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Covid-19 acute responses and possible long term consequences: What nanotoxicology can teach us

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ABSTRACT

Long-term effects of Covid-19 disease are still poorly understood. However, similarities between the responses to SARS-CoV-2 and certain nanomaterials suggest fibrotic pulmonary disease as a concern for public health in the next future. Cross-talk between nanotoxicology and other relevant disciplines can help us to deploy more effective Covid-19 therapies and management strategies.

SARS-CoV-2 and nanoparticles in the airways

The coronavirus SARS-CoV-2 is responsible for the ongoing pandemic of atypical pneumonia Covid-19. While many aspects of the acute physiopathological changes occurring in the respiratory system have been already disentangled, substantial uncertainties remain concerning the possible long term, residual effects of resolved Covid-19 disease. However, many of the molecular, cellular and systemic alterations caused by SARS-CoV-2 are well known to particle toxicologists as they present a striking similarity to the mechanisms of toxicity caused by nanomaterials. Interestingly, initial evidence also points to an association between Covid-19 disease severity and exposure to particulate matter pollution. Thus, exposure to particulates might have a potential role in priming the immune system while facilitating the aggravation of SARS-CoV-2 infection symptoms [1–4].

As known for the coronavirus SARS-CoV, the surface protein spike S1 of SARS-CoV-2 mediates cell entry [5]. The viral spike protein acquires a round tip shape *via* priming by the serine protease protein TMPRSS2. This proteolytic reaction is essential for the effective recognition of spike with the receptor angiotensin converting enzyme II (ACE2) binding on the surface of target cells [5]. Consequently, SARS-CoV-2 enters the target cell by a molecular mechanism known as endocytosis through the endosomal molecular pathway [6]. From the endo-lysosomal intracellular waste disposal compartments, the virus escapes into the cell cytoplasm and releases its genome. Comparably, one-dimensional nanomaterials similar in size enter the cell by tip recognition [7]. Nanomaterials can be internalized through energy-dependent molecular processes such as endocytosis, non-phagocytic mechanisms as well as receptor mediated endocytosis [8]. Outstandingly, cationic polyamidoamine dendrimer (PAMAM) nanoparticles are shown to bind also ACE2, decrease its enzymatic activity and downregulate its expression in lung tissue [9]. Similarly to viral particles, after cell internalization, also nanoparticles can escape the endo-lysosomal intracellular compartment by dissociation with their protein corona in the acidic lysosomal microenvironment [6,10]. An acute consequence of lysosomal leakage is the production of reactive oxygen species (ROS), also observed in many viral infections [11,12].

In the distal lung, pneumocytes and resident macrophages are the first cell populations to encounter SARS-CoV-2 [5]. These interactions lead to activation of NF-κB and STAT3 *via* the MyD88 signalling cascade [13]. This, in turn, leads to production of pro-inflammatory cytokines and chemokines, such as IL-6, TNFα, IFNγ and IP-10, eliciting strong Th1 response [5,14].

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Also rigid multi-walled carbon nanotubes (rMWCNT), among other nanomaterials, induce innate immune response by activation of NF-κB, STAT3 and HIF-1/2, and consequent cytokine cascade [15,16]. Interferons (IFNs) are pivotal molecules in the defense response to viral infections, often deploying inhibitory mechanisms, like in the case of the coronavirus ORF3b protein [14]. Not surprisingly, also a number of carbon and metal nanoparticles have been reported to alter the expression of IFN signalling pathways both *in vivo* and *in vitro* [17,18].

As the Covid-19 disease progresses, massive damage of the pulmonary tissue occurs by induction of an uncontrolled innate immune response, mainly mediated by M1 pro-inflammatory macrophages and granulocytes. In the most severe cases, a cytokine release syndrome, with IL-6, IL-1 and TNFα-storm is observed [14]. Similarly, inhalation or aspiration of CNTs can stimulate the recruitment of inflammatory monocyte derived macrophages as well as over-produce IL-1β and TNFα together with strong neutrophil influx [19,20].

As recently described, high IgG and IgM titers are found in the blood of Covid-19 patients within 19 days from the onset [21]. In addition to the strong innate immune responses to nanoparticles, also antibodies against nanomaterials have been discovered [22]. Moreover, up-regulation of antigen processing pathways, RIG-1 and several viral-induced human disease pathways have been reported consequently to carbon nanomaterial exposure, both *in vitro* [23] and in murine lung *in vivo* [19,24]. Furthermore, SARS-CoV-2 response also includes pneumocytes hyperplasia, multinucleated giant cell formation, and fibrin clusters in the pulmonary tissue [25]. Goblet cell hyperplasia and foreign-body giant cells are also observed consequently to rMWCNTs exposure [19].

Covid-19 related, sudden and strong cytokine influx as well as excess of pus in the alveoli promote acute lung injury. When the acute phase of inflammation fades away, the tissue repair process is mediated by regulatory M2 macrophages and deactivating cytokines TGFβ and IL-10 [26]. Healing is required to resolve the inflammation but becomes pathological if uncontrolled or prolonged, leading to tissue scarring. Different macrophage phenotypes are essential in determining deleterious and beneficial effects during the tissue remodeling and repair processes [26]. Several viruses are known to regulate macrophage polarization [27], similarly to carbon nanomaterial exposure [15]. Moreover, nanomaterial-induced fibrosis can be observed in less than a month in murine models *post* acute exposure [28].

Following the outbreak of SARS-CoV epidemic in 2003, several survivors were reported to develop pulmonary fibrosis through activation of TGFβ and down-regulation of the anti-fibrotic ACE2 gene [29]. At the damaged tissue, also type II pneumocytes proliferate and contribute to the elevated levels of proinflammatory cytokines. These, in turn, recruit fibroblasts and induce their transdifferentiation into myofibroblasts [29]. Similar chronic effects have been also suggested as a consequence of the SARS-CoV-2 infection, but robust observations are still missing as the first wave of acute disease is still increasing in the global population. Nonetheless, recent evidence from CT scan imaging of Covid-19 patients has highlighted initial signs of possible pulmonary fibrosis already shortly after disease onset [30]. Interestingly, a major theme in nanosafety concerns the assessment of pulmonary fibrosis as a possible long-term effect of nanomaterial exposure. In fact, interstitial fibrosis and granulomas have been observed consequently to inhalation of certain carbon nanotubes. In these cases, nanomaterial exposure leads to increased production of PDGF and TGFβ [31]. Moreover, these nanomaterials cause transcriptomic changes in the lung similar to those caused by bleomycin, a known fibrogenic agent [31]. However, different exposures might cause lung fibrosis through distinct mechanisms. Small molecules, such as bleomycin, might induce fibrosis by primarily inducing tissue damage through metabolic stress [32]. This, consequently, activates the immune system as a secondary event. On the other hand, certain nanoparticles might induce lung fibrosis by a combination of metabolic tissue damage and primary activation of the innate immune cells. Combinations of different nanoparticle properties, such as shape as well as size, surface charge, hydrophobicity, surface area and reactivity can trigger mechanisms leading to immune responses. Moreover, biopersistence, together with the fibrous structure of the nanoparticles (the fibre paradigm), is known to induce fibrosis and granuloma formation, like for example in the case of rMWCNT [33].

Here we summarized noticeable cellular and molecular similarities between the acute responses to both SARS-CoV-2 infection and certain nanomaterials exposure. Altogether, they suggest long term consequences, such as pulmonary fibrosis. Considering the large number of Covid-19 cases currently occurring worldwide, the possibility that fibrotic pulmonary disease will prominently come to the attention of public health in the future should not be neglected. Cross-talk between nanotoxicology, pneumology, immunology and virology can facilitate faster advancement of our understanding of the Covid-19 disease as well as our ability to deploy effective therapeutic and management strategies. At the same time, the ever increasing data emerging on the effects of SARS-CoV-2 infection can lead to better predictions of the effects of nanomaterials on human health.

CRediT authorship contribution statement

Pia A.S. Kinaret: Data curation, Formal analysis, Investigation, Validation, Visualization, Writing - original draft, Writing - review & editing. **Giusy del Giudice:** Investigation, Validation, Writing - original draft, Writing - review & editing. **Dario Greco:** Conceptualization, Supervision, Funding acquisition, Project administration, Resources, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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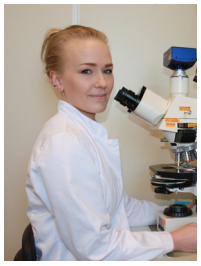

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