International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Immunological aspects of COVID-19

Alaa Khalifa Mohamed Mahdy[1,], Amel Mahmoud Kamal El-din[1], Emad Allam Abdel-Naeem[1], Rania Bakry[2], Ahmed Fathy Madi[3], Manal Mohamed Saber[1]*

[1]*Faculty of medicine; Minia University, Egypt.*

[2]*South Egypt Cancer Institute; Assiut University, Egypt.*

[3]*Faculty of medicine; Minia University, Egypt.*

Abstract

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by the virus SARS-CoV-2. The first known case was identified in Wuhan, China, in December 2019. The disease quickly spread worldwide, resulting in the COVID-19 pandemic. Although SARS-CoV-2 has a tropism for ACE2-expressing epithelial cells of the respiratory tract, people with severe COVID-19 have symptoms of systemic hyperinflammation. Clinical laboratory findings of elevated IL-2, IL-7, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein 1-alpha (MIP-1-alpha), and tumor necrosis factor (TNF-α) indicative of cytokine release syndrome (CRS) suggest an underlying immunopathology.

Keywords: Covid-19, SARS-CoV-2.

Full length article ** Corresponding Author, alaa.khalifa89@gmail.com*

1. Introduction

Coronavirus belongs to the family Corona viridae which is known to produce mild form of respiratory diseases in humans. In recent times, there have been three major disease outbreaks, beginning with the severe acute respiratory syndrome coronavirus (SARS–CoV) in 2002, followed by the Middle East respiratory syndrome coronavirus (MERS–CoV) in 2012, and now the severe acute respiratory syndrome coronavirus 2 (SARS–CoV2), In December 2019, China reported an outbreak of pneumonia of unknown causes in Wuhan [1]. An unknown beta corona virus was discovered from lower respiratory tract samples of these patients using next-generation sequencing. Human airway epithelial cells were used to isolate the virus that was named 2019–novel Coronavirus (2019–nCoV) [2].

1.1. Structure of SARS-CoV2

SARS-CoV2 is a virus that has an envelope and crown like spikes. Its diameter is ranging from 65nm to125 nm. It contains single strands of RNA. SARS-CoV-2 consists of four main structural proteins including spike (S) glycoprotein, small envelope (E) glycoprotein, membrane (M) glycoprotein, and nucleocapsid (N) protein, and also many accessory proteins [3].

1.2. Pathophysiology and immunopathogenesis of Covid-19

The pathogenesis of COVID-19 begins with the recognition of an angiotensin receptor (ACE2), especially common on the surface of type 2 alveolar cells and capillary endothelial cells. These cells are infected by the SARS-CoV-2 virus, assisted by the viral protease TMPRSS212, thus allowing the virus to replicate easily [4]. Because ACE2 is highly expressed on the apical side of lung epithelial cells in the alveolar space [5]. This virus can likely enter and destroy them. This matches with the fact that the early lung injury was often seen in the distal airway [6]. Epithelial cells, alveolar macrophages and dendritic cells are three main components for innate immunity in the airway, T cell responses are initiated by antigen presentation via such dendritic cells and macrophages [7]. In severe cases, patients showed lymphopenia, particularly the reduction in peripheral blood T cells [8]Patients were reported to have increased plasma concentrations of proinflammatory cytokines, including interleukin (IL)-6, IL-10, granulocyte-colony stimulating factor (G-CSF), monocyte chemo-attractant protein1 (MCP1), macrophage inflammatory protein (MIP)1α, and tumor necrosis factor (TNF)-α [9]. Severe patients also showed pathological cytotoxic T cells derived from CD4+ T cells, these cytotoxic T cells can kill virus but also contribute to lung injury[10]. In addition to

respiratory symptoms, thrombosis and pulmonary embolism have been observed in severe diseases. This is in line with the finding that elevated d-dimer and fibrinogen levels were observed, hypercoagulable profiles seen in severe diseases likely indicate significant endothelial injury as endothelial cells also express ACE2 and such endothelial injury causes microvascular permeability that can facilitate viral invasion [11].

1.3. The immunopathology of covid 19

In severe SARS-CoV2 infections, the virus appears to first suppress the immunity of the host. This results in viremia then a hyper inflammatory response may occur ***[12].*** It has been also reported that in SARS-CoV-2 infection the normal immune response is disrupted, resulting in impairment of immune system and unexpected inflammatory reactions in critical COVID-19 patients. These patients show lymphopenia, activation and dysfunction of lymphocytes, abnormal granulocytes and monocytes, high levels of cytokines, and an increased immunoglobulin G (IgG) and total antibodies [13]. Lymphocyte percentages were detected to be lower than 20% in severe infection .More analysis showed an important decrease in T cell counts, especially CD8+ T cells in severe infection compared with mild infection [14].Patients expressed higher percentages, but not absolute numbers, of activated cells expressing HLA-DR and CD38 [15]. Increased neutrophils and the neutrophil-to-lymphocyte ratio is usually significant indicator for critical cases and poor clinical outcome [16].Also a reduced percentage of eosinophils, basophils, and monocytes was reported in severe patients [17]. $CD4^+$ and $CD8^+$ T cells elicited by SARS-CoV-2 infection are directed against a range of antigens including structural and nonstructural proteins and are significantly associated with milder disease [18]. Ab-mediated depletion of $CD8^+$ T cells in convalescent macaques partially abrogates protection against rechallenge with SARS-CoV-2, suggesting a role for $CD8^+$ T cells in the face of waning Ab responses [19]. In view of its relevance for the induction of neutralizing Abs, the $CD4^+$ T cell response to the S protein has been studied in great detail in convalescent individuals and vaccinees with prediction algorithms, peptide or protein stimulation, and isolation of T cell clones [19]. Levels of total lymphocytes , $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells ,$CD19^+$ B cells, and $CD56^+$ NK cells remained lower than normal[20]. In COVID-19 infection, an aggressive inflammatory response may occur with the release of many inflammatory cytokines in an event known as “cytokine storm”. It was observed in severe COVID-19 patients. It results from the release by immune and non-immune effector cells of substantial amounts of pro-inflammatory cytokines and appears to contribute to SARS-CoV-2 pulmonary affection and massive lung damage [21].

High levels of cytokines (interleukin IL-1, IL-2, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)γ, and tumor necrosis factor (TNF)) cooperate with the complement and coagulation systems to stimulate disseminated intravascular coagulation (DIC), respiratory failure and multi-organ failure [22]. Critically ill COVID-19 patients suffering from cytokine storm are believed to have a bad prognosis and high fatality rate. The hypercytokinemia in the lungs leads to diffuse alveolar affection, hyaline membrane formation, thrombus formation , fibrin exudates, and healing by fibrosis [23]. IgM levels become increased during the first week after COVID-19 infection, reached the peak after 2 weeks and then returned to near-background levels in most cases. IgG can be detectable after 1 week and remained at an elevated level for a long time. Patients with severe and critical infection exhibit higher levels of IgM than patients with mild infection, however the level of IgG in patients with critical infection was lower than those in patients with mild and severe infection [17].

1.4. Mechanisms of immunity

The main aim of the innate immunity is to prevent more spread of any foreign pathogen. It works by initiating a signaling cascade after the recognition of what is called “pathogen-associated molecular patterns.” The pattern recognition receptors (PRRs) are responsible for this cascade [24]. SARS-CoV-2 binds host pattern recognition receptors (PRR), and toll-like receptors (TLR) which begin downstream signaling pathways stimulating secretion of cytokines and IFN which are considered the most effective mechanism to limit CoV infections [25]. SARS-CoV-2, similar to other CoV, have developed many mechanisms to suppress IFN induction and signaling .SARS-CoV-2 membrane (M) protein inhibits the production of type I and III IFNs induced by the cytosolic dsRNA-sensing pathway mediated by retinoic acid-inducible gene I (RIG-I) / melanoma differentiation-associated gene 5 (MDA5) – mitochondrial antiviral signaling protein (MAVS) signaling [26]. M protein interacts with RIG-I, MAVS, and TBK1(TANK Binding Kinase 1), so preventing the formation of the multiprotein complex containing RIG-I, MAVS, TRAF3 (TNF receptor-associated factor) , and TBK1 and subsequently impending the phosphorylation and activation of IRF3 (Interferon regulatory factor 3). Consequently, ectopic expression of the SARS-CoV-2 M protein allows the replication of virus and reduces antiviral immunity [27]. The adaptive immune response is not started until the innate immune alarms occur. The adaptive immune system has three major cell types: B cells, $CD4^+$ T cells, and $CD8^+$ T cells. B cells are responsible of release antibodies. $CD4^+$ T cells consist of helper and effector cells. $CD8^+$ T cells kill the infected cells [28].

1.5. Laboratory diagnosis of COVID-19

Reliable laboratory diagnosis represents one of the main tools for the promotion, prevention, and control of infectious diseases, diagnostic methods for COVID-19 fall under two main categories: immunological and molecular. Immunological tests can be serological tests that mainly detect antibodies in blood or viral antigens in respiratory secretions. Regarding molecular tests, they are based on the detection of SARS-CoV-2 RNA mainly in nasopharyngeal samples, which in most cases require adequate laboratory infrastructure. In addition to the cited tests, other laboratory parameters have been used as an aid in the clinical monitoring of patients with COVID-19 [29]. Serological tests are especially important for the diagnosis of patients with mild to moderate disease, in the absence of molecular diagnostics [30]. These tests can have several benefits, such as estimating the transmissibility and lethality rates, assessing individual and community immunity, and valuing the need and effectiveness of no pharmaceutical

interventions (e.g., social isolation). Furthermore, the plasma of convalescents with high levels of antibody production could be used as a therapeutic support [31]. IgM and IgG antibodies detected on ELISA have more than 95% specificity in the diagnosis of COVID-19 [32]High titers of IgG antibodies detected by ELISA demonstrate a positive correlation with neutralizing antibodies [33]. Lateral flow immunochromatography (LFI) devices have been developed by different companies worldwide and have been widely used. In general, this method detects IgM and IgG antibodies in approximately 20 minutes, individually or simultaneously. Antibodies to glycoprotein S (spike) are analyzed from blood samples obtained by finger puncture without the need for sophisticated equipment or specialized professionals [34] However, these tests are purely qualitative and can only indicate the presence or absence of SARS-CoV-2 antibodies [30] . Despite its potential value as a tool for pandemic control, the validation of LFI tests remains challenging [35].

2. Molecular tests

Most molecular tests, unlike serological ones, are performed in a specialized laboratory using cutting-edge equipment and highly qualified staff. Nasopharyngeal (NP) swabs are considered the standard samples for the detection of SARS-CoV-2. In addition to the NP swabs, the use of samples from the lower respiratory tract (sputum or bronchial lavage) and oropharyngeal (OP) swabs are used as alternatives to improve the biosafety of health care workers [32]. The ability to assess their accuracy (sensitivity and specificity) as well as their ability to monitor immunity over time remains insufficient [36]. Currently, antibody responses against SARS-CoV-2 remain poorly understood, and the clinical usefulness of the serological test is still unclear[37]. Although the detection of IgM and IgG by ELISA is positive even on the fourth day after the onset of symptoms, high levels of these antibodies are produced in the second and third weeks of the disease [30] . From the time of onset, the IgM antibody titer increases, two weeks after the onset of symptoms, both IgG and IgM are present and their levels start to decrease after the fourth week. An interesting aspect is that the most severe cases have higher levels of IgM and IgG than the mild cases [38]. Samples must be collected when the patient is in the acute phase of infection, preferably up to 5 days after the onset of symptoms[39]. This method has the advantage of being both quantitative and highly specific [32]. Reverse transcription PCR (RT-PCR) assay is widely used in the early diagnosis and monitoring of SARS-CoV-2 infections. RT-PCR was positive in 59 to 78.2% of cases [40] This assay may detect the viral RNA in the early stage of clinical symptoms even with negative CT findings, Gene sequencing can identify SARS-CoV-2 accurately, but the major disadvantages are that it is very time-consuming and expensive [41] . Reverse transcription followed by real-time reverse transcription polymerase chain reaction (RT-PCR) is considered the gold standard for the diagnosis of COVID-19, gene sequencing is more important for research on the variants and origin of SASR-CoV-2 [41].

2.1. Non-specific laboratory tests

Results of laboratory tests can increase the support for the diagnosis, prognosis, and monitoring of patients through the detection and measurement of different biomarkers. Although nonspecific, some biomarkers have been reported to be associated with the infectious process of SARS-CoV-2. Therefore, low lymphocyte and platelet counts; low serum albumin levels; and increased serum levels of C-reactive protein, D-dimer, ferritin, lactate dehydrogenase, transaminases, and interleukin-6 can be used in risk stratification to predict the severity of COVID-19[31] .

References

[1] C. Li, Y. Yang, L. Ren. (2020). Genetic evolution analysis of 2019 novel coronavirus and coronavirus from other species. Infection, Genetics and Evolution. 82: 104285.

[2] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu. (2020). A novel coronavirus from patients with pneumonia in China, 2019. New England Journal of Medicine. 382(8): 727-733.

[3] I. Astuti. (2020). Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 14(4): 407-412.

[4] C.-C. Lai, T.-P. Shih, W.-C. Ko, H.-J. Tang, P.-R. Hsueh. (2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. International journal of antimicrobial agents. 55(3): 105924.

[5] I. Hamming, W. Timens, M. Bulthuis, A. Lely, G.v. Navis, H. van Goor. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland. 203(2): 631-637.

[6] T. Carvalho, F. Krammer, A. Iwasaki. (2021). The first 12 months of COVID-19: a timeline of immunological insights. Nature Reviews Immunology. 21(4): 245-256.

[7] J. Furlong-Silva, P.C. Cook. (2022). Fungal-mediated lung allergic airway disease: The critical role of macrophages and dendritic cells. PLoS pathogens. 18(7): e1010608.

[8] P. Zhou, X.-L. Yang, X.-G. Wang, B. Hu, L. Zhang, W. Zhang, H.-R. Si, Y. Zhu, B. Li, C.-L. Huang. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 579(7798): 270-273.

[9] H. Qing, Z. Li, Z. Yang, M. Shi, Z. Huang, J. Song, Z. Song. (2020). The possibility of COVID-19 transmission from eye to nose. Acta ophthalmologica. 98(3): e388.

[10] S. Turcan, D. Rohle, A. Goenka, L.A. Walsh, F. Fang, E. Yilmaz, C. Campos, A.W. Fabius, C. Lu, P.S. Ward. (2012). IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature. 483(7390): 479-483.

[11] M. Sashindranath, H.H. Nandurkar. (2021). Endothelial dysfunction in the brain: setting the stage for stroke and other cerebrovascular

complications of COVID-19. Stroke. 52(5): 1895-1904.
[12] G. Schiuma, S. Beltrami, D. Bortolotti, S. Rizzo, R. Rizzo. (2022). Innate immune response in SARS-CoV-2 infection. Microorganisms. 10(3): 501.
[13] M. Merad, C.A. Blish, F. Sallusto, A. Iwasaki. (2022). The immunology and immunopathology of COVID-19. Science. 375(6585): 1122-1127.
[14] J. Liu, S. Li, J. Liu, B. Liang, X. Wang, H. Wang, W. Li, Q. Tong, J. Yi, L. Zhao. (2020). Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. E Bio Medicine. 55.
[15] D. Moser, K. Biere, B. Han, G. Schelling, A. Choukér. (2021). COVID-19 impairs immune response to Candida albicans. Frontiers in immunology. 12: 640644.
[16] B. Zhang, X. Zhou, C. Zhu, F. Feng, Y. Qiu, J. Feng, Q. Jia, Q. Song, B. Zhu, J. Wang Sr. (2020). Immune phenotyping based on neutrophil-to-lymphocyte ratio and IgG predicts disease severity and outcome for patients with COVID-19. Frontiers in Molecular Biosciences.
[17] R.J. Martens, A.J. van Adrichem, N.J. Mattheij, C.G. Brouwer, D.J. van Twist, J.J. Broerse, C. Magro-Checa, C.M. van Dongen, R.L. Mostard, S. Ramiro. (2021). Hemocytometric characteristics of COVID-19 patients with and without cytokine storm syndrome on the sysmex XN-10 hematology analyzer. Clinical Chemistry and Laboratory Medicine (CCLM). 59(4): 783-793.
[18] C.R. Moderbacher, S.I. Ramirez, J.M. Dan, A. Grifoni, K.M. Hastie, D. Weiskopf, S. Belanger, R.K. Abbott, C. Kim, J. Choi. (2020). Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. Cell. 183(4): 996-1012. e19.
[19] K. McMahan, J. Yu, N.B. Mercado, C. Loos, L.H. Tostanoski, A. Chandrashekar, J. Liu, L. Peter, C. Atyeo, A. Zhu. (2021). Correlates of protection against SARS-CoV-2 in rhesus macaques. Nature. 590(7847): 630-634.
[20] J. Liu, S. Li, J. Liu, B. Liang, X. Wang, H. Wang, W. Li, Q. Tong, J. Yi, L. Zhao. (2020). Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. E Bio Medicine. 55.
[21] D. Darif, I. Hammi, A. Kihel, I.E.I. Saik, F. Guessous, K. Akarid. (2021). The pro-inflammatory cytokines in COVID-19 pathogenesis: What goes wrong? Microbial pathogenesis. 153: 104799.
[22] T. Iba, T.E. Warkentin, J. Thachil, M. Levi, J.H. Levy. (2021). Proposal of the definition for COVID-19-associated coagulopathy. Journal of Clinical Medicine. 10(2): 191.
[23] S. Bhaskar, A. Sinha, M. Banach, S. Mittoo, R. Weissert, J.S. Kass, S. Rajagopal, A.R. Pai. (2020). Cytokine storm in COVID-19—immunopathological mechanisms, clinical considerations, and therapeutic approaches: the REPROGRAM consortium position paper. Frontiers in immunology. 11: 569067.
[24] M.D. Hollenberg, M. Epstein. (2022). The innate immune response, microenvironment proteinases, and the COVID-19 pandemic: pathophysiologic mechanisms and emerging therapeutic targets. Kidney international supplements. 12(1): 48-62.
[25] M.S. Diamond, T.-D. Kanneganti. (2022). Innate immunity: the first line of defense against SARS-CoV-2. Nature immunology. 23(2): 165-176.
[26] D.K. Beyer, A. Forero. (2022). Mechanisms of antiviral immune evasion of SARS-CoV-2. Journal of molecular biology. 434(6): 167265.
[27] J.-H. Lee, L. Koepke, F. Kirchhoff, K.M. Sparrer. (2023). Interferon antagonists encoded by SARS-CoV-2 at a glance. Medical Microbiology and Immunology. 212(2): 125-131.
[28] A. Sette, S. Crotty. (2021). Adaptive immunity to SARS-CoV-2 and COVID-19. Cell. 184(4): 861-880.
[29] S. Cimerman, A. Chebabo, C.A.d. Cunha, A.J. Rodríguez-Morales, Deep impact of COVID-19 in the healthcare of Latin America: the case of Brazil. Scientific Electronic Library Online. Vol. 24, pp 93-95.
[30] N. Sethuraman, S.S. Jeremiah, A. Ryo. (2020). Interpreting diagnostic tests for SARS-CoV-2. Journal of the American Medical Association. 323(22): 2249-2251.
[31] N. Chen, M. Zhou, X. Dong, J. Qu, F. Gong, Y. Han, Y. Qiu, J. Wang, Y. Liu, Y. Wei. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet. 395(10223): 507-513.
[32] M.J. Loeffelholz, Y.-W. Tang. (2020). Laboratory diagnosis of emerging human coronavirus infections–the state of the art. Emerging microbes & infections. 9(1): 747-756.
[33] S. Zhao, Q. Lin, J. Ran, S.S. Musa, G. Yang, W. Wang, Y. Lou, D. Gao, L. Yang, D. He. (2020). Preliminary estimation of the basic reproduction number of novel coronavirus (2019-nCoV) in China, from 2019 to 2020: A data-driven analysis in the early phase of the outbreak. International Journal of Infectious Diseases. 92: 214-217.
[34] F. Amanat, D. Stadlbauer, S. Strohmeier, T.H. Nguyen, V. Chromikova, M. McMahon, K. Jiang, G.A. Arunkumar, D. Jurczyszak, J. Polanco. (2020). A serological assay to detect SARS-CoV-2 seroconversion in humans. Nature medicine. 26(7): 1033-1036.
[35] S.E.F. Yong, D.E. Anderson, W.E. Wei, J. Pang, W.N. Chia, C.W. Tan, Y.L. Teoh, P. Rajendram, M.P.H.S. Toh, C. Poh. (2020). Connecting clusters of COVID-19: an epidemiological and serological investigation. The Lancet infectious diseases. 20(7): 809-815.
[36] A.K. Winter, S.T. Hegde. (2020). The important role of serology for COVID-19 control. The Lancet infectious diseases. 20(7): 758-759.
[37] B. Tang, N.L. Bragazzi, Q. Li, S. Tang, Y. Xiao, J. Wu. (2020). An updated estimation of the risk of

transmission of the novel coronavirus (2019-nCov). Infectious disease modelling. 5: 248-255.

[38] F. Zeng, Y. Huang, Y. Guo, M. Yin, X. Chen, L. Xiao, G. Deng. (2020). Association of inflammatory markers with the severity of COVID-19: A meta-analysis. International Journal of Infectious Diseases. 96: 467-474.

[39] R. Wölfel, V.M. Corman, W. Guggemos, M. Seilmaier, S. Zange, M.A. Müller, D. Niemeyer, T.C. Jones, P. Vollmar, C. Rothe. (2020). Virological assessment of hospitalized patients with COVID-2019. Nature. 581(7809): 465-469.

[40] T. Ai, Z. Yang, H. Hou, C. Zhan, C. Chen, W. Lv, Q. Tao, Z. Sun, L. Xia. (2020). Correlation of chest CT and RT-PCR testing for coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology. 296(2): E32-E40.

[41] Y. Wu, X. Xu, Z. Chen, J. Duan, K. Hashimoto, L. Yang, C. Liu, C. Yang. (2020). Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain, behavior, and immunity. 87: 18-22.