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COMMENTARY

Scale of Health: Indices of Safety and Efficacy in the Evolving Environment of Large Biological Datasets

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ABSTRACT The interdependent relationship between pharmacology and toxicology is fundamental to the concepts of efficacy and safety of both drugs and xenobiotics. The traditional concept of establishing efficacious and tolerated doses to define a 'therapeutic window' appears simplistic in the context of an exponentially increasing database on molecular mechanisms and cell biology that inform our understanding of homeostasis. Recent advances in nano medicine illustrate the convergence of efficacy and safety considerations that are central to establishing a clear pathway for regulatory review. The following overview considers biological responses to the administration of nanoparticles and the scale of balanced, within a range that might be considered 'normal', to unbalanced, abnormal responses associated with health and disease.

KEY WORDS drugs · immunity · inflammation · nanoparticles · vaccines

INTRODUCTION

There are a variety of biological measures along the spectrum of a "healthy" to a "diseased" individual. This spectrum, which may be considered as a continuous scale of health, represents a holistic approach to assessing safety of materials. Materials that impact on the position of an individual in this spectrum could include traditional and non-traditional pharmaceuticals, personal health care products or components of new technologies found in the environment. The framework discussed here suggests a procedure for information across the range of toxic and efficacious responses to be gathered for informed decision making in either the regulatory or industrial environment. For the purposes of discussion the focus has been limited to pulmonary exposures. Pulmonary immunological and inflammatory markers comprise a portion of a larger comprehensive predictive model of toxicological responses following exposure to particles. However, because the lungs are often the primary route of exposure for particulates and because they often determine the ultimate physiological response, they are deserving of the focus of this discussion.

Therapeutic and environmental exposures to nanoparticles are examples of the need for good approaches to interpreting biological responses. Nanoparticles that have been used in arrange of industrial processes in which exposure is a toxicological consideration are now being considered for drug and vaccine delivery. This appears to be a direct practical convergence of efficacy and safety considerations [1–3].

These systems require consideration of defined input variables within a manageable study design, resulting in biological response outputs within the hierarchical stress model (Fig. 1). The input variables are inorganic and organic nanoparticles with defined physicochemical properties. The output variables are predominantly immunological and inflammatory responses. Utilizing carefully crafted hypothesis-based experiments and associated risk mitigation, the production of data suitable for the development of industry and regulatory standards is possible. Furthermore, this data is critical in the development of a critical-path decision process in safety and efficacy assessment sufficient to guide technology development. A multidisciplinary approach is essential to succeed in both research and development efforts (Fig. 2).

THE CONVENTIONAL WISDOM ON NOAEL

There is a need to transition the paradigm of safety and toxicity related to exposure to nanoparticle aerosols for

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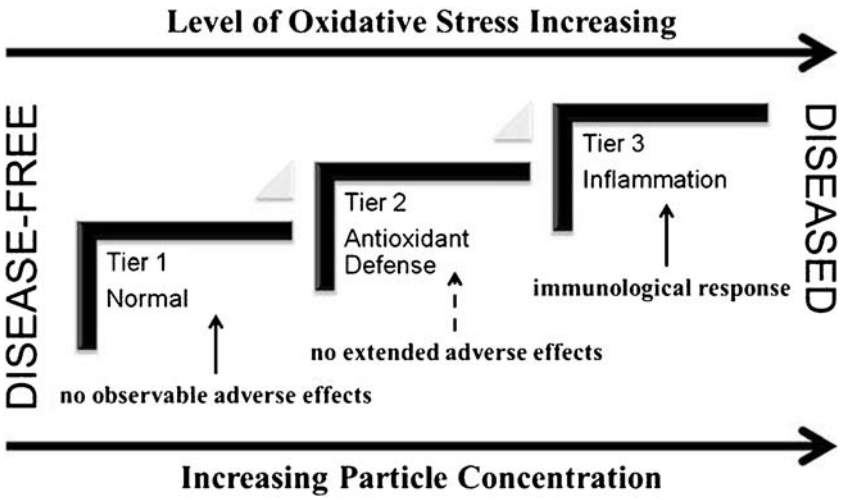

Fig. 1 Hierarchal Oxidative Stress Model. Similar to increasing particle concentration, other physicochemical properties, such as surface charge or aggregate size, can influence oxidative stress. It is noteworthy to mention that some observed inflammatory responses can also be classified with "no extended adverse events".

pharmaceuticals. One approach is to move beyond the traditional no observed adverse effect level (NOAEL) to safety. For the purpose of discussion, a new standard might be proposed for a no extended effect level (NEEL) relevant to common human experiences [4]. Since not all effects can be considered adverse this shift enables rational design of therapeutic particles and defines trends towards toxicity. The resultant data and data interpretation could balance the understanding derived from cell culture and animal experiments. Cell culture, in which human cell lines or primary cells are employed, offers the advantage of occupational or clinical relevance but also the limitation of isolation from tissue/organism with respect to biochemistry and biophysics. Animal studies demonstrate phenomena related to the whole organism while having the limitation of species-specific biology. The scientific validity of these models and cost and time considerations affect decision-making. The assumption that 'no observed adverse effect' is the criterion for safety requires reassessment as it has implications for individual and population health, regulation and control of medicines, and industrial or consumer emissions. Clearly, the definition of 'observed' and 'adverse' in this context has great significance. With current technology many things can be observed and detection levels can often be at the molecular level. Since much can be observed then how does a change in a particular observation rise to the level of an adverse effect? Addressing these questions would seem to be a philosophical necessity in this rapidly changing period of scientific and technical achievement. Table 1 compares and contrasts the quantitative and qualitative natures of NOAEL *versus* NEEL.

As this approach evolves it will move decision-making from historically mandated empirical testing protocols for data collection and pragmatic interpretation to holistic scientific observations that support clear but more informed interpretations. This would potentially allow products to proceed to later stages of development where a more thorough understanding of the effects they elicit may result in higher probabilities of success without incurring additional risk.

The NOAEL approach has been very valuable through most of the last century when large, continuous data sets could not be measured or stored due to the limitations of methodology and information science. It facilitated a very cautious risk management approach that has served society well. The last two decades has seen a revolution in real-time data collection and the ability to store and retrieve data that makes the potential for a new, NEEL, approach attainable. It is hoped that the new approach will give greater sensitivity to biological events and improve the decision making process by increasing the amount of data being considered and its underlying meaning and reducing the potential for error in the risk/benefit assessment.

Nanotechnology broadly, and nano medicine in particular, has raised many questions regarding the balance between efficacy and safety. These have yet to be adequately answered

	Hemostasis	Inflammation	Proliferation	Maturation	
	MINUTES	HOURS	DAYS	MONTHS	
EXPOSURE	blood coagulation	phagocytosis			RECOVERY
	neutrophil recruitment		macrophage clearance		
	cytokine production			growth factors	
			tissue strengthening		

Fig. 2 The observed time scale from exposure to recovery. The table below charts the biochemical responses that are measured and recorded immediately after exposure through time until recovery. The time scale ranges from minutes to months and included biomarkers on the molecular to cellular to tissue scale.

Table I Comparison of features of measurement and use of existing NOAEL and proposed NEEL approach. It should be noted that while some aspects of the NEEL approach can be derived from existing literature further consideration of database integration and metadata analysis will be required to implement this approach fully

Item	NOAEL	NEEL
Decision paradigm	Digital	Analog
Quantitative endpoint	Threshold	Response range
Time of event	Instantaneous or aggregated	Continuous
Basis for decision making *(in terms of observed biological phenomenon[a])*	Single independent measures	Concurrent multiple variable
Interpretation	Assigned numerical limit for significance	Multivariate statistical analyses

[a] Aggregate events associated with a measure of toxicity, e.g. inflammation, may be considered separately or in conjunction

as we explore the seemingly enormous potential for positive impact on human health, which is overshadowed by considerations of safety. In the following exposition consideration will be given to a relatively narrowly defined set of circumstances that it is presumed could be expanded to related physicochemical and biological conditions.

Initial experiments in a single organ system, such as the lungs, exposed daily to nanoparticles and aerosols of various origins can later be extended to other organs and tissues throughout the body and ultimately to the health and well-being of the entire organism.

TRANSITION FROM WHOLE ORGANISM TO CELLULAR SYSTEMS AND MOLECULAR TECHNIQUES

There is a need to transition from testing exclusively in whole organisms to screening in cellular systems. And, there are many teams of researchers building cell culture models that could not only supplement animal models, but also be predictive of human responses [5]. The study by Li *et al.* sets a standard for using a library of engineered nanomaterials (i.e. functionalized carbon nanotubes) to assess the impact of a specific physicochemical property (i.e. surface charge) in an effort to establish a predictive toxicological model that relates the material's inflammatory effect at cellular level to the development of pulmonary fibrosis in the lung. The study concluded that the *in vitro* hazard ranking was validated by the fibrogenic potential of the engineered nanomaterial *in vivo*. The goal of this on-going effort is to not simply develop predictive *in vitro* models; instead, the spotlight should be on data collection at the interface of *in vitro* and *in vivo* assessments to substantially increase knowledge that will allow integration of these apparently divergent fields. Both models have merit. Maximizing the convergence of their advantages while minimizing their disadvantages will drive progress in both pharmacological and toxicological sciences. Although it is premature to consider this approach as redefining toxicology, actively integrating data into a framework of information and knowledge suitable for decision-making is a significant, paradigm-shifting step. The linchpin in this process is to integrate time (i.e. kinetics, time points, age, and recovery time) into all experimental designs.

Exposure to ambient nanoparticle aerosols is known to cause toxicity following pulmonary exposure. Nanoparticles have been proposed as therapeutic delivery systems and the decade's long knowledge of ambient exposures has been overlaid on the relatively new field of nano medicine. This is a poor extrapolation because ambient nanoparticle composition, frequently including carcinogenic substances and long residence time of months to years are incomparable to therapeutic nanoparticles of efficacious composition that are rapidly cleared. Case in point: the human body is in homeostasis. At any point in time, it is resolving trends toward toxicity or disease of which the organism is largely unaware. Given this understanding, subtle questions remain unanswered by traditional toxicological inference:

> The conventional notion in toxicology, that there should be no observable adverse effects, does not account for normal excursions in "threat" resolution. This is important when considering vaccines for which the adjuvancy of the particles initiates an immune response that is beyond the norm. The "normal" response to an upper respiratory tract infection is inflammation followed by resolution. However, many people would consider any measurable "inflammatory" response to an adjuvant in this sensitive organ system "unacceptable".
> There is a scale of health between the extremes of disease-free and diseased that is epitomized by a variety of biological responses (cell recruitment and population regulation, inflammatory mediators, reactive species, secreted enzyme production). Defining the nature and magnitude of these responses facilitates a more holistic approach to safety and toxicity.

Consequently, much more can be done to increase the understanding of the phenomena by which we define the transition to toxicity. If the biological responses in cells and

animals to nanoparticles of different composition and residence time were followed, then the data could be curated to create a detailed database. Using these data, the fields of pharmacology and toxicology could elucidate appropriate models collaboratively for applying both cell and animal systems for safety and efficacy testing for our next generation (and previous generations) of medicines.

NANOTOXICOLOGY AND ITS INFLUENCE ON NANOPHARMACOLOGY

When reviewing the current literature in the field of nano toxicology, it is apparent that establishing a single mechanism for nanoparticle-induced toxicity has proven to be a challenging endeavor. For example, elucidating the mechanism of action of polymer-based particles *versus* metal particles in biology is especially complicated. Nano toxicologists have proposed many linear relationships relating a unique nanoparticle physicochemical property with an observed biological response. However, most of these proposed linear relationships are contradictory when searching through the nano toxicity literature. In short, simple explanations may not describe the results in nano toxicology research. However, nano toxicology research is imperative to the success of nano medicine.

To date, the particle, including nanoparticle, toxicology literature has established that the size, composition, and origin of particles result in a range of responses that may be inflammatory; macrophages communicate with dendritic cells by active transfer of particles or degraded components; and dendritic cells are the major cells involved in trans locating particles to lymph nodes. These findings align very well with the current knowledge in pharmaceutical sciences. Clearance of macrophages or dendritic cells filled with particles, as well as clearance of the particle themselves, induces a moderate inflammatory response and happens in a relatively short time frame. These two characteristics are well suited for particle-comprised vaccine systems [6, 7]. The recent work of Schanen *et al.* describes studies exploring some of the most widely manufactured engineered nanomaterials and their effects on fully human autologous MIMIC immunological constructs – a non-animal alternative to diagnose nanoparticle immunogenicity. Further studies show that certain nanoparticles potentiate dendritic cell maturation and encourages T (H) 1-biased responses, induces antigen presenting cells to secrete the anti-inflammatory cytokines, and then subsequently induces a T (H) 2-dominated T-cell profile. This demonstration partially explains the differential effects of nanoparticles to modulate oxidative stress and prompt inflammatory responses through an ROS-inflammasome-IL-1β pathway.

The balance of knowledge derived from *in vitro* and *in vivo* experiments presented as integrated responses serving a decision process has not been fully explored. Immune responses to nanoparticles can be employed as a model to develop such a balanced approach. The immunogenicity of particles is known to elicit both toxic (inflammatory) and protective (immunizing) responses. Inorganic particles are thought to be more immunogenic than soluble organic particles *in vivo*; it is unclear whether this effect can be explained solely through particle uptake by phagocytic or antigen-presenting cells. The important physicochemical properties of particles (inorganic or organic) involved in cell recruitment, the type of cell, and differential particle-cell interactions are central to understanding the immuno toxicity in the lungs. It is possible that particles mediate communication between different immunological cell types in the lungs with potentially toxic effects. More specifically, phagocytosed particles, of different composition, could be sequestered to different degrees by immunological cells in the lungs. This rationale is paramount to prevention of toxicity and development of vaccine adjuvants. The particles elicit their toxic or efficacious responses by engagement in a spatial-temporal matrix of interactions with a wide range of elements of the biological environment in the lungs, the kinetic nature of which has not been thoroughly elucidated.

INFLAMMATORY RESPONSE AS A BIOMARKER IN VACCINATION

In broad terms localized early onset immune responses may be characterized under the heading of 'inflammation'. For vaccine in particular, where it is intended to stimulate the immune response, these reactions need to be interpreted carefully since the risk may be far outweighed by the benefit. Indeed, if the response resolves quickly this would be no different than any of a number of daily exposures that humans experience [8–10].

There is also a large body of literature available on the factors that contribute to the ability of a substance to deposit in and translocate through the lung. Aerosol deposition data have been summarized by the International Commission on Radiation Protection (ICRP) and National Commission on Radiation Protection (NCRP) [11]. Nanoparticle deposition and clearance have been studied by a number of researchers, led notably by Oberdorster [12]. In contrast to what is known about aerosolized liquids, not much is known about aerosolized engineered nanoparticles. The properties of these aerosolized particles, especially engineered nanoparticles that make them so desirable in many different industries may also cause adverse pathology in tissues. The stability of particles is unknown, which may be the very link between exposure and adverse pathology [13]. Because some nanoparticles have been shown to enter the interstitial spaces and persist within cultured cells and tissue and in the whole animal, the stability/durability of the particle is of great importance [14, 15]. These and other questions must be carefully examined before nanoparticle applications can be approved for human clinical use.

Many research groups have published evidence of nano aggregates internalized within a cell *via* a vesicle or endosome [16–21]. Some have postulated that there could be translocation of individual nanoparticles to the circulatory system from these internalized nano aggregates (Fig. 3). Phagocytes are an essential component of the immune system. In drug delivery applications, these cell types hinder the effectiveness for intravenous delivery of nanoparticles; these particles easily aggregate on the cytoplasmic membrane and enter the cell in endosomes. Currently, some researchers are studying the efficacy of nanoparticles to deliver therapeutic agents to specific cells in the body [22].

Nano aggregates are recognizable to mononuclear phagocytes; the particles could be cleared from the body by phagocytosis. It has been hypothesized, however, that the binding affinity and subsequent immune pathways differ. Phagocytic cell uptake and humoral activity work in parallel; and information on these processes would allow for an increase in our understanding of various aspects of the cellular immune response. It is generally accepted that the physical and chemical properties of nanoparticles, such as size, surface charge, and type and degree of surface modification, can affect their uptake by phagocytes. However, the mechanisms and relative contributions of different properties are not known.

Research has begun to define the relationship between physical and chemical nanoparticle properties, cellular uptake, and mechanistic toxicology. Currently, it is generally accepted that most nanoparticles aggregate when brought into physiological or environmental matrices [23]. Over time, some particles de aggregate or dissociate into ions, depending on surrounding conditions. No single nanoparticle property contributes to observed toxicity or biocompatibility; instead it is a combination of physical and chemical properties that influence the biological response due to nanoparticle exposure [24].

NANOPARTICLE TO CELL INTERACTIONS MAY FOLLOW ONE OR MORE PATHWAYS

In this section, factors that influence activation of the innate immune system after lung exposure to nanoparticles will be discussed. Although nanoparticles are being developed for delivery by a number of different routes, we will focus on lung delivery due to the extensive use of this route of delivery in the nanoparticle field and the ease of monitoring lung inflammation. Activation of the innate immune system in response to exposure to nanoparticles may vary depending on specific nanoparticle properties (i.e., size, charge, and composition), the concentration of the nanoparticle exposure and the route of exposure. The innate immune system rapidly responds to insults to the host (i.e., infection or tissue damage) with the production of proinflammatory cytokines and chemokines and the recruitment of innate immune cells such as neutrophils and macrophages [25–30]. Host innate responses to nanoparticles may involve a variety of pathways. Direct cytotoxicity of host tissue by nanoparticles may induce release of genomic DNA that under some circumstances activates innate immune responses and enhances adaptive immune responses *via* toll-like receptor 9 (TLR9) dependent and independent pathways [31–33]. Innate immune responses induced by nanoparticles may require Myd88, an adaptor protein involved in many innate signaling pathways [34]. Activation of the inflammasome may be involved with nanoparticle activation of innate immune responses [35]. Mast cells may also be activated by nanoparticles to release preformed mediators that enhance innate and adaptive immune responses [26, 36].

Carbon nanotubes with varying surface modification were evaluated for their ability to activate innate immunity *in vivo* and *in vitro* [5]. Carbon nanotubes were tested as prepared or after surface modifications to include cationic or anionic surfaces. This study demonstrated that nanotubes with a strong cationic surface modification exhibited activation of innate immune responses (interleukin 1 beta, IL-1β; transforming growth factor beta, TGF-β and platelet derived growth factor PDGF) after *in vitro* treatment of macrophage like cells (THP-1) despite the lack of cytotoxicity [5]. Oropharyngeal installation of the cationic nanotubes induced production of IL-1β and PDGF in the lung bronchoalveolar lavage (BAL) fluid at 40 h post exposure although TGF-β was not elevated. At 21 days, BAL IL-1β levels had returned to normal while PDGF and TGF-β levels were elevated. Collagen deposition at 21 days was increased after cationic nanotube exposure. The same nanotube with an anionic surface modification exhibited decreased activation of innate immunity *in vitro*

Stages of Pathway	Toxicant	Reactions with Biomolecules	Response			
			Cellular	Organ	Organism	Population
Example Outcome	Material	Oxidation/ Reduction	Leaky membrane	Physiological changes	Disease/ Degeneration	Growth/Decline
			Apoptosis			
	Chemical	Binding/ Bonding	Gene activation	Disrupted homeostatis	Death	Termination

Fig. 3 Relating exposure, to molecular reactions, to eventual effect.

and decreased collagen deposition as compared to the unmodified nanotube *in vivo* [5].

The shape of nanoparticles may also influence activation of innate immunity and inflammation. Aluminum oxyhydroxide nanoparticles were prepared as nanorods, nano plates and nano polyhedra and compared for activation of innate immune responses and adaptive immune responses *in vitro* and *in vivo* [35]. Nanorods with a larger hydrodynamic size were more potent than nanorods with a smaller hydrodynamic size for the *in vitro* activation of innate immune responses as determined by IL-1β production by THP-1 cells [35]. The larger nanorods induced greater IL-1β production that the aluminum based adjuvant alum, nano plates and nano polyhedra. The IL-1β production was dependent on the NLRP3 inflammasome pathway. The larger nanorods also induced cytokine production (IL-1β, IL-6 and IL-12) by bone marrow-derived dendritic cells (BMDC) and also increased their expression of antigen-presenting (MHC II) and costimulatory molecules (CD40, CD80 and CD86) [35]. The larger nanorods also exhibited vaccine adjuvant activity *in vivo* that was superior to alum demonstrating that in this nanoparticle system, *in vitro* activation of innate responses predicted *in vivo* responses [35].

It is important to evaluate both *in vitro* and *in vivo* activation of innate immunity. The studies mentioned above demonstrated that *in vitro* activation of innate immune responses correlated with *in vivo* inflammation and fibrosis. Other studies have demonstrated that *in vitro* and *in vivo* innate immune responses to nanoparticles may not always agree [37]. Copper nanoparticles coated with chitosan exhibited decreased cellular cytotoxicity *in vitro* when compared to uncoated copper nanoparticles. However, when compared to uncoated nanoparticles, the chitosan coated nanoparticles exhibited increased cytotoxicity and induction of innate immune responses in the lung after nasal delivery [37]. The authors conclude "…coating metal NPs with mucoadhesive polysaccharides (e.g. chitosan) decreases their ability to be cleared from the lungs, prolonging the exposure of cells and tissue to toxic metal oxides and producing a dramatic acute inflammatory response." This study emphasizes the importance of evaluating nanoparticle toxicity using both *in vitro* and *in vivo* studies.

When using nanoparticle delivery systems, the impact of activation of the innate immune system is dependent upon the desired use of the nanoparticle delivery system. For example, if a nanoparticle delivery system is developed to delivery therapeutic proteins such as insulin, activation of the innate immune system would be undesirable. In contrast, a nanoparticle system developed to deliver vaccine antigens would be expected to activate the innate immune system for optimal induction of antigen-specific adaptive antibody and T cell responses. However, activation of the innate immune system for a vaccine application should be sufficient to enhance the induction of protective adaptive immune responses (i.e., neutralizing antibody, T lymphocytes) while not inducing persistent innate immune responses that could lead to permanent adverse effect such as lung fibrosis [5]. To define innate immune response parameters that are not toxic to the host, it may be helpful to evaluate natural conditions that are expected to result in activation of innate immune responses while not producing long term adverse effects in humans. For example, human upper respiratory tract infection is known to induce activation of the innate immune system as demonstrated by local cytokine production in the nasal lavage fluid including IL-1β, IL-8, IL-6 and TNF-α [38–40]. Nanoparticle vaccine adjuvants that activate the innate immune system in a manner that mimics natural infection may provide an adjuvant system that induces potent and long-lasting adaptive immune responses similar to those induced by natural infection without long-term adverse effects. In contrast, nanoparticle systems for delivery of therapeutics would be expected to deliver their therapeutic cargo without activation of innate immunity. Depending on the proposed use of the nanoparticle formulation, additional studies are needed to evaluate the impact of nanoparticle composition, shape, charge, dose and frequency of delivery on host innate immune responses induced. Additional studies are needed to better define the innate immune system activating properties of nanoparticles and how these properties could be successfully applied to medical applications.

Recently, it was reported that the commonly held assumptions of distribution and clearance may not be so apparent [41–44]. The role of recruited macrophages and the mononuclear phagocyte system responds to different particle types in unique ways. Additionally, the nanoparticle–cellular mechanisms that determine particle deposition, accumulation, or localization are not intuitive or predictable [45–47]. The conclusions of these studies highlight that nanoparticles with varying surface chemistry, chemical composition, and size would not behave similarly in localized tissues or systemically in the circulatory system. Deposition of nanoparticles also has not been widely studied. Some have postulated that nanoparticles accumulate in lymph nodes; others have suggested that these novel materials accumulate in the liver. Others have reported on the significant role of excretion organs, like the kidney, spleen, or liver, after delivery of nanoparticles [48].

After nanoparticles are internalized by phagocytes, the inflammatory cascade may be triggered [24]. Inflammation is the complex biological response of cells and tissues to harmful pathogens and other toxicants. It is a proactive mechanism to both remove these harmful pathogens and initiate production of repair enzymes. Unchecked inflammation can lead to a host of diseases, such as asthma, atherosclerosis, and rheumatoid arthritis; therefore, it is normally tightly regulated by the body. We hypothesize that phagocytosis of nano aggregates can cause toxic tissue inflammation mediated by the

ingesting phagocytes *via* cytokines and other chemicals such as nitric oxide.

Although "small" is usually equated to "nonimmunogenic" in immunology, it is possible that nanoparticles could activate a specific immunoglobulin on a B cell and cause an adaptive humoral response. For a nanoparticle-induce adaptive response, specific properties are needed. First, there must be the delivery of an identifiable foreign antigen. If this foreign antigen is a protein, it should contain both B and T cell epitopes needed to induce both cellular and humoral adaptive immune responses. Second, there must be evidence of activation of the innate immune system (i.e., inflammation). Foreign antigen delivery in the absence of inflammation may induce either no adaptive response or it may induce antigen-specific tolerance. This could occur through conjugation with a carrier protein, providing both B cell receptor aggregation and linked recognition of a peptide for T-cell help. If antibodies specific for nanoparticles are being produced (as opposed to antibodies cross-reactive with nanoparticles preexisting as described above), they would not only clear the first nanoparticle introduction but would provide an augmented memory response with faster kinetics.

TIME SCALE OF SCRUTINY

The nature of a response to an exogenous or endogenous stimulus can be considered with respect to a time scale. It is frequently the case that responses enter a cascade that beyond a certain point becomes irreversible and would classically be considered the realm of toxicity. However, the reversible responses may be transient and controlled to limit the prospects of proceeding to deleterious effects on the health of the individual.

THE SCALE OF HEALTH

The framework discussed has already been recognized with regard to one element of pulmonary response to particulate exposure, reduction/oxidation of glutathione, and the notion of resolvable inflammation as differentiated from inflammation that is a precursor of disease. There are questions that need to be asked, such as when is something stressed to the point of irrecoverable toxicity *versus* when can stress be recoverable? The hierarchical oxidative stress model considers the different tiers of toxicity relative to oxidative stress and increasing particle dosing concentrations. Tier 1 represents the state of cells or tissues in a basal expression (i.e. normal condition). Tiers 2 and 3 represent the state of cells or tissues undergoing oxidative stress states, including a less detrimental but measurable antioxidant defense and a generally regarded toxic state of inflammation, respectively. The difference between Tier 2 and Tier 3 is that effects observed in Tier 2 are, potentially, reversible and not prolonged.

There has been a concerted effort to characterize the interactions between lung fluids and particles (i.e., dissolution rates, absorption and adsorptions, mobility). However, little information has been disseminated on creating an array of responses that define effects—specifically for the purpose of risk decision making for engineered nanoparticles to be used as aerosolized medicines. The framework described here could provide information at the interface of nano toxicology and nano medicine with a foundational approach that can be (a) replicated in other fields of safety and efficacy and (b) become a component of a higher-level integrated structure allowing transitions from data to information to knowledge. This strategy fundamentally underpins rational decision making [49].

We recognize a new paradigm that is perhaps implicit in the current literature but has to our knowledge not been formally stated. Classic toxicological and pharmacological principles have been extended by current analytical capability in all fields of science and the capacity to store and interpret data. Among the key points that should be noted are:

- All biological and biochemical responses exist on a continuous (not necessarily linear) scale; responses are not simple and discrete functions;
- Toxicologists and pharmacologists (and those in related disciplines) have had the most interest in negative and positive responses after exposure. In decision making, they have found utility in simple threshold values;
- Traditional methodologies in this field were limited due to inability to compute large data sets but in the absence of alternative approaches were valuable;
- In the present environment, we can capture the range of responses along continuous (but not necessarily linear) scales; and because we can, *we should*, as this will lead to better informed decision making and contribute in an unprecedented manner to knowledge in these important field of public health.

It is now possible for researchers and health specialists to consider data along a scale of health rather than discrete and limited data sets, to which threshold values have been ascribed meaning. This paradigm is evident in a variety of disciplines, including but not limited to, microarray technologies, epigenetics, and the "omics" sciences (genomics, proteomics, and metabolomics). Large data sets are pulled together using informatics and pattern recognition coupled with classification and cluster analyses.

Current research and development efforts in nano medicine have focused on formulating an antigen in or on the surface of a particle [50–53]. Decorated particles have been demonstrated to qualitatively affect the immune response.

Depending on properties of the particle, such as dissolution rate or residence time or size, the heightened immune response could favor either Type 1 T helper (Th1) or Type 2 (Th2) cells [54]. To this end, specific research questions, such as the following, should be posed:

> Can nanotechnology be exploited to promote one response with respect to another?
> Can a particle-type composed of a zero valent metal favor Th1 type response, while a dendrimer particle elicits a Th2 response?

The Th1/Th2 paradigm provides the rationale for developing new types of vaccines against infectious agents and of novel strategies for the therapy of allergic and autoimmune disorders [55]. Th1 cells produce interferon-gamma, interleukin-2, and tumor necrosis factor-beta, which activate macrophages and are responsible for cell-mediated immunity and phagocyte-dependent protective responses. Th2 cells produce IL-4, IL-5, IL-10, and IL-13, which are responsible for strong antibody production, eosinophil activation, and inhibition of several macrophage functions, thus providing phagocyte-independent protective responses. These responses have been measured in cell culture and animal experiments and represent a confounding of responses to particles, adjuvants, and antigens. What is lacking in the collective body of knowledge of nanoparticles in pharmacological sciences, however, are the tedious correlations of any one antigen (or its concentration) having an adjuvant effect on the entire system. Or, it can be postulated that the particle formulation and its ability to activate the innate immune system/inflammation can provide an "adjuvant effect". The nature and magnitude of the immunological/inflammatory responses should be considered along a scale of health as opposed to absolutes of "safe" or "toxic".

It has been the convention in scientific endeavor to study a single element of any system with the view that we can only understand its importance by holding all other variables constant. Except in the simplest of systems this is not only time consuming but also unlikely to result in significant or definitive observations. It has been demonstrated (in engineering predominantly) that multivariate statistical design examines all areas of the experimental space in sufficient detail to draw meaningful conclusions in a timely fashion. These research principles opt to be applied to modern toxicology. A radical change in the perception of safety and toxicity is needed in order to rapidly bring new therapies to ailing patients. It is imperative that an approach is adopted that allows cost-effective innovation, technological advance, and product development by industry and rational, flexible, efficient, and timely decision making by regulators. Only a new approach – different than the one currently being used - will promote innovation and contribute positively to competition in the global marketplace.

The alarming linkage between nanoparticle health effects and the use of nanotechnology in vaccine and drug development rests on the fate of particles in the human body (particularly in and from the lungs). It is evident that this linkage has implications for both toxicology and medicine. Through recent technological innovations in nanoparticle sample preparation, imaging, and immunological screening assays in cells and in the lungs, the fields of nano toxicology and nano biotechnology can overlap.

Understanding the results (and interpretation of the results) of studies is imperative to the utilization of R&D efforts translating to consumer use. This requires knowledge of not only the raw data, but also the transparency of the meta-data, as well (i.e. instrumentation parameters, replicates, and statistics). The diversity of experimental design and analytical techniques used today hinders the discovery and evaluation of data. There is an emerging opinion that favors harmonization of the way in which data is collected and these methods become available.

Mechanistic toxicology linked to cause and effect relationships has emerged as a field of study with several functions related to the use of predictive toxicity testing [56]. Researchers have defined these types of relationships as constructs that enable existing knowledge to be linked to exact molecular reactions that can in turn be used to populate risk assessment paradigms. The relationships between initial point (and type) of exposure to mode of action to eventual effect can either be a linear sequence of events or a set of events that branch from individual initiating events. One potential use of reporting on these relationships may be identifying other potential adverse mechanisms of action or routes to detoxification or recovery after cellular, tissues, or organ injury. The stages of the pathway begin at identifying and characterizing the toxicant (i.e., material or chemical), the reactions with biomolecules (i.e. oxidation, reduction, binding, or bonding), and the response to cells, organs, organisms, and populations. The description outlined herein is not a revolution, but an evolution of understanding the relationship between toxicology and pharmacology in the evolving environment of large biological databases.

There is a need for a framework that organizes and integrates available information about the molecular interactions that could lead to an adverse outcome on an organism or population. These data can include: structural properties, laboratory methods, or *in vitro*/*in vivo* study data [57]. In addition, sharing information from large biological databases has the potential to provide a narrative for modes of action, allow for chemicals to be grouped, enable experimental designs using a tiered testing approach, or decipher between relevant *vs.* irrelevant assays.

CONCLUSION

Large databases allow the continuum of biological responses to stimuli to be described in great detail and patterns to be

observed from which an individual's state of health can be determined. Functioning in this complex data rich environment brings into question traditional threshold limits for both efficacy and toxicity as these were developed to allow decision making with limited data. The foregoing discussion raises the prospect of considering a continuous scale of health with regard to a variety of interconnected biological processes and pathways that if considered thoroughly would allow for more informed decision making both is therapeutics, risk assessment and regulatory oversight. The current trends to manage 'big data' with the intent of deriving knowledge from large databases from which to benefit society may make its first major impact in the arena of human health. We believe that considering complex phenomena that impact on health or disease as continuous rather than discrete processes may be the first area in which this impact will be seen. Integration of knowledge obtained from specific pathways and there correlation with irreversible phenonomena will aid in our understanding of future interventions.

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