sequences were identified in metagenomes generated from bats, rodents, dromedaries and culicoides, and faustovirus-like and pandoravirus-like sequences were detected in metagenomes generated from culicoides [21•,28•] (reviewed Halary *et al.* [21•]).

Evidence for a causative role of giant viruses of amoebas in pathogenicity

Causality criteria

An increasing body of data supports the presence of GVA in humans, and in addition, the question of the putative pathogenic role of these viruses has been addressed and documented, mainly for mimiviruses, and more recently for marseilleviruses. Establishing a causative role of viruses in diseases can be a long journey. Criteria developed since 1840 by Henle, Loeffler and Koch to prove the etiologic association between an infectious agent and a specific disease have been deemed less and less appropriate over time [29]. Other criteria for causative relationships were proposed [30], including some specifically applied to viruses in 1937, 1957 and 1976 (Box 1) [31–33]. However, newly discovered viruses can challenge existing postulates, as, for instance, viruses determining chronic or latent infections. Thus, with the advent of new technologies and improved knowledge in microbiology and virology, criteria considered for suspecting or establishing a causality link have drifted considerably. Notably, sequence-based criteria were introduced in 1996, and metagenomic Koch's postulates were finally proposed in 2012 [34,35]. Since 2003, the presence and possible pathogenic role of GVA has been serendipitously observed or investigated using a broad range of technological approaches including culture, electron microscopy, serology and various techniques based on molecular biology, including metagenomics (Table 1). The findings fulfill several of the criteria considered as proofs of viral disease causation (Figures 1 and 2).

Host cells other than phagocytic amoebas for giant viruses of amoebas

All GVA have been isolated on cultures of *Acanthamoeba castellani*, *Acanthamoeba polyphaga*, or *V. vermiformis* [13,36]. Numerous cell lines have been tested for their permissivity to mimiviruses or marseilleviruses. In experimental inoculation tests, Mimivirus was capable of entering professional phagocytes, among which various human myeloid cells including circulating monocytes, monocyte-derived macrophages and myelomonocytic cells, and also mouse myeloid cells [37•]. Further experiments conducted with mouse macrophages showed a significant increase in Mimiviral DNA load during a 30-hour period of incubation; in addition, only approximately one quarter of the macrophages were viable after 30 hours, and macrophage extracts led to Mimivirus replication within amoebae and to amoebal lysis. These findings indicated productive infection of macrophage by Mimivirus post-internalization. In addition, Mimivirus was demonstrated to replicate in total human peripheral blood mononuclear cells (PBMC), as measured by the tissue culture infective dose method [38•]. Furthermore, Mimivirus was revealed to induce type I IFN production in infected human PBMC and to inhibit interferon stimulated genes expression in these cells. These findings question if amoebae are the exclusive hosts for the giant Mimivirus. Moreover, inoculation of Jurkat cells, which are immortalized human T lymphocyte cells, with a serum sample positive for Giant blood Marseillevirus (GBM) DNA led to detection of this virus by PCR in the culture supernatant, and viral DNA and virions were detected within Jurkat cells 21 days post-infection by PCR, fluorescence *in situ* hybridization, or transmission electron microscopy [39••]. Although GBM was not propagated, these results indicated productive infection of these cells. It should be considered that the host barrier may be far more limited for GVA than for other viruses, because GVA infect their hosts by phagocytosis [37•]. This was exemplified by the capability of Mimivirus to enter human macrophages through phagocytosis, and this closely resembled Mimivirus entry in amoebas [37•]. In addition, mimiviruses, marseilleviruses or faustoviruses have been isolated from different phagocytic protists, including amoebozoa, and stramenopiles, and also mammals, including humans, and also insects [9,26,27,28,39].

Mimivirus

Serological-only evidence

Concomitantly with the initial attempts to identify the giant Mimivirus, serological testing of sera from patients with unexplained pneumonia showed that the strongest reactivities were against this amoeba-resisting microbe [40]. Subsequently, the prevalence of antibodies to Mimivirus was assessed using microimmunofluorescence in several studies, in most cases in pneumonia patients hospitalized in intensive care units (ICU) (Table 1). IgG prevalence was most often ≈10–20% in pneumonia patients, ranging from 0% to 25% [41•,42–44]. In contrast, it was 0% and 2.3% in intubated control patients without pneumonia and healthy controls, respectively [40,41•]. Moreover, IgG and IgM elevations or seroconversions were observed in patients with hospital-acquired pneumonia [44]. The first strong evidence of infection with a GVA was in a laboratory technician who handled large amounts of Mimivirus and developed unexplained pneumonia [45••]. He exhibited seroconversion to 23 Mimivirus proteins, as assessed by 2-dimensional gel