ABSTRACT:
The novel coronavirus disease, COVID-19, impose a challenge on scientists and physicians in terms of understanding the phases of viral pathogenesis and host immunopathogenesis that lead to the fatal complications of the disease. Three pillars have been thought to play essential role in determining the prognosis of COVID-19, namely the viral load of SARS-CoV-2, the count and functionality of T lymphocytes and the cross-reactivity of T lymphocytes with common cold coronaviruses. Collectively, these three factors might constitute a triad governs the time-based and phase-based progression of COVID-19 patients. This review discusses the role of this triad in COVID-19 and presents the so far accumulated knowledge and research that justify the central role of this triad of factors in disease prognosis. Understanding the factors control disease progression is most important in helping physicians to treat the multi-phased host-microbe disease.

KEYWORDS: Coronavirus, COVID-19, SARS-CoV-2, T cells, viral load, T cells cross reactivity

INTRODUCTION:
Kinetics of the viral infection and the implications of the viral load on the disease and on the immune response

Viral infections differ from bacterial and parasitic infections that viruses are intracellular microbes which trigger a lot of mess inside host cells by favoring viral replicative cycle over cellular antiviral defenses\(^1\). Furthermore, unlike, bacteria, viruses replicate at far higher exponential power than bacteria do. Bacterial agents duplicate in binary fission; this means every single bacterium yields two daughter bacteria after certain time, called duplication time. Viruses replicate by different method; a single or few viruses enter susceptible host cells and turn them into factories for replication\(^2\). Hirano et al stated that the average per-cell yield of active virus, or the burst size, was estimated to be about \(1-6 \times 10^2\) plaque-forming units\(^3\). And this replication takes place after 12 to 36h from the host cell infection, called the eclipse period\(^4,5\). In this context, the exponential replicative curve of viruses is far unmatched and infective viruses replicate to trillions inside host body within few days. A model for SARS-CoV-2 replication using minimal probable burst size (100 PFU/cell) and the longest probable eclipse period (36h) reveal that the viral load of SARS-CoV-2 after 7 days of infection starting with the minimum infective dose, 1000 virions, will be at least 10 trillions virus. It is estimated that a single cough from infectious COVID-19 patient spews up to 5 million viruses; therefore, if the infective dose of SARS-CoV-2 was 5x10^5 virions, then the viral load after 7 days of infection will be 5000 times, or 3-4 logs higher than the calculated minimum viral load, namely 10 trillions. Accordingly, it is speculated that such explosive replication of pathogenic viruses is not possible to be matched but by two strategies: a robust and early immune counterattack and a very early and efficient antiviral therapy\(^6\). The immune system is very capable to tackle viral infections and it is the best tool to combat the stellar number of attacking viruses. Our immune system is a highly complicated and multi-arm defense instrument used to fight back intruding microbial agents. For viral infections, the first line of immune defense is the innate immunity, or the non-specific
immune response(7). Once there is an established viral infection, this means that the innate immunity did not contain or diminish the infection at its very beginning. Within 4-6 days after infection, cell-mediated immunity (CMI) arm is activated specifically towards the pathogenic virus. CMI is mainly mediated by T-lymphocytes, or T-cells to which antigen presenting cells provide samples of the viral proteins to positively select, activate and clonally expand the virus-specific T-cells. After few hours, T helper cells will activate CD8+ cytotoxic T cells that wipe out infected cells to minimize the reservoir of the replicating virus. B cells follows T-cells in few days to specifically expand and convert to plasma cells to produce virus-targeting IgM, then IgG antibodies(8). In regard to SARS-CoV-2, it is a very malicious virus that causing COVID-19 with 15% of population infected might develop severe or deadly pulmonary and multi-organ complications(9). This is attributed to the wide tropism of SARS-CoV-2; it infects most of human body tissue which express angiotensin converting enzyme 2 (ACE2). Moreover, SARS-CoV-2 infects cells express cell surface CD147 proteins; therefore, it is found that SARS-CoV-2 bind abundantly to red blood cells that express thousands of CD 147 on their surface; this is thought to participate in the observed coagulopathies in COVID-19 patients(10). However, SARS-CoV-2 is an enveloped virus which does not burst out infected cells, instead newly assembled SARS-CoV-2 progenies bud from cells without direct damage(11). So what makes SARS-CoV-2 so dangerous; the answer lies in the most ominous facet of SARS-CoV-2 pathogenesis, its ability to deregulate immune system. Like all other enveloped viruses, the main pathogenic effect comes from the inflammation driven by the innate and adaptive immune response to the viral infection(12). Nevertheless, SARS-CoV-2 excels more than other viruses in annoying and exhausting the immune system in an unprecedented way leading in some patients to severe hyperinflammatory phase of the disease, called cytokine storm or macrophage activation syndrome (MAS). This phase of the disease is the responsible to almost all morbidity and mortality of COVID-19(13).

Back to the viral load, what the load of SARS-CoV-2 has to do with the prognosis of COVID-19 patients? From virology point of view, it is well known that the higher viral load attained in a shorter duration of time is the more difficult for the immune system to contain the infection efficiently(12). Moreover, the higher viral load, the less likely any antiviral therapy might succeed with clinically observed response(8). Thus, the viral load of SARS-CoV-2 is most likely one of the most important factors govern the fate of the disease. The peak viral load of SARS-CoV-2 was found to be 5-7 days after infection; then, most patients show progressive decline in viral load reaching baseline at day 8 to 10 after infection; this is typically the viral phase of the disease(13). It has been shown that the higher viral load, or the lower PCR Ct, the worst prognosis was observed for COVID-19 patients. This implies on the strict relationship between the viral load of SARS-CoV-2 and the later hyperinflammatory complications(8). Hence, it is hypothesized that the higher viral load of SARS-CoV-2 in the body, the more likely the adaptive immune system later will be deregulated and/or exhausted where the innate system tries to compensate the deficit by pumping up very high levels of pro-inflammatory cytokines and chemokines that in turn might result in further negative feedback in the already exhausted adaptive immune cells. In this context, the inoculum of SARS-CoV-2 seems one of the factors that govern the prognosis of the disease. Back to the model of the viral load of SARS-CoV-2 calculated earlier in this article, it is evident that exposure to 5 millions viral particles leads to 5000 folds higher viral load than the minimal infective dose (1000 viral particle) in just 5-7 days post-infection. Thus, the amount of inoculum of SARS-CoV-2 during the primary infection is crucial because there are no memory cells to fight back and the adaptive immune response takes at least one week to mount a response(6-15). At that time, a stellar number of viral invaders have to be dealt by the immune system(14); this is not an easy task to accomplish. Hence, some patients fail to contain the infection properly and timely and this is maybe due to the size of the viral inoculums(13). Based on what is explained so far, wearing masks is an essential tool not to protect from infection only, but to lower the viral inoculum as well; low viral inoculum subsequently enhances greatly the chances of having asymptomatic or mild-moderate COVID-19 without progressing to the immune deregulation/hyperinflammatory phase of the disease where all fatalities occur(5,16). This explains the higher percent of severe COVID-19 cases among front-line health workers who are consistently exposed to high loads of SARS-CoV-2.
More to the point, an early and efficient antiviral therapy seems mandatory to lower the viral load during the first week of SARS-CoV-2 infection during which the virus replicates progressively to dangerous high levels. In this endeavor, a question might be raised, what is the definition of the proper and efficient antiviral therapy. The answer is more complicated that it seems. To explain this, there is a need to clarify some points. First, it is very uncommon to find a single antiviral agent able to efficiently kill pathogenic viruses in vivo; therefore, most of successful antiviral therapies are combinations of drugs rather than a single drug. Second, the successful antiviral therapy does not necessarily equal to the therapy that wipes out the virus completely; any viral agent or combination of agents capable of lowering the viral load 1 to 2 log or slowing the viral replication might be in some instances highly successful. It is prudent to take into account that, in the majority of viral infections, the real killer of the viral pathogens is the immune system; hence, any antiviral therapy that could lower the viral load by partial killing or slowing replication would offer a great opportunity to the immune system to face much easier encounter with far less probable immune deregulations.

Surprisingly, throughout COVID-19 pandemic, numerous clinical trials on novel or old repurposed drugs, which were tested as potential antiviral agents for SARS-CoV-2, were trialed improperly. From virological point of view, the most common faulty setting wastring potential antiviral therapy during the late phase of the disease, the hyperinflammatory phase, not during the early viral replicative phase of the disease. In addition, combinational antiviral therapies were not always of primary focus. Third, antiviral agents that lower 1-2 log of the viral load were not considered successful therapies. Log reduction of microbial pathogens has always been underestimated. It is noteworthy to mention that one log reduction of one trillion viruses is equal to killing 900 billion viruses while the left is only 100 million viruses. In other words, one log reduction equals to 90% of the viral pathogen reduction; one log reduction provides far better chance to the immune system to deal safely with the remaining 10% of the virus. Hence, 1-2 log reduction antiviral therapy is really a game changer especially if taken at the beginning of the infection.

The role of T-cells count and SARS-CoV-2-cross-reactivity of T-cells in the course of COVID-19 disease

In almost all patients with COVID-19, including severely ill patients, it has been found that no viable virus was isolated from respiratory tissues after day 10 to 14 of infection. On the other hand, COVID-19 positive PCR results are seen from day 7 till beyond day 14 of infection; this actually is attributed to the detection of RNA fragments coming from live and later from dead SARS-CoV-2 virions. It is bewildering to find out that the majority of severely ill COVID-19 patients with severe pulmonary involvement have no viable virus after 10 to 14 days of infection. In this instance, what is causing the severe pulmonary disease or other organs diseases, such as blood clotting, liver, heart, and kidney disorder? The answer is the out-of-control escalated immune response which was already triggered by the viral replicative phase during the first 10 to 14 days of the disease. The hyperinflammatory phase of the disease is the cornerstone for the prognosis of COVID-19 patients. Anyhow, it is not yet clear what is the main triggering factor(s) to the hyperinflammatory response, is it the live viruses or the viral debris from hundreds of trillions of dead viruses. By all means, both possibilities depend directly on the maximal viral load of infection.

Five days from the primary exposure, SARS-CoV-2 replicates in the host ACE2-expressing cells without major immune resistance. T-cells start kicking back at the 5th day of infection. On the other hand, SARS-CoV-2-specific IgM antibodies start to appear in very low titers at the 8th–10th day of infection, and IgG antibodies at the >14th day of infection. Accordingly, humeral immune response kicks off at the end of the phase of the viral replication. Hence, it is T-cells, rather than B cells, which mount the most effective and decisive immune response to SARS-CoV-2. It was stated previously, the main immune response to SARS-CoV-1, a close relative to SARS-CoV-2, is T-cell immune response. By observing the timeline of anti-SARS-CoV-2 IgM and IgG, it seems, like SARS-CoV-1, that T-cells immune response is the one which terminates SARS-CoV-2 infection. There are numerous clues affirm the central role of T-cells, not B cells, in the fight against SARS-CoV-2. Patients with COVID-19, who were on CD20-depleting antibody therapy, show similar recovery rates to healthy COVID-19 patients.
Furthermore, lab animals with crippled B cell function responded well to SARS-CoV-1 infection\(^\text{27}\). In addition, 10% of the recovered COVID-19 patients did not develop SARS-CoV-2 specific antibodies\(^8,28\). All of these clues support the idea that T-cell arm of CMI, along with innate immunity, bear the whole burden for clearing SARS-CoV-2 infection during the first 1-2 weeks of the infection.

Knowing the central role of T-cells, mainly CD4+ T-helper 1 and CD8+ T-lymphocytic cells could solve the mystery of why children and adolescents rarely develop symptomatic or severe COVID-19 and why older patients suffer worse prognosis of the disease. Lymphocytes mature and develop in the thymus and in bone marrow\(^29\). Thymus activity is highest during the first two decades of life and consequently lymphocytes count is highest during this age. It was revealed that the peripheral lymphocyte count declines in the first 2 decades from 5000 to 2000 cells/μl\(^29\). For the following three decades, the peripheral lymphocyte count decline to 1800-1600 cells/μl. From age 50s to 90 year, peripheral lymphocyte count declines to 1500-1200 cells/μl\(^26\). The age-related decline of lymphocytes count and activity might be one of the main factors for the observed predilection of older COVID-19 patients to severe pulmonary and multi-organ affection.

It is interesting to mention that most of severe COVID-19 patients who enter hyperinflammatory phase of the disease and who have deteriorating pulmonary function show sharp decline in peripheral blood lymphocytes, lymphopenia, and increased white blood cell (WBC) count, leukocytosis. And neutrophil: lymphocyte ratio (NLR) is sharply increased\(^30\). Hence, lymphopenia and increased NLR have become important prognostic factors for COVID-19. Moreover, it has been found that CD8+ T-cells decline most followed by CD4+ T-helper 1 cells while T-helper 2 cells decline minimally and B cells do not decline\(^30,31\). This differential decline in the lymphocytes indicates that the cytotoxic CMI is most affected in severe COVID-19 patients not the humoral immunity. Interestingly, most of ill-progressing COVID-19 patients with lymphopenia and high NLR showed very high titer of SARS-CoV-2 during the first 10 days of the infection\(^16,22\). Thus, an observed link between the viral load of SARS-CoV-2 and the host lymphopenia and high NLR does exist. Interestingly, almost zero cases in symptomatic COVID-19 children showed lymphopenia or increased NLR.

According to the knowledge gained from the pathogenesis of COVID-19 and the accompanied immune deregulation, a question still needs an answer. Why CD8+ cytotoxic and CD4+ T-helper1 cells die and their count decline in severely ill COVID-19 patients.

Furthermore, what is the relationship between cytokine storm, or macrophage activation syndrome (MAS), and the observed lymphopenia. There have been many speculations and theories, but so far nothing is confirmed. Some scientists stated that lymphopenia occurs because of immune exhaustion phenomenon\(^32\); others speculated that the very high viral load might lead to inability of CMI to contain the virus and subsequently fail to limit the infection, therefore the innate arm of the immune system tries to compensate the deficit by increasing the secretion of pro-inflammatory cytokines/chemokines mainly by monocytes, macrophages and neutrophils leading to leukocytosis; moreover, soaring pro-inflammatory cytokines result in a negative feedback on the already exhausted lymphocytes\(^8,20,23\). Other researchers explains that the high level of pro-inflammatory cytokines, especially IL-6, induce apoptosis in lymphocytes via FasL and IL-6 receptors leading to sharp lymphopenia\(^23,24,25\).

Collectively, it seems the poorly progressing patients enter to a vicious cycle where too high viral load impose an unprecedented challenge to T-cells which face an extreme difficulty to contain the huge number of live viruses or viral debris leading to massive necrosis of lymphocytes in spleen, lymph nodes, and lung accompanied with soaring levels of secreted pro-inflammatory cytokines that would quell further the count of lymphocytes resulting in destructive systemic inflammation. Another piece of the riddle needs to be addressed though. Why some old patients with multiple comorbidities unexpectedly show mild to moderate illness. In fact, there could be genotypic variations in some of the host genes that have a role in the susceptibility of COVID-19 patients to immune deregulation\(^33\). However, it was revealed that up to 60% of blood samples collected before COVID-19 pandemic showed SRAS-CoV-2 cross-reactive T-cells. In other words, more than half of population might have memory T-cells to SRAS-CoV-2 before the primary exposure to SARS-CoV-2\(^34,35\). The importance of these findings roots from the notion that blood samples were collected before
the advent of coronavirus pandemic in December 2019. This means that SRAS-CoV-2 cross-reactive T-cells were previously generated toward closely related coronaviruses, which most likely the four strains of common cold coronaviruses16,34. What supports this notion is the observed lower morbidity and mortality of COVID-19 patients in the third world countries compared to the first world countries; moreover, the lower morbidity and mortality found in slum neighborhoods compared to high socio-economic neighborhoods in India. It is well known, the dwellers of overcrowded areas with poor health system are more prone to frequent viral and bacterial infections. Common cold coronaviruses, SARS-CoV-1, and MERS infection provide humeral protection for only 6 months to 2 years26,37. Therefore, reinfection can occur later; hence, the more frequent reinfections with coronaviruses, the higher number of clonally expanded memory T-cells to coronaviruses. Thus, people live in poor conditions undergo frequent respiratory coronavirus infections; in this way they have more chance to possess high number of SARS-CoV-2 cross-reactive T-cells.

**CONCLUSION:**

Taken together, a triad composed of three factors seems to govern the fate of COVID-19 patients: the viral load of SARS-Cov-2, the count and activity of T lymphocytes, and the presence or absence of SARS-CoV-2 cross-reactive T-cells. These three factors are thought to control the course of the disease and explain the wide clinical spectrum of COVID-19 disease which ranges from asymptomatic to a severe pulmonary and multi-organ disease. Again, these three factors explain why children are not severely affected by SARS-CoV-2 and why elderly with or without comorbidities are the most adversely affected.

Accordingly, it is recommended to prevent or lower high viral load of SARS-CoV-2. Wearing masks are therefore important not only to protect from infection but also to lower the infectious inoculum which has a strong impact on the maximal viral load of infection. Moreover, early antiviral therapy of COVID-19 patients, especially those of age >30 years, seems a good protocol, taken into consideration it is not easy to predict precisely who patients will progress best and who will progress worst. In this instance, the idea behind starting early antiviral therapy to all COVID-19 patients older than 30 years is to slow down as could as possible the viral replication; in this way, T-cells can contain the viral infection with less risky course of immune response which unlikely results in cytokine storm and macrophage activation syndrome which are very dangerous immune deregulatory conditions where all deaths from COVID-19 occur.

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