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Review Article

Immune cell interactions and lung damage in SARS-CoV-2 special attention on monocytes/macrophages, T and B lymphocytes, dendritic cells and Toll-like receptors in SARS-CoV-2

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Abstract: Coronavirus disease 2019 (COVID-19) is caused by a novel human virus - SARS-CoV-2, is characterized in severe cases by dysregulated innate and adaptive immune responses leading to lung injury and systemic hyperinflammation. This review summarizes the roles of monocyte/macrophages, T and B lymphocytes, dendritic cells, and Toll-like receptor signaling in the immunopathogenesis of severe COVID-19. Severe COVID-19 is characterized by light neutrophilia, reduced numbers of transitional and non-classical monocytes, lymphopenia and diminished conventional and plasmacytoid dendritic cells (DCs). Inflammatory monocytes/macrophage activation and excessive cytokine production contribute to alveolar damage and cytokine storm. Alveolar macrophages (AMs) are abortively infected by the virus in an ACE2-dependent manner, but still no virus replication has been detected. AMs produce high quantities of IL-6 TNF-α, and IL-1β promoting cytokine storm. T lymphocytes dysregulation is associated with lymphopenia, impaired antiviral responses, and T-cell exhaustion. Severe COVID-19 infection causes a disproportionate decrease of $CD4^+$ T cells, reduced $CD8^+$ T cells and alteration of T cell subsets i.e. Tregs decrease and stimulation of Th1, Th2 and Th17. TLR3 and TLR7 are broadly expressed on human immune cells and recognize dsRNA and ssRNA in the endosome. Since TLR7 duplicated genes are localized on the X-chromosome, the disease is more expressed in men. B cell responses are characterized by impaired germinal center formation and extrafollicular antibody production. B cells produce neutralizing antibodies having little somatic hypermutations (SHM) that block the virus in the extra-follicular space. The activation of B cells via contact with Tfh cells antigen and BCR antigen is described. The reduction of germinal centers in thoracic lymph nodes and spleen is discussed. DCs depletion and dysfunction reduce antigen-presentation and interferon responses. A significant reduction of conventional DCs1 and DCs2 and of pDCs in blood is detected. Simultaneously, $CD1c^+$ DCs are recruited and increased in the lung in severe COVID-19. Toll-like receptor pathways further amplify inflammatory signaling and immune imbalance. Collectively, these immune alterations contribute to defective viral clearance and progressive lung injury. Understanding the interactions between innate and adaptive immune responses in COVID-19 may support the development of targeted immunomodulatory therapies and provide broader insights into viral immunopathology.

Keywords: COVID-19, monocytes/macrophages, T lymphocytes, B lymphocytes, dendritic cells, Toll-like receptors

Introduction

Coronavirus disease 2019 (COVID-19) is caused by a novel human virus - severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. About 5% of cases experienced severe diseases presented by acute respiratory syndrome and systemic hyper-inflammation. In severe disease, increased levels of various cytokines and chemokines constitute a cytokine storm [2]. These cytokines and chemokines trigger recruitment of inflammatory cells such as neutrophils and monocytes, lymphocytes and dendritic cells to lung tissue

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and direct dysregulated immune responses [3, 4].

Coronaviruses are enveloped, non-segmented, positive-sense, single-stranded RNA (ssRNA) viruses with a genome of 26.32 kilobases encoding four structural proteins i.e. spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins, SARS-CoV-2 enter target cells through angiotensin-converting enzyme 2 (ACE2) receptor [5]. Viral entrance is supported also by transmembrane serine protease, serine 2 (TMPRESS2) [6].

The pathophysiology of COVID-19 is mainly attributed to dysfunction of innate and adaptive immune responses by SARS-CoV-2. The uncontrolled immune response leads to delayed viral clearance, inflammation and tissue damage in the lungs. One of the hallmarks of COVID-19 is lymphopenia (decrease of T and B cells) in blood and an increase of aberrant activation and recruitment of myeloid cells [7] that may contribute to immune pathology. Resident alveolar macrophages (AMs) are depleted and replaced by inflammatory monocyte-derived macrophages [8]. SARS-CoV-2 virus evades the innate immune response by suppressing the antiviral type I IFN responses [9]. The various viral variants have different pathogenicity concerning Delta variant have significant mortality risk than preceding variants, and the Omicron variant is less pathogenic than other variants [9]. AMs can retain viruses for long periods following altered antigen-presentation, poor INF responses to subsequent infections and altered cellular organelles [10]. In addition, long covid-19 has served as a multi-organ disease which shows symptoms from many systems such as respiratory, cardiovascular, digestive, neuropsychic etc. [11]. DCs as antigen-presenting cells are a bridge between innate immunity and adaptive immunity. In severe cases some of them are depleted and this causes defective and delayed T cell activation [12, 13]. Many viruses activate the innate immune system via Toll-like receptors (TLRs), which contributes to the elimination of viruses [14]. Since TLRs are very important for SARS-CoV-2 encountering by immune system this review discusses the main TLRs in the pathogenesis of the disease.

Mild COVID-19 has been characterized by focal early interstitial pneumonia, active $CD8^+$/$CD4^+$ T cells and increased Th17 T cells, robust controlled adaptive immune response. Severe COVID-19 is defined by diffuse alveolar damage (DAD), thrombo-inflammation and uncontrolled innate immune response. T cells and B cells are exhausted. There is marked cytokine storm.

In this review, we have summed up some of the recent findings on the innate and adaptive arms of the immune response in severe COVID-19. Lung has a heterogeneous population of monocytes, macrophages and DCs that monitor the interface between external environment and our body [15]. Altered homeostasis of these cell populations has been linked to immune dysregulation. Severe COVID-19 has been characterized by light neutrophilia, lymphopenia, reduced numbers of classical ($CD14^{++}CD16^-$) and transitional monocytes and recruitment of non-classical monocytes ($CD14^+CD16^{++}$) and diminished conventional and plasmacytoid DC numbers in blood with an increase of $CD1c^+$ DCs in lung tissue.

Therefore, an integrative view of the myeloid and lymphoid populations altered in lung tissue in severe COVID-19 is clearly needed.

Monocytes/Macrophages

Since their discovery in 1880s by Nobel Laureate Elie Metchnikoff, and of their ability to engulf foreign entities (phagocytosis) [16]. They are called mononuclear phagocytes (MNF) by van Furth (1960s) [17]. Human lung alveolar macrophages (AMs) have local proliferative capacity, and they can be derived from yolk sac macrophages and fetal liver macrophages [18]. Together with DCs AMs reside in lungs and monitor foreign substances [15]. The immunohistochemical and flowcytometric markers of human mononuclear phagocytes (MNPs) are $CD11c^+$ and $HLA\text{-}DR^+$. Additional markers for identification of AMs are CD206 (mannose receptor), CD163 and CD 169 [19]. Recently, it has been revealed that human AMs, DCs and monocytes have superior function in phagocytosis [20]. AMs internalize and present antigens to naïve T lymphocytes locally, while DCs present antigens in regional lymph nodes and tertiary lymphoid structures [21, 22]. AMs located in the alveoli are maintaining local homeostasis by engulfing the senescent surfactant, produced by alveolocytes type II (ATII) [23]. ATII cells build a proper microenvironment

for AMs in exchange for macrophage scavenging functions, a great example of symbiosis at alveolar level [23]. The development of AMs is realized through ATII-derived granulocyte-macrophage colony stimulating factor (GM CSF). The sources of lung GM-CSF include immune cells i.e. basophils innate lymphocytes type 2 (ILC2s) and non-immune cells, especially ATII [24].

At the initial stages of lung COVID-19 infection the immune response is triggered by recruited and local antigen-presenting cells including macrophages and DCs that release great quantity of pro-inflammatory cytokines TNFα, IL1β, and IL-6 [25].

COVID-19 causes significant alterations to immune cells in the blood. In healthy humans' blood monocytes are three subpopulations including classical ($CD14^{++}CD16^{-}$) that are about 90%; non-classical ($CD14^{+}CD16^{++}$), about (5-10%) and intermediate ($CD14^{++}CD16^{+}$) (1-5%) [26]. The classical monocytes are increased in mild blood disease [20] and intermediate and classical are decreased, because they are recruited to lung tissue in severe COVID-19 [27]. The "classically" activated macrophages are designated M1 type, and "alternatively" activated ones - M2 type [25]. Macrophages in COVID-19 control T-cell priming, modulate Th1/Th2 balance and induce Th17 cell differentiation [26]. Infected macrophages by SARS-CoV-2 virus impair the adaptive immune response in the lung [20, 28]. M1 macrophages cooperate with Th1 cells to produce pro-inflammatory cytokines. M2 macrophages make antigen-presentation to Th2 cells, secrete anti-inflammatory cytokines, clear apoptotic cells [29]. In COVID-19 macrophages via their FcγRs are linked to membrane viral antigens on the infected cells and aggravate immune response [29].

In bronchoalveolar lavage fluid (BALF) pro-inflammatory monocyte-derived macrophages are abundant in severe COVID-19 [27].

Activated classical monocytes ($CD14^{++}CD16^{-}$) show increased expression of HLA-DR (an antigen presentation marker) and CD86 (a co-stimulatory molecule) in mild COVID-19, which are absent in severe disease and in control groups [30]. CD163 marker increased in severe disease [30, 31]. The expression profile of blood intermediate monocytes ($CD14^{++}CD16^{+}$) in COVID-19 patients includes pro-inflammatory ($CD86^{+}$) and anti-inflammatory ($CD163^{+}CD206^{+}$) markers. During acute progression of the disease monocytes differentiate into macrophages still in circulation [32]. These cells begin to produce IL-6, TNF-α, and IL-10, efficiently promoting the cytokine storm [32] (**Figure 1**). The hallmark of severe COVID-19 is decreased levels of HLA-DR and CD68 in circulating monocytes and CD163 positivity [33, 34].

In severe COVID-19, AMs expand alveoli with mixed infiltrate of lymphocytes and hyperplastic ATII [35]. The M1 macrophages induce cytokine release initiated by recognition of disease-associated molecular patterns (DAMPs) from virus-infected cells, or by recognition of pathogen-associated molecular patterns (PAMPs) via toll-like receptors (TLR2, TLR4) [36]. AMs are in alveoli, maintaining alveolar homeostasis. AMs are $CD64^{+}$, HLA-DR^{+} $CD11c^{+}$ and Siglec F^{+} expression. The induction of TLR2 (via S protein), TLR3, TLR7, TLR8 (via viral ssRNA and dsRNA) leads to the release of pro-inflammatory cytokines and IFNs through the induction of transcription factors (TFs) NF-κB, IRF3, and IRF7 [20]. Upon infection, monocytes change their cytokine/chemokine secretion and migrate to alveoli as macrophages and become infected by phagocytosed apoptotic viral infected epithelial cells [37]. Alveolar macrophages (AMs) are abortively infected by the virus in an ACE2-dependent manner, but no virus replication has been detected by TEM [37] (**Figure 1**). Authors show that levels of intracellular viral RNA and protein were very low in AMs and find no evidence that DCs, T cells, B cells, or NK cells were infected by virus [37]. According to some authors, although macrophages have numerous anti-microbial activities, they may favor preferential bacterial replication [28], the so called "Trojan horse" phenomenon that can direct cell-to-cell contact allowing viral spread through all tissues [38]. It has been reported that monocytes-macrophages can disseminate some viruses such as influenza, Chikungunya, Zika and human herpes viruses [38].

The term "cytokine storm" has been historically described as an influenza-like syndrome associated with systemic infections and immune therapies [36]. The concentrations of serum TNF-α, IL-6 and IL-8 are less strong compared

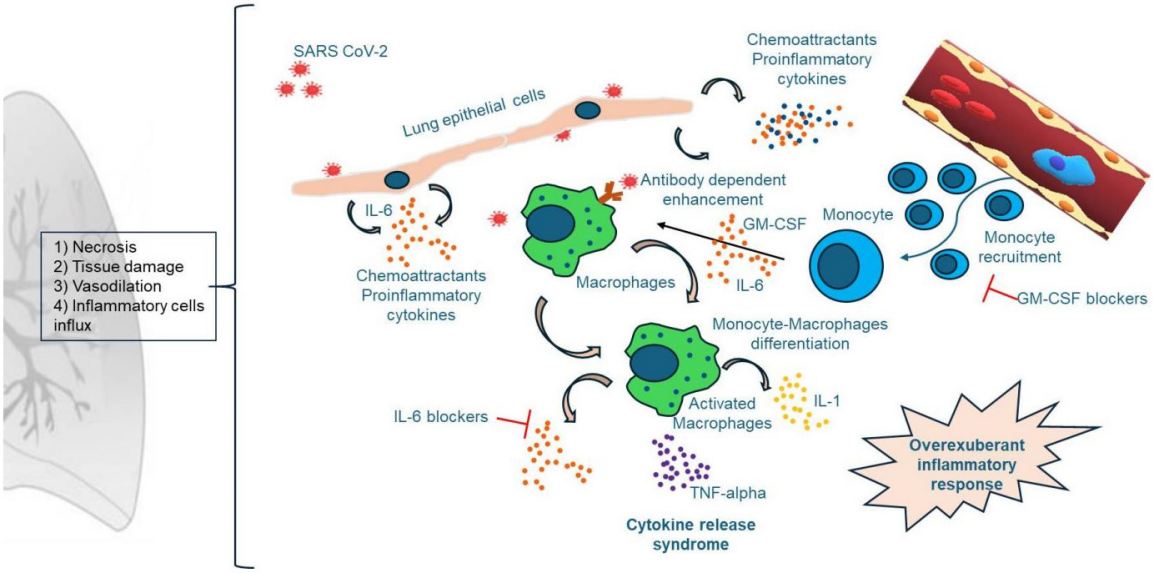

Figure 1. Role of monocytes/macrophages in COVID-19 pathogenesis: 1) SARS-CoV-2 infection induces release of pro-inflammatory cytokines such as IL-6 in the lung; 2) Antibody-dependent enhancement induces macrophage activation and differentiation and release of IL-1, TNF-α, IL-6 GM-CSF; 3) Recruitment of monocytes from peripheral blood; 4) Overexuberant inflammatory response - cytokine storm. The schema is adapted after Gomez-Rial et al., 2020, [40].

to other pathologies such as trauma, cardiac arrest [37, 39]. In alveolar space of COVID-19 pneumonia there is persistent enrichment of AMs monocytes and T cells [37].

T lymphocytes

Virus attaches to and invades cells expressing its receptor to replicate (for example, ATII cells). Viral peptides are presented to $CD8^+$ CTLs. $CD8^+$ T cells become activated and start to divide, show clonal expansion and develop virus-specific effector and memory T cells [10]. Antigen-presenting cells such as DCs and macrophages present viral peptides to $CD4^+$ T cells via MHC II molecules [10].

The adaptive immune response begins with fine recognition of the virus through TCR of CD8 and T helper cells [41]. SARS-CoV-2 infection has been associated with significant lymphopenia in blood [5], and the lymphocyte infiltrate is mild to moderate in alveolar septa. In the acute phase of SARS-CoV-2 in humans, 80% of symptomatic patients show a dramatic loss of $CD4^+$ T cells and of $CD8^+$ T cells [42]. Severe SARS-CoV-2 infection causes a disproportionate decrease of $CD4^+$ T cells and alteration of T cell subsets i.e. Tregs decrease and stimulation of Th1, Th2 and Th17 and elaboration of pro-inflammatory cytokines occurs [5]. In peripheral blood some authors find a significant reduction in the levels of $CD3^+$, $CD4^+$, $CD8^+$ T cells in moderate and severe infection [42]. In BALF $CD8^+$ T cells reduction has been shown in severe COVID-19 patients [43].

The abnormal and excessive immune response to SARS-CoV-2 infection partly depends on T cell immunological memory [44]. COVID-19 pathogenesis is dependent on an aberrant host immune responses i.e. overactive T cells that cannot neutralize the virus and cross-reactive memory B cells and naïve B cells that initiate production of neutralizing antibodies against SARS-CoV-2 Spike protein having little somatic hypermutation (SHM) [4].

$CD8^+$ T cells directly kill infected cells via CTL granules. Severe SARS-CoV-2 infection may preferentially impair $CD8^+$ T cells especially from the effector memory population characterized with lack of CD45RA and CCR7 expression [45]. The reduction of $CD8^+$ T cells could be due to increased migration of T cells to the site of infection or to apoptosis. Another reason for diminished $CD8^+$ T cell levels might be defects in type 1 immune responses (Th1) and skewing towards Th2 memory cells leading to delayed viral clearance. Alteration in type I IFN

signaling, decline of antigen presenting potential of APCs, and bystander activated CTLs contribute also to impaired $CD8^+$ T cells function in severe COVID-19 [45]. The bystander effect of $CD4^+$ T cells and $CD8^+$ T cells is an induction of T cell proliferation via cytokines IL-18 and IL-15, instead of TCR antigen triggering [46]. For instance, bystander $CD8^+$ T cells rapidly produce IFN-γ, but are not specifically pathogen-specific, however bystander memory $CD4^+$ T cells produce IL-17A in the absence of TCR engagement in COVID-19 [44, 45, 47].

In contrast to their activation, there is a significant T cell exhaustion in COVID-19. T lymphocytes failed to produce Th1 cytokines (IFN-γ, IL-2 and lymphotoxin), to accumulate cytotoxic granules and express NKG2A inhibitory receptor [48]. T cell persistent stimulation induces both $CD4^+$ and $CD8^+$ exhaustion, confirmed by the expression of three exhaustion markers i.e. PD-1, mucin domain-containing protein-3 (TIM-3), and ITIM-bearing receptor NKG2A [44]. Exhausted T cells in parallel show decreased expression of co-stimulatory molecule CD28 [48].

There is data of SARS-CoV-2 infected mainly $CD4^+$ T lymphocytes via LFA-1 adhesion molecule [44, 49]. $CD4^+$ T cells, responsible for IFN-γ production, are generally inhibited. Upon acute viral infection, virus-specific $CD4^+$ T cells commonly differentiate into Th1 cells producing IFN-γ, TNF-α and IL-2 and related cytokines [4]. $CD4^+$ T cells also differentiate into Tfh cells that help B cells to develop neutralizing antibodies against viral S protein [50]. Virus-specific $CD4^+$ T cells produce limited proportions of Th2, Th17 and Th9 in the context of type 1-like inflammation. Th1 effector cells help CTL and B cells and drive B cell proliferation. In COVID-19 Tregs show diminished numbers in blood and tissues and limit the host antiviral responses. In severe COVID-19, Th2 immune responses can prevail over Th1 ones. For example, in the mucosa of airways, in COVID-19 there are evidence of high levels of basophil and mast cell degranulation and increased expression of Th2 cytokines such as IL-4 and IL-13 [51]. Th17 cells increase in severe COVID-19 and stimulate many signaling pathways leading to the production of many cytokines like IL-6, IL-1β, TNF-α, G-CSF, GM-CSF and TGF-β [52]. Therefore, the T helper cell lymphocyte subsets that predominate in lung tissue in COVID-19 are Th1, Th2, and Th17 [44] (**Figure 2**). $CD4^+$ CTLs bear cytotoxic activity like $CD8^+$ CTLs and can directly kill MHC-II-expressing infected cells such as lung endothelial and epithelial cells infected with COVID-19 [53]. Mechanisms leading to peripheral lymphopenia and severe COVID-19 remain unclear. There is hypothesis that SARS-CoV-2 can destroy splenic and lymph node follicles [54]. Another opinion is that activated lymphocytes can be sequestered in the injured lung [54].

B lymphocytes

Through $CD4^+$ T helper cells (Tfh) results in B cell activation and production of antibodies [55, 56]. Naïve B cells rather than cross-reactive memory B cells produce neutralizing antibodies in COVID-19 [57]. Neutralizing antibodies block viral infection and alveolar macrophages, recognize neutralized viruses and apoptotic cells and clear them by phagocytosis [58].

B-2 cells are produced in bone marrow and egress in blood to spleen and lymph nodes (LNs) [58]. Naïve follicular B cells encounter their cognate antigen in lymph node follicular dendritic cells (LN FDCs) and migrate to the light zone, where they contact Tfh cells' TCR to encounter the antigen delivered by DCs. This interaction is supported also by CD40 on B cells and CD40L on T cells. Later activated B cells migrate to the dark zone of germinal center (GC), where B cells accrue productive mutations within their BCR, which captures and presents antigen to T cells upon their return in light zone. There B cells differentiate into memory B cells and Plasma cells [59].

The initial diversity of the BCR repertoire is a result of somatic recombination process called V(D)J recombination [61]. After encountering antigen, B cells undergo a process of affinity maturation and of rapid somatic hypermutation (SHM) and class switch recombination (CSR) in the GCs [62, 63] (**Figure 3**).

In severe COVID-19 infection there is an impaired GC B cell response and robust extrafollicular response [59]. Compared to other APCs, antigens are detected more sensitively by the BCR. B cells activate, especially $CD4^+$ T cells and mediate the $CD4^+$ T cells APCs, $CD8^+$ T cells

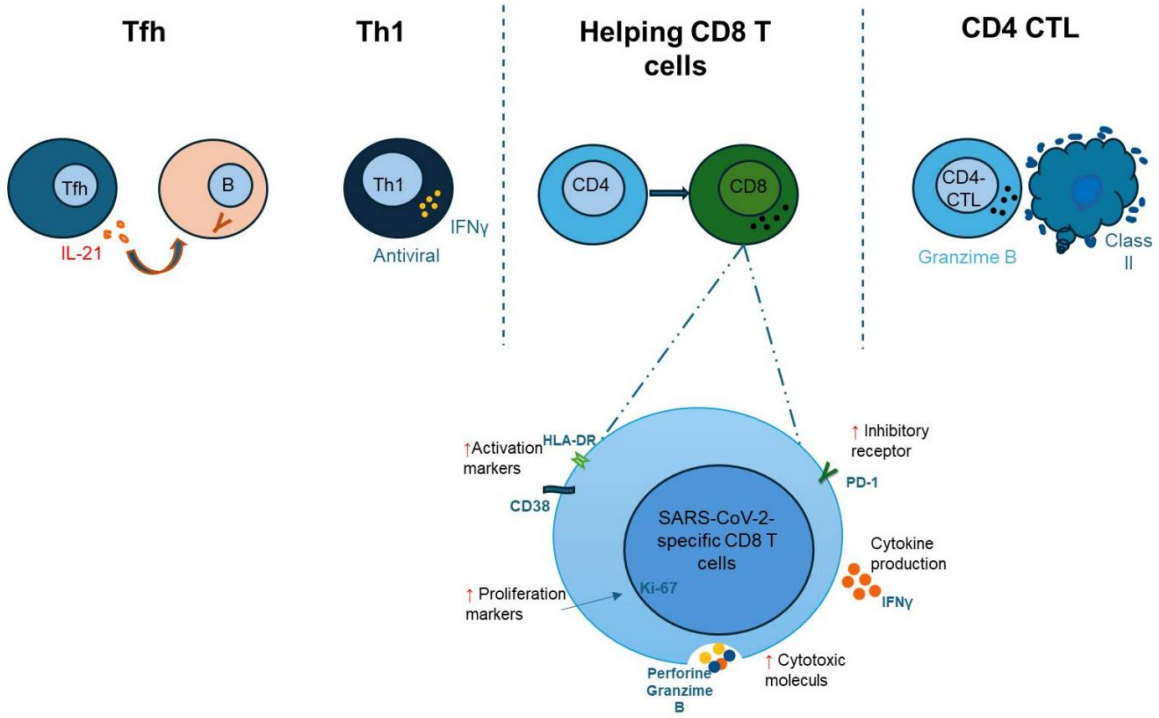

Figure 2. CD4⁺ T helper cell function in COVID-19. Tfh cells provide help to B cells via IL-21 for affinity maturation and antibody production. Th1 cells have direct antiviral functions via cytokine secretion IFN-γ and recruitment of innate immune cells. CD8⁺ T cells express activation markers (CD38 and HLA-DR), a proliferation marker (Ki-67), and cytotoxic molecules (perforin and granzyme B), and checkpoint inhibitory receptors (PD-1 and TIM-3). The schema is adapted after Sette and Crotty, 2021 [4] and Jung and Shin, 2021 [47].

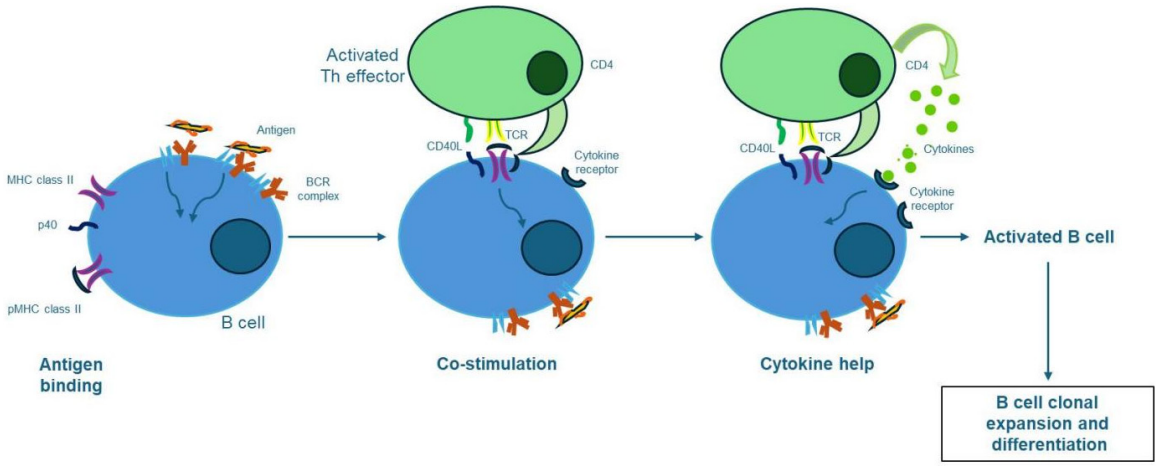

Figure 3. B cell activation. The activation of resting mature naïve B cells occurs in three steps namely antigen-binding, costimulation and cytokine help. 1) Signal 1 is delivered when multiples molecules of antigen bind to multiple surface BCR complexes (peptide-MHCII complex) that can engage TCR of activated Tfh cells. Binding of CD40L on Tfh cells to CD40 on B cells delives co-stimulatory signal; 2) Signal 2 cytokine receptor expression; 3) Signal 3 Tfh cytokines bind to their receptors on B cells. The schema is adapted after Proverb, 2014 [60].

to differentiate into different functional subsets [64]. B cells enhance the ability to present antigens by DCs and macrophages [65]. B cells can exist in the extra-follicular tissue and at the site of inflammation [66]. Some B cells follow activation traditionally in germinal centers in mild COVID-19 leading to appearance of short-living plasma cells that produce ear-

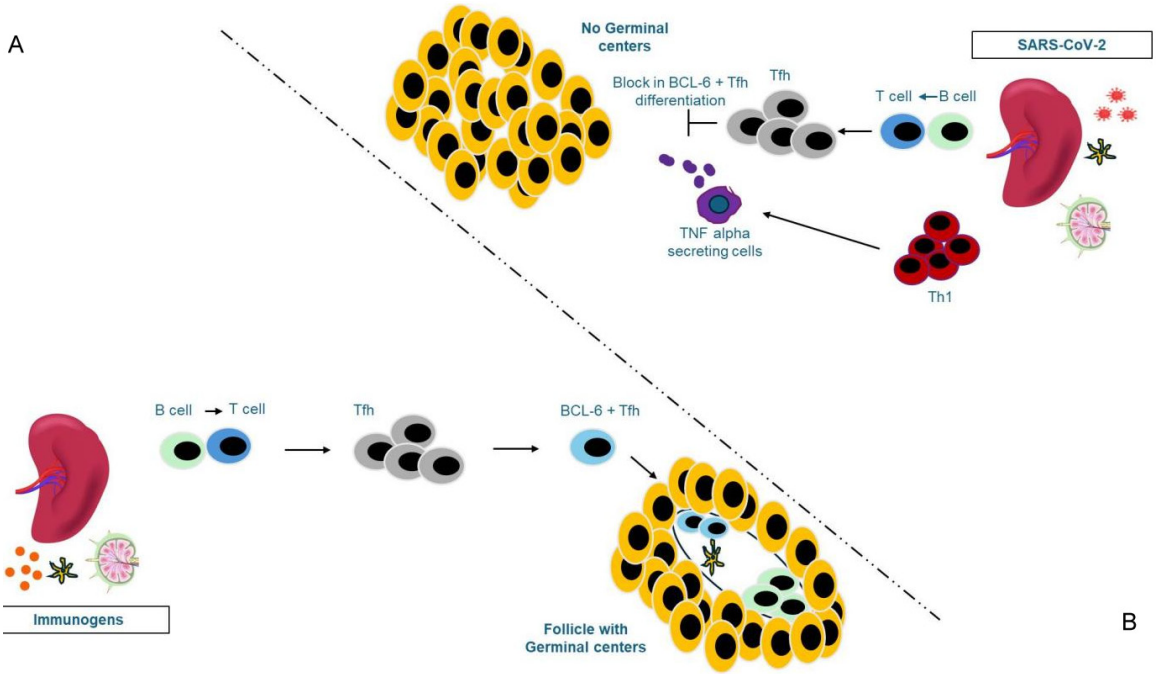

Figure 4. Loss of Bcl6$^+$ Tfh cells and germinal centers in COVID-19. A. When other immunogens contact with antigen-presenting cells (APCs) in spleen and lymph nodes we have Tfh cells in contact with B cells and germinal center formation; B. When SARS-CoV-2 infection occurs germinal centers are lost in lymph nodes and spleen; Bcl6$^+$ GC B cells and Bcl6$^+$ Tfh cells are markedly diminished; Abundant Th1 cells and aberrant TNF-α production occurs. The schema is adapted after Kaneko et al., 2014 [53].

ly low-affinity antibodies (IgM/IgG) (**Figure 3**). In severe COVID-19 B cells undergo non-classical pathway of activation in the extrafollicular zone. Overtime, recovery or vaccination leads to the formation of robust, stable B memory cells that recognize SARS-CoV-2 spike protein [66].

Several studies analyzed the morphological changes in pulmonary LNs in COVID-19 patients, collected during autopsy [67]. Using multicolor histological assessment of post-mortem thoracic LNs and spleens, impaired organization of B cell zones has been observed by reduction of the volume of GCs or their complete absence [53, 68]. A significant decrease of GC cells such as FDCs, Bcl6$^+$ Tfh cells and B lymphocytes has been detected [67]. Kaneko et al. have reported that severe SARS-CoV-2 infection blunts the GC response that dampens the long-lived antibody response [53] (**Figure 4**). GCs are formed after antigen-activated B cells that receive help from Tfh cells. Within GCs, B cells undergo clonal expansion and affinity maturation, receive further help from Tfh cells and differentiate into memory B cells and plasma cells. The lack of GCs is associated by the absence of Bcl6$^+$ B cells and Tfh cells [53]. The analysis shows increased Th1 (T-bet$^+$) T cells and increase in TNF-α content. Notably, authors find preserved AID$^+$ B cells, that indicate that activated Th cells are still in contact with antigen-specific B cells [53, 68, 69]. Kaneko et al. have found that in the absence of GCs in COVID-19, the extra-follicular zones support class-switched antibody B cell response [53].

Dendritic cells

DCs are sentinel leukocytes that orchestrate innate and adaptive immune responses. DCs are discovered by Ralf Steinman in 1973 [70]. The major populations of DCs identified are conventional DCs (type 1 - cDC1s and type 2 - cDC2s), plasmacytoid DCs (pDCs) [71], Langerhans cells (LCs) in the skin, and monocyte-derived DCs (mo-DCs) [72, 73]. In peripheral blood some authors find a significant reduction in the levels of cDC1, DC3 and cDC2 cells in COVID-19 infection [83]. In BALF immature DCs, cDCs, active DCs, pDCs are reduced [74].

DCs are professional antigen-presenting cells (APCs) together with macrophages and B lym-

phocytes [12, 20, 75]. Upon stimulation, phagocytosis of PAMPs and DAMPs were released after pyroptosis from epithelial and endothelial cells after viral infection [76]. DCs undergo maturation and migrate to secondary lymphoid organs to activate and shape T cell polarization and differentiation [2, 77].

In humans, myeloid cDC1 CD141^{hi} DCs (BDCA-3^{+} DCs) represent the functional homolog of murine cDC1s cells (CD8α^{+} and CD103^{+} DCs). Human myeloid cDC1s are approximately one-tenth the frequency of cDC2s in steady state blood and tissues [73, 78]. Other markers should be used for cDC1s identification such as CLEC9A, CADM1 (NECLR), XCR1. The cDC1s lack expression of cDC2s and mo-DCs markers. They show enhanced cross-presentation to CD8^{+} T cells, polarize Th1 and Th2 response and secret IL12p70 in response to TLR3 stimulation [73, 79, 80].

Myeloid or conventional cDC2s express CD1c, FcεR1, SIRPA, and the myeloid antigens CD11c, CLEC10A (CD301a) [80]. The cDC1c^{+} DCs are the human homolog of CD11b^{+} murine cDC2s. Human cDC1c^{+} DCs prime CD4^{+} T cells but also can polarize them towards a Th1 subset and secrete IL-12 [80]. Human cDC2s are excellent antigen-presenting cells in bacterial, viral and fungal infections [79].

Human pDCs are found mainly in blood and secrete high amounts of IFN-α and type I IFN. They are mainly CD123^{+} (IL-3Rα), CD303^{+} (BDCA-2^{+}) and CD304^{+} (BDCA-4^{+}) and have restricted potential to prime T cells [79].

The newest DC type, DC3, has been described in human blood and express genes from both cDC2s and monocytes and express CD1c and CD163 markers [80].

Using multicolor flow-cytometry has been determined to have a significant reduction of CD123^{hi} pDCs and CD141^{+} DCs. Dramatic decrease of CD1c^{+} DCs and of classical Mo and non-classical Mo was more pronounced in severe COVID-19 in blood and are associated with increased inflammatory markers such as PCT, CRP and weakly with IL-6 levels. Simultaneously, CD1c^{+} DCs are recruited and increased in lung tissue in severe COVID-19 [3]. Subsequently, human cDC2s are capable of cross-presentation of viral antigens to CD8^{+} T cells quite efficiently [81, 82] (**Figure 5**). Thus, given their PRR repertoire and their cytokine secretion profile CD1c^{+} DCs behave as excellent APCs in SARS-CoV-2 infection, as in other bacterial, fungal and viral infections [71, 79]. Moreover, cDC2s can efficiently prime Tfh cells and can efficiently polarize Th2 and Th17 responses [79].

In COVID-19 the number of DCs, their ability to secrete anti-viral cytokines (IFN type I) and their capability of antigen-presentation decline unexpectedly in severe disease [83-85]. It is known that SARS-CoV-2 enters DCs and macrophages via DC-SIGN and furin rather than ACE2 and TMPRSS2 [85]. This is a PRR/adhesion receptor capturing antigens and presenting them to CD4^{+} T cells to trigger immune response [86]. DC-SIGN carries SARS-CoV-2 antigen in tissues - a Trojan horse effect [87] like other viruses (e.g. HIV, Ebola, dengue, cytomegalovirus) and other pathogens (e.g. Leishmenia species, Candida albicans, Mycobacterium tuberculosis, Aspergilus fumigatus, Streptococcus pneumonie [76]. There are three mechanisms that lead to the decline in the number of DCs in COVID-19. First, there is altered distribution in DCs in lungs: a decline of cDC1s and pDCs [3] and increased number of cDC2s migrating from blood to lung tissue [85]. Moreover, DC apoptosis is increased during SARS-CoV-2 via TNF-related mechanism [88]. In addition, MDSCs inhibit immature DCs to differentiate into mature ones [89]. Second, the expression of co-stimulatory molecules (CD80, CD86) and HLA-DR decreased after stimulation with cytokines (IL-6, TNF-α, IL-1β, and PGE2), [104] inhibiting of mTOR and Wnt5a oncogene expression [85]. Third, type I IFN reduced significantly in corona virus infections and there is a congenital deletion of TLR7 in critically ill patients [14].

Toll-like receptors (TLR) signaling

The innate immune system recognizes microorganisms and responds to them via TLR recognition. Activation of TLRs triggers intracellular pathways that support the release of inflammatory cytokines and chemokines. TLR3, -7, -8, AND-9 are in the endosome membranes and recognize nucleic acids from SARS-CoV-2 RNA [90]. Six human TLRs have altered expression in COVID-19 patients. These are homodi-

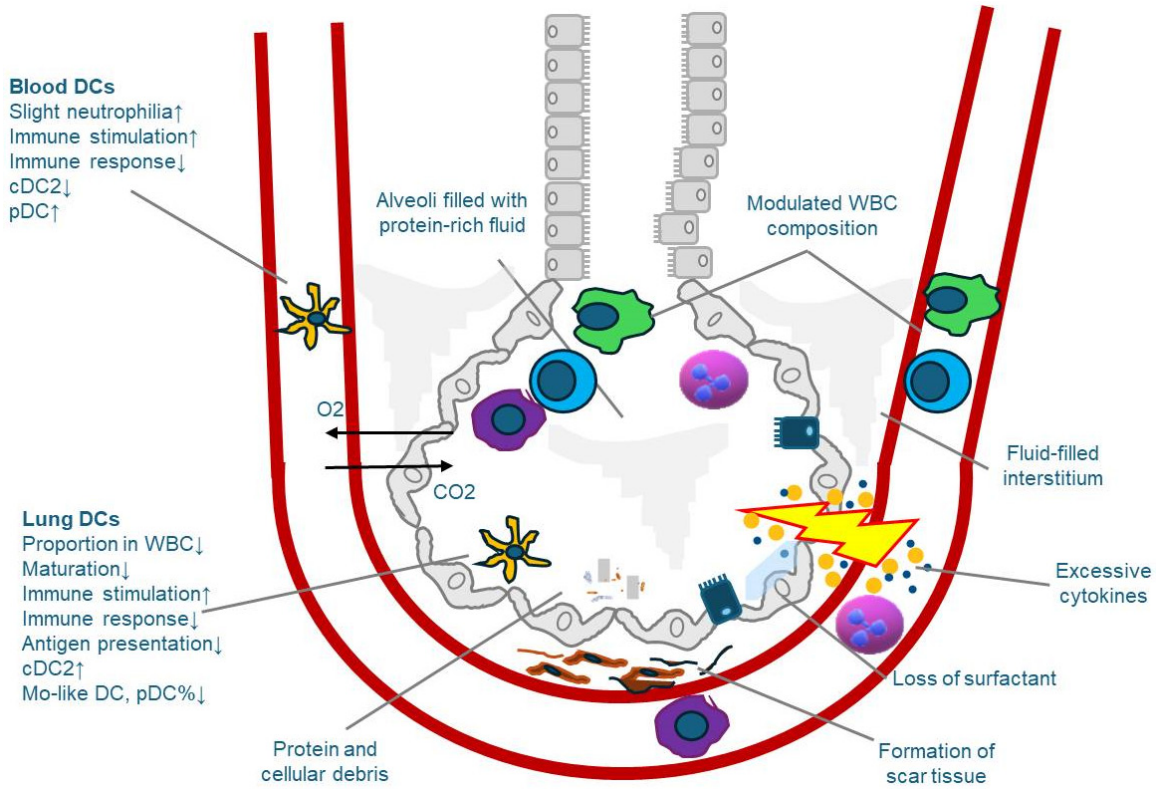

Figure 5. Severe COVID-19 in alveoli and cDC2 CD1c$^+$ dendritic cells. SARS-CoV-2 modulates DCs proportions and DC-SIGN expression in peripheral blood and in the lung. In severe infection DCs are less capable of antigen presentation, MHCII expression and maturation. In the lung cDC2 CD1c$^+$ prevailed, while other DC subsets decreased, and mainly immature subsets were recruited.

mers (TLR3, TLR4, TLR7, TLR8, and TLR9) or homodimers (TLR2/1 and TLR2/6). Some of them are located at the cell surface (TLR2 and TLR4), and others (TLR3, TLR4, TLR7, TLR8, and TLR9) are found in the endosomes [91] (**Figure 6**). Some pattern recognition receptors (PRRs) are secreted in the cytosol including NOD-like receptors (NLRs), RIG-like receptors (RLRs), and increased level of cytosolic nucleic acid sensors [92].

The TLRs are activated by PAMPs or DAMPs produced by SARS-CoV-2 infection [91]. Activation of TLRs triggers a cascade of molecular interactions between factors of signaling pathways, which lead to the activation of pro-inflammatory gene expression and the initiation of innate immunity [93]. After ligand-induced dimerization, the cytoplasmic TIR domain associates with TIR domain-containing adaptor molecules to transmit signaling. The adaptor proteins transmitting signals in COVID-19 are myeloid differentiation primary- esponse gene 88 (MyD88) and TIR-domain-containing adaptor protein inducing IFN-β (TRIF) [34]. All TLRs, except TLR3, are associated with MyD88 proteins. Nevertheless, TLR3 and TLR4 use a TRIF-dependent pathway. Both pathways lead to the activation of downstream molecules: nuclear factor-κB (NFκB), activating protein-1 (AP-1), and members of the IFN-regulatory factor (IRF) family [94]. TF NF-κB is the main regulator of TLR responses and triggers inflammatory cytokines TNF-α and IL-6 release [95].

TLRs and COVID-19

In the pathogenesis of COVID-19 several TLRs i.e. TLR2, TLR3, TLR4, TLR7, TLR8 and TLR9 take place. These TLRs are expressed mainly on immune cells such as T and B, DCs, Mono-Macros, NK cells and T and B cells [14].

TLR4 is innate immune sensor that has been strongly upregulated in lungs in severe COVID-19. TLR4 recognizes SARS-CoV-2 spike (S) (S1 Subunit) protein since it is localized on the cellular membrane, acting as agonist, driving

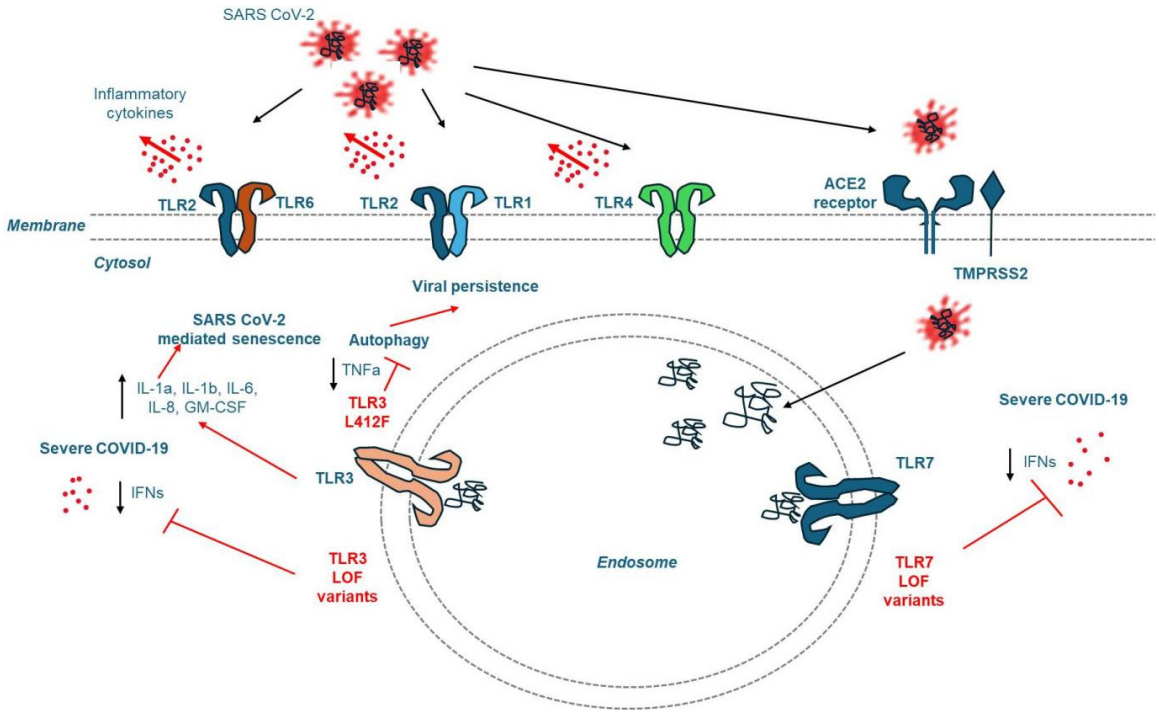

Figure 6. TLRs and the pathogenesis of COVID-19. Several TLRs such as TLR2, TLR3, TLR4 and TLR7 and heterodimers TLR2/TLR1 and TLR2/TLR6 have been associated with the pathogenesis of COVID-19. The TLR2/TLR6, TLR2/TLR1, TLR2 and TLR4 are located at cellular surface of innate immune cells and on some other cells such as lung epithelial cells, endothelial cells. LOF TLR3 and L412F polymorphisms and TLR7 LOF variants mainly in men, negatively regulate anti-SARS-CoV-2 immune responses, induce cytokine storm, reduce IFNs and TNF-α. TLR3 and TLR7 are in the endosome and sense ssRNA and dsRNA. The schema is adapted after Mantovani et al., 2023, [34].

hyperinflammation and leads to NETosis and activation of inflammasome [96]. TLR4 mRNA is expressed only in myeloid cells (Mono-Macro, DCs) and is undetectable in lymphoid cells. TLR4 is attached to alveolar and bronchial epithelial cells. SARS-CoV-2 interacts most highly with TLR4 compared to other TLRs [41]. NLRs in the cytosol of immune cells (Macro-Mono) trigger the inflammasome [55, 97] where there is activation of procaspase-I into caspase-I that leads to activation of IL-1β (the main activator of IL-6 that aggravates the cytokine storm [41]. TLR4-antagonists like metformin or glycyrrhizin reduce severe inflammation [97].

TLR3 is broadly expressed in human immune cells as mDCs, NK cells, effector $CD8^+$ T cells, and non-immune cells like intestinal epithelial cells, lung and dermal fibroblasts and recognizes viral dsRNA [32]. It recognizes viral dsRNA and uses TRIF-dependent pathway and leads to the activation of downstream molecules such as NF-κB that induce cytokine release [95]. TLR3 is critical innate immune sensor for SARS-CoV-2 virus. When properly activated it supports protection, but when dysregulated it leads to hyperinflammation. In peripheral blood lower TLR3 expression is associated with severe COVID-19, whereas in enhanced expression of TLR4 often compensates, causes inflammatory cytokine storm. Rare loss-of-function (LOF) mutations in TLR3 have been identified in patients with severe COVID-19 leading to impaired type 1 IFN production. TLR3 agonists (Poly I:C) usually boost innate immunity [98].

TLR7 in COVID-19 are tandem duplicated genes on the X-chromosome, located in the endosome membrane that detect ssRNA and synthetic oligoribonucleotides, such as Imidazoquinolne, Imiquimod, and R-848 [14]. Its activation leads to the production of IL-1, IL-6, MCP-1, MIP-1A, TNF-α, and Type I IFN [63]. TLR7 can trigger NET formation in COVID-19 patients [99]. The activation of TLR7/8 induces strong inflammatory response in patients and induces lung injury [14]. TLR7/8 induces release of IL-6 that inactivates cell-mediated antiviral response, by inhibiting CTLs [41]. TLR7 is lo-

calized in the membranes of endosomes and lysosomes of immune cells. It encounters the ssRNA of viruses. TLR7 is expressed on monocytes, macrophages, pDCs, and B cells, where it triggers high type 1 IFN production [14]. TLR7 receptor gene is located on the X chromosome in humans, specifically at Xp 22.2 near the TLR8 gene. TLR7 particularly escapes X-inactivation and is highly expressed in females. Because TLR7 gene is on the X chromosome, men (XY) are more susceptible to the impact of a single mutated copy of LOF mutations than women. Rare deletions of TLR7 variants exist in up to 2% of males under 60 years old in severe COVID-19 [100].

TLR8 genes are located on the endosomal membrane together with TLR7 in X chromosome. TLR8 is in monocytes, macrophages, mDCs and neutrophils. Lung and secondary lymphoid organs (spleen and lymph nodes) have an upregulated expression of TLR8. TLR8 is marker of severity in critical COVID-19 in peripheral blood mononuclear cells (PBMCs). Males exhibit significantly higher TLR8 expression compared to women. TLR8 mediates harmful hyperinflammation [14].

TLR9 receptors are abundantly expressed on lung epithelial cells, nasal mucosa and immune cells. TLR9 is primarily located on the endosomal membranes of immune cells (pDCs, B lymphocytes and neutrophiles), in fibroblasts and pneumocytes type II. TLR9 is a sensor of unmethylated CpG DNA motifs. In COVID-19 TLR9 recognizes mitochondrial DNA (mtDNA) released by host cells damaged by SARS-CoV-2 virus. In lungs TLR9 is highly expressed in lung epithelial cells and neutrophils in severe pneumonia [101].

The immune system is mainly targeted by different SARS-CoV-2 variants, by vaccines themselves and is dysregulated in long COVID-19.

The purpose of vaccines against SARS-CoV-2 have been to strengthen an effective immune response, both cellular and humoral. The existing vaccines are two main types. The vaccines developed by Pfizer and Moderna use mRNA technology and lipid nanoparticle (LNP) delivery system. The formulations by AstraZeneka, Johnson and Johnson and Gam-COVID-vac (Sputnik V) contain DNA delivered with non-replicating recombinant adenovirus (AdV) vector system [102, 103]. Both vaccines encode production of the SARS-CoV-2 spike (S) protein, which is the target of neutralizing antibodies for several months post vaccination [102]. The information coming from clinical trials show that both the Pfizer/BioNTech (BNT162b2) and Moderna (mRNA-1273) mRNA vaccines achieved 90-95% efficacy in the protection against COVID-19. The AdV vaccines (ChAdOx1 nCoV-19) and Gam-COVID-vac (Sputnik V) show protection at a slightly lower efficacy about 70% and 91%, respectively. Both vaccines generate significant neutralizing antibody titres and virus-specific T cell responses. DCs producing type I IFN that have taken the vaccine-derived nucleic acids encoding S protein deliver signal to T cells in LNs and mobilize adaptive immunity $CD4^+$ and $CD8^+$ T cells, and Tfh^+ cells promoting B cell differentiation [102].

Vaccines against SARS-CoV-2 are associated with diminished disease severity, but its effect is reduced over time. Despite the decreased vaccine effectiveness, the risk of severe disease remains dramatically lower in vaccinated adults even of the emergence of new variants [104].

Alpha and Delta variants of the SARS-CoV-2 coronavirus were highly responsive to original COVID-19 vaccines, retaining significant antibody protection. In contrast, the heavily mutated Omicron variant caused widespread immune escape. However, cellular immunity (T cells) generated by vaccines remained robust across all three variants. Omicron is the first VOC that is less virulent [105, 106].

Post-acute Sequelae of SARS-CoV-2 Infection (PASC) or Post-COVID Syndrome are long term consequences after COVID-19 disease formulated by World Health Organization (WHO) [107]. From 2022, over 200 symptoms and at least 50 clinical conditions persisted for more than three months, which determine long COVID (LC), have been identified [108]. There were found long-term fatigue, dyspnea, memory difficulties, and concentration challenges occurring after SARS-CoV-2 infection. Several key pathogenetic mechanisms and associated risk factors for LC, such as persistent virus and SARS-CoV-2 antigen presence, widespread autoimmunity (long-term presence of antinuclear-ANA and antineutrophil-ANCA antibodies), reactivation of latent viruses, mast cell activa-

tion, chronic low-grade systemic inflammation, disruption of the intestinal microbiome, neurological complaints, metabolic dysfunction [109]. Five early symptoms including dyspnea, prior psychiatric disorders, and specific biomarkers such as D-dimer, CRP, and lymphocyte count [110] are announced. Low-grade inflammation, in metabolic syndrome and type 2 diabetes, is discussed as a major risk factor for severe forms and critical complications of COVID-19 [111]. Prolonged COVID-19 infection increases insulin resistance, destroys pancreatic endocrine cells, and promotes chronic para-inflammatory phenomena in type 2 diabetes [112].

Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, is characterized in severe cases by dysregulated immune responses, vaccines that stimulate both cellular and humoral immune responses, and appearance of long COVID-19 in many patients suffered from the disease.

In conclusion we may state, that in COVID-19 there existed an exuberant and dysregulated innate immune response with decreased monocytes in blood and increased interstitial and alveolar macrophages in lung tissue, and with decreased cDC1s and DCs and increased cDC2s there. The adaptive immune response is poor and is presented with lymphopenia. Such knowledge is crucial to develop targeted therapies in management of COVID-19 infection.

Conclusions

Exuberant and dysregulated innate immune response with classical monocytes increased in mild disease in blood and intermediate and non-classical decreased, because they are recruited to lung tissue in severe COVID-19. SARS-CoV-2 infection has been associated with significant lymphopenia in blood presented with dramatic loss of $CD4^+$ T cells and of $CD8^+$ T cells. There is a significant T cell exhaustion in COVID-19. SARS-CoV-2 infected mainly $CD4^+$ T lymphocytes via LFA-1 adhesion molecule. The T helper cell lymphocyte subsets that predominate in lung tissue in COVID-19 are Th1, Th2, and Th17. Naïve B cells rather cross-reactive memory B cells produce neutralizing antibodies in COVID-19. A significant decrease of GC cells such as FDCs, $Bcl6^+$ Tfh cells and B lymphocytes has been detected. The lack of GCs is associated by the absence of $Bcl6^+$ B cells and Tfh cells. In severe COVID-19 B cells undergo non-classical pathway of activation in the extrafollicular zone. It is known that SARS-CoV-2 enters DCs and macrophages via DC-SIGN and furin rather than ACE2 and TMPRSS2. There is altered distribution in DCs in lungs: a decline of cDC1s and pDCs and increased number of cDC2s migrating from blood to lung tissue. TLR4 is innate immune sensor in myeloid cells and recognizes SARS-CoV-2 spike (S) (S1 Subunit) protein. TLR3 recognizes viral dsRNA, TLR7 receptor gene is located on the X chromosome in humans, specifically at Xp 22.2 near the TLR8 gene, and detects ssRNA, while TLR9 recognizes mitochondrial DNA (mtDNA). The understanding of TLRs and their role in neutralizing virus is main in the management of SARS-CoV-2 viral infection.

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Disclosure of conflict of interest

None.

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