Review Article

COVID-19 Subclinical Infection and Immunity: A Review

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Abstract

The aetiologic agent of COVID-19 is a novel coronavirus, SARS-CoV-2. Like other coronaviruses, it generally induces enteric and respiratory diseases in animals and humans. COVID-19 may be subclinical, and symptomatic, ranging from mild–to-severe disease. The spectrum of presentation is the result of several factors ranging from the inoculum size, inherent host susceptibility, possible cross-reacting circulating antibodies. Subclinical viral infections are associated with widespread community transmission and in some cases like Polio, herd immunity. An understanding of the biology and immune behavior in subclinical coronavirus disease 2019 (COVID-19) might be useful in the quest for vaccine development as well as the current control efforts against the COVID-19 pandemic. We carried out a narrative review of the available literature on the biology, etiopathogenesis, clinical manifestation of SARS-CoV-2 viral infection, focusing on our current understanding of the disease mechanisms and its clinical manifestation, and the host immune response to the infection. We also highlighted some of the research gaps regarding subclinical infection in COVID-19 and its potential application for vaccine development and other preventive efforts toward containing the current COVID-19 pandemic.

Keywords: Coronaviruses, coronavirus disease 2019, immunity, SARS-CoV-2, subclinical infection

Introduction

In December 2019, China informed the World Health Organization of novel viral pneumonia in the city of Wuhan.[1] This was later named coronavirus disease 2019 (COVID-19). Subsequently, on January 30, 2020, the WHO declared COVID-19 as a Public Health Emergency of International Concern. Following this, the WHO on March 11, 2020, declared COVID-19 a global pandemic.[1,2] Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) etiologic is the etiologic agent of COVID-19 and has spread worldwide with resultant high morbidity and mortality globally.[2,3] This high morbidity and mortality have also resulted in fear and apprehension that has substantially crippled social and economic activities globally.[3]

COVID-19, similar to other infectious diseases results in both subclinical and overt symptomatic infection. The overt disease may range from mild to severe depending on several factors ranging from the inoculum size, the inherent host susceptibility, possible cross-reacting circulating antibodies, and comorbidity.[3] Although severe COVID-19 can be a fatal disease, asymptomatic or mild cases have been reported suggesting a role for subclinical infection in the community spread of the infection.[3] However considering COVID-19 being a new disease, it is not certain what role this asymptomatic or subclinical infection may have in optimizing natural immunity and by extension halting the community spread of the disease.[4,5] However, we know that in some infectious diseases, especially in situations where cure is not in sight, the symptomatic disease may be the tip of the iceberg largely because only severe diseases present to the attention of the health system and are therefore the ones counted and characterized, while subclinical infection, which largely resides in the community, away from the focus of the health system stays beneath, thereby forming the body of the unseen disease burden.[6] People with subclinical infection usually take little or no precaution and thus are liable to contribute to the widespread transmission

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of the virus in the community. Recent reports have shown that immunoglobulin (Ig) production in individuals with subclinical infection is not any different from that in individuals with the mild-to-severe disease.[7,8] Although the clinical relevance of naturally acquired Igs in conferring protecting against reinfection with the virus in the same individual or in persons transfused with convalescent serum have been advanced.[8,9] However, the duration and quality of such immune response appears to be short which may limit its extensive clinical utility. Subclinical infection in some viral infections have been shown to contribute to community-level protection through immunologic responses against the virus from low grade, but the widespread transmission of the virus by asymptomatic carriers contributes significantly to the development of herd immunity as seen in poliovirus infection.[8,9] In epidemics and pandemics especially in developing countries where resources for health is in short supply, subclinical infections are largely not tracked making it difficult to correctly characterize and quantify the burden, behavior, and the natural course of the infection or disease.[7-9] This is even more so that COVID-19, being a new disease with high morbidity and significant mortality makes public health efforts and attention to be focused on containing and curtailing the huge human and economic catastrophe it brought in the wake of the pandemic.[8,9] The overall aim of this narrative review is to evaluate the current evidence on the biology and epidemiology of the virus as well as immune response in individuals with subclinical COVID-19 with the aim of identifying areas for potential application in the current push toward strategies for containing the pandemic through effective vaccine production.

Methods

We conducted searches of the PubMed, Web of Sciences, AJOL, and Google Scholar database for all English language literature using the following search terms or phrases: "COVID-19," "SARS-CoV-2," "immunity," "immune response," "antibody," "antibody response," "subclinical infection," "asymptomatic infection," "biology," "pathogenesis" and "aetiopathogenesis." We included all articles written in English irrespective of the date of publication. We included electronic articles, ahead-of-print publications available in the PubMed database. Unpublished data were not solicited from authors. Articles that were judged not to be applicable were excluded based on consensus between two of the authors (CSY and NYS). We additionally identified articles that may be relevant from the reference list of other articles identified by our PubMed searches. This was done for publications through April 2021.

Etiopathogenesis: Biology of the virus, mechanism of infection, immunologic response to infection

Biology of the virus

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the cause of COVID-19 pandemic. It is still a great concern to both global health and the economy of nations. in the 21st century, three coronaviruses have emerged within the human population as spillover infections they include: Severe acute respiratory syndrome coronaviruses-2 (SARS-CoV-2), SARS-CoV-1, and Middle East respiratory syndrome coronavirus (MERS-CoV).[10] However, SARS-CoV-2 is the most pathogenic among the three coronaviruses. Most cases are asymptomatic, where infected persons experience mild symptoms which include cough, fever, fatigue, headache, and mild or no pneumonia. Severe clinical manifestations can occur with hypoxia, dyspnoea, it can also result in multi-organ, respiratory failure, and shock.[11]

Coronaviruses are large enveloped positive-stranded RNA viruses, they generally induce enteric and respiratory diseases in animals and humans.[3] They are crown-like with spikes on the surface of the virus, positive-sense, single-stranded, and enveloped RNA that belong to the family Coronaviridae.[1] The entire SARS-CoV-2 genome is >30 kb in length and consists of 14 open reading frames (ORFs) that encode 27 proteins.[12] On the 5' end of the viral genome is ORFa/b that encodes for a polyprotein that undergoes posttranslational cleavage into 16 nonstructural proteins (nsp1–16) that forms the replicase/transcriptase complex. The 3' end of the genome contains ORFs that encode five structural proteins (envelope [E], spike [S], membrane [M], and nucleocapsid [N]) and 9 accessory factors.[13,14]

After cellular entry, the virus utilizes the host machinery to produce its lipid envelop on the S proteins that give the virus its characteristic crown-like appearance. The M protein which is the most abundant, structural protein enables the attachment of the viral membrane to the viral nucleic acid within the virion. Changes in the virus are facilitated by the N protein. In addition, the S protein plays a critical role in promoting attachment and fusion of the virus to the host membrane.[15]

Pathogenesis

In general, pattern recognition receptors on the surface of macrophages, dendritic cells (DCs), endothelial cells, mucosal epithelial cells, and lymphocytes function in recognition of invading pathogens. Initial infection evokes the release of resident dendritic cells, which are activated and bind the pathogen in the respiratory epithelium and are drained to the mediastinal and cervical lymph nodes.[13,14] It is processed and presented to naïve circulating T-cells as major histocompatibility complex (MHC)/peptide complex, this activates the T-cells resulting in proliferation and migration of T-cells to the site of infection.[13,14] The viral structural spike (S) protein of SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor. The serine protease, Type 2 Transmembrane Serine Protease (TMPRSS2) in the host cell further promotes viral entry into the host cell by cleaving ACE2 and activating the SARS-CoV-2 S protein.[13,14] Naïve CD8 cells activation depends on the presentation of antigen by DCs, this, in turn, depends on a number of factors: number of cells present, the concentration of antigen, and the expression

of cell surface molecules such as MHC, CD80, CD86, and cell adhesion molecules.[13,14]

Mechanism of infection

The mechanism of infection of the virus can be viewed in three stages. Stage I starts with the inhalation of the SARS-CoV-2 and its attachment to the epithelial cells within the nasal cavity where replication starts. This binding is mediated by the spike protein that is made up of two subunits S1 and S2. The angiotensin-converting enzyme-2 (ACE2 is the main receptor for both SARS-CoV-2 and SARS-CoV-1 on the epithelial cells where the S protein shows affinity to and binds to SARS-CoV-2 in either open or closed conformation.[16] The S protein on the surface of the virus is cleaved and activated by an intracellular protease called TMPRSS2 to generate unlocked, fusion-catalyzed forms on the cell that supports early entry of the virus.[16,17] Local propagation of the virus goes on with limited innate immune response but the virus can be detected using nasal swabs.[18] The viral load may be low and the patient is mostly asymptomatic for 1–2 days but the individuals are infectious.[12,18]

Previous reports suggested recombination of the receptor-binding domain (RBD) with furin or TMPRSS2 cleavage sites with a unique amino acid insertion. This activity with the S protein allows effective cleavage by furin and other proteases making the virus more infectious as a result of cleavage sites acquisition.[12,19]

In the second stage, the virus propagates and migrates down the respiratory tract and a robust innate immune response is triggered. This is also the stage where clinically the individual manifests symptoms of COVID-19. A high percentage of infected patients present with mild disease restricted to the upper and conducting airways. They can be monitored at home with conservative and symptomatic therapy.[12,18]

The third stage may be seen in 20% of infected patients where it progresses to severe disease due to pulmonary infiltrates. Fatality and morbidity rates increase because the virus reaches the gas exchange units of the lungs and infects the alveolar type II cells. Infected alveolar units may be peripheral and subpleural. SARS-CoV propagates within type II cells where the viral particles are released, and the cells undergo apoptosis and death.[2] The severity of COVID-19 is determined by the high expression of ACE2 by organs involvement. Tissue and organ infections and damage have been associated with ACE2 expression. The expression of ACE on the heart contributes to myocardial injury and cardiovascular damage.[20] In addition, organs that express furin predispose themselves to infection by the virus.[21-23]

Immunologic response to infection

SARS- CoV-2 entry into the host cell is followed by replication, transcription, assembly, and release. These activities are associated with cells damage leading to acute inflammatory and immune responses by the host. Rapid cellular activity in the early stages of infection results in cell death increased vascular permeability and the release of inflammatory cells in large quantities.[24]

Severe cases demonstrate elevated plasma levels of interleukin (IL)-2, IL-7, IL-10, Granulocyte colony-stimulating factor (G-CSF), IP-10, MCP-1, MIP-1A and tumor necrosis factor-α which is considered as cytokine storms which facilitate the pathogenesis of the virus and relate to clinical outcome.[25,26] This directly leads to tissue and organ damage due to hyper-inflammatory response. In an asymptomatic or nonsevere patient, recruitment of immune cells occurs in the blood before symptoms are resolved. Antibody cells appear briefly before recovery and persist for 7 days thereby defining the utility of early antibody test. However, the detection of Igs can help in determining immune components for protection.[27] Sometimes in asymptomatic patients, there may be minimum pro-inflammatory mediators when they become symptomatic, this may be related to the expression of SARS-CoV-2 S protein which downregulates ACE2.[28] Reduce expression of ACE2 on the cells surface affects the physiological function of the lungs due to its effect on the renin-angiotensin-system which modulates the inflammatory response.[29] The binding of SARS-CoV-2 to ACE2 results in its depletion which may lead to generalized inflammation.[30]

The innate immune response from coronaviruses infections can either be protective or destructive to the host. Protection can be from the role of type I interferons (IFN) response and or its downstream components that control viral replication and subsequent inducement of an effective adaptive immune response. Contrarily, SARS-CoV-2 infection can also lead to reduced macrophages and lymphocytes levels in the blood due to cell death.[31,32] However, immune failure and immunosuppression have been associated with the downward regulation of type I IFN response.

Sub-clinical Infection and coronavirus disease 2019 immunity

SARS-CoV-2 infection activates both innate and acquired immune responses. Virus infection activates a number of host immune responses such as induction and maturation of dendritic cells DCs, thus elaborating the release of inflammatory factors, including the synthesis of type I IFNs, which are critical in limiting viral spread. CD4+ T-cells stimulate B-cells to produce virus-specific antibodies like Ig G and IgM, whereas CD8+ T-cells are cytopathic to virus-infected cells. T helper cells produce cytokines and other mediators of inflammation to help mount an immune response, antibodies and complement factors such as C3a and C5a production are really important in combating the viral infection. SARS-CoV-2 can also block the host immune defense inducing the apoptosis of T-cells.

Subclinical COVID 19 infection refers to COVID 19 infection with clear evidence of SARS COV 2 infection without clinical symptoms. This differs from COVID 19 patients who are in the pre-symptomatic spectrum of COVID 19 that eventually develop symptoms. The magnitude of subclinical COVID

19 infection is diverse depending on the population studied, inclusion criteria which may range from clearly asymptomatic to mildly asymptomatic disease. This is made complex by the possibility of symptoms developing from other concomitant infections like seasonal flu and other endemic infections. In a large study in Japan of 1600 patients with COVID 19, subclinical infection was seen in 2.4%.[4] Another report showed a high prevalence of 45%.[33] It is generally challenging to determine the proportion of subclinical infection. A modeling study using variables in high- and middle-income countries have demonstrated varying proportions of subclinical infection according to age, individuals who are <20 years have about 79% subclinical infection this decreased to about 31% in those >70 years.[34] In low- and middle-income countries, given the proportionally younger age demographics, theoretically, the proportion of subclinical infection may be high. However, the susceptibility of this population to COVID 19 may really be different.

Subclinical infection is generally seen in several infectious diseases. Susceptibility or resistance to infections may be related to the inoculum dose host susceptibility and pathogen's virulence. Any infection that has high sub-clinical infection may contribute significantly to the development of herd immunity. Conversely, it has been demonstrated that subclinical COVID 19 infection may be infectious, thus contributing to the spread of COVID 19.[4] It has been demonstrated that viral load is high both in clinical and subclinical infection.[35] Interestingly, the median duration of viral shedding in subclinical COVID 19 infection was shown to be 19 days, this is even longer than 14 days seen in symptomatic patients.[36] This is instructive and suggests that symptomatic individuals can mount a stronger and qualitative immune response to reduce the duration of viral shedding. Subclinical infection poses a significant challenge in isolation contact tracing as a means of controlling epidemics.[6,37] This shows there is great limitation in the control of COVID 19 using this strategy, whereas diseases without significant subclinical infections that are infectious may readily be controlled by isolation and contact tracing. This is especially challenging depending on the proportion of subclinical infection in COVID 19. In general, antibody response to SARS COV2 infection is complex and the duration of development of specific antibody response may be highly variable. Although there is variability in the onset of demonstrable antibody response following SARS COV 2 infection, a study demonstrated 100% of patients develop such antibody response 20 days postinfection and the levels wane over time.[9] The duration of IgG and neutralizing antibodies may last 2–3 months.[36] This is much shorter than the duration seen in SARS COV and MERS infection which may last for about 1–2 years.[36,38,39] However, Gaebler *et al*., in a study that consist of both symptomatic and subclinical infection in New York demonstrated SARS COV2 neutralizing antibodies of up to 6.2 months. This correlated well with the titers of IgM and IgG antibodies against the RBD of the spike protein of SARS-CoV-2, but there was a five-fold reduction between two-time points of measurements at 1.3 and 6.2 months.[40] This is instructive, as 44% of their cohort had persistent post COVID symptoms, this shows that antibody response may be directly proportional to clinical symptoms. The same study also demonstrated SARS CO2 antigen persistence in enterocytes as immune complexes on the follicular dendritic cellular surface. This antigen persistence is crucial for memory B-cell stimulation and may be helpful in the prevention of re-infection.

Patients with subclinical SARS COV 2 infection appear to have comparatively lower immune responses characterized by lower pro and anti-inflammatory responses. Specifically, these include: IL-6, macrophage colony-stimulating factor, tumor necrosis factor-related apoptosis-inducing ligand, G-CSF, and growth-regulated oncogene-α.[36] Conversely, higher levels of IL-12, leukemia inhibitory factor, and stem cell factor have been demonstrated in patients with subclinical infection.[36]

It is pertinent to have a cursory look at factors associated with subclinical infection to understand the putative factors that may potentiate overt and severely symptomatic disease. Matsuba and colleagues evaluated a number of factors to determine if they are significantly associated with subclinical COVID 19 infection.[4] Such factors include the use of Angiotensin 2 converting enzyme (ACE2) and Angiotensin 2 receptor blocker this was not found to be associated with subclinical diseases. Although, theoretically, SARS COV 2 binds to ACE on the cell surface to facilitate viral entry. This is not surprising as viral entry into cells is facilitated by serine protease Type 2 Transmembrane Serine Protease (TMPRSS2) in the host cell which actually cleaves ACE2 leading to the activation of SARS-CoV-2 S protein and eventual viral entry. It may be scientifically plausible to consider higher inoculum dose exposure to SARS COV2 linked to symptomatic COVID 19. Possibly via higher exposure to SARS COV 2 in crowded places or close contacts of patients confirmed COVID 19. However, the study determined the antibody response of passengers in a crowded train in Kanagawa, Japan, and close contacts of patients with confirmed COVID 19 but there was no significant difference in SARS COV 2 antibodies status. Similarly, no antibody differences were seen in patients' demographics, BCG vaccination status, body mass index, cigarette smoking, and comorbid diseases (hypertension, diabetes, dyslipidemia, and lung diseases).[4] Although, a systematic review has shown that comorbidities such as hypertension, diabetes, and chronic pulmonary disease are significantly associated with severe disease.[41] There may yet be several other factors that may contribute to susceptibility to symptomatic disease.

It is important to consider the role of subclinical infection in conferring immunity. Since the medieval era it is known that variolation tends to confer immunity. It has been demonstrated that contacts of patients who had smallpox developed subclinical infection twice greater than those who developed the overt disease. This was demonstrated by the presence of neutralizing antibodies against smallpox,

it is thought that subclinical smallpox may play a role in conferring herd immunity[42] Herd immunity generally refers to conferring of immunity to specific infections when a critical proportion of individuals of a given population have been vaccinated. The importance of herd immunity following vaccinations to measles, chickenpox, mumps, polio, and some other infections have been described to be really important in protecting populations against these diseases.[43] It is also possible that some infections including subclinical infections may contribute to herd immunity. In general, there is paucity of data on antibody kinetics in subclinical SARS COV 2 infection, which constitutes a significant research gap in SARS COV 2 knowing that this constitutes a significant burden of the disease.[44] It has been shown that when the absolute number of people infected in a country is high and there is higher effective reproduction number, higher proportion of persons in the country are needed to recover from COVID 19 to confer herd immunity.[45] Due to the possibility of cross-reactivity and development of some immunity from seasonal coronaviruses which include 229E, OC43, NL63, and HKU1 this may contribute to protection against severe COVID 19. It has been known that viruses that share the same epitopes or common structure may trigger cross-reactivity and ultimately the development of neutralizing antibodies.[46] Unlike in other infections, COVID 19 has a relatively short duration of neutralizing antibodies, hence the role of subclinical infection in contributing to herd immunity very low. It is, therefore, needful to harp more on robust vaccination for COVID 19 for effective control and other preventive measures. Another challenge with vaccination is the breakthrough COVID 19 infections. This breakthrough infections have been attributed largely to the wide circulation of SARS COV 2 variants of concern which may be up to 70% in circulation in the United States and subclinical infection constitutes 27%.[47] This again would contribute to the magnitude of subclinical COVID 19 infections. More research is needed on subclinical infections in resource-limited settings and its long-term sequelae.

Conclusions

The COVID 19 pandemic has posed a monumental challenge globally regarding containment measures, due to its nonspecific clinical and laboratory profile and effective treatment strategies. In-depth research in COVID 19 immunity and subclinical infection is invaluable to better understand the disease and guide disease prevention and control. It is, therefore, needful to carry out in-depth research on subclinical infection of coronaviruses to utilize its possible therapeutic or preventive potential in future coronavirus-related epidemics and pandemics.

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Conflicts of interest

There are no conflicts of interest.

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