Epidemiology of Monkeypox

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Acanthamoeba is well known to host a variety of microorganisms such as viruses, bacteria, protozoa, and yeast. Given the number of cases of monkeypox infection, it is speculated that amoebae may be aiding viral transmission to the susceptible hosts. Although there is no confirmatory evidence to suggest that *Acanthamoeba* is a host to monkeypox (a double-stranded DNA virus), the discovery of mimivirus (another double-stranded DNA virus) from *Acanthamoeba*, suggests that amoebae may shelter monkeypox virus.

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1. *Acanthamoeba* in the Environment

Acanthamoeba is known to have an extensive global distribution that spans both aquatic and terrestrial habitats [1][2]. In fact, *Acanthamoeba* is one of the most prevalent protists in the soil. As significant grazers of the bacterial biomass in these settings, these amoebae are thought to regulate not only the variety but also the abundance and turnover of bacterial communities in the soil and plant rhizospheres [3][4][5]. *Acanthamoeba* also releases nutrients locked in the microbial biomass in the soil microbial loop, ultimately promoting plant growth [6].

Acanthamoeba can survive in a variety of environments and have been found in swimming pools, bottled water, seawater, ponds, stagnant water, freshwater lakes, saltwater lakes, river water, distilled water bottles, ventilation ducts, the water–air interface, air-conditioning units, sewage, compost, sediments, soil, beaches, vegetables, air, surgical instruments, contact lenses and their cases, the atmosphere (recent decontamination studies), etc., and they have been isolated from the continent of Antarctica [2][7]. Moreover, free-living *Acanthamoeba* spp. have been discovered in a diverse range of animals, including monkeys, dogs, lizards, kangaroos, Indian buffaloes, reptiles, mice etc. [8], and even marine creatures, including fish, amphibians, etc. [9][10][11]. Furthermore, reports of keratitis due to *Acanthamoeba* have been described in animals; however, prospective studies are warranted to comprehend the prevalence of amoebae in animals [12].

2. *Acanthamoeba:* The Microbial World's Trojan Horse

The Trojan horse nature of amoebae, together with their ubiquitous presence in the environment, strengthens the researchers' hypothesis. The life cycle of *Acanthamoeba* is comprised of a vegetative trophozoite stage during which amoebae divides mitotically and an inactive dormant cyst stage [2][13][14]. Of note, amoebae cysts are highly impervious to chemicals and physical and radiological conditions and can also be air-borne [15]. This ability of

amoebae to phenotypically transform from an active trophozoite form into an air-borne cyst form is of additional concern. *Acanthamoeba* is already known to shelter a wide range of viruses such as poliovirus, mimivirus, enterovirus, coxsackievirus, adenoviruses, and echovirus, amongst others [16], as well as bacteria such as *Coxiella*, *Legionella*, *Mycobacterium*, *Helicobacter*, *Salmonella*, *Pseudomonas*, *Escherichia coli*, *Vibrio*, *Listeria*, *Rickettsia*, *Shigella*, *Pasteurella*, to name a few (reviewed in [17]). Of note, *Acanthamoeba* act as an incubator-type reservoir for microbes, as well as pathogens, where such microbes utilize the amoebae's defense system to withstand harsh environments and/or elude host defenses and antimicrobial therapy while multiplying within amoebae. Moreover, amoebae are known as a "genetic melting pot", where the exchange of genes leading to the adaptation of microbes, possibly resulting in greater pathogenicity, may occur [18].

3. *Acanthamoeba* as a Training Ground for Pathogens

It is well known that pathogens may have used their capacity to thrive and proliferate inside *Acanthamoeba* as a tool for learning how to avoid the assault of macrophage-mediated death. This is due to the striking similarities observed between how different pathogens such as *Mycobacterium* or *Legionella pneumophila* survive inside human macrophages and *Acanthamoeba*, including the use of similar transcriptional, post-transcriptional, and cellular mechanisms. This suggests that both amoebae and human macrophages may share characteristics that enable the intracellular pathogens to spread infection [16][19].

Interestingly, the natural reservoir of pathogenic *Chlamydiae* is not known [20]. The discovery that *Chlamydophila pneumoniae* can multiply in free-living amoebae in addition to environmental *Chlamydiae* [20] suggests that protozoa may act as an environmental reservoir for *Chlamydiae* in this situation. These studies indicate that *Chlamydiae* may flourish in both amoebae and humans, even though the longevity of this relationship during the amoeba encystment is not yet known [20]. In line with this, it was also shown that the amoeba symbiont *Parachlamydia acanthamoebae* can enter and grow in human macrophages and may therefore have the ability to infect humans [17][21][22].

In addition, *Acanthamoeba* is capable of taking in and housing *Mycobacterium leprae* (known to cause leprosy, a neurological and dermatological disease), and the bacteria can keep both their viability and growth characteristics within the amoebae [23]. Moreover, it has been reported that the quiescent encysted amoebae can shield *M. leprae* from harmful circumstances such as desiccation and high temperature and pH [24]. Similar and prospective studies to determine if the monkeypox virus remains viable and can grow within amoebae trophozoites and cysts are warranted.

4. The Role of "One Health" in Monkeypox Infection

The need for improved collaboration between the human, animal, and environmental health sectors has been underlined by the apparent inability of global health security to avoid or prepare for the recent COVID-19 pandemic [25]. The monkeypox epidemic is a resurgent viral zoonosis that naturally occurs in highly forested areas in Africa.

Monkeypox virus inter-human transmission, albeit rare, is what causes epidemics, particularly in residential and medical settings. The information that is now available, however, points to the possible cessation/reduction of human infections in the absence of recurring zoonotic incursions. Therefore, a key area to focus on in fighting this infection would be to prevent viral transmission from animals to humans [26]. During the 2003 monkeypox outbreak in the US, which was caused after patients came in contact with imported animals (prairie dogs), two patients presented with severe illness [27][28]. One patient had encephalitis that improved during a 14-day hospital stay, and the second had diffused pox lesions, including oropharyngeal lesions that resulted in difficulty in breathing and swallowing [27][28].

To this end, the use of an amalgamation of approaches may well be the way onward in targeting viruses such as the monkeypox virus that may be using amoebae to shelter and persist in the environments. This notion is further strengthened by the findings from a recent study that investigated different amoebae species that are frequently observed in the environment (namely: *Acanthamoeba* spp., *Dictyostelium discoideum*, *Vermamoeba vermiformis*) as potential reservoirs for the plague causing bacteria reservoir *Yersinia pestis* [29]. Interestingly, these amoebae were isolated from the soil in plague-affected prairie dog burrows. Field-based and laboratory studies were carried out to evaluate the environmental co-occurrence of the study amoebae species with plague epidemics, the prevalence and severity of experimental infections in amoebae, the location of bacteria within amoebae, their viability following phagocytosis, and the replication of bacteria inside trophozoite amoebae [29]. The authors demonstrated the natural phenomena of the co-occurrence of plague-causing bacteria and various amoebae species during an active plague epizootic, suggestive of the complex interactions between bacteria, amoebae, and host immune factors and the environment. Furthermore, amoebae were isolated from prairie dog burrows, which were also the same species that were implicated in monkeypox infection in 2003 [27][28][29].

5. Monkeypox Virus Interaction with Its Hosts

Monkeypox virus interactions with *Acanthamoeba* and how these interactions impact each symbiont for their prevalence and species dominance are important questions that remain undetermined. The roles of symbionts within their hosts can range from mutually beneficial to parasitic, depending on the endosymbiont and its host. *Acanthamoeba* is considered as the Trojan horse of the microbial world. However, amoebae interactions with smaller microbes is primarily driven by its nutritional needs to ensure that it remains as a vegetatively and metabolically active and multiplying trophozoite. In addition to an increase in its species, this property of *Acanthamoeba* is attributed to the regulation of microbial communities in the environment, in particular for the soil composition/fertility. However, when it feeds on a few selected bacteria/viruses, a selective pressure is exerted that favors phagocytic microbe with mechanisms to resist digestion. Hence, in these cases, amoebae serve as intracellular training grounds for bacteria/viruses to evolve resistance mechanisms against phagocytic killing as well as allowing selected bacteria/viruses to be housed inside *Acanthamoeba* as endosymbionts [17]. Although the impact of *Acanthamoeba*–microbe symbiosis is often described as beneficial to the endosymbionts in terms of enhancing pathogenicity, survival under harsh conditions such as the presence of disinfectants/chemicals as well as resisting physiological/radiological conditions and/or fulfilling their energy needs, the effect of endosymbionts on

the host species such as *Acanthamoeba* remains unclear. Among a few studies, it is suggested that the endosymbionts provide benefits to their host *Acanthamoeba* by enhancing *Acanthamoeba* motility and growth that could enhance its environmental prevalence, protecting *Acanthamoeba* from the pathogenic *Legionella* spp. and enhancing *Acanthamoeba* pathogenicity (reviewed in [30]). Using advanced molecular "omics" technologies, there is a need for a holistic approach to understand the microbial community dynamics in natural environments to comprehend effect/function of each symbiont and their prevalence in complex ecological populations.

The pathogenesis of monkeypox virus infection is not well understood, but it is believed to involve a complex interplay between viral and host factors. Monkeypox virus is an enveloped, double-stranded DNA virus that belongs to the genus Orthopoxvirus in the family Poxviridae. Members of the Poxviridae family, such as the monkeypox virus, are thought to exhibit diverse spectra of living and surviving in a host cell [31]. The cellular entry receptor for the monkeypox virus has not been identified, but it is believed to be a member of the laminin-binding integrin family. Once inside the host cell, the virus undergoes a series of replication steps, including the expression of early and late viral genes, DNA replication, and the assembly of virions [32]. The virus replicates in the cytoplasm of infected cells and produces two distinct forms of infectious particles, intracellular mature virions and extracellular enveloped virions [33]. The immunohistochemical and histopathological tests found that the monkeypox virus antigens were identified in ovarian, brain, heart, kidney, liver, pancreatic, and lung tissues [34], suggesting extensive tissue infection and damage. The host immune response to monkeypox virus infection is complex and involves both innate and adaptive immune mechanisms. Innate immune cells, such as dendritic cells, macrophages, and natural killer cells, are activated early in the infection and secrete pro-inflammatory cytokines and chemokines, which recruit additional immune cells to the site of infection. The adaptive immune response to the monkeypox virus involves the production of virus-specific antibodies and T cells, which can recognize and eliminate infected cells [35]. The Poxviridae family virus develops many strategies to escape the host's immune response to infection. Natural killer (NK) cells are supposed to kill virus-infected cells by secreting cytokines that would stimulate the activity of other cell types, such as T cells and dendritic cells [36]. Monkeypox virus infection can induce NK cell changes such as an increment in the number of all NK subsets in non-human primates [37]. Moreover, following the monkeypox virus infection a delayed or reduced expression of chemokine receptors on each NK cell subset suggested its immune evasion response [38]. It was also reported that the monkeypox virus has a safe avoidance component and the avoidance process utilized by the monkeypox virus ensures the viral store is resistant by repressing the activation of CD4+ and CD8+ T cells after interaction with monkeypox-virus-infected cells [39].

Although the precise mechanisms of monkeypox evasion of *Acanthamoeba* phagocytic killing require experimental investigations, based on genome analysis and earlier investigation using human cells, several viral proteins have been found to be essential for entry, release, and host cell modulation. In this regard, several cell modulatory proteins have been identified that may also play a role in monkeypox evasion of *Acanthamoeba* intracellular killing. These include: J3R (chemokine binding protein), J2L (cytokine response-modifying protein B), D9L (ankyrin repeat domain containing protein), CP77 (type I interferon (IFN) evasion protein), F3L (RNA-binding protein E3), H1L (dual specificity protein phosphatase H1), D3R (EGFR binding protein), D11L (Protein C6), C7L (Protein F1), B16R (soluble IFN-alpha receptor), C1L (IFN antagonist K1L), B13R (protein B13), B9R (soluble IFN-γ receptor B8), P1L (protein N1), C6R (protein K7), A37R (MHC modulating protein), A41L (protein A41 (chemokine binding protein),

and A47R (TLR inactivating protein); however, studies are needed to determine the role of the aforementioned proteins in evading phagocytic killing of *Acanthamoeba* [40].

As in the case of other microorganisms, amoebae have been recognized as biological “Trojan horses” for viruses and have been charged with increasing virulence and protecting human viruses against environmental harshness. Amoeba-infecting viruses are not uncommon, and several have been isolated from amoeba cultures, such as adenoviruses [41] and enteroviruses [42]. Additionally, previously the researchers reported that the presence of heat shock proteins in *Acanthamoeba* might be allowing long-term survival and long-distance transmission of the SARS-CoV-2 virus [43]. In any case, the receptors and the internalization pathways for amoeba–monkey virus interaction have not been described. However, recent genomic analysis of *Acanthamoeba* showed the presence of laminin-binding protein as one of the major parasites’ adhesins [44]. Given the fact that the entry of the monkeypox virus into host cells is mediated by the viral envelope glycoproteins, the researchers suggest this could interact with laminin-binding proteins on the surface of *Acanthamoeba* allowing its entry and facilitating its transmission. Notably, monkeypox virus has a relatively large genome of about 196,858 base pairs, encoding 190 open reading frames needed for viral replication. It is logical that viral replication can only take place during the metabolically-active trophozoite stage of the *Acanthamoeba* as the virus requires the functional host cell machinery to synthesize its DNA, early and later proteins, virion assembly, trafficking, etc. The cyst stage is inactive or exhibit minimal metabolic activity. For example, viral entry into cells is likely dependent on phagocytic uptake (absent in the cyst stage) and involves actin. Once inside, viral proteins and enzymes promote synthesis of early proteins, DNA replication, transcription factors, late genes, structural proteins, and enzymes and virion genomes are processed and assembled into nascent virions that contain all enzymes, factors, and genetic information needed for a new infectious cycle. Hence, it is likely that, once infected with monkeypox, the metabolism of *Acanthamoeba* and monkeypox virus are functionally intertwined; however, future experimental studies are needed to determine this.

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