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Review Article

Phenotypical and Functional Status of Natural Killer (NK) Cells in COVID-19

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Abstract

COVID-19 pandemic which is caused by SARS-CoV-2 is a world-wide emergency. Scientists, researchers are totally involved to find out a solution for this highly contagious disease. Understanding the immunopathology in COVID-19 is of utmost importance. A number of recent data shows that the dysregulated immune response and its inflammatory component as the main cause of morbidity and mortality in COVID-19. Natural Killer (NK) cells function is critical in the first-line of defence against viral infection. Deficiencies in the NK cell compartment shows increased susceptibility to certain viral infections in humans. This review summarizes the phenotypical changes as well as functional status of Natural Killer (NK) cells, a critical component of early antiviral immunity, in COVID-19. Most of the studies reveal that the number of NK cells is significantly decreased along with downregulation of CD16 in severe COVID-19 patients but in case of moderate or mild COVID-19 patients, the frequency of NK cells is increased.

Keywords: *Natural Killer Cells; CD16; SARS-CoV-2; COVID-19*

Abbreviations

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2; COVID-19: Coronavirus Disease 2019; HCoVs: Human Coronaviruses; MERS: Middle East Respiratory Syndrome Coronavirus; WHO: World Health Organization; NK Cells: Natural Killer Cells; NKR: Natural Killer Cell Receptor; Ksp37: Killer-specific Secretory Protein 37; DC: Dendritic Cell; TIGIT: T cell Immunoreceptor with Ig and ITIM domains; IFN-γ: Interferon-γ; MHC: Major Histocompatibility Complex; ISG15: Interferon Stimulated Gene15

Introduction

COVID-19 has spread rapidly all over the world and is one of the hardest challenges that modern science is facing today. The coronavirus infection mainly induces severe respiratory illnesses. Many strategies have been adopted to gain control over the rapid spread of this viral disease [1]. This unexpected pandemic, caused by a coronavirus SARS-CoV-2, has affected the population throughout the globe and has raised the demand to develop either an anti- COVID-19 therapeutic drug or vaccine or any other immunotherapeutic approach [2]. Worldwide there are 3,24,29,965 confirmed cases and 9,85,823 deaths are recorded till 26th September, 2020 [3].

Recent data suggested that T lymphocyte and inflammatory cytokines in the peripheral blood are correlated with the severity of COVID-19 [4,5]. The significance of lymphocyte subsets in peripheral blood and in the diagnosis and prognosis of COVID-19 are still an area

of vast research. A number of recent data shows that the dysregulated immune response and its inflammatory component as the main cause of morbidity and mortality [6]. NK cells are the innate immune cells whose unique ability is to rapidly destroy virally infected cells and tumors without prior sensitization [7]. NK cell deficiencies in human body causes increased susceptibility to certain viral infections [8-10]. This review describes the role of Natural Killer (NK) cells, a critical component of early antiviral immunity, in COVID-19.

Natural killer (NK) cells

NK cell function is critical in the first-line of defence against viral, bacterial and parasitic infection [11] and their functional exhaustion has been correlated to disease progression [12]. NK cells are activated by a plethora of cytokines including IL-2, IL-12, IL-15 and type I INF [13-18]; once triggered, they produce several chemokines such as CCL3/MIP1α, CCL4/MIP1β CCL5/RANTES, and cytokines such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and granulocyte/macrophage colony-stimulating factor (GM-CSF) [19]. These soluble factors play an important regulatory role in haematopoiesis and also contribute to priming or activation of cellular networks.

NK cells stochastically express a unique set of activating and inhibitory receptors. NK cell receptors are highly polymorphic in closely related species. The diversity of NKRs suggests an acute selective pressure from the pathogens and viruses they encounter. Microbial substances possess multiple genes encoding immunoevasins that interfere with antigen presentation by degrading, retaining, or preventing the assembly of MHC class I on the cell surface of infected cells [20]. Downregulation of MHC class I cause infected cells to be susceptible to NK cell recognition of "missing self".

Current studies show that NK cells are engaged in an active bi-directional cross-talk with autologous dendritic cells (DCs). This cross-talk needs both NK-cell –DC interaction and secretion of specific cytokines [10,20-22]. In addition to this monocytes/macrophages and neutrophils have been shown to regulate the recruitment and activation of NK cells, which eliminate over-stimulated macrophages. This "menage a trois" involves direct reciprocal interactions as well as positive amplification loops mediated by cell-derived cytokines, with the aim of inducing IFN-γ production by NK cells [23,24].

NK cells in COVID-19

Several studies reported that the number of NK cells is significantly decreased in severe COVID-19 patients [25,26]. NK cells along with Cytotoxic T lymphocytes are indispensable for mounting an appropriate antiviral response in SARS-CoV-2-infected patients [27]. It has been observed that a significant increase of NKG2A expression was occurred in COVID-19 patients. Upregulation of NKG2A was associated with the exhaustion of NK cells and cytotoxic T cells at the early stage of SARS-CoV-2 infection, and therefore, was associated to severe disease progression [27]. However, increased frequency of NK cells were observed in convalescent patients (CPs) as compared to healthy donors [28]. Evidences from several studies suggested that the peripheral blood NK cell number is much lower in both severe and moderate COVID-19 patients [29-33]. Contrast to this view, two recent reports suggested that NK cell numbers are increased in BAL samples from COVID-19 patients [34,35]. It has been found from BAL samples of moderate COVID-19 patients that NK cells are strongly activated along with stronger interferon response [36]. This group also confirmed that like peripheral blood NK cells, a similarly activated phenotype of NK cells was observed in BAL fluid. An increase in adaptive NK cells was almost exclusively found in severe COVID-19 patients [36].

The vast majority of adaptive NK cells co-expresses NKG2C and CD57 [37]. Higher frequencies of NKG2C+CD57+ CD56dim NK cells were observed in severe, but not in moderate COVID-19 patients, in comparison to healthy controls (Maucourant., *et al.* 2020). Interestingly, the absolute number of NKG2C-CD57- cells was decreased in COVID-19 patients. The most interesting finding by Maucourant., *et al.* 2020; was that they identified two immunotypes associated with COVID-19 severity. (i) One immunotype related to severe disease showed higher expression of perforin, Ksp37, and NKG2C. In addition to this perforin and Ksp37 is part of the compound phenotype of armed CD56bright NK cells. These NK cells are associated with severe disease. (ii) The other immunotype related to moderate disease

showed higher expression of MIP-1β, CD98, and TIGIT. As the armed CD56bright NK cell phenotype negatively correlated with TIGIT expression, so the association between high expression of the inhibitory checkpoint TIGIT and moderate disease was confirmed by the fact [36].

According to Wen., *et al.* 2020; NK cells have two lineages: (i) CD56+CD16− NK cells (NK1), which express high levels of CD56 and low levels of CD16 and (ii) C56−CD16+ NK cells (NK2), which express high levels of CD16 and low levels of CD56. The absolute number of NK cells decreased in COVID-19 patients, whereas the relative ratio of NK cells in ERS (early recovery stage) patients was higher than that in the healthy controls. In particular, LRS (late recovery stage) patients group would have an increase in T and NK cells, with a lower expression of inflammatory genes [38]. Both the NK cell lineages CD56brightCD16− and CD56dimCD16+ NK cells were significantly decreased in severe COVID-19 patients in comparison to healthy donors. In the recovered group, the proportion of NK cells was comparable with HDs [39].

CD161 has been reported to be a marker of inflammatory monocytes and NK cells [40,41]. It was observed that severe COVID-19 patients had significantly lower circulating CD16+ NK cells in comparison to healthy donors [39]. They also noticed that the down-regulation of CD16 was occurred rather than specific depletion of CD56low CD16+NK cells. In addition to it, they suggested that the cause of loss of cell surface CD16 expression during COVID-19 is not only due to receptor internalization but also other mechanisms may be responsible, such as transcriptional down-regulation or shedding [39]. Wilk., *et al*. 2020; recently reported a decrease in CD16 mRNA on NK cells during COVID-19 [30].

Bats are reservoirs of many infectious viruses, such as Ebola, Marburg, Hendra, Nipah and SARS corona viruses [42,43]. Type I IFNs provide a strong activation signal for NK cells. A dysregulation in the IFN-NK cell axis in bats could have deleterious immunological effects. ISG15, an extracellular cytokine, has been shown to boost the effector functions of NK cells [44], which may contribute to control viral replication in bats. [Although NK cells have been characterized in bat peripheral blood using cross-reacting antibodies [45], but their functionality and responsiveness against bat viruses are yet to be tested. According to current evidences, if NK cell activation threshold in bats can be boosted then it can avoid damaging immune responses.

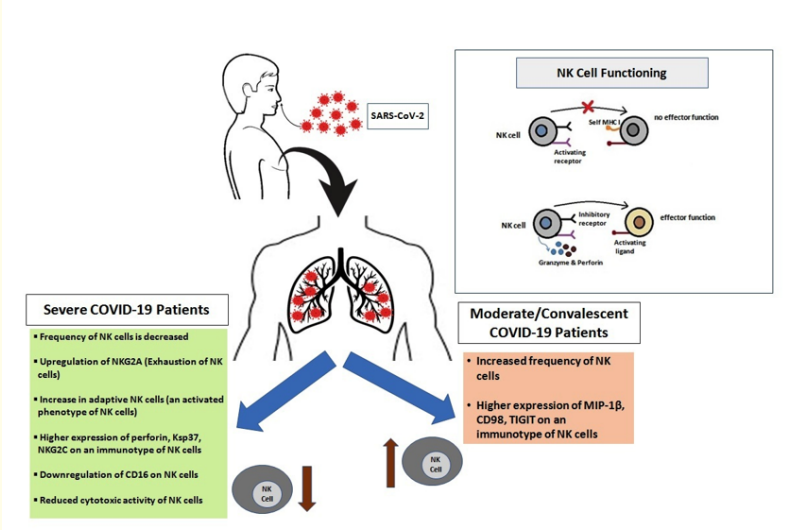

Figure 1: *Brief outline of role and status of natural killer (NK) cells in COVID-19-infected patients. [] in inset box, it depicts the functioning of NK cell.*

Discussion

Most of the studies reported that the NK cell count reduces remarkably during SARS-CoV-2 infection, mostly in severe COVID-19 patients [27,29,38]. There is contrasting view too. Some researchers advocated that the frequency of NK cells was increased in convalescent patients (CPs) or moderate COVID-19 patients as compared to healthy donors [28,36]. It has been suggested that the loss of NK cell's effector functions is the most prominent immunological feature of the macrophage activation syndrome (also referred to as hemophagocytic lymphohistiocytosis, HLH), a condition that can be triggered by infections and that closely resembles the "hyperferritinemic syndrome", which can be compared to SARS-CoV-2-related 'cytokine storm' [46]. The local and systemic inflammation contributes to reduce NK cell effector functions in HLH. Similar to it, the elevated IL-6 and IL-10 levels (as the ones observed in COVID-19) are capable to inhibit NK cytotoxic activity as the expression of perforin and granzyme B decreases. It has been assumed that IL-6 may further impair NK cells activity by reducing the expression of NKG2D which is important in the killing of infected cells [47].

CD16 down-regulation is very much related to NK cell activation, maturation and development [48], resulting in antibody-dependent cell cytotoxicity and tumor necrosis factor–α secretion. CD16 Down-regulation after interaction with IgG-immune complexes may also check excessive immune responses after influenza vaccination [49,50]. Kuri-Cervantes., *et al*. 2020; observed a marked down-regulation of CD16 on NK cells in severe COVID-19 patients and suggests substantial mobilization of these cells [39].

According to current sources, the United States Food and Drug Administration (FDA) have approved one US-based drug company to use NK cell-based immunotherapy as an alternative treatment of COVID-19 in future [51,52]. The available data suggests that NK cell-targeted therapy may be beneficial to control viral disease and its progression. These data also suggest that NK cells can intensify the severity of the disease at later time points. Thus, extreme caution should be practised when designing a treatment strategy with NK cell. Another possibility may be the inhibition of the GSK-3 pathway which drives the maturation and function of natural killer (NK) cells [53]. And if the GSK-3 pathway is inhibited, it will play a central role in boosting the adaptive and innate immune responses against viruses.

Conclusion

In conclusion, NK cells have significant role in COVID-19. Various studies reveal that the number of NK cells is significantly decreased in severe COVID-19 patients but in case of moderate or mild COVID-19 patients, the frequency of NK cells is increased. Two immunotypes of NK cell has been identified. One is linked to severe COVID-19 and other is linked to moderate COVID-19 patients.

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Conflict of Interests

Author declares there is no conflict of interests exists.

Author Contributions

KC planned the idea for the review article. KC and PCG performed all the literature search and data analysis, and finally drafted and critically revised the work.

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