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Perforin and resistance to SARS coronavirus 2

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In this brief perspective, we outline the role of perforin in the host response to viral infections and draw parallels between morbidity and mortality patterns associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the functionality of perforin (Fig 1). We also reference known genetic disorders relating to perforin and their potential relevance. Finally, based on our hypothesis, suggestions are made regarding how the immune response may be augmented to increase resistance to SARS-CoV-2–related illness.

Perforin is a potent pore-forming protein and permits cytotoxic proteases, such as granzyme B, to enter the cytoplasm of virally infected target cells. Upon recognition of a target cell by cytotoxic cells, an immune synapse is formed and perforin and granzymes are secreted into the synaptic cleft. Perforin then forms pores in the target cell membrane, which allows granzyme proteases to enter the target cell cytosol, leading to cell death.[1] Supporting the pivotal role of this protein in immune responses to viral infections, perforin knockout mice cannot protect themselves against viruses.[1] In addition, perforin is known to be an important component of the human immune response to usual respiratory viral infections such as those that produce common cold symptoms (known etiological pathogens to include coronaviruses).[2]

Several factors affect variance in perforin expression (Fig 1) including age. There appears to be easier 'perforin exhaustion' in the elderly, which was shown in a series of studies by Mariani et al.[3] Compared with those from younger donors, peripheral natural killer (NK) cells from elderly subjects consumed up to 12 times more perforin following culture with target cells (K562 leukemic tumor cells), and synthesized significantly less perforin in response to a stimulus (PHA).[3] Similarly, it has been shown that the expression of perforin by NK cells declines significantly after the age of 70 years and children have higher levels than adults.[4] Sex differences are also reported, with adult women having consistently higher levels of perforin than do men of equivalent age.[4]

These patterns of perforin expression reflect what is known of varying susceptibility to severe symptoms associated with SARS-CoV-2 infection. That is, evidence to date suggests that serious adverse effects are more likely to occur in adults older than 70 years, and are more common in men than in women. Moreover, it appears that children rarely present with severe symptoms, and are less symptomatic overall compared with adults.

Another determinant of perforin levels may be an individual's body mass index,[5] and recognized risk factors for poor outcomes from SARS-CoV-2 infection include not only obesity but also diabetes mellitus (DM). Perforin genes have been identified to be significantly downregulated in obesity, and NK cells from obese individuals have shown reduced cytolytic activity in comparison with those from lean individuals.[5] In relation to diabetes, in a study by Kumar et al,[6] patients with type 2 DM and pulmonary tuberculosis (PTB) had decreased perforin expression by cytotoxic T lymphocytes (CTLs) compared with those with PTB but without DM.

Known human disorders of perforin, or *perforinopathies,* have been associated with protracted viral infections (mainly EBV) but are more commonly complicated by cytokine release syndromes (CRS), which include hemophagocytic lymphohistiocytosis and macrophage activation syndrome.[1] Some patients with severe SARS-CoV-2 infection have certain clinicopathological overlap with these CRS.[7] Failed target cell death has been postulated to be an inciting factor for CRS in patients with *perforinopathies*. Extended attachment between the CTL and its target cell, with unsuccessful attempts at cytolysis, leads to hypersecretion of cytokines and chemokines.[1]

An example of a gene polymorphism resulting in compromised perforin function is the highly prevalent Ala91Val amino acid substitution, present in 8% to 9% of white populations.[1] Even monoallelic inheritance of Ala91Val is associated with a significant impairment of perforin activity and a reduction of a least 35% in the cytotoxic potential of NK cells.[1,8] Although attention has focused previously on the association of Ala91Val with malignancies,[1] it is proposed that this common hypomorphic mutation may have an impact on susceptibility to severe SARS-CoV-2 infection. Indeed, mutations in perforin and related genes have previously been associated with secondary hemophagocytic lymphohistiocytosis/macrophage activation syndrome–like presentations in patients who died from H1N1 influenza.[8]

NK-cell function can be boosted by vaccines such as those for yellow fever virus, Ebola virus, BCG, and cytomegalovirus. BCG vaccination in children was shown to be associated with increased NK-cell activity against unrelated candida infections and increased production of both perforin and granulysin.[9] Indeed, at the time of writing this article, the authors have become aware of the commencement of a double-blind trial in Australia, initially in health care workers, which is investigating the benefit

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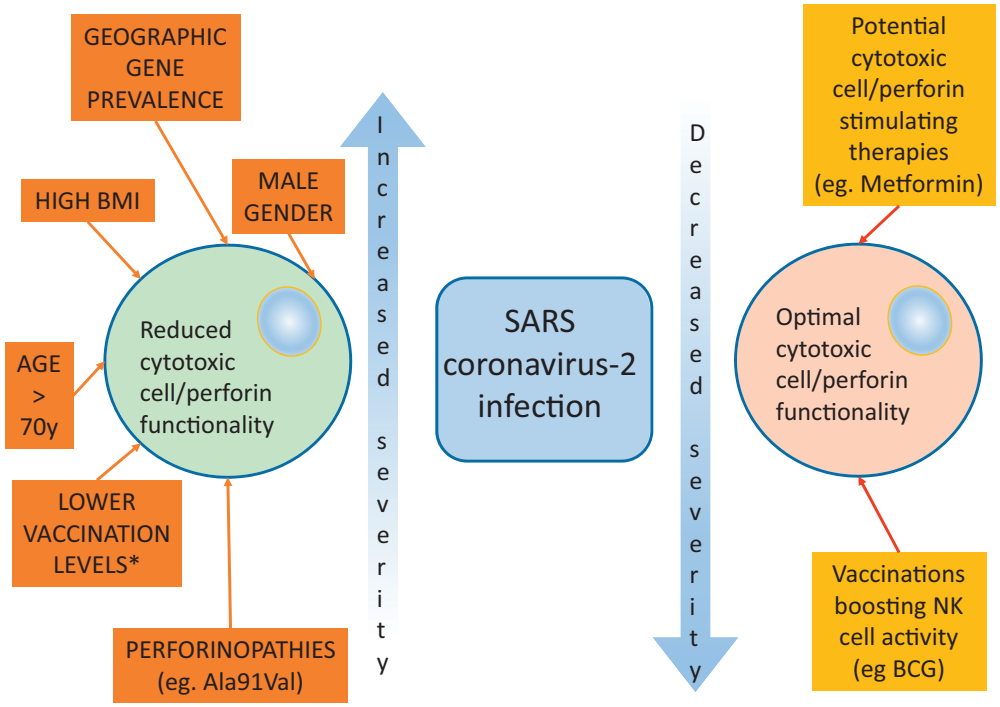

FIG 1. Factors with the potential to affect perforin expression and functionality. *BMI*, Body mass index. *Specifically live attenuated vaccines that boost NK-cell function.

of BCG vaccination in enhancing resistance to SARS-CoV-2. Another potential strategy to boost innate perforin levels might be to consider the relatively well-tolerated drug metformin. This has been shown to increase perforin and granzyme B expression at doses used in the treatment of diabetes.[10]

In conclusion, we suggest that variations in NK-cell/perforin function may be one important factor in driving differences in the severity of symptoms and mortality rates associated with SARS-CoV-2 infection. It is plausible that vaccine history, genetic profiles, and certain current medications may all exert some effect on the host response to SARS-CoV-2 infection. Importantly, genetic testing for mutations in perforin and related genes may identify other at-risk populations. Further data collection would assist in the clarification of such effects and therapeutic recommendations should be based on outcomes from robust clinical trials.

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