freedomfordoctors.org. 2020 Aug
Published online 2020 Aug 08. http://freedomfordoctors.org/pdf/ScExTsVz.pdf

SARS-CoV-2, Exosomes, Tetraspanins (CD9/CD63/CD81), and Vaccine Zeal

Brian T. Sweeney, M.D.[1]
[1] FOUNDATION TO RECOGNIZE EDUCATE AND EMPLOY DOCTORS OF MEDICINE LLC, Delaware, U.S.A.

Abstract

A purported global pandemic attributed to an RNA strand of approximately 29,903 bases called SARS-CoV-2 has created an environment of anxiety, fear, and populace control regardless of affected status. SARS-CoV-2 has been suggested as the cause of an alleged disease state called COVID-19 with an initial mortality rate of approximately 0.3 percent which is now combined with pneumonia and influenza deaths as PIC (Pneumonia, Influenza, and COVID-19) yielding a mortality rate of 8.6% (as of July 30, 2020) according to the latest CDC report. This exponential increase is largely a product of health agencies attributing nearly any death to COVID-19 if that person tested positive—even post-mortem—regardless of the near-term circumstances surrounding the death (e.g., myocardial infarction, hypoglycemia, crush injury from a large bookcase).

SARS-CoV-2 is a lipid-bilayered vesicle ranging in diameter between 30-150 nm. which coincidentally is the same approximate size of an exosome. Exosomes are ubiquitous Extracellular Vesicles (EVs) generated in cells throughout the human life span. Initially thought to be involved mostly as transporters for intracellular waste products, research has demonstrated over subsequent decades that they are capable of storing and transporting proteins, lipids, and even 'virus components' (e.g., RNA) intercellularly. Recent research has focused on the biogenesis, structure, and pathways that are inherent to exosomes and SARS-CoV-2 to suggest that the latter may be confused with the former or that the former may show promise as a candidate for vaccine inclusion. In the first case, the entire pandemic may be seen as a serious error that has lead to lockdowns, economic devastation, personal calamities (including suicides), and iatrogenic deaths (especially through the use of ventilators). In the second case, some may argue that producing an inoculation that is nearly the same as the protagonist should be examined seriously; in effect, a 'vaccine zeal' may supplant 'vaccine hesitancy' as worthy of primary concern in the vaccine debate. Further elucidation of the situation may be found in the fact that exosomes are identified by the presence of at least one of the three surface markers called CD9, CD63, or CD81.

In the interest of clarification, a test for the CD9/CD63/CD81 markers should be undertaken since the presence of any of these three tetraspanins (i.e., proteins found in cells and EVs) would demonstrate that the particle in question is an exosome and indicate that a significant flaw has been made in the identification of and treatment modalities for SARS-CoV-2. As an exempt (sometimes known as non-profit) organization that has no monetary relationship with the Centers for Disease Control (CDC), World Health Organization (WHO), or other established societies and foundations medical or otherwise except for the FOUNDATION TO RECOGNIZE EDUCATE AND EMPLOY DOCTORS OF MEDICINE (FREEDOM), this author declares no conflicts of interest. This article has not been peer-reviewed.

Introduction

SARS-Cov-2 was first identified in December, 2019 through bronchoalveolar secretions of seven patients who became ill in or around a 'wet market' in Wuhan, China. Doctors and researchers focused almost exclusively on finding an etiologic agent that was viral in origin. Malnutrition, toxicity, physical or emotional trauma, and genetics were given little regard despite the apparent poor sanitation, pollution, and electromagnetic radiation affecting the indigenous people of the area. (Note: Much of this and what follows is derived from research performed by Andrew Kaufman, M.D. whose work can be found at andrewkaufmanmd.com.)

Many researchers have explored our area of study, but few stray from the commonly-held belief that there is nothing more than a coincidence. Dr. James Hildreth, a world-renowned expert in virology from Meharry Medical College, is quoted as stating, 'A virus is fully an exosome in every sense of the word', in the context of Human Immunodeficiency Virus (HIV) fusion to and evasion of detection by target cells (Wells, 2003). His view is more nuanced in the research paper underlying the subject of the aforementioned journal article where he focuses primarily on the possibility of exosomes being exploited in the biogenesis, infectivity, and traffic of HIV (Gould et al., 2003). Recently, Dr. Hildreth steadfastly has clarified his position as fully consistent with the existence of a novel coronavirus. Others have pointed out the striking similarities between SARS-CoV-2 and exosomes, but have stopped short of calling them identical.

A similar refrain implores additional research on the potential diagnostic and therapeutic modalities that may be afforded through the use of exosomes with regard to viral infection. Tangentially, some researchers noted the presence of at least four (4) HIV insertions within the SARS-CoV-2 single-stranded RNA, but withdrew their findings after further scrutiny. The implication of such a discovery would be that any related patents for treatment could generate a windfall; however, this line of investigation is beyond the scope of this treatise. To return back to the primary area of examination, the characteristics (e.g., size, cargo, role) of the two aforementioned entities necessitate further differentiation if we are to determine them as being distinct. Testing for the presence of the tetraspanins CD9, CD63, and CD81 would provide such a means before premature pursuit of additional vaccine research, development, and manufacture.

SARS-CoV-2 and Exosomes

SARS-CoV-2 is suggested to have surface proteins on its' lipid envelope that is specific for fusion with target cell membrances (Hoffmann et al., 2020) similarly to Extracellular Vesicles (Hoen et al., 2016). Prevailing scientific opinion indicates that viruses inject their contents into the cell and 'hijack' the cell's apparatus. A sequence of cytoplasmic release of genetic material, translation into proteins, and replication within the Endoplasmic Reticulum-Golgi Intermediate Compartment (ERGIC) takes place. Regardless of your position on the existence of viruses, most researchers acquiesce to the stance that Extracellular Vesicles may be imbued with mRNAs, non-coding RNAs, and proteins that can regulate cell functions and gene expression. While this area of expertise is beyond the scope of this treatise, the shared generation and pathways of the two entities invites further investigation. The reader may want to refer to the work by Mr. Pierre Arsène referenced later in this article for detailed 'possible assumptions' of how exosomes may have evolved from 'defective viruses' or

'viruses' may have developed from EVs (e.g., exosomes). This leads us to examine how we might differentiate particles of approximately the same size as shown by electron microscopy using methods such as flow cytometry. Royal Raymond Rife and darkfield microscopes as superior alternatives is a topic for another day.

CD9/CD63/CD81 tetraspanins

Tetraspanins are proteins that may be found on the membranes of cells. Steve McClellan, the Manager of Basic & Translational Research Operations at the University of South Alabama Mitchell Cancer Institute and Chief, Flow Cytometry Core Laboratory, is an expert on exosomes with thirty (30) years of experience in flow cytometry. He had the following to say during a webinar for Thermo Fisher entitled, *Analysis of Surface Antigens on Exosomes using the Invitrogen Attune NxT Flow Cytometer:*

> Now, if we're going to use antibodies to look at three gold standard reference molecules, those being CD9, CD63, and CD81, you cannot call a particle an exosome unless it has at least one of those markers on the surface. Let me repeat that. If you don't see at least one of those three CD markers on the surface of the exosome, you cannot call it an exosome.

This would seem to be the most appropriate dividing line between exosomes and SARS-CoV-2 assuming that the latter exists. This may be the appropriate avenue for those who would appeal to 'settled science' being personified as the paramount arbiter in this issue. Proponents of 'evidence-based medicine' (e.g., physicians, public health officials, hospital administrators) also may be satisfied by this approach. In this way, research that relies heavily upon explaining such things as viral structure, uptake, fusion, replication, pathogenesis, mode of transport, genetic material sequence coding, and mechanism for cell entry and exit could be rendered moot if detection is accomplished of any of the three tetraspanins of CD9, CD63, or CD81.

Subsequent to this author envisioning this line of thinking, the following was discovered in the preprint of a research article by Pierre Arsène of Mursla Ltd., Cambridge, UK entitled, "*Lessons for SARS-CoV-2 study (COVID-19 disease) from its exosome relatives*":

> The most documented tetraspanins found on the surface of EVs are CD9, CD63 & CD81. They have an important role in cell targeting and fusion (van Dongen et al., 2016). Some retroviruses, such as HIV-1, were mentioned to be enriched in CD63 and CD81 (Mathieu et al., 2019). It suggests that they could possess an additional "cloaking" ability that could make them more infectious: targeted cell and the immune system may confuse it with naïve EVs (Sato et al., 2008), (Hoen et al., 2016). For coronaviruses, tetraspanins on the host cell membrane can promote entry by binding to the virus receptors and proteases contributing to the infection and pathogenesis. For instance, CD9 on the cell membrane facilitates the entry of MERS-CoV after interacting with MERS-CoV receptor dipeptidyl peptidase 4 (DPP4) (Earnest et al., 2017), (Hantak et al., 2018). Interestingly, CD81 has a role in the entry of Hepatitis C virus (HCV) into the cell as being the first identified receptor (Farquhar et al., 2011). Having said that, it has not been confirmed yet which host cell molecules are also present on the envelope of SARS-CoV-2.

The last line is the most important as it alludes to the same line of inquiry that this author is

pursuing. Proponents of the discovery of a 'novel coronavirus' should be eager to respond to questions about their discovery so that the alleged pandemic could be resolved as quickly as possible. The reader may argue that the presence of CD9, CD63, or CD81 does not mean exclusively that a 'virus' has been confused with an exosome because these markers independently could be found on viruses and exosomes. This author counters that the historical inability to isolate and purify any 'virus' excludes it from consideration and ipso facto demonstrates that what we have called a 'virus' is an exosome if any of these three tetraspanins are discovered. Counter-arguments to this demand that the disputer prove the existence of a virus through evidentiary means including, but not limited to: black-and-white microscopy; darkfield examination; and, satisfaction of Koch's postulates.

Vaccines

Prior to the emergence of COVID-19, the grassroots movement for parents, adults, and children to decline the administration of vaccines had grown significantly. Major motion pictures and web-based documentaries had fueled this sentiment resulting in adherents being labeled as 'anti-vaxxers' or people with 'vaccine hesitancy'. Despite considerable evidence, including vaccine injury cases heard before 'vaccine courts' (largely financed through taxpayer money), an earnest, vibrant debate about vaccine safety and efficacy was lacking. Literature dating back to nearly one-hundred years ago or more had detailed concerns about the underlying germ theory proposed by such luminaries as the chemist Louis Pasteur. Proponents of terrain theory, such as the distinguished chemist and physician Antoine Béchamp, were marginalized due to their shortcomings in dominating public opinion in the same way that Pasteur did. Early trials of smallpox vaccine were plagued with the death of large numbers of animals. Some professional societies and political bodies expressed concern about the use of vaccines on human beings. Pasteur postulated that viruses were the causative agent in some disease processes despite the fact that their size (i.e., approximately $1/100^{th}$ to $1/1000^{th}$ of that of bacteria) limited them from being seen under the light microscopes prevalent at that time.

Doubts about the existence of pathogenic 'viruses' (as we commonly think of them) has intensified in recent years. Stefan Lanka, a respected virologist, has proven in a high court of Germany that the measles virus does not exist. Researchers at INSERM (The Institut national de la santé et de la recherche médicale), which is the French National Institute of Health and Medical Research, claimed in 2016 that they had seen the Hepatitis C virus for the first time after nearly twenty-five (25) years, but stated three years later (in 2019) that it was instead a 'misunderstood particle'. (Original articles about this on isserm.org and stopru.org as well as a subsequent article indicating their mistake seem to be 'disappeared' at this time for some reason; this link alludes to the 'discovery'). This entails that physicians and other health professionals have been diagnosing viral infections and disease for decades utilizing indirect tests such as surface antigens (e.g., p24 in the case of HIV), antibodies (e.g., anti-HCV), and viral loads. SARS-CoV-2 shares similar concerns due to the use of RT-PCR testing for the RNA strand (which the Nobel Prize-winning developer of the method, Dr. Kary Mullis, had warned against) and the recent reliance on antibody testing of IgM and IgG which purportedly are specific to part of the S1 subunit of SARS-CoV-2. Further clarification on how a determination was made that there are IgM and IgG isotopes specific enough to rule out other causes of infection or disease states is imperative. Research that has ruled-out cross-reactivity with the antibodies of other microorganisms is available, but the methodology is

lacking. An analogy to specific IgM antibodies only binding to the agent causing tuberculosis may be cited, but how that relationship was proven is unknown at this time by this researcher. In the cases of early HIV and SARS-CoV-2 research, a parallel may be drawn where subsequent researchers graduated correlations into causations without substantial proof.

Discussion

The interplay of SARS-CoV-2, exosomes, tetraspanins, and vaccines is fertile territory for exploration. Engineered exosomes may play a promising role in the transport of proteins and molecules in the treatment of oncogenic and infectious processes. The temptation to utilize them as a panacea for a 'virus' that has not been isolated and purified is inadvisable. Vaccines have a long and problematic history with regard to demonstrating effectiveness in creating antibodies to viral and bacterial microorganisms. Mr. Arsène proposes the consideration of Extracellular Vesicles (EVs) as prospective agents for bioengineering and therapeutic purposes:

> Considering the proviral effect that EVs from infected cell might have, the use of engineered EVs with non-pathogenic viral proteins or molecules could beconsidered as a vaccine strategy (Hoenet al., 2016), (Urbanelliet al., 2019). One of these strategies was already studied for SARS-CoV where an EV vaccine containing Spikeprotein of the virus could induce high levels of neutralizing antibodies in mouse models (Kuate et al., 2007).
> In terms of antiviral, engineered EVs can also be used for targeted drug delivery by using the same viral protein that binds cells susceptible to being infected. Alternatively, some EVs have natural affinity to inflammation sites. Platelet-derived EVs loaded with anti-inflammatory TPCA-1 were shown to calm cytokine storm, often associated with severe COVID-19 cases, in mice model with acute lung injury (Ma et al., 2020). EVs expressing specific proteins, such as ACE2, could act as virus binders and thereby reduce infectivity.
> Another promising prospect of EV-based therapeutics is the administration of mesenchymal stem cells (MSCs)-derived EVs to reduce inflammation and injury in respiratory diseases. This is currently being tested in clinical trials in China for the treatment of COVID-19 related severe pneumonia (Shah et al., 2019), (NCT04276987) (ChiCTR2000030484) (ChiCTR2000030261). International Society for Extracellular Vesicles (ISEV) and International Society for Cell and Gene Therapy (ISCT) have recently made a joint statement about its potential under the right conditions (Börger et al., 2020).

The primary concern that this author has about the use of exosomes (which vary widely in size and cargo) as vaccines (subcutaneous, intramuscular, or intraepidermal), antivirals, or stem cells is that we simply do not know what deleterious effects may be encountered. Vaccine inserts almost universally state (usually in Section 13) that they have not been tested for carcinogenic, mutagenic, or fertility risks. The CDC website indicates that the 'effectiveness' of the influenza vaccination for 2018 was twenty-nine percent (29%). Hydroxychloroquine, the anti-malarial du jour being touted as a treatment for COVID-19, has been used historically for lupus, rheumatoid arthritis, and porphyria cutanea tarda, but has a wide array of side effects including vision, hearing, and breathing problems. Stem cells have ethical considerations including unintended consequences. Earnest critique of these

concerns is welcome. This author is well aware that 'debunkers', lobbyists, and bots may cease upon this information to critique without earnestness. Perhaps they would be better served to look into the eyes of their significant others, children, and friends and examine their motives before embarking on such an undertaking. This is not a time for financial, political, or personal considerations to trump care for humanity regardless of race, color, or creed.

Conclusion

While previous researchers and this author may deviate with respect to the willingness to accept that exosomes might show promise in the treatment of viral infection, mutual support exists recognizing the uncanny resemblance of SARS-CoV-2 and exosomes such that even consideration could be made for utilizing the latter as a 'Trojan horse' for the former. This makes it even more obligatory that any historical or novel 'viruses' must be ruled out as being exosomes.

Methodology, including advanced microscopy and flow cytometry, could ameliorate any confusion between exosomes and 'viruses' through the detection of any of the three tetraspanins called CD9, CD63, and CD81. In fact, each of these proteins has been suggested as playing a role in the propagation of MERS-CoV, HIV-1, and HCV, respectively, as previously described. No isolation and purification of any virus has been made despite the redefinying of the word 'isolation' as it relates to PCR, which is prone to egregious error.

The risk of vaccine zealotry blinding researchers, microbiologists, medical practitioners, private foundations, and health agencies on the local, national, and global levels is an oppressive force against which we must guard. The commercial development and administration of a vaccine for a non-existent virus could endanger any stakeholder of involved pharmaceutical companies of being in violation of “The Nuremburg Code”. Minimally, any proponent of such an undertaking runs the risk of being labeled as “an opportunist...and severely opinionated even when the opinion he holds is absurd.” (Kildall, 1993).

Acknowledgments:

This author would like to thank Amandha Vollmer, Andrew Kaufman, Ethel Hume, Stefan Lanka, Robert Willner, Bryan (Baruch) Ellison, Peter Duesburg, and Gary Kildall for inspiration and education.

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