

Chapter 10

Nanomaterial Effects on Viral Infection



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Abstract The potential for environmental and occupational exposures of populations to nanomaterials (NMs) has fostered concerns of associated adverse health effects, with a particular emphasis on pulmonary injury and disease. Many studies have revealed that several types of NMs can evoke a variety of biological responses, such as pulmonary inflammation and oxidative stress, which contribute to allergy, fibrosis, and granuloma formation. Less attention has been paid to health effects that may result from exposure to NMs and additional stressors such as pathogens, with a particular focus on susceptibility to viral infection. This chapter will summarize the current body of literature related to NMs and viral exposures with a primary focus on immune modulation. A summary of the studies performed and major findings to date will be discussed, highlighting proposed molecular mechanisms behind NM-driven host susceptibility, challenges, limitations, and future research needs. Specific mechanisms discussed include direct interaction between NMs and biological molecules, activation of pattern recognition receptors (PRRs) and related signaling pathways, production of oxidative stress and mitochondrial dysfunction, inflammasome activation, and modulation of lipid signaling networks.

Keywords Nanomaterials · Carbon nanotubes · Pathogens · Viruses · Pulmonary · Inflammation · Infection · Oxidative stress · Immune response

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10.1 Introduction

It has been demonstrated that exposure to small particles and fibers (i.e., particulate matter and asbestos), most notably via inhalation, can enhance host susceptibility to microbial infections. The ability of nanomaterials (NMs) to alter the health outcomes associated with infections, specifically those associated with viruses, has not been well characterized; however, several recent reports suggest that select types of NMs have the capacity to modulate and, in some cases, worsen the outcomes of viral infections. In this context, a few reports have begun to address the molecular mechanisms involved that contribute to the impacts that these NMs have on viral infectivity, primarily through modulation of the host immune system. In this chapter, the current state of knowledge regarding these mechanisms in response to varied NMs and viruses will be discussed, highlighting gaps in the science and suggesting where future research is needed.

10.2 Multiple Environmental Stressors

The notion of “multiple environmental stressors” is not new, and several lines of evidence support that exposure to environmental chemicals/particulates and microbes can induce adverse health effects that are distinct from exposure to single agents alone (Feingold et al. 2010). For example, results of epidemiological studies demonstrate that exposure to air pollution, which contains a mixture of chemical and biological agents, is a risk factor for increased incidence of pulmonary infections (Ciencewicky and Jaspers 2007), co-exposure to pesticides and heavy metals is suspected to have a direct impact on the gastrointestinal tract and disrupt the normal microbiome, leading to persistent inflammation and infection (Mapesa et al. 2016), and exposure to both aflatoxin and hepatitis B virus (HBV) (Groopman et al. 2005) can lead to 59.4 times higher risk of developing liver cancer compared to only 7.3 and 3.4 times the risk for HBV or aflatoxin exposure alone (Qian et al. 1994). Small particulates, especially those that are inhaled, have additionally been shown to increase susceptibility to respiratory viruses. Epidemiological studies have demonstrated that human exposure to inhaled diesel exhaust, cigarette and wood smoke, coal dust, silica, particulate matter 2.5 (PM_{2.5}), and air pollution of varied composition increases the severity and chance of acquiring respiratory infections (Ciencewicky and Jaspers 2007; Speizer et al. 1980; Clifford et al. 2015; Huang et al. 2016; Jaspers et al. 2009; Chen et al. 2012; Becker and Soukup 1999; Ma et al. 2017; Rabovsky et al. 1986; Rebuli et al. 2018). There is now consistent evidence to support that exposure to air pollution leads to severe lower respiratory infections, especially in children under 5 years of age. Furthermore, this association has been observed after children are exposed to short-term spikes of PM_{2.5} (Horne et al. 2018).

Due to their small size and resemblance to previously mentioned particulates and fibers (i.e., asbestos) that are associated with adverse pulmonary effects, there is

remarkable interest in inhalation as a route of exposure to NMs and subsequent toxicity. Airborne exposure to NMs is likely to occur in occupational settings, by direct consumer use of products and indirectly through environmental contamination, and several studies have reported that certain types of NMs, such as carbon nanotubes (CNTs), are present above the National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit in workplace environments (Boonruksa et al. 2016; Thompson et al. 2015; Hedmer et al. 2015). Other potential exposures could arise through release from consumer products (i.e., tires, electronics, cosmetics), product end-of-life, and avenues of disposal (Nowack et al. 2013).

Most of the pulmonary toxicity studies performed to date investigate well-described adverse effects of particulate exposure such as sub-chronic tissue damage, fibrogenesis, granulomatous changes, impaired clearance, robust inflammation, airway hyperreactivity, and airflow obstruction (Guzman et al. 2006; Li et al. 2010; Teeguarden et al. 2010; Saber et al. 2013). These data originate primarily from studies that assess the toxicity of exposure to single-component NMs in isolation of other imposed stressors. Because the scenario of co-exposure to NMs and infectious agents in the ambient environment is likely, understanding the combined health effects caused by exposure to NMs and viruses is highly significant to the field of public health.

10.3 Viral Infections and Public Health Impacts

Viral infections account for a considerable global health burden. Several viruses contribute to this burden and include human immunodeficiency virus (HIV), Ebola, corona, Zika, West Nile, dengue, and hepatitis A–C, among others. Several common viruses, such as influenza viruses, are associated with significant infection rates annually, which include seasonal epidemics and pandemics. Emerging and reemerging viruses, such as those that cause severe acute respiratory syndrome (SARS), can also contribute significantly to health burdens. Acute lower respiratory tract (LRT) infections associated with viruses are a leading cause of death and disability among children and adults globally and are consistently considered one of the top ten global health challenges. Epidemiological evidence shows that lower respiratory tract infections caused 2.74 million deaths and 103 million disability-adjusted life years (DALYs) based on the mortality and morbidity data from 195 countries in 2015 (GBD 2015 LRI Collaborators 2017). Other common respiratory viruses, including respiratory syncytial virus and rhino virus, also contribute considerably to the global health burden (Forum of International Respiratory Societies 2017). In the USA, upper and lower respiratory tract infections are estimated to be responsible for about \$15 billion in direct treatment and \$9 billion due to missed work (Dixon 1985). It is evident that viral infections remain a top priority in the field of public health, and thus understanding how exposure to contaminants, such as nanoparticles (NPs), may impact the morbidity and mortality associated with these infections is vitally important.

10.4 Nanoparticle Exposure and Viral Infection

A literature search on PubMed using terminologies “nanomaterials” and “viruses” revealed 3639 papers. Addition of the term “toxicity” produced 265 hits and the subsequent addition of “infection” resulted in 60 papers. More careful assessment of these latter publications led to three papers that were excluded because they did not actually study viruses. Of the remaining 57, only 15 published from 2010 to 2018 focused specifically on the toxicity of NMs and viral infections (9%) where live viruses were employed. More details for these 15 studies are shown in Table 10.1. The remaining studies investigated nonpathogen-related toxicity (26%) or the application of NMs to therapeutic delivery, vaccine development, and viral detection (79%) which will not be discussed in detail in this chapter (Fig. 10.1).

The studies that have investigated the toxicity of NMs and their ability to modulate viral infections focused on a few select types of NMs that included carbon nanotubes, spherical carbon and carbon black, fullerenes, titanium dioxide (TiO₂), silver, and gold. Specific viruses employed were herpes simplex virus type 2 (HSV-2), influenza A virus (IAV), respiratory syncytial virus (RSV), HIV-1, dengue virus type 2, murine gamma herpesvirus 68 (MHV-68), Epstein–Barr virus, and foot-and-mouth disease virus (FMDV). Interestingly, only six of the published articles addressed NM exposure and increased viral susceptibility, whereas results of the remaining nine papers showcased the ability of NMs to inhibit virus titers either through blocking viral entry or through replication. For example, cells exposed to silver nanoparticles were shown to inhibit dengue virus serotype 2 (DENV-2) viral replication and production of progeny viruses (Murugan et al. 2015), and inhibit replication of HSV-2 (Hu et al. 2014), HIV-1 (Trefry and Wooley 2012), and HSV-2 (Sopova et al. 2010), whereas foot-and-mouth disease virus (FMDV) titers were reduced by gold NP (Rafiei et al. 2016). Another interesting aspect of these studies showed that the effects of carbon NMs on viral infection varied by the type of nanoparticles tested. Exposure to both single-walled carbon nanotubes (SWCNTs) was able to increase virus titers of IAV (Sanpui et al. 2014; Chen et al. 2017). Interestingly, in another study, exposure of mice to multiwalled carbon nanotubes (MWCNTs) exacerbated pneumonia and immune cell infiltration in lungs of RSV-infected mice but did not influence the virus titers compared with RSV treatment only (Hashiguchi et al. 2015a). Our group has also observed a similar effect with MWCNTs in a mouse model of IAV infection where titers of IAV were not increased (in review), despite the significant increase in titers observed for SWCNTs in the same mouse model. Spherical carbon nanoparticles (Barras et al. 2016) and fullerene (C₆₀) derivatives (Shoji et al. 2013) were shown to interfere with HSV-1 and IAV, respectively, whereas exposure to a single dose of TiO₂ nanoparticles exacerbated pneumonia in RSV-infected mice (Hashiguchi et al. 2015a). Lastly, a more recent report showed for the first time the ability of double-walled carbon nanotubes (DWCNTs) to reactivate latent herpesvirus (MHV-68) in cells and mice (Sattler et al. 2017). They also discovered that these DWCNTs could induce more pronounced effects in terms of reactivating latent virus than carbonaceous spherical nanoparticles.

Table 10.1 Literature summary of the impact of NMs on viral susceptibility

NP type	Prepared with	Avg. diameter (nm)	NM exposure	Dose	Experimental model	Virus type	Virus exposure	Dose	Exposure time	Overall findings	Reference
<i>Carbon</i>											
Single-walled CNT	1% Pluronic F-127	0.805	Cell culture media	50 µg/mL	Human small airway epithelial cells	IAV H1N1 strain A/Mexico/4108/2009	Cell media	MOI 0.1–0.5	CNT 24 h + LAV 24 h	CNT increased IAV titers; repressed antiviral genes	Sanpui et al. (2014)
Single-walled CNT	1% Pluronic F-127	0.805	IT	20 µg/mouse	C57BL/6 mouse	IAV H1N1 strain A/Mexico/4108/2009	IN	3.12×10^4 TCID ₅₀ /mouse	CNT 4d + IAV 3d	CNT increased IAV titers and injury in lungs; altered immune markers and repressed antiviral genes	Chen et al. (2017)
Multiwalled CNT	PBS	40–50	IN	0–0.25 mg/kg	BALB/c mouse	Human respiratory syncytial virus (RSV)	IN	3.5×10^5 PFU/mouse	CNT 5d + RSV 5d	CNT exacerbated pneumonia and immune cell infiltration in lungs; no change in virus titers compared to RSV only	Hashiguchi et al. (2015a)
Double-walled CNT	Unknown	1000, 7000, and 15,000 nm length	IN	0–0.25 mg/kg	BALB/c mouse	Human respiratory syncytial virus (RSV)	IN	3.5×10^5 PFU/mouse	CNT 1, 3 5 d + RSV 5d	CNT increased CCL5 in BALF of RSV-infected mice; increased monocyte infiltration in lung tissue	Watanabe et al. (2018)
Double-walled spherical carbon CNT;	1% Pluronic F-127	10	Cell culture media in vitro; IT in vivo	50 µg/mL for in vitro; 50 µg per mouse	BHK-21 cells, MHV-68-infected B cell line S11, ANA-1, MH-S; C57BL/6 mouse	Murine gamma herpesvirus 68	Cell media; IN	MOI 1.0 for in vitro; 5.0×10^4 PFU/mouse	MHV-68 2 h + CNT 2 h in vitro; MHV-68 28d + CNT 24 h in vivo	CNT and spherical carbon restored acute herpesvirus infection; boosted production of lytic viral proteins and inflammatory responses in lungs	Sattler et al. (2017)

(continued)

Table 10.1 (continued)

NP type	Prepared with	Avg. diameter (nm)	NM exposure	Dose	Experimental model	Virus type	Virus exposure	Dose	Exposure time	Overall findings	Reference
Fullerene (C60) derivatives	DMSO	N/A	Cell culture media	25–100 µM	MDCK cells	IAV H1N1 strain A/PR/8/34 or A/Aichi/2/68	Cell media	MOI 1.0	Mix fullerenes + IAV; expose cells 24 h	Fullerenes inhibited endonuclease activity of PA protein (subunit of influenza RNA polymerase); inhibited IAV infection and the expression of nucleoprotein and nonstructural protein 1	Shoji et al. (2013)
Carbon nanodots	Water	10–30	Cell media	100, 200, 300 µg/mL	Vero cells, human lung cancer cells (A549)	Herpes simplex virus type 1	Cell media	10 ³ TCID ₅₀ /mL	C-dots and HSV-1 cocultured 2 h	Carbon dots interfered with the virus entry; inhibited infection	Barras et al. (2016)
Carbon black	Ultrapure water	14	Incubate with human cathelicidin (LL-37); cell media	100 µg/mL	WI-38 cells	Rhinovirus (RV1B)	Cell media	Dose determined during assay	Carbon black 1 h + RV1B 1 h	Carbon black altered host defense peptide structure and function	Findlay et al. (2017)
<i>Metal oxides</i>											
TiO ₂ NP	PBS	35	IN	0.25 or 2.5 mg/kg	BALB/c mice	Respiratory syncytial virus (RSV)	IN	3.5 × 10 ⁵ PFU/mouse	TiO ₂ 5d + RSV 5d	TiO ₂ NP exacerbated pneumonia in RSV-infected mice; increased IFN-γ, CCL5; increased infiltration of lymphocytes into the alveolar septa	Hashiguchi et al. (2015b)
Silicon dioxide NP; silver NP	N/A	N/A	Cell media	0.1 and 1.0 mg/mL	Vero cells	Herpes simplex virus type 2 (HSV-2)	Cell media	N/A	N/A	SiNP exposure had no influence on HSV-2; AgNP reduced virus titers	Sopova et al. (2010)

Metals

Silver NP	Ultrapure water	25	Cell media	10, 100, 1000 µg/mL	293 T/17 human embryonic kidney cells; Vero cells	HIV-1 vector (pNL4-3Δ + casset)	NP mixed with HIV-1	1 – 11 × 10 ² TCID ₅₀ /ml	Mix NP and HIV-1 vector and add to cells 1 h	AgNP inhibited early stage infection	Trefry and Wooley (2012)
Silver NP	Cell media	30–40	Cell media	25 µg/mL	Vero cells	Herpes simplex virus 2 (HSV-2)	NP mixed with HSV-2	5 × 10 ⁴ TCID ₅₀ /mL	Cell media 1 h	AgNP inhibited production of progeny viruses and virus replication	Hu et al. (2014)
Silver NP	Deionized water	30–70	Cell media	10, 20, 30 µg/mL	Vero cells	Dengue virus (DEN-2)	Cell media	5 × 10 ⁴ TCID ₅₀ per mL	NP 1 h + DEN-2 1 h and culture for 5d	AgNP inhibited virus replication	Murugan et al. (2015)
Gold NP	Cell media	13	Cell media	1.56 µg/mL	BHK-21 cells	Foot-and mouth disease virus (FMDV)	Cell media	100 PFU	Mix NP and FMDV 1 h and add to cells for 24 h	NP reduced virus titer; inhibition of viral growth at the early stages of infection; inhibited postentry stages of virus replication concomitant with the onset of intracellular viral RNA synthesis	Rafiei et al. (2016)
Gold Nanorods	PBS	10	Cell media; IN	1.25 and 2.5 µg/mL; 20 µg/mouse	Hep-2 cells; BALB/c mice	RSV	Gold nanorods mixed with RSV	100 PFU/mL; 6 × 10 ⁶ PFU / mouse	24 h in vitro; 5d in vivo	Gold nanorods inhibited RSV infection via upregulation of TLR, NOD-like receptor, and RIG-I-like receptors	Bawage et al. (2016)

IT intratracheal aspiration, *IN* intranasal, *IAV* influenza virus, *CNT* carbon nanotubes, *MOI* multiplicity of infection, *PFU* plaque forming units

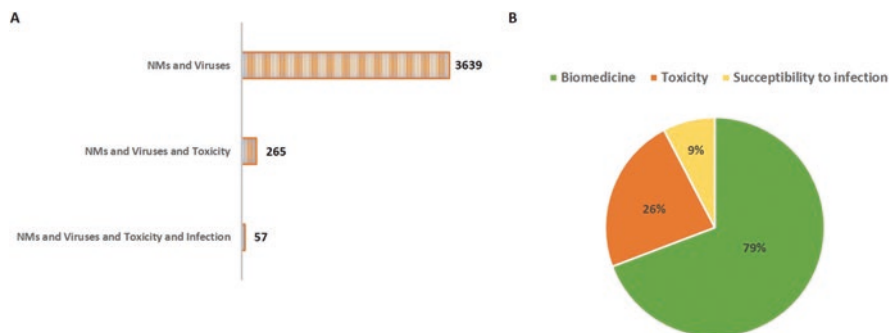


Fig. 10.1 (a) Number of articles based on select search terms in the NCBI PubMed database. (b) Percentage of scientific articles (out of 57) that investigated NM exposures and susceptibility to infection (9%) compared to those that focused on other toxic endpoints (26%) and biomedical applications (79%)

10.5 Nanoparticle and Viral Exposure Routes and Scenarios

There are several ways whereby individuals can be exposed to NMs and viruses which likely influence resultant health effects: (1) nanoparticles and viruses can impact an organism through separate exposures sequentially (in either order) or (2) they can interact in the environment to form an “exposure mixture,” leading to a simultaneous dual exposure scenario. While epidemiological data support the association between particulate exposures (via inhalation) and respiratory infections, it is difficult to define exactly how and when the exposures occurred. There is some evidence however to suggest that the order of exposure is important. For example, it has been demonstrated that small airway epithelial cells infected with IAV produce increased titers only when exposed to pristine SWCNTs prior to infection. The noted increase in IAV titers could not be duplicated with a simultaneous dual exposure or by exposing the cells to the SWCNTs after infection (Sanpui et al. 2014). However, in another study, DWCNTs were able to reactivate latent herpesvirus, suggesting that the infections occurred prior to NP exposure (Sattler et al. 2017). There is not an abundance of evidence to suggest that classic additive, synergistic, or antagonistic toxicity occurs under any of these exposure scenarios, but this observation likely depends on the endpoints that are quantified, which have been limited. However, mixed injury profiles may be much more detrimental compared with effects caused by exposure to single agents (Sanpui et al. 2014; Chen et al. 2017; Walling and Lau 2014; Shvedova et al. 2008). Noteworthy, the exposure scenarios described all likely occur in occupational and environmental settings on some level.

10.6 Immune Response to Viruses

With the knowledge that select NMs can alter host susceptibility to viral infections, it is necessary to understand the molecular mechanisms by which these particles elicit such responses. There is a large focus on modulation of the immune system as a primary target of NMs which requires an understanding of how the host immune system typically responds to viral infections. Viruses depend on host cells to reproduce and the type of targeted host cell depends on the specific strain of virus. For example, cold and flu viruses infect respiratory epithelial cells, whereas HIV attacks T cells of the immune system (Owen et al. 2013). Despite diverse cell types, most viruses go through a lytic cycle that encompasses viral entry into the host cell (a few viruses do not enter the cell but inject their contents), replication using the host cell machinery, and packaging and release of new viral progeny (Owen et al. 2013). As an example, influenza A viruses primarily enter lung cells by binding directly to cell surface receptors (e.g., sialic acid receptors) through which they then enter the host cell through various uptake means such as receptor-mediated endocytosis, clathrin-coated vesicles, and/or macropinocytosis. The virus then releases its contents, including genetic material, into the cell and utilizes host machinery to replicate. Progeny viral particles are assembled beneath the host cell's outer membrane and then released from the cell to initiate a new infection cycle (Edinger et al. 2014).

Once infected, the host immune response to viruses occurs in two phases. The first is a rapid-onset “innate” response that involves the synthesis of proteins called interferons (IFNs) and the stimulation of natural killer lymphocytes. When viruses enter epithelial cells or are phagocytized by immune cells such as macrophages or neutrophils, the cells produce multiple cytokines and chemokines to activate additional innate immune cells. Type I interferons (IFNs) are the principal cytokines that are involved in these responses (Koyama et al. 2008). Once IFNs and downstream cytokines/chemokines are produced, they act as signaling molecules that allow infected cells to warn nearby cells of a viral presence – this signal makes neighboring cells increase the numbers of major histocompatibility complex (MHC) class I molecules upon their surfaces, so that T cells surveying the area can identify and eliminate the viral infection. As infection proceeds, the “adaptive immune response” is initiated, which ultimately results in the synthesis of virus-specific antibodies by B lymphocytes and the cell-mediated response (the synthesis of specific cytotoxic T lymphocytes that kill infected cells).

Upon activation of T cells and B cells specific to the certain types of viruses, an adapted and long-term immune response to the virus will be developed. Antibodies produced by B cells are specific to viral surface antigens and are very important in neutralizing viruses and preventing spread during the acute infection phase (Owen et al. 2013). For example, antibodies can bind to and neutralize viral antigens that have important functions in cellular membrane penetration. CD8⁺ cytotoxic T (Tc) cells and CD4⁺ helper T (Th) cells are required for cell-mediated antiviral defense. Activated Th cells produce IFNs, interleukin 2 (IL-2), and other cytokines while activated Tc cells can directly kill infected cells.

10.7 Viral-Related Immune Mechanisms Perturbed by NMs

With the knowledge that select NMs can increase host susceptibility to viral infections, it is necessary to understand the molecular mechanisms by which these particles elicit such responses. While the response can vary somewhat between virus types, we will focus on several distinct immune mechanisms perturbed by NMs that have been reported and may contribute to altered viral infection status. These include: (1) direct interaction between NMs and biological molecules, (2) activation of pattern recognition receptors (PRRs) and related signaling pathways, (3) production of oxidative stress and mitochondrial dysfunction, (4) inflammasome activation, and (5) modulation of lipid signaling networks.

10.7.1 *Direct Interaction Between NMs and Biological Molecules*

There is limited information regarding the direct interactions between NMs and viruses and how such actions contribute to altered host immunity and/or disrupted viral life cycles, and hence, infection status. The physicochemical properties of NMs, that is, hydrophobicity, surface moieties generated through surface reactions, and the inherent electronic structure that promotes pi-pi interaction with aromatic organics, can influence favorable interactions with biomaterials (Otyepkova et al. 2016; Li et al. 2013a; Wepasnick et al. 2011; Smith et al. 2009; Guo et al. 2014). Certain NMs, such as those that are carbonaceous in composition (i.e., CNT, graphene), have a propensity to interact with biomacromolecules due to these inherent properties, and much work has been focused on the ability of such NPs to form a “corona” in biological systems (Saleh et al. 2010). It is therefore reasonable to assume that NPs can interact with natural bio-particulates, that is, viruses. Due to their size and surface chemistry, these ultrafine bio-particulates may have elongated residence time in environmental media, such as air, water, or soil, which can greatly impact environmental fate and transport and thus human exposure.

10.7.1.1 Interaction of NPs with Protein Corona

“Protein coronas” are a complex of proteins in cell culture media or biological fluids that adsorb to the surface of NMs. Such proteins and other molecules can hitch a ride on NMs, using them as vehicles for transport either to the cell surface or to aid in cell entry. This may also be the case for viruses. For example, sorption of pathogen moieties may be transported by NMs to receptors, such as pathogen recognition receptors (PRRs), to initiate antiviral responses (Monopoli et al. 2011; Yoneyama et al. 2016); however, the opposite could also occur, whereby direct interaction of

NMs and pathogen moieties could limit interaction with immune surveillance systems, impairing normal host defenses.

Protein corona-coated NMs have been recently termed nanomaterial-associated molecular patterns (NAMPs). NAMPs have been shown to be directly recognized by PRRs based on their protein composition (Silva et al. 2017). Proteins undergoing adsorption to NMs can experience possible conformational and functional changes, leading to altered immune responses to either the NMs or pathogens. For example, the epitope of a protein in a corona mixture may be more exposed and thus more easily recognized by the PRR (Saptarshi et al. 2013). Another study discovered very distinctive adsorption and binding patterns of protein corona based on profiles of amino acids (serine, tyrosine, etc.) that may have distinct effects on the activation of immune pathways (Cai et al. 2013).

10.7.1.2 Interaction of NMs with Viruses

Numerous studies have shown that NMs are excellent vehicles for targeting viruses as part of their design (i.e., adding antibodies to select viruses), and as a result are widely employed in diagnostic or therapeutic platforms (Lehner et al. 2013). However, very few studies have addressed the ability of NMs in their native form to interact directly with viruses or viral particles, either in the ambient environment or within a host. A recent study utilized MS2 bacteriophages as a model surrogate for norovirus, which is known to cause 18% of all diarrheal disease incidences worldwide (Ahmed et al. 2014), to investigate their ability to interact with carbon NMs (Hoelzer et al. 2013). MS2 capsids consist of small icosahedral shells ($T = 3$), composed of 90 dimers of the coat protein, which protects the genomic RNA inside (Valegard et al. 1990). The viral coat proteins include various amino acid residues, both on the interior (20–90 residues) and the exterior (1–20 and 90–129 residues) of the capsids (Penrod et al. 1996). These amino acids present positively (e.g., protonated amine and diaminoiminium groups) and negatively charged moieties (e.g., carboxylates), which display a pH-dependent net surface charge on the MS2 (Penrod et al. 1995). Furthermore, a number of residues on the capsid exterior exhibit positive hydropathy index values, indicating strong hydrophobicity (Penrod et al. 1995, 1996; Kyte and Doolittle 1982). Strong hydrophobicity and presence of surface moieties on these colloiddally stable MS2 capsids can facilitate attachment to carbon NMs. A recent report from our groups showed that interaction between MS2 capsids and multiwalled carbon nanotubes occurs in water and that this interaction was stable, even in high salinity conditions (Merryman et al. 2019). The implication of this work may extend beyond MS2 capsids and into other similar waterborne viruses (e.g., rotavirus, adenovirus, etc.) since many such virus capsids are decorated with similar amino acid residues, containing similar functional moieties (Dawson et al. 2005; Armanious et al. 2016). Similar studies to address NM–viral interactions in air or in hosts have not been documented. Such interactions in the environmental space will likely have a profound impact in controlling the fate and transport of viruses.

10.7.1.3 Interaction of NMs with Host Cells

NMs have been shown to interact with many cell types through a variety of different mechanisms. In some cases, NMs interact with the cell surface directly through spontaneous membrane penetration, essentially piercing the cell membrane, which can lead to complete internalization (Li et al. 2013b; Shi et al. 2011). This has been primarily studied for carbon-based NMs that are long and fiber-like. In contrast, there are numerous studies that support the cellular uptake of NMs through receptor-mediated mechanisms that lead to endocytosis.

Several cell types that are known targets for viruses and/or provide defense against viral infections have been shown to engulf NMs where the cell fate depends on the type of particle, dose, and cell type. For example, NMs can be phagocytosed and degraded by pulmonary macrophages (Lahiani et al. 2017; Meng et al. 2015), epithelial cells (Foldbjerg et al. 2014), brain microglia cells (Shigemoto-Mogami et al. 2016), and lymphocytes (Brown et al. 2007), among others. Macrophages and epithelial cells usually serve as first-line defense barriers for viral infections of the respiratory tract, and elsewhere, where they engulf NMs and activate cellular signaling pathways to induce inflammatory responses (Pondman et al. 2015). Once engulfed, both macrophages (and eosinophils) can degrade and clear NMs through peroxidase-driven mechanisms (Kotchey et al. 2013; Andon et al. 2013). However, the degradation is largely dependent on the specific NM-type and is highly influenced by size, surface chemistry, and surface area. For example, SWCNTs with oxygen moieties, but not the pristine form of SWCNTs, can undergo degradation more readily (Allen et al. 2009). Thus, CNTs with a lower degradability may stay longer and induce prolonged impacts on disease susceptibility within a host. It can be hypothesized that the ability of NMs to alter the abundance and response of cell populations to viral infections could lead to more (or less) severe infections. For example, limiting the number of macrophages available to clear virus particles or impairing their ability to recruit additional immune cells would essentially diminish the normal host antiviral defenses.

Specific to viruses, the ability of NMs to influence specific viral entry receptors has only recently been studied. For example, in order to enter cells, IAV binds primarily to sialic acid receptors which are small sugars attached to proteins within cell membranes. A number of chemically distinct forms of sialic acids exist which differ in the linkage of sialic acid to the sugar galactose. Joining the carbon atom at position 2 of the sialic acid hexose via an oxygen atom to the carbon at position 3 or position 6 of the hexose of galactose creates alpha 2,3 or alpha 2,6 linkages, respectively. These linkages differ in both their distribution in tissues (i.e., lung) and affinity for human IAV strains with preferential affinity for alpha 2,6 linkages (Ramos and Fernandez-Sesma 2012). Once viruses enter cells through these receptors, they form a replication complex and using the host cell machinery generate copies of their genomes that are subsequently packaged into viral progeny particles that are released from the cell. Recent observations by our group (manuscript in preparation) have shown that while CNTs do not alter the expression of the enzymes that add the receptor-specific moieties in lung epithelial cells, they can alter the number

of receptors on the cell surface. This is the first observation that NPs can influence select viral-specific receptor localization which may help to explain why these same NPs alter IAV infectivity. In other cases, NPs have been shown to significantly interfere with viral entry, although precise mechanisms have not been identified. For example, carbon nanodots formed with 4-aminophenylboronic acid hydrochloride impaired entry of herpesvirus into both Vero and A549 cells that was most prominent during the early stages of interaction between the virus and cell receptors (Barras et al. 2016).

10.7.2 Activation of Pattern Recognition Receptors (PRRs) and Related Signaling Pathways

Initial recognition of viruses by host cells is mediated by intracellular pathogen recognition receptors (PRRs) that interact directly with conserved molecular patterns present in lipid, lipoprotein, protein, carbohydrates, and nucleic acid moieties called pathogen-associated molecular patterns (PAMPs Fig. 10.2) (Kawai and Akira 2009). Activation of these receptors, including toll-like receptors (TLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors

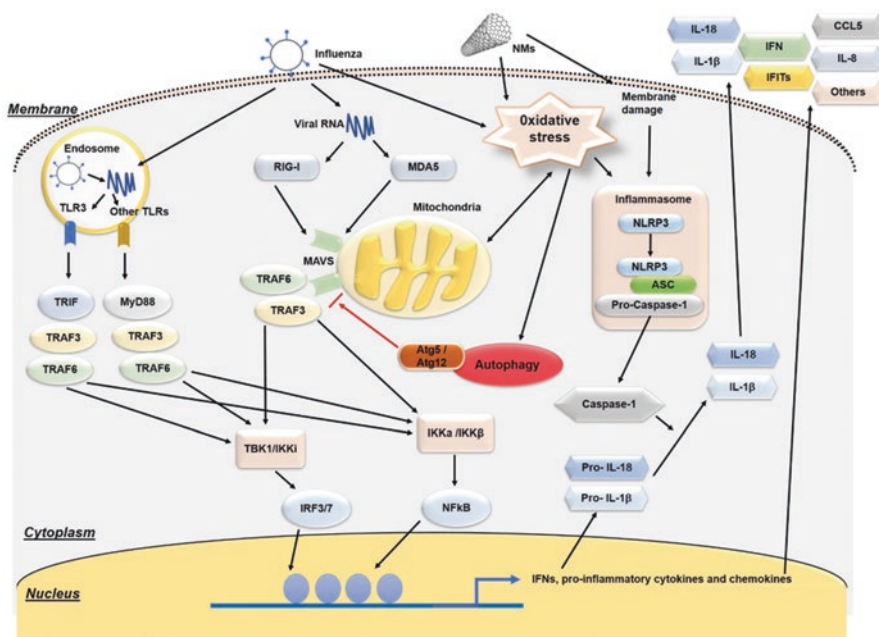


Fig. 10.2 Diagram showing the interplay of several signaling mechanisms that may be perturbed by NMs and viruses including endosomal PRRs (TLRs) and PRRs that work through the mitochondria (RIG-I-MAVS), inflammasomes, and how ROS may serve as a trigger

(RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and absent in melanoma 2-like receptors (AIM2) (Silva et al. 2017; Kawai and Akira 2009), leads to the production and release of IFNs that interrupt viral life cycles by directly interfering with their ability to replicate within an infected host cell. For example, both viral DNA and RNA can be recognized by several PRRs. Endosomal TLRs, such as TLR3, can recognize double-stranded RNA of viruses, while TLR7 and TLR8 can recognize single-stranded viral RNA (Owen et al. 2013). Surface TLRs such as TLR2 and TLR4 have also been shown to detect components of viruses that cause measles, hepatitis C, herpes, and respiratory syncytial virus which initiate pro-inflammatory immune responses (Koyama et al. 2008). While TLRs detect extracellular DNA and RNA endocytosed by cells, RLRs, including RIG-I and Melanoma Differentiation-Associated protein 5 (MDA5), are cytosolic receptors required for detection of intracellular viral RNA (and sometimes viral DNA) from actively replicating cells (Dixit and Kagan 2013). RIG-I and MDA5 can form a complex that activates mitochondrial antiviral signaling (MAVS) to induce type I IFNs and pro-inflammatory cytokines.

An interesting hypothesis has emerged that suggests NMs can modulate PAMP signaling networks either through direct interaction with receptors or indirectly through production of endogenous ligands. With respect to the former, one study proposed that CNTs interact directly with TLR homo- and heterodimer extracellular domains based on a computational model (Turabekova et al. 2014). By using X-ray structuring and mathematical modeling, they discovered that the internal hydrophobic pockets of some TLRs may be capable of binding select carbon nanostructures, such as SWCNTs, containing 11 carbon atom layers (Turabekova et al. 2014). Furthermore, they showed this relationship only for select TLR subtypes, specifically TLR1/TLR2, TLR2/TLR6, and TLR4/myeloid differentiation factor-2 (MD-2). They suspected that this interaction would lead to activation of the receptor and initiation of subsequent downstream immune responses (Turabekova et al. 2014). While this report did not test TLR activity, a few alternate studies have done so and have shown that both MWCNTs and SWCNTs can activate the TLR2/4–MyD88–NF κ B signaling pathway in macrophages which leads to chemokine secretion (Meng et al. 2015; Mukherjee et al. 2018). Additionally, noninfectious stimuli from either “self” or the environment, including hydrophobic regions of CNTs, may be categorized as danger-associated molecular patterns (DAMPs), which are involved in the activation of NLR pathways (Silva et al. 2017; Mason et al. 2012). Interestingly, one study found different profiles of gene expression for TLR signaling, NLR signaling, and downstream signal transduction between CNTs and their depyrogenized versions, indicating the possibility of endotoxin or microbial contamination of CNTs leading to misinterpretation of PRR activation by CNTs (Lahiani et al. 2017). Importantly, the ability of NMs to impact PRRs specific to viruses (i.e., TLR3) has not been as well studied. Recent work by our group has shown that SWCNTs can impair TLR3 activity that is normally induced by the viral analog polyinosinic:polycytidylic acid (poly I:C) and IAV which leads to downstream expression of genes that combat viral replication. Furthermore, these same NMs also impact RIG-I–MDA5 signaling by impairing the ability of the RIG-I–MDA5 complex to “dock” with mitochondrial antiviral signaling (MAVS) protein on

the mitochondrial membrane (Chen et al. 2019). Interruption of this docking mechanism leads to the reduced expression of antiviral genes, leaving the host cells more susceptible to increased virus titers.

10.7.3 Production of Oxidative Stress and Mitochondrial Dysfunction

Oxidative stress occurs when antioxidant mechanisms in a cell or body fail to scavenge overproduced reactive oxygen species (ROS), causing further organelle damage and cell apoptosis (Dong and Ma 2015; Shvedova et al. 2012). Such cellular stress can cause oxidation of biomolecules, such as lipids, proteins, and DNA, and can either activate or impair signal transduction of immune responses (Weinberg et al. 2015).

As mentioned above, RIG-I-MDA5 controls antiviral signaling pathways through docking with MAVS which is located on the mitochondrial membrane, and its function is highly dependent on mitochondrial structure and function (Weinberg et al. 2015). Because the mitochondria are known to serve as a source of ROS production, they can also be damaged by ROS. As many NMs are known to generate ROS production, it is possible that they can induce mitochondrial dysfunction and as a result, affect downstream innate immune responses (Kim et al. 2015). In a recent study, ROS produced by SWCNTs was shown to alter mitochondrial membrane potential and the expression of genes involved in mitochondrial function (fission, fusion). Furthermore, these same SWCNTs impaired mitochondrial-dependent antiviral pathways (RIG-I-MAVS) which led to high IAV titers (Chen et al. 2019).

The literature is not consistent on the role of ROS in response to viral infections as some strains cause ROS and others do not. However, it is known that oxidative stress-related protein complexes called stress granules (SGs) commonly assemble in infected cells. These SGs contain several ribonucleoproteins (mRNPs) and also the same RIG-I-MDA5 proteins found in the mitochondria which can induce the activation of protein kinase receptor (PKR)/eukaryotic translation initiation factor 2a (eIF2a) pathways (Yoneyama et al. 2016). Thus, oxidative stress induced by NMs may also affect the formation and/or function of SGs, formed in response to viruses which also may interfere with the normal defense response to an infection.

10.7.3.1 Stimulation of Autophagy

Autophagy is a cellular process that promotes energy efficiency and mediates damage control of cells that experience various kinds of stimuli or stresses, such as oxidative stress (Glick et al. 2010). Autophagy is also associated with disease susceptibility and related outcomes to pathogenic infections; for example, the hemagglutinin protein of influenza virus can lead to autophagy-related cell death and exacerbate

viral infections in mice (Sun et al. 2012). Autophagy is proven to be a mechanism for negative-stranded RNA viruses to evade host's immune responses as the viruses can regulate and exploit the cellular state of autophagy (Wang et al. 2018).

Studies have shown that NMs can induce autophagy. Park and colleagues found that exposure of BEAS-2B cells to SWCNTs led to higher expression of autophagic genes and proteins with increased formation of autophagosome-like vacuoles (Park et al. 2014a). Similarly, another study discovered that high-pressure carbon monoxide conversion (HiPco SWCNTs) can induce autophagosome formation in a macrophage cell line, RAW264.7 (Park et al. 2014b). As discussed earlier, functionalization of CNTs is a major factor in determining their toxicity. SWCNTs functionalized with carboxylic acid induced formation of autophagosomes through the protein kinase B (AKT) - tuberous sclerosis complex 2 (TSC2) - mammalian target of rapamycin (mTOR) pathway in A549 cells (Liu et al. 2011). An autophagy study on 81 CNTs with different functional groups showed that the modified surface chemistry on CNTs determined the capacity for and specific pathways of inducing autophagy (Wu et al. 2014). Thus, autophagy as a result of NM exposure may push cells to a state where pathogens can benefit, leading to exacerbation of infection. Interestingly, some key proteins that regulate autophagy are also important in controlling pathogen-induced innate immune pathways. For example, the higher expression of *Atg5* and *Atg12* is negatively associated with interferon production through the RLR/MAVS signaling pathway (Takeshita et al. 2008).

10.7.4 *Inflammasome Activation*

Several types of NMs, such as CNTs, can cause pulmonary inflammation through activation of several innate immune sensors associated with oxidative stress, such as nucleotide-binding oligomerization domain (NOD)-like receptor pyrin domain-containing 3 (NLRP3) inflammasome (Girtsman et al. 2014; Dong and Ma 2016; Palomaki et al. 2011). The NLRP3 inflammasome is the most extensively studied inflammasome complex, and is activated by a wide range of environmental stimuli, including bacteria, viruses, and ROS (Dong and Ma 2015; Palomaki et al. 2011; Chen et al. 2015; Sarvestani and McAuley 2017). The main function of NLRP3 is cleaving protease-1 to activate caspase-1 and trigger the secretion of downstream pro-inflammatory cytokines, interleukin (IL)-1 β and IL-18 (Sarvestani and McAuley 2017).

Studies have shown that *Chlamydia pneumoniae* and SWCNT co-treatment synergistically enhanced IL-1 β secretion in macrophages in a time- and dose-dependent manner via the NLRP3 inflammasome and caspase-1 activation pathways (Matsuo et al. 2015). This IL-1 β secretion can be reduced by either inhibition of NLRP3 expression or blocking ROS production by N-acetyl-L-cysteine (Matsuo et al. 2015; Geiler et al. 2010). Another study indicated that CNTs and TLR agonists synergistically promoted activation of NLRP3 inflammasome and production of IL-1 β , an essential process to enhance inflammatory activity (Yang et al. 2013). Interestingly,

the secretion of downstream IL-1 β was not affected by SWCNTs, either alone or in combination with IAV in mouse bronchoalveolar lavage fluid (BALF) (Chen et al. 2017). In this study, the length of exposure to CNTs might be a factor (Chen et al. 2017; Shvedova et al. 2008), since other studies indicated a much earlier peak (day 1) in secreted IL-1 β in the BALF of mice treated with SWCNTs (Shvedova et al. 2005). SWCNTs and *Listeria monocytogenes* or *Toxoplasma gondii* have also been shown to synergistically induce IL-1 β (Shvedova et al. 2008; Swedin et al. 2012), but with lowered ROS production compared to those treated with CNTs only. SWCNTs can also serve as a negative regulator of NLRP3 inflammasome activation via ROS, leading to reduced inflammatory responses in Caco-2 cells infected by *Escherichia coli* and *Staphylococcus aureus* (Chen et al. 2015). The synergism of NLRP3 activation in the co-exposure scenario might result from mechanisms that include PRR pathways with ROS production (Sarvestani and McAuley 2017). This also indicates that the mechanism of NM impacts on pathogen infection likely involves multiple signaling pathways. It is noteworthy to mention that most of the studies performed to date related to inflammasome regulation by NMs and pathogens involve bacteria and fungi, with little focus on viruses.

10.7.5 Modulation of Lipid Signaling Networks

Lipids are naturally occurring hydrophobic biomolecules that play an essential role in cellular life from membrane formation to energy storage (Voelker 1991). In addition, lipids also serve as effectors and second messengers in a variety of biological processes that are associated with the immune system, both innate and adaptive (Cabral 2005; Getz and Reardon 2014). For example, the Class I PI3-kinase (PI3K) signaling pathway can be activated by a variety of viruses, such as influenza virus, to dock proteins carrying lipid domains and is also essential for viral entry and fusion (Mazzon and Mercer 2014). The activation, differentiation, and function of immune cells, such as macrophages and T lymphocytes, is also highly regulated by lipids such as cholesterol and fatty acids (Hubler and Kennedy 2016). In response to respiratory viruses, specifically, lipids are known to modulate host–pathogen interactions in several ways, acting as major players in viral-induced membrane fluidity, lipid rafts and viral entry receptors, formation of viral replication complexes, and modulation of host cell metabolism – which in turn afford advantages to the virus in completing its life cycle (Garcia-Sastre 2013; Heaton and Randall 2011; Tanner et al. 2014).

The importance of the lipid landscape in lung disease states has only recently been underscored. Several contemporary studies have begun to decipher the major classes of lipids, and in some cases, quantify select lipids that are present in normal lung cells and tissues. In the lung, significant changes of the lipidome are associated with asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and pathogenic infection (Zehethofer et al. 2015; Zhao et al. 2014; Köberlin et al. 2015). In fact, lipids are now recognized as major regulators of immune responses, and

lipid networks are being considered as predictive markers of pathogen responses in patients, as well as in rodents (Köberlin et al. 2015). Recent advancements in liquid chromatography-mass spectrometry (LC-MS) technologies have allowed for global “omic” analysis of lipids. Studies performed to date utilizing this technology have shown that lipid biosynthesis, metabolism, and the profile of the lipids present can all be perturbed by disease state. Sphingolipid metabolism was strongly regulated when pathogen sensing pathways were perturbed by commercial agonists (to Toll-like receptors) in human lung cells or in a mouse model of experimental asthma (Zehethofer et al. 2015; Köberlin et al. 2015). Furthermore, changes in biosynthesis of these perturbed lipid profiles correlated with the expression of related lipid biosynthesis genes. Specific to influenza, exposure of ferrets to both historic and contemporary strains of pandemic influenza A virus revealed relationships between altered lipid metabolism and inflammation (Tisoncik-Go et al. 2016). For example, the sphingomyelin module (group of species with similar co-expression relationships) was positively correlated with innate immune and lipid metabolism gene modules, as well as virus titer and levels of viral mRNA (Tisoncik-Go et al. 2016). In mouse models, distinct patterns of lipid metabolites were associated with the pathogenic (5-lipoxygenase metabolites) and resolution phases (12/15-lipoxygenase metabolites) of influenza A infection (Tam 2013). Several reports now show that cells infected specifically with IAV increase *de novo* fatty acid and sphingolipid biosynthesis and unique cholesterol compositions while lowering levels of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) species and omega-3 polyunsaturated fatty acid (PUFA)-derived lipid mediator, protectin D1 (Tanner et al. 2014; Murray et al. 2009). These changes have been linked with regulation of viral budding, viral replication and pathogenicity.

Additionally, there is substantial interest in the use of lipids as diagnostic and prognostic biomarkers (t'Kindt et al. 2015). Several studies have provided evidence that lipids (and/or metabolites) can be used to differentiate between disease states, such as community acquired and noncommunity-acquired pneumonia cases (To et al. 2016), and between healthy individuals and asthmatic patients (Kang et al. 2014). Previous work by t'Kindt and colleagues was able to identify 1584 unique lipids in induced sputum and demonstrated that sphingolipid profiles in sputum samples were similar between smokers without COPD and never-smokers but were very different from profiles of COPD patients (t'Kindt et al. 2015). Taken together, these studies demonstrate a substantial role for alterations in lipid metabolism during the course of viral infection and disease progression, and suggest that these patterns of alterations are distinct enough to have clinical relevance in diagnosis, prognosis, and treatment.

Though little is known, a few studies provide evidence to support that NMs are capable of altering lipid profiles in cells with impacts on the immune system. For example, long and stiff CNTs can damage membranes of lysosomes, which are responsible for breaking down diverse biomolecules and debris, including phagocytosed pathogens (Zhu et al. 2016). The interaction between pristine or functionalized CNTs and cellular membranes was investigated using computational models, and the results indicated that the distribution and orientation of CNTs within the

lipid bilayer membranes were largely dependent on the functionalization of the CNTs (Baoukina et al. 2013). A lipidomic study on all major phospholipid classes revealed that lipid peroxidation in mouse lung tissues exposed to SWCNTs through inhalation was the major oxidative damage mechanism (Tyurina et al. 2011). The characterized specific phospholipid peroxidation pathway pointed to the mitochondria as the target of CNT toxicity (Tyurina et al. 2011). Another study in which mice were exposed to SWCNTs via a single intratracheal instillation revealed an upregulation of fatty acid β -oxidation 13 weeks postexposure, a change that is likely due to stress in the endoplasmic reticulum and mitochondria (Park et al. 2016). These studies support and circle back to the notion of how oxidative stress induced by CNTs may play an important role in immune responses to pathogenic infections, which was previously discussed. Another report investigated the ability of CNTs to change global phospholipids in SWCNT-exposed cells and demonstrated the modification of several lipid classes (Wu et al. 2011) that are highly relevant to IAV infections. No studies to date have studied the changing lipid landscape in response to varied carbonaceous NMs and how such changes modulate viral infections.

Nano-TiO₂ exposure has also been shown to impact lipid metabolism and profiles in ways that could have downstream impacts on immune function. For example, mice exposed intranasally to nano-TiO₂ exhibited increased peroxidation of lipids as a result of ROS production, as well as increased expression of arachidonate 5-lipoxygenase-activating proteins (ALOX5AP) that regulate biosynthesis of leukotrienes, pro-inflammatory lipid mediators secreted by inflammatory cells (Li et al. 2013c). A study on liver cells (HepG2) of several nano-TiO₂ particles of differing sizes and surface areas demonstrated impacts on cholesterol biosynthesis, bile acid synthesis, zymosterol (cholesterol precursor) biosynthesis, free fatty acid availability, and fatty acid activation after exposure to high doses of nano-TiO₂ (Thai et al. 2016).

10.8 Future Perspectives

The advent of emergent technologies, such as the development of NMs, has and will continue to greatly enhance technological advancements in industrial, consumer, environmental, and biomedical sectors, among others. However, it is important that we understand the potential health and safety impacts that are relevant to real work settings. These settings include both single exposures to NMs and co-exposures with relevant viruses that carry tremendous health burden. As discussed, NMs have the ability to modulate normal immune responses to viruses and some reports suggest that this leads to worsened infections. However, the number of studies that have analyzed such outcomes are still relatively small, despite the potential consequences to exposed populations. Much of the work to date has focused heavily on CNTs and a few select viruses (i.e., IAV, RSV) but there is much work to be done regarding the commonalities in immune modulation between different types of NMs and viruses. Defining which stages of the viral life cycle are impacted by NMs would offer

tremendous knowledge that is not only useful to the health and safety field, but would uncover mechanisms that could be essential in developing vaccines or other viral therapeutics. Very little data exist regarding the ability of NMs to interact directly with viruses or viral moieties and how such interactions alter environmental fate and transport (and ultimately exposure) and manipulation of normal immune responses to NMs or viruses singly which are likely distinct to some extent. Furthermore, the ability of NMs to alter interactions of viruses with their cell surface receptors and PRRs is an intriguing mechanism that warrants further investigation. The development of technology for generation of lipid profiles has highlighted the importance of lipids as both structural and signaling molecules that play important roles in viral life cycles. This suggests yet another mechanism that can be considered in NM–viral studies. Finally, model systems for testing viral infections with nanoparticulates need further development as standard models used to assess toxicity of one stressor may not be suitable for the other (i.e., some viruses cannot be adapted to mice). In addition, testing of dual stressors has not yet made use of more complex (i.e., 3D) tissue culture systems or organoids which may provide a valuable platform for testing the wide array of NMs and viruses.

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