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Factors preventing materno-fetal transmission of SARS-CoV-2

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ARTICLE INFO

Keywords:

SARS-CoV-2
Epithelial barrier
Caveolin
Placenta
Lung
Syncytium

ABSTRACT

Although many pregnant women have been infected by coronavirus, the presence of intrauterine vertical transmission has not been conclusively reported yet. What prevents this highly contagious virus from reaching the fetus? Is it only the presence of a strong placental barrier, or is it the natural absence of the some receptor that the viruses use for transmission? We, therefore, need to comprehensively understand the mechanism of action of the mammalian epithelial barriers located in two different organs with functional similarity. The barriers selected as potential targets by SARS-CoV-2 are the alveolo-capillary barrier (ACB), and the syncytio-capillary barrier (SCB). Caveolae are omega-shaped structures located on the cell membrane. They consist of caveolin-1 protein (Cav-1) and are involved in the internalisation of some viruses. By activating leukocytes and nuclear factor- κ B, Cav-1 initiates inflammatory reactions. The presence of more than one Cav-1 binding sites on coronavirus is an important finding supporting the possible relationship between SARS-CoV-2-mediated lung injury. While the ACB cells express Cav-1 there is no caveolin expression in syncytiotrophoblasts. In this short review, we will try to explain our hypothesis that the lack of caveolin expression in the SCB is one of the most important physiological mechanisms that prevents vertical transmission of SARS-CoV-2. Since the physiological Cav-1 deficiency appears to prevent acute cell damage treatment algorithms could potentially be developed to block this pathway in the non-pregnant population affected by SARS-CoV-2.

1. Introduction

Maternal viral infection does not always equate to placental and fetal viremia because only a few viruses are able to cause both placental and fetal infections [1–3]. Very little is known about SARS-CoV-2 and whether it can even colonise the placenta. Although many pregnant women have been infected by coronavirus, the presence of intrauterine vertical transmission has not been conclusively reported yet. What prevents this highly contagious virus from reaching the fetus? Is it only the presence of a strong placental barrier, or is it the natural absence of

the pathways that the viruses use for transmission? In order to comment on whether SARS-CoV-2 is able to cross the placenta, we need to comprehensively understand the mechanism of action of the mammalian epithelial barriers located in two different organs with functional similarity. The barriers selected as potential targets by SARS-CoV-2 are (i) the alveolo-capillary barrier (ACB), and (ii) the syncytio-capillary barrier (SCB). In addition to the functional similarity between the ACB and the SCB, the presence of direct contact sites between maternal-fetal cells and having the necessary syncytiotrophoblast receptors for the binding of the virus makes the SCB a good potential target for

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<https://doi.org/10.1016/j.placenta.2020.05.012>

Received 2 May 2020; Accepted 28 May 2020

Available online 29 May 2020

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SARS-CoV-2.

Despite its strong physical properties and cellular immune mechanisms, the SCB is not able to prevent all pathogens from damaging and crossing the barrier. In the early and late stages of pregnancy, viral transmission may be possible due to a weaker placental barrier. The presence of diseases such as hypertension and preeclampsia that disrupt the intercellular fusion and syncytium formation may leave the fetus vulnerable to viral attack [1–3]. So far no precise data on vertical transmission has been found in pregnant women affected by SARS-CoV-2. How might the SCB overcome a SARS-CoV-2 attack? Does the mechanism, that appears to counteract vertical transmission of the virus, only depend on the physical properties of the SCB, or are other factors also playing a role? Caveolae are omega-shaped morphological structures located on the plasma membrane [4]. They consist of caveolin-1 protein (Cav-1) and are responsible for the rapid transport of extracellular substances to intracellular organelles [5]. They are also involved in the internalisation of some viruses. Both alveolar and endothelial cells of the lungs express Cav-1 isoforms [6]. Although members of coronavirus family do not use the caveolin pathway to enter the lung cells, they trigger the Cav-1 system can lead to acute alveolar damage [7]. During the early phase of cell damage, by activating polymorphonuclear leukocytes (PMNs) and nuclear factor- κ B (NF- κ B), Cav-1 initiates inflammatory reactions [8]. The presence of more than one caveolin binding sites on coronavirus is an important finding supporting the possible relationship between SARS-CoV-2-mediated cell injury and caveolins [9]. Placental endothelium and stroma cells as well as smooth muscles and pericyts can express caveolin [10]. However, caveolin expression is not observed in the cells forming the SCB, especially syncytiotrophoblasts [10,11]. Hence, caveolin deficiency may be a physiological defence mechanism developed by the SCB against virus-mediated cell damage and vertical transmission. In this short review, we will try to explain our hypothesis that the lack of caveolin expression in the SCB is one of the most important physiological mechanisms that prevents vertical transmission of SARS-CoV-2.

2. Formation of the SCB

The haemochorial placenta, consisting of fetal and maternal tissues, has three main functions: (i) nourishing and eliminating waste from the fetus, (ii) preventing fetal rejection, and (iii) preventing microorganisms from reaching the fetus. The existence of a strong and selective barrier system is essential for all these functions to be performed flawlessly [2,3]. The basic group of cells that makeup the placental barrier are mononuclear and specialised cytotrophoblasts with stem cell character. In order for a maternal circulating virus to reach the fetus, it must either pass through the area where extravillous trophoblast cells are located in the decidua, or the area where syncytiotrophoblasts are in contact with maternal blood. Well formed multinucleated syncytium is involved in establishing the natural barrier between the mother and fetal circulation. During a mechanism called syncytialisation, cytotrophoblasts fuse with the overlying multinucleated syncytiotrophoblasts and form the outer layer of the placental microvilli [2,3]. Finger-like pseudopodial cytoplasmic protrusions from cytotrophoblast project into the overlying syncytiotrophoblast. The syncytiotrophoblast cells are supplied with nutrients and organelles from the fusing cytotrophoblasts. Syncytiotrophoblasts are more resistant to pathogens than cytotrophoblasts. For syncytiotrophoblast to become infected, it must be physically or enzymatically damaged. Since the fused multinucleated trophoblast layer is constantly interacting with maternal blood, syncytiotrophoblast can be physically damaged. However, cytotrophoblasts are capable of regenerating damaged syncytiotrophoblast. Similarly, an excessive viral load or multiple pathogens attacking the barrier at the same time may lead to the breakdown of the syncytiotrophoblast defence system. Moreover, pathogens that make the mother immunocompromised can disrupt the physiological functions of the placental barrier and allow fetal transmission. The placental barrier may not prevent the passage of some

pathogens in the early stages of pregnancy, where intercellular fusion is not fully realised or in the late stages of pregnancy where the syncytium formation begins to deteriorate [1–3]. Placental colonisation of pathogens during the seperiods may lead to miscarriage, premature birth or reinfection in the mother [12,13]. Since the fused multinucleated syncytiotrophoblast layer does not contain intercellular gap junctions, it is not uncommon for pathogens to cross the barrier through leukocytes to reach the fetus [2]. Any pathogen that crosses the barrier is usually captured by Hofbauer cells. These cells are macrophage-like cells containing vacuoles and granules located around the chorionic villi and appear to be more during early pregnancy.

3. ACE2 and TMPRSS2 expression in the SCB

SARS-CoV-2 uses the ACE2 as the main receptor binding site for viral entry into the target cell [14–16]. However, binding of the SARS-CoV-2 spike protein to ACE2 is a process that requires serine protease activation [16]. TMPRSS2 provides basic protease activity for the spike protein of SARS-CoV-2 [14,15] [bib14]. The most important difference of the SCB from the ACB is that receptor expression levels in the SCB vary according to the trimester of pregnancy. The extravillous trophoblast does not express ACE2 in the first trimester, and therefore viral transmission through the decidua would not seem possible. In addition, there is also low TMPRSS2 activation in the SCB during the first trimester that may restrict the transmission of the virus to the fetus [17]. From the 24th gestational week, both ACE2 and TMPRSS2 expressions increase in the maternal-fetal interfaces. This data would suggest that viral transmission increases with advancing gestational age. However, clinical data does not support this. To date, the virus has not been isolated in any of the babies born from mothers infected with SARS-CoV-2. The fact that IgM was detected in several neonates does not confirm the presence of vertical transmission.

SARS-CoV-2 infects the lung cells through ACE2 and TMPRSS2 receptors. However, it does not cause a similar clinical picture in the placenta, which contains the same receptors [18]. In light of this, we believe that the virus may not always produce clinical signs of infection in cells with ACE2 and TMPRSS2 expression. We postulate that ACE2 and TMPRSS2 interaction is necessary for viral entry into a target cell but there could be other pathways and receptors involved. Of note, protease inhibitors have been shown to help prevent the entry of SARS-CoV-2 into target cells but they cannot prevent viral entry completely [16]. Despite the moderate ACE2 and TMPRSS2 expression in the ACB, the rapid and severe course of lung injury suggests that the virus uses different pathways, such as clathrin-mediated endocytosis, to enter the target cells. In the previous two pandemics, we know that SARS-CoV and MERS-CoV infiltrated into target cells through clathrin-mediated endocytosis and cathepsin-mediated S protein cleavage [19].

4. Comparison of the ACB and the SCB

In terms of physiological functions, we can compare the ACB with the SCB. However, the ACB and the SCB are completely different both in terms of barrier thickness and their resistance mechanisms against pathogens. The thickness of the barrier separating the maternal and fetal cells is about 1–2 μ m. The thickness of the ACB is 0.65 μ m. This means that the SCB has twice the thickness of the ACB [3]. Although both barriers consist of more than one specialised cell group coming together to make tight connections, the placental barrier differs from the ACB in many respects. While the syncytiotrophoblasts that form the front line of the SCB are multi-nuclear, mono-nuclear cells gain density as they progress down the barrier. These two different specialised groups of cells that form the barrier are fused to form a syncytium. Syncytiotrophoblasts are constantly regenerated by cytotrophoblasts where they are fused. Syncytium formation plays an important role in the barrier's resistance against pathogens along the SCB line. However, the fusion

properties and regeneration capacities of the cells in the ACB are negligible. The intracellular organelles, such as golgi and endoplasmic reticulum, in cytotrophoblasts are larger than those in alveoli, making the SCB stronger. Compared to the thin basement membrane in the ACB, the basement membrane lying below the SCB presents an additional physical barrier. The fact that most placental infections originate from the decidual region is one of the most important indicators of the SCB's resistance to pathogens [20]. The absence of gap junctions in the SCB creates another important resistance point against viruses that want to pass into the fetus in maternal leukocytes.

In both barriers, ACE2 and TMPRSS2 proteins are expressed. However, while the expression of these two proteins is moderate in the ACB, it is weaker in the SCB and it varies according to the trimester of pregnancy. Another important factor that distinguishes the SCB from the ACB is the absence of E-cadherin and toll-like receptors on the syncytiotrophoblasts that mediate the entry of viruses or other pathogens into the cell [2,21]. This difference in receptor expressions, as well as the strong barrier properties of the placenta, can minimise the possibility of SARS-CoV-2 colonising the syncytiotrophoblast. For all these reasons, the ACB is more likely to be damaged in the presence of any pathogen than the SCB. In order for the strong SCB to be damaged, a different set of conditions must occur. The viral load that colonises the SCB would need to be very high; and more than one pathogen must attack the SCB at the same time –this could be in the form of virus-virus, virus-bacteria, and/or virus-parasite combinations [2,3]. The presence of concurrent maternal systemic diseases or immunocompromisation may further facilitate vertical transmission.

5. Breakdown of the SCB

Damage of natural syncytial resistance for the above discussed reasons can enable pathogens, including coronavirus, that are free in maternal-fetal interface, or inside of maternal leukocytes, to cross into the fetus. Viral or bacterial colonisation in cytotrophoblasts, following the enzymatic hydrolysis of the syncytial layer suggests that the inner parts of the barrier are more susceptible to infections [2]. However, it is not easy for pathogens to cross the cytotrophoblast layer and reach the basement membrane. The destruction of the syncytial or decidual barrier triggers a strong innate host defence reaction against pathogens that are both free-flowing in maternal blood and within the cell [21]. Natural killer cells, macrophages and lymphocytes located in maternal-fetal contact sites form a strong but controlled defence against pathogens. The regulation of the NF- κ B pathway is necessary for a controlled immune response against colonised pathogens. Thus, it may be possible to avoid uncontrolled cell damage [22]. NF- κ B is the main transcription factor that provides activation of genes responsible for immune and inflammatory events [23]. Since open reading frame (ORF) 9c interacts with many proteins that regulate the NF- κ B pathway [24], there may be a close relationship between Orf3b, Orf10 of SARS-CoV-2 and the activation of NF- κ B [25]. Activation of NF- κ B by pathogens can lead to the destruction of cytotrophoblasts, the release of reactive oxygen species, and many cytokines and the collection of polymorphonuclear leukocytes [7,26]. Neutrophil extracellular traps, a degradation product resulting from leukocytes degradation, also increases the protease activity, leading to the expansion of tissue damage. Placental cells have a powerful weapon, progesterone, to protect against NF- κ B and leukocyte-mediated cell damage. Progesterone, produced abundantly in placental cells, is a physiological inhibitor of NF- κ B [27]. Therefore, activation of the NF- κ B pathway in any immune response to any pathogen can be kept within certain limits, ensuring that the trophoblast cell damage is at the lowest level. Despite all these physical and cellular immune mechanisms, and even if a pathogen reaches the basement membrane, it still may not be able to cross the capillary endothelium and spread to the fetus. As detailed above the placental barrier has many mechanisms to prevent pathogen transmission. In addition to these natural barriers located one after another, the greatest obstacle for the pathogen to reach the fetus

may as a result of insufficient caveolin expression in the SCB.

6. Caveolin and SARS-CoV-2

According to our hypothesis, the most important reason why the sensitivity of the SCB and the ACB to SARS-CoV-2 is different, is that caveolae proteins, known as specialised plasma membrane microdomains with a diameter of 40–90 nm, are expressed differently in the lung and placental cells [11]. Caveolin-1 (Cav-1) was the first marker protein for caveolae to be identified. The three caveolin genes lead to four different caveolin protein expressions known as CAV-1 α , CAV-1 β , CAV-2, and CAV-3 [28]. Due to its close relationship with cholesterol and sphingolipids, as well as many signals and effector molecules, caveolins play an important role in intracellular and intercellular signal transduction [29]. The role of caveolins in endocytosis and transcytosis also allows some viruses to enter cells. The coronavirus family, including SARS-CoV-2, does not appear to use caveolins to enter cells. This is despite SARS-CoV carrying at least eight caveolin-binding sites that are located on the spike and M proteins [9]. So why does SARS-CoV carry so many caveolin-binding sites if it does not use them to enter target cells? Explaining the answer to this question through an analogy will make it more understandable. If we accept that the cell membrane is a well-designed war-line, the easiest way for the virus to cross the line will be at the location of the ACE2 receptor. Whilst the viruses that enter a target cell through ACE2 proliferates and tries to integrate its genome into the nuclear genome of the host cell, the viruses that are left outside begin to cluster on the caveolins. The high density of Cav-1 in the septa of the ACB will allow colonisation of coronavirus in these areas, triggering stimulation of post-receptor mechanisms and the onset of inflammatory events [6]. The use of caveolae-mediated-endocytosis inhibitors or Cav-1 knockout prevents viral entry and the subsequent improvement of clinical outcome supports our hypothesis [30].

7. Caveolin and the ACB

We find it helpful to briefly summarise the changes in the ACB following binding of the coronavirus to the caveolins. In acute lung injury, disruption of albumin transportation in the ACB is a typical finding. Since the endothelial cell Cav-1 regulates the efficient uptake and transport of albumin in the ACB, the colonisation of Cav-1 by coronavirus may disrupt albumin transport and initiate lung oedema [31]. The disrupted transport of albumin in the ACB of Cav-1^{-/-} (lacking caveolae) mouse lungs is important data that supports caveolins role in SARS-CoV-2 mediated lung injury [32]. As Cav-1 inhibits eNOS activity, it leads to increased pulmonary vascular resistance and impaired vascular permeability [32,33]. The mice with Cav-1^{-/-} phenotype also showed thickening of the ACB, narrowing of the alveolar volume, storage of hematopoietic cells and collagen fibrils in the alveolar septum, and consequently reduced lung compliance [34].

Cav-1 is one of the main stimulants of proinflammatory events in lung cells. Cav-1's inflammatory initiating effect is mainly through the NF- κ B pathway. Activation of Cav-1 for any reason increases the release of cytokines such as IL-6 and TNF by stimulating inflammation in the ACB [35]. The rapid reduction of NF- κ B-mediated inflammation in Cav-1^{-/-} mice reveals the close relationship between Cav-1 and the NF- κ B pathway [8]. Overexpression of Cav-1 also strongly triggers leukocyte-mediated inflammation. Deletion of Cav-1 on leukocytes inhibits the inflammatory events mediated by leukocytes in lung cells and protects against hyperoxia-induced cell death [8]. All of this is strong data supporting the critical role of Cav-1 in acute lung injury. As SARS-CoV carries both 1) eight Cav-1 binding sites on S and M proteins, with the clear evidence of colonisation of Cav-1; and 2) the orf3a in the genome of the virus, we strongly suggest that SARS-CoV-2-mediated acute lung injury may be a Cav-1 dependent process [36].

8. Caveolin and the SCB

When we evaluate the SCB in terms of structural design and caveolin content, we encounter a completely different situation from what we see in the ACB. This important difference between the two barriers may be the main mechanism that prevents, or reduces, the fetal vertical transmission of coronavirus. Studies have shown that none of the Cav-1, Cav-2 and Cav-3 proteins are expressed in syncytiotrophoblast cells [10]. Although, as discussed previously, the concentration varies according to the trimester of pregnancy, syncytiotrophoblasts express ACE2 and TMPRSS2 moderately [14,15]. Consequently, syncytiotrophoblasts have receptor and protease activity to bind SARS-CoV-2. However, even if the virus binds to the receptors in syncytiotrophoblasts and enters the cell, it can not release its destructive effect on the placenta. For the syncytiotrophoblasts to be damaged by the virus, leukocytes and the NF- κ B pathway must be activated. It is well known that Cav-1 is required for leukocyte adhesion, chemotaxis, epithelial and endothelial cell apoptosis, and NF- κ B activation during virus-mediated injury [8]. Therefore for the activation of leukocytes and the NF- κ B pathway, Cav-1 protein must be expressed on syncytiotrophoblasts. However, there is no caveolin expression in syncytiotrophoblasts from the two basic cells that make up the SCB. In cytotrophoblasts, although weak Cav-1 expression is observed in the early placentation stage, with the syncytialisation of the cells, this expression decreases significantly and becomes undetectable after the 24th week of gestation [37]. Leyden et al. could not detect caveolins in either syncytiotrophoblasts or cytotrophoblasts in their immunofluorescence and immunoelectron microscopic studies [10]. Thus, since Cav-1 is required for the formation of caveolae, trophoblastic cells lacking sufficient Cav-1 will lack these structures. Due to the lack of sufficient Cav-1 expression in the trophoblast cells forming the placental barrier, all cells in the barrier should, in theory, be protected from virus-related cell damage. As a result, in addition to the excellent structural properties of the SCB, trophoblastic cells do not contain caveolin, which appears to be the most important mechanism that prevents vertical transmission of SARS-CoV-2.

9. Conclusion

This review summarises the possible mechanisms that prevent vertical transmission of SARS-CoV-2. The SCB has many specialised defence mechanisms to prevent viral transmission. Cells forming the SCB do not contain most of the receptors necessary for the attachment of viruses. Besides the SCB's thickness of around 1 to 2 μ m; the strong physical properties of the basement membrane; and the absence of intercellular gap junctions limiting vertical transmission of viruses within leukocytes. Lack of caveolin expression in the SCB appears to prevent virus-mediated cell damage, allowing the barrier to remain intact. Even if the virus enters the cell, a Cav-1 mediated inflammatory reaction will not emerge and no significant damage will occur in the barrier. Of note, the absence of caveolin in the SCB inhibits both leukocyte and NF- κ B mediated inflammatory reactions, which are activated by coronavirus, and prevents vertical transmission. Since the physiological Cav-1 deficiency appears to prevent vertical transmission by protecting the barrier integrity, treatment algorithms could potentially be developed to block this pathway in the non-pregnant population affected by SARS-CoV-2.

Author contribution

OC and AS contributed to conception, design and writing; BB, NC, MA, SNA, MU, AE, ATT contributed to acquisition, analysis, interpretation and revision of the manuscript; IED BD and SA critically revised the manuscript; All authors, gave final approval and they agree to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of competing interest

We declare no competing interests.

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