Oxidative Stress and Inflammation Induced by Environmental and Psychological Stressors: A Biomarker Perspective

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Abstract

Significance: The environment can elicit biological responses such as oxidative stress (OS) and inflammation as a consequence of chemical, physical, or psychological changes. As population studies are essential for establishing these environment-organism interactions, biomarkers of OS or inflammation are critical in formulating mechanistic hypotheses.

Recent Advances: By using examples of stress induced by various mechanisms, we focus on the biomarkers that have been used to assess OS and inflammation in these conditions. We discuss the difference between biomarkers that are the result of a chemical reaction (such as lipid peroxides or oxidized proteins that are a result of the reaction of molecules with reactive oxygen species) and those that represent the biological response to stress, such as the transcription factor NRF2 or inflammation and inflammatory cytokines.

Critical Issues: The high-throughput and holistic approaches to biomarker discovery used extensively in large-scale molecular epidemiological exposome are also discussed in the context of human exposure to environmental stressors.

Future Directions: We propose to consider the role of biomarkers as signs and to distinguish between signs that are just indicators of biological processes and proxies that one can interact with and modify the disease process.

Keywords: exposome, nanomaterials, cytokines, proteomics, genomics, NRF2, xenobiotics, emotions, neuroendocrinology

Biomarkers of Inflammation and Oxidative Stress

THE THEORY THAT OXIDATIVE STRESS (OS)—an imbalance between production of toxic oxygen species, reactive oxygen species (ROS), and endogenous antioxidants (164)—may be at the basis of a disease was first put forward by Harman in 1956 with the ''free radical theory of aging,'' in which he concluded, ''This theory is suggestive of chemical means of prolonging effective life (64).'' Despite this, there are no antioxidants currently approved by regulatory agencies

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for any disease (59), with the possible exception of edaravone, an antioxidant approved in Japan and India for aiding neuroprotection in stroke patients (96).

Inflammation is also postulated as a pathogenic mechanism in most diseases or as a major risk factor (44). Its oldest definition is by Aulus Cornelius Celsus in the first century AD, who defined the four hallmarks of inflammation: ''*rubor, et tumor, cum calore, et dolore*,'' redness, swelling, heat, and pain (29). These are, for Celsus, ''*notae vero inflammationis*.'' ''*Notae*'' is usually translated in English with ''signs'' (''the cardinal signs of inflammation''). This is our first encounter, in our review, of the concept of biomarkers. In a way, biomarkers are ''signs.'' The father of semiotics, Charles Sanders Peirce, described a semiotic triad where he defines the relationship between a sign, the object it stands for, and the interpretant (9). This concept is shown in Figure 1, where when the interpretant sees smoke, she knows that that sign indicates that somewhere there is a wildfire.

From Celsus' perspective, the signs of inflammation were viewed mainly with a diagnostic or classification purpose. However, in clinical and preclinical studies, as well as in epidemiological studies, biomarkers are also used to gain insights into the causal mechanisms underlying diseases. Biomarkers have been classified into biomarkers for the etiology of the disease (risk factors; including biomarkers of exposure), and biomarkers of disease used in the screening or diagnosis, or to monitor disease progression (prognosis) (116). Even if not implicit in their definition, one desirable criterion for a biomarker is to be accessible—that is, measurable in biological fluids that can be obtained in a minimally invasive manner (such as urine, blood, or synovial fluid). In animal models, biomarkers can also be measured in tissues and organs, possible only in human patients in the few cases where biopsy samples are obtained for diagnostic purposes.

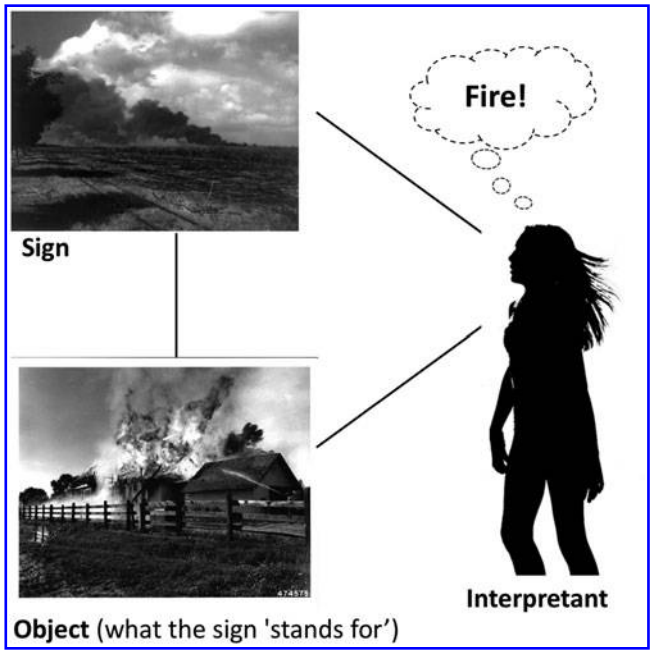

FIG. 1. The semiotic triad according to Peirce. Images from Wikimedia Commons.

The study of diseases can, in its turn, lead to the definition of new biomarkers and to the refinement of the criteria for disease classification. For instance, an inflammatory response often results in tissue damage (*e.g.*, joint damage in arthritis) and loss of function, the ''*functio laesa*'' described by Galen (145). The study of the molecular mechanisms of inflammation led to infiltration of white blood cells, their recruitment in the tissues as a result of inflammatory mediators known as chemokines (197), being considered an additional criterion of inflammation, more than the classical cardinal signs. Cytokines and chemokines, inflammatory mediators that are causative of many features of inflammatory diseases, including the old five cardinal signs, are now used as biomarkers of inflammation.

The identification of cytokines as mediators of inflammation in the mid 1980s led to what Tracey called the ''cytokine theory of disease'' (176). Contrary to the OS theory of disease, this led to major advances in the treatment of chronic inflammatory diseases, and less than 15 years after the identification of the cytokine tumor necrosis factor-α (TNF-α), anti-TNF-α antibodies were approved in the therapy of chronic inflammatory diseases and are now the top-selling biologicals.

Here, we review both biomarkers of OS and inflammation; this is not a random choice, as the two fields are tightly linked. ROS can activate the transcription factor nuclear factor kappa B (NF-κB) (156), which has many inflammatory cytokines among its target genes. Conversely, inflammation can induce OS (147), as, for instance, in the case of ROS production by polymorphonuclear neutrophils (125).

Although the two pathways are so intertwined, they present entirely different challenges in terms of biomarkers. When studying disease mechanisms, we want to be able to measure the effectors of inflammation. The development of anti-TNF-α drugs was possible as researchers could measure TNF-α levels in patients with commercially available immunoassays that detect TNF-α in stored blood samples.

All this is very difficult when studying OS in disease. Here, the effector molecules are ROS, which have short half-lives, ranging from nanoseconds to milliseconds (85). This makes it impossible to measure ROS in biological samples and we must rely on signs, chemicals that are produced by the interaction of ROS with various cellular molecules (57).

Another important aspect to consider is the difference between biomarkers that measure the formation of the effector molecule and those that measure the response of the organism to an inflammatory stimulus; inflammation is a defense/repair reaction of the organism to an infection or injury. The process is complex, as the effector cytokines in inflammation are produced after a series of steps. As mentioned earlier, there are several biomarkers of OS that indicate the exposure of the organism to ROS by measuring oxidative breakdown products of cellular molecules. However, exposure to OS can be inferred by measuring the cellular defense response to it and, in this review, we will give the example of the transcription factor nrf2/antioxidant response element (ARE) transcription factor that is activated by ROS and other electrophiles. There are many ways in which environmental stressors induce disease by common pathways (39, 123). The sections in this review will deal with psychological, environmental, or noise-induced stress, trying to focus on how their effects on inflammation or OS were detected.

Engineered Nanomaterials as Environmentally Borne Agents Inducing Inflammation and OS

Engineered nanomaterials (ENM) are manufactured materials in which at least one dimension is in the nanometer range (<100 nm). The higher surface area increases the material's reactivity (126). Redox interactions of ENM are major mechanisms of toxicity, particularly for metal and metal oxide nanomaterials, quantum dots, and carbon nanotubes (CNT).

Typically, OS is induced by ENM in a three-tiered hierarchical sequence (Fig. 2) (126, 131). A mild production of ROS induced by ENM (Tier 1) lowers the reduced glutathione (GSH)/oxidized GSH (GSSG) ratio and upregulates genes encoding type II anti-oxidant enzymes, thus re-establishing homeostasis. In Tier 2, the defensive reaction is more complex; ENM induce the production of ROS, triggering the production of inflammatory cytokines and chemokines. However, the inflammatory response is transient, as the elimination of the triggering event (*e.g.,* the phagocytosed ENM) and inflammation-damping feedback mechanisms re-establish homeostasis. In Tier 3, the GSH/GSSG ratio is completely imbalanced and essential components of cells are damaged, causing genotoxicity and cytotoxicity.

This sequence does not distinguish between the capacity of ENM to generate ROS in cell-free systems (131) and ROS generation consequent to nano-bio interaction. In a complex system (tissue, organ), a different three-pronged model can be proposed for the inflammatory response to ENM (Fig. 3). In this model, Prong 1 represents the lack of response, due to either ''ignorance'' or ''tolerance,'' in which the living system eliminates ENM immediately, for example, by excretion with urine. Prong 2 is the classical protective inflammatory response, in which a tissue reacts to ENM while sending alarm signals, with recruitment of blood immune cells to eliminate the ENM. The reaction is, however, limited in time and circumscribed; after eliminating the ENM, inflammation is resolved. Prong 3 is the pathological situation of an inflammatory reaction that cannot be resolved, as in the case of persistent materials (*e.g.,* fiber-like particles). This may result in persistent inflammation with tissue destruction and development of non-functional neo-tissue (granuloma, scarring tissue, pannus, fibrotic tissue). Only Prong 3 can be eventually harmful for the organism and cause irreparable damage.

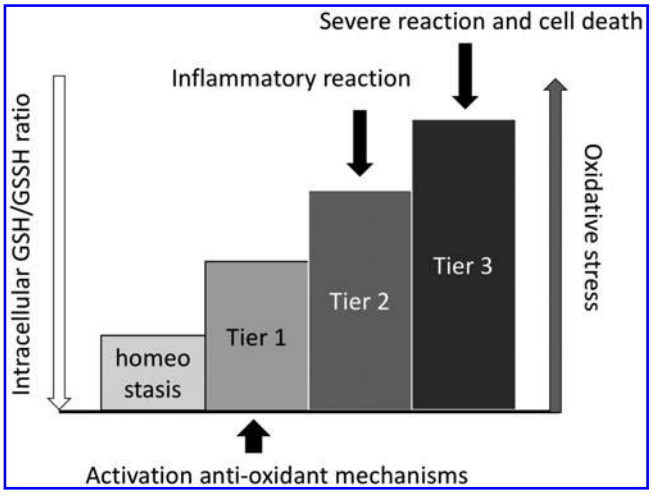

FIG. 2. The three-tiered paradigm of OS induced by nanomaterials at the single-cell level. OS, oxidative stress.

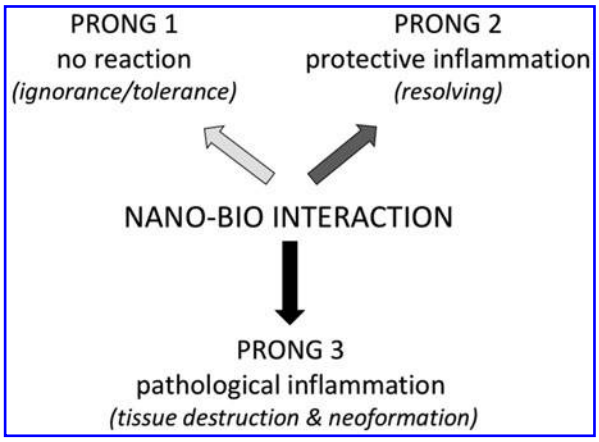

FIG. 3. The three-pronged response of complex living systems to nanomaterials.

The three-tiered model of ENM-induced OS does not reflect the three-pronged model of ENM-induced inflammation because the entire organism considers the death of single cells as an acceptable event that may be important in the ''cleaning'' and healing reaction.

ROS production by nanomaterials

Unstable ENM can undergo oxidation, reduction, and dissolution in biological media, releasing reactive-free ions (in the case of metal ENM such as silver), or excitation of electrons and generation of ROS (as in the case of titania ENM and fullerenes on UV irradiation). In addition, the reactive surface of ENM may absorb transition metals that catalyze ROS-generating reactions (Fenton, Fenton like, Haber-Weiss) and produce cytotoxic and genotoxic hydroxyl radicals (126, 131, 137). Production of ROS resulting from the interaction of ENM with living systems is mainly indirect, due to damage or alterations caused by ENM to membranes, which trigger an alarm inflammatory reaction.

The production of ROS is one of the defensive mechanisms initiated by innate defense cells such as phagocytes, aiming at killing microorganisms. Silica and polycationic particles can interact with plasma membrane phospholipids, leading to membrane destabilization and ROS production (3, 103). Urate crystals can also bind plasma membrane cholesterol and lead to aggregation of receptors and other molecules within lipid rafts, thus activating Syk-dependent inflammatory signaling and ROS generation (128, 202). ROS induction can also be indirect, as, for instance, in the case of crystalline silica particles that can induce TNF-α production that stimulates ROS generation (15). Once internalized, ENM can destabilize and rupture the membranes of organelles such as phagolysosomes and mitochondria, causing ROS production and inflammation.

High-aspect ratio ENM (rigid fiber-like or needle-like ENM) are particularly challenging for phagocytes and generate a phenomenon known as ''frustrated phagocytosis,'' with waves of phagocytes attempting to take up the material, and persistent inflammation, including ROS production. ROS production induced by ENM is in most cases associated with

Akt/mTOR pathway, autophagy, and apoptosis (5). This can evolve into a Tier 3 reaction, with ROS production inducing lipid peroxidation, membrane destabilization, and DNA and protein damage. Rigid needle-like ENM can also cause phagocyte death by literally perforating the cell membrane (121).

Unlike apoptosis, necrotic cell death implies membrane damage and the release of intracellular components, which perpetuates the inflammatory reaction (187). This may lead to tissue damage or, in a ''pathological'' attempt to contain the danger, can result in fibrotic or granulomatous reactions (Prong 3). In some cases, leukocytes can successfully degrade high-aspect ratio EMN without phagocytosing them, as in the case of multi-walled CNT, which are sensitive to several leukocytic enzymes, and can, therefore, be eliminated rapidly without causing excessive ROS-mediated tissue damage (183). This would, therefore, be a classical Prong 2 inflammatory reaction that is resolved without causing permanent damages.

Inflammatory reactions induced by nanomaterials

Inflammation (Prong 2) is, therefore, a central event in ENM-induced OS (24). At the organism level, inflammation is a defensive mechanism that succeeds in tagging and eliminating potentially dangerous agents (including ENM). Inflammation, however, always induces some collateral damage, that is, the death of some cells (including both the effector cells and innocent bystanders). In terms of ENM cytotoxicity, at the single-cell level, inflammation can be in Tier 2 (resolves without cell death) or in Tier 3 (ending with cell death). Both events can be included in Prong 2, an inflammatory reaction at the tissue/organ/organism level that succeeds in eliminating the danger despite some cell death and tissue damage, and that succeeds in repairing the collateral damage and restoring functionality. Tier 2 and Tier 3 cellular reactions, on the other hand, are also included in Prong 3, the unresolving inflammation that fails in reestablishing tissue homeostasis, so that organ function is eventually compromised.

One aspect of ENM-induced inflammation is the capacity of activating the inflammasome, in particular NLRP3, which is the main inflammasome complex. This is a complex of proteins that assembles in the cytoplasm in response to inflammatory stimuli and leads to the activation of the enzyme caspase-1, which is responsible for cleavage and activation of the precursor forms of two important inflammatory cytokines, interleukin (IL)-1β and IL-18 (82). Caspase-1 can also auto-activate and mediate cell death (172). Several studies have shown that ENM can activate the NLRP3 inflammasome [reviewed in Sun *et al.* (171)], similarly to other particulate agents (*e.g.*, hydroxyapatite crystals, cholesterol crystals, and aluminum hydroxide particles) (52, 74, 83). Inflammasome activation by ENM can occur through different mechanisms, including generation of ROS, which participate in inflammasome activation (63, 190).

Another mechanism is the destabilization of phagolysosomal membranes with consequent release of lysosomal enzymes, in particular cathepsin B, that activate the inflammasome either directly or *via* ROS (82). Other putative mechanisms of ENM-induced inflammasome activation include activation of the NADPH oxidases (NOX), K^+ efflux, purinergic receptor P2X7 (P2X7R)-mediated ATP depletion, decrease of mitochondria membrane potential, and thioredoxin-interacting protein (TXNIP)-induced NALP3 activation (82, 148). A summary of typical data of ENM-induced NLRP3 inflammasome activation is presented in Table 1. The general conclusion is that crystals and high-aspect particles (such as fibers) are excellent activators of the inflammasome. However, since inflammasome activation is not *per se* a sign of toxicity or pathological inflammation, it is a reaction that can be included both in Prong 2 and in Prong 3, and only a deeper kinetic analysis may allow us to discriminate between protective and pathological activation.

A final note of caution, when studying OS and inflammation induced by RNM, regards the possibility that ENM are inadvertently contaminated with endotoxin. This is a very common event when ENM synthesis and handling are not carried out in endotoxin-free conditions (180). The presence of endotoxin can cause *per se* inflammation that may be erroneously attributed to ENM (106).

Induction of OS and inflammation by particulate matter

All that has been said earlier for ENM applies as well to particulate matter (PM) collected from the environment. Indeed, studies on the capacity of PM to induce OS and inflammation are extensive and date back many years. Diesel exhaust particles (DEP), concentrated ambient particles, and ultrafine particles are some of the many types of PM that have been extensively studied in this direction [see, for instance, Li *et al.* (105)]. Although many studies show that PM can induce significant pulmonary inflammation on inhalation *in vivo*, still we are unable to associate PM characteristics with the ability to induce OS and inflammation. In fact, ambient PM is typically morphologically and chemically heterogeneous, very much depending on the specific environmental conditions (such as temperature and humidity) and concomitant emissions.

In addition, the presence of biologically active molecules such as bacterial lipopolysaccharide (see earlier for ENM) is practically never tested in the many studies published so far [see, for instance, Ying *et al.* (204)], leaving open the possibility that several of the effects caused by PM can be attributed to the presence of bacterial moieties, which typically trigger the same OS and inflammatory effects. Thus, the PM ability of inducing OS and inflammation is most likely the result of intrinsic toxicity of the chemicals present in the PM, of its state of aggregation and consequent changes in morphology (which may cause mechanical stress to cells), and of the presence of bystander biological substances (such as pollens, animal allergens, and bacterial fragments of whole micro-organisms). In this context, although we can list many biomarkers of both OS and inflammation induced by inhalation of PM, it is impossible to associate any of them to particle-specific effects.

NRF2 as an Indicator of Response to OS

One approach to monitoring OS as well as environmental electrophilic chemicals is to use biomarkers based on the response of the organism, as opposed to measuring oxidized products of cellular components. NRF2 is the main transcriptional regulator of cellular homeostasis and protects against multiple stress conditions. On dimerization with small MAF proteins, it recognizes an enhancer in the promoter

Table 1. Inflammasome Activation Induced by Particles *Versus* Engineered Nanomaterials

Material	*Main findings*	*References*
MSU crystals	NLRP3-dependent induction of IL-1β release *in vitro*	(115)
Cholesterol crystals	Cholesterol crystals activate NLRP3 and induce IL-1β production in human macrophages	(52, 143)
Hydroxyapatite crystals	Induction of IL-1β and IL-18 production in mouse macrophages is induced by needle-like and clumped nanocrystals, but not spherical and larger crystals, and depends on potassium efflux, generation of ROS, and lysosomal damage/cathepsin B *in vitro*, and on various NLRP3 components *in vivo* (knockout mice)	(83)
Crystalline silica	Crystalline silica induces NLRP3 activation and IL-1β production through phagolysosome destabilization	(50, 74, 195)
Amyloid β	Induction of IL-1β release *in vitro* in LPS-primed primary mouse microglial cells, NLRP3 inflammasome dependent, and ATP dependent	(61)
Asbestos	Asbestos induces NLRP3 inflammasome activation *in vitro* (human primary macrophages), dependent on ROS production, cathepsin B activity, P2X7Rs, and Src/Syk kinases	(50, 132)
Aluminum salts	Aluminum salts induce NLRP3 activation and IL-1β production through phagolysosome destabilization	(74)
CeO_2 nanowires of various size	Correlation between nanowires' length and lysosomal damage, cathepsin B release, and IL-1β release *in vitro* (human THP-1)	(81)
Polystyrene and PLG nanospheres	Smaller particles are taken up better by mouse BMDC *in vitro* and induce more IL-1β release, in an NLRP3-, cathepsin B-, and phagosomal acidification-dependent manner	(159)
Silver nanospheres	Smaller particles induce IL-1β release in monocytes better than larger ones, dependent on mitochondrial superoxide, cathepsin release, and K^+ efflux	(201)
TiO_2 nanobelts	Induction of IL-1β release *in vitro* by long but not by short or spherical particles (human THP-1, murine alveolar macrophages)	(62)
TiO_2 nanospheres	Phagocytosis-independent induction of IL-1β release *in vitro* (mouse BMDM, human THP-1 and primary keratinocytes). Smaller particles more active than larger ones in murine DC, in an actin-, ROS-, NLRP3-, and caspase-1-dependent fashion.	(195, 203)
SiO_2 nanospheres	Phagocytosis-independent induction of IL-1β release *in vitro* (mouse BMDM, human THP-1, and primary keratinocytes). Amorphous silica NPs induce IL-1β production in mouse DC in an actin-, ROS-, NLRP3-, and caspase-1-dependent fashion.	(195, 203)
Carbon nanotubes	Long CNT induce NLRP3 inflammasome activation *in vitro* (human primary macrophages), dependent on ROS production, cathepsin B activity, P2X7R, and Src/Syk kinases	(132)

BMDC, bone marrow-derived dendritic cells; BMDM, bone marrow-derived macrophages; CNT, carbon nanotubes; IL, interleukin; LPS, lipopolysaccharide; P2X7R, purinergic receptor P2X7; PLG, poly(d,l-lactide-co-glycolide); ROS, reactive oxygen species.

region of target genes, termed ARE/electrophile responsive element (EpRE) genes. These account for about 1% of human genome and encode phase I, II, and III detoxification enzymes, GSH, peroxiredoxin (PRDX) and thioredoxin (TXN) metabolism, intermediary metabolism related to pentose phosphate pathway, *etc*. (67).

The main mechanism of regulation is protein stability by the ubiquitin/proteasome system (UPS) illustrated in Figure 4. Under non-stress conditions, the E3 ubiquitin ligase adapter Kelch-like ECH-associated protein 1 (KEAP1) drives NRF2 to ubiquitination by the Cul3/RBX complex and rapid proteasomal degradation. However, KEAP1 contains several cysteine residues that have a low pKa value, making them highly suited for acting as a sensor for oxidative and electrophilic stress (112). Oxidant or electrophilic modification of critical cysteines in KEAP1, mainly C155, C273, and C288, prevents the protein from connecting NRF2 to the UPS, thus resulting in the accumulation of nuclear NRF2 and transcriptional activation of ARE genes.

Another crucial mechanism for control of NRF2 stability is by phosphorylation. Several kinases phosphorylate NRF2 with different outcomes. Activating phosphorylation by MAP kinases, PKC or PERK at Ser40, and other residues appears to free NRF2 from KEAP1 control. On the other hand, the Ser/Thr protein kinase glycogen synthase kinase 3 (GSK-3) phosphorylates at least Ser 335 and 338 in murine NRF2, thereby creating a recognition site for the E3 ubiquitin ligase adapter β-TrCP, leading to ubiquitination by Cul1/RBX and proteasome degradation of NRF2 (40, 141, 142). Importantly, several phosphatases, such as phosphatase and tensin homolog (PTEN), contain thiol reactive cysteines in their catalytic center, which become inactive on oxidation or reaction with electrophiles (139, 149). In this case, PTEN inhibition leads to sustained activation of AKT and inhibition of GSK-3. As a result, NRF2 escapes GSK-3/β-TrCP-mediated degradation (40). Later, we will discuss advances on its role for protection against several environmental forms of stress.

Heavy metals

Occupational or environmental exposure to heavy metals generates OS that, depending on the route of entrance and

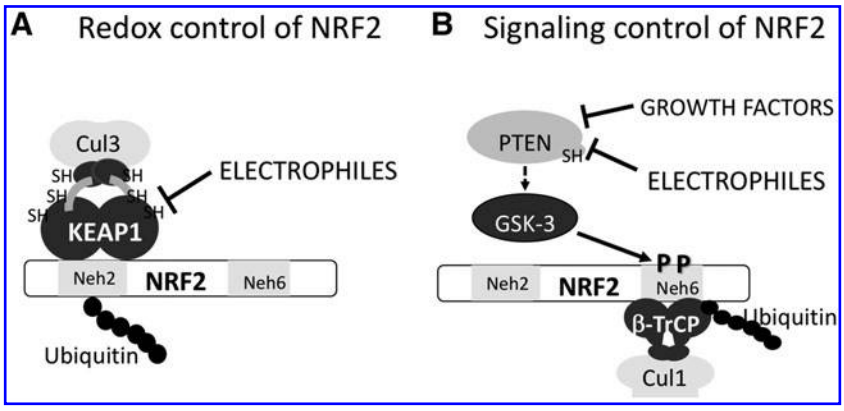

FIG. 4. Regulation of NRF2 by protein stability. (A), KEAP1/NRF2 interaction. Thiol reactive groups in KEAP1 provide a mechanism for sensing the levels of ROS and electrophiles. **(B)** β-TrCP/GSK-3/NRF2 interaction. Phosphorylation of NRF2 by GSK-3 provides a layer of regulation by signaling pathways and by electrophilic compounds and alters the balance kinase/phosphatase, exemplified here with PTEN [modified from Schmidt *et al.* (154)]. GSK-3, glycogen synthase kinase 3; KEAP1, Kelch-like ECH-associated protein 1; NRF2, nuclear factor (erythroid-derived 2)-like 2; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species.

clearance, may produce liver, kidney, or lung damage among others. Chromium, arsenic, cadmium, mercury, and lead can interact with nucleophilic thiol groups, for example, cysteine residues in proteins. Cells have a thiol buffering capacity represented by the GSH and PRDX/TXN systems. Exposure to heavy metals will compromise these systems and alter a significant fraction of thiols in critical enzymes. Formation of sulfur-metal bonds in redox-sensitive cysteines of KEAP1 will result in its stabilization. Also, signaling pathways are altered, with phosphatases such as PTEN being inhibited, resulting in increased activation of AKT, inhibition of GSK-3, and further stabilization of NRF2. The result is upregulation of genes involved in GSH synthesis and maintenance of reduced TRX and GSH, both of which are important to tolerate metal exposure.

Furthermore, as reported for cadmium, chromium, arsenic, and others, low-level chronic exposure to metals may induce cancer. Somatic mutations in the interface of interaction between KEAP1 and NRF2 have been correlated with several types of tumors (162), and NRF2 levels are elevated in cancer cells, resulting in a metabolic reprogramming that allows to withstand OS and adverse growth conditions. It is, therefore, possible that part of the tumorigenic activity associated with exposure to heavy metals might be due to dysregulation of NRF2 (165).

Most of the exposure to heavy metals is in the form or reactive molecules. For example, intracellular reduction of hexavalent chromium generates highly reactive pro-oxidant intermediates, together with superoxide, hydrogen peroxide, and hydroxyl radical. These compounds irreversibly inhibit TXN reductase and deplete TXN and PRDX (124). Although this situation is typical of KEAP1 inhibition, it is also recognized that TXN depletion has additional effects on signaling pathways such as activation of ASK1, leading to upregulation of NRF2 by MAPK kinases by yet-unknown mechanisms.

Noise injury

As expected, noise-induced OS also results in the activation of NRF2 and induction of some of its target genes, including superoxide dismutase (SOD) and heme oxygenase (54, 73). These studies also show that NRF2 deficiency increases noise-induced injury and hearing loss, whereas its induction has protective effects (54, 73).

Exposure to PM

Similar to other forms of environmental stress, exposure to PM results in activation of NRF2 and increased transcription of its target genes (48, 77). NRF2 induces not only transcription of antioxidant genes but also in phase 2 enzymes of xenobiotic metabolism. As a consequence, NRF2 deficiency increases the genotoxic actions of diesel exhaust (6). This has led to the suggestion that the levels of expression of NRF2 target genes could represent markers of exposure to PM (196). Because NRF2 also regulates secondary effects of environmental pollutants, such as inflammation and allergy, the NRF2 response to PM could also be used to predict those at risk of developing asthma (104), cardiovascular disease (CVD), and atherosclerosis (99).

Mental stress

Recent studies have correlated depression, typically caused by mental stress and social defeat, with low-grade chronic inflammation that affects critical regions of the brain (14). NRF2 modulates inflammation by downregulating the NF-κB pathway (41, 78). In addition, NRF2 inhibits expression of IL-6 and IL-1β by directly binding the proximal promoter of these genes (90). *Nrf2*-knockout mice exhibit a depressive-like behavior, with reduced levels of dopamine and serotonin and increased levels of glutamate in the prefrontal cortex, altered levels of proteins associated to depression such as VEGF and synaptophysin, as well as microgliosis. Importantly, depressive-like behavior elicited by endotoxin in wild-type mice could be attenuated with the NRF2 activator sulforaphane (114).

Ionizing and UV radiation

Exposure to cosmic, ionizing radiations and UV radiation, either from natural sources of from devices, represents a significant challenge to homeostatic redox mechanisms and

nucleic acids integrity. Recent studies have demonstrated that NRF2 promotes DNA repair and drives detoxification of superoxide that is generated after irradiation (158). In particular, NRF2 regulates the expression of RAD51, many DNA repair genes, including those of homologous recombination repair pathway, and have putative AREs (80).

Noise-Induced Hearing Loss

Noise-induced hearing loss (NIHL) can be induced by exposure to loud sound. Affected individuals may have inability to hear certain frequencies of sound, cognitive impairment of sound perception, including sensitivity to sound and ringing in the ears (tinnitus) (1). The association between noise exposure and hearing loss was first recognized by Sir Francis Bacon (1561–1626) (65).

NIHL is caused by acute (*e.g.*, sudden exposure to loud noise, explosion) or chronic acoustic trauma (*e.g.*, loud music). NIHL is the most common occupational disease and its severity differs among individuals, and increases with age, compromising the quality of life that extends beyond hearing loss. Unfortunately, unlike birds and amphibians, the ability to regenerate hearing is lost in mammals. Thus, in human beings, any damage to the hearing organ from any sound source over time leads to permanent hearing loss.

Cochlea is located within the inner ear and houses the specialized peripheral end organ of the auditory system, which mediates the transduction of sound waves into electrical nerve impulses that travel to the brain for central processing of auditory information. Acoustic insults to the cochlea cause mechanical and metabolic changes affecting almost all cell types, but particularly the sensory hair cells and neurons. Temporary and permanent threshold shifts occur from mechanical and metabolic changes caused by the exposure to different noise levels (34, 70, 92, 95, 100). Mechanical damages occur with exposure to the noise levels of 115–125 dB sound pressure level (SPL), whereas metabolic damages occur with exposure to the noise level of less than 115 dB SPL. These changes in threshold shifts are related to noise-induced neural degeneration, which begins shortly after noise exposure and can progress for several years post-exposure (94, 119). Importantly, early noise exposure can intensify age-related hearing loss (94).

Beginning at 85 dB ($\sim$300 times the energy level of 60 dB), long or repeated exposures may result in a notable loss of hearing. This level of 85 dB and higher includes some everyday sounds, for example, music on personal listening devices or emanating from small machinery such as lawnmowers. Noise coming from urban traffic, household appliances, personal listening devices, or occupational noise can also elicit hearing loss. Importantly, long or repeated exposure to moderate noise levels is often associated with alterations in behavior, as well as with changes in neuroendocrine, cardiovascular, and immune systems (45, 189). Cochlear damage and hearing loss associated with chronic environmental noise exposure may be linked to an increase in ROS levels as well as with inflammatory processes in the cochlea.

NIHL: pathology

Early studies of NIHL demonstrated mechanical damages of the cochlear structures, including the disruption of Reissner's and basilar membranes, damage and loss of stereocilia bundles, damage of the inner hair cells (IHCs) and outer hair cells (OHCs), *stria vascularis*, spiral ganglion cells, and lateral wall of the OHCs (23, 92, 100, 168). OS and inflammation are major contributors to NIHL.

Cell death induced by acoustic overexposure occurs primarily through apoptosis and necrosis. Apoptosis is a programmed cell death, with no inflammatory response (70). As discussed later, the apoptosis can be mediated by the activity of enzymes, induced by increased production of ROS and reactive nitrogen species (RNS) (92, 129, 188). The second cell death pathway is necrosis, a passive unprogrammed cell death that is identified by swollen and pale-staining cytoplasm resulting in rupture of the cell, spillage of the cell contents, damage to surrounding tissue, and evocation of an inflammatory response (70). Both apoptotic and necrotic pathways have been observed in the cochlea immediately after noise exposure (76), as discussed later and illustrated in Figure 5.

OS-induced apoptosis in hearing loss

The cochlea is a highly metabolically active sensory organ that receives 0.5 ml/min of blood flow in normal conditions (92). Metabolically, noise exposure can decrease cochlear blood flow, leading to cochlear ischemia-reperfusion injury, induce cell death by producing ROS, and contribute to injury and death of hair cells and spiral ganglion cells (33, 70, 92). Noise-induced OS causes lipid peroxidation in the spiral ganglion, organ of Corti, and *stria vascularis*, leads to oxidation of actin filaments of stereocilia and cell membrane lipids, protein oxidation, damage to nuclear and mitochondrial DNA, swelling and degeneration of afferent nerve endings, and release of toxic lipid peroxidation products such as 4-hydroxy 2,3-nonenal (HNE) (51, 92).

OS biomarkers in hearing loss

OS occurs immediately and is present up to 30 days after noise exposure (70, 200). In the first 10 days, the formation of ROS reaches its peak (200). OS has been identified by a variety of biomarkers of ROS and RNS activity in the cochlea and the brain (51). These biomarkers include HNE, nitrotyrosine (NT), malondialdehyde (MDA), inducible nitric oxide synthase (iNOS), cytochrome-C, caspase-3, -8, -9, and p66shc. Formation of HNE was observed after noise exposure in the lateral wall and Claudius cells, the Deiter's cells, and the OHC bodies (33, 51, 200). Formation of NT, a biomarker of NO production formed by nitration of a tyrosine residue in proteins, occurs in the hair cells after noise exposure (33, 51, 200). MDA was observed in cochlea immediately after noise exposure (33). The expression of iNOS in the hair cells, wall of the blood vessels of *stria vascularis*, and marginal cells was observed immediately after noise exposure (160).

Noise exposure induces cytochrome-C release from mitochondria and caspase-3, -8, or -9 activation in both apoptotic and necrotic OHCs, whereas caspase activation occurs only in apoptotic OHCs (129). Another important issue is the cause of ROS production in NIHL. Animal studies have shown that local application of NOX inhibitors has protective effects (21), and a genome-wide association study (GWAS) (98) showed that NOX3 is critical in the development of

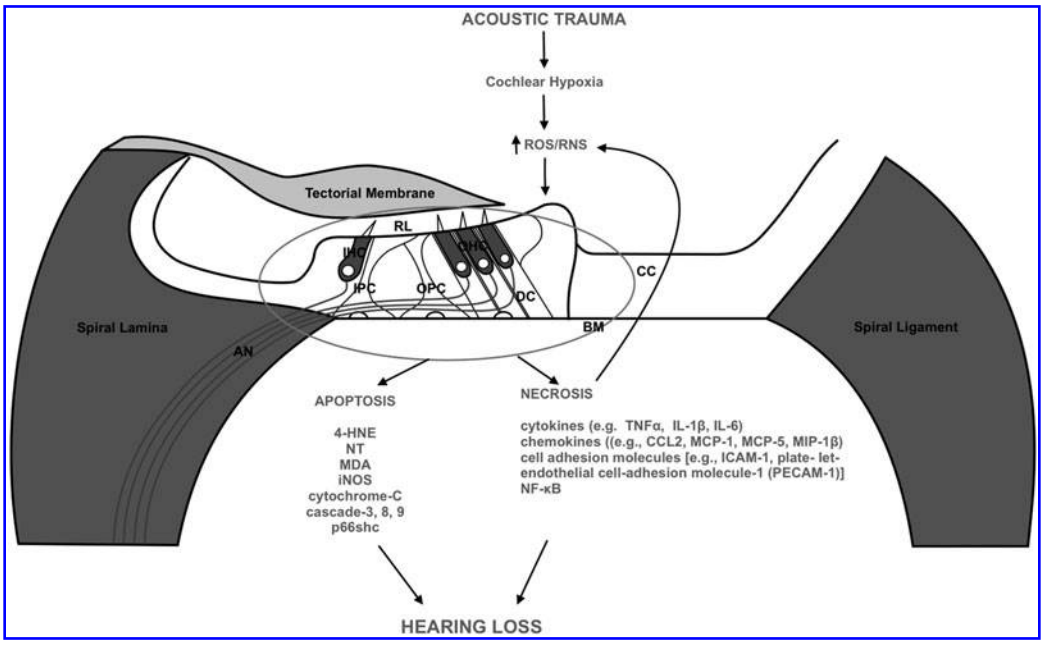

FIG. 5. Mechanisms of ototoxicity induced by acoustic trauma.

NIHL. These studies led to the suggestion of a potential therapeutic approach to inner ear pathologies (150).

Inflammatory biomarkers in hearing loss

Acoustic trauma can also initiate inflammation in the *stria vascularis*, compromising blood supply to the cochlea and causing hypoxia and injury to HCs (166). Injuries to the *stria vascularis* and spiral ligament occur after noise trauma, damaging type II and IV fibrocytes and leading to permanent hearing loss (71).

As a response to acoustic trauma, the cochlear cells express cytokines, such as TNF-α and IL-1β, and chemokines, which cause leukocyte migration (86). These inflammatory cells, producing cytokines, chemokines, ROS, and RNS, then propagate the inflammatory process to the inner ear.

After acoustic trauma, an influx of inflammatory cells occurs (71, 173, 174, 186). These cells are mostly found in the spiral ligament (type I, III, and IV fibrocytes), and in the perilymph of the *scala tympani* and *scala vestibule* (71, 173, 174, 186).). IL-6 immunoreactive cells were observed initially in the cytoplasm of type III and IV fIbrocytes, then throughout the spiral ligament and the *stria vascularis* (58). Double labeling with the neuronal marker NeuN showed IL-6 expression in the spiral ganglion neurons after noise exposure. Chemokines (such as MCP-1/CCL2, MCP-5/CCL12, and MIP-1β/CCL4) are upregulated by acoustic trauma (174). ICAM-1/CD54, a vascular adhesion molecule that mediates leukocyte extravasation, as well as other adhesion molecules, including P-selectin, PECAM-1, and VCAM-1, are also increased after noise exposure (161, 174, 199).

The measurement of cytokines as biomarkers on inflammation in hearing loss may have translational implication. For instance, the IL-1 receptor antagonist drug anakingra improves hearing loss associated with autoinflammatory diseases (120) and autoimmune diseases, including in glucocorticoid (GC)-resistant patients (181).

Brain–Body Interactions of Stress, OS, and Inflammation

Although stress and inflammation are often implicated in disease, there is a bidirectional regulation between the two. Figure 6 outlines the main regulatory pathways. The classical pathway is mediated by GC. The fact that stress activates the hypothalamus-pituitary-adrenal axis (HPAA) to increase GC has been known for a long time, and GC are probably the oldest anti-inflammatory drugs. The finding that GC inhibit the expression of inflammatory cytokines provided an important mechanism of action (20). It soon became clear that inflammatory cytokines activate the HPAA and, thus, increase GC that not only inhibit cytokine synthesis but also protect from their toxicity, in a classical feedback (18, 19, 35, 179). This raises an important point in the interpretation of the scheme in Figure 6: Inflammatory cytokines and inflammation are also stressors themselves, sometimes defined as ‘‘immune stressors.’’

It comes, therefore, as no surprise that corticosteroids are used as biomarkers of stress. Because their levels in the blood vary with time, the measurement of hair cortisol, which accumulates over weeks and months, has been proposed as a

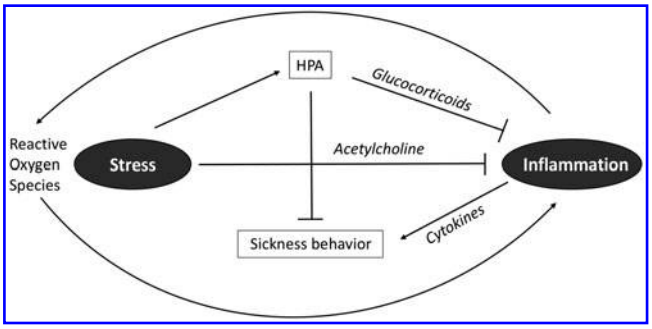

FIG. 6. The main neuroendocrine-immune pathways.

biomarker of stress (117) and has been successfully used in the study of post-traumatic stress disorder (PTSD) (111). More recent studies have identified in the cholinergic response mediated by vagus nerve stimulation another mechanism of regulation of peripheral inflammation by the brain, which also led to the development of novel therapeutic options (133, 177).

The fact that infection induces a sickness behavior (lethargy, anorexia, social isolation) is an old observation, but findings by Dantzer [reviewed in Dantzer *et al.* (47)] that this is mediated by cytokines have provided a molecular mechanism for what is believed to be an adaptive response of the organism to better survive an infection (46). Similar to their effect on IL-1-induced lethality (19), GC inhibit the anorexigenic response to IL-1 (140), indicating that they act by inhibiting not only cytokine production but also their action.

As mentioned earlier, OS can induce inflammation and, vice versa, inflammation induces OS. This has been hypothesized to be the case in PTSD, where a study measuring levels of inflammatory cytokines and markers of OS suggests that inflammatory cytokines induce ROS production, which then amplifies the inflammatory response (194).

Fitting GC and the HPAA in this bi-directional autostimulatory loop between inflammation and OS is more difficult. Clearly, GC, by blocking production of inflammatory cytokines, could remove a major stimulus of OS. On the other hand, activation of the HPAA, by increasing metabolic rate and glucose availability, can result in increased OS (167) and, in agreement with this hypothesis, administration of corticosterone to rats causes an increase in biomarkers of OS, lipid peroxides, and protein carbonyls (153).

It should be pointed out, however, that the latter study as well as the study on PTSD cited earlier (194) were performed by measuring, among other biomarkers, superoxide levels in plasma and blood. Because, as mentioned earlier, superoxide has a half-life in milliseconds, some aspects of the methodological approach might be questioned.

Effect of emotional states on OS and inflammation

Emotions, inflammation, and OS share three fundamental features: (i) They help the host to adjust to different environmental challenges and maintain a status of homeostasis; (ii) they can have both protective and deleterious effects for the host; and (iii) they are deeply intertwined in ways that we have only started to appreciate and are not fully explored. The few examples given later should be sufficient to support these statements.

There is evidence that basic emotions, such as laughter and joy, improve immunological competence of the host through natural killer cells, which are important in cancer surveillance (60). It is possible that the negative emotional state often experienced after a diagnosis of cancer might contribute to the development of this disorder, and that ''laughing therapy'' (60) and patient support groups might have a biological therapeutic value for cancer patients undergoing chemotherapy.

Negative emotions (anger and rage) can be protective as they not only represent an immediate reaction to real or imaginative dangers but also contribute to the exacerbation of chronic inflammatory diseases (25, 88). Anger, rage, and aggressive behavior cause a significant increase in the serum levels of IL-1 (136).

Several studies (11, 26, 32, 42, 43, 113, 138) have highlighted the link between emotion and immunity.

Studies have shown how external conditions (massage-like stroking or enriched environment) can improve the host resilience to immunosuppression (107, 138).

We think that the implications of these studies go beyond the simple duality of emotions and immunity as an example of body–mind continuum. Indeed, the ''mirroring effect'' that we have proposed to explain how emotions influence immune response and vice versa (26, 42, 43) might as well work for another system.

There is increasing evidence that emotional state and personality affects inflammation and OS. Interestingly, several risk factors for CVD (high-fat diet, sedentary lifestyle, and smoking) are associated with elevated OS, and they are lifestyle choices associated with depression (7, 97).

Many recent studies highlighted links between outlook on life and outcome in disease. For example, people who have Type D personality, a pessimistic and socially inhibited outlook, do worse during CVD. Heart failure patients with this personality type have elevated levels of xanthine oxidase and reduced heat shock protein 70. These factors combined might increase OS and inflammation, leading to a worse prognosis (89). On the other hand, higher optimism correlates with lower levels of inflammation, IL-6, and markers of endothelial dysfunction. Similarly, there is a positive correlation between higher optimism scores and increased carotenoid and antioxidant levels, which is suggestive that optimists may have lower OS (22).

These studies might help with the stratification of patients based on their personality traits. People who practice meditation have lower levels of lipid peroxidation in serum but higher levels of NO, which is indicative of normalized endothelial function (69). The risk of all-cause mortality, stroke, and myocardial infarction can be lowered by as much as 45%, due to a reduction in psychosocial stress and in blood pressure, both of which are linked to OS (151). Similarly, yoga seems to attenuate OS, possibly by increasing glutathione reductase (GR) and glutathione peroxidase (GPx) and by decreasing serum lipid peroxides and F2-isoprostane. Yoga practice in type 2 diabetics decreases MDA, increases GSH and vitamin C, and improves glycemic control (66). However, studies done in this area are small and, therefore, should be interpreted with caution until larger randomized clinical trials are done.

Social isolation is an increasingly worrying threat, as socially isolated people are at increased risk of several diseases, including atherosclerosis and dementia (56, 157). Social isolation downregulates the genes required for the GC response, which could impair ability to dampen immune responses, and inflammation is exacerbated by increased expression of NF-κB (38). Similarly, in rats that have been chronically isolated, OS is observed, accompanied by decreased GPx and GR (49).

Opposed to social isolation, environmental enrichment normalizes levels of TNF-α, CCL3, and CCL4 by preventing changes in microglial expression in Alzheimer's disease models (184), and it lowers hippocampal damage and OS during chronic cerebral hypoperfusion (113).

Most animal models of environmental enrichment use physical exercise. Depressed patients who exercise have lower markers of OS (155). In rats, GSH depletion causes anxiety-like behavior, whereas moderate exercise on a

treadmill prevents OS-induced anxiety and decreases OS markers in the hippocampus, amygdala, and the *locus coeruleus* (146).

Biomarkers of OS and inflammation in mental disorders

There is a lot of clinical evidence to support a role of OS and inflammation in mental disorders. Depression causes elevated total OS in plasma and serum, with increased plasma NO levels associated with suicidal thoughts (88, 101). Depressive patients are found to have lower total antioxidant activity, with scores being indicative of poor response to pharmacological treatments (13). A meta-analysis study looking at C-reactive protein (CRP), IL-1, and IL-6 found that these factors were positively correlated with depression (75). Serum TNF-α and CRP are elevated during depression; treatment with SSRIs returned levels to those seen in non-depressed patients, and this was associated with decreased clinical scores of depression (178). In social phobia patients, studies have shown that alongside having increased MDA there was increased SOD, GSH-Px, and catalase and lipid peroxidation. Interestingly, a positive correlation was seen between anxiety levels and MDA, SOD, and GSH-Px as well as a positive correlation was seen between MDA, SOD, and CAT and the duration of illness (10). CRP levels are elevated in men who have anxiety disorders and are higher in those who have late-onset anxiety disorders (>50 years), but no difference is seen in TNF-α and IL-6 (184).

The Human Exposome

Proposed by Wild in 2005 as an environmental counterpart to the human genome, the human exposome represents the totality of environmental (*i.e.*, non-genetic) exposure individual experiences between conception and death (191). As a ''comprehensive description of lifelong exposure history'' (192), the exposome effectively provides a new framework for bringing together interdisciplinary teams to understand the environmental determinants of chronic disease risk, the influence of which are estimated to exceed those related to genetic predisposition (144). Wild subsequently elaborated on his definition of the exposome, emphasizing the dynamic nature of exposures over a lifetime and categorizing components of the exposome into three domains; internal, specific external, and general external (192).

Specific reference was made to factors that influence the internal or cellular environment (*e.g.*, metabolism, aging, gut microflora activity), vary at the individual level (*e.g.*, occupation, lifestyle components, mental stress, noise, air pollutants), or influence populations (*e.g.*, climate, education, urban/rural surroundings) (192). The definition was also expanded to include behavioral interactions and products of endogenous exposure (*e.g.*, epigenetic changes), and to account for the cumulative nature of exposures and their corresponding biological adaptations (118). Although there have been several proposed revisions of this original definition, the practical implementation of this general concept has led researchers to embrace more holistic and integrated methods for assessing the external and internal environments.

Central to the implementation of this idea is the application of data-dense omics techniques that report on various aspects of the internal chemical milieu (in most cases focused on the blood) to provide complementary datasets related to biochemical status in individual biosamples. Combined with access to mature biobanking resources, the recent radical advances in molecular biology approaches, multivariate data analysis tools, biomonitoring technology, and the proliferation of inexpensive mobile devices have enabled exposome studies to become a reality.

The exposome proposal came in response to the limitations in epidemiological studies attempting to link environmental exposure assessments with disease endpoints—effectively ''bottom-up'' approaches, focused on a small number of priority exposures. By contrast, exposome studies seek to benefit from ''top-down,'' data-driven, agnostic approaches that can uncover previously unknown and/or complex relationships, as well as guide subsequent hypothesis-based investigations to provide mechanistic insight about disease etiology. Complete characterization of the exposome—which would require high-resolution, real-time monitoring of all exposures throughout life—is clearly unfeasible for multiple obvious reasons (8). However, it is proposed that understanding the status of the exposome, particularly that of the internal chemical environment, at the individual level during critical periods of life may help delineate the contributions of various genetic and environmental factors.

Studying the exposome

An improved ability to characterize the external chemical environment can enhance exposure models both spatially and temporally, but it is the extensive characterization of an internal chemical environment that represents the most significant advance in recent years, and an opportunity to delineate the contributions made by multiple, interacting factors, to the biological changes observed at an individual level. High-throughput platforms now exist for profiling the metabolome (metabolites), proteome (proteins), transcriptome (gene transcripts), and adductome (typically endogenous-xenobiotic conjugates) and they collectively provide a wealth of information about the status of biological samples [reviewed in the context of the human exposome by (193), summarized briefly in Table 2].

These platforms (mostly, now) provide broad coverage of their biomolecular target classes, and, therefore, permit the application of data-driven approaches that mirror those used in the GWAS. The techniques may be performed on cells, tissues, or biofluids, making novel biomarker selection possible for *in vitro*, *in vivo*, and human studies and integrated analyses may be performed across the omics platforms to cross-validate or integrate findings. Initial studies have indicated that suitably collected/stored biobanked samples are amenable to analysis by multiple omics platforms (68), and large-scale initiatives to conduct exposome studies are now underway [including the EXPOsOMICS (182), HELIX (185), and HEALS projects in the EU].

Unpicking the exposome: cellular inflammatory responses to DEP

Although there is considerable activity in molecular epidemiological analysis to characterize biological samples archived in biobanks, the validation and mechanistic understanding that accompanies these largely correlative analyses is both complementary and vital; tying together evidence from

Table 2. Summary of Common Omics Approaches in Exposome Studies

Omics	*Target molecule*	*Analytical platform*	*Typical profile content (~ no. of molecules)*	*Advantages and limitations of technique*
Transcriptome	Gene transcripts	Microarray	<50,000	Custom arrays available Suitable for cellular, tissue, and biofluid samples Limited target number Limited detection limit
		Next-generation sequencing	Full transcriptome	High sensitivity (single-nucleotide level) Low limit of detection Qualitative and quantitative Suitable for cellular, tissue, and biofluid samples High cost
Proteome	Proteins	Mass spectrometry		High throughput, specificity, and sensitivity Qualitative and quantitative Multiple methods required to cover proteome Complex feature annotation
		Protein array	>20,000	High throughput and sensitivity Low sample consumption Efficient feature annotation Limited target number
Metabolome	Small-molecule metabolites	Nuclear magnetic resonance spectroscopy	>100	Minimal sample preparation Suitable for cellular, tissue, and biofluid samples Metabolite annotation/assignment typically straighforward
		Liquid chromatography–mass spectrometry	>1000	Suitable for cellular, tissue, and biofluid samples Derivatization required for polar and high-molecular-weight metabolites Metabolite annotation/assignment can be time-consuming
Adductome (serum albumin)	Endogenous-xenobiotic conjugates	Mass spectrometry	>100	Ability to capture information on reactive intermediates and/or short-lived exposures Variety of techniques available for targeting with different specificity and resolution Time-consuming annotation

multiple levels of analysis is required to corroborate the correlative analyses that are conducted on these omics readouts (144, 182). For example, although many of the ongoing exposome studies include aspects regarding air pollutants, validation of these studies requires a coherent molecular context, whereas conversely targeted analyses need to be directed appropriately. For example, DEP are considered important environmental causes of OS and pulmonary inflammation due to their ubiquitous presence in air (84), their concentration in populated areas (206), and the breadth of sub-populations that are susceptible to their inflammatory and oxidative effects (12, 17, 27, 37, 53).

Adverse responses to DEP exposure have traditionally been assessed by quantifying specific inflammatory cells, cytokines, or cell adhesion molecules (CAM) after exposures (2, 93, 127, 152, 175). These targets are well-characterized mediators of inflammation that provide strong evidence of DEP induction of inflammatory cellular response, but they are limited in their potential to expand our mechanistic knowledge of the observed toxicity. In contrast, agnostic approaches for omics screening report on both characterized and uncharacterized markers, offering chances to explore a wider range of associations with DEP toxicity. Xiao *et al.* used a proteomic screening approach, showing that DEP induced OS in RAW 264.7 macrophages; the response was accompanied by more than eightfold increases in new protein expression. Furthermore, the biological functions of the proteins reflected a hierarchical response to OS, with the macrophages expressing antioxidant enzymes after low-dose exposures ($\leq$10 μg/ml), pro-inflammatory signaling proteins after mid-dose exposures (10–50 μg/ml), and regulators of mitochondrial function at cytotoxic doses ($\geq$50 μg/ml) (198). Although many

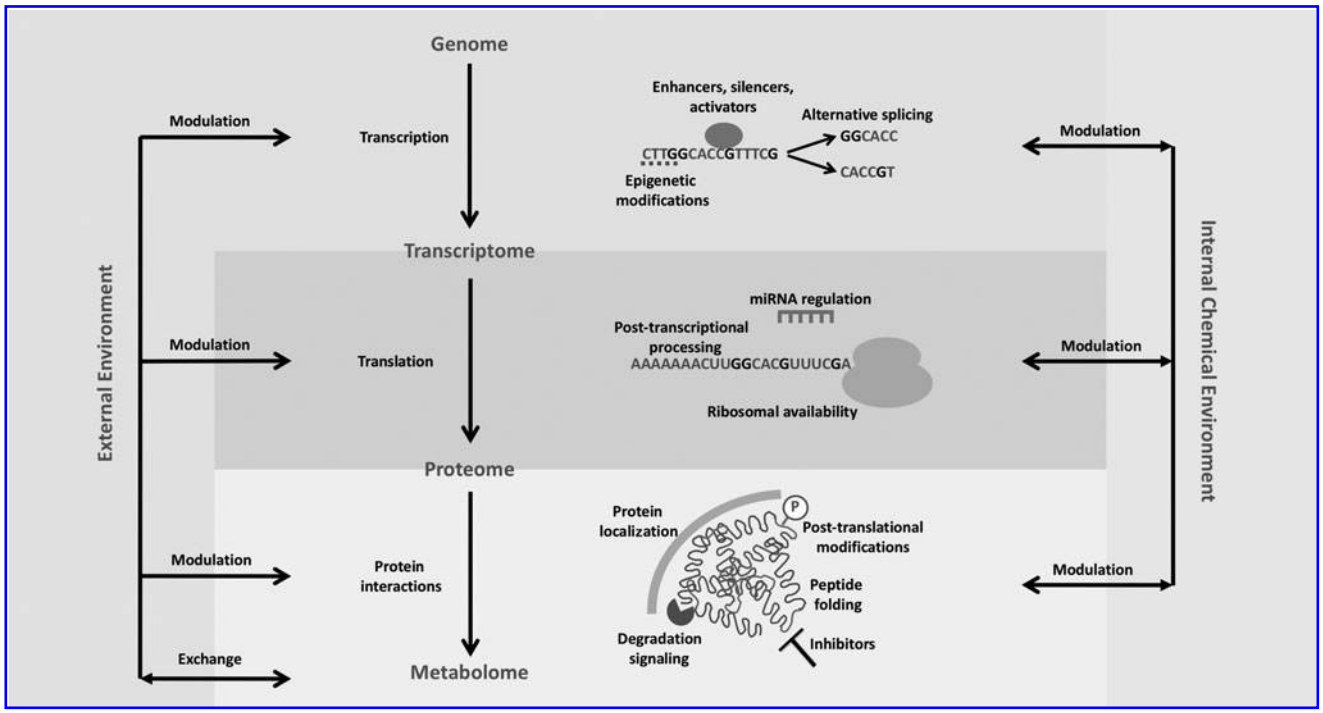

FIG. 7. Interactions between genes and the environment are mediated by a complex network of biological entities. Environmental exposures occur in the context of all previous exposures and the multifactorial responses elicited by the biological network. Characterization of the biological complexity of the internal chemical milieu during key periods of life (*e.g.*, small molecules that constitute the metabolic phenotype; status of gene expression; *etc.*) by using high-throughput omics platforms provides a window on the human exposome, and an opportunity to start dissecting the contributions of various factors to the etiology of chronic diseases.

of these proteomic changes validated existing hypotheses of how macrophages respond to DEP, others (including the induction of receptor-induced apoptosis) were previously uncharacterized (198). These changes revealed novel pathways that are triggered by DEP-induced OS, advancing our understanding of the response.

Metabolomic, transcriptomic, and proteomic profiles are strongly dependent on the expression and behavior of the other molecular species as well as experience a dynamic interaction with the external chemical environment (Fig. 7). Proteomic responses to DEP-induced OS (198) are mirrored by transcriptomic studies showing changed expression of oxidative response genes after DEP exposure (91, 134) as well as micro RNA (79).

Supporting the hypothesis that surface-bound metals and polycyclic aromatic hydrocarbons (PAHs) contribute to particulate toxicity (87, 135), metal and PAH-rich heavy fuel oil particles induce inflammatory and OS pathways more than carbonaceous DEP (130).

Redox status, inflammation, and ROS are all intricately linked with biotransformation of compounds across this continuum. For example, dietary components have been linked with modulation of cytokine levels, with a concomitant impact on the metabolome (36), with other recent examples including herbal medicine (205), involvement in arthritis [reviewed by Chimenti *et al.* (31)], and broader implication in pathways identified by cross-omics analysis (16, 163). In addition, a comprehensive survey focused on the role of metabolome studies in characterizing oxidative studies was previously published by Liu *et al.* (108).

Exposome and adverse outcome pathways: inflammation and ROS are key integrators of complex exposure-response relationships

One exemplar that elegantly illustrates the interplay of inflammatory mediators and ROS with other components of the internal chemical milieu is the analgesic/antipyretic compound paracetamol (also known as acetaminophen), one of the most commonly used over-the-counter drugs worldwide. Self-administration is the main cause for personal exposure (exposure in the wider environment is negligible), and large-scale, cross-sectional metabolic phenotyping studies of humans have shown a high prevalence of significant (*e.g.*, therapeutic dose) exposure (109, 110).

Characterization of population-level use of therapeutics (*e.g.*, anti-inflammatory agents) provides an overall profile of the exposome, and it helps contextualize and validate observed responses at the individual level (*e.g.*, when addressing research questions related to chronic inflammation that may be confounded by unreported pharmaceutical use). Intense scrutiny has allowed many of the toxicological consequences of paracetamol exposure and metabolism to be elucidated (including depletion of cellular antioxidants, increased ROS formation, and formation of reactive intermediates).

However, despite such widespread, long-term, and consistent use, the complexities of the exposure-response relationship continue to be uncovered (including metabolite conjugation to arachidonic acid to produce an active metabolite AM404 (72) and metabolite modulation of the nociceptive response (4)). This also illustrates the challenge that

is faced when considering how to dissect the complex, multicomponent exposures that occur throughout life; considerations of additive or non-linear effects arising from co-exposures all add up.

Attempts to bring together the diverse pieces of evidence that relate to exposure-response-disease relationships, and to address the challenge of this inherent complexity have resulted in the formulation of systems toxicology tools such as adverse outcome pathways (AOPs), as summarized by Burden *et al.* (28). By establishing a series of causal steps from an initial molecular initiating event onward, the AOP approach may help integrate knowledge about multiple environmental exposures that share common pathways, described by using an agreed ontology. The combination of the exposome and AOP frameworks is likely to be particularly useful when the complexity of exposure-response relationships is considered; exposures do not occur in isolation, but in a context that encodes previous exposures and responses, and they mediate the dynamic relationship between co-exposures.

In the context of OS, several redox proteomics techniques and their application to the field of inflammation and neuroinflammation have led to the identification of specific oxidized proteins undergoing carbonylation (170) or glutathionylation (30, 122). The extension of omics to oxidative post-translational modifications may add a dimension to the information obtained and eventually provide a more precise way of identifying exposure to agents that cause OS.

The strong underlying biological network connections between certain sets of pathologies—particularly those related to systemic inflammatory status that underpin multiple chronic disease conditions—may mean that we move away from attempts to find correspondence between individual exposures and outcomes, and toward using biological networks to link and explain complex exposure patterns with (multi)morbidities defined by detailed molecular phenotyping (102, 169).

Conclusions

The use of biomarkers of inflammation or OS has been instrumental to formulating causal hypotheses on disease mechanisms (such as the effect of environmental stressors).

We mentioned earlier that, in a way, biomarkers are signs. However, there are different types of signs and so, if biomarkers are really signs in a non-metaphorical sense, there must be different types of biomarkers as well, depending on the relationship they have with their referent. In fact, we do not just measure biomarkers; in some cases, we can measure the ''real thing.''

For instance, to quantify exposure to heavy metals, we can measure their level in the organism. When this is not possible, we can measure signs that are indicators of the exposure of the body to heavy metals. Some of them have a direct relationship to the object; for example, a product of lipid peroxidation is directly formed by a chemical reaction between an ROS and a lipid. Others can be an indicator of the response of the organism to the object that we want to measure, and this is the case of NRF2 or the markers of exposure described in the section on the exposome; all these will have various degrees of separation from the object that we want to measure, and this needs to be considered as some may require just transcription (if we measure an mRNA), others transcription, and translation (if we measure a protein).

This is particularly important if we are measuring exposure to a physical stressor, such as noise, or a psychological stressor, that is not present at the time of the examination.

Interestingly, even if not obvious, inflammation itself is a response to a foreign body or to an endogenous or exogenous stressor. It is important to note that measuring biomarkers is not always a second, imperfect choice when we cannot measure what we need (like in the case of short-lived radicals). Often, measuring a biological response adds a second dimension, that of biological relevance. But in the case of inflammatory biomarkers, we observe a further level of relevance.

Let us consider inflammatory cytokines. As mentioned earlier, they are easily measurable biomarkers of inflammation, many of which are measurable in plasma or serum. However, some of them are not simple signs but proxies. A proxy of something is a sign that has a vicarious relationship with that something: It both ''stands for'' its referent and ''stands *in* for'' it.

This means that not only there is a link between proxy and referent; but one can also interact with the referent by operating on the proxy and, as a result, affect the object (or process, in this case) that it stands for (55). In short, they have a bidirectional relationship with the referent. If MDA is elevated in a disease because of increased lipid peroxidation, you cannot improve the disease by administering an anti-MDA antibody, because it is just a sign of OS.

However, in inflammatory diseases, IL-6 and TNF-α are not only signs of the inflammatory process but also proxies, and so their inhibition, for instance with antibodies, improves the disease in patients with chronic inflammation. This concept is exemplified in Figure 8, where the mediators and processes described in this article are assigned a value of biomarkers (considering their ease of measurement) or proxies.

Researchers often classify biomarkers differently. For instance, MDA, IL-6, and TNF would be considered mechanism-based biomarkers because they reflect a potential disease mechanism. On the other hand, IL-6 and TNF are also defined as ''pharmacological targets,'' although not all pharmacological targets are biomarkers as some of them are not measurable. The definition of biomarkers as signs and their value, whether they are proxies or just signs, will need to be considered when considering their role in disease.

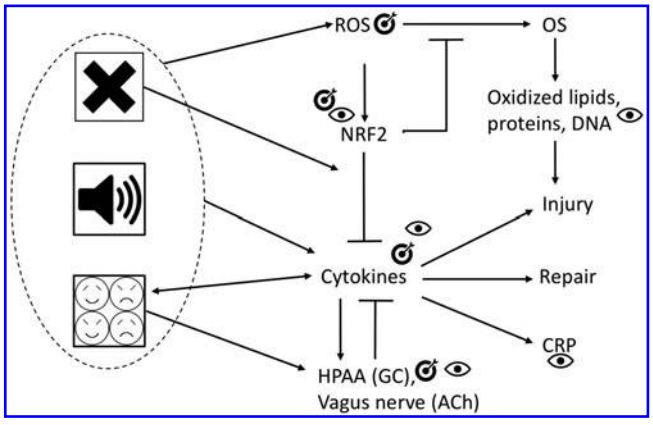

FIG. 8. Biomarkers for stress-induced inflammation and OS. The symbols denote whether the biomarker is a sign (that indicates the activation of a process) or a proxy (that are also targets we can interact with to modify the disease process).

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Authors' Contributions

All authors contributed equally. Contributors to each section—first section on biomarkers and the conclusions: P.G. and L.F.; nanomaterials: D.B.; NRF2: A.C. and G.M.; noise: S.L.; brain–body interactions: F.D.A., A.H., and P.G.; exposome: T.A. and L.S. P.G. edited and coordinated the text.

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Abbreviations Used

AOP = adverse outcome pathway
ARE = antioxidant response element
BMDM = bone marrow-derived macrophages
CAM = cell adhesion molecules
CCL = CC chemokine ligand
CNT = carbon nanotubes
CRP = C-reactive protein
CVD = cardiovascular disease
DEP = diesel exhaust particles
ENM = engineered nanomaterials
EpRE = electrophile responsive element
GC = glucocorticoids
GPx = glutathione peroxidase
GR = glutathione reductase
GSH = reduced glutathione
GSK-3 = glycogen synthase kinase 3
GSSG = oxidized glutathione
GWAS = genome-wide association studies
HNE = 4-hydroxy 2,3-nonenal
HPAA = hypothalamus-pituitary-adrenal axis
IHC = inner hair cells
IL = interleukin
iNOS = inducible nitric oxide synthase
KEAP1 = Kelch-like ECH-associated protein 1
MAF = musculoaponeurotic fibrosarcoma oncogene homolog
MAPK = mitogen-activated protein kinase
MDA = malondialdehyde
NF-κB = nuclear factor kappa B
NIHL = Noise-induced hearing loss
NLP3 = NACHT, LRR and PYD domains-containing protein 3
NRF2 = nuclear factor (erythroid-derived 2)-like 2
NT = nitrotyrosine
OHC = outer hair cells
OS = oxidative stress
P2X7R = purinergic receptor P2X7
PAH = polycyclic aromatic hydrocarbons
PM = particulate matter
PRDX = peroxiredoxin
PTEN = phosphatase and tensin homolog
PTSD = post-traumatic stress disorder
RNS = reactive nitrogen species
ROS = reactive oxygen species
SOD = superoxide dismuates
SPL = sound pressure level
TNF = tumor necrosis factor
TXN = thioredoxin
TXNIP = thioredoxin-interacting protein
UPS = ubiquitin/proteasome system