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Com base no texto transcrito abaixo, extraído do artigo científico "*The role of giant viruses of amoebas in humans"*, responda as questões de número 1 (um) a 5 (cinco).

THE ROLE OF GIANT VIRUSES OF AMOEBAS IN HUMANS

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Abstract

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.

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 geo-graphical 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 Un-completed 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=1TmFZ3DBnD3jNl3lwjyS6TOa 741M&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 mm-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]).

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 mam-mals, including humans, and also insects [9,26,27,28,39].

Conclusion

The presence and impact of GVA and virophages in humans undoubtedly represent an important field that deserves further investigation in medicine. Such investigations are difficult. However, it has been increasingly demonstrated that GVA can be present in humans. Evidence is particularly strong for mimiviruses and marseilleviruses, which were isolated from human feces, bronchoalveolar fluid and blood. Regarding the potential pathogenic role of these viruses in humans, the link

between amoebal mimiviruses and pneumonia has been the most documented, whereas marseilleviruses have mostly been described in asymptomatic persons, and in an adenitis patient. Furthermore, for all these GVA, one must consider that their tremendous gene repertoires confer on them a strong potential for interaction with other organisms. It is also noteworthy that the closest relatives to faustoviruses are asfarviruses, which cause a common and severe disease in pigs [13]. Regarding other megaviruses, they include poxviruses, which are pathogenic in insects and mammals, including humans [65], and Acanthocystis turfacea chlorella virus, a phycodnavirus that was found in human pharyngeal samples and tentatively associated with cognitive disorders [66]. Until recently, the belief that all viruses are small entities probably limited the detection of GVA in humans. As this paradigm has been crumbling for a decade, future research should clarify the prevalence and consequences of their presence in humans. It appears particularly relevant to continue searching for mimiviruses in respiratory samples and stools, and for marseilleviruses in the blood and in lymph nodes. Nevertheless, a broader panel of human samples from healthy and sick people should be tested; for instance, urine samples might be studied. In addition, investigations should involve a broad range of technological approaches, including serology, immunehistochemistry, immunofluorescence, FISH, targeted and random nucleic acid amplification, Sanger and next-generation sequencing, cytometry, microscopy, and high throughput culture isolation. Particularly, metagenomes currently extensively generated from human samples should be more exhaustively, thoroughly and recurrently screened for the presence of sequences best matching these GVA. Finally, experimental models on cells or animals would be helpful to gain a better understanding of the consequences of GVA presence in humans.

QUESTÃO 1

Casos de pneumonia em humanos foram responsáveis pela descoberta acidental dos vírus gigantes Mimivírus que vieram desafiar o conceito da definição de vírus.

a) Qual a estratégia usada que possibilitou a descoberta dos mimivírus?

A estratégia consistiu no uso de uma cultura axênica da Acanthamoeba spp. implementada para isolar microrganismos resistente à ameba como Legionella spp.

b) Como eles foram descritos?

Como micróbios resistentes a ameba, visível na microscopia e luz e semelhante a coco gram positivo.

QUESTÃO 2

Novas descobertas advieram com a descoberta dos vírus gigantes, como os virófagos. Quais as suas características?

São caracterizados como (i) vírus de vírus, que se (ii) replicam nas fábricas virais dos hospedeiros dos mimivírus e (iii) podem prejudicar seu ciclo replicativo e morfogênese.

QUESTÃO 3

Enumere os achados que sugerem que os seres humanos são expostos aos vírus gigantes ou que fortemente indica que estão presentes em humanos.

- ***Comuns na biosfera (água mar, água fresca, amostras de solo de vários países do mundo);***
- ***Estudos de metagenômica revelam sequências compatíveis com esses vírus em amostras ambientais similares provenientes de áreas geográficas diversas;***
- ***Seus hospedeiros (Acanthamoeba e Vermamoeba) são comuns em ambientes humanos;***
- ***Frequência de 12% de DNA de mimivírus em amostras de superfícies inanimadas em hospital no Brasil;***
- ***Mimivírus e marseillevírus foram isolados de fezes humanas, fluido broncoalveolar e sangue.***

QUESTÃO 4

Enumere 2 (duas) evidências laboratoriais que indicam que as amebas não são os hospedeiros exclusivos do mimivírus.

- ***Permissividade de macrófagos à infecção in vitro (aumento carga DNA viral, lise ameba com extrato de macrófagos infectados)***
- ***Replicação em células mononucleares de sangue periférico humano (PBMC)***
- ***Indução da produção de IFN tipo 1***
- ***Inibição da expressão de genes estimulados pelo IFN***
- ***Células Jukart (linf T imortalizado) inoculadas com amostra soro+ para DNA de vírus gigante resultam em PCR+ no sobrenadante, DNA viral e vírions detectados 21 dias após infecção, fluorescência na HIS ou MET.***

QUESTÃO 5

Na conclusão da revisão, os autores apontam que pesquisas futuras devem esclarecer a prevalência e consequência da presença destes vírus em humanos. Em quais amostras clínicas eles sugeriram que a pesquisa deve investiga-los?

- ***Amostras respiratórias (mimivírus)***
- ***Fezes (mimivírus)***
- ***Sangue (marseillevirus)***
- ***Nódulos linfáticos (marseillevirus)***
- ***Urina***
- ***Amplo painel de indivíduos doentes e saudáveis***

Com base no estudo de Casotti *et al.*: "*Factors associated with paradoxical immune response to antiretroviral therapy in HIV infected patients: a case control study*", cujo resumo encontra-se abaixo, responda as questões de número 6 (seis) a 10 (dez).

FACTORS ASSOCIATED WITH PARADOXICAL IMMUNE RESPONSE TO ANTIRETROVIRAL THERAPY IN HIV INFECTED PATIENTS: A CASE CONTROL STUDY

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BMC Infect Dis. 2011; 11: 306.

Background: A paradoxical immunologic response (PIR) to Highly Active Antiretroviral Therapy (HAART), defined as viral suppression without CD4 cell-count improvement, has been reported in the literature as 8 to 42%, around 15% in most instances. The present study aims to determine, in a cohort of HIV infected patients in Brazil, what factors were independently associated with such a discordant response to HAART.

Methods: A case-control study (1:4) matched by gender was conducted among 934 HIV infected patients on HAART in Brazil. Cases: patients with PIR, defined as CD4 < 350 cells/mm3 (hazard ratio for AIDS or death of at least 8.5) and undetectable HIV viral load on HAART for at least one year. Controls: similar to cases, but with CD4 counts ≥ 350 cells/mm3. Eligibility criteria were applied. Data were collected from medical records using a standardized form. Variables were introduced in a hierarchical logistic regression model if a p-value < 0.1 was determined in a bivariate analysis.

Results: Among 934 patients, 39 cases and 160 controls were consecutively selected. Factors associated with PIR in the logistic regression model were: total time in use of HAART (OR 0.981; CI 95%: 0.96-0.99), nadir CD4-count (OR 0.985; CI 95%: 0.97-0.99), and time of undetectable HIV viral load (OR 0.969; CI 95%: 0.94-0.99).

Conclusions: PIR seems to be related to a delay in the management of immunodeficient patients, as shown by its negative association with nadir CD4-count. Strategies should be implemented to avoid such a delay and improve the adherence to HAART as a way to implement concordant responses.

QUESTÃO 6

Quais são os objetivos do estudo?

Determinar, em uma coorte de pacientes infectados pelo HIV no Brasil, quais fatores são independentemente associados com uma resposta discordante ao HAART.

QUESTÃO 7

Como o estudo foi delineado?

Caso-controle pareado por gênero na proporção de 1:4. Casos e controles foram selecionados entre 934 pacientes infectados pelo HIV em tratamento com HAART. Casos eram pacientes com resposta paradoxal definida como CD4<350 células/mm3 e carga viral indetectável. Controles eram semelhantes aos casos, mas com CD4>350 células/mm3. Os dados foram coletados de prontuários médicos usando um instrumento padronizado de coleta. Trinta e nove casos e 160 controles foram selecionados consecutivamente.

QUESTÃO 8

Quais foram os fatores encontrados em associação com o desfecho em exame?

Tempo total em uso de HAART, nadir e tempo de carga viral indetectável.

QUESTÃO 9

Quais são as conclusões possíveis tendo em vista os resultados do estudo?

A resposta paradoxal parece estar relacionada a um atraso no manejo dos pacientes imunodeficientes, como demonstrado por sua associação negativa com o nadir.

QUESTÃO 10

Em síntese, qual a principal providência a ser tomada para que o desfecho não ocorra em pacientes com a doença em questão?

Evitar o atraso no início do tratamento, que deve ser instituído o mais cedo possível.