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REVIEW ARTICLE

The Impact of Nanomaterials in Immune System

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As a nanotechnology has been actively applied to the overall areas of scientific fields, it is necessary to understand the characteristic features, physical behaviors and the potential effects of exposure to nanomaterials and their toxicity. In this article we review the immunological influences induced by several nanomaterials and emphasize establishment of the animal models to estimate the impact of these nanomaterials on development of immunological diseases.
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INTRODUCTION

A nanotechnology which has appeared since the mid-twenties century has provided the methods by which the limitations in the industrial application of each scientific technology could be overcome. It has also brought another revolutionary change to the overall areas in the scientific technology and the related industries. A nanotechnology has become a basis of the integrated development of scientific technology which has independently been developed. It has also been actively applied to the overall areas of scientific fields. The characteristics of nanomaterials are subject to their surface property, according to which the desirable physical property can be given deliberately to them.

As described here, products manufactured using nanomaterials are characterized to overcome the areas which cannot be resolved with the previous technology. It can therefore be stated that they have a higher degree of applicability. Synchronously, there is an increasing concern that they would be new hazardous factors for both humans and nature (1). In identifying and then clarifying risks due to exposure to nanomaterials, it is extremely important to clarify the physicochemical property and physical behavior of nanomaterials and to understand the biological and physiological actions of them in an *in vivo* setting (2). Based on this, a systematic approach should be made to examine the possible detrimental effects on the production, environment and health. In general, methods for assessing the detrimental effects of conventional types of chemicals are divided into the definition of detrimentalness, the assessment of dose-response, that of exposure and that of detrimental characteristics. These methods can also be applied to nanomaterials. In nanomaterials, however, as their surface area is increased, their responsiveness and toxicity to viable tissues are also increased (3). It is of prime importance to clarify new physicochemical property and to evaluate the resulting toxicity of them. Accordingly in the assessment of detrimentalness of nanomaterials, it is necessary to understand the characteristic features, physical behaviors, the potential effects of exposure to nanomaterials and their toxicity (4).

IMMUNOLOGICAL EFFECTS OF NANOMATERIALS

Nanomaterials have a structure that the size of one of three surfaces is 1～100 nm according to the definition made by ISO/TC 229. As described here, based on a smaller size of ＜100 nm, nanomaterials have physicochemical properties which are greatly different from general types of other substances. Due to a higher degree of surface reactivity and the cell membrane permeability, an *in vivo* influx of nanomaterials produces the stress on a cellular level.

Until the year of 2006, 69% of total manuscripts published on a peer-reviewed journal are related to the toxicity and the remaining 31% are related to the exposure. In particular,

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since 2008, research articles about the toxicity have been abruptly increased. These articles have reported the pulmonary toxicity, neurotoxicity and the permeability to blood-brain-barrier. Besides, peer-reviewed articles about the immunotoxicity have also been increasingly published.

Nanomaterials are crystalline, fine particles with a large surface area, and they have a higher degree of surface charge and proton exchangeability. Besides, it is also expected that they might have a positive effect on the environment because they facilitate the synthesis and transportation of contaminants in the environment. In addition, the potential problems of nanomaterials are not limited to the environmental pollution. By various routes such as an inhalation, they are absorbed into the body and then induce the biological toxicity (5,6). This makes nanomaterials further interesting. The possible routs by which nanomaterials migrate in the human body include the following:

1) Endocytosis: Nanomaterials enter the cells when they are surrounded by the cell membrane without passing it.

2) Cell membrane penetration occurring with the action of hydrophobic particles.

3) Transportation of nanoparticles with a size of <5 nm across the cell membrane channel.

As described here, it has been proposed that the contactable nanoparticles might stimulate the immune system in the body. By contrast, recent studies have been conducted to examine industrial nanoparticles. Industrial nanoparticles are mainly contacted in a working place by daily commodities or pharmaceutical products in customers. The total amount of exposure may be relatively lower or the actual one occurring in the local tissue might be relatively higher. Nanoparticles produce oxygen radicals (7,8). Besides, the mitochondrial perturbation and apoptosis (9) are induced, which are followed by the cytotoxicity. With the reactions with body proteins and other biological substances, various types of hazardous reactions might occur.

One of the advantages of nanoparticles, a biological permeability makes an intracellular and intranuclear influx of nanomaterials possible. Based on these findings, it has therefore been postulated that the oxidative stress and inflammatory responses induce the occurrence of immunotoxicity directly or indirectly. It is therefore imperative that the technology for assessing the exposure be established to examine the physicochemical properties of nanomaterials and to clarify the related immunotoxic mechanisms.

Peer-reviewed articles about the toxicity of nanomaterials

Table I. Immunotoxicity of carbon-based nanomaterials

Nanomaterials	Summary	Reference no.
Carbon black (<100 nm)	Induction of MCP-1, CCL2, IL-6, C-reactive protein and exaggeration of arthrosclerosis in animals	25
Carbon black (14 nm)	Induction of slight expression of CD80 and MHC class II and significant expression of CD86 and DEC205 in endothelial cells	26
Single-walled carbon nanotubes (PEG coating 1~5 nm in diameter, 50~200 nm in length)	Persistence of SWNT for several months in kidney and liver without obvious toxicity	27
Single-walled carbon nanotubes (1~4 nm in diameter)	Biodegradation of single-walled carbon nanotubes by hypochlorite and ROS mediated byf human neutrophil myeloperoxidase	28
Single-walled carbon nanotubes (1~2 nm in diameter 20 nm-several μm in length)	Induction of ROS, inflammatory cytokines and expression of apoptosis related genesin macrophages	29
Single-walled carbon nanotubes (800 nm length)	Inhibition of production of IL-8, 6, TNF-α and MCP-1 in A549 cells	30
Multi-walled carbon nanotubes (10~30 nm in diameter 30~50 nm in length)	Induction of fibrosis in asthma animal model and suggestion of the role of TGF-β and PDGF	31
Multi-walled carbon nanotubes (20~40 nm in diameter, 5~30 μm in length)	Induction of ROS, inflammatory cytokines and activation of NF-kB in A549 or BEAS-2B cells	32
C_{60} fullerene (0.7 nm in diameter)	No toxicity in animal lung	33

have been summarized based on the chemical constituents. Based on the classification system into carbon nanomaterials, metals, metal-oxide nanomaterials and other silicas, a review of the above peer-reviewed journals was made. Results were represented in Tables I, II and III. For example, of carbon nanomaterials, carbon black induces the immune responses. In particular, multi-walled carbon nanotubes (MWCNT) rather than single-walled carbon nanotubes (SWCNT) induce the activation of immune responses to a greater extent (Table I). Of metals and metal oxides, iron oxide nanoparticles strongly induce the immune responses. Besides, gold nanoparticles induce a moderate degree of the immune responses. Furthermore, TiO_2 induces a lower degree of the immune responses (Table II). In addition, it has also been reported that such substances as silica, polystyrene and latex nanomaterials have an immunotoxicity (Table III).

Table II. Immunotoxicity of metal-based nanomaterials

Nanomaterials	Summary	Reference no.
TiO_2 (0.02~0.03 μm)	Induction of ROS but not TNF-α in respiratory epithelial cells	34
TiO_2 (4~6 nm, rutile)	Induction of monocytes and lung inflammation, cardiac edema and systemic inflammation but decrease of platelets	35
TiO_2 (20 nm)	Engulfment of TiO_2 by alveolar macrophages within24 hours in animal treatment	36
TiO_2 (15, 50, 100 nm)	Induction of histamine release without allergens	37
TiO_2 (<100 nm)	Induction of apoptosis and necrosis in macrophage cells	38
Gold (13 nm)	Induction of apoptosis and acute inflammation in liver and localization of nanoparticles in Kupffer cells of liver and macrophages in spleen	39
Gold (2, 40 nm)	Internalization by both microglial cells and primary hippocampal neurons and toll-like receptor 2 up-regulation in the olfactory bulb; up-regulation of TLR-2, IL-1α, GM-CSF and nitric oxide in microglia	40
Gold (0.8~15 nm)	No changes in mRNA induction of pro-inflammatory (TNF-α, IL-8, iNOS) and oxidative stress markers (HO-1, SOD) as well as IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, GM-CSF, TNF-α, INF-γ	41
Fe_2O_3	Induction of pro-inflammatory cytokines (IL-1, TNF-α, IL-6), T_H0 cytokine (IL-2), T_H1 type cytokine (IL-12), T_H2 type cytokines (IL-4, IL-5), TGF-α and IgE	42
Fe_2O_3	Decrease in cell viability associated with significant increases in lactate dehydrogenase activity, IL-1α and ferritin expression	43
Zinc oxide (11 nm), cerium oxide (8 nm)	Induction of ROS, apoptosis and inflammation by ZnO but inhibition of ROS by CeO_2	44

Table III. Immunotoxicity related of silica or polystyrene-based nanomaterials

Nanomaterials	Summary	Reference no.
Silicon	No acute irritation in HaCaT keratinocytes, a human skin equivalent model (HSEM), and *in vivo* mouse model	45
Silica particles (12 nm)	Increased blood level of IL-1β and TNF-α in blood, and increased release of nitric oxide released from peritoneal macrophages; increased. mRNA expressions of IL-1, IL-6, TNF-α, iNOS, and COX-2	46
Silica (70, 300, 1,000 nm)	Induction of liver damage and inflammatory cytokines by 70 nm particles, but not 300 or 1,000 nm particles	47
Polystyrene (60 nm)	Highly toxic to BEAS-2B cells, human microvascular endothelial cells, hepatoma cells, microvascular endothelial cells and macrophages	48
Polystyrene nanoparticles (20, 500, 1,000 nm)	Uptake of polystyrene nanoparticles by dendritic cells and migration of dendritic cells to lymph nodes	49
Latex nanomaterial (25, 50, 100 nm)	Induction of fibrinogen, MCP-1 by 25 or 50 nm particles, but not by 100 nm particles	50

THE NECESSITY FOR CONDUCTING STUDIES ABOUT THE RELATEDNESS TO DISEASE THROUGH AN ANIMAL EXPERIMENTAL MODEL OF PULMONARY DISEASES IN AN *IN VIVO* SETTING

Relatedness of nanomaterials to diseases can be presumed based on studies about the particulate matter (PM) associated with air pollution and the diseases (10-12). A long-term exposure to particulate matter (PM) increases the incidence of complications associated with pulmonary (13,14) and cardiovascular diseases (15) and the resulting mortality. The occurrence of malignant mesothelioma due to an inhalation of asbestos fiber (16,17), which has become a socially issue in recent years, well illustrates the hazardousness of microparticles. Particulate matters (PM10) of $<10\,\mu$m in size are associated with respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), acute/chronic bronchitis and lower respiratory tract diseases and cardiovascular diseases such as myocardial infarction, arrhythmias and arteriosclerosis. Besides, particulate matters (PM2.5) of $<2.5\,\mu$m in size have a poorer effect on the respiratory system and cardiovascular one as compared with PM10 and they also raise the mortality. An analysis was performed for the correlation between the concentration of PM2.5 and the mortality in the USA, according to which 100,000 people are found to die directly or indirectly due to PM2.5 every year.

The number of particulate matters in the atmosphere has been reported to be associated with the occurrence of respiratory diseases. Since then, it has been proposed that nanoparticles inhaled through a mathematical model of the expansion of human lungs might be deposited in an area ranging from the trachea to terminal bronchioles. The surface area of particles rather the amount of particles are associated with the occurrence of inflammatory responses. At the present, no studies have provided the possibility that carbon nanotubes, nano-fibers and nano-wire products might induce the detrimentalness to such an equivalent extent to asbestos. Nevertheless, to rule out this possibility, it is imperative that the safety following a long-term exposure to above materials be audited. An experimental animal model of a long-term exposure would therefore be mandatory.

Nanoparticles contained in particulate matter are known as a strong inducer of oxidative stress in macrophages and epithelial cells lining the airway tract. They increase the activity such molecules as MAP kinase, NF-κB and AP-1 and also promotes the synthesis of oxygen radicals (18,19). This phenomenon is explained as one of the key pathophysiologic mechanisms by which COPD occurs, one of the representative chronic inflammatory respiratory tract diseases. It has been hypothesized that COPD might be a type of dust-induced diseases. In this regard, the use of particulate matters and biomass fuel which has recently been of increasing interest has been considered as a pathogenesis of COPD. Nanoparticles contained in the atmosphere have a possibility for aggravating the respiratory diseases.

In a murine model of asthma, one of the respiratory diseases, particulate matter of $0.2\sim10\,\mu$m in size was experimentally administered. According to this experiment, as the size of particles was relatively smaller, the deposition to the lower respiratory tracts was increased. Following the administration of microparticles into the airway tract in the above experimental model, the concentration of protein in the bronchoalveolar lavage is increased (20) and this is also accompanied by the increased occurrence of eosinophilic and neutrophilic inflammations occurring in the airway tract and the elevated levels of T_H2 cytokines (IL-4, IL-5 and eotaxin) (21,22) as well as T_H1 cytokines (IFN-γ, IL-6 and TNF-α) (23). Besides, following an intranasal administration of oil flash ash, a key substance for pathological phenomena due to particulate matters in a murine model of asthma, the occurrence of respiratory hypersensitivity and eosinophilic inflammation was further increased (24).

In regard to the effects of nanoparticles on the asthma, a murine model of albumin-induced asthma has shown that the inflammatory responses and the production of immunoglobluins were increased by nanoparticles in the respiratory tract (Fig. 1). These inflammatory phenomena are explained by the expression of IL-5 and eotaxin which was induced in the

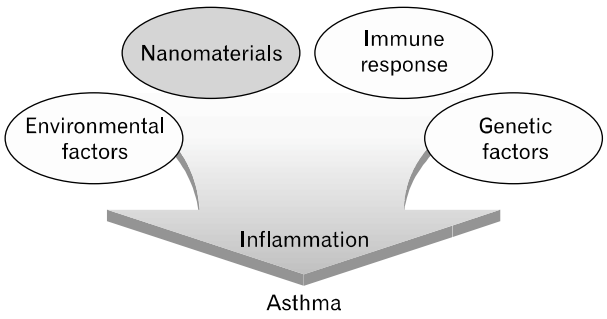

Figure 1. Potential contribution of nanomaterials on asthma pathophysiology.

early stage of inflammation. Subsequently, with the induced expression of such cytokines as IL-13, RANTES and MCP-1, the degree of inflammation was further increased. Moreover, based on the reports that TiO_2 which has frequently been used for cosmetic products aggravates the atopic dermatitis, it has been proposed that nanoparticles might aggravate the immune diseases.

In conclusion, with the development of nanotechnology, a great deal of nanoproducts and the related materials have been produced. Due to a lack of the clarification of the potential hazard of technical development, however, demand and supply of the products have not been activated up to present. At the present, therefore, if guidelines or landmarks should be provided for safety assessment or data about the safety range including the immune toxicity of nanomaterials, this would greatly contribute to the activation of nanoindustry.

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CONFLICTS OF INTEREST

The author have no financial conflict of interest.

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