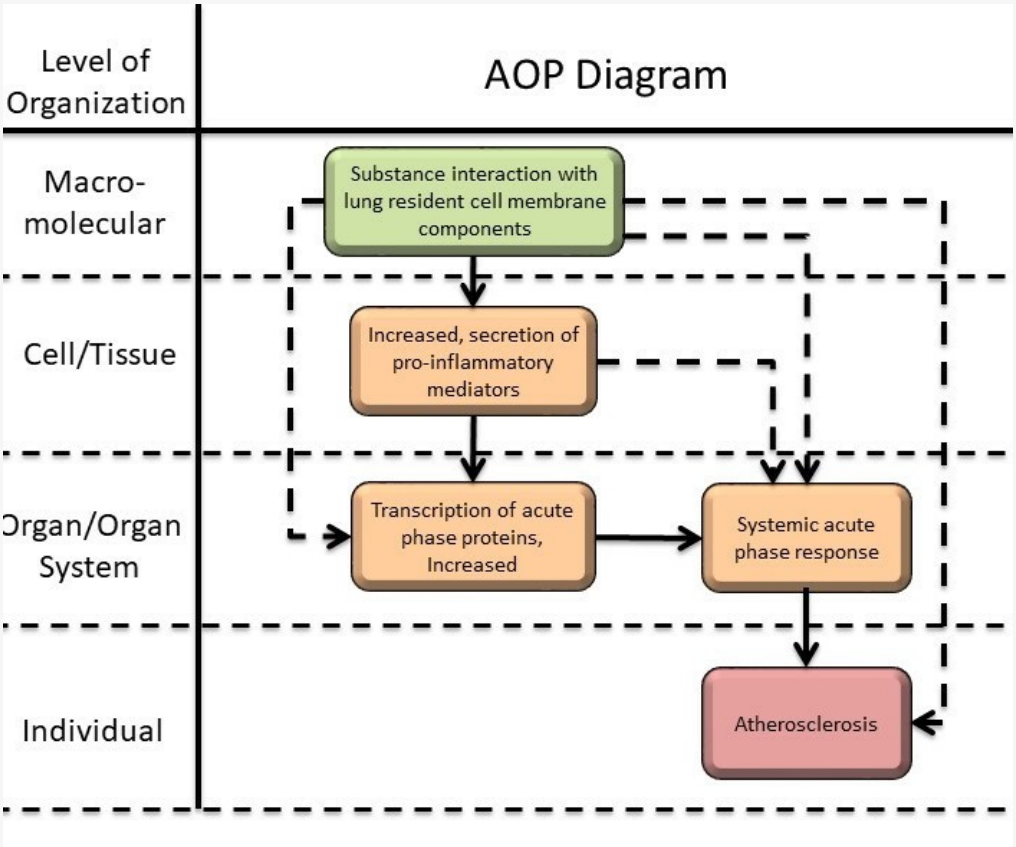
AOP ID and Title:

AOP 237: Substance interaction with lung resident cell membrane components leading to atherosclerosis
Short Title: Interaction with lung cells leading to atherosclerosis

Graphical Representation



Authors

Claudia Torero Gutierrez[1], Sarah Søs Poulsen[1], Jorid Birkelund Sørli[1], Håkan Wallin[2], Sabina Halappanavar[3], Carole L. Yauk[4], Ulla Vogel[1,*]

[1]The National Research Centre for the Working Environment, Denmark

[2]Statens Arbeidsmiljøinstitutt, Norway

[3]Health Canada, Canada

[4]University of Ottawa, Canada

*Corresponding author: Ulla Vogel (ubv@nfa.dk)

Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite	Under Development	1.55	Included in OECD Work Plan

Abstract

The present adverse outcome pathways (AOP) presents the link between the interaction of stressors of the pulmonary system and atherosclerosis. After interaction with the lung cell membrane, stressors can induce the release of pro-inflammatory factors, which in turn triggers the expression of acute phase proteins genes in the lungs and other tissues. Serum amyloid A (SAA) and C-reactive protein (CRP) are the major acute phase proteins in humans, and are considered risk factors for cardiovascular disease (Table 1 presents selected differences between acute phase response in humans and mice). In particular, serum amyloid A restricts the transport of cholesterol to the liver, allowing the accumulation of cholesterol in arteries and the formation of foam cells, an early

marker of atherosclerosis.

Table 1. Selected differences in acute phase response between humans and mice.

Characteristic	Humans	Mice
Number of identified genes involved in acute phase reponse	62	62
Major acute phase proteins	CRP, SAA	Haptoglobin, SAA, serum amyloid P
Moderate and minor acute phase proteins	Haptoglobin, fibrinogen, α_1 acid glycoprotein	CRP, fibrinogen
SAA isoforms	*Saa1, Saa2* and *Saa4*	*Saa1, Saa2, Saa3* and *Saa4*

References: [1-4].

This AOP mainly focus on particles or particulate matter as stressors, however other inflammatory conditions that induce acute phase response, can be consider stressors and lead to atherosclerosis. In addition, most of the evidence is based on animal studies (mice) as a model for the human system, however the adverse outcome of the present AOP, atherosclerosis, is only applicable for humans. The AOP can be used for regulatory purposes and to risk assess inhalable materials having acute phase response as the critical effect.

Background

Cardiovascular disease (CVD) is the leading cause of death worldwide, being responsible for 32% of all deaths in 2019 (WHO: http://www.who.int). The term CVD covers all diseases of the cardiovascular system, including atherosclerosis, which is manifested as increased plaque deposition or build-up in the arteries. Although, atherosclerosis is not a cause of death, it can lead to fatal conditions as stroke and myocardial infarction. Atherosclerosis is normally asymptotic disease and is initiated by a biological, chemical or physical insult to the artery walls. This leads to the expression of cell adhesion molecules (selectins, VCAM-1 and ICAM-1) on the endothelial lining of the arteries, which facilitates the activation, recruitment, and migration of monocytes through the endothelial monolayer [5,6]. Inside the intima layer, the monocytes differentiate into macrophages and internalize fatty deposits (mainly oxidized low-density lipoprotein). This results in them transforming into foam cells, which is a major component of the atherosclerotic fatty streaks. The fatty streaks reduce the elasticity of the artery walls and the foam cells promote a pro-inflammatory environment by secretion of cytokines and ROS. In addition, foam cells also induce the recruitment of smooth muscle cells to the intima. Added together, these changes lead to the formation of plaques on the artery walls. A fibrous cap of collagen and vascular smooth muscle cells protects the necrotic core and stabilizes the plaque [7,8]. However, blood clots can be formed if the plaque ruptures. These may travel with the bloodstream and obstruct the blood flow of smaller vessels, eg. the coronary arteries, which ultimately can lead to myocardial infarction.

Inhalation of particulate matter, chemicals and pathogens have been related to increased pulmonary inflammation. Whereas a normal immune reaction is crucial for effective elimination of threats to the body, chronic and unresolved inflammation has been linked to both adverse pulmonary and adverse systemic effects in humans. In concordance with this, various retrospective and prospective epidemiological studies have linked pulmonary exposure to respirable air particulates with increased the risk of developing CVD [9-12]. Inhalation of particles has been proposed to affect the cardiovascular system in several different ways, including through disruption of vasomotor function and through acceleration of plaque progression in atherosclerosis [13,14].

The development of the present AOP was supported by the EU project NanoPASS (Grant number: 101092741) and the Focused Research Effort on Chemicals in the Working Environment (FFIKA) form the Danish Government.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
	MIE	1495	Substance interaction with the lung resident cell membrane components	Interaction with the lung cell membrane
	KE	1496	Increased, secretion of proinflammatory mediators	Increased proinflammatory mediators
2	KE	1438	Transcription of acute phase proteins, Increased	Increased transcription of APP

Sequence	Type	Event ID	Title	Short name
3	KE	1439	Systemic acute phase response	Systemic APR
7	AO	1443	Atherosclerosis	Atherosclerosis

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Substance interaction with the lung resident cell membrane components	adjacent	Increased, secretion of proinflammatory mediators	High	Low
Increased, secretion of proinflammatory mediators	adjacent	Transcription of acute phase proteins, Increased	High	Moderate
Transcription of acute phase proteins, Increased	adjacent	Systemic acute phase response	High	Moderate
Systemic acute phase response	adjacent	Atherosclerosis	High	High
Substance interaction with the lung resident cell membrane components	non-adjacent	Transcription of acute phase proteins, Increased	High	Moderate
Substance interaction with the lung resident cell membrane components	non-adjacent	Systemic acute phase response	High	Moderate
Increased, secretion of proinflammatory mediators	non-adjacent	Systemic acute phase response	High	Moderate
Substance interaction with the lung resident cell membrane components	non-adjacent	Atherosclerosis	High	Moderate

Stressors

Name	Evidence
Lipopolysaccharide	Not Specified
Graphene oxide nanoparticles	Not Specified
Carbon nanotubes	Not Specified
Insoluble nano-sized particles	Not Specified
Virus	Not Specified

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adult	High

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI
mouse	Mus musculus	High	NCBI

Sex Applicability

Sex	Evidence
Male	High
Female	High

This AOP is applicable to adult humans of both sexes. Although atherosclerosis is a condition that begins during childhood and progresses through life, its clinical manifestation is mostly observed in older individuals [15].

The AOP is applicable to all stressors that can be inhaled and, therefore, interact with the pulmonary system, and induce pulmonary inflammation if the dose is high enough.

Essentiality of the Key Events

	Defining question	**High**	**Moderate**	**Low**
Support for essentiality of KEs	What is the impact on downstream KEs and/or the AO if an upstream KE is modified or prevented?	Direct evidence from specifically designed experimental studies illustrating prevention or impact on downstream KEs and/or the AO if upstream KEs are blocked or modified	Indirect evidence that modification of one or more upstream KEs is associated with a corresponding (increase or decrease) in the magnitude or frequency of downstream KEs	No or contradictory experimental evidence of the essentiality of any of the KEs.
MIE: Substance interaction with the lung resident cell membrane components	**Moderate.** It has been observed that there is a dose-response relationship between the dose of the stressor (i.e. substance interaction with lung cells), and acute phase response outcomes (KE2 and KE3). In addition, Danielsen et al. showed that Toll-like receptor 4 (*Tlr4)* knockout mice exposed to LPS, known to be a agonist for TLR4, did not induce an increase in cytokine/chemokines mRNA levels in lung and liver tissues (KE1) and did not produce a systemic acute phase response (KE3) [16]. Toll-like receptor 2 (*Tlr2)* knockout mice exposed to multiwalled carbon nanotubes did not induce increased *Saa1* mRNA levels in liver tissue (KE2) and did not induce increased SAA1 levels in plasma (KE3) [16].			
KE1: Increased, secretion of proinflammatory mediators	**Strong.** Mice presenting IL-6 gene disruption (IL-$6^{-/-}$) shown a reduced response in liver mRNA levels (KE2) and serum levels (KE3) of the acute phase proteins haptoglobin, α1-acid glycoprotein and serum amyloid a, after challenged by turpentine, lipopolysaccharide and bacterial infection [17]. In an *in vitro* study, blocking IL-6 receptors in hepatic cell lines resulted in a reduction of SAA1 mRNA (KE2), while blocking IL-1β and TNF-α receptors partially reduced the expression of SAA1 mRNA [18]. In a clinical trial study, patients with a history of myocardial infarction where administered with a monoclonal antibody for IL-1β (canakinumab). The results showed that the treatment significantly reduced blood CPR levels in a dose-dependent manner (KE2			

	and KE3) after 48 months, and there was a decrease in incidence rate of recurrent cardiovascular events (AO) [19].
KE2: Acute phase proteins transcription, Increased	**Strong.** Gene transcription is necessary for the synthesis of proteins (KE3). Thompson et al. showed that suppression of SAA3 in SAA1/SAA2 double knockout mice produced a significant reduction of atherosclerotic plaque area (AO) [20].
KE3: Systemic acute phase response	**Strong.** Studies using animal model of atherosclerosis have shown that elevated levels of SAA induces plaque progression (AO) [20,21]. In prospective epidemiological studies, CRP and SAA levels are predictive of risk of cardiovascular disease [22,23].

Uncertainties or Inconsistencies

Atherosclerosis is a disease influenced by multiple factors including high levels of lipoproteins in blood, elevated blood pressure, smoking, obesity, type 2 diabetes, diet, physical activity, among others [15,24,25]. As described by Libby, inflammation is also involved in atherosclerosis, providing mechanisms for the risk factors to induce atherosclerotic plaque formation and progression [26,27]. Therefore, although inflammation and acute phase response are not the only causes of atherosclerosis, the early key events (KE1, KE2 and KE3) can be used to evaluate the particle-induced risk to developing atherosclerosis.

CRP and SAA are risk factors for cardiovascular disease [23]. However, Mendelian randomization studies have shown that CRP genotypes are not associated with risk of coronary heart disease and that genetically elevated levels of CRP are not associated with coronary heart disease risk [28,29]. In humans, measuring gene expression of acute phase proteins is not very common, as a tissue sample is needed, while measuring acute phase protein in blood is more common.

In mice studies, it is possible to measure both SAA gene expression and protein levels, however the dynamic range for Saa gene expression is larger. Although it is suggested that acute phase proteins are mainly produced in the liver [2], it has been shown that in mice the liver has little upregulation of *Saa* genes after exposure to ultrafine carbon particles or diesel exhaust particle. On the other hand, the lung shows a marked expression of *Saa3* mRNA [30,31].

There is an inconsistency with the results from human studies. It has been observed that in most controlled human studies, an increase in CRP and/or SAA was observed after exposure to particulate matter [32-37]. However, in other human studies the exposure did not induce acute phase response [38,39], maybe due to low levels of exposure [40] or limited statistical power.

In the case of nanomaterials, it has been shown that physicochemical characteristics as size, surface area, surface functionalization, shape, composition, among others, affect the magnitude and duration of acute phase response in mice [41-43]. In animal models, both inflammatory and acute phase response are predicted by the total surface area of the retained, insoluble particles [42,44]

Weight of Evidence Summary

Biological plausibility of each KER

	Defining question	**High**	**Moderate**	**Low**
Support for Biological Plausibility of KERs	Is there a mechanistic (i.e., structural or functional) relationship between KEup and KEdown consistent	Extensive understanding based on extensive previous documentation and broad acceptance - Established mechanistic	The KER is plausible based on analogy to accepted biological relationships but scientific understanding is not	There is empirical support for a statistical association between KEs (See 3.), but the structural or functional

	with established biological knowledge?	basis	completely established.	relationship between them is not understood.

MIE => KE1: Interaction with the lung cell membrane leads to Increased proinflammatory mediators	Biological Plausibility of the MIE => KE1 is **High**. Rationale: There is extensive evidence showing that interaction of stressors with the respiratory system induces the release of proinflammatory markers.
KE1 => KE2: Increased proinflammatory mediators leads to Increased transcription of acute phase proteins	Biological Plausibility of the KE1 => KE2 is **High**. Rationale: Acute phase proteins are induced by pro-inflammatory cytokines. These cytokines are produced at sites of inflammation mainly by monocytes and macrophages.
KE2 => KE3: Increased transcription of acute phase proteins leads to Systemic acute phase response	Biological Plausibility of the KE2 => KE3 is **High**. Rationale: After gene expression of acute phase proteins in tissues during inflammatory conditions, mRNA is translated and folded into proteins. These proteins are then release to the systemic circulation.
KE3 => AO: Systemic acute phase response leads to Atherosclerosis	Biological Plausibility of the KE3 => KE2 is **High**. Rationale: During acute phase response, serum amyloid A replaces apolipoprotein A-1 from high-density lipoprotein. This replacement obstructs the reverse transport of cholesterol to the liver, allowing the formation of foam cells, an early marker of atherosclerotic lesions.
Non-adjacent MIE => KE2: Interaction with the lung cell membrane leads to Increased transcription of acute phase proteins	Biological Plausibility of the MIE => KE2 is **High**. Rationale: Acute phase response occurs during inflammatory condition, including the interaction of stressor with the airways. There is extensive evidence that nanomaterials induce the expression of acute phase response genes in mice.
Non-adjacent MIE => KE3: Interaction with the lung cell membrane leads to Systemic acute phase response	Biological Plausibility of the MIE => KE3 is **High**. Rationale: There is plenty of evidence showing that inhalation or instillation of stressors induces systemic acute phase response in humans and mice.
Non-adjacent KE1 => KE3: Increased proinflammatory mediators leads to Systemic APR	Biological Plausibility of the KE1 => KE3 is **High**. Rationale: Pro-inflammatory cytokines induce the release of acute phase proteins. These proteins are releases from inflammatory sites to the systemic

	circulation
Non-adjacent MIE => AO: Interaction with the lung cell membrane leads to Atherosclerosis	Biological Plausibility of the MIE => AO is **Moderate**. Rationale: There is evidence that the interaction of the lungs with stressor induces atherosclerotic plaque progression; however, the mechanistic relationship has not been clarified.

Please also refer to AOP173: Substance interaction with the pulmonary resident cell membrane components leading to pulmonary fibrosis, which shares MIE and KE1 with the present AOP.

Empirical support for each KER

	Defining question	**High**	**Moderate**	**Low**
Empirical Support	Does KEup occur at lower doses and earlier time points than KE down and at the same dose of prototypical stressor, is the incidence of KEup > than that for KEdown? Are there inconsistencies in empirical support across taxa, species and prototypical stressor that don't align with expected pattern for hypothesised AOP?	Multiple studies showing dependent change in both events following exposure to a wide range of specific prototypical stressors. (Extensive evidence for temporal, dose-response and incidence concordance) and no or few critical data gaps or conflicting data	Demonstrated dependent change in both events following exposure to a small number of specific prototypical stressors and some evidence inconsistent with expected pattern that can be explained by factors such as experimental design, technical considerations, differences among laboratories, etc.	Limited or no studies reporting dependent change in both events following exposure to a specific prototypical stressor (i.e., endpoints never measured in the same study or not at all); and/or significant inconsistencies in empirical support across taxa and species that don't align with expected pattern for hypothesised AOP
MIE => KE1: Interaction with the lung cell membrane leads to Increased proinflammatory mediators	Empirical Support of the MIE => KE1 is **Moderate**. Rationale: There are limited *in vitro* studies which show a temporal and dose-dependent relationship between these two events.			
KE1 => KE2: Increased proinflammatory mediators leads to Increased transcription of acute phase proteins	Empirical Support of the KE1 => KE2 is **High**. Rationale: There are is a large number of studies showing a dose concordance and temporal concordance.			
KE2 => KE3: Increased	Empirical Support of the KE2 => KE3 is **High**.			

transcription of acute phase proteins leads to Systemic acute phase response	Rationale: There are is a large number of studies showing a dose concordance and temporal concordance. However, there are inconsistencies between gene expression and translation of acute phase proteins.
KE3 => AO: Systemic acute phase response leads to Atherosclerosis	Empirical Support of the KE3 => AO is **Moderate**. Rationale: There is a limited number of animal studies showing the relationship between the key events, in addition of epidemiological studies showing association between the key events.
Non-adjacent MIE => KE2: Interaction with the lung cell membrane leads to Increased transcription of acute phase proteins	Empirical Support of the MIE => KE2 is **Moderate**. Rationale: There are is a large number of studies showing a dose concordance and temporal concordance in animal studies. However, in the case of nanomaterials it has been shown that physicochemical characteristics affect the magnitude and duration of the expression of acute phase proteins in mice.
Non-adjacent MIE => KE3: Interaction with the lung cell membrane leads to Systemic acute phase response	Empirical Support of the MIE => KE3 is **Moderate**. Rationale: There are plenty of studies showing a dose concordance and temporal concordance in animal and controlled human studies. It has been observed that systemic acute phase response is not always observed after exposure.
Non-adjacent KE1 => KE3: Increased proinflammatory mediators leads to Systemic APR	Empirical Support of the KE1 => KE3 is **Moderate**. Rationale: There is plenty of studies showing a dose concordance and temporal concordance. However, there are inconsistencies between changes in blood levels of pro-inflammatory mediators and systemic APR.
Non-adjacent MIE => AO: Interaction with the lung cell membrane leads to Atherosclerosis	Empirical Support of the MIE => AO is **Moderate.** Rationale: There is a number of studies showing the relationship between the key events.

Quantitative Consideration

The table below presents a characterization of every KER.

It is important to clarify that when assessing stressors in mice studies, it is possible to measure the gene expression of acute phase proteins (KE2) in different tissues, however in humans this is not likely as a tissue sample would be required. On the other hand, in humans it is much more common and easier to measure systemic acute phase response (KE3) through a blood sample. In mice, it has been shown that *Saa3* mRNA in lung tissue and blood levels of SAA3 are correlated [42]. In addition, SAA levels in mice and humans seem to be in level in magnitude after exposure to zinc oxide nanoparticles [42]. This suggest, that systemic acute phase response in humans can be estimated from mice studies.

In the case of nanomaterials and mice studies, *Saa3* mRNA in lung tissue is also correlated to pulmonary inflammation measured as neutrophil numbers, and both of these endpoints can be estimated by calculating the dosed surface area (specific surface area multiplied by dose level) [42].

Finally, the relative risk of people developing a cardiovascular disease can be calculated from blood level of acute phase proteins in epidemiological studies.

KER	Quantitative understanding
MIE => KE1: Interaction with the lung cell membrane leads to Increased proinflammatory mediators	The quantitative understanding of MIE => KE1 is **Low**. Rationale: The quantitative prediction of the release of proinflammatory factors can be made from the interaction of the stressors with the pulmonary system. In the case of some stressors (nanomaterials) it is possible to make a prediction using the dosed surface area of the materials and neutrophil numbers as an indirect marker of the release of pro-inflammatory factors.
KE1 => KE2: Increased proinflammatory mediators leads to Increased transcription of acute phase proteins	The quantitative understanding is of KE1 => KE2 is **Moderate**. Rationale: In mice, the gene expression of the acute phase protein SAA after exposure to metal oxide nanomaterials can be estimated using an indirect marker of the release of pro-inflammatory factors (neutrophil numbers).
KE2 => KE3: Increased transcription of acute phase proteins leads to Systemic acute phase response	The quantitative understanding of KE2 => KE3 is **Moderate**. Rationale: In mice, the systemic levels of the acute phase protein SAA after exposure to metal oxide nanomaterials can be estimated from the gene expression in lung tissue.
KE3 => AO: Systemic acute phase response leads to Atherosclerosis	The quantitative understanding is of KE3 => AO is **High**. Rationale: The risk of developing a cardiovascular disease at population level can be calculated from blood levels of acute phase proteins.
Non-adjacent MIE => KE2: Interaction with the lung cell membrane leads to Increased transcription of acute phase proteins	The quantitative understanding of MIE => KE2 is **Moderate.** Rationale: In mice, the gene expression of the acute phase protein SAA after exposure to metal oxide nanomaterials can be estimated from the dosed surface area.
Non-adjacent MIE => KE3: Interaction with the lung cell membrane leads to Systemic acute phase response	The quantitative understanding of MIE => KE3 is **Moderate**. Rationale: In mice, the blood levels of the acute phase protein SAA after exposure to metal oxide nanomaterials can be estimated from the dosed surface area.
Non-adjacent KE1	The quantitative understanding of KE1

Non-adjacent KE1 => KE3: Increased proinflammatory mediators leads to Systemic APR	The quantitative understanding of KE1 => KE3 is **Moderate**. Rationale: In mice, the blood levels of the acute phase protein SAA after exposure to metal oxide nanomaterials and multiwalled carbon nanotubes can be estimated from neutrophil numbers in broncheoalveolar lavage fluid.
Non-adjacent MIE => AO: Interaction with the lung cell membrane leads to Atherosclerosis	The quantitative understanding of MIE => AO is **Moderate**. Rationale: Epidemiological studies have shown the risk ratios of having a cardiovascular event per increase or decrease of exposure to particulate matter.

Considerations for Potential Applications of the AOP (optional)

Particle-induced acute phase response can be regarded as a critical effect linking particle-exposure to cardiovascular disease. Dose-response relationships can be used to establish no-observed-adverse-effect levels (NOAEL) for regulatory purposes and occupational exposure limits for inhalable materials can be determined through health-based risk assessments. This approach was taken by the Danish National Research Centre for the Working Environment at request of the Danish Working Environment Authority and an occupational exposure limit for zinc oxide was proposed based on the induction of acute phase response as the critical effect (the report can be found in: Dokumentation for helbredsbaserede grænseværdier for kemiske stoffer i arbejdsmiljøet (nfa.dk)).

As mentioned previously, not all KE can easily be measured in humans, therefore animal studies can be used to measure early KE and perform a risk assessment of different stressors. Additionally, physicochemical properties, such as specific surface area and dissolution, are important predictors of particle-induced acute phase response that can be used for hazard assessment [42]

References

1 Cray, C. Acute phase proteins in animals. *Prog Mol Biol Transl Sci* **105**, 113-150, doi:10.1016/B978-0-12-394596-9.00005-6 (2012).

2 Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* **340**, 448-454, doi:10.1056/NEJM199902113400607 (1999).

3 Medicine, N. L. o. Acute phase response related genes.

4 Tannock, L. R. *et al.* Serum amyloid A3 is a high density lipoprotein-associated acute-phase protein. *J Lipid Res* **59**, 339-347, doi:10.1194/jlr.M080887 (2018).

5 Cybulsky, M. I. *et al.* A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. *J Clin Invest* **107**, 1255-1262, doi:10.1172/JCI11871 (2001).

6 Hansson, G. K. & Libby, P. The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol* **6**, 508-519, doi:10.1038/nri1882 (2006).

7 Libby, P. Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol* **32**, 2045-2051, doi:10.1161/ATVBAHA.108.179705 (2012).

8 Virmani, R. *et al.* Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. *Arterioscler Thromb Vasc Biol* **25**, 2054-2061, doi:10.1161/01.ATV.0000178991.71605.18 (2005).

9 Clancy, L., Goodman, P., Sinclair, H. & Dockery, D. W. Effect of air-pollution control on death rates in Dublin, Ireland: an intervention study. *Lancet* **360**, 1210-1214, doi:10.1016/S0140-6736(02)11281-5 (2002).

10 Dockery, D. W. *et al.* An association between air pollution and mortality in six U.S. cities. *N Engl J Med* **329**, 1753-1759, doi:10.1056/NEJM199312093292401 (1993).

11 Pope, C. A., 3rd *et al.* Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. *Circulation* **109**, 71-77, doi:10.1161/01.CIR.0000108927.80044.7F (2004).

12 Pope, C. A., 3rd *et al.* Particulate air pollution as a predictor of mortality in a prospective study of U.S. adults. *Am J Respir Crit Care Med* **151**, 669-674, doi:10.1164/ajrccm/151.3_Pt_1.669 (1995).

13 Cao, Y. *et al.* Vascular effects of multiwalled carbon nanotubes in dyslipidemic ApoE-/- mice and cultured endothelial cells. *Toxicol Sci* **138**, 104-116, doi:10.1093/toxsci/kft328 (2014).

14 Moller, P. *et al.* Atherosclerosis and vasomotor dysfunction in arteries of animals after exposure to combustion-derived particulate matter or nanomaterials. *Crit Rev Toxicol* **46**, 437-476, doi:10.3109/10408444.2016.1149451 (2016).

15 Raitakari, O., Pahkala, K. & Magnussen, C. G. Prevention of atherosclerosis from childhood. *Nat Rev Cardiol* **19**, 543-554, doi:10.1038/s41569-021-00647-9 (2022).

16 Danielsen, P. H. *et al.* Nanomaterial- and shape-dependency of TLR2 and TLR4 mediated signaling following pulmonary exposure to carbonaceous nanomaterials in mice. *Part Fibre Toxicol* **18**, 40, doi:10.1186/s12989-021-00432-z (2021).

17 Kopf, M. *et al.* Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature* **368**, 339-342, doi:10.1038/368339a0 (1994).

18 Hagihara, K. *et al.* IL-6 plays a critical role in the synergistic induction of human serum amyloid A (SAA) gene when stimulated with proinflammatory cytokines as analyzed with an SAA isoform real-time quantitative RT-PCR assay system. *Biochem Biophys Res Commun* **314**, 363-369, doi:10.1016/j.bbrc.2003.12.096 (2004).

19 Ridker, P. M. *et al.* Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med* **377**, 1119-1131, doi:10.1056/NEJMoa1707914 (2017).

20 Thompson, J. C. *et al.* Serum amyloid A3 is pro-atherogenic. *Atherosclerosis* **268**, 32-35, doi:10.1016/j.atherosclerosis.2017.11.011 (2018).

21 Christophersen, D. V. *et al.* Accelerated atherosclerosis caused by serum amyloid A response in lungs of ApoE(-/-) mice. *FASEB J* **35**, e21307, doi:10.1096/fj.202002017R (2021).

22 Pai, J. K. *et al.* Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med* **351**, 2599-2610, doi:10.1056/NEJMoa040967 (2004).

23 Ridker, P. M., Hennekens, C. H., Buring, J. E. & Rifai, N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* **342**, 836-843, doi:10.1056/NEJM200003233421202 (2000).

24 Herrington, W., Lacey, B., Sherliker, P., Armitage, J. & Lewington, S. Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. *Circ Res* **118**, 535-546, doi:10.1161/CIRCRESAHA.115.307611 (2016).

25 Libby, P. *et al.* Atherosclerosis. *Nat Rev Dis Primers* **5**, 56, doi:10.1038/s41572-019-0106-z (2019).

26 Libby, P. The changing landscape of atherosclerosis. *Nature* **592**, 524-533, doi:10.1038/s41586-021-03392-8 (2021).

27 Libby, P. Inflammation during the life cycle of the atherosclerotic plaque. *Cardiovasc Res* **117**, 2525-2536, doi:10.1093/cvr/cvab303 (2021).

28 Collaboration, C. R. P. C. H. D. G. *et al.* Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. *BMJ* **342**, d548, doi:10.1136/bmj.d548 (2011).

29 Elliott, P. *et al.* Genetic Loci associated with C-reactive protein levels and risk of coronary heart disease. *JAMA* **302**, 37-48, doi:10.1001/jama.2009.954 (2009).

30 Saber, A. T. *et al.* Lack of acute phase response in the livers of mice exposed to diesel exhaust particles or carbon black by inhalation. *Part Fibre Toxicol* **6**, 12, doi:10.1186/1743-8977-6-12 (2009).

31 Saber, A. T. *et al.* Particle-induced pulmonary acute phase response correlates with neutrophil influx linking inhaled particles and cardiovascular risk. *PLoS One* **8**, e69020, doi:10.1371/journal.pone.0069020 (2013).

32 Monse, C. *et al.* Concentration-dependent systemic response after inhalation of nano-sized zinc oxide particles in human volunteers. *Part Fibre Toxicol* **15**, 8, doi:10.1186/s12989-018-0246-4 (2018).

33 Monse, C. *et al.* Health effects after inhalation of micro- and nano-sized zinc oxide particles in human volunteers. *Arch Toxicol* **95**, 53-65, doi:10.1007/s00204-020-02923-y (2021).

34 Walker, E. S. *et al.* Acute differences in blood lipids and inflammatory biomarkers following controlled exposures to cookstove air pollution in the STOVES study. *Int J Environ Health Res* **32**, 565-578, doi:10.1080/09603123.2020.1785402 (2022).

35 Wyatt, L. H., Devlin, R. B., Rappold, A. G., Case, M. W. & Diaz-Sanchez, D. Low levels of fine particulate matter increase vascular damage and reduce pulmonary function in young healthy adults. *Part Fibre Toxicol* **17**, 58, doi:10.1186/s12989-020-00389-5 (2020).

36 Baumann, R. *et al.* Systemic serum amyloid A as a biomarker for exposure to zinc and/or copper-containing metal fumes. *J Expo Sci Environ Epidemiol* **28**, 84-91, doi:10.1038/jes.2016.86 (2018).

37 Haase, L. M. *et al.* Cross-sectional Study of Workers Employed at a Copper Smelter-Effects of Long-term Exposures to Copper on Lung Function and Chronic Inflammation. *J Occup Environ Med* **64**, e550-e558, doi:10.1097/JOM.0000000000002610 (2022).

38 Andersen, M. H. G. *et al.* Association between polycyclic aromatic hydrocarbon exposure and peripheral blood mononuclear cell DNA damage in human volunteers during fire extinction exercises. *Mutagenesis* **33**, 105-115, doi:10.1093/mutage/gex021 (2018).

39 Andersen, M. H. G. *et al.* Assessment of polycyclic aromatic hydrocarbon exposure, lung function, systemic inflammation, and genotoxicity in peripheral blood mononuclear cells from firefighters before and after a work shift. *Environ Mol Mutagen* **59**, 539-548, doi:10.1002/em.22193 (2018).

40 Andersen, M. H. G. *et al.* Health effects of exposure to diesel exhaust in diesel-powered trains. *Part Fibre Toxicol* **16**, 21, doi:10.1186/s12989-019-0306-4 (2019).

41 Poulsen, S. S. *et al.* Multi-walled carbon nanotube-physicochemical properties predict the systemic acute phase response following pulmonary exposure in mice. *PLoS One* **12**, e0174167, doi:10.1371/journal.pone.0174167 (2017).

42 Gutierrez, C. T. *et al.* Acute phase response following pulmonary exposure to soluble and insoluble metal oxide nanomaterials in mice. *Part Fibre Toxicol* **20**, 4, doi:10.1186/s12989-023-00514-0 (2023).

43 Bengtson, S. *et al.* Differences in inflammation and acute phase response but similar genotoxicity in mice following pulmonary exposure to graphene oxide and reduced graphene oxide. *PLoS One* **12**, e0178355, doi:10.1371/journal.pone.0178355 (2017).

44 Cosnier, F. *et al.* Retained particle surface area dose drives inflammation in rat lungs following acute, subacute, and subchronic inhalation of nanomaterials. *Part Fibre Toxicol* **18**, 29, doi:10.1186/s12989-021-00419-w (2021).

Appendix 1

List of MIEs in this AOP

Event: 1495: Substance interaction with the lung resident cell membrane components

Short Name: Interaction with the lung cell membrane

Key Event Component

Process	Object	Action
pattern recognition receptor signaling pathway		increased
toll-like receptor signaling pathway	Toll-like receptor	increased
toll-like receptor 4 signaling pathway	Toll-like receptor 4	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:173 - Substance interaction with the pulmonary resident cell membrane components leading to pulmonary fibrosis	MolecularInitiatingEvent
Aop:451 - Interaction with lung resident cell membrane components leads to lung cancer	MolecularInitiatingEvent
Aop:237 - Substance interaction with lung resident cell membrane components leading to atherosclerosis	MolecularInitiatingEvent

Biological Context

Level of Biological Organization

Molecular

Cell term

Cell term
eukaryotic cell

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

As stated earlier, there are many different ways by which pro-fibrotic stressors can interact with the components of cell membrane and often involve multiple interactions at the same time. Few studies investigate the exact interaction between the stressor and the cellular membrane components. Asbestos and silica crystals engage scavenger receptors present on the macrophages (Murthy et al., 2015). Bleomycin binds high affinity bleomycin binding sites present on rat alveolar macrophage surfaces, leading to macrophage activation (Denholm and Phan, 1990). However, the consequences of such interactions such as, the release of PRR agonists DAMPs (alarmins) from dying or injured cells, increased gene or protein synthesis downstream of receptor binding or in the case of NMs, their cellular uptake, are measured routinely as indicative of occurrence of such interactions (Nel et al., 2009; Cheng et al., 2013). Because of the phys-chem properties such as surface charge, NMs and asbestos like materials can bind to cellular macromolecules and cell surface/membrane components, which in turn, facilitate their uptake and intracellular sequestration by the cells (NIOSH, 2011a; Pascolo et al., 2013). Several DAMPs that can be effectively measured in biological samples and cultured cells include High Mobility Group Binding 1 (HMGB1) protein, Heat Shock proteins (HSPs), uric acid, annexins, and S100 proteins (Bianchi, 2007). Of all DAMPs, interleukin (IL)-1α is the most commonly measured alarmin. IL-1α is the principal pro-inflammatory moiety and is a designated 'alarmin' in the cell that alerts the host to injury or damage (Di Paolo and Shayakhmetov, 2016). It is shown that administration of necrotic cells to mice results in neutrophilic inflammation that was entirely mediated by IL-1α released from the dying or necrosed cells and consequent activation of IL-1 Receptor 1 (IL-1R1) signalling (**Suwara** et al., 2014). IL-1α is released following exposure to MWCNTs (Nikota et al., 2017) and silica (Rabolli et al., 2014). Although IL1-b is not a designated alarmin, its secretion following exposure to stressors is routinely assessed and is linked to initiation of cell or tissue injury.

Other high aspect ratio fibres such as asbestos and CNTs induce frustrated phagocytosis and acute cell injury (Boyles et al., 2015; Dörger et al., 2001; Brown et al., 2007; Kim et al., 2010; Poland et al., 2008), leading to DAMP release (Nikota et al, 2017), inflammation and immune responses.

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
rat	Rattus norvegicus	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adults	High

Sex Applicability

Sex	Evidence
Male	High

Human, mouse, rat.

Although the expression of DAMPs following exposure to pro-fibrotic substances is not assessed across species, it is known that alarmins are released after trauma or injury, and their release is important for initiating the inflammatory response in all species including humans. The immediate acute inflammatory response involving DAMP signalling is also observed in human idiopathic pulmonary fibrosis (IPF); however, anti-inflammatory drugs have proven ineffective for treating IPF. Danger signalling axis including uric acid, adenosine triphosphate and IL-33/ST2 has been proven to promote lung fibrosis in animals.

Key Event Description

The human lung consists of approximately 40 different resident cell types that play different roles during homeostasis, injury, repair and disease states (Franks et al., 2008; Luettich et al., 2021). Of these, resident airway epithelial cells, alveolar/interstitial macrophages and dendritic cells are well characterised for their ability to sense the danger upon interaction with harmful substances and relay the message to mount the necessary immune/inflammatory response. The resident macrophages are

present in all tissues, and in a steady state, macrophages contribute to epithelial integrity, survey the tissue for invading pathogens or chemicals and maintain an immunosuppressive environment. Their main function is to clear the incoming irritants and microbes. They are named differently based on the tissue type and their specific functions (Kierdorf et al., 2015).

Substance interactions:

The chemicals or pathogens interact with cellular membrane to gain access to the organisms' interior. A predominant interaction mechanism involves the recognition of innate immune response agonists by pattern recognition receptors (PRRs) present on resident cells such as epithelial and alveolar macrophages. PRRs are also present on other immune and parenchymal cells. PRRs can be activated by two classes of ligands. Pathogen associated molecular patterns (PAMPs) are microbial molecules derived from invading pathogens. PAMPs will not be discussed further as pathogens are not the focus for the AOP presented here. The other class of ligands are called danger associated molecular patterns (DAMPs) that include cellular fragments, nucleic acids, small molecules, proteins and even cytokines released from injured or dying cells (Bianchi, 2007). Most fibrogenic stressors discussed in this AOP act via DAMPs-driven PRR activation. High aspect ratio (HAR) materials such as asbestos or carbon nanotubes (CNTs) pierce the cellular membrane of epithelial cells or resident macrophages resulting in cell injury or non-programmed cellular death. Alveolar macrophages trying to engulf HAR fibres that are long and stiff undergo frustrated phagocytosis because of their inability to engulf the piercing fibres and subsequently lead to cell injury (Boyles et al., 2015; Brown et al., 2007; Donaldson K et al., 2010; Dörger et al., 2001; Mossman and Churg, 1998). The cellular debris from injured or dying cell then serves as ligands for PRRs (Nakayama, 2018), leading to cell activation. In case of pro-fibrotic insoluble particles such as silica, coal dust and nanomaterials (NMs), the particle adsorbed opsonins such as immunoglobulins, complement proteins, or serum proteins act as ligands to the receptors on the macrophage cell surface (Behzadi et al., 2017). The tissue response to these materials resembles that observed following foreign body invasion in lungs.

Toll-like receptors (TLRs) are highly conserved PRRs that are associated with fibrogenic stressors (Desai et al., 2018). Inhibition of TLR-4 is protective against bleomycin-induced fibrosis (Li et al., 2015). However, the exact role and mechanisms by which TLRs mediate lung fibrosis are yet to be uncovered and some studies have shown TLRs to be protective against lung fibrosis (Desai et al., 2018). Asbestos and silica crystals are suggested to engage scavenger receptors present on the macrophages. Mice deficient in class A scavenger macrophage receptor with collagenous structure (MARCO) are shown to induce reduced fibrogenic response following chrysotile asbestos exposure; although, the direct binding of MARCO by asbestos is not investigated in the study (Murthy et al., 2015). In case of soluble substances such as bleomycin, paraquat (Dinis-Oliveira et al., 2008) (N,N'-dimethyl-4, 4´-bipyridinium dichloride) and other soluble fibrogenic chemicals, direct damage of lung epithelial cells and resulting cellular debris or secreted cytokines (DAMPs) serve as triggers for downstream cascading pro-inflammatory events, tissue injury and fibrosis. Engagement of PRRs and consequent cell activation is observed in various organisms including flies and mammals (Denholm and Phan, 1990; Matzinger, 2002).

How it is Measured or Detected

Detection of DAMPs or homeostasis-altering molecular processes:

Cellular interaction with substances or particles can be measured by assessing the release of DAMPs from stressed, injured or dying cells - indicative of binding of PRRs on the cell surface. Release of DAMPs is reflective of substance interaction with resident cells and their activation, a key step in the process of inflammation.

The release of DAMPs can be measured by the techniques listed in the published literature (Nikota et al., 2017; Rabolli et al., 2014; Suwara et al., 2014).

Targeted enzyme-linked immunosorbent assays (ELISA) (routinely used and recommended):

ELISA – permits quantitative measurement of antigens in biological samples. For example, in a cytokine ELISA (sandwich ELISA), an antibody (capture antibody) specific to a cytokine is immobilised on microtitre wells (96-well, 386-well, etc.). Experimental samples or samples containing a known amount of the specific recombinant cytokine are then reacted with the immobilised antibody. Following removal of unbound antibody by thorough washing, plates are reacted with the secondary antibody (detection antibody) that is conjugated to an enzyme such as horseradish peroxidase, which when bound, will form a sandwich with the capture antibody and the cytokine (Amsen and De Visser, 2009). The secondary antibody can be conjugated to biotin, which is then detected by addition of streptavidin linked to horseradish peroxidase. A chromogenic substrate can also be added, which is the most commonly used method. Chromogenic substrate is chemically converted by the enzyme coupled to the detection antibody, resulting in colour change. The amount of colour detected is directly proportional to the amount of cytokine in the sample that is bound to the capture antibody. The results are read using a spectrophotometer and compared to the levels of cytokine in control samples where cytokine is not expected to be secreted or to the samples containing known recombinant cytokine levels.

Interleukin (IL)-1α and -1β is activated or secreted into the cytosol following stimulus (Di Paolo and Shayakhmetov, 2016). Targeted ELISA can be used to quantify IL-1α or IL-1β that is released in the culture supernatant of the cells exposed to toxicants, in bronchoalveolar lavage fluid and serum of exposed animals. The assay is also applicable to human serum, cerebrospinal fluid, and peritoneal fluids.

Similarly, other alarmins can also be quantified by ELISA. Western blot is another method that can be used to quantify the release of various alarmins using specific antibodies. ELISA or real-time reverse transcription-polymerase chain reaction (qRT-PCR) assays can also be used to quantify the expression of genes or proteins that are regulated by the receptor binding – e.g. downstream of

TLR binding.

Frustrated phagocytosis and cellular uptake of NMs:

In vitro, interaction of NMs with the cellular membrane is investigated by assessing their uptake by lysosomes (Chen et al., 2013; Nel et al., 2009; Varela et al., 2012). Immunohistochemistry methods targeting lysosome specific proteins are regularly employed for this purpose. In co-localisation experiments, lysosomal marker Lysosomal-associated membrane protein 1 (LAMP1) antibody is used to detect particle co-localisation with lysosomes. A combination of Cytoviva hyperspectral microscope and immunolocalisation (Decan et al., 2016) or confocal microscopy to visualise co-localisation of fluorescence labelled nanoparticles with lysosomal markers have been used.

Frustrated phagocytosis is assessed using microscopic techniques such as time-lapse microscopy, backscatter electron microscopy and others (Donaldson et al., 2010; Murphy et al., 2012; Padmore et al., 2017; Pascolo et al., 2013; Schinwald et al., 2012). In addition, MIE 1668 of AOP303 notes other indirect methods for measuring frustrated phagocytosis.

Cellular co-culture models of the pulmonary epithelium:

Complex co-culture systems, such as those containing epithelial cells and immune cells, better model the environment of the lung epithelium and can be used to study the interaction of potentially pro-fibrotic fibres and particles with resident lung cells. This type of model has been used, alongside electron microscopy, to study lung cell interactions with CNTs following 24 h *in vitro* exposure (Clift *et al.,* 2014). More recently, the EpiAlveolar model, which contains primary human alveolar epithelial cells, endothelial cells, as well as fibroblasts was assessed for its ability to predict fibrosis induced by CNTs (Barasova *et al.,* 2020). Using laser scanning, fluorescence, and enhanced darkfield microscopy, CNT interaction with the resident cells of the model was shown, and this interaction induced the formation of holes in the epithelial model (Barasova *et al.,* 2020). While new co-culture models are a better recapitulation of the native lung environment as compared to traditional mono-cultures, the increased complexity necessitates enhanced expertise in tissue culture techniques, and can make them less practical as compared to submerged mono culture methods.

Ex vivo model of the lung – Precision cut lung slices (PCLS):

Even closer to the *in vivo* condition than co-culture models, PCLS techniques capture the native lung architecture, cell-cell communication and cellularity of the lung. Advancement in culturing and cryopreservation techniques has increased accessibility and use of PCLS for longer term studies (Bai *et al*., 2016, Neuhaus *et al*., 2017). These slices can be cultured *ex vivo* for up to a week with minimal reduction in viability, and the technique has recently been assessed for its applicability to assess nanomaterial induced fibrosis *ex vivo* (Rahman *et al.,* 2020). Using multi-walled carbon nanotubes (MWCNTs) and darkfield microscopy, interaction between the nanofibers and the lung epithelium could be determined. The main downside of this technique is the animal requirement, which precludes their use in a first-pass screening context for the MIE.

References

1. Amsen D, de Visser KE, Town T. Approaches to determine expression of inflammatory cytokines. Methods Mol Biol. 2009;511:107-42. doi: 10.1007/978-1-59745-447-6_5.

2. Bai Y, Krishnamoorthy N, Patel KR, Rosas I, Sanderson MJ, Ai X. Cryopreserved Human Precision-Cut Lung Slices as a Bioassay for Live Tissue Banking. A Viability Study of Bronchodilation with Bitter-Taste Receptor Agonists. Am J Respir Cell Mol Biol. 2016 May;54(5):656-63. doi: 10.1165/rcmb.2015-0290MA.

3. Barosova H, Maione AG, Septiadi D, Sharma M, Haeni L, Balog S, O'Connell O, Jackson GR, Brown D, Clippinger AJ, Hayden P, Petri-Fink A, Stone V, Rothen-Rutishauser B. Use of EpiAlveolar Lung Model to Predict Fibrotic Potential of Multiwalled Carbon Nanotubes. ACS Nano. 2020 Apr 28;14(4):3941-3956. doi: 10.1021/acsnano.9b06860.

4. Behzadi S, Serpooshan V, Tao W, Hamaly MA, Alkawareek MY, Dreaden EC, Brown D, Alkilany AM, Farokhzad OC, Mahmoudi M. Cellular uptake of nanoparticles: journey inside the cell. Chem Soc Rev. 2017 Jul 17;46(14):4218-4244. doi: 10.1039/c6cs00636a.

5. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol. 2007 Jan;81(1):1-5. doi: 10.1189/jlb.0306164.

6. Boyles MS, Young L, Brown DM, MacCalman L, Cowie H, Moisala A, Smail F, Smith PJ, Proudfoot L, Windle AH, Stone V. Multi-walled carbon nanotube induced frustrated phagocytosis, cytotoxicity and pro-inflammatory conditions in macrophages are length dependent and greater than that of asbestos. Toxicol In Vitro. 2015 Oct;29(7):1513-28. doi: 10.1016/j.tiv.2015.06.012.

7. Brown DM, Kinloch IA, Bangert U, Windle AH, Walter DM, Walker GS, et al. An in vitro study of the potential of carbon nanotubes and nanofibres to induce inflammatory mediators and frustrated phagocytosis. Carbon. 2007;45(9):1743-56. doi: https://doi.org/10.1016/j.carbon.2007.05.011.

8. Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. (2011). Current Intelligence Bulletin 62: Asbestos Fibers and Other Elongate Mineral Particles: State of the Science and Roadmap for Research. Retrieved from https://www.cdc.gov/niosh/docs/2011-159/.

9. Cheng LC, Jiang X, Wang J, Chen C, Liu RS. Nano-bio effects: interaction of nanomaterials with cells. Nanoscale. 2013 May

7;5(9):3547-69. doi: 10.1039/c3nr34276j.

10. Clift MJ, Endes C, Vanhecke D, Wick P, Gehr P, Schins RP, Petri-Fink A, Rothen-Rutishauser B. A comparative study of different in vitro lung cell culture systems to assess the most beneficial tool for screening the potential adverse effects of carbon nanotubes. Toxicol Sci. 2014 Jan;137(1):55-64. doi: 10.1093/toxsci/kft216.

11. Decan N, Wu D, Williams A, Bernatchez S, Johnston M, Hill M, Halappanavar S. Characterization of in vitro genotoxic, cytotoxic and transcriptomic responses following exposures to amorphous silica of different sizes. Mutat Res Genet Toxicol Environ Mutagen. 2016 Jan 15;796:8-22. doi: 10.1016/j.mrgentox.2015.11.011.

12. Denholm EM, Phan SH. Bleomycin binding sites on alveolar macrophages. J Leukoc Biol. 1990 Dec;48(6):519-23. doi: 10.1002/jlb.48.6.519.

13. Desai O, Winkler J, Minasyan M, Herzog EL. The Role of Immune and Inflammatory Cells in Idiopathic Pulmonary Fibrosis. Front Med (Lausanne). 2018 Mar 20;5:43. doi: 10.3389/fmed.2018.00043.

14. Di Paolo NC, Shayakhmetov DM. Interleukin 1α and the inflammatory process. Nat Immunol. 2016 Jul 19;17(8):906-13. doi: 10.1038/ni.3503.

15. Dinis-Oliveira RJ, Duarte JA, Sánchez-Navarro A, Remião F, Bastos ML, Carvalho F. Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. Crit Rev Toxicol. 2008;38(1):13-71. doi: 10.1080/10408440701669959.

16. Donaldson K, Murphy FA, Duffin R, Poland CA. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. Part Fibre Toxicol. 2010 Mar 22;7:5. doi: 10.1186/1743-8977-7-5.

17. Dörger M, Münzing S, Allmeling AM, Messmer K, Krombach F. Differential responses of rat alveolar and peritoneal macrophages to man-made vitreous fibers in vitro. Environ Res. 2001 Mar;85(3):207-14. doi: 10.1006/enrs.2001.4234.

18. Franks TJ, Colby TV, Travis WD, Tuder RM, Reynolds HY, Brody AR, Cardoso WV, Crystal RG, Drake CJ, Engelhardt J, Frid M, Herzog E, Mason R, Phan SH, Randell SH, Rose MC, Stevens T, Serge J, Sunday ME, Voynow JA, Weinstein BM, Whitsett J, Williams MC. Resident cellular components of the human lung: current knowledge and goals for research on cell phenotyping and function. Proc Am Thorac Soc. 2008 Sep 15;5(7):763-6. doi: 10.1513/pats.200803-025HR.

19. Kierdorf K, Prinz M, Geissmann F, Gomez Perdiguero E. Development and function of tissue resident macrophages in mice. Semin Immunol. 2015 Dec;27(6):369-78. doi: 10.1016/j.smim.2016.03.017.

20. Kim JE, Lim HT, Minai-Tehrani A, Kwon JT, Shin JY, Woo CG, Choi M, Baek J, Jeong DH, Ha YC, Chae CH, Song KS, Ahn KH, Lee JH, Sung HJ, Yu IJ, Beck GR Jr, Cho MH. Toxicity and clearance of intratracheally administered multiwalled carbon nanotubes from murine lung. J Toxicol Environ Health A. 2010;73(21-22):1530-43. doi: 10.1080/15287394.2010.511578.

21. Luettich K, Sharma M, Yepiskoposyan H, Breheny D, Lowe FJ. An Adverse Outcome Pathway for Decreased Lung Function Focusing on Mechanisms of Impaired Mucociliary Clearance Following Inhalation Exposure. Front Toxicol. 2021 Dec 14;3:750254. doi: 10.3389/ftox.2021.750254.

22. Li XX, Jiang DY, Huang XX, Guo SL, Yuan W, Dai HP. Toll-like receptor 4 promotes fibrosis in bleomycin-induced lung injury in mice. Genet Mol Res. 2015 Dec 21;14(4):17391-8. doi: 10.4238/2015.

23. Matzinger P. The danger model: a renewed sense of self. Science. 2002 Apr 12;296(5566):301-5. doi: 10.1126/science.1071059.

24. Mossman BT, Churg A. Mechanisms in the pathogenesis of asbestosis and silicosis. Am J Respir Crit Care Med. 1998 May;157(5 Pt 1):1666-80. doi: 10.1164/ajrccm.157.5.9707141.

25. Murphy FA, Schinwald A, Poland CA, Donaldson K. The mechanism of pleural inflammation by long carbon nanotubes: interaction of long fibres with macrophages stimulates them to amplify pro-inflammatory responses in mesothelial cells. Part Fibre Toxicol. 2012 Apr 3;9:8. doi: 10.1186/1743-8977-9-8.

26. Murthy S, Larson-Casey JL, Ryan AJ, He C, Kobzik L, Carter AB. Alternative activation of macrophages and pulmonary fibrosis are modulated by scavenger receptor, macrophage receptor with collagenous structure. FASEB J. 2015 Aug;29(8):3527-36. doi: 10.1096/fj.15-271304.

27. Nakayama M. Macrophage Recognition of Crystals and Nanoparticles. Front Immunol. 2018 Jan 29;9:103. doi: 10.3389/fimmu.2018.00103.

28. Nel AE, Mädler L, Velegol D, Xia T, Hoek EM, Somasundaran P, Klaessig F, Castranova V, Thompson M. Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater. 2009 Jul;8(7):543-57. doi: 10.1038/nmat2442.

29. Neuhaus V, Schaudien D, Golovina T, Temann UA, Thompson C, Lippmann T, Bersch C, Pfennig O, Jonigk D, Braubach P, Fieguth HG, Warnecke G, Yusibov V, Sewald K, Braun A. Assessment of long-term cultivated human precision-cut lung slices as an ex vivo system for evaluation of chronic cytotoxicity and functionality. J Occup Med Toxicol. 2017 May 26;12:13. doi: 10.1186/s12995-017-0158-5.

30. Nikota J, Banville A, Goodwin LR, Wu D, Williams A, Yauk CL, Wallin H, Vogel U, Halappanavar S. Stat-6 signaling pathway and not Interleukin-1 mediates multi-walled carbon nanotube-induced lung fibrosis in mice: insights from an adverse outcome pathway framework. Part Fibre Toxicol. 2017 Sep 13;14(1):37. doi: 10.1186/s12989-017-0218-0.

31. Padmore T, Stark C, Turkevich LA, Champion JA. Quantitative analysis of the role of fiber length on phagocytosis and inflammatory response by alveolar macrophages. Biochim Biophys Acta Gen Subj. 2017 Feb;1861(2):58-67. doi: 10.1016/j.bbagen.2016.09.031.

32. Pascolo L, Gianoncelli A, Schneider G, Salomé M, Schneider M, Calligaro C, Kiskinova M, Melato M, Rizzardi C. The interaction of asbestos and iron in lung tissue revealed by synchrotron-based scanning X-ray microscopy. Sci Rep. 2013;3:1123. doi: 10.1038/srep01123.

33. Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WA, Seaton A, Stone V, Brown S, Macnee W, Donaldson K. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotechnol. 2008 Jul;3(7):423-8. doi: 10.1038/nnano.2008.111.

34. Rabolli V, Badissi AA, Devosse R, Uwambayinema F, Yakoub Y, Palmai-Pallag M, Lebrun A, De Gussem V, Couillin I, Ryffel B, Marbaix E, Lison D, Huaux F. The alarmin IL-1α is a master cytokine in acute lung inflammation induced by silica micro- and nanoparticles. Part Fibre Toxicol. 2014 Dec 13;11:69. doi: 10.1186/s12989-014-0069-x.

35. Rahman L, Williams A, Gelda K, Nikota J, Wu D, Vogel U, Halappanavar S. 21st Century Tools for Nanotoxicology: Transcriptomic Biomarker Panel and Precision-Cut Lung Slice Organ Mimic System for the Assessment of Nanomaterial-Induced Lung Fibrosis. Small. 2020 Sep;16(36):e2000272. doi: 10.1002/smll.202000272.

36. Schinwald A, Donaldson K. Use of back-scatter electron signals to visualise cell/nanowires interactions in vitro and in vivo; frustrated phagocytosis of long fibres in macrophages and compartmentalisation in mesothelial cells in vivo. Part Fibre Toxicol. 2012 Aug 28;9:34. doi: 10.1186/1743-8977-9-34.

37. Suwara MI, Green NJ, Borthwick LA, Mann J, Mayer-Barber KD, Barron L, Corris PA, Farrow SN, Wynn TA, Fisher AJ, Mann DA. IL-1α released from damaged epithelial cells is sufficient and essential to trigger inflammatory responses in human lung fibroblasts. Mucosal Immunol. 2014 May;7(3):684-93. doi: 10.1038/mi.2013.87.

38. Varela JA, Bexiga MG, Åberg C, Simpson JC, Dawson KA. Quantifying size-dependent interactions between fluorescently labeled polystyrene nanoparticles and mammalian cells. J Nanobiotechnology. 2012 Sep 24;10:39. doi: 10.1186/1477-3155-10-39.

List of Key Events in the AOP

Event: 1496: Increased, secretion of proinflammatory mediators

Short Name: Increased proinflammatory mediators

Key Event Component

Process	Object	Action
cytokine production involved in inflammatory response	Cytokine	increased
chemokine secretion	Chemokine	increased
complement activation		increased
	Interleukin	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:173 - Substance interaction with the pulmonary resident cell membrane components leading to pulmonary fibrosis	KeyEvent
Aop:320 - Binding of SARS-CoV-2 to ACE2 receptor leading to acute respiratory distress associated mortality	KeyEvent
Aop:382 - Angiotensin II type 1 receptor (AT1R) agonism leading to lung fibrosis	KeyEvent
Aop:392 - Decreased fibrinolysis and activated bradykinin system leading to hyperinflammation	KeyEvent
Aop:409 - Frustrated phagocytosis leads to malignant mesothelioma	KeyEvent
Aop:377 - Dysregulated prolonged Toll Like Receptor 9 (TLR9) activation leading to Multi Organ Failure involving Acute Respiratory Distress Syndrome (ARDS)	KeyEvent

AOP ID and Name	Event Type
Aop:39 - Covalent Binding, Protein, leading to Increase, Allergic Respiratory Hypersensitivity Response	KeyEvent
Aop:319 - Binding to ACE2 leading to lung fibrosis	KeyEvent
Aop:451 - Interaction with lung resident cell membrane components leads to lung cancer	KeyEvent
Aop:468 - Binding of SARS-CoV-2 to ACE2 leads to hyperinflammation (via cell death)	KeyEvent
Aop:237 - Substance interaction with lung resident cell membrane components leading to atherosclerosis	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term
eukaryotic cell

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
rats	Rattus norvegicus	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adults	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Human, mouse, rat

Cytokines are the common pro-inflammatory mediators secreted following inflammogenic stimuli. Cytokines can be defined as a diverse group of signaling protein molecules. They are secreted by different cell types in different tissues and in all mammalian species, irrespective of gender, age or sex. A lot of literature is available to support cross species, gender and developmental stage application for this KE. The challenge is the specificity; most cytokines exhibit redundant functions and many are pleotropic.

Key Event Description

Pro-inflammatory mediators are the chemical and biological molecules that initiate and regulate inflammatory reactions. Pro-inflammatory mediators are secreted following exposure to an inflammogen in a gender/sex or developmental stage independent manner. They are secreted during inflammation in all species. Different types of pro-inflammatory mediators are secreted during innate or adaptive immune responses across various species (Mestas and Hughes, 2004). Cell-derived pro-inflammatory mediators include cytokines, chemokines, and growth factors. Blood derived pro-inflammatory mediators include vasoactive amines, complement activation products and others. These modulators can be grouped based on the cell type that secrete them, their cellular localisation and also based on the type of immune response they trigger. For example, members of the interleukin (IL) family including IL-2, IL-4, IL-7, IL-9, IL-15, IL-21, IL-3, IL-5 and Granulocyte-macrophage colony stimulating factor (GM-CSF) are involved in the adaptive immune responses. The pro-inflammatory cytokines include IL-1 family (IL-1α_, IL-1β, IL-1rα, IL-18, IL-36α, IL-36β, IL-36γ, IL-36Rα, IL-37), IL-6 family, Tumor necrosis factor (TNF) family, IL-17, and Interferon gamma (IFN-γ) (Turner et al., 2014). While IL-4 and IL-5 are considered T helper (Th) cell type 2 response, IFN-γ is suggested to be Th1 type response.

Different types of pro-inflammatory mediators are secreted during innate or adaptive immune responses across various species (Mestas and Hughes, 2004). However, IL-1 family cytokines, IL-4, IL-5, IL-6, TNF-α, IFN-γ are the commonly measured mediators in experimental animals and in humans. Similar gene expression patterns involving inflammation and matrix remodelling are

observed in human patients of pulmonary fibrosis and mouse lungs exposed to bleomycin (Kaminski, 2002).

Literature evidence for its perturbation:

Several studies show increased proinflammatory mediators in rodent lungs and bronchoalveolar lavage fluid, and in cell culture supernatants following exposure to a variety of carbon nanotube (CNT) types and other materials. Poland et al., 2008 showed that long and thin CNTs (>5 μm) can elicit asbestos-like pathogenicity through the continual release of pro-inflammatory cytokines and reactive oxygen species. Exposure to crystalline silica induces release of inflammatory cytokines (TNF-α, IL-1, IL-6), transcription factors (Nuclear factor kappa B [NF-κB], Activator protein-1 [AP-1]) and kinase signalling pathways in mice that contain NF-κB luciferase reporter (Hubbard et al., 2002). Boyles et al., 2015 found that lung responses to long multi-walled carbon nanotubes (MWCNTs) included high expression levels of pro-inflammatory mediators Monocyte chemoattractant protein 1 (MCP-1), Transforming growth factor beta 1 (TGF-β1), and TNF-α (Boyles et al., 2015). Bleomycin administration in rodents induces lung inflammation and increased expression of pro-inflammatory mediators (Park et al., 2019). Inflammation induced by bleomycin, paraquat and CNTs is characterised by the altered expression of pro-inflammatory mediators. A large number of nanomaterials induce expression of cytokines and chemokines in lungs of rodents exposed via inhalation (Halappanavar et al., 2011; Husain et al., 2015a). Similarities are observed in gene programs involving pro-inflammatory event is observed in both humans and experimental mice (Zuo et al., 2002).

How it is Measured or Detected

The selection of pro-inflammatory mediators for investigation varies based on the expertise of the lab, cell types studied and the availability of the specific antibodies.

Real-time reverse transcription-polymerase chain reaction (qRT-PCR) – will measure the abundance of cytokine mRNA in a given sample. The method involves three steps: conversion of RNA into cDNA by reverse transcription method, amplification of cDNA using the PCR, and the real-time detection and quantification of amplified products (amplicons) (Nolan et al., 2006). Amplicons are detected using fluorescence, increase in which is directly proportional to the amplified PCR product. The number of cycles required per sample to reach a certain threshold of fluorescence (set by the user – usually set in the linear phase of the amplification, and the observed difference in samples to cross the set threshold reflects the initial amount available for amplification) is used to quantify the relative amount in the samples. The amplified products are detected by the DNA intercalating minor groove-binding fluorophore SYBR green, which produces a signal when incorporated into double-stranded amplicons. Since the cDNA is single stranded, the dye does not bind enhancing the specificity of the results. There are other methods such as nested fluorescent probes for detection, but SYBR green is widely used. RT-PCR primers specific to several pro-inflammatory mediators in several species including mouse, rat and humans, are readily available commercially.

Enzyme-linked immunosorbent assays (ELISA) – permit quantitative measurement of antigens in biological samples. The method is the same as described for the MIE. Both ELISA and qRT-PCR assays are used *in vivo* and are readily applicable to *in vitro* cell culture models, where cell culture supernatants or whole cell homogenates are used for ELISA or mRNA assays. Both assays are straight forward, quantitative and require relatively a small amount of input sample.

Apart from assaying single protein or gene at a time, cytokine bead arrays or cytokine PCR arrays can also be used to detect a whole panel of inflammatory mediators in a multiplex method (Husain et al., 2015b). This method is quantitative and especially advantageous when the sample amount available for testing is scarce. Lastly, immunohistochemistry can also be used to detect specific immune cell types producing the pro-inflammatory mediators and its downstream effectors in any given tissue (Costa et al., 2017). Immunohistochemistry results can be used as weight of evidence; however, the technique is not quantitative and depending on the specific antibodies used, the assay sensitivity may also become an issue (Amsen and De Visser, 2009).

Cell models - of varying complexity have been used to assess the expression of pro-inflammatory mediators. Two dimensional submerged monocultures of the main fibrotic effector cells – lung epithelial cells, macrophages, and fibroblasts – have routinely been used *in vitro* due to the large literature base, and ease of use, but do not adequately mimic the *in vivo* condition (Sharma *et al.,* 2016; Sundarakrishnan *et al.,* 2018). Recently, the EpiAlveolar *in vitro* lung model (containing epithelial cells, endothelial cells, and fibroblasts) was used to predict the fibrotic potential of MWCNTs, and researchers noted increases in the pro-inflammatory molecules TNF-α, IL-1β, and the pro-fibrotic TGF-β using ELISA (Barasova *et al.,* 2020). A similar, but less complicated co-culture model of immortalized human alveolar epithelial cells and idiopathic pulmonary fibrosis patient derived fibroblasts was used to assess pro-fibrotic signalling, and noted enhanced secretion of Platelet derived growth factor (PDGF) and Basic fibroblast growth factor (bFGF), as well as evidence for epithelial to mesenchymal transition of epithelial cells in this system (Prasad et al., 2014). Models such as these better capitulate the *in vivo* pulmonary alveolar capillary, but have lower reproducibility as compared to traditional submerged mono-culture experiments.

References

1. Amsen D, de Visser KE, Town T. Approaches to determine expression of inflammatory cytokines. Methods Mol Biol. 2009;511:107-42. doi: 10.1007/978-1-59745-447-6_5.

2. Barosova H, Maione AG, Septiadi D, Sharma M, Haeni L, Balog S, O'Connell O, Jackson GR, Brown D, Clippinger AJ, Hayden P, Petri-Fink A, Stone V, Rothen-Rutishauser B. Use of EpiAlveolar Lung Model to Predict Fibrotic Potential of Multiwalled Carbon Nanotubes. ACS Nano. 2020 Apr 28;14(4):3941-3956. doi: 10.1021/acsnano.9b06860.

3. Boyles MS, Young L, Brown DM, MacCalman L, Cowie H, Moisala A, Smail F, Smith PJ, Proudfoot L, Windle AH, Stone V. Multi-

walled carbon nanotube induced frustrated phagocytosis, cytotoxicity and pro-inflammatory conditions in macrophages are length dependent and greater than that of asbestos. Toxicol In Vitro. 2015 Oct;29(7):1513-28. doi: 10.1016/j.tiv.2015.06.012.

4. Costa PM, Gosens I, Williams A, Farcal L, Pantano D, Brown DM, Stone V, Cassee FR, Halappanavar S, Fadeel B. Transcriptional profiling reveals gene expression changes associated with inflammation and cell proliferation following short-term inhalation exposure to copper oxide nanoparticles. J Appl Toxicol. 2018 Mar;38(3):385-397. doi: 10.1002/jat.3548.

5. Halappanavar S, Jackson P, Williams A, Jensen KA, Hougaard KS, Vogel U, Yauk CL, Wallin H. Pulmonary response to surface-coated nanotitanium dioxide particles includes induction of acute phase response genes, inflammatory cascades, and changes in microRNAs: a toxicogenomic study. Environ Mol Mutagen. 2011 Jul;52(6):425-39. doi: 10.1002/em.20639.

6. Hubbard AK, Timblin CR, Shukla A, Rincón M, Mossman BT. Activation of NF-kappaB-dependent gene expression by silica in lungs of luciferase reporter mice. Am J Physiol Lung Cell Mol Physiol. 2002 May;282(5):L968-75. doi: 10.1152/ajplung.00327.2001.

7. Husain M, Kyjovska ZO, Bourdon-Lacombe J, Saber AT, Jensen KA, Jacobsen NR, Williams A, Wallin H, Halappanavar S, Vogel U, Yauk CL. Carbon black nanoparticles induce biphasic gene expression changes associated with inflammatory responses in the lungs of C57BL/6 mice following a single intratracheal instillation. Toxicol Appl Pharmacol. 2015a Dec 15;289(3):573-88. doi: 10.1016/j.taap.2015.11.003.

8. Husain M, Wu D, Saber AT, Decan N, Jacobsen NR, Williams A, Yauk CL, Wallin H, Vogel U, Halappanavar S. Intratracheally instilled titanium dioxide nanoparticles translocate to heart and liver and activate complement cascade in the heart of C57BL/6 mice. Nanotoxicology. 2015b;9(8):1013-22. doi: 10.3109/17435390.2014.996192.

9. Kaminski N. Microarray analysis of idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol. 2003 Sep;29(3 Suppl):S32-6.

10. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. J Immunol. 2004 Mar 1;172(5):2731-8. doi: 10.4049/jimmunol.172.5.2731.

11. Nolan T, Hands RE, Bustin SA. Quantification of mRNA using real-time RT-PCR. Nat Protoc. 2006;1(3):1559-82. doi: 10.1038/nprot.2006.236.

12. Park SJ, Im DS. Deficiency of Sphingosine-1-Phosphate Receptor 2 ($S1P_2$) Attenuates Bleomycin-Induced Pulmonary Fibrosis. Biomol Ther (Seoul). 2019 May 1;27(3):318-326. doi: 10.4062/biomolther.2018.131.

13. Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WA, Seaton A, Stone V, Brown S, Macnee W, Donaldson K. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotechnol. 2008 Jul;3(7):423-8. doi: 10.1038/nnano.2008.111.

14. Prasad S, Hogaboam CM, Jarai G. Deficient repair response of IPF fibroblasts in a co-culture model of epithelial injury and repair. Fibrogenesis Tissue Repair. 2014 Apr 29;7:7. doi: 10.1186/1755-1536-7-7.

15. Sharma M, Nikota J, Halappanavar S, Castranova V, Rothen-Rutishauser B, Clippinger AJ. Predicting pulmonary fibrosis in humans after exposure to multi-walled carbon nanotubes (MWCNTs). Arch Toxicol. 2016 Jul;90(7):1605-22. doi: 10.1007/s00204-016-1742-7.

16. Sundarakrishnan A, Chen Y, Black LD, Aldridge BB, Kaplan DL. Engineered cell and tissue models of pulmonary fibrosis. Adv Drug Deliv Rev. 2018 Apr;129:78-94. doi: 10.1016/j.addr.2017.12.013.

17. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochim Biophys Acta. 2014 Nov;1843(11):2563-2582. doi: 10.1016/j.bbamcr.2014.05.014.

18. Zuo F, Kaminski N, Eugui E, Allard J, Yakhini Z, Ben-Dor A, Lollini L, Morris D, Kim Y, DeLustro B, Sheppard D, Pardo A, Selman M, Heller RA. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. Proc Natl Acad Sci U S A. 2002 Apr 30;99(9):6292-7. doi: 10.1073/pnas.092134099.

Event: 1438: Transcription of acute phase proteins, Increased

Short Name: Increased transcription of APP

Key Event Component

Process	Object	Action
acute-phase response	Acute phase proteins	increased

AOPs Including This Key Event

AOP ID and Name	Event Type

AOP ID and Name	Event Type
Aop:237 - Substance interaction with lung resident cell membrane components leading to atherosclerosis	KeyEvent

Biological Context

Level of Biological Organization

Organ

Organ term

Organ term

lung

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

- Taxonomic applicability: APR is part of the immune reponse and is obseved in vertebrate species[3].
- Life stage applicability: This key event is applicable to all life stages.
- Sex applicability: This key event is applicable to male and female sexes.

Key Event Description

Acute phase response is characterized by the change in plasma concentration of acute phase proteins (APP), along with other physiological changes during inflammatory conditions [1,2]. In humans, the major APPs are C-reactive protein and serum amyloid A, while in mice the major APPs are serum amyloid A, haptoglobin and serum amyloid P [1,3].

It is widely accepted than APPs are mainly produced by the liver, however several other tissues have been showed to express APPs; these include lungs, intestines, kidneys, skin and adipose tissue in humans [4-7], and kidney, spleen, brain, lung and testis in mice [8,9]. According to National Center for Biotechnology Information (NCBI) serum amyloid A isoforms and C-reactive protein genes in mice have been shown to be expressed in tissue from adrenal gland, bladder, central nervous system, colon, duodenum, genital fat pad, heart, kidney, large intestine, limbs, liver, lung, mammary gland, ovary, placenta, small intestine, subcutaneous fat pad, testis and thymus[10]. In the case of humans, these genes have been shown to be expressed in tissue from adrenal gland, appendix, fat, gall bladder, heart, kidney, liver, lung, placenta, prostate, salivary gland, small intestine, stomach, thymus, thyroid, trachea and uterus[10].

How it is Measured or Detected

Gene expression of APPs can be measured from tissue samples using quantitative Polymerase Chain Reaction (PCR). Humans and mice express four SAA isoforms (*Saa1, Saa2, Saa3* and *Saa4*), however *Saa3* is a pseudogene in humans[11]. CRP is expressed in humans and mice, although only moderately expressed in mice[12].

It has been shown that in mice the *Saa3* isoform is the most differentially expressed APP gene in lung tissue and it is not highly expressed in the liver, while *Saa1* gene is the most differentially expressed in liver tissue after exposure to particles [13-15]. In humans, it has been shown that *crp*, *saa1*, *saa2* and *saa4* gene expression can be measured in lung samples taken during surgery [16]. In addition, microarray analysis can be used to evaluate the gene expression of several APPs at the same time [13].

References

1 Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* **340**, 448-454, doi:10.1056/NEJM199902113400607 (1999).

2 Mantovani, A. & Garlanda, C. Humoral Innate Immunity and Acute-Phase Proteins. *N Engl J Med* **388**, 439-452, doi:10.1056/NEJMra2206346 (2023).

3 Cray, C., Zaias, J. & Altman, N. H. Acute phase response in animals: a review. *Comp Med* **59**, 517-526 (2009).

4 Schrödl, W. *et al.* Acute phase proteins as promising biomarkers: Perspectives and limitations for human and veterinary medicine. **10**, 1077-1092, doi:https://doi.org/10.1002/prca.201600028 (2016).

5 de Dios, O. *et al.* C-reactive protein expression in adipose tissue of children with acute appendicitis. *Pediatr Res* **84**, 564-567, doi:10.1038/s41390-018-0091-z (2018).

6 Urieli-Shoval, S., Cohen, P., Eisenberg, S. & Matzner, Y. Widespread expression of serum amyloid A in histologically normal human tissues. Predominant localization to the epithelium. *J Histochem Cytochem* **46**, 1377-1384, doi:10.1177/002215549804601206 (1998).

7 Venteclef, N., Jakobsson, T., Steffensen, K. R. & Treuter, E. Metabolic nuclear receptor signaling and the inflammatory acute phase response. *Trends Endocrinol Metab* **22**, 333-343, doi:10.1016/j.tem.2011.04.004 (2011).

8 Kalmovarin, N. *et al.* Extrahepatic expression of plasma protein genes during inflammation. *Inflammation* **15**, 369-379, doi:10.1007/BF00917353 (1991).

9 Saber, A. T. *et al.* Particle-induced pulmonary acute phase response correlates with neutrophil influx linking inhaled particles and cardiovascular risk. *PLoS One* **8**, e69020, doi:10.1371/journal.pone.0069020 (2013).

10 NCBI. <https://www.ncbi.nlm.nih.gov/gene> (2023).

11 Shridas, P. & Tannock, L. R. Role of serum amyloid A in atherosclerosis. *Curr Opin Lipidol* **30**, 320-325, doi:10.1097/MOL.0000000000000616 (2019).

12 Pepys, M. B. & Hirschfield, G. M. C-reactive protein: a critical update. *J Clin Invest* **111**, 1805-1812, doi:10.1172/JCI18921 (2003).

13 Halappanavar, S. *et al.* Pulmonary response to surface-coated nanotitanium dioxide particles includes induction of acute phase response genes, inflammatory cascades, and changes in microRNAs: a toxicogenomic study. *Environ Mol Mutagen* **52**, 425-439, doi:10.1002/em.20639 (2011).

14 Poulsen, S. S. *et al.* Multi-walled carbon nanotube-physicochemical properties predict the systemic acute phase response following pulmonary exposure in mice. *PLoS One* **12**, e0174167, doi:10.1371/journal.pone.0174167 (2017).

15 Saber, A. T. *et al.* Particle-induced pulmonary acute phase response may be the causal link between particle inhalation and cardiovascular disease. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* **6**, 517-531, doi:10.1002/wnan.1279 (2014).

16 Calero, C. *et al.* Differential expression of C-reactive protein and serum amyloid A in different cell types in the lung tissue of chronic obstructive pulmonary disease patients. *BMC Pulm Med* **14**, 95, doi:10.1186/1471-2466-14-95 (2014).

Event: 1439: Systemic acute phase response

Short Name: Systemic APR

Key Event Component

Process	Object	Action
acute-phase response	Acute phase proteins	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:237 - Substance interaction with lung resident cell membrane components leading to atherosclerosis	KeyEvent

Biological Context

Level of Biological Organization

Organ

Organ term

Organ term

blood

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

- Taxonomic applicability: APR is part of the immune response and is observed in vertebrates[6].
- Life stages applicability: This key event is applicable to all life stages.
- Sex applicability: This key event is applicable to male and females sexes.

Key Event Description

During acute phase response, the plasma concentration of acute phase proteins (APP) changes in more than 25%. APPs that increase their concentration during APR are called positive APP, while negative APP are decreased during APR [1]. In humans, positive APPs include C-reactive protein, serum amyloid A, C3 and C4 complement system, mannose-binding lectin, fibrinogen, fibronectin, ferritin, haptoglobin, hemopexin, among others [1,2]. In humans the two major APPs are C-reactive protein (CRP) and serum amyloid A (SAA), whose concentration can increase in more than 1000-fold during acute phase response [1]. SAA and CRP have been shown to be correlated in humans [3-5].In mice, the major APP are serum amyloid A, haptoglobin and serum amyloid P[6].

How it is Measured or Detected

Systemic acute phase response is assessed by measuring APPs concentrations in blood plasma or serum, most often CRP and SAA. In humans, these proteins are measured by immunoassays detecting single or multiple proteins [4,7-12]. In addition, CRP is measured by turbidimetric [13-15] and nephelometric assays [16].

In mice, CRP is not a major APP [6], therefore SAA isoforms are measured using ELISA assays or western blot [17-21].

References

1 Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* **340**, 448-454, doi:10.1056/NEJM199902113400607 (1999).

2 Mantovani, A. & Garlanda, C. Humoral Innate Immunity and Acute-Phase Proteins. *N Engl J Med* **388**, 439-452, doi:10.1056/NEJMra2206346 (2023).

3 Baumann, R. *et al.* Human nasal mucosal C-reactive protein responses after inhalation of ultrafine welding fume particles: positive correlation to systemic C-reactive protein responses. *Nanotoxicology* **12**, 1130-1147, doi:10.1080/17435390.2018.1498930 (2018).

4 Monse, C. *et al.* Concentration-dependent systemic response after inhalation of nano-sized zinc oxide particles in human volunteers. *Part Fibre Toxicol* **15**, 8, doi:10.1186/s12989-018-0246-4 (2018).

5 Ridker, P. M., Hennekens, C. H., Buring, J. E. & Rifai, N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* **342**, 836-843, doi:10.1056/NEJM200003233421202 (2000).

6 Cray, C., Zaias, J. & Altman, N. H. Acute phase response in animals: a review. *Comp Med* **59**, 517-526 (2009).

7 Meier, R. *et al.* Associations of short-term particle and noise exposures with markers of cardiovascular and respiratory health among highway maintenance workers. *Environ Health Perspect* **122**, 726-732, doi:10.1289/ehp.1307100 (2014).

8 Baumann, R. *et al.* Systemic serum amyloid A as a biomarker for exposure to zinc and/or copper-containing metal fumes. *J Expo Sci Environ Epidemiol* **28**, 84-91, doi:10.1038/jes.2016.86 (2018).

9 Adetona, A. M. *et al.* Impact of Work Task-Related Acute Occupational Smoke Exposures on Select Proinflammatory Immune Parameters in Wildland Firefighters. *J Occup Environ Med* **59**, 679-690, doi:10.1097/JOM.0000000000001053 (2017).

10 Andersen, M. H. G. *et al.* Health effects of exposure to diesel exhaust in diesel-powered trains. *Part Fibre Toxicol* **16**, 21, doi:10.1186/s12989-019-0306-4 (2019).

11 Walker, E. S. *et al.* Acute differences in blood lipids and inflammatory biomarkers following controlled exposures to cookstove air pollution in the STOVES study. *Int J Environ Health Res* **32**, 565-578, doi:10.1080/09603123.2020.1785402 (2022).

12 Wyatt, L. H., Devlin, R. B., Rappold, A. G., Case, M. W. & Diaz-Sanchez, D. Low levels of fine particulate matter increase vascular damage and reduce pulmonary function in young healthy adults. *Part Fibre Toxicol* **17**, 58, doi:10.1186/s12989-020-00389-5 (2020).

13 Kim, J. Y., Chen, J. C., Boyce, P. D. & Christiani, D. C. Exposure to welding fumes is associated with acute systemic inflammatory responses. *Occup Environ Med* **62**, 157-163, doi:10.1136/oem.2004.014795 (2005).

14 Barregard, L. *et al.* Experimental exposure to wood-smoke particles in healthy humans: effects on markers of inflammation, coagulation, and lipid peroxidation. *Inhal Toxicol* **18**, 845-853, doi:10.1080/08958370600685798 (2006).

15 Sikkeland, L. I. B. *et al.* Systemic and Airway Inflammation after Exposure to Fumes from Military Small Arms. *Am J Respir Crit Care Med* **197**, 1349-1353, doi:10.1164/rccm.201709-1857LE (2018).

16 Brand, P. *et al.* Relationship between welding fume concentration and systemic inflammation after controlled exposure of human subjects with welding fumes from metal inert gas brazing of zinc-coated materials. *J Occup Environ Med* **56**, 1-5, doi:10.1097/JOM.0000000000000061 (2014).

17 Hadrup, N. *et al.* Acute phase response and inflammation following pulmonary exposure to low doses of zinc oxide nanoparticles in mice. *Nanotoxicology* **13**, 1275-1292, doi:10.1080/17435390.2019.1654004 (2019).

18 Poulsen, S. S. *et al.* Multi-walled carbon nanotube-physicochemical properties predict the systemic acute phase response following pulmonary exposure in mice. *PLoS One* **12**, e0174167, doi:10.1371/journal.pone.0174167 (2017).

19 Gutierrez, C. T. *et al.* Acute phase response following pulmonary exposure to soluble and insoluble metal oxide nanomaterials in mice. *Part Fibre Toxicol* **20**, 4, doi:10.1186/s12989-023-00514-0 (2023).

20 Christophersen, D. V. *et al.* Accelerated atherosclerosis caused by serum amyloid A response in lungs of ApoE(-/-) mice. *FASEB J* **35**, e21307, doi:10.1096/fj.202002017R (2021).

21 Halappanavar, S. *et al.* Pulmonary response to surface-coated nanotitanium dioxide particles includes induction of acute phase response genes, inflammatory cascades, and changes in microRNAs: a toxicogenomic study. *Environ Mol Mutagen* **52**, 425-439, doi:10.1002/em.20639 (2011).

List of Adverse Outcomes in this AOP

Event: 1443: Atherosclerosis

Short Name: Atherosclerosis

Key Event Component

Process	Object	Action
Atherosclerosis		increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:237 - Substance interaction with lung resident cell membrane components leading to atherosclerosis	AdverseOutcome

Biological Context

Level of Biological Organization

Individual

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

- Taxonomic applicability: Human.
- Life stages applicability: All life stages. Although atherosclerosis is mostly observed in adult humans, this condition begins early in life, and progresses through adulthood [7,8]. Children with chronic inflammation diseases have shown to develop atherosclerosis in early childhood [9,10].
- Sex applicability: Unspecific, atherosclerosis is manifested in males and females [11].

Key Event Description

Atherosclerosis is defined as the accumulation of fatty and fibrous material in the intima layer of arteries [1]. Atherosclerosis initiates with the alteration of the endothelium homeostasis and accumulation of modified low density lipoproteins (LDL) in the intima layer. The activation of endothelial cells leads to the recruitment and translocation of monocytes to the intima, where monocytes differentiate into macrophages. Following this, macrophages internalize oxidized LDL becoming foam cells. Several factors enhance plaque progression including continuous accumulation of foam cells and lipoproteins, and migration and proliferation of smooth muscle cells in the intima layer. Extracellular matrix macromolecules produced by smooth muscle cells results in the thickening of the intima. A necrotic core rich in lipids inside the atherosclerotic plaque is formed when macrophages and smooth muscle cells go through apoptosis. During progression, atherosclerotic plaques can develop calcification regions and expand toward the lumen of the artery. Advanced atherosclerosis can diminish the arteries lumen and/or form a thrombus (i.e. blood clot), reducing the blood flow, and leading to ischemia. The rupture of atherosclerotic plaques can also lead to embolism [1,2].

How it is Measured or Detected

Atherosclerosis can be detected through direct and indirect methods. Techniques that allow direct visualization of atherosclerotic plaques include ultrasonography, computed tomography angiography, magnetic resonance imaging, and optical coherence tomography [1]. These techniques can measure the intima thickness of arteries, along with detection of calcified components [3,4]. Techniques that allows the evaluation of atherosclerosis without direct visualization of plaques include angiography, aortic pulse wave velocity and the ankle-arm systolic blood pressure index [1,3,5]. Finally, although nonspecific, inflammatory markers are also use to evaluate atherosclerosis including blood levels of IL-6, CRP and TNF-α [5].

The induction and/or progression of atherosclerosis after exposure to a stressor can be studied through animal models. Examples of these are the *ApoE*$^{-/-}$ and *LdLr*$^{-/-}$ mouse models and Watanabe rabbit model, where the development of atherosclerotic can be assessed [6].

Regulatory Significance of the AO

Atherosclerosis is the principal cause of cardiovascular diseases including myocardial infarction, stroke and angina pectoris [1,2,12].

In turn, cardiovascular diseases are the principal cause of deaths worldwide and measures have been made by many countries to control risk factors and prevent this disease.[13].

References

1 Libby, P. *et al.* Atherosclerosis. *Nat Rev Dis Primers* **5**, 56, doi:10.1038/s41572-019-0106-z (2019).

2 Jebari-Benslaiman, S. *et al.* Pathophysiology of Atherosclerosis. *Int J Mol Sci* **23**, doi:10.3390/ijms23063346 (2022).

3 van der Meer, I. M. *et al.* Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. *Circulation* **109**, 1089-1094, doi:10.1161/01.CIR.0000120708.59903.1B (2004).

4 Poyrazoglu, H. G., Vurdem, U. E., Arslan, A. & Uytun, S. Evaluation of carotid intima-media thickness in children with migraine: a marker of subclinical atherosclerosis. *Neurol Sci* **37**, 1663-1669, doi:10.1007/s10072-016-2648-0 (2016).

5 Rodondi, N. *et al.* Markers of atherosclerosis and inflammation for prediction of coronary heart disease in older adults. *Am J Epidemiol* **171**, 540-549, doi:10.1093/aje/kwp428 (2010).

6 Gistera, A., Ketelhuth, D. F. J., Malin, S. G. & Hansson, G. K. Animal Models of Atherosclerosis-Supportive Notes and Tricks of the Trade. *Circ Res* **130**, 1869-1887, doi:10.1161/CIRCRESAHA.122.320263 (2022).

7 McGill, H. C., Jr., McMahan, C. A. & Gidding, S. S. Preventing heart disease in the 21st century: implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. *Circulation* **117**, 1216-1227, doi:10.1161/CIRCULATIONAHA.107.717033 (2008).

8 McMahan, C. A. *et al.* Risk scores predict atherosclerotic lesions in young people. *Arch Intern Med* **165**, 883-890, doi:10.1001/archinte.165.8.883 (2005).

9 Yamamura, K. *et al.* Early progression of atherosclerosis in children with chronic infantile neurological cutaneous and articular syndrome. *Rheumatology (Oxford)* **53**, 1783-1787, doi:10.1093/rheumatology/keu180 (2014).

10 Tyrrell, P. N. *et al.* Rheumatic disease and carotid intima-media thickness: a systematic review and meta-analysis. *Arterioscler Thromb Vasc Biol* **30**, 1014-1026, doi:10.1161/ATVBAHA.109.198424 (2010).

11 Libby, P. The changing landscape of atherosclerosis. *Nature* **592**, 524-533, doi:10.1038/s41586-021-03392-8 (2021).

12 Frostegard, J. Immunity, atherosclerosis and cardiovascular disease. *BMC Med* **11**, 117, doi:10.1186/1741-7015-11-117 (2013).

13 Vaduganathan, M., Mensah, G. A., Turco, J. V., Fuster, V. & Roth, G. A. The Global Burden of Cardiovascular Diseases and Risk: A Compass for Future Health. *J Am Coll Cardiol* **80**, 2361-2371, doi:10.1016/j.jacc.2022.11.005 (2022).

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 1702: Interaction with the lung cell membrane leads to Increased proinflammatory mediators

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Substance interaction with the pulmonary resident cell membrane components leading to pulmonary fibrosis	adjacent	Moderate	Moderate
Interaction with lung resident cell membrane components leads to lung cancer	adjacent	Moderate	Moderate
Substance interaction with lung resident cell membrane components leading to atherosclerosis	adjacent	High	Low

Key Event Relationship Description

Innate immune response is the first line of defence in any organism against invading infectious pathogens and toxic substances. It involves tissue triggered startle response to cellular stress and is described by a complex set of interactions between the toxic

stimuli, soluble macromolecules and cells (reviewed in Nathan, 2002). The process culminates in a functional change defined as inflammation, purpose of which is to resolve infection and promote healing. In lungs, the interaction of toxic substances with resident cells results in cellular stress, death or necrosis (Pouwels et al., 2016) leading to release of intracellular components such as alarmins (Damage associated molecular patterns [DAMPs], Interleukin (IL)-1α, High mobility group box 1 [HMGB1]). Released alarmins (danger sensors) bind cell surface receptors such as Interleukin 1 Receptor 1 (IL-1R1), Toll Like Receptors (TLRs) or others leading to activation of innate immune response signalling.

For example, binding of IL-1α to IL-1R1 can release Nuclear factor kappa B (NF-κB) resulting in its translocation to nucleus and transactivation of pro-inflammatory genes including cytokines, growth factors and acute phase genes. The signalling also stimulates secretion of a variety of pro-inflammatory mediators. Overexpression of IL-1α in cells induces increased secretion of pro-inflammatory mediators. Products of necrotic cells are shown to stimulate the immune system in an IL-1R1-dependent manner (Chen et al., 2007).

The secreted alarmins activate resident cells pre-stationed in the tissues such as mast cells or macrophages leading to propagation of the already initiated immune response by releasing more eicosanoids, cytokines, chemokines and other pro-inflammatory mediators. Thus, secreted mediators signal the recruitment of neutrophils, which are the first cell types to be recruited in acute inflammatory conditions. Neutrophil influx in sterile inflammation is driven mainly by IL-1α (Rider P, 2011). IL-1 mediated signalling regulates neutrophil influx in silica-induced acute lung inflammation (Hornung et al., 2008). IL-1 signalling also mediates neutrophil influx in other tissues and organs including liver and peritoneum. Other types of cells including macrophages, eosinophils, and lymphocytes are also recruited in a signal-specific manner. Recruitment of leukocytes induces critical cytokines associated with the T helper type 2 immune response, including Tumor necrosis factor alpha (TNF-α), IL-1β, and IL-13.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility of this relationship is high. There is a mechanistic relationship between the MIE (Event 1495) and KE1 (Event 1496) which has been evidenced in a number of both *in vitro* and *in vivo* model systems in response to stressors such as, asbestos, silica, bleomycin, carbon nanotubes, and metal oxide nanoparticles (NPs) (Behzadi *et al.,* 2017; Denholm & Phan 1990; Dostert et al., 2008; Mossman & Churg 1998).

Increased expression of IL-1α or IL-1β following lung exposure to multi-walled carbon nanotubes (MWCNTs), bleomycin, micro silica particles, silica crystals, and polyhexamethylene guanidine phosphate has been shown to be associated with neutrophil influx in rodents (Gasse et al., 2007; Girtsman et al., 2014; Hornung et al., 2008; Nikota et al., 2017; Rabolli et al., 2014; Suwara et al., 2014). Inhibition of IL-1 function by knocking out the expression of IL-1R1 using IL-1R1 knockout mice or via treatment with IL-1α or IL-1β neutralising antibodies results in complete abrogation of lung neutrophilic influx following exposure to MWCNTs (Nikota et al, 2017), cigarette smoke (CS) (Halappanavar et al., 2013), silica crystals (Rabolli et al., 2014) and bleomycin (Gasse et al., 2007). IL1-R1, Myeloid differentiation primary response protein (Myd88) or the IL-33/St2 signaling are involved in pulmonary fibrosis induced by bleomycin (Gasse et al., 2007; Xu et al., 2016).

Empirical Evidence

Empirical support for this KER is moderate. There are limited *in vitro* studies, which show a temporal and dose-dependent relationship between these two events, using the upregulation of specific surface receptors as a proxy for direct membrane interaction (Chan et al., 2018; Denholm & Phan, 1990; Roy et al., 2014). There are also studies that provide general support for the idea that an interaction with the lung resident cell membrane components leads to increased, secretion of pro-inflammatory and pro-fibrotic mediators (Table 1).

Dose-Response Evidence:

There are a few studies which provide evidence for a dose-response relationship in this KER. An *in vitro* study demonstrated a concentration-response relationship, in which silica exposure induced increases in pro-inflammatory cytokines through scavenger receptors in cultured bone marrow-derived murine mast cells. Cells were exposed to 6.25, 12.5, 25 or 50 μg/cm^2 silica dioxide (SiO_2) for 24 h. Macrophage scavenger receptor (MSR2) expression increased over time at 50 μg/cm^2 and in a concentration-dependent relationship. Moreover, Tumor necrosis factor alpha (TNF-α), IL-13 and Monocyte chemoattractant protein-1 (MCP-1) increased in a concentration-dependent manner (Brown et al., 2007). This provides indications that at higher concentrations of the stressor, the interaction with the lung resident cell membrane components (Event 1495) leads to an increased secretion of pro-inflammatory mediators (Event 1496).

Temporal Evidence:

In vitro and *in vivo* studies have demonstrated temporal concordance of the KEs.

TLR4 signal pathway was evaluated in differentiated macrophages exposed to silica at 2.5 μg/cm^2. After 16 and 24 h, the mRNA expression level of TLR4 increased. Moreover, the protein expression level of TLR-4 and related MyD88/Toll-interleukin-1 receptor domain containing adaptor protein (TIRAP) pathway increased at 24 h. Release of IL-1β, IL-6, IL-10, and TNF-α was induced by silica exposure at 24 h. Pre-treatment with resatorvid (TAK-242), an inhibitor of TLR4 signaling, suppressed the release of the cytokines (Chan et al., 2018).

Macrophages exposed to zinc oxide (ZnO) NPs at 2.5 μg/mL for 24 h increased the expression level of TLR6 and MyD88, TNF receptor-associated factor (TRAF), and IL-1 receptor-associated kinase (IRAK). At 24 h, they also observed an increase in the mRNA and protein levels of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α. These results demonstrated that ZnO NPs induced pro-inflammatory mediators by TLR stimulation and Mitogen-activated protein kinases (MAPKs) activation (Roy et al., 2014).

The pro-inflammatory IL-1β induced granulocyte migration and can be produced as a result of cellular detection of pathogen associated molecular patterns (PAMPs). Mice exposed to 2.5 mg/mouse of silica by instillation showed an increase of mRNA expression of pro-IL-1β in bronchoalveolar lavage fluid (BALF) at 6, 12, and 24 h post-exposure in a time-dependent manner. At early time points (1 h, 3 h, 6 h), there was an increase in the release of an alarmin (IL-1α) which indicates that the alarmin was released due to cell damage leading to cytokine production and an inflammatory reaction. Moreover, at 24 h, the levels of mature IL-1β and neutrophil accumulation in BALF increased. Neutralization or deletion of IL-1α reduced the observed responses (Rabolli et al., 2014).

Epithelial damage can lead to the release of alarmins. In this stead, conditioned media from primary human bronchial epithelial cells (PBECs) exposed to thapsigargin was able to induce a pro-inflammatory response in primary human lung fibroblasts. PBECs were exposed to thapsigargin (a tumor promoter in mammalian cells) 20 μM for 2 h. After that, the cell culture medium was replaced, and cells were incubated for 24 h. At this time, the medium was recovered and used to culture lung fibroblast for 5 h. This conditioned media from epithelial cell damage contains the alarmin IL-1α, which induced increased gene expression of IL-6, IL-8, MCP-1, and Granulocyte-macrophage colony-stimulating factor (GM-CSF) in fibroblasts. These responses were reduced with anti-IL-1α treatment (Suwara et al., 2014).

Heijink et al. 2015 conducted a similar strategy to identify the relationship between DAMPs and pro-inflammatory mediator release after exposure to CS. Neutrophils treated with CS bubbled for 1 min, released high levels of HMGB1 as a consequence of necrotic cell death. The cell-free supernatant, which contains HMGB1, was used to culture human bronchial epithelial cells, and after 24 h it promoted the production of the C-X-C motif chemokine ligand (CXCL)8 or IL-8 by lung epithelial cells. Pharmacological inhibitors, such as 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (OxPAPC) and Receptor for advanced glycation endproducts (RAGE) antagonist peptide (RAP), reduced the effect of CXCL8 release.

HMGB1 and Heat shock protein 70 (HSP-70) can be released by damaged hepatocytes. In a study, mice were treated with acetaminophen 350 mg/Kg for 3 and 6 h. At these time points, the liver perfusate was obtained and an increase in HSP-70 and HMGB1 protein levels was observed. RAW 264.7 cells (a macrophage cell line) treated with the liver perfusate exhibited increased mRNA expression levels of MCP-1 and IL-1β (Martin-Murphy et al. 2010).

Female mice were intratracheally administered with bleomycin at 5 mg/kg to represent idiopathic pulmonary fibrosis. IL-33, a molecule that can act as a DAMP, increased in lungs after 3 and 7 days of treatment. In serum, at 7-, 14- and 28-days post-exposure, IL-4 and IL-13 increased. It was concluded that IL-33/ST2 signaling pathway is involved in pulmonary fibrosis by bleomycin (Xu et al., 2016).

Uncertainties and Inconsistencies

Attenuation or complete abrogation of KE1 (Event 1496) and KE2 (Event 1497) following inflammogenic stimuli is observed in rodents lacking functional IL-1R1 or other cell surface receptors that engage innate immune response upon stimulation. However, following exposure to MWCNTs, it has been shown that absence of IL-1R1 signalling is compensated for eventually and neutrophil influx is observed at a later post-exposure time point (Nikota et al., 2017). In another study, acute neutrophilic inflammation induced by MWCNTs was suppressed at 24 h in mice deficient in IL-1R1 signalling; however, these mice showed exacerbated neutrophilic influx and fibrotic response at 28 days post-exposure (Girtsman et al., 2014). The early defence mechanisms involving DAMPs is fundamental for survival, which may necessitate activation of compensatory signaling pathways. As a result, inhibition of a single biological pathway mediated by an individual cell surface receptor may not be sufficient to completely abrogate the lung inflammatory response. Forced suppression of pro-inflammatory and immune responses early after exposure to substances that cannot be effectively cleared from lungs, may enhance the injury and initiate other pathways leading to exacerbated response.

Quantitative Understanding of the Linkage

A majority of the *in vivo* studies are conducted with only one dose and thus, it is difficult to derive quantitative dose-response relationships based on the existing data. However, it is clear from the studies referenced above that greater concentrations or doses of pro-fibrotic substances result in higher release of alarmins, and consequently, higher pro-inflammatory signalling. The above studies also demonstrate strong temporal relationships between the individual KEs.

Response-response relationship

One study has demonstrated a response-response relationship for this KER.

Human intervertebral disc cells were treated with 0, 0.5, 1, or 2 mg/ml of recombinant HMGB1 for 24 h. Protein levels were determined in cell medium supernatant by enzyme-linked immunosorbent assay (ELISA). HMGB1 stimulates the expression of IL-6 and Matrix metalloproteinase 1 (MMP-1) in a response-response relationship. A strong correlation was observed by Spearman's rank correlation coefficient between HMGB1 treatment and IL-6 or MMP-1 levels (Shah et al., 2019).

Other reports have studied both KEs, but they do not indicate if the response-response relationship was linear or not (coefficient or

correlation is not shown) (Chakraborty et al., 2017; Fukuda et al. 2017; Kim et al., 2020, Piazza et al., 2013; Yang et al., 2012;).

Time-scale

Some studies have described how long after a change in the MIE (Event 1495; interaction substance and components), KE1 (Event 1496; pro-inflammatory mediators are secreted) is impacted (Table 2).

Table 2. Time-scale related studies relevant to the MIE (Event 1495) - KE1 (Event 1496) relationship.

Reference	*In vitro*/*in vivo*/population study	Design	MIE (Event 1495)	KE1 (Event 1496)
			Timepoint	Timepoint
Xu et al., 2016	*In vivo*	40 Female Kunming strain mice Bleomycin was intratracheally administered 5 mg/Kg. Days post-exposure	IL-33 3, 7 days	IL-4, IL-13 7, 14, and 28 days
Roy et al., 2014	*In vitro*	Primary mice macrophages exposed to 2.5 mg/ml ZnO for 24 hrs.	Increased TLR6 expression 0.5, 3, 6, 12, and 24 h	Increased IL-6, TNF-α 24 h
Rabollli et al., 2014	*In vivo*	Female C57BL/6 mice Exposed to silica 2.5 mg/mouse by instillation	Increased the release of IL-1α 1, 3, and 6 h	Increased mRNA expression of pro-IL-1β 6, 12, and 24 h

Known Feedforward/Feedback loops influencing this KER

Pancreatic cancer cells stimulated with S100 calcium-binding protein A8 (S100A8) and S100 calcium-binding protein A9 (S100A9) released pro-inflammatory cytokines IL-8, TNF-α, and Fibroblast growth factor (FGF). Cancer cell-derived conditioned media and the individual cytokines (TNF-α and Transforming growth factor beta [TGF-β]) induced the protein expression of S100A8 and S100A9 in HL-60 monocytic cell line and primary human monocytes (Nedjadi et al. 2018).

References

1. Behzadi S, Serpooshan V, Tao W, Hamaly MA, Alkawareek MY, Dreaden EC, Brown D, Alkilany AM, Farokhzad OC, Mahmoudi M. Cellular uptake of nanoparticles: journey inside the cell. Chem Soc Rev. 2017 Jul 17;46(14):4218-4244. doi: 10.1039/c6cs00636a.

2. Brown JM, Swindle EJ, Kushnir-Sukhov NM, Holian A, Metcalfe DD. Silica-directed mast cell activation is enhanced by scavenger receptors. Am J Respir Cell Mol Biol. 2007 Jan;36(1):43-52. doi: 10.1165/rcmb.2006-0197OC.

3. Chakraborty D, Zenker S, Rossaint J, Hölscher A, Pohlen M, Zarbock A, Roth J, Vogl T. Alarmin S100A8 Activates Alveolar Epithelial Cells in the Context of Acute Lung Injury in a TLR4-Dependent Manner. Front Immunol. 2017 Nov 13;8:1493. doi: 10.3389/fimmu.2017.01493.

4. Chan JYW, Tsui JCC, Law PTW, So WKW, Leung DYP, Sham MMK, Tsui SKW, Chan CWH. Regulation of TLR4 in silica-induced inflammation: An underlying mechanism of silicosis. Int J Med Sci. 2018 Jun 14;15(10):986-991. doi: 10.7150/ijms.24715.

5. Chen CJ, Kono H, Golenbock D, Reed G, Akira S, Rock KL. Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. Nat Med. 2007 Jul;13(7):851-6. doi: 10.1038/nm1603.

6. Denholm EM, Phan SH. Bleomycin binding sites on alveolar macrophages. J Leukoc Biol. 1990 Dec;48(6):519-23. doi: 10.1002/jlb.48.6.519.

7. Dostert C, Pétrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science. 2008 May 2;320(5876):674-7. doi: 10.1126/science.1156995.

8. Fukuda K, Ishida W, Miura Y, Kishimoto T, Fukushima A. Cytokine expression and barrier disruption in human corneal epithelial cells induced by alarmin released from necrotic cells. Jpn J Ophthalmol. 2017 Sep;61(5):415-422. doi: 10.1007/s10384-017-0528-7.

9. Gasse P, Mary C, Guenon I, Noulin N, Charron S, Schnyder-Candrian S, Schnyder B, Akira S, Quesniaux VF, Lagente V, Ryffel B, Couillin I. IL-1R1/MyD88 signaling and the inflammasome are essential in pulmonary inflammation and fibrosis in mice. J Clin Invest. 2007 Dec;117(12):3786-99. doi: 10.1172/JCI32285.

10. Girtsman TA, Beamer CA, Wu N, Buford M, Holian A. IL-1R signalling is critical for regulation of multi-walled carbon nanotubes-induced acute lung inflammation in C57Bl/6 mice. Nanotoxicology. 2014 Feb;8(1):17-27. doi: 10.3109/17435390.2012.744110.

11. Halappanavar S, Nikota J, Wu D, Williams A, Yauk CL, Stampfli M. IL-1 receptor regulates microRNA-135b expression in a negative feedback mechanism during cigarette smoke-induced inflammation. J Immunol. 2013 Apr 1;190(7):3679-86. doi: 10.4049/jimmunol.1202456.

12. Heijink IH, Pouwels SD, Leijendekker C, de Bruin HG, Zijlstra GJ, van der Vaart H, ten Hacken NH, van Oosterhout AJ, Nawijn MC, van der Toorn M. Cigarette smoke-induced damage-associated molecular pattern release from necrotic neutrophils triggers proinflammatory mediator release. Am J Respir Cell Mol Biol. 2015 May;52(5):554-62. doi: 10.1165/rcmb.2013-0505OC.

13. Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, Rock KL, Fitzgerald KA, Latz E. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. Nat Immunol. 2008 Aug;9(8):847-56. doi: 10.1038/ni.1631.

14. Kim DH, Gu A, Lee JS, Yang EJ, Kashif A, Hong MH, Kim G, Park BS, Lee SJ, Kim IS. Suppressive effects of S100A8 and S100A9 on neutrophil apoptosis by cytokine release of human bronchial epithelial cells in asthma. Int J Med Sci. 2020 Feb 4;17(4):498-509. doi: 10.7150/ijms.37833.

15. Martin-Murphy BV, Holt MP, Ju C. The role of damage associated molecular pattern molecules in acetaminophen-induced liver injury in mice. Toxicol Lett. 2010 Feb 15;192(3):387-94. doi: 10.1016/j.toxlet.2009.11.016.

16. Mossman BT, Churg A. Mechanisms in the pathogenesis of asbestosis and silicosis. Am J Respir Crit Care Med. 1998 May;157(5 Pt 1):1666-80. doi: 10.1164/ajrccm.157.5.9707141.

17. Nathan C. Points of control in inflammation. Nature. 2002 Dec 19-26;420(6917):846-52. doi: 10.1038/nature01320.

18. Nedjadi T, Evans A, Sheikh A, Barerra L, Al-Ghamdi S, Oldfield L, Greenhalf W, Neoptolemos JP, Costello E. S100A8 and S100A9 proteins form part of a paracrine feedback loop between pancreatic cancer cells and monocytes. BMC Cancer. 2018 Dec 17;18(1):1255. doi: 10.1186/s12885-018-5161-4.

19. Nikota J, Banville A, Goodwin LR, Wu D, Williams A, Yauk CL, Wallin H, Vogel U, Halappanavar S. Stat-6 signaling pathway and not Interleukin-1 mediates multi-walled carbon nanotube-induced lung fibrosis in mice: insights from an adverse outcome pathway framework. Part Fibre Toxicol. 2017 Sep 13;14(1):37. doi: 10.1186/s12989-017-0218-0.

20. Piazza O, Leggiero E, De Benedictis G, Pastore L, Salvatore F, Tufano R, De Robertis E. S100B induces the release of pro-inflammatory cytokines in alveolar type I-like cells. Int J Immunopathol Pharmacol. 2013 Apr-Jun;26(2):383-91. doi: 10.1177/039463201302600211.

21. Pouwels SD, Zijlstra GJ, van der Toorn M, Hesse L, Gras R, Ten Hacken NH, Krysko DV, Vandenabeele P, de Vries M, van Oosterhout AJ, Heijink IH, Nawijn MC. Cigarette smoke-induced necroptosis and DAMP release trigger neutrophilic airway inflammation in mice. Am J Physiol Lung Cell Mol Physiol. 2016 Feb 15;310(4):L377-86. doi: 10.1152/ajplung.00174.2015.

22. Rabolli V, Badissi AA, Devosse R, Uwambayinema F, Yakoub Y, Palmai-Pallag M, Lebrun A, De Gussem V, Couillin I, Ryffel B, Marbaix E, Lison D, Huaux F. The alarmin IL-1α is a master cytokine in acute lung inflammation induced by silica micro- and nanoparticles. Part Fibre Toxicol. 2014 Dec 13;11:69. doi: 10.1186/s12989-014-0069-x.

23. Rider P, Carmi Y, Guttman O, Braiman A, Cohen I, Voronov E, White MR, Dinarello CA, Apte RN. IL-1α and IL-1β recruit different myeloid cells and promote different stages of sterile inflammation. J Immunol. 2011 Nov 1;187(9):4835-43. doi: 10.4049/jimmunol.1102048.

24. Roy R, Singh SK, Das M, Tripathi A, Dwivedi PD. Toll-like receptor 6 mediated inflammatory and functional responses of zinc oxide nanoparticles primed macrophages. Immunology. 2014 Jul;142(3):453-64. doi: 10.1111/imm.12276.

25. Shah BS, Burt KG, Jacobsen T, Fernandes TD, Alipui DO, Weber KT, Levine M, Chavan SS, Yang H, Tracey KJ, Chahine NO. High mobility group box-1 induces pro-inflammatory signaling in human nucleus pulposus cells via toll-like receptor 4-dependent pathway. J Orthop Res. 2019 Jan;37(1):220-231. doi: 10.1002/jor.24154.

26. Suwara MI, Green NJ, Borthwick LA, Mann J, Mayer-Barber KD, Barron L, Corris PA, Farrow SN, Wynn TA, Fisher AJ, Mann DA. IL-1α released from damaged epithelial cells is sufficient and essential to trigger inflammatory responses in human lung fibroblasts. Mucosal Immunol. 2014 May;7(3):684-93. doi: 10.1038/mi.2013.87.

27. Xu J, Zheng J, Song P, Zhou Y, Guan S. IL-33/ST2 pathway in a bleomycin-induced pulmonary fibrosis model. Mol Med Rep. 2016 Aug;14(2):1704-8. doi: 10.3892/mmr.2016.5446.

28. Yang D, Postnikov YV, Li Y, Tewary P, de la Rosa G, Wei F, Klinman D, Gioannini T, Weiss JP, Furusawa T, Bustin M,

Oppenheim JJ. High-mobility group nucleosome-binding protein 1 acts as an alarmin and is critical for lipopolysaccharide-induced immune responses. J Exp Med. 2012 Jan 16;209(1):157-71. doi: 10.1084/jem.20101354.

Relationship: 2053: Increased proinflammatory mediators leads to Increased transcription of APP

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Substance interaction with lung resident cell membrane components leading to atherosclerosis	adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Acute phase response is present in vertebrate species [14]. In addition, serum amyloid A, one of the major acute phase proteins, has been conserved in mammals throughout evolution and has been described in humans, mice, dogs, horses, among others [4].

Key Event Relationship Description

This KER presents the association between the secretion of pro-inflammatory mediators and transcription of acute phase protein in different tissues, mainly lungs and liver. The evidence of the KER presented is based on animal studies (mice), human studies and *in vitro* studies.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility is high. It is known that acute phase proteins are induced by pro-inflammatory cytokines, primary interleukin (IL)-6, IL-1β, and tumor necrosis factor α (TNF-α). These cytokines are produced a sites of inflammation mainly by monocytes and macrophages [1-4]. Following cytokine release, signaling cascades and transcription factors are activated, regulating the expression of acute phase reaction genes [3].

Immune cells are recruited to inflammatory sites by inflammatory mediators (i.e. cytokines and chemokines)[5]. Pulmonary inflammation in mice is commonly assessed as the number or fraction of neutrophils in the broncheoalveolar lavage fluid (BALF) [6] and can be used as indirect marker of the release of pro-inflammatory factors.

Empirical Evidence

- Il-1 (IL-1α and IL-1β, 10 ng/mL each) and IL-6 (500 units/mL), both in presence of 1μM dexamethasone, increased the relative levels of serum amyloid a (SAA) mRNA in cultured human adult aortic smooth muscle cells [7].
- Human hepatoma cells exposed to IL-6, IL-1β and TNF-α for 20 h showed a reduced synthesis of albumin and increased synthesis of the acute phase proteins C3 and ceruloplasmin. In addition, mice exposed to IL-1β and TNF-α showed an increase of *Saa* mRNA in liver tissue [8].
- After pulmonary exposure to lipopolysaccharide (LPS) (300 μg/mL), lung tissue from female C57BL/6 mice showed upregulation of several cytokines and chemokines genes and upregulation of the acute phase proteins genes serum amyloid A and α_1-protease inhibitor [9].

- Mice presenting IL-6 gene disruption (IL-$6^{-/-}$) shown a reduced response in liver mRNA levels of acute phase proteins haptoglobin, α1-acid glycoprotein and SAA, after challenged by turpentine, lipopolysaccharide and bacterial infection [10].
- After repeated instillation of carbon black nanoparticles, female C57BL/6BomTac mice showed increased expression of chemokine genes along with increased *Saa3* gene expression in lung tissue. In addition, there were dose-response relationships with several cytokine proteins in lung tissue[11].
- Intratracheal instillation of titanium dioxide in female C57BL/6 showed that 28 days after exposure, several genes of cytokines, chemokines and acute phase proteins were upregulated. Additionally, there were significant increases in inflammatory mediators in lung tissue [12].

The table in the following link presents evidence of the relationship using neutrophil numbers in BALF as indirect measurement of the release of pro-inflammatory mediators: Empirical evidence KER2.

Quantitative Understanding of the Linkage

Response-response relationship

A Pearson's correlation coefficient of 0.82 ($p<0.001$) has been calculated between log-transformed neutrophil numbers in brochoalveolar lavage fluid and log-transformed *Saa3* mRNA levels in lung tissue, in female C57BL/6J mice 1 and 28 days after intratracheal instillation of metal oxide nanomaterials [13] (Figure 1).

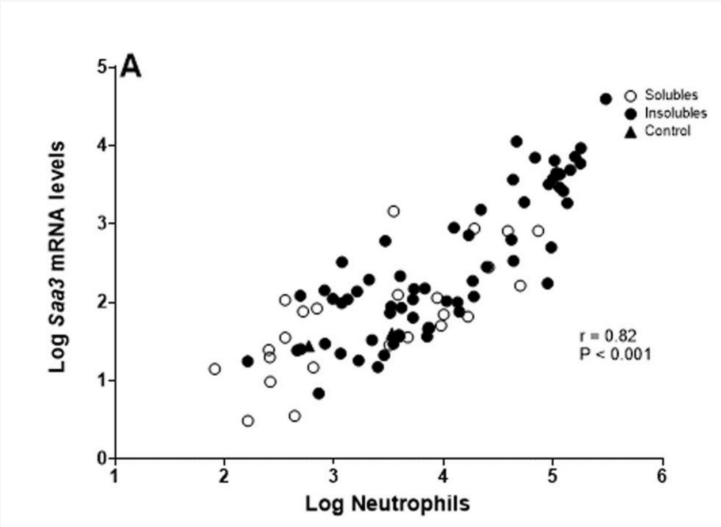


Figure 1. Correlations between neutrophil numbers and *Saa3* mRNA levels in lung tissue, including data from 1 and 28 days after exposure to nanomaterials. Reproduced from Gutierrez et al. (2023)[13].

Time-scale

It has been shown that pro-inflammatory mediators concentrations increase before acute phase proteins:

- Upregulation of cytokine genes (IL-1α, IL-1β, IL-6 and TNF- α) was shown to peak around 2h after pulmonary exposure to LPS in female C57BL/6J mice, while upregulation SAA genes showed their highest upregulation at 8-12h after exposure [9].

Known Feedforward/Feedback loops influencing this KER

Some acute phase proteins (f. ex. C-reactive protein, serum amyloid A and complement components) have pro-inflammatory functions, including induction of inflammatory cytokines, chemotaxis and activation of immune cells. On the other hand, other acute phase proteins present anti-inflammatory functions (f. ex. Haptoglobin and fibrinogen) as antioxidative and tissue repair inducer[1].

References

1 Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* **340**, 448-454, doi:10.1056/NEJM199902113400607 (1999).

2 Mantovani, A. & Garlanda, C. Humoral Innate Immunity and Acute-Phase Proteins. *N Engl J Med* **388**, 439-452, doi:10.1056/NEJMra2206346 (2023).

3 Venteclef, N., Jakobsson, T., Steffensen, K. R. & Treuter, E. Metabolic nuclear receptor signaling and the inflammatory acute phase response. *Trends Endocrinol Metab* **22**, 333-343, doi:10.1016/j.tem.2011.04.004 (2011).

4 Uhlar, C. M. & Whitehead, A. S. Serum amyloid A, the major vertebrate acute-phase reactant. *Eur J Biochem* **265**, 501-523, doi:10.1046/j.1432-1327.1999.00657.x (1999).

5 Janeway, C., Murphy, K. P., Travers, P. & Walport, M. *Janeway's immunobiology*. 7. ed. / Kenneth Murphy, Paul Travers, Mark Walport. edn, (Garland Science, 2008).

6 Van Hoecke, L., Job, E. R., Saelens, X. & Roose, K. Bronchoalveolar Lavage of Murine Lungs to Analyze Inflammatory Cell Infiltration. *J Vis Exp*, doi:10.3791/55398 (2017).

7 Meek, R. L., Urieli-Shoval, S. & Benditt, E. P. Expression of apolipoprotein serum amyloid A mRNA in human atherosclerotic lesions and cultured vascular cells: implications for serum amyloid A function. *Proc Natl Acad Sci U S A* **91**, 3186-3190, doi:10.1073/pnas.91.8.3186 (1994).

8 Ramadori, G., Van Damme, J., Rieder, H. & Meyer zum Buschenfelde, K. H. Interleukin 6, the third mediator of acute-phase reaction, modulates hepatic protein synthesis in human and mouse. Comparison with interleukin 1 beta and tumor necrosis factor-alpha. *Eur J Immunol* **18**, 1259-1264, doi:10.1002/eji.1830180817 (1988).

9 Jeyaseelan, S., Chu, H. W., Young, S. K. & Worthen, G. S. Transcriptional profiling of lipopolysaccharide-induced acute lung injury. *Infect Immun* **72**, 7247-7256, doi:10.1128/IAI.72.12.7247-7256.2004 (2004).

10 Kopf, M. *et al.* Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature* **368**, 339-342, doi:10.1038/368339a0 (1994).

11 Jackson, P. *et al.* Exposure of pregnant mice to carbon black by intratracheal instillation: toxicogenomic effects in dams and offspring. *Mutat Res* **745**, 73-83, doi:10.1016/j.mrgentox.2011.09.018 (2012).

12 Husain, M. *et al.* Pulmonary instillation of low doses of titanium dioxide nanoparticles in mice leads to particle retention and gene expression changes in the absence of inflammation. *Toxicol Appl Pharmacol* **269**, 250-262, doi:10.1016/j.taap.2013.03.018 (2013).

13 Gutierrez, C. T. *et al.* Acute phase response following pulmonary exposure to soluble and insoluble metal oxide nanomaterials in mice. *Part Fibre Toxicol* **20**, 4, doi:10.1186/s12989-023-00514-0 (2023).

14 Cray, C., Zaias, J. & Altman, N. H. Acute phase response in animals: a review. *Comp Med* **59**, 517-526 (2009).

Relationship: 1589: Increased transcription of APP leads to Systemic APR

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Substance interaction with lung resident cell membrane components leading to atherosclerosis	adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Acute phase response is present in vertebrate species [17]. In addition, serum amyloid A, one of the major acute phase proteins, has been conserved in mammals throughout evolution and has been described in humans, mice, dogs, horses, among others [18].

Key Event Relationship Description

This KER presents the association between the transcription of acute phase protein genes in different tissues and induction of systemic acute phase response. The evidence of the KER presented is based on animal studies (mice).

Evidence Supporting this KER

Biological Plausibility

The biological plausibility is high. After gene expression of acute phase proteins in tissues during inflammatory conditions, mRNA is translated and folded into proteins [1]. These proteins are then release to the systemic circulation [2].

Empirical Evidence

Species	Stressor	Acute phase protein expression	Systemic acute phase response	Reference
Mouse	Carbon black nanoparticles	Yes, significant *Saa1, Saa2* and *Saa3* gene expression increase in lung tissue, at days 1, 3 and 28 after exposure. *S a a 3* gene expression increase in liver tissue at day 1 after exposure.	Yes, significant increase of plasma SAA at 1 and 28 days after exposure.	3
Mouse	Multiwalled carbon nanotubes (referred as CNT_{small})	Yes, increased differential expression of acute phase response genes in liver tissue 1 and 3 days after exposure to 162 µg. Increased differential expression of acute phase response genes in lung tissue 3 days after exposure to 18 and 162 µg, and 1 and 3 days after exposure to 54 µg.	Yes, increased plasma SAA3 1, 3 and 28 days after exposure to 162 µg, and 3 days after exposure to 18 and 54 µg.	4,5
Mouse	Multiwalled carbon nanotubes (referred as CNT_{large})	Yes, increased differential expression of acute phase response genes in liver tissue 1 and 3 days after exposure to 162 µg. Increased differential expression of acute phase response genes in lung tissue 1 and 3 days after exposure to 54 and 162 µg.	Yes, increased plasma SAA3 1 and 3 days after exposure to 162 µg, and 3 days after exposure to 54 µg.	4,5
Mouse	Graphene oxide	Yes, increased mRNA expression of *Saa3* in lung tissue, at all dose 1 and 3 days after exposure. Increased gene expression of *Saa1* in liver tissue 1 day after exposure to 18 µg, and 3 days after exposure to 162 µg.	Yes, increased SAA3 plasma levels 3 days after exposure to 54 and 162 µg.	6
Mouse	Reduced graphene oxide	Yes, increased mRNA expression o f *Saa3* in lung tissue, 3 days after exposure to 162 µg. No changes in gene expression of *Saa1* in liver tissue.	No, no change in SAA3 plasma concentration 3 days after exposure.	6
Mouse	Multiwalled carbon nanotubes (NM-400 to NM-403)	Yes, increased *Saa1* mRNA expression in liver tissue with all MWCNTs, 1 day after exposure to 54 µg, and after exposure to 18 µg in the case of NM-401 and NM-403. After 28 days, only NM-400 (54 µg) produced an increase i n *Saa1* mRNA levels in liver tissue. Increased *Saa3* mRNA expression in lung tissue with all MWCNTs, 1 day after exposure to 54 µg, and after exposure to 6 and 18 µg in the case of NM-402 and NM-403.	Yes, increased SAA1/2 plasma levels 1 day after exposure to NM-400, NM-401 and NM-403. No change in SAA1/2 28 and 92 days after exposure. Increased SAA3 plasma levels 1 days after exposure to all MWCNT. Increased SAA3 plasma levels 28 and 92 days after exposure to NM-401.	7

Species	Stressor	Acute phase protein expression	Systemic acute phase response	Reference
		After 28 days, NM-400 (18 and 54), NM-402 (54 µg) and NM-403 (54 µg) produced an increase in *Saa3* mRNA levels in lung tissue.		
Mouse	Particulate matter from non-commercial airfield	Yes, increased expression of *Saa3* mRNA in lung tissue and *Saa1* mRNA in liver tissue after 1 day of exposure to 54 µg. No effect after 28 and 90 days.	Yes, increased plasma SAA3 levels after exposure to 54 µg after 3 days.	8
Mouse	Particulate matter from commercial airport	Yes, increased expression of *Saa3* mRNA in lung tissue after 1 day of exposure to 18 and 54 µg. No effect after 28 and 90 days.	No change in plasma SAA3.	8
Mouse	Diesel exhaust particles	Yes, increased expression of *Saa3* mRNA in lung tissue after 1 day of exposure to 54 and 162 µg, and increased expression of *Saa1* mRNA in liver tissue 1 day after exposure to 162 µg. No effect after 28 days.	Yes, increased plasma SAA3 levels after exposure to 54 µg, at 3 days.	8
Mouse	Carbon black	Yes, increased expression of *Saa3* mRNA in lung tissue at day 1 and day 90.	No change in plasma SAA3.	8
Mouse	Uncoated zinc oxide nanoparticles	Yes, increase on Saa3 mRNA in lung tissue 1 day after exposure to 2 µg. No effect 3 and 28 days after exposure.	No effect on plasma SAA3.	9
Mouse	Coated zinc oxide nanoparticles	Yes, increase on *Saa3* mRNA in lung tissue 1 day after exposure to 0.7 and 2 µg. No effect 3 and 28 days after exposure.	No effect on plasma SAA3.	9
Mouse	Zinc oxide	Yes, increased *Saa1* mRNA expression in liver tissue 1 day after exposure to 0.7 µg. No change in *Saa3* mRNA expression in lung tissue.	No change in plasma SAA3 or SAA1/2 levels.	10
Mouse	Copper oxide	Yes, increased *Saa3* mRNA expression in lung tissue 1 day after exposure to 2 and 6 µg. Increased *Saa1* mRNA expression in liver tissue 1 day after exposure to 6 µg.	Yes, increased plasma SAA1/2 level after exposure to 6 µg, 1 day after exposure.	10
Mouse	Tin dioxide	Yes, increased *Saa3* mRNA expression in lung tissue and *Saa1* mRNA expression in liver tissue, 1 day after exposure to 162 µg.	Yes, increased plasma SAA3 after exposure to 162 µg, 1 day after exposure.	10
Mouse	Titanium dioxide	Yes, increased *Saa3* mRNA expression in lung tissue and *Saa1* mRNA expression in liver tissue 1 day after exposure.	Yes, increased plasma SAA3 and SAA1/2 after exposure to 162 µg, 1 day after exposure.	10
Mouse	Carbon black	Yes, increased *Saa3* mRNA expression in lung tissue 1 and 28 days after exposure. Increased *Saa1* mRNA expression in liver tissue 1 day after exposure	Yes, increased plasma SAA3 and SAA1/2 after exposure to 162 µg, 1 day after exposure.	10
Mouse	Singlewalled carbon nanotubes	Yes, increased SAA1, SAP and haptoglobin gene expression in liver tissue, 1 day after exposure.	Yes, increase serum CRP, haptoglobin and SAP 1 day after exposure.	11
Mouse	Multiwalled carbon nanotubes	Yes, increased SAA1, SAP and haptoglobin gene expression in liver tissue, 1 day after exposure.	Yes, increase serum CRP, haptoglobin and SAP 1 day after exposure. No changes after 28 days.	11
Mouse	Serum amyloid A	Yes, significantly increase of *Saa3*	Yes, increased levels of	12

Species	Stressor	Acute phase protein expression	Systemic acute phase response	Reference
		mRNA levels in lung tissue and endogenous SAA3. Expression levels in liver tissue.		

Uncertainties and Inconsistencies

Although it is suggested that acute phase proteins are mainly produced in the liver [13], it has been shown that in mice, the liver has little upregulation of *Saa* genes after exposure to ultrafine carbon particles or diesel exhaust particle, while it is in the lung where there is a marked expression of *Saa3* mRNA [14,15].

It has been observed in some studies that the increase of *Saa* genes in lung or liver tissue does not translate into an increase in plasma SAA concentration [6,8,9]. This might be due to a protein concentration below the methods detection levels[9], while measuring gene expression provides a larger dynamic range.

Quantitative Understanding of the Linkage

Response-response relationship

A Pearson's correlation coefficient of 0.89 ($p<0.001$) has been calculated between log-transformed *Saa3* mRNA levels in lung tissue and log-transformed SAA3 plasma protein levels, in female C57BL/6J mice 1 day after intratracheal instillation of metal oxide nanomaterials [10] (Figure 1).

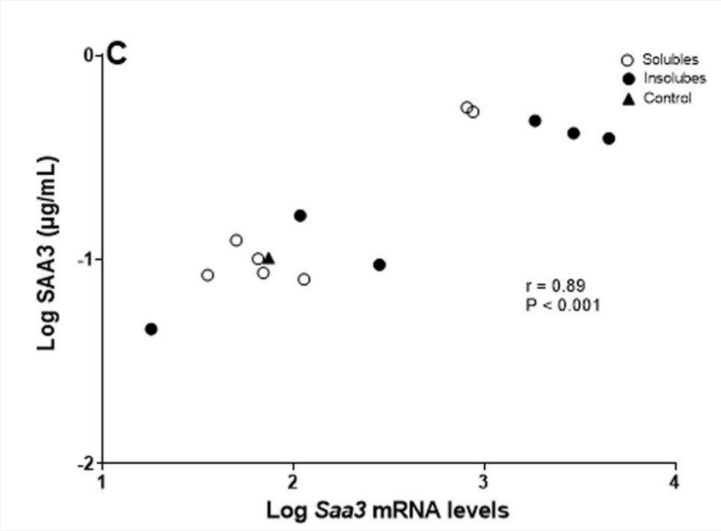


Figure 1. Correlations between Saa3 mRNA levels in lung tissue and SAA3 plasma protein levels, including data from 1 day after exposure to nanomaterials. Reproduced from Gutierrez et al. (2023)[10].

Time-scale

After exposure to titanium dioxide nanoparticles in mice, expression of *Saa1* mRNA in the liver is short lasting, while expression of *Saa3* mRNA in lung tissue is longer lasting, as it has been observed 28 day after exposure [16].

After exposure to multiwalled carbonanotubes, it has been observed that expression of *Saa1* and *Saa3* in liver and lung tissue can be elevated 28 days after exposure, however in most cases there is no increase in plasma SAA1/2 nor SAA3 levels past day 1 after exposure [7].

References

1 Alberts, B. *Molecular biology of the cell*. Sixth edition. edn, (CRC Press, an imprint of Garland Science, 2017).

2 Van Eeden, S., Leipsic, J., Paul Man, S. F. & Sin, D. D. The relationship between lung inflammation and cardiovascular disease. *Am J Respir Crit Care Med* **186**, 11-16, doi:10.1164/rccm.201203-0455PP (2012).

3 Bourdon, J. A. *et al.* Hepatic and pulmonary toxicogenomic profiles in mice intratracheally instilled with carbon black nanoparticles reveal pulmonary inflammation, acute phase response, and alterations in lipid homeostasis. *Toxicol Sci* **127**, 474-484, doi:10.1093/toxsci/kfs119 (2012).

4 Poulsen, S. S. *et al.* Changes in cholesterol homeostasis and acute phase response link pulmonary exposure to multi-walled carbon nanotubes to risk of cardiovascular disease. *Toxicol Appl Pharmacol* **283**, 210-222, doi:10.1016/j.taap.2015.01.011 (2015).

5 Poulsen, S. S. *et al.* MWCNTs of different physicochemical properties cause similar inflammatory responses, but differences in transcriptional and histological markers of fibrosis in mouse lungs. *Toxicol Appl Pharmacol* **284**, 16-32,

doi:10.1016/j.taap.2014.12.011 (2015).

6 Bengtson, S. *et al.* Differences in inflammation and acute phase response but similar genotoxicity in mice following pulmonary exposure to graphene oxide and reduced graphene oxide. *PLoS One* **12**, e0178355, doi:10.1371/journal.pone.0178355 (2017).

7 Poulsen, S. S. *et al.* Multi-walled carbon nanotube-physicochemical properties predict the systemic acute phase response following pulmonary exposure in mice. *PLoS One* **12**, e0174167, doi:10.1371/journal.pone.0174167 (2017).

8 Bendtsen, K. M. *et al.* Airport emission particles: exposure characterization and toxicity following intratracheal instillation in mice. *Part Fibre Toxicol* **16**, 23, doi:10.1186/s12989-019-0305-5 (2019).

9 Hadrup, N. *et al.* Acute phase response and inflammation following pulmonary exposure to low doses of zinc oxide nanoparticles in mice. *Nanotoxicology* **13**, 1275-1292, doi:10.1080/17435390.2019.1654004 (2019).

10 Gutierrez, C. T. *et al.* Acute phase response following pulmonary exposure to soluble and insoluble metal oxide nanomaterials in mice. *Part Fibre Toxicol* **20**, 4, doi:10.1186/s12989-023-00514-0 (2023).

11 Erdely, A. *et al.* Identification of systemic markers from a pulmonary carbon nanotube exposure. *J Occup Environ Med* **53**, S80-86, doi:10.1097/JOM.0b013e31821ad724 (2011).

12 Christophersen, D. V. *et al.* Accelerated atherosclerosis caused by serum amyloid A response in lungs of ApoE(-/-) mice. *FASEB J* **35**, e21307, doi:10.1096/fj.202002017R (2021).

13 Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* **340**, 448-454, doi:10.1056/NEJM199902113400607 (1999).

14 Saber, A. T. *et al.* Lack of acute phase response in the livers of mice exposed to diesel exhaust particles or carbon black by inhalation. *Part Fibre Toxicol* **6**, 12, doi:10.1186/1743-8977-6-12 (2009).

15 Saber, A. T. *et al.* Particle-induced pulmonary acute phase response correlates with neutrophil influx linking inhaled particles and cardiovascular risk. *PLoS One* **8**, e69020, doi:10.1371/journal.pone.0069020 (2013).

16 Wallin, H. *et al.* Surface modification does not influence the genotoxic and inflammatory effects of TiO2 nanoparticles after pulmonary exposure by instillation in mice. *Mutagenesis* **32**, 47-57, doi:10.1093/mutage/gew046 (2017).

17 Cray, C., Zaias, J. & Altman, N. H. Acute phase response in animals: a review. *Comp Med* **59**, 517-526 (2009).

18 Uhlar, C. M. & Whitehead, A. S. Serum amyloid A, the major vertebrate acute-phase reactant. *Eur J Biochem* **265**, 501-523, doi:10.1046/j.1432-1327.1999.00657.x (1999).

Relationship: 2860: Systemic APR leads to Atherosclerosis

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Substance interaction with lung resident cell membrane components leading to atherosclerosis	adjacent	High	High

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Although atherosclerosis is mostly observed in adult humans, this condition begins early in life, and progresses through adulthood [23,24]. Children with chronic inflammation diseases have shown to develop atherosclerosis in early childhood. [25,26]. In addition, atherosclerosis is manifested in males and females [27].

Key Event Relationship Description

This KER presents the association between systemic acute phase response and atherosclerosis as adverse outcome. The evidence of the KER presented is based on animal studies (mice), epidemiological studies and *in vitro* studies.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility is high. During acute phase response, serum amyloid A (SAA), one of the major acute phase proteins, replaces Apolipoprotein A-1 from high density lipoprotein (HDL). This replacement obstructs the reverse transport of cholesterol to the liver, allowing the accumulation of cholesterol in cells, denominated foam cells [1-3]. Foam cells are early markers of atherosclerotic lesions [4], and it has been shown that macrophages have a higher uptake of HDL containing SAA than HDL alone [2].

The two major human acute phase response, SAA and C-reactive protein (CRP), have been shown to be correlated in humans [5-7], and both are predictors of future cardiovascular event risks [7].

Empirical Evidence

- SAA was moderately associated with angiographic coronary artery disease in women (21-86 years old) suspected on having myocardial ischemia [8].
- High levels of CRP were associated with an increased risk of coronary heart disease in men and women [9].
- In mouse model of periodontal disease, ApoE$^{-/-}$ mice were infected with a polymicrobial consortium (*Porphyromonas gingivalis, Treponema denticola,* and *Tannerella forsythia*). 16-weeks after infection, the infected mice presented elevated levels of SAA in comparison to control mice, in addition of increased plaque progression [10].
- Male ApoE$^{-/-}$ mice overexpressing SAA1 presented higher levels of plasma SAA and an increase in atherosclerotic lesions (plaques) than non-SAA1 overexpressing ApoE$^{-/-}$ mice [11].
- After one injection of adenoviral vector enconding human SAA1, ApoE$^{-/-}$ mice presented elevated and transient levels of human SAA along with an increase in atherosclerotic lesions [12].
- Overexpression of SAA3 led to increased levels of SAA3 and atherosclerosis lesions in ApoE$^{-/-}$ mice in comparison to control mice. In addition, when SAA3 was suppressed in ApoE$^{-/-}$ × SAA1.1/2.1-DKO (ApoE$^{-/-}$ mice deficient in SAA1 and SAA2), there was a significant decreases in atherosclerotic lesions [13].
- In an *in vitro* study, Increasing concentrations of SAA (0 – 2 µM) produced a dose-response relationship of foam cells in RAW264.7 cells [14].
- Intratracheal instillation of human serum amyloid A once a week for 10 weeks in ApoE$^{-/-}$ mice (on Western-type diet) induced an increase in plasma SAA3 and atherosclerotic plaque progression [15].

Uncertainties and Inconsistencies

Mendelian randomization studies have shown that CRP genotypes are not associated with risk of coronary heart disease and that genetically elevated levels of CRP are not associated with coronary heart disease risk [16,17].

Quantitative Understanding of the Linkage

Response-response relationship

The association between CRP and SAA levels and risk of nonfatal myocardial infarction or fatal coronary heart disease (i.e. acute events due to the progression of atherosclerosis) can be calculated from prospective, epidemiological studies [7,9]. This approach was used by the Dutch Expert Committee on Occupational Safety (DECOS) when establishing a health-based occupational exposure limit for diesel engine exhaust based on risk of lung cancer (https://www.healthcouncil.nl/documents/advisory-reports/2019/03/13/diesel-engine-exhaust).

The Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) are prospective cohort investigations respectively involving 121,700 female U.S. registered nurses who were 30 to 55 years old at baseline in 1976 and 51,529 U.S. male health professionals who were 40 to 75 years old at baseline in 1986 [9]. In the NHS, among women without cardiovascular disease or cancer before 1990, 249 women had a nonfatal myocardial infarction or fatal coronary heart disease between the date of blood drawing and follow-up in June 1998. In the HPFS, 266 men had a nonfatal myocardial infarction or fatal coronary heart disease between the date of blood drawing and the return of a follow-up questionnaire in year 2000.

In the NHS and HPFS studies, the associations between CRP in blood and risk of nonfatal myocardial infarction or fatal coronary

heart disease for women and men were reported in Pai et al. (2004)[9], whereas the association for both SAA and CRP in NHS was reported in Ridker et al. (2000)[7].

The dose-response relationships are shown in Figure 1. Here, plasma levels of CRP and SAA were closely associated with future risk of coronary heart disease (CHD).

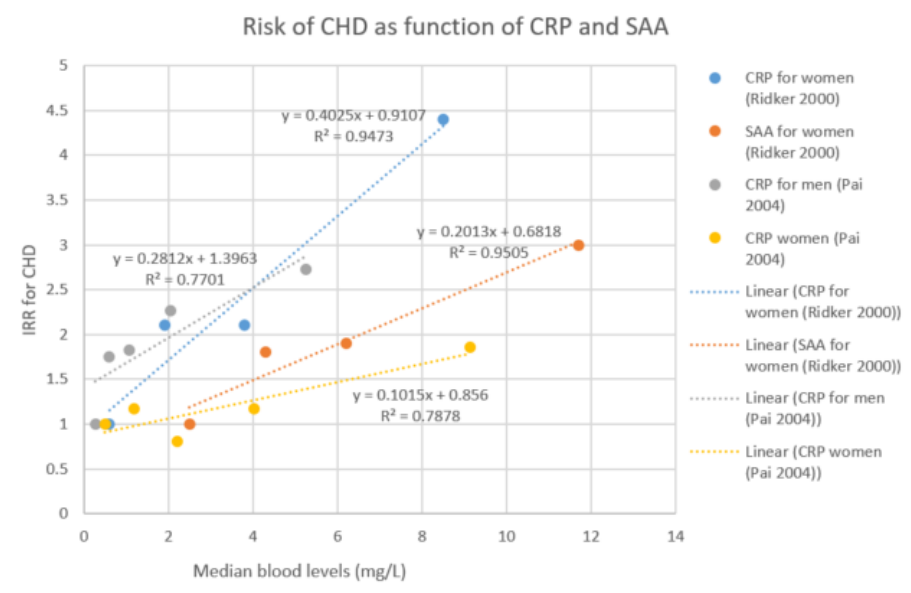


Figure 1. Association between the relative risk (RR) of CHD in NHS as function of quartiles of serum levels of CRP and SAA from Ridker et al. [7] and quintiles of CRP from the NHS and the HPFS studies from Pai et al.[9]. The trend lines are linear associations, as these gave the highest R^2 values.

According to the Danish Heart Foundation (https://hjerteforeningen.dk/alt-om-dit-hjerte/noegletal/), when a person reached the age of 55 years, the lifetime risk of a cardiovascular event is 67% in men and 66% in women. 56,379 Danes are diagnosed with a cardiovascular disease each year. Of these, 15,087 were diagnosed with are apoplexy and 16,050 with ischemic heart disease. These diagnoses are here regarded as manifestations of plaque progression. Thus, 55% of the cardiovascular diagnoses are relate to plaque progression. The lifetime risk of these diseases is thus 0.66X0.55= 0.363 = 36%.

The relative risk of 1:100 excess cardiovascular disease was calculated as

RR= (1 + 36)/36= 1.02778

The relative risk of 1:1000 excess cardiovascular disease was calculated as

RR= (1+360)/360= 1.00278

If the relative risk of 1.02778 excess is used in the equations obtained in Figure 1 and presented in the next table, it is observed that in the studies by Ridker et. al and Pai et al., 6-54% increases in blood levels of CRP or SAA were associated with 1% increased risk of cardiovascular disease.

Biomarker	Equation of increased IRR	Increase of biomarker associated with 1% increased risk(1)	Baseline levels	Increase of biomarker in % of baseline level associated 1% increased risk
CRP women [7]	ΔIRR = 0.4025 CRP (mg/L)	0.07 mg/L	0.6 mg/L	0.07/0.6= 12%
SAA women [7]	ΔIRR= 0.2013 SAA (mg/L)	0.138 mg/L	2.5 mg/L	0.138/2.5=6%
CRP women[9]	ΔIRR= 0.1015 CRP (mg/L	0.27 mg/L	0.5 mg/L	0.27/0.5=54%
CRP men[9]	ΔIRR= 0.2812 CRP	0.099 mg/L	0.27	0.099/0.27=37%

CRP (high)	(mg/L)		mg/L	

(1) The biomarker level is calculated as 0.02778/slope. For example, for CRP level in women CRP = 0.02778/0.4025 = 0.07 mg/L.

Known modulating factors

Modulating factor	Specification	Effects on the KER	References
Life style	High body mass index	Increased level of SAA and CRP, therefore increased risk of atherosclerosis.	8
Life style	Smoking	Increased level of CRP, therefore increased risk of atherosclerosis.	8,18
Medication	Intake of non-steroidal anti-inflammatory drugs	Reduction of CRP and other pro-inflammatory markers, decrease risk of atherosclerosis.	4
Medical conditions	Chronic inflammatory diseases	Increased level of acute phase proteins, therefore increased risk of atherosclerosis.	19
Medical conditions	Infectious diseases	Increased levels of CRP, therefore increased risk of atherosclerosis.	18

Known Feedforward/Feedback loops influencing this KER

Atherosclerosis is an inflammatory condition [20,21], therefore there are increased levels of pro-inflammatory factors, including acute phase proteins, than can sustain the progression of atherosclerosis [22].

References

1 Meek, R. L., Urieli-Shoval, S. & Benditt, E. P. Expression of apolipoprotein serum amyloid A mRNA in human atherosclerotic lesions and cultured vascular cells: implications for serum amyloid A function. *Proc Natl Acad Sci U S A* **91**, 3186-3190, doi:10.1073/pnas.91.8.3186 (1994).

2 Lindhorst, E., Young, D., Bagshaw, W., Hyland, M. & Kisilevsky, R. Acute inflammation, acute phase serum amyloid A and cholesterol metabolism in the mouse. *Biochim Biophys Acta* **1339**, 143-154, doi:10.1016/s0167-4838(96)00227-0 (1997).

3 McGillicuddy, F. C. *et al.* Inflammation impairs reverse cholesterol transport in vivo. *Circulation* **119**, 1135-1145, doi:10.1161/CIRCULATIONAHA.108.810721 (2009).

4 Libby, P. *et al.* Atherosclerosis. *Nat Rev Dis Primers* **5**, 56, doi:10.1038/s41572-019-0106-z (2019).

5 Baumann, R. *et al.* Human nasal mucosal C-reactive protein responses after inhalation of ultrafine welding fume particles: positive correlation to systemic C-reactive protein responses. *Nanotoxicology* **12**, 1130-1147, doi:10.1080/17435390.2018.1498930 (2018).

6 Monse, C. *et al.* Concentration-dependent systemic response after inhalation of nano-sized zinc oxide particles in human volunteers. *Part Fibre Toxicol* **15**, 8, doi:10.1186/s12989-018-0246-4 (2018).

7 Ridker, P. M., Hennekens, C. H., Buring, J. E. & Rifai, N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* **342**, 836-843, doi:10.1056/NEJM200003233421202 (2000).

8 Johnson, B. D. *et al.* Serum amyloid A as a predictor of coronary artery disease and cardiovascular outcome in women: the National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). *Circulation* **109**, 726-732,

doi:10.1161/01.CIR.0000115516.54550.B1 (2004).

9 Pai, J. K. *et al.* Inflammatory markers and the risk of coronary heart disease in men and women. *N Engl J Med* **351**, 2599-2610, doi:10.1056/NEJMoa040967 (2004).

10 Rivera, M. F. *et al.* Polymicrobial infection with major periodontal pathogens induced periodontal disease and aortic atherosclerosis in hyperlipidemic ApoE(null) mice. *PLoS One* **8**, e57178, doi:10.1371/journal.pone.0057178 (2013).

11 Dong, Z. *et al.* Serum amyloid A directly accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. *Mol Med* **17**, 1357-1364, doi:10.2119/molmed.2011.00186 (2011).

12 Thompson, J. C. *et al.* A brief elevation of serum amyloid A is sufficient to increase atherosclerosis. *J Lipid Res* **56**, 286-293, doi:10.1194/jlr.M054015 (2015).

13 Thompson, J. C. *et al.* Serum amyloid A3 is pro-atherogenic. *Atherosclerosis* **268**, 32-35, doi:10.1016/j.atherosclerosis.2017.11.011 (2018).

14 Lee, H. Y. *et al.* Serum amyloid A stimulates macrophage foam cell formation via lectin-like oxidized low-density lipoprotein receptor 1 upregulation. *Biochem Biophys Res Commun* **433**, 18-23, doi:10.1016/j.bbrc.2013.02.077 (2013).

15 Christophersen, D. V. *et al.* Accelerated atherosclerosis caused by serum amyloid A response in lungs of ApoE(-/-) mice. *FASEB J* **35**, e21307, doi:10.1096/fj.202002017R (2021).

16 Collaboration, C. R. P. C. H. D. G. *et al.* Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. *BMJ* **342**, d548, doi:10.1136/bmj.d548 (2011).

17 Elliott, P. *et al.* Genetic Loci associated with C-reactive protein levels and risk of coronary heart disease. *JAMA* **302**, 37-48, doi:10.1001/jama.2009.954 (2009).

18 Willeit, J. *et al.* Distinct risk profiles of early and advanced atherosclerosis: prospective results from the Bruneck Study. *Arterioscler Thromb Vasc Biol* **20**, 529-537, doi:10.1161/01.atv.20.2.529 (2000).

19 Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* **340**, 448-454, doi:10.1056/NEJM199902113400607 (1999).

20 Ross, R. Atherosclerosis--an inflammatory disease. *N Engl J Med* **340**, 115-126, doi:10.1056/NEJM199901143400207 (1999).

21 Balci, B. The modification of serum lipids after acute coronary syndrome and importance in clinical practice. *Curr Cardiol Rev* **7**, 272-276, doi:10.2174/157340311799960690 (2011).

22 Kobiyama, K. & Ley, K. Atherosclerosis. *Circ Res* **123**, 1118-1120, doi:10.1161/CIRCRESAHA.118.313816 (2018).

23 McGill, H. C., Jr., McMahan, C. A. & Gidding, S. S. Preventing heart disease in the 21st century: implications of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. *Circulation* **117**, 1216-1227, doi:10.1161/CIRCULATIONAHA.107.717033 (2008).

24 McMahan, C. A. *et al.* Risk scores predict atherosclerotic lesions in young people. *Arch Intern Med* **165**, 883-890, doi:10.1001/archinte.165.8.883 (2005).

25 Yamamura, K. *et al.* Early progression of atherosclerosis in children with chronic infantile neurological cutaneous and articular syndrome. *Rheumatology (Oxford)* **53**, 1783-1787, doi:10.1093/rheumatology/keu180 (2014).

26 Tyrrell, P. N. *et al.* Rheumatic disease and carotid intima-media thickness: a systematic review and meta-analysis. *Arterioscler Thromb Vasc Biol* **30**, 1014-1026, doi:10.1161/ATVBAHA.109.198424 (2010).

27 Libby, P. The changing landscape of atherosclerosis. *Nature* **592**, 524-533, doi:10.1038/s41586-021-03392-8 (2021).

List of Non Adjacent Key Event Relationships

Relationship: 2958: Interaction with the lung cell membrane leads to Increased transcription of APP

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Substance interaction with lung resident cell membrane components leading to atherosclerosis	non-adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

The expression of *Saa* mRNA in lung and liver tissue has been shown in mice after pulmonary exposure to a variety of nanomaterials (see Empirical evidence), and in humans in different tissues as lung, liver and arteries [13,14].

Key Event Relationship Description

This KER presents the association between the interaction of stressors with the lungs cells and transcription of acute phase proteins in different tissues, mainly lungs and liver. The evidence of the KER presented is based on animal studies (mice).

Evidence Supporting this KER

Biological Plausibility

The biological plausibility is high. Production of acute phase proteins is triggered by cellular pattern-recognition molecules after sensing pathogens, tissue damage or dysmetabolism, through a cytokine cascade [1]. In the lungs, this cytokine cascade is produced by epithelial cells and resident macrophages [2]. In the table below it is shown that a variety of stressors produced an increase in Serum amyloid A (SAA) gene isoforms in mice tissue.

Empirical Evidence

For this KER, exposure through the respiratory system (inhalation or intratracheal instillation) of stressors is considered as interaction with lung resident cell membrane components. The table in the following link presents evidence of KER: EMPIRICAL EVIDENCE KER5.

Uncertainties and Inconsistencies

Although it is suggested that acute phase proteins are mainly produced in the liver [3], it has been shown that in mice, the liver has little upregulation of *Saa* genes after exposure to ultrafine carbon particles or diesel exhaust particle, while it is in the lung where there is a marked expression of *Saa3* mRNA [4,5].

In the case of nanomaterials, it has been shown that physicochemical characteristics as size, surface area, surface functionalization, shape, composition, among others, affect the magnitude and duration of the expression of acute phase proteins in mice [6-12].

In humans, measuring gene expression of acute phase proteins is not very common as a tissue sample is needed, while measuring acute phase protein in blood in more common. However, *Saa* mRNA has been shown expressed in different tissues including lung, liver and arteries [13,14].

Quantitative Understanding of the Linkage

Response-response relationship

In the case of some insoluble nanomaterials, it has been observed that log-transformed dosed surface area (dosed mass multiply by specific surface area) and log-transformed *Saa3* mRNA levels in mice lung tissue presented a Pearson's correlation coefficient of 0.70 ($p < 0.001$) 1 day post-exposure. The linear regression formula obtained was Log *Saa3*mRNA = 1.080*Log Dosed surface area + 0.9415 ($p<0.001$)[7] (Figure 1).

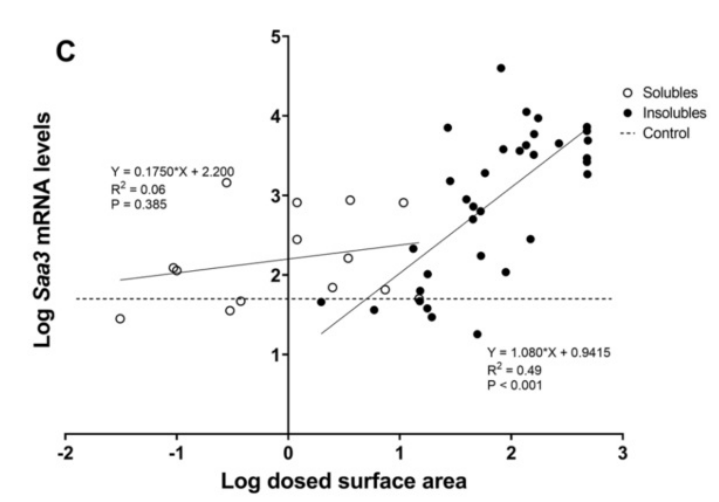


Figure 1. Correlations between dosed surface area and *Saa3* mRNA levels in lung tissue, 1 day after exposure to nanomaterials. Reproduced from Gutierrez et al. (2023)[7].

Time-scale

After exposure to titanium dioxide nanoparticles in mice, expression of *Saa1* mRNA in the liver is short lasting, while expression of *Saa3* mRNA in lung tissue is longer lasting, as it has been observed 28 day after exposure [10].

References

1 Mantovani, A. & Garlanda, C. Humoral Innate Immunity and Acute-Phase Proteins. *N Engl J Med* **388**, 439-452, doi:10.1056/NEJMra2206346 (2023).

2 Moldoveanu, B. *et al.* Inflammatory mechanisms in the lung. *J Inflamm Res* **2**, 1-11 (2009).

3 Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* **340**, 448-454, doi:10.1056/NEJM199902113400607 (1999).

4 Saber, A. T. *et al.* Lack of acute phase response in the livers of mice exposed to diesel exhaust particles or carbon black by inhalation. *Part Fibre Toxicol* **6**, 12, doi:10.1186/1743-8977-6-12 (2009).

5 Saber, A. T. *et al.* Particle-induced pulmonary acute phase response correlates with neutrophil influx linking inhaled particles and cardiovascular risk. *PLoS One* **8**, e69020, doi:10.1371/journal.pone.0069020 (2013).

6 Poulsen, S. S. *et al.* Multi-walled carbon nanotube-physicochemical properties predict the systemic acute phase response following pulmonary exposure in mice. *PLoS One* **12**, e0174167, doi:10.1371/journal.pone.0174167 (2017).

7 Gutierrez, C. T. *et al.* Acute phase response following pulmonary exposure to soluble and insoluble metal oxide nanomaterials in mice. *Part Fibre Toxicol* **20**, 4, doi:10.1186/s12989-023-00514-0 (2023).

8 Barfod, K. K. *et al.* Increased surface area of halloysite nanotubes due to surface modification predicts lung inflammation and acute phase response after pulmonary exposure in mice. *Environ Toxicol Pharmacol* **73**, 103266, doi:10.1016/j.etap.2019.103266 (2020).

9 Bengtson, S. *et al.* Differences in inflammation and acute phase response but similar genotoxicity in mice following pulmonary exposure to graphene oxide and reduced graphene oxide. *PLoS One* **12**, e0178355, doi:10.1371/journal.pone.0178355 (2017).

10 Wallin, H. *et al.* Surface modification does not influence the genotoxic and inflammatory effects of TiO2 nanoparticles after pulmonary exposure by instillation in mice. *Mutagenesis* **32**, 47-57, doi:10.1093/mutage/gew046 (2017).

11 Hadrup, N. *et al.* Acute phase response and inflammation following pulmonary exposure to low doses of zinc oxide nanoparticles in mice. *Nanotoxicology* **13**, 1275-1292, doi:10.1080/17435390.2019.1654004 (2019).

12 Danielsen, P. H. *et al.* Effects of physicochemical properties of TiO(2) nanomaterials for pulmonary inflammation, acute phase response and alveolar proteinosis in intratracheally exposed mice. *Toxicol Appl Pharmacol* **386**, 114830, doi:10.1016/j.taap.2019.114830 (2020).

13 Meek, R. L., Urieli-Shoval, S. & Benditt, E. P. Expression of apolipoprotein serum amyloid A mRNA in human atherosclerotic lesions and cultured vascular cells: implications for serum amyloid A function. *Proc Natl Acad Sci U S A* **91**, 3186-3190, doi:10.1073/pnas.91.8.3186 (1994).

14 Urieli-Shoval, S., Cohen, P., Eisenberg, S. & Matzner, Y. Widespread expression of serum amyloid A in histologically normal human tissues. Predominant localization to the epithelium. *J Histochem Cytochem* **46**, 1377-1384, doi:10.1177/002215549804601206 (1998).

Relationship: 2959: Interaction with the lung cell membrane leads to Systemic APR

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Substance interaction with lung resident cell membrane components leading to atherosclerosis	non-adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Systemic acute phase response measured as elevation of CRP and SAA in humans, and SAA in mice has been shown after exposure to several stressors (see Empirical evidence).

Key Event Relationship Description

This KER presents the association between the interaction of stressors with the lungs and the induction of systematic acute phase response. The evidence of the KER presented is based on animal studies (mice), controlled human studies and epidemiological studies.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility is high. Pulmonary inflammation occurs when stressor interact with the airways [1] and acute phase response is induced during inflammatory conditions [2]. It has been shown (see table below) that exposure to different stressors produces an increase of acute phase proteins in blood [i.e. C-reactive protein (CRP) and serum amyloid A (SAA)] in humans and mice.

Empirical Evidence

For this KER, exposure through the respiratory system (inhalation or intratracheal instillation) of stressors is considered as interaction with lung resident cell membrane components. The table in the following link presents evidence of KER: EMPIRICAL EVIDENCE KER6.

Uncertainties and Inconsistencies

In the case of nanomaterials, it has been shown that physicochemical characteristics as size, surface area, surface functionalization, shape, composition, among others, affect the magnitude and duration of acute phase response in mice [3-5].

It has been observed that in most controlled human studies, an increase in CRP and/or SAA was observed after exposure to particulate matter [6-10]. However, in other human studies the exposure did not induce acute phase response[11,12], maybe due to a low level of exposure [13]

Quantitative Understanding of the Linkage

Response-response relationship

In the case of some insoluble nanomaterials, it has been observed that log-transformed dosed surface area (dosed mass multiply by specific surface area) and log-transformed SAA3 plasma levels in mice presented a Pearson's correlation coefficient of 0.92 ($p < 0.001$) 1 day post-exposure [4] (Figure 1). The linear regression formula obtained was Log SAA3 = 0.9459 *Log Dosed surface area – 2.854 ($p=0.01$). The correlation coefficient between log-transformed dosed surface area and log-transformed SAA1/2 plasma levels was 0.83 ($p<0.05$) and the linear regression formula was Log SAA1/2 = 0.6368 *Log Dosed surface area +0.09524 ($p=0.01$) [4] (Figure 2).

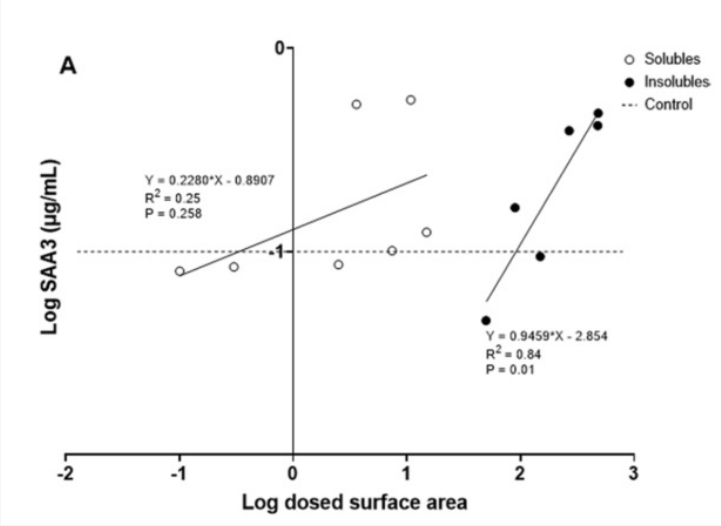


Figure 1. Correlations between pulmonary dosed surface area and SAA3 protein in plasma, 1 day after exposure to nanomaterials. Reproduced from Gutierrez et al. (2023) [4].

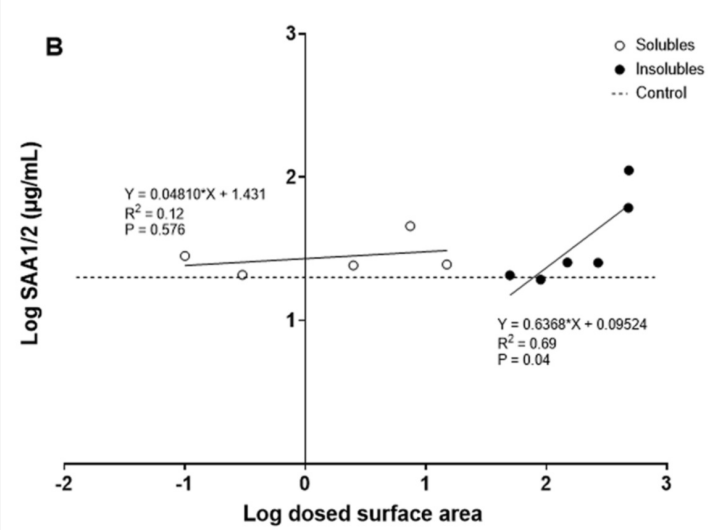


Figure 2. Correlations between pulmonary dosed surface area and SAA1/2 protein in plasma, 1 day after exposure to nanomaterials. Reproduced from Gutierrez et al. (2023) [4].

Time-scale

In mice, increased SAA levels are observed 1 and 3 days after most exposures, however increased SAA levels are not frequently observed 28 or 90 days after exposure [3,14-17].

In humans, increased SAA and CRP has been observed 22h and 2 days after exposure to zinc oxide, but not 3 days after exposure [7]. After exposure to zinc oxide, copper oxide or a mix both, SAA levels were elevated 24h after exposure in humans, but not 6h after exposure [10].

References

1 Moldoveanu, B. *et al.* Inflammatory mechanisms in the lung. *J Inflamm Res* **2**, 1-11 (2009).

2 Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* **340**, 448-454, doi:10.1056/NEJM199902113400607 (1999).

3 Poulsen, S. S. *et al.* Multi-walled carbon nanotube-physicochemical properties predict the systemic acute phase response following pulmonary exposure in mice. *PLoS One* **12**, e0174167, doi:10.1371/journal.pone.0174167 (2017).

4 Gutierrez, C. T. *et al.* Acute phase response following pulmonary exposure to soluble and insoluble metal oxide nanomaterials in mice. *Part Fibre Toxicol* **20**, 4, doi:10.1186/s12989-023-00514-0 (2023).

5 Bengtson, S. *et al.* Differences in inflammation and acute phase response but similar genotoxicity in mice following pulmonary exposure to graphene oxide and reduced graphene oxide. *PLoS One* **12**, e0178355, doi:10.1371/journal.pone.0178355 (2017).

6 Monse, C. *et al.* Concentration-dependent systemic response after inhalation of nano-sized zinc oxide particles in human volunteers. *Part Fibre Toxicol* **15**, 8, doi:10.1186/s12989-018-0246-4 (2018).

7 Monse, C. *et al.* Health effects after inhalation of micro- and nano-sized zinc oxide particles in human volunteers. *Arch Toxicol* **95**, 53-65, doi:10.1007/s00204-020-02923-y (2021).

8 Walker, E. S. *et al.* Acute differences in blood lipids and inflammatory biomarkers following controlled exposures to cookstove air pollution in the STOVES study. *Int J Environ Health Res* **32**, 565-578, doi:10.1080/09603123.2020.1785402 (2022).

9 Wyatt, L. H., Devlin, R. B., Rappold, A. G., Case, M. W. & Diaz-Sanchez, D. Low levels of fine particulate matter increase vascular damage and reduce pulmonary function in young healthy adults. *Part Fibre Toxicol* **17**, 58, doi:10.1186/s12989-020-00389-5 (2020).

10 Baumann, R. *et al.* Systemic serum amyloid A as a biomarker for exposure to zinc and/or copper-containing metal fumes. *J Expo Sci Environ Epidemiol* **28**, 84-91, doi:10.1038/jes.2016.86 (2018).

11 Andersen, M. H. G. *et al.* Association between polycyclic aromatic hydrocarbon exposure and peripheral blood mononuclear cell DNA damage in human volunteers during fire extinction exercises. *Mutagenesis* **33**, 105-115, doi:10.1093/mutage/gex021 (2018).

12 Andersen, M. H. G. *et al.* Assessment of polycyclic aromatic hydrocarbon exposure, lung function, systemic inflammation, and genotoxicity in peripheral blood mononuclear cells from firefighters before and after a work shift. *Environ Mol Mutagen* **59**, 539-548, doi:10.1002/em.22193 (2018).

13 Andersen, M. H. G. *et al.* Health effects of exposure to diesel exhaust in diesel-powered trains. *Part Fibre Toxicol* **16**, 21, doi:10.1186/s12989-019-0306-4 (2019).

14 Bourdon, J. A. *et al.* Hepatic and pulmonary toxicogenomic profiles in mice intratracheally instilled with carbon black nanoparticles reveal pulmonary inflammation, acute phase response, and alterations in lipid homeostasis. *Toxicol Sci* **127**, 474-484, doi:10.1093/toxsci/kfs119 (2012).

15 Poulsen, S. S. *et al.* Changes in cholesterol homeostasis and acute phase response link pulmonary exposure to multi-walled carbon nanotubes to risk of cardiovascular disease. *Toxicol Appl Pharmacol* **283**, 210-222, doi:10.1016/j.taap.2015.01.011 (2015).

16 Poulsen, S. S. *et al.* MWCNTs of different physicochemical properties cause similar inflammatory responses, but differences in transcriptional and histological markers of fibrosis in mouse lungs. *Toxicol Appl Pharmacol* **284**, 16-32, doi:10.1016/j.taap.2014.12.011 (2015).

17 Hadrup, N. *et al.* Pulmonary effects of nanofibrillated celluloses in mice suggest that carboxylation lowers the inflammatory and acute phase responses. *Environ Toxicol Pharmacol* **66**, 116-125, doi:10.1016/j.etap.2019.01.003 (2019).

Relationship: 3052: Increased proinflammatory mediators leads to Systemic APR

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Substance interaction with lung resident cell membrane components leading to atherosclerosis	non-adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	NCBI
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Acute phase response is conserved in vertebrate species [13].

Key Event Relationship Description

This KER presents the association between the secretion of pro-inflammatory mediators and induction of systemic acute phase response. The evidence of the KER presented is based on animal studies (mice), controlled human studies and epidemiological studies.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility is high. The production of acute phase proteins during acute phase response is induced by the release of pro-inflammatory markers as interleukin (IL)-6, IL-1β, and tumor necrosis factor α (TNF-α) at inflammatory sites [1,2]. The release of inflammatory markers also induces the recruitment of immune cells to inflammation sites [3].

Neutrophils in the broncheoalveolar lavage fluid (BALF) are frequently used to measure pulmonary inflammation in mice [4] and can be utilized as an indirect indicator of the release of pro-inflammatory factors.

Empirical Evidence

Evidence of the secretion of pro-inflammatory mediators is presented as change in concentration of pro-inflammatory markers in blood, or increase neutrophil numbers in blood or BALF. The table in the following link presents evidence of KER: EMPIRICAL EVIDENCE KER8.

Uncertainties and Inconsistencies

Wyatt et al. observed a decrease in blood neutrophil numbers in humans after exposure to ambient particulate matter although an increase in SAA and CRP was observed. It was mentioned this might be due to the translocation of neutrophil from major vessels to smaller arteries [5].

In the study by Meier et al., the authors obtained a negative association between $PM_{2.5}$ exposure and blood levels of TNF-α and IL-6, while SAA and CRP were positive associated with the exposure. The authors mentioned these results might be due the time point where the samples were taken [6].

Barregard et al. also observed that IL-6 levels were lower after exposure to wood smoke than after exposure to clean air. The discussed this response as a possible sequestering of cytokines in the pulmonary capillary bed [7].

Quantitative Understanding of the Linkage

Response-response relationship

A Pearson's correlation coefficient of 0.79 ($p<0.001$) has been calculated between log-transformed neutrophil number in BALF and log-transformed SAA3 plasma protein levels, in female C57BL/6J mice 1 day after intratracheal instillation of metal oxide nanomaterials [8] (Figure 1).

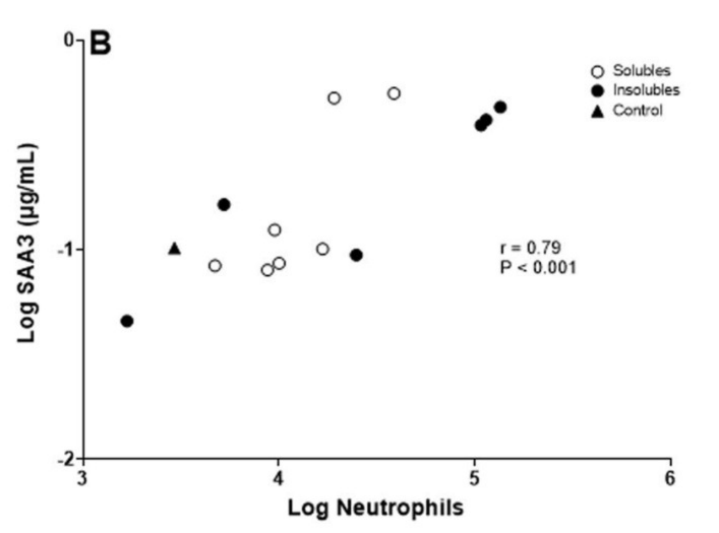


Figure 1. Correlations between neutrophil numbers and SAA3 plasma protein levels, including data from 1 day after exposure to nanomaterials. Reproduced from Gutierrez et al. (2023)[8].

A linear dose-response has also been found between log10-transformed neutrophil numbers and log2-transformes SAA3 plasma protein levels in mice, 1 day after exposure to multiwalled carbon nanotubes (Figure 2) [9].

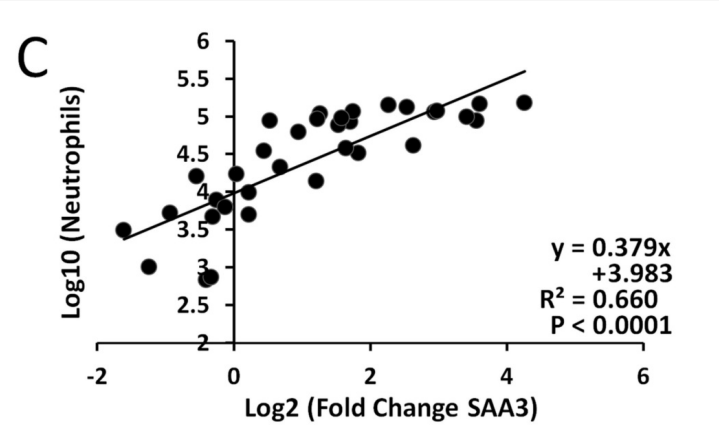


Figure 2. Transformed SAA3 protein vs. transformed neutrophil influx. Reproduced from Poulsen et al. (2017) [9].

Time-scale

It has been shown that pro-inflammatory mediators concentrations increase before acute phase proteins:

- In humans patients with atherosclerotic renal stenosis, blood IL-6 increased in the first hour after renal artery stenting and reach its highest concentration at 6h, while C-reactive protein (CRP) increased 6h after the treatment, peaking at 24h after treatment [10].
- In human infants undergoing cardiopulmonary bypass, it has been observed that blood concentrations of IL-6 significantly increased after cessation of the procedure and remained elevated 24h later, while CRP started increased 6h after bypass and kept increasing at 12h and 24h after bypass [11].

Known Feedforward/Feedback loops influencing this KER

IL-1, IL-6 and TNF- α can decrease acute phase response by decreasing their own production through the induction of corticosteroids [12].

References

1 Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* **340**, 448-454, doi:10.1056/NEJM199902113400607 (1999).

2 Mantovani, A. & Garlanda, C. Humoral Innate Immunity and Acute-Phase Proteins. *N Engl J Med* **388**, 439-452, doi:10.1056/NEJMra2206346 (2023).

3 Janeway, C., Murphy, K. P., Travers, P. & Walport, M. *Janeway's immunobiology*. 7. ed. / Kenneth Murphy, Paul Travers, Mark Walport. edn, (Garland Science, 2008).

4 Van Hoecke, L., Job, E. R., Saelens, X. & Roose, K. Bronchoalveolar Lavage of Murine Lungs to Analyze Inflammatory Cell Infiltration. *J Vis Exp*, doi:10.3791/55398 (2017).

5 Wyatt, L. H., Devlin, R. B., Rappold, A. G., Case, M. W. & Diaz-Sanchez, D. Low levels of fine particulate matter increase vascular damage and reduce pulmonary function in young healthy adults. *Part Fibre Toxicol* **17**, 58, doi:10.1186/s12989-020-00389-5 (2020).

6 Meier, R. *et al.* Associations of short-term particle and noise exposures with markers of cardiovascular and respiratory health among highway maintenance workers. *Environ Health Perspect* **122**, 726-732, doi:10.1289/ehp.1307100 (2014).

7 Barregard, L. *et al.* Experimental exposure to wood-smoke particles in healthy humans: effects on markers of inflammation, coagulation, and lipid peroxidation. *Inhal Toxicol* **18**, 845-853, doi:10.1080/08958370600685798 (2006).

8 Gutierrez, C. T. *et al.* Acute phase response following pulmonary exposure to soluble and insoluble metal oxide nanomaterials in mice. *Part Fibre Toxicol* **20**, 4, doi:10.1186/s12989-023-00514-0 (2023).

9 Poulsen, S. S. *et al.* Multi-walled carbon nanotube-physicochemical properties predict the systemic acute phase response following pulmonary exposure in mice. *PLoS One* **12**, e0174167, doi:10.1371/journal.pone.0174167 (2017).

10 Li, J. J. *et al.* Time course of inflammatory response after renal artery stenting in patients with atherosclerotic renal stenosis. *Clin Chim Acta* **350**, 115-121, doi:10.1016/j.cccn.2004.07.013 (2004).

11 Allan, C. K. *et al.* The relationship between inflammatory activation and clinical outcome after infant cardiopulmonary bypass. *Anesth Analg* **111**, 1244-1251, doi:10.1213/ANE.0b013e3181f333aa (2010).

12 Uhlar, C. M. & Whitehead, A. S. Serum amyloid A, the major vertebrate acute-phase reactant. *Eur J Biochem* **265**, 501-523, doi:10.1046/j.1432-1327.1999.00657.x (1999).

13 Cray, C., Zaias, J. & Altman, N. H. Acute phase response in animals: a review. *Comp Med* **59**, 517-526 (2009).

Relationship: 2960: Interaction with the lung cell membrane leads to Atherosclerosis

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Substance interaction with lung resident cell membrane components leading to atherosclerosis	non-adjacent	High	Moderate

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
Adults	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Mouse models of human atherosclerosis has been shown to present atherosclerotic lesion progression after exposure to concentrated ambient particles, welding fumes and diesel exhaust particles [3,5,7].

In humans, epidemiological studies have shown that air pollution, as a stressor that interacts with the lungs, is a risk factor for cardiovascular diseases [14].

Key Event Relationship Description

This KER presents the association between the interaction of stressors with the lungs and atherosclerosis as the outcome. The evidence of the KER presented is based on mouse models of human atherosclerosis.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility is moderate. Exposure to different stressors have been shown to induce the progression of atherosclerotic in mouse models of human atherosclerosis (see below). In humans, it has been hypothesized that air pollution, an example of stressor that interacts with the lungs, and cardiovascular diseases are linked by three pathways: i) translocation of inflammatory mediators from the lungs to the systemic circulation, ii) activation of alveolar receptors that results in the alteration of autonomic response and changes in cardiovascular function, and iii) translocation of particles (stressors) from the lungs to the systemic circulation [1,2].

Empirical Evidence

For this KER, exposure through the respiratory system (inhalation or intratracheal instillation) of stressors is considered as interaction with lung resident cell membrane components.

- $ApoE^{-/-}$ and double knockout ApoE-/-$LDLr^{-/-}$ mice exposed to concentrated ambient particles (110 µg/m^3) for 6h/d, 5d/week for 5 months develop severe atherosclerosis. $ApoE^{-/-}$ mice exposed to concentrated ambient particles presented a 57% increase in aortic intima surface coverage by atherosclerotic lesion than mice exposed to air [3].
- Intrapharyngeal aspiration of singlewalled carbon nanotubes into $ApoE^{-/-}$ mice (20 µg/mouse every 2 weeks for 8 weeks) induced a significant increase in plaque progression [4].
- $ApoE^{-/-}$ mice on a Western diet showed an increase in atherosclerotic lesion area after exposure to fumes from gas metal arc-stainless steel welding (40 mg/m^3) for 3h/day for 10 days [5].
- A modest increase in atherosclerotic plaque area was observed in $ApoE^{-/-}$ mice after intratracheal instillation of titanium dioxide nanoparticles (0.5 mg/kg) once a week for four weeks [6].
- $ApoE^{-/-}$ mice, fed a Western diet and exposed to diesel exhaust particles through oropharyngeal aspiration (35 µg) twice a week for four weeks, presented an increased atherosclerotic lesions area [7].
- Intratracheal instillation of human serum amyloid A once a week for 10 weeks in $ApoE^{-/-}$ mice (on Western-type diet) induced atherosclerotic plaque progression [8].

In addition, several epidemiological studies have shown that exposure to particulate matter from air pollution is associated to cardiovascular diseases:

- A prospective study in six cities from USA showed that air pollution was associated with death from cardiopulmonary diseases [9].
- In Dublin, there was a decrease in black smoke concentration in air, along with a significant decrease in the number of cardiovascular deaths, after the 1990 ban on coal sales [10].

Uncertainties and Inconsistencies

$ApoE^{-/-}$ mice seem to have a moderate plaque progression when feed a normal diet, instead of high-fat diet, and exposed to the stressor for a short period [6].

Quantitative Understanding of the Linkage

Response-response relationship

- Following the ban of coal in Dublin, a decrease of 70% of black smoke (35 µg/m^3) was observed along with a 10.3% decrease ($p<0.0001$) in cardiovascular deaths [10].
- A prospective study following postmenopausal women from USA for 6 years observed that an increase of 10 µg of $PM_{2.5}$ (particulate matter with a diameter of less than 2.5 µm) was associated with 24% increased risk of cardiovascular event and a 76% increased risk of death from a cardiovascular disease [11].
- Beelen et al. analyzed data from 22 European cohort studies on long-term exposure to air pollution and associations with cardiovascular diseases mortality. It was obtained that a $PM_{2.5}$ increase of 5 µg/m^3 was associated with 21% increased risk of death from cerebrovascular disease, while an increase of 10 µg/m^3 of PM_{10} (particulate matter with a diameter of less than 10 µm) was associated with an 22% increased risk of death from cerebrovascular disease [12].
- Results from 11 cohort studies on long-term exposure to air pollution and incidence of acute coronary events showed a 13% increased risk of coronary events associated to 5 µg/m^3 increase of $PM_{2.5}$, and a 12% increased risk of coronary events associated to 10 µg/m^3 increase of PM_{10} [13].
- A cohort study of population living in Denmark between 2005 and 2017, and aged more than 50 years old, showed that a 5 µg/m^3 increase of $PM_{2.5}$ was associated to a 22% increased risk of stroke, while an increase of 1.85 µg/m^3 increase of $PM_{2.5}$ was associated to a 5.3% increased risk of myocardial infarction [14,15].

References

1 Miller, M. R. & Newby, D. E. Air pollution and cardiovascular disease: car sick. *Cardiovasc Res* **116**, 279-294, doi:10.1093/cvr/cvz228 (2020).

2 Van Eeden, S., Leipsic, J., Paul Man, S. F. & Sin, D. D. The relationship between lung inflammation and cardiovascular disease. *Am J Respir Crit Care Med* **186**, 11-16, doi:10.1164/rccm.201203-0455PP (2012).

3 Chen, L. C. & Nadziejko, C. Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice. V. CAPs exacerbate aortic plaque development in hyperlipidemic mice. *Inhal Toxicol* **17**, 217-224, doi:10.1080/08958370590912815 (2005).

4 Li, Z. *et al.* Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. *Environ Health Perspect* **115**, 377-382, doi:10.1289/ehp.9688 (2007).

5 Erdely, A. *et al.* Inhalation exposure of gas-metal arc stainless steel welding fume increased atherosclerotic lesions in apolipoprotein E knockout mice. *Toxicol Lett* **204**, 12-16, doi:10.1016/j.toxlet.2011.03.030 (2011).

6 Mikkelsen, L. *et al.* Modest effect on plaque progression and vasodilatory function in atherosclerosis-prone mice exposed to nanosized TiO(2). *Part Fibre Toxicol* **8**, 32, doi:10.1186/1743-8977-8-32 (2011).

7 Miller, M. R. *et al.* Diesel exhaust particulate increases the size and complexity of lesions in atherosclerotic mice. *Part Fibre Toxicol* **10**, 61, doi:10.1186/1743-8977-10-61 (2013).

8 Christophersen, D. V. *et al.* Accelerated atherosclerosis caused by serum amyloid A response in lungs of ApoE(-/-) mice. *FASEB J* **35**, e21307, doi:10.1096/fj.202002017R (2021).

9 Dockery, D. W. *et al.* An association between air pollution and mortality in six U.S. cities. *N Engl J Med* **329**, 1753-1759, doi:10.1056/NEJM199312093292401 (1993).

10 Clancy, L., Goodman, P., Sinclair, H. & Dockery, D. W. Effect of air-pollution control on death rates in Dublin, Ireland: an intervention study. *Lancet* **360**, 1210-1214, doi:10.1016/S0140-6736(02)11281-5 (2002).

11 Miller, K. A. *et al.* Long-term exposure to air pollution and incidence of cardiovascular events in women. *N Engl J Med* **356**, 447-458, doi:10.1056/NEJMoa054409 (2007).

12 Beelen, R. *et al.* Long-term exposure to air pollution and cardiovascular mortality: an analysis of 22 European cohorts. *Epidemiology* **25**, 368-378, doi:10.1097/EDE.0000000000000076 (2014).

13 Cesaroni, G. *et al.* Long term exposure to ambient air pollution and incidence of acute coronary events: prospective cohort study and meta-analysis in 11 European cohorts from the ESCAPE Project. *BMJ* **348**, f7412, doi:10.1136/bmj.f7412 (2014).

14 Poulsen, A. H. *et al.* 'Source-specific' air pollution and risk of stroke in Denmark. *Int J Epidemiol* **52**, 727-737, doi:10.1093/ije/dyad030 (2023).

15 Poulsen, A. H. *et al.* Source-Specific Air Pollution Including Ultrafine Particles and Risk of Myocardial Infarction: A Nationwide Cohort Study from Denmark. *Environ Health Perspect* **131**, 57010, doi:10.1289/EHP10556 (2023).

16 Vaduganathan, M., Mensah, G. A., Turco, J. V., Fuster, V. & Roth, G. A. The Global Burden of Cardiovascular Diseases and Risk: A Compass for Future Health. *J Am Coll Cardiol* **80**, 2361-2371, doi:10.1016/j.jacc.2022.11.005 (2022).