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LETTER TO THE EDITOR

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High COVID-19 virus replication rates, the creation of antigen–antibody immune complexes and indirect haemagglutination resulting in thrombosis

A new pathogenic virus, COVID-19, appeared in 2019, in Wuhan, China, typically causing fever, cough, diarrhoea and fatigue and significant mortality (Mao, 2020). COVID-19 has also shown about 80% genetic similarity to the severe acute respiratory symptom (SARS) virus, which is already known to be derived from a bat virus (Ye, 2020). Arterial thrombosis and venous thrombosis, variously attributed to long-term patient immobilizations, inflammation, autoimmune reactions or endothelial cell damage to the blood vessels, have also been reported for COVID-19 infections (Bikdeli, 2020; Kollias et al., 2020). However, another explanation for thrombosis (blood clots) in some patients infected with COVID-19 is discussed.

Viruses and other pathogens evolve traits to best endure in whatever conditions they normally encounter. And these viral traits can carry over to infections of other species. For example, some bat viruses can display a high replication rate in host cells after transmission to secondary hosts of other species, such as in the case of viruses that evolved their replication while they infected animals such as bats and thereby were selected by the immune responses of bats (Brook, 2020). This is characteristic of several enveloped RNA viruses from bats, including severe acute respiratory syndrome (SARS) virus of the genus *Betacoronavirus* (Brook, 2020).

If a bat origin of COVID-19 is a starting premise and the immune characteristics of some bat species can be extrapolated to bat species generally, the COVID-19 virus will have evolved to best endure the immune system of a bat. There are several potential ways for viruses in general to evade immune responses, such as interfering with interferon signalling, inhibiting antiviral NK cells (e.g. by production of inhibiting ligands or interference with activating ligand production), inducing an elevation in immunosuppressive T_{REG} cells (which can elevate production of immunoregulatory cytokines interleukin-10 and transforming growth factor β), exhausting virus specific T cells through persistent antigenic stimulation or production of exhaustion marker ligands (e.g. galectin-9 produced by T_{REG} cells), mutating T-cell (especially CD8 T cell)-targeted antigenic determinants (epitopes) on the virus, inducing CD4 T cells to lose proliferative capacity and cytokine production (interleukin-2, interleukin-21, etc.), impairing cytotoxic effector functions of CD8 T cells, mutating or shielding viral epitopes targeted by antibodies (Abdel-Hakeem, 2014; Jonjic, 2008; Schountz, 2017).

Among the antiviral defences of some bat immune systems, a continuous production of interferon-α is one the main characteristics (Zhou, 2016). Continuous production of interferon-α, mainly interferon-α2 and interferon-α3, with a lesser amount of interferon-α1, has been linked to interferon regulatory factors IRF3, IRF7 and other factors that bind to promoter regions of interferon-stimulated genes to induce expression of antiviral proteins in bats, such as bone marrow stromal cell antigen 2 (BST2, also called tetherin) that restricts viral replication, including Ebola and Marburg viruses, and Mx1, which has a broad-spectrum antiviral action against RNA viruses and some DNA viruses, including restriction of viral replication, without inducing interferon acute inflammatory responses (Zhou, 2016).

Interferon-α, BST2 and Mx1 could be primary reasons why some bat viruses would adapt their replication in bat cells to overcome BST2 and Mx1 restriction of viral replication. This would also explain why some bat viruses replicate very quickly in secondary host cells (e.g. human and other mammalian cells) that are slower to produce these antiviral proteins. Individuals will vary by age, health and genetics in the speed and quantity of interferons, NK cells, T cells, and antibodies that their immune systems will be able to mobilize against bat virus infections.

Some bat immune systems are not proinflammatory and achieve infection tolerance, with even antiviral NK cells having expression of inhibiting receptors that could restrain the NK cells from attacking virus-infected cells (as discussed above, viruses can inhibit NK cells by regulating NK cell ligands, and a feeble antiviral NK cell and T-cell response to COVID-19 has been widely seen in humans) (Pavlovich, 2018; Zhang, Zhao, et al., 2020; Zhou, 2016). Other than antiviral antibodies, the other remaining bat immune response threat to the virus-infected cells would be from CD4 T cells enabling cytotoxic CD8 T cells to induce the demise (apoptosis) of the virus infected cells (Abdel-Hakeem, 2014; Hislop, 2007; Pardy, 2019).

Almost all T-cell activations require that an antigen (i.e. a molecular pattern that a patient's immune system recognizes as foreign) be presented on a specific surface protein known as a major histocompatibility complex (MHC) (Abdel-Hakeem, 2014). T cells predominantly are α:β T cells with this MHC requirement for antigen presentation to activate α:β T cells, using MHC class II for presentation to CD4 T cells and MHC class I for presentation to cytotoxic CD8 T cells (Abdel-Hakeem, 2014).

Another major antiviral consequence of continuous production of interferon-α is an increased expression of MHC class I for antigen presentation of the virus to cytotoxic CD8 T cells (Murphy, 2012).

Therefore, some bat viruses subject to natural selection in bats could evolve to avoid or minimize T-cell attacks by broadening their targeted cells to include cells that have little or no MHC class I expression that could present antigens of the virus to cytotoxic CD8 T cells.

Neurons and red blood cells (i.e. erythrocytes) normally express little or no MHC class I proteins that could be used to present viral antigens to cytotoxic CD8 T cells (Giles, 1987; Roe, 2020). Therefore, neurons, erythrocytes and any other cells with low MHC class I expression could be included among the cellular infection targets for bat viruses. Could COVID-19 virus bind to human erythrocytes? It is already known that many species of human coronavirus, including the SARS virus of the genus *Betacoronavirus*, have a spike (S) glycoprotein that can bind to sialic acid residues on human cells, such as 9-O-acetylated-sialic acid (9-O-Ac-Sia), and human erythrocytes extensively express sialic acid residues; thus, COVID-19 binding to human erythrocytes is possible (Aoki, 2017; Tortorici, 2019).

It should be noted that human brain cells have the highest level of sialic acid residues in the body; the luminal sides of endothelial cells in the blood vessels, epithelial cells of the lungs, respiratory tract and gastrointestinal tract of humans also heavily express sialic acid residues; sialic acid residues are targeted by many viral pathogens in addition to the SARS virus; and these cells rich in sialic acid residues have been extensively targeted by many COVID-19 infections (Mao, 2020; Tortorici, 2019; Varki, 2008; Fantini, 2020; Zhang, Zhao, et al., 2020). It is unlikely for this to be a mere coincidence. In fact, it has been recently reported that structural and molecular modelling of COVID-19 suggests that its spike (S) glycoprotein can attach to sialic acid residues on host cell surfaces (such as luminal epithelial cells of the respiratory tract) in addition to binding to angiotensin-converting enzyme-2 (ACE2) (Fantini, 2020).

The viral infection of cells having little or no MHC can go undetected by CD8 T cells, but mammalian erythrocytes are non-nucleated cells that are theoretically unable to support transcription and translation, so mammalian erythrocytes are a dead end for viral infection (Anderson, 2018). But since replication is possible in neurons and other cells with little or no MHC, a virus trait including cells with little or no MHC as targets can still survive by natural selection in nucleated cells with little or no MHC, such as neurons.

The remaining defence will be antibodies, which, unlike T cells, can bind to antigens that lack MHC protein presentation (Jazayeri, 2019). The next step after viral binding to erythrocytes (regardless of viral entry or replication) could be B-2 or B-1 cell-derived antibodies (primarily IgM, some IgG or IgA) binding to viral antigens on viruses bound to the sialic acid residues on the erythrocytes (red blood cells) that would create antigen–antibody immune complexes (Agarwal, 1995; Punt, 2019a). Antigen–antibody immune complexes are normally destroyed by phagocytes and cleared by the spleen or liver without symptoms, but if an individual's immune system cannot clear them quickly, they can interact with platelets and cause the formation of tiny clots, leading to microvascular thrombosis, and also cause various type III hypersensitivity symptoms, such as fever, joint pain, glomerulonephritis, vasculitis, vascular purpura and rashes (Agarwal, 1995; Punt, 2019a). Uncleared antigen–antibody immune complexes can bind to mast cells, neutrophils and macrophages, triggering large releases of several inflammatory cytokines and an increased blood vessel permeability. This allows antigen–antibody immune complexes to deposit in tissues and create localized inflammation and complement activation leading to widespread production of inflammatory chemokines, cytokines, prostaglandins and proteases (Punt, 2019a). Proteases can attack epithelium, mesothelium and endothelium basement membrane proteins including collagen and elastin, functionally critical to lungs, blood vessels and other luminal tissues (Punt, 2019a). Symptoms consistent with uncleared antigen–antibody immune complexes have been widely observed in COVID-19 infected patients, including an extensive array of inflammatory cytokines, vasculitis, microvascular thrombosis, and spleen and lymph node damage (Zhang, Zhao, et al., 2020).

However, in a subset of humans in certain circumstances, when the antigen–antibody immune complexes deposit in tissues and ultimately cause the release of proteases, the protease destruction of epithelium, mesothelium and endothelium basement membranes (including matrix glycoproteins called laminins that are major constituents of basement membranes) can make some released constituents immunogenic and lead to the creation of antiphospholipid antibodies (Coppo, 2004; Punt, 2019b). Thus, uncleared antigen–antibody immune complexes can result in a clumping reaction called an indirect haemagglutination by such antibodies (Agarwal, 1995; Derksen, 2004). Antiphospholipid antibodies have already been linked to thrombosis in COVID-19 patients (Zhang, Xiao, et al., 2020).

Extensive indirect haemagglutination with antiphospholipid antibodies can induce thrombosis (blood clots), microvascular thrombosis, embolisms (obstructions in blood vessels), ischaemia (insufficient blood flow from a blocked artery) or cardiac infarction (heart attack) (Atkinson, 2008; Shah, 2014). However, sialic acid residues on erythrocytes will attract very strong negative regulators of complement, including factor H and other regulators, which in combination should prevent complement attack on the erythrocytes and avoid haemolysis by antibody-dependent activation of the complement system by the classical pathway (Bajic, 2015).

Since luminal endothelial cells of blood vessels also express sialic acid residues, it should be noted that antigen–antibody immune complexes and protease release could also result from the binding of antibodies to COVID-19 viral antigens from virions released during the budding replication stage of virally infected endothelial cells, so the COVID-19 infection of endothelial cells lining the luminal side of blood vessels could also be a contributing factor to indirect haemagglutination with antiphospholipid antibodies causing thrombosis (Tortorici, 2019; Varki, 2008; Fantini, 2020; Zhang, Zhao, et al., 2020; Zhang, Xiao, et al., 2020).

In conclusion, a continuous production of interferon-α, BST2 and Mx1 in some bat immune systems is probably a reason why some bat viruses would adapt their replication in bat cells to evade BST2 and Mx1 restriction of viral replication. This would also explain why some bat viruses can replicate quickly and extensively in secondary viral host cells (e.g. human and other mammalian cells) that are slower to produce interferon-α, BST2 and Mx1. Another

antiviral consequence of continuous production of interferon-α is an increased expression of MHC class I for viral antigen presentation to cytotoxic CD8 T cells. Therefore, some bat viruses could adapt to minimize T-cell attacks by broadening their targets to include cells that have little or no MHC class I expression for presenting viral antigens to cytotoxic CD8 T cells. Neurons, erythrocytes and any other cells with low MHC class I expression could be among the COVID-19 infection targets. COVID-19 virus is probably able to bind to human erythrocytes using the same spike (S) glycoprotein that can bind to sialic acid residues on human cells, such as 9-O-acetylated-sialic acid (9-O-Ac-Sia), in addition to binding to ACE2 expressed on human cells. Extensive binding of COVID-19 virus to erythrocytes, even without viral entry or replication, and antibodies binding to viral antigens, would create antigen–antibody immune complexes, which if not cleared in certain individuals could cause inflammatory type III hypersensitivity symptoms, including protease releases that can destroy epithelium, mesothelium and endothelium basement membranes and create antiphospholipid antibodies, which in some individuals could transition into extensive indirect haemagglutination of erythrocytes by antiphospholipid antibodies, ultimately inducing thrombosis. Blood clotting in the major arteries, veins and smaller blood vessels of the lungs of this subset of patients could result if their immune systems had slow or weak mobilization of interferons, NK cells, T cells to suppress viral replication and depended on antiviral antibodies, while being unable to quickly clear the resulting antigen–antibody immune complexes.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

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